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## Effect of crude protein level in high grain diets on calf growth and feed utilization

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## Efecto del nivel de proteína en raciones alto grano sobre el crecimiento de terneros y eficiencia de utilización del alimento

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## Efeito do nível de proteína em rações alto grão no crescimento dos bezerros e eficiência de uso da ração

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## Abstract

This study assessed the effect of crude protein level in the diet on animal performance, feed conversion ratio and N utilization, in early-weaned lot-fed calves. Four iso-energetic rations were formulated (ME: 12.0 MJ/kg DM) differing in the crude protein content (CP: 12%, 15%, 17% and 21%, DM basis), and offered in a random experimental design to 24 Hereford calves ( $107.2 \pm 21.5$  kg). Animals were individually housed and fed ad libitum twice a day for 84 days. Increasing CP levels in the diet promoted a quadratic response ( $P < 0.01$ ) for dry matter intake (kg/d) and liveweight gain (LWG, kg/d), with maximum LWG of 1.36 kg/d for CP= 17.5%, without differences in subcutaneous back fat thickness ( $3.70 \pm 0.73$  mm,  $P = 0.25$ ) or feed conversion ratio ( $4.44 \pm 0.26$ ,  $P > 0.10$ ). As CP increased in the diet, N consumption increased quadratically ( $P < 0.01$ ), and an improvement was observed for CP ( $P = 0.053$ ) and NDF ( $P = 0.020$ ) apparent digestibility. Daily excretion of N in feces tended to decrease ( $P = 0.06$ ), while urea-N concentration both in blood ( $P = 0.013$ ) and urine ( $P = 0.046$ ) increased. These results show that a level of 17% CP in the diet would optimize the performance of lot-fed early-weaned beef calves while controlling the excretion of N to the environment.

**Keywords:** calves, early weaning, growth, intake, N utilization

## Resumen

En el presente trabajo se evaluó el efecto del nivel de proteína bruta en la dieta sobre el crecimiento, eficiencia de conversión del alimento y utilización del N en terneros de destete precoz alimentados en confinamiento. Fueron formuladas cuatro raciones isoenergéticas (ME: 12,0 MJ/ kg MS), difiriendo en el contenido de proteína bruta (PB: 12 %, 15 %, 17 % y 21 % de la MS), y ofrecidas en un diseño completamente aleatorizado a 24 terneros Hereford ( $107,2 \pm 21,5$  kg). Los animales fueron estabulados individualmente y alimentados *ad libitum* en dos comidas diarias durante 84 días. El consumo de materia seca (kg/d) y la ganancia de peso vivo (GP, kg/d) aumentaron en forma cuadrática ( $P < 0,01$ ) al incrementarse el contenido de PB, registrándose GP máximas de 1,36 kg/d para PB= 17,5 %, sin diferencias en el espesor de grasa dorsal ( $3,70 \pm 0,73$  mm,  $P=0,25$ ) ni en el índice de conversión ( $4,44 \pm 0,26$ ;  $P > 0,10$ ). Conforme aumentó la PB en la dieta, el consumo de N aumentó de forma cuadrática ( $P < 0,01$ ), y mejoró la digestibilidad aparente de la PB ( $P=0,053$ ) y la FDN ( $P=0,020$ ). La excreción diaria de N en heces tendió a disminuir ( $P= 0,06$ ), mientras que la concentración de N ureico en sangre ( $P=0,013$ ) y en orina ( $P=0,046$ ) aumentó en ambos casos. Los resultados demostraron que un nivel de 17 % de PB en la dieta optimizaría la performance de terneros destetados precozmente a corral, simultáneamente controlando la excreción de N al ambiente.

**Palabras clave:** terneros, destete precoz, crecimiento, consumo, utilización de N

## Resumo

No presente trabalho avaliou-se o efeito do nível de proteína bruta na dieta sobre o crescimento, a eficiência da conversão alimentar e a utilização de N em bezerros confinados ao desmame precoce. Quatro rações isoeenergéticas foram formuladas (ME: 12,0 MJ / kg de MS) diferindo no teor de proteína bruta (PB: 12%, 15%, 17% e 21% de MS) e oferecidas num delineamento inteiramente casualizado a 24 bezerros Hereford ( $107,2 \pm 21,5$  kg). Os animais foram alojados individualmente e alimentados ad libitum em 2 refeições diárias durante 84 dias. O consumo de matéria seca (kg / d) e o ganho de peso vivo (GP, kg / d) aumentaram quadraticamente ( $P < 0,01$ ) com o aumento do teor de PB, registrando GP máximo de 1,36 kg / d para PB = 17,5%, sem diferenças na espessura da gordura dorsal ( $3,70 \pm 0,73$  mm,  $P = 0,25$ ) ou no índice de conversão ( $4,44 \pm 0,26$ ;  $P > 0,10$ ). Com o aumento da PB na dieta, o consumo de N aumentou quadraticamente ( $P < 0,01$ ), e a digestibilidade



aparente da PB ( $P = 0,053$ ) e da FDN ( $P = 0,020$ ) melhorou. A excreção diária de N nas fezes tendeu a diminuir ( $P = 0,06$ ), enquanto a concentração de N da uréia no sangue ( $P = 0,013$ ) e na urina ( $P = 0,046$ ) aumentou em ambos os casos. Os resultados mostraram que um nível de PB de 17% na dieta otimizaria o desempenho de bezerros desmamados precocemente no confinamento, controlando simultaneamente a excreção de N para o ambiente.

**Palavras-chave:** bezerros, desmame precoce, crescimento, consumo, uso de N

## 1. Introduction

Early weaning of calves at two months of age significantly improves the reproductive performance of primiparous and multiparous cows in poor body condition at the beginning of the service period<sup>(1)(2)(3)</sup>, but it challenges the nutrition of the early-weaned calf. During its first eight weeks of life, the calf depends greatly on its mother's milk<sup>(4)</sup>, which gradually declines as lactation progresses and, with it, the specific contribution of milk in the calf's diet<sup>(5)(6)</sup>. Supplementation with high energy-protein ration (ME 12 MJ/kg, CP 19%) on temperate pastures in British breed calves weaned at 60 days of age has been enough to achieve weight gains in the range of 0.6 to 0.7 kg/d<sup>(7)(8)</sup>, and a bodyweight of around 150 kg at 6 months of age, similar to those reported for lactating calves on native pastures<sup>(7)(8)</sup>. Calves' early weaning associated with lot feeding with concentrated diets allows, in addition to improving cows' reproductive rate, to significantly increase calves' live weight at 180 days of age, compared to that achieved in grazing<sup>(9)</sup>, positively impacting productivity (total kilograms of weaned weight/cow exposed to bull) and economic result of the cow-calf system<sup>(10)</sup>.

Given the early development stage of the early-weaned calves, protein requirements for the expression of growth potential are maximum compared to more advanced development stages<sup>(11)</sup>, which leads to increasing the CP concentration in diets offered to this category. However, although it is essential to ensure the daily intake of this nutrient, excessive CP in rations is undesired, both because of the negative effect on its cost, and the environmental impact of more N excretion in feces and urine<sup>(12)(13)</sup>. Although grazing animals need concentrates with 18-19% CP to complement the lower quality forage base, probably, when feeding high

concentrate total mixed rations the daily supply of metabolizable protein (MP) needed to meet calves' requirements to gain around 1.0-1.2 kg/d is achieved with less CP in the ration<sup>(14)</sup>.

There is little information quantifying the response of early-weaned calves of beef breeds to the CP level in the diet, varying in the magnitude and type of response in weight gain (WG) and feed conversion efficiency, depending on animal factors, such as weaning age and breed, evaluated CP range<sup>(15)(16)(17)</sup> and concentrate level in the diet<sup>(18)</sup>. On the other hand, the ability of nutritional models to predict the expected daily intake for this animal category, a fundamental input in the prediction of the net protein supply for growth, is not sufficiently validated. Pascoal and others<sup>(18)</sup> report an underestimation of the necessary CP intake by NRC (1984), compared to the observed values, given the same WG in early-weaned calves. A more precise adjustment between protein requirements and supply would contribute to improving the efficiency of the whole process.

This study aimed to evaluate the effect of increasing levels of CP in the diet on WG and feed efficiency of early-weaned Hereford calves, lot-fed with a high concentrate total mixed ration.

The hypothesis suggests that there is a level of CP contribution in the ration that optimizes the WG and conversion ratio, minimizing the N excretion.

## 2. Material and methods

The experiment was carried out in Cassinoni Experimental Station (EEMAC) of the Agronomy College (Paysandú, 32° 38' S; 58° 04' W) in the summer-autumn of 2013. Twenty-four open-air pens were used for individual housing (10 m<sup>2</sup>), with 30% of



shaded area and provided with a drinker with *ad libitum* water supply and a feeder each.

### 2.1 Animals, experimental design and treatments

Twenty-four Hereford calves castrated at birth and early-weaned at  $80 \pm 10$  days of age ( $90.1 \pm 13.6$  kg) were randomly assigned to one of four feeding treatments, after a 14-day transition period to solid diet. Four totally mixed rations were formulated (TMR, 82% concentrate, 18% roughage) differing in CP concentration: 12, 15, 18, or 21% (dry matter basis). The increase in CP content was generated by substituting ground corn grain for soybean meal, keeping the urea and the other ingredients constant, resulting in iso-energetic rations ( $ME = 12.0 \pm 0.03$  MJ/kg; Table 1).

Calves were group fed during the transition to solid diet according to the protocol reported by Simeone and Beretta<sup>(7)</sup>. After this process, the calves, already assigned to their treatment, were transferred to the experimental facilities, and for three weeks they gradually adapted to the individual lot housing and were introduced to their experimental diets. By the end of this period animals reached *ad libitum* intake and the initial LW was recorded ( $107.2 \pm 21.5$  kg).

The calves were treated against internal and external parasites with injectable 1% ivermectin (Ivomec® from Laboratorio MERIAL), and constantly monitoring their health status from then on.

During the 12-weeks experimental period, food was offered *ad libitum* in two daily meals of the same amount (8 am and 7 pm), adjusting the supply through bunk reading, so that residual food was not less than 10% of the offered.

Table 1. Ingredient and nutritional composition of the experimental rations

	Treatments <sup>I</sup>			
	P12	P15	P18	P21
<i>Ingredients</i> (% DM)				
Alfalfa hay	10.0	10.0	10.0	10.0
Rice husk	7.9	7.9	7.9	8.0
Corn grain	72.4	64.7	57.1	49.1
Soybean meal	6.6	14.3	21.9	29.8
Urea	0.53	0.53	0.53	0.53
Premix <sup>II</sup>	2.56	2.56	2.56	2.57
<i>Nutritional composition</i> (% DM)				
Organic matter	93.8	93.7	92.8	91.8
Crude protein (CP)	11.9	15.2	17.4	20.9
Neutral detergent fiber	24.3	23.7	20.7	25.3
Acid detergent fiber	10.2	11.1	9.7	11.6
Fats/oils	3.9	4.3	4.6	5.0
NSC <sup>III</sup>	63.3	59.4	55.4	50.8
RDP, % CP <sup>III</sup>	52.2	54.0	55.1	56.1
ME, MJ/kg DM <sup>III</sup>	11.97	12.01	12.00	11.95

I. Crude protein level in the ration: 12, 15, 18 and 21%, dry matter (DM) basis. II. Premix including: CaCO<sub>3</sub> (57.2%), NaCl (17.1%), Rumensin-10% monensin (2.3%); Zoodry feedlot (vitamin-mineral supplement) (11.7%); Beef-sacc yeasts (11.7%). III. Values based on BCNRM (14), RDP: rumen degradable protein; NSC: non-structural carbohydrates; ME: metabolizable energy



## 2.2 Measurements and sampling

Live weight (LW) was recorded every 14 days without fasting, before the first meal of the day. The dry matter intake (DMI) was estimated daily as the difference in dry weight between the offered feed and the residual food present in the feeder, which was weighed and discarded. Samples of the offered feed were taken weekly to determine the DM content, while individual leftovers were sampled every 15 days. Samples were dried in a forced-air stove at 60 °C to constant weight, then ground to 2 mm and preserved for further chemical analysis.

Rump height<sup>(19)</sup>, *Longissimus dorsi* muscle area (REA), and subcutaneous dorsal fat thickness (DFT) were measured at the beginning and end of the experimental period. These last two were determined by ultrasonography at 12.<sup>a</sup> and 13.<sup>a</sup> rib height, placing the transducer laterally in that area, and specific software for beef breed evaluation (BioSoft Tool Box II, Biotronic) processed the images.

The TMRs apparent digestibility was estimated *in vivo* in week 11 of the experiment, in two animals per treatment, through the total collection of feces<sup>(20)</sup>. They were collected for three consecutive days, directly from the floor of each lot, by directly observing the animals during the day and collecting the night production in the following morning before the first feeding. Fresh feces were weighed every 24 hours and one subsample per animal was dried for DM content determination, and then preserved for further chemical analysis. Blood and urine samples were taken on day 4 of week 11 from the same animals 4 hours after the first feeding of the day. The urine samples were taken by spontaneous urination according to the technique described by Santos and others<sup>(21)</sup>, keeping a subsample of 10 ml per animal diluted in 0.036 N sulfuric acid, so the pH is lower than 3. Blood samples were extracted by a puncture on the jugular vein and put in a test tube containing EDTA as anticoagulant, immediately centrifuged at 5000 rpm for 15 minutes, and the plasma conserved at -15 °C. In week 11, daily samples were taken from the supplied and leftover food. Samples were dried in a forced-air stove at 60 °C to constant weight to determine DM content, and preserved for further chemical analysis.

## 2.3 Chemical analysis

The chemical composition of the offered and leftover feed was analyzed on composite samples by treatment for the entire experimental period, determining ash content<sup>(22)</sup>, neutral detergent fiber using  $\alpha$ -amylase and correcting by ash contamination (aNDFom), acid detergent fiber (ADF)<sup>(23)</sup> and CP (total N\*6.25)<sup>(22)</sup>. Ash, CP and aNDFom concentration were also analyzed on composite samples of food and feces per animal corresponding to week 11 of the experimental period, and urea concentration was determined in plasma (BUN) and urine (UUN) samples<sup>(22)</sup>.

## 2.4 Calculated variables

The feed conversion ratio was calculated as the ratio between the daily average DMI (kg/d) and the WG (kg/d). The apparent digestibility of DM (DMD, %), organic matter (OMD), CP and NDF were calculated as the difference between daily intake of each fraction (FI) and the product of feces concentration (FC) by daily feces production (FP):  $D = (FI \text{ g/d} - FC\% \times FP \text{ g/d}) / FI \text{ g/d}$ . The FC was estimated from nutrient concentration of the (NC) in the offered feed (OF) and in the leftovers (CF, g/d = OF kg/d  $\times$  NC g/kg - leftovers kg/d  $\times$  NC g/kg).

The urinary excretion of N (NU, g/d) was estimated based on the prediction equation reported by Kohn and others<sup>(24)</sup>, from BUN (g/L) concentration and the urea elimination rate through urine (CR, L filtered blood per day/kg LW):  $NU \text{ g/d.kg LW} = CR \times BUN$ . The considered CR value was  $1.3 \pm 0.12 \text{ L/d.kg LW}$  reported for cattle by the same authors.

To interpret the observed response, the contribution of metabolizable protein (MP) and metabolizable energy (ME) in the different treatments was estimated from the BCNRM model (Beef Cattle Nutrient Requirements Model)<sup>(14)</sup>. The observed values of DMI and LW were used as inputs, and the tabular values of degradability of CP and the carbohydrates provided by the model were considered for the ingredients of the diet (under the assumption that they present the same amino acid composition and fermentation pattern as the ingredients used in TMRs).



## 2.5 Statistical analysis

The experiment was analyzed using linear models corresponding to a completely randomized design considering the animal as the experimental unit. Variables with repeated measures over time (LW and DMI) were analyzed using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC), including the initial LW as covariate. The WG was analyzed according to a linear mixed model of slopes heterogeneity of LW as a function of time, and the slopes of the fitted lines were compared by simple contrasts. Variables without repeated measures over time were analyzed using the SAS GLM procedure. When the treatment effect was significant, the linear and quadratic effects associated with the level of inclusion of CP in the TMR were evaluated. An effect was considered significant when the probability of type I error was lower than 5%, and it was considered a trend when it was lower than 10%.

## 3. Results

### 3.1 Animal growth

The effect of treatments on the variables that describe growth and development of calves is presented in Table 2.

The evolution of LW showed a linear adjustment ( $P < 0.01$ ) and the slope varied according to the treatment ( $P = 0.039$ ). The WG and final LW increased quadratically; registering maximum values of WG and final LW (predicted from adjusted regressions) of 1.36 kg/d and 219.4 kg for CP levels in the TMR of 17.5% and 18.0%, respectively (Figure 1). No differences in final rump height were detected ( $P > 0.10$ ), so the LW:height ratio had the same quadratic response as the final LW. There was no significant effect for the CP level on the REA, nor on the DFT ( $P > 0.10$ ).

Table 2. Effect of protein level in diet on growth and development parameters of early-weaned calves (84 days post-weaning)

	Treatments <sup>I</sup>					P-Value	
	P12	P15	P18	P21	SD	Linear	Quadratic
Live weight gain (kg/d) <sup>II</sup>	1.12	1.27	1.38	1.23	0.08	0.02	0.001
Final live weight (kg) <sup>III</sup>	202.0	215.1	220.9	209.3	6.62	0.039	<0.0001
Final Height (cm)	104.9	107.3	106.5	107.0	2.18	0.195	0.335
Final LW:final height <sup>IV</sup>	1.90	2.00	2.10	2.00	0.08	0.247	0.014
Final area of <i>Longissimus dorsi</i> (cm <sup>2</sup> )	40.6	42.9	42.5	39.4	4.08	0.591	0.135
Final subcutaneous back-fat thickness (mm)	3.6	3.3	3.7	4.0	0.73	0.265	0.358

I. Increasing levels of crude protein in diet 12, 15, 18 and 21% of ration in dry matter basis. II.  $WG, \text{kg/d} = -0.0083x^2 + 0.2897x - 1.167$ ;  $R^2 = 0.93$ . III.  $LW, \text{kg} = -0.6861x^2 + 23,565x + 17,515$ ;  $R^2 = 0.97$ . IV.  $LW:\text{Height} = -0.0056x^2 + 0.1967x + 0.33$ ;  $R^2 = 0.90$

### 3.2 Intake, apparent digestibility and conversion ratio

As the ration CP increased, DMI and N consumption increased quadratically ( $P < 0.01$ ), estimating a maximum DMI of 5.75 kg/d for a CP level of 17.6% (Table 3), while the maximum N consumption of 190 g/day was reached at CP = 21.3% (Figure 2). Intake expressed every 100 kg of LW did not vary with the treatment ( $3.84 \pm 0.16 \text{ kg}/100 \text{ kg LW}$ ;  $P > 0.10$ ), it only varied with the experimental week ( $P < 0.01$ ) regardless of the type of ration ( $P > 0.10$ ).

The DMD and OMD tended to increase linearly with the CP content of the diet ( $P < 0.10$ ), as did the digestibility of NDF and CP ( $P < 0.05$ ). As a consequence of the intake response and the observed digestibility coefficients, digestible CP and NDF intakes increased quadratically as the CP content in the diet increased ( $P < 0.01$ ; Table 3), as did the digestible OM intake ( $P < 0.01$ ; Figure 1).

The estimated daily contribution of MP based on BCNRM<sup>(14)</sup> was 481, 537, 612 and 617 g/d for CP levels in the TMR of 12, 15, 18 and 21%, respectively (Figure 1). As the proportion of soybean meal in the



ration increased and the corn grain decreased, the daily contribution of rumen degradable protein in P21 doubled compared to P12 (0.33 vs 0.60 kg/d). This did not affect the synthesis of microbial protein between treatments (0.33 vs 0.38 kg/d) but modified the N balance in rumen (-9.1, 4.5, 20.5 and 34.9 g N/d, for CP 12, 15, 18 and 21%, respectively).

The feed conversion ratio, was not significantly affected by the protein level in the ration. Nonetheless, a numerical tendency to improve this indicator was observed as the protein concentration increased in the diet up to CP = 18.0%.

Table 3. Effect of the level of protein in the diet on consumption, apparent digestibility and feed conversion efficiency

	Treatments <sup>1</sup>				SD	P-Value	
	P12	P15	P18	P21		Linear	Quadratic
<b>DM consumption (kg/d)</b>							
DM	5.24	5.50	5.89	5.51	0.094	<0.0001	<0.0001
OM	4.77	5.16	5.47	5.06	0.089	<0.0001	<0.0001
CP	0.62	0.83	1.02	1.15	0.016	<0.0001	<0.0001
NDF	1.27	1.30	1.22	1.39	0.023	<0.0001	<0.0001
<b>Apparent digestibility (%)</b>							
DM	80.60	88.30	89.30	89.70	3.98	0.090	0.265
OM	84.30	91.00	92.30	92.00	3.39	0.087	0.216
CP	84.10	91.20	94.60	94.60	1.86	0.004	0.053
NDF	78.10	84.60	89.20	90.40	3.49	0.020	0.343
<b>Conversion ratio (kg consumed/kg WG)</b>							
	4.60	4.36	4.33	4.44	0.26	0.319	0.120

1. Crude protein level in diet 12, 15, 18 and 21% of ration in dry basis. DM Dry matter, OM: organic matter, CP: Crude protein, NDF: neutral detergent fiber, LWG: live weight gain.

Figure 1. Nitrogen consumption (NC), total N in feces (NF), urea N in blood (BUN) and urine (UUN), in early-weaned calves fed with iso-energetic rations (ME 12.0 MJ/kg) differing in crude protein concentration.

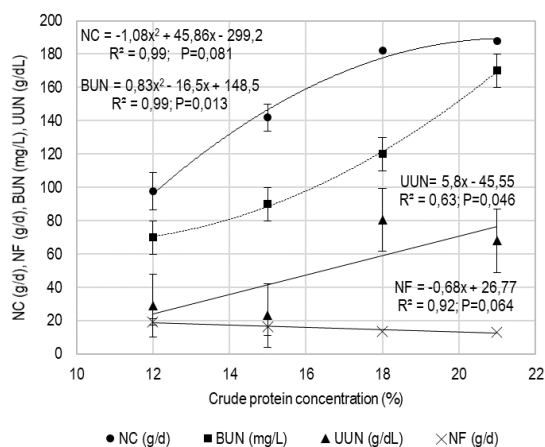
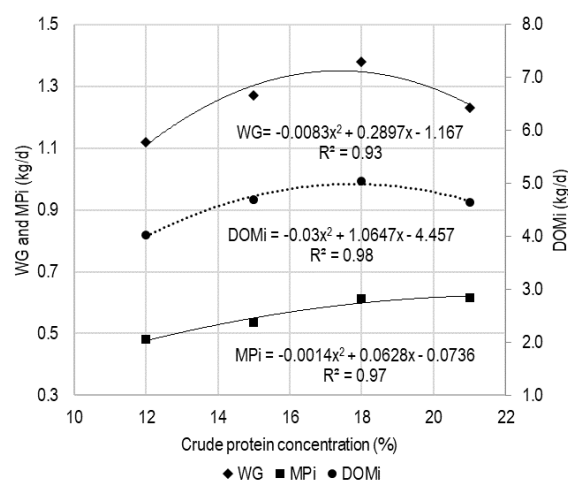


Figure 2. Weight gain (WG), metabolizable protein intake (MPI) and digestible organic matter intake (DOMi) in early-weaned calves fed with iso-energetic rations (ME 12.0 MJ/kg), differing in crude protein concentration







### 3.3 N in blood, feces, and urine

During the measurement period of BUN concentration and N excretion, the daily N consumption tended to increase quadratically with the CP level in the diet ( $P < 0.08$ ), as it did during the entire experimental period. Daily N fecal excretion decreased linearly ( $P < 0.06$ ), while urinary excretion increased linearly ( $NU \text{ g/d} = 2.29x - 16.19 \text{ R}^2 = 0.98; P < 0.01$ ). In parallel, BUN and UUN concentration increased quadratically ( $P = 0.013$ ) and linearly ( $P = 0.046$ ), respectively, with the CP level in the diet (Figure 2).

## 4. Discussion

### 4.1 Intake, digestibility, contribution of metabolizable protein and efficiency of N use

The DMI increased quadratically when the CP level increased in the diet, maximizing at 5.75 kg/d for a level of CP=17.5% in the diet. Expressed every 100 kg of LW, the DMI was of  $3.84 \pm 0.84\%$  ( $P > 0.10$ ), a value within the reported for this category, with *ad libitum* intake of concentrated rations such as the used in the present study<sup>(9)</sup>. Several authors also report quadratic-type increases in DMI in response to the CP level in the diet. Veira and others<sup>(16)</sup> registered a significant increase in the DMI up to 12% of CP, stabilizing from this value. These authors evaluated the effect of the CP level in similar types of diet and calves' age at weaning (8 to 12 weeks of age, 80 kg LW), although using a range of values lower than those of the present study (10% to 16% CP). Neville and others<sup>(17)</sup> also worked with concentrated diets (14% forage) and increasing CP levels in the diet of Hereford and Angus calves, but younger at weaning than those of the present study (48 days, varying between 21 to 60 days) and 74 kg of LW. They report a stable DMI for CP values in the diet between 14.5% and 23.5%, falling significantly when the CP increased to 28.5% (2.93 kg/d, 3.02 kg/d, 2.77 kg/d, respectively); however, N consumption increased linearly. Acayezu and others<sup>(25)</sup> also report a quadratic type response in the DMI against increasing CP levels in the diet (15% to 22%) when working with even lighter Holando calves (40 kg) but reached the maximum at higher values of CP (19%), falling significantly at 22%. On the contrary, other authors that measured the effect of the CP level in

less concentrated rations (roughage > 40%) found no response in DMI, both in early-weaned calves<sup>(18)(26)(27)</sup> and in finishing steers<sup>(12)(28)(29)</sup>.

The regulation of voluntary DMI depends on a group of factors with short and long-term effects, being the quality of the diet one of the main involved in short-term<sup>(11)</sup> regulations, mainly in feedlot conditions. In the present study, an increase in DMI was associated with an improvement in the apparent digestibility of OM, CP and NDF, a similar response to that observed by Obeid and others<sup>(28)</sup>, Veira and others<sup>(16)</sup>, and Neville and others<sup>(17)</sup>. Likewise, this increase in DMI associated with the CP level could contribute to modifying the site of digestion for part of the diet's starch and protein, through a positive effect on the passage rate<sup>(30)</sup>.

As a result of the response in DMI and the increase in the CP concentration in the diet, the N consumption ( $\text{g/kg LW}^{0.75}$ ) increased 80% when going from 12% to 21% of CP in the diet, significantly improving the apparent digestibility of DM, OM, CP and NDF. Other authors also report an improvement in the digestibility of CP<sup>(16)(25)(27)</sup>, and NDF<sup>(16)</sup> by increasing the concentration of N in the diet, while other studies do not report an effect on the fiber<sup>(25)(27)</sup>.

Increasing substitution levels of corn grain for soybean meal resulted in different protein concentration among the TMRS, therefore, differences in the fermentation characteristics between ingredients could have also contributed to generate differences in the synthesis of microbial biomass and the contribution of non-degradable protein in the rumen, differentially affecting the MP available in each treatment. The daily contribution of MP (kg/d), estimated based on BCNRM<sup>(14)</sup> from the observed intake and ingredient composition of each diet, increased 28% when the CP in the diet went from 12% to 21%. In parallel, the N balance calculated at ruminal level showed a linear increase, going from negative values in P12, where the contribution of rumen degradable protein was the limiting factor for the synthesis of microbial protein, to maximum levels in P21, where the balance of N in rumen represented a 57% excess according to the requirements. Furthermore, the contribution of the microbial protein to MP fell from 51.5% in P12 to 39.7% in P21, probably modifying the amino acid profile of MP as well.



From the point of view of the N use efficiency, the excess of N in the rumen resulting from the positive balance observed would increase the concentration of ruminal ammonium, its absorption through the rumen walls, and later, at a metabolic level, it would be converted into urea and excreted through urine<sup>(11)</sup>. This would explain the increase observed in the BUN and UUN concentration in the present study, as N consumption increased (Figure 2). High N consumption has been related to high BUN concentration<sup>(31)</sup> and high urinary excretion<sup>(32)</sup>. Cole and others<sup>(31)</sup> report a limit BUN concentration of 9 mg/dL, above which it is considered that the consumption of N would be excessive. In the present study, minimum levels of BUN were registered in P12, followed by a sustained increase in BUN concentration for each variation percentage unit in the CP concentration of the diet (7, 9, 12 and 17 mg/dL for ration CP levels of 12%, 15%, 18% and 21%, respectively), showing that CP levels above 15% would already be excessive (Figure 2). Kohn and others<sup>(24)</sup> report a close linear relationship between BUN concentration (mg/dL) and excretion rate of N in urine (NU, g/d.kg LW) in all species. Daily NU excretion estimated for the present study was minimal in P12 (13.9 g/d), increasing 2.5 times in P21 (35.0 g/d). This elimination process is not only undesirable from an environmental point of view<sup>(12)</sup>, but it would also result in an increase in energy costs for maintenance<sup>(33)</sup>.

#### 4.2 Growth and feed conversion ratio

Calves' WG increased quadratically, registering decreasing increments up to a CP diet inclusion level of 17.5%, and a decrease from this value, accompanying the variation observed digestible OM intake and MP contribution (Figure 1). Other authors also observed such response with early-weaned calves fed with concentrated diets; the optimal level differing according to age (19-20% in younger calves)<sup>(17)(25)</sup>, and breed (around 14-16% in Holando calves)<sup>(15)(16)</sup>.

Weight gain is determined by the contribution of energy substrates and intestinal amino acid absorption. Furthermore, protein retention will depend on the MP usage efficiency, also conditioned by the availability of energy substrates and essential amino acids that might be limiting<sup>(34)</sup>. The nutritional

evaluation of the diets based on the BCNRM<sup>(14)</sup> model showed that, in all treatments, the daily MP intake limited the expression of higher WG rates, which could have been achieved according to the energy intake. It is important to highlight the good fit between WG predicted by the model and the observed for the CP levels in the diet of 12%, 15% and 18% ( $R^2 = 0.97$ ). However, this adjustment occurred when the observed intake was used, which was in all treatments, well above the one predicted by the model. The positive WG response to increases in the contribution of MP up to P18 would be a consequence of the high protein requirements during the early phase of the animal's life<sup>(11)</sup> when it registers an important growth in lean body mass, and a lower increase rate of adipose tissue, which deposits later<sup>(35)</sup>. Bartlett and others<sup>(36)</sup>, and Donnelly and others<sup>(37)</sup> document an increase in muscle deposition in calves as a response to the increase in the CP content of the diet, simultaneously registering an increase in water and protein content, and a decrease in fat content<sup>(37)</sup>. The response observed in REA and DFT, as indicators of the gain composition, was numerically consistent with the reported by the aforementioned authors. Greater muscle deposition (higher REA values) was recorded in P15 and P18, while DFT was not affected; both variables presented a high negative correlation ( $r = -0.79$ ). Gleghorn and others<sup>(29)</sup> reported that even when increasing CP levels in the ration did not affect the DMI of finishing steers, a quadratic increase in carcass weight was observed with a maximum of 13% CP, with no effect of the CP level on DFT. WG fall as of P18 (-11%) would partly respond to the DMI drop (-6%), but it could also be due to lower efficiency in the use of MP<sup>(37)</sup>, added to a change in the partition of the ME consumed. The latter would be attributable to the probable increase in maintenance requirements resulting from a higher energy cost for the detoxification of N<sup>(33)</sup> excess in P21, and the consequent reduction in the proportion of consumed ME available for WG.

The conversion ratio did not show statistically significant variations when varying CP concentration in the diet, which was consistent with the same type of quadratic response observed for the DMI and the WG. However, at a numerical level, more favorable values were observed as the protein in the ration



increased to around 17-18%. In this range, although DMI increases, WG would do so more than proportionally. This could be associated with the positive impact of CP level on the digestibility of OM, CP and NDF, the increase in MP contribution and the characteristics of amino acids absorbed regarding the requirements for WG<sup>(14)</sup>. Above 18% CP, not only an improvement in CP digestibility is no longer observed (Table 3), but the metabolic cost associated with the excretion of excess N<sup>(33)</sup> would be much higher than that registered for lower CP levels in the diet, while BUN concentration increased more than proportionally when the concentration of CP in the diet was of 21%.

## 5. Conclusions

Early-weaned Hereford calves (80 ± 10 days-old) fed with concentrated rations (18% roughage, ME: 12.0 MJ/kg) during post-weaning, maximized their growth when the CP content of the diet ranged between 17% and 18%, achieving WG of 1.36 kg/d and weights of 219 kg when leaving the feedlot. However, a lower CP level of around 15% would not significantly affect the feed conversion and would maintain a good WG (1.31 kg/d), while simultaneously reducing N excretion, presenting a benefit from an environmental point of view.

### Authors contribution statement

VB: Conceptualization, data analysis, article writing and editing; AS: conceptualization, supervision, data collection, and manuscript review; IM: data collection; IY: data collection.

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