

VICTOR VALÉRIO DE CARVALHO

**PREWEANING NUTRITIONAL EFFECTS OF SUPPLEMENTS ON PERFORMANCE
OF SUCKLING BEEF CALVES GRAZING TROPICAL PASTURES**

Thesis submitted to the Animal
Science Graduate Program of the
Universidade Federal de Viçosa, as
partial fulfillment of the requirements
for the degree of *Doctor Scientiae*

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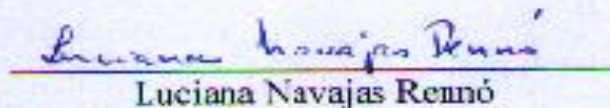
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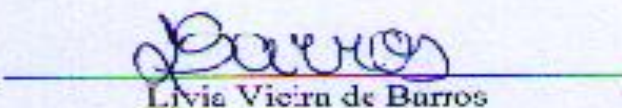
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Dedicated to:

My grandparents Wilson, Belarmino and Odilia (in memorian) and Wany,

My parents Robson and Cleone,

And brothers Otávio, Robson Jr, Ananda and Lucas

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BIOGRAPHY

VICTOR VALÉRIO DE CARVALHO, son of Robson Dutra de Carvalho and Cleone Valério de Oliveira, was born in Manhumirim, Minas Gerais, on July 11th of 1987.

In March of 2007, he joined the Animal Science undergrad at Universidade Federal de Viçosa; between 2010 and 2011, he participated of an exchange program in Denmark, and then concluded his course in July of 2012. In August of 2012, he started the Master's degree in the Animal Science Department of Universidade Federal de Viçosa, with major in Ruminant Nutrition and Production, concluding this course in March of 2014. Then, he started his PhD course in the same area and department, concluding the thesis in July of 2018, after a period of research scholar program in the Texas A&M University, from January of 2017 to July of 2017.

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ABSTRACT

CARVALHO, Victor Valério de, D.Sc., Universidade Federal de Viçosa, July, 2018. **Prewaning nutritional effects of supplements on performance of suckling beef calves grazing tropical pastures.** Adviser: Mário Fonseca Paulino.

For this thesis, two chapters were prepared based on studies with Nellore cow-calf pairs, submitted to different creep-feeding supplementation strategies. In the first chapter forty Nellore cow-calf pairs (iBW= 131±3.5 kg, 3 mo of age) and their respective dams (iBW = 457±9.3 kg, 7 yr of age) were randomly assigned to *Brachiaria decumbens* pastures (CP, 89 g/kg DM; NDF, 572 g/kg DM) to evaluate the effects of supplements with increasing RUP contents on nutritional and productive performance, and metabolic responses. The treatments were: control (no supplement) and supplements with similar CP content (230 g/kg DM) and increasing levels of RUP (Low = 94; Medium = 132 and High = 170 g RUP/kg DM) fed at 10 g/kg BW. Supplemented calves had greater intake of DM, CP and TDN intake compared with control calves, but there were no differences among calves fed supplements with the increasing RUP levels. The supplements RUP levels had no adverse effect on forage intake nor in fibre and organic matter digestibility, neither on rumen microbial protein synthesis. Increasing the supplement RUP content linearly decreased mRNA expression of genes associated with liver ureagenesis, carbamoyl phosphate synthetase (CPS) and argininosuccinate synthetase (AS), with a concurrent linear decrease in serum urea-N concentration (SUN) and urinary urea-N excretion (U). However, there was no effect mRNA expression of genes associated with liver gluconeogenesis (phosphoenolpyruvate carboxykinase, PEPCK) among supplemented calves, which parallels the lack of treatment effect for serum glucose and insulin concentrations. Moreover, no differences were detected for T3 and T4 serum concentration. Supplementation increased (<0.01) calves average daily gain (~ 0.997 vs. 0.793 kg/d), and weaning weight (~270 vs. 241 kg), in which ultrasound carcass traits (REA and SFT) traits were also greater than non-supplemented calves, however, these variables were similar among supplemented calves. These results indicate that increasing the supplement RUP contents had no adverse effect on calves' intake and digestibility. In addition, as supplement RUP level increased, mRNA expression liver ureagenesis, UUN and SUN decreased, which indicates a better N use efficiency, though all supplements provided similar performance. The second chapter aimed to evaluate the effects of creep-feeding supplementation on growth performance and efficiency, feed intake and digestibility by Nellore calves grazing tropical pastures, using a meta-analytical

approach. The dataset were collected from 18 studies, which were carried out between 2007 and 2017, totaling 75 treatment means that represented 832 cow-calf pairs. All studies had a control treatment with no creep-feeding supplementation (**non creep-fed calves**), and a wide variety of creep-feeding treatments (**creep-fed calves**), that were evaluated herein as daily amount of supplement intake (SUP, g/kg BW, DM basis) and supplemental crude protein intake (SCPI, g/kg BW); whereas dependent variables were evaluated as differential values relative to non creep-fed calves. Forage DM intake (g/kg BW) decreased linearly as SUP increased, whereas milk DM intake (g/kg BW) were not affected, though total DM intake increased linearly. The fibre digestibility responded quadratically to SUP and SCPI; however, DM digestibility and TDN intake increased linearly as SUP increased. The supplemental weight gain (SWG) responded quadratically as SUP ($P < 0.01$) and SCPI ($P < 0.01$) increased, and there was an influence of calf sex. The maximum responses were estimated at 7.6 g/kg BW (0.2 kg/d) for bull calves, and at 5.5 g/kg BW (0.1 kg/d) for heifer calves. The calculated supplement crude protein content for maximal responses were 225 and 224 g CP/kg DM for bull and heifer calves respectively. Likewise, supplemental feed efficiency responded quadratically to SUP and SCPI levels. We conclude that creep-feeding improves preweaning weight gain, in which calves responds differently according to sex. Nonetheless, the best results in supplemental weight gain is achieved with moderate amounts of protein supplements.

RESUMO

CARVALHO, Victor Valério de, D.Sc., Universidade Federal de Viçosa, julho de 2018. **Efeitos nutricionais de suplementos no desempenho de bezerros de corte lactentes em pastagens tropicais.** Orientador: Mário Fonseca Paulino.

Para esta tese, dois capítulos foram elaborados com base em estudos com vacas e bezerros Nelore, submetidos a diferentes estratégias de suplementação em creep-feeding. No primeiro capítulo, quarenta bezerros Nelore ($PCi = 131 \pm 3,5$ kg, 3 meses de idade) e suas respectivas fêmeas ($PCi = 457 \pm 9,3$ kg, 7 anos de idade) foram aleatoriamente distribuídos em pastagens de *Brachiaria decumbens* (PB, 89 g / kg de MS; FDN, 572 g / kg de MS) para avaliar os efeitos de suplementos com concentrações crescentes de PNDR no desempenho nutricional e produtivo e nas respostas metabólicas. Os tratamentos foram: controle (sem suplementação) e suplementos com teor de PB similar (230 g / kg MS) e níveis crescentes de PNDR (Baixo = 94; Médio = 132 e Alto = 170 g PNDR / kg MS) fornecidos na quantidade de 10 g / kg de peso corporal. Os bezerros suplementados tiveram maior consumo de MS, PB e NDT em comparação com os bezerros controle, mas não houve diferenças entre os bezerros suplementados com os níveis de PNDR. Os níveis de PNDR não apresentaram efeito adverso no consumo de forragem, nem a digestibilidade da fibra e da matéria orgânica, tampouco na síntese de proteína microbiana no rúmen. O aumento do conteúdo de PNDR nos suplementos diminuiu linearmente a expressão de genes associados à ureagênese hepática, carbamoil fosfato sintetase (*CPS*) e argininosuccinato sintetase (*AS*), com uma diminuição linear concomitante na concentração sérica de N ureico (SUN), assim como na excreção de N-ureico na urina. No entanto, não houve efeito na expressão de mRNA de genes associados à gliconeogênese do fígado (fosfoenolpiruvato carboxiquinase, *PEPCK*) entre os bezerros suplementados, o que se assemelha à falta de efeito do tratamento para as concentrações séricas de glicose e insulina, e não foram detectadas diferenças para T3 e T4. A suplementação aumentou ($<0,01$) o ganho médio diário de bezerros ($\sim 0,997$ vs. $0,793$ kg / d) e o peso à desmama (~ 270 vs 241 kg), nos quais características de ultra-som (AOL E EGS) também foram maiores do que os bezerros não suplementados, no entanto, essas variáveis foram semelhantes entre os bezerros suplementados, indicando que o aumento do conteúdo do suplemento não afetou o consumo e digestibilidade de bezerros. Além disso, como o nível suplementar de PNDR aumentou, a expressão de mRNA da ureagênese do fígado, NUU e NUS diminuiu, o que indica uma melhor eficiência de uso de N, entretando não resultou em maior desempenho dos bezerros. O segundo

capítulo teve como objetivo avaliar os efeitos da suplementação com creep-feeding no desempenho e na eficiência de crescimento, e no consumo e digestibilidade de bezerros Nelore em pastagens tropicais, utilizando uma abordagem meta-analítica. O conjunto de dados foi coletado de 18 estudos, que foram realizados entre 2007 e 2017, totalizando 75 médias de tratamento que representaram 832 vacas e bezerros. Todos os estudos tiveram tratamento controle, sem suplementação (bezerros não alimentados em creep-feeding), e uma grande variedade de tratamentos de creep-feeding (bezerros alimentados em creep-feeding), que foram avaliados como quantidade diária de suplemento (SUP, g / kg de PC, com base na MS) e consumo suplementar de proteína bruta (CSPB, g / kg PC); enquanto as variáveis dependentes foram avaliadas como valores diferenciais em relação aos bezerros não alimentados em creep-feeding. O consumo de MS de forragem (g/kg de PC) diminuiu linearmente com o aumento da suplementação, enquanto o consumo de MS (g/kg de PC) não foi afetado, embora o consumo total de MS tenha aumentado linearmente. A digestibilidade da fibra respondeu quadraticamente a SUP e CSPB; no entanto, a digestibilidade da MS e a ingestão de NDT aumentaram linearmente com o aumento do SUP. O ganho de peso suplementar (GPS) respondeu de forma quadrática, com o aumento da SUP ($P < 0,01$) e de CSPB ($P < 0,01$) e houve influência do sexo em bezerros. As respostas máximas foram estimadas em 7,6 g / kg de peso vivo (0,2 kg / dia) para bezerros, e em 5,5 g / kg de peso vivo (0,1 kg / dia) para bezerras. O teor de proteína bruta do suplemento calculado para as respostas máximas foi de 225 e 224 g de PB / kg de MS para bezerros e bezerras, respectivamente. Da mesma forma, a eficiência alimentar suplementar respondeu de forma quadrática aos níveis SUP e CSPB. Conclui-se que a suplementação em creep-feeding melhora o ganho de peso durante a fase de cria, onde os bezerros apresentam diferentes respostas de acordo com o sexo. No entanto, os melhores resultados em ganho de peso suplementar são obtidos com quantidades moderadas de suplementos protéicos.

GENERAL INTRODUCTION

Although Brazil has a significant production of beef cattle, the age at which the animals are slaughtered is still high, which compromises the efficiency of the production system and the quality of the meat. One of the factors that negatively affect these parameters is the low productive performance of the calves during the suckling phase, which also contributes to the cow-calf operations to present the lowest profitability among the production phases of beef cattle in Brazil.

Protein supplementation has been widely recommended for cattle consuming tropical forage. The main objective is to meet the requirements of rumen degradable protein (RDP), providing sufficient amounts of nitrogen to optimize ruminal microbial growth, in order to maximize the fermentation of fibrous carbohydrates from forage and microbial protein flow to the small intestine (Detmann et al., 2014).

In beef cattle, milk is not sufficient to meet the requirements of calves after three months of age (Henriques et al., 2011) which occurs simultaneously with pasture quantity and quality declining due to rainy to dry season transition, which can present one or more nutritional limitations that will compromise forage intake and digestion, or the metabolism of absorbed substrates (Detmann et al. 2014). Thus, strategies to supplement nutrients for calves, which are not fully obtained by the intake of pasture and milk, have been effectively increased the productive performance of the animals (Paulino et al., 2018). Therefore, creep-feeding supplementation may increase cow-calf operation efficiency, reduce age at first conception in heifers, and reduce slaughter age and improve meat quality in males, which all can lead to a better use of land and sustainability of beef cattle production.

Therefore, the objective of this thesis was to consistently evaluate the effects of different supplements amounts and composition on nutritional and productive performance of suckling beef calves grazing on tropical pastures.

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CHAPTER 1

Performance and metabolic responses of nursing beef calves fed supplements with increasing contents of rumen undegradable protein

ABSTRACT. Forty Nellore cow-calf pairs (iBW= 131±3.5 kg, 3 mo of age) and their respective dams (iBW = 457±9.3 kg, 7 yr of age) were randomly assigned to *brachiaria decumbens* pastures (CP 89 g/kg DM; NDF, 572 g/kg DM) to evaluate the effects of supplements with increasing RUP contents on nutritional and productive performance, and metabolic responses. The treatments were: control (no supplement) and supplements with similar CP content (230 g/kg DM) and increasing levels of RUP (Low = 94; Medium = 132 and High = 170 g RUP/kg DM) fed at 10 g/kg BW. Supplemented calves had greater intake of DM, CP and TDN intake compared with control calves, but there were no differences among calves fed supplements with the increasing RUP levels. The supplements RUP levels had no adverse effect on forage intake nor in fibre and organic matter digestibility, neither on rumen microbial protein synthesis. Increasing the supplement RUP content linearly decreased mRNA expression of genes associated with liver ureagenesis, carbamoyl phosphate synthetase (*CPS*) and argininosuccinate synthetase (*AS*), with a concurrent linear decrease in serum urea-N concentration (SUN) and urinary urea-N excretion. However, there was no effect mRNA expression of genes associated with liver gluconeogenesis (phosphoenolpyruvate carboxykinase, *PEPCK*) among supplemented calves, which parallels the lack of treatment effect for serum glucose, insulin concentrations, T3 and T4 concentrations. Supplemented calves had greater (<0.01) average daily gain (~ 0.997 vs. 0.793 kg/d), and weaning weight (~270 vs. 241 kg), in which ultrasound carcass traits (REA and SFT) traits were also greater than non-supplemented calves, however, these variables were not improved by the increasing RUP levels. These results indicates that increasing the supplement RUP contents had no adverse effect on calves' intake and

digestibility. In addition, as supplement RUP level increased, mRNA expression liver ureagenesis, UUN and SUN decreased, which indicates a better N use efficiency, though all supplements provided similar performance.

1. Introduction

Protein supplementation have been recommendend to increase weaning weight of suckling beef calves grazing on tropical pasture, and the optimum crude protein concentrations in supplements have already been studied (Lopes et al. 2014). However the optimal adjustments of rumen undegradable protein (RUP) content have not been studied, which could improve the protein use efficiency and leads to greater performance. Supplements should be properly formulated in order to present balanced amounts of rumen degradable protein (RDP) and rumen undegradable protein (RUP) to maximize microbial growth and protein utilization. Supplements containing rumen degradable protein (RDP) in excess may cause an overly ammonia production in the rumen due to protein degradation rates higher than the bacterial protein synthesis. The surplus of N-NH₃ is converted in urea in the liver and can be partially excreted in the urine (Lapierre & Lobley, 2001). Therefore, the ruminal degradation of protein is one of the main factors that contributes to the low efficiency of this nutrient in ruminants, which can impact negatively the environment due to the urea excretion via urine (Van den Borne et al. 2006; Dijkstra et al. 2013), and also compromise the feasibility of supplementation.

The supplementaion with RUP sources have improved the efficiency of protein utilization (Titgemeyer et al. 1989) and the N retention in beef cattle (Batista et al. 2016), as well as the N retention and productive performance in calves (Wankhede & Kalbande, 2001; Pattanaik et al. 2003), likely due to greater flow of aminoacids to the intestine that were not degraded in the rumen. Even though milk represents an important source of metabolizable protein, the lower milk

production in late lactation may not be sufficient to support beef calves potential growth (Lardy 2001; Costa e Silva et al. 2015). Additionally, it has been pointed out that young cattle needs RUP besides the microbial protein to supply their metabolizable protein requirements (Klopfenstein, 1996). Thus, we hypothesized that providing supplements with increasing RUP contents would improve calves performance during the suckling phase. Therefore, the objective of this study was to evaluate the effects of feeding supplements with increasing RUP levels on nutritional and productive performance as well as on metabolic responses of Nellore calves grazing on tropical pastures.

2. Material and methods

All practices involving the use of animals were approved by the Institutional Animal Care and Use Committee of the Universidade Federal de Viçosa (protocol CEUAP-UFV number 05/2016).

2.1. Animals, experimental design and supplements

This experiment was carried out at the Beef Cattle Section of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (20° 45' S 42° 52' W), between January and June, which corresponded to the rainy-dry transition season. The average temperature and precipitation values were 22.6°C and 521 mm, respectively (Department of Agricultural Engineering - UFV).

Forty Nellore calves (iBW= 131±3.5 kg, 3 mo of age) and their respective dams (iBW = 457±9.3 kg, 7 yr of age) were used in this experiment. The cow-calf pairs were distributed in a completely randomized design with 4 treatments and 10 replicates. The treatments were as follows: Control (no supplementation), and supplement formulated to contain similar amounts of crude protein (230 g CP/kg DM) and increasing levels of RUP (Low = 94; Medium = 132 and High = 170 g RUP/kg DM) fed at 10 g/kg BW (as-fed, Table 1).

The cow-calf pairs were allocated in four paddocks of 10 hectares each (one for each treatment), covered with *Brachiaria decumbens*, and provided with covered troughs and drinkers. Next to dams' troughs, there were troughs with access restricted to the calves (creep feeders) in which the different treatments were provided. Cows and calves were adapted to experimental feeding and management over 14 days prior the evaluation period, when all calves received similar supplement amount and composition (CP, 250 g/kg DM, fed at 5g/kg BW). The experimental period lasted 140 d, when the calves were weaned with approximately 8 mo of age.

The supplement was delivered daily at 10h00. Cows received mineral mixture *ad libitum*. Animals were weighed every 30 d without fasting at 6h00 in order to adjust the amount of supplement to be provided to each group. To prevent effects of the plots on the experimental treatments, animals were rotated among the four paddocks every seven days, so each group stayed for the same period of time on each plot.

2.2. Experimental procedures and sampling

Cows and calves were weighed after a 14-hour fasting period at the beginning and at the end of the experimental period to evaluate weight gain. The pasture was sampled every 28 days during the experiment to quantify the availability of DM by cutting biomass at ground level in four randomly selected 0.5 × 0.5 m quadrats in each paddock. All pasture samples were oven-dried (60°C) and ground through 1 and 2 mm screens prior to analysis. Qualitative evaluations of the pasture consumed by the animals were performed by the hand-plucking method every 14 days.

To evaluate the nutritional characteristics of the calves' diet, a 10-days digestibility trial was carried out on day 70 using the three marker method. The first six days were used to stabilize the markers flow in the gastrointestinal tract of the calves, and the last 4 days for faecal collection at 16h00 on day 7; at 14h00 on day 8; at 10h00 on day 9 and at 06h00 on day 10. To estimate faecal excretion, calves received 10g of chromic oxide (Cr₂O₃) as external marker (Detmann et al. 2001).

The Cr₂O₃ was weighed into paper cartridges, which were inserted directly into the esophagus of each calf at 10h00 daily during the digestibility period. Titanium dioxide (TiO₂) was included in the supplement (Titgemeyer et al. 2001) at 10 g/kg of supplement offered to estimate supplement intake by individual calves. The indigestible neutral detergent fibre (iNDF) content was used as an internal marker to estimate forage DM intake (Detmann et al., 2001).

The milk intake by the calves was assumed to be equal to dams' milk yield, which was estimated using oxytocin and machine milking method, performed at three different periods throughout each experiment, following similar procedures described by Almeida et al. (2018).

Spot urine samples were obtained four hours after supplementation, from calves on the final day of each digestibility period after spontaneous urination. Urine samples were diluted with 0.036 N H₂SO₄ and stored at -20°C prior to analysis. Blood samples were collected from calves approximately four hours after supplementation at the end of each digestibility period, and at the end of the experiment (weaning). Blood samples were collected from the jugular vein into containing a clot activator and separator gel (BD Vacutainer®, SST II Advance) and were immediately centrifuged at 3,600 x g for 15 min. Serum was collected and stored until subsequent analysis.

On the final day of the experiment, rib eye area (REA), subcutaneous fat thickness (SFT), and fat thickness at the P8 site (P8TF) was measured, using an Aloka Ultrasound (model SSD 500V, Aloka Co., Ltd., Tokyo, Japan.) with a 18 cm linear probe. The images were analyzed in the Biosoft Toolbox® II Beef program (Biotronics Inc., Ames, Iowa, USA). Subsequently, liver samples (average 100 mg of tissue, wet weight) were collected from 5 random calves per treatment, between the 11th and 12th ribs by percutaneous needle biopsy using similar procedures described

by Arthington and Corah, (1995), rinsed and snap-frozen in liquid nitrogen and stored at -80°C until total RNA isolation and quantitative real-time PCR analysis.

2.3. Analytical procedures

All samples were oven-dried at 60°C for 72 h and ground through a 2-mm screen in a Wiley mill (model 3, Arthur H. Thomas, Philadelphia, PA). Subsequently, half of each sample was ground through a 1-mm screen. Samples of forage, feces, and supplement processed through 1-mm screen sieve were analyzed according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al. 2012) for dry matter (DM; INCT-CA method G-003/1), ash (INCT-CA method M-001/1), crude protein (CP; INCT-CA method N-001/1) by Kjeldahl method, ether extract (EE; INCT-CA method G-004/1) by Randall method, neutral detergent fiber (NDF; INCT-CA method F-002/1), using alpha thermostable amylase without addition of sodium sulfite and corrected for ash and protein.

Indigestible neutral detergent fiber (iNDF; INCT-CA method F-009/1) was quantified in samples processed at 2 mm sieve by in situ incubation procedures with F57 bags (Ankom®) for 288 hours. In addition, fecal samples were evaluated for the contents of chromium (INCT-CA method M-005/1) and titanium (INCT-CA method M-007/1).

Fecal excretion was estimated by rationing the quantity of chromic oxide offered and the concentration in feces. Individual supplement DM intake was estimated by relation of excretion of TiO₂ in feces and marker concentration in the supplement. The forage DM intake was estimated using iNDF as an internal marker according to Detmann et al. (2001).

The milk samples were analyzed for protein, fat, lactose, and total solids content by infrared spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark). Then, average milk yield was calculated and corrected to 4% of fat (fat-corrected milk yield, FMY, kg/d; NRC 2001).

In the urine samples, analyses were carried out for creatinine, uric acid and urea by colorimetric kinetic, enzymatic colorimetric and kinetic fixed time methods, respectively, using automatic biochemical analyzer (Mindray, BS200E, China) and tests kits (Bioclin, Belo Horizonte, Brazil). In the serum from blood samples, the concentrations of urea and glucose were analysed using an automatic biochemical analyser (Mindray, BS200E, China). In addition, the concentrations of insulin, T3 and T4 were analyzed using tests kits (Beckman Coulter®, USA) and determined by immunoassay techniques (Access® 2, Beckman Coulter, USA).

The daily urinary volume was estimated using the relationship between the daily creatinine excretion (CE), calculated using the equation proposed Silva et al. (2012), and its concentration in the spot samples.

Allantoin was analyzed by the colorimetric method as described by Chen and Gomes (1992). The total excretion of purine derivatives was calculated by the sum of the amounts of allantoin and uric acid excreted in urine. The absorbed purine were calculated based on the excretion of purine derivatives using the equation proposed by Barbosa et al., (2011). Then, the ruminal synthesis of microbial nitrogen compounds (MICN) was calculated as a function of absorbed by the following equation:

$$MICN = 70 \times AP / 0.93 \times 0.137 \times 1.000$$

where MICN= ruminal synthesis of nitrogen compounds (g/d), AP = absorbed purines (mmol/d), 70 = purine N content (mg/mol), 0.93 = purine digestibility and 0.137 = relation of purine N:total N of microorganisms (Barbosa et al. 2011).

2.4. Total RNA isolation, primers design, and RT-qPCR analysis

Total RNA extraction was performed from 50 mg of liver samples using RNeasy Mini Kit (Qiagen, Valencia, CA). After that, total RNA was subsequently treated with DNase I Amplification Grade (Invitrogen, Waltham, MA) and RNA concentration was estimated by NanoVue Plus spectrophotometer (GE Healthcare, Freiburg, Germany). The RNA integrity was evaluated through agarose gel electrophoresis. The first strand of cDNA synthesis was performed using a GoScript Reverse Transcriptase kit (Promega, Madison, WI, USA). Samples were stored at -20°C for further analysis.

The liver urea synthesis associated genes used in this study were *Carbamoyl phosphate synthetase* (CPS) and *Argininosuccinate synthetase* (AS), and the liver gluconeogenesis associated genes were *phosphoenolpyruvate carboxykinase* (PEPCK). Primers for target gene amplification and endogenous amplification were designed using PrimerQuest program (www.idtdna.com/Scitools/Applications/PrimerQuest) with sequences obtained from Gen-Bank database. Primer sequences of each gene are presented in Table 2. The *18S ribosomal RNA* (*18S*; NR_036642.1) was used as the endogenous control gene. Serial dilution of cDNA was used to determinate the amplification efficiency and optimal primer concentration for each gene (Livak and Schmittgen, 2001). Quantitative real-time PCR reactions were performed in an ABI Prism 7300 Sequence Detection Systems thermocycler (Applied Biosystems, Foster City, CA, USA) using a GoTaq qPCR Master Mix (Promega Corporation, Madison, WI, USA). The qPCR reaction consisted of three cycle parameters: 95°C for 3 min, 40 cycles at 95°C for 10 s, and 60°C for 30 s. The amplification efficiency was 0.90 to 0.99. After amplification, a melting curve (0.01°C/s) was used to confirm product purity. Results are expressed relative to 18S using the ΔC_t method (Livak and Schmittgen, 2001).

Statistical Analysis

Data were analyzed by ANOVA using a model that encompassed the fixed effects of treatments and using calf BW as a covariate. After the initial ANOVA, the residuals from the analysis of each variable were assessed for normality using Shapiro-Wilk's test. As expected, the gene expression data were not found normal and it was transformed using $\ln(2^{-\Delta\Delta Ct} + 1)$ (Voge et al., 2004). The comparisons among treatments were performed out by a set of orthogonal contrasts, which encompassed a comparison between the control treatment and the treatments with supplementation, and the linear and quadratic effects of the increasing RUP content in the supplements. All statistical procedures were conducted using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and statistical significance was considered at $P < 0.10$.

3. Results

3.1. Intake and apparent digestibility

The chemical compositions of the forage, supplement and milk are shown in Table 1. The average forage DM availability throughout the experiment was 3792 kg/ha. The supplement DM intake occurred as planned, and there was no difference among supplemented calves.

Supplemented calves had greater intake of DM, CP and TDN compared with non-supplemented calves. Supplementation decreased ($P < 0.02$) forage DM intake, but had no effect ($P = 0.38$) on milk DM intake. The forage DM intake was similar among calves fed supplements with increasing RUP levels. Likewise, total DM, CP and TDN intake was not affected by the supplement RUP levels (Table 3). Feeding supplements increased OM digestibility, without affecting the NDF digestibility. However, increasing RUP level had no adverse effect on diet digestibility (Table 3).

3.2. Nitrogen compounds flow, serum metabolites and hepatic mRNA expression

Supplemented calves had greater ($P<0.05$) ruminal microbial N production (MICN) than control, although MICN was not affected by the increasing supplement RUP levels (Table 4). The efficiency of microbial protein production (EMP) was similar among all treatments and averaged 137 g of microbial CP/kg of Digested OM.

Supplemented calves had greater ($P<0.05$) mRNA expression of argininosuccinate synthetase (*AS*) and greater serum urea-N concentration and urinary urea N-excretion compared to non-supplemented calves. As supplement RUP levels increased the mRNA expression of ureagenic genes linearly decreased, *CPS* ($P<0.04$) and *AS* ($P<0.09$; Fig.1), followed by a linear decrease in serum urea-N concentration and urinary urea-N excretion.

Serum insulin concentration was greater for supplemented calves, as well as serum glucose and T3 concentration. However no effects were observed in mRNA expression of *PEPCK* and on serum T4 concentration between control and supplemented calves. Increasing supplements RUP level had no effect on mRNA expression of *PEPCK*, which parallels with lack of difference on serum glucose and insulin concentrations. Likewise, T3 and T4 serum concentration did not differ among supplemented calves.

3.3. Performance and ultrasound carcass traits

Supplemented calves had greater ADG ($P<0.01$; 0.997 vs 0.793 kg/d) and greater final BW ($P<0.01$; 270 vs 241 kg/d) than non-supplemented calves. In addition, the rib eye area (REA, 48 vs 40 cm²) and subcutaneous fat thickness (2.04 vs 1.51 mm) were greater for supplemented calves. However, neither weight gain nor ultrasound carcass traits were improved by the increasing supplement RUP levels (Table 6).

Calves supplementation had no effect on dams' performance and fat-corrected milk production ($P = 0.88$), which averaged 6.5 kg/d (Table 6).

4. Discussion

The forage CP content in this study of 89 g/kg DM was higher than the minimum required to maximise microbial growth on fibre in beef cattle consuming tropical forages (70–80 g CP/kg DM; Sampaio et al. 2009). However, these values is not well established for suckling beef calves. The greater microbial N production production in response to supplementation indicates that the protein intake from forage and milk was not sufficient for optimum ruminal microbial growth. However, microbial N production was not affected by the increasing supplement RUP levels, indicating that $\text{NH}_3\text{-N}$ concentration was not limiting microbial growth.

The total DM intake was greater for supplemented calves compared to non-supplemented calves. Even though calves supplementation decreased forage DM intake, the milk intake was not affected. Therefore the greater intake of concentrate feeds with higher digestibility contributed for greater DM intake. As a result of similar DMI and apparent digestibility of diets, the intake of total digestible nutrients was unaffected by the supplement RUP levels. The absence of RUP effects on intake on apparent digestibility of DM and OM have been previously reported in beef cattle diets (Devant et al. 2000; Duarte et al. 2011; Batista et al. 2016). This suggests that protein degradability had little effect on site and extent of OM digestion when the diets contained adequate levels of CP, which is consistent with the results of the current study

Dietary strategies to reduce N losses should focus on an optimal relation between RDP and RUP and an optimal efficiency of absorbed AA utilization for tissue deposition. Losses of N may be reduced by decreasing protein degradation in the rumen, and increasing N use by ruminal

microorganisms (Bach et al., 2005). Therefore the supplementation of grazing cattle should provide additional N to maximize forage digestion, and provide additional protein to meet animal requirements; however, providing N in excess should be prevented in order to improve protein utilization, by reducing the N loss via feces and urine. An imbalanced protein supplementation can cause excess in ruminal N-NH₃ that is absorbed through the rumen wall and can be partially lost in urine (Bach et al. 2005).

Previous studies suggest that urea irreversible loss and recycling rates gradually increase with age as well as with the development of the reticulorumen in calves (Hayashi et al 2006). Increased activities of urea cycle enzymes may also be secondarily caused by the demand on urea synthesis after weaning, such as by an increased production of ammonia by ruminal microbes or gluconeogenesis from amino acids. (Takagi et al., 2008). At weaning phase in the present study, the increasing supplement RUP levels resulted in a linear decrease in the mRNA expression of genes associated with ureagenesis in liver, *CPS* and *AS*, which were followed by a linear decrease in serum urea concentration and urinary urea N excretion. These data suggest that ammonia absorption across rumen wall was increased in calves fed low RUP supplement, due to greater amount of protein being degraded in rumen, which in turn was metabolized to urea in liver synthesis resulting in greater SUN and increased excretion in urine. Similar to our results, Larmann et al. (2012) reported a concurrent increase in mRNA expression of hepatic ureagenesis enzymes and serum urea concentrations. Accordingly, Sultan et al. (2009) observed a linear decrease in SUN and urinary N excretion when decreasing the RDP:RUP ration in buffalo calves diets, which resulted in improved protein use efficiency.

A portion of the ammonia produced is utilized in the foregut for the synthesis of bacterial proteins, which are digested and absorbed as peptides or amino acids in the small intestine, and

are finally utilized as body proteins, nucleic acids, and substrates of gluconeogenesis (Lapierre and Lobley, 2001). Additionally the additional metabolizable protein could provide more aminoacids to be used for gluconeogenesis. However, there was no effect on mRNA expression of *PEPCK*, a key enzyme for liver gluconeogenesis, which parallels the lack of treatment effect for serum glucose and insulin concentrations. Positive responses to RUP supply in dairy heifers performance were followed by increase in glucose and insulin serum concentrations (Silva et al. 2018), likely to a better protein and energy metabolism. In addition, the decreased liver urea production can improve the energy status of the animal by energy salvage by less N excretion (Reed et al. 2017; Jennings et al. 2018) and/or due to greater provision of aminoacids used for gluconeogenesis (Takagi et al., 2008) .

The decrease in liver urea synthesis indicates that ammonia rumen loss were decreased when increasing RUP levels, which led to decrease in N loss in urine. Because the treatments did not affect the microbial protein synthesis, it would be expected a greater metabolizable protein availability for absorption in the intestine, resulting in greater N availability for anabolic purposes, as reported by Batista et al (2016), which could result in higher growth rates. However, no responses in calves' performance were observed in the present study. Similar to our results, Moriel et al, (2017), replaced soybean meal with by-pass soybean meal for suckling beef calves and did not observe any potential benefit of increasing RUP supply in calf performance. According to Santos et al. (1998) one of the reasons for the absence of responses in performance when bovines is supplemented with RUP sources is when the control diet provides enough metabolizable protein to support potential performance. Considering the milk CP content and the milk DM intake in the present study, the calves consumed approximately 210 of CP from milk, which presents a high biological value (95% true protein, INRA, 1993), and it has been demonstrated to by pass rumen

through esophageal groove (Rodrigues et al. 2002), thereby representing an important source of metabolizable protein.

Neither muscle nor fat deposition were enhanced by the increasing supplement RUP levels, as shown by the ultrasound measurements results, which is consistent with the lack of difference in serum metabolites that could promote increases in carcass muscle and fat deposition (glucose, insulin, T3 and T4). In agreement with our results, previous researches did not observed improved performance when feeding by-pass soybean meal for early-weaned calves (Vendramini et al 2011,2013). Some studies that reported improved performance when feeding diets with increasing RUP supply for calves, but they used animal sources of protein (Sultan et al. 2009). Therefore, it is difficult to indicate whether the improvement in performance is a result from changes in the site of digestion, or by changes in protein profile quality characteristics (digestibility and amino acid composition).

The present study has confirmed previous findings (Lopes et al. 2016) indicating that beef calves does not reduce milk intake when fed supplement. Thereby, in the current study, calves supplementation did not impact dam performance, as measured by the average daily gain, final body weight and fat-corrected milk production.

5. Conclusion

Supplementation increased average daily gain by 204 g/d, resulting in calves 29 kg heavier at weaning compared to non-supplemented calves, but had no effect on dams productive performance, as measured by final BW and fat-corrected milk production. Increasing supplements RUP contents had no adverse effects on intake and diet digestibility, nor in microbial protein synthesis. As supplement RUP content increased, the mRNA expression of genes associated with ureagenesis in liver linearly decreased, followed by a linear decrease in serum urea-N

concentration and urinary urea-N excretion, which indicates a potential to reduce N losses and thereby improve N use efficiency; however, calves' performance was not improved by the supplements RUP levels.

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Table 1.

Feed and chemical composition of the supplements, and chemical composition of the forage and milk

Item	Control	Supplement RUP levels			Forage ¹
		Low	Medium	High	
<i>Ingredients (g/kg as-fed)</i>					
Ground corn	-	417	417	417	-
Soybean meal	-	553	276	-	-
By-pass soybean meal	-	-	276	553	-
Minerals ²	1000	3	3	3	-
<i>Chemical composition (g/kg DM)</i>					
Crude protein	-	226	237	235	89
RUP ³	-	94	132	170	27
NDF	-	133	150	152	572
EE	-	20	22	24	16
NFC	-	557	510	507	239
Dry matter (g/kg as-fed)	-	881	892	886	339
<i>Milk components (g/kg)</i>					
Protein	43	46	45	47	-
Fat	35	36	35	34	-
Lactose	45	46	44	45	-
Total solids	135	140	137	139	-

¹ Mean values of the samples obtained by hand-plucking (grazing simulation) throughout the experiment.

² Mineral mix composition (g/kg): dicalcium phosphate, 500; sodium chloride, 477; zinc sulfate, 14; copper sulfate, 7; cobalt sulfate, 0.5; potassium iodate, 0.5 and magnesium sulfate, 0.25.

³ Calculated using NRC (2001) and BR-CORTE (2016) tabular values as a percentage of crude protein.

Table 2

Primer sequence used for real-time quantitative PCR

Gene ¹	Accession No. NCBI ²	Primer sequences (5'-3')	Amplicon size, bp
<i>CPS</i>	XM_587645	F- ACACTGGCTGCAGAATACCC R- TTCTTGCCAAGCTGACGCAA	191
<i>AS</i>	M26198	F- GAAGAAGGCGCTGAAGCTTG R- GGTGAGCTCAAACCGGATCT	250
<i>PEPCK</i>	AY145503.1	F- CCCCCAGAGATCAAGAATCA R- ATTGGAGGTGGACAGTCAGG	86

¹ Genes: Carbamoyl phosphate synthetase (*CPS*), Argininosuccinate synthetase (*AS*), phosphoenolpyruvate carboxykinase (*PEPCK*).

² National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov).

Table 3

Intake and apparent digestibility of suckling beef calves fed supplements with increasing rumen undegradable protein (RUP) levels

Item ¹	Control	Supplement RUP level ²			SEM	P-value ³		
		Low	Medium	High		C vs. S	L	Q
<i>Intake (kg/d)</i>								
Dry matter	3.87	4.91	4.76	4.81	0.312	0.021	0.830	0.820
Supplement DM	-	1.76	1.78	1.77	0.185	-	0.969	0.945
Milk DM	0.73	0.70	0.61	0.72	0.056	0.380	0.756	0.181
Forage DM	3.14	2.45	2.38	2.29	0.239	0.016	0.637	0.982
CP	0.48	0.82	0.80	0.84	0.052	<0.001	0.664	0.566
EE	0.30	0.32	0.30	0.35	0.022	0.383	0.297	0.116
TDN	2.60	3.55	3.24	3.68	0.220	0.002	0.470	0.131
<i>Intake (g/kg BW.d)</i>								
Dry matter	17.79	21.47	21.64	21.83	1.326	0.027	0.855	0.995
Forage DM	14.37	10.70	10.74	10.42	0.980	0.004	0.844	0.878
<i>Digestibility (g/kg DM)</i>								
OM	678	714	715	740	11.6	0.001	0.103	0.371
NDF	649	640	653	640	11.2	0.771	0.990	0.409
CP	590	635	633	628	22.7	0.166	0.824	0.938

¹CP, crude protein; EE, ether extract; OM, organic matter; NDF, neutral detergent fiber.

²Supplement RUP levels (low = 94, medium = 132, High = 170 g RUP/kg DM)

³Contrasts: C vs. S = Non-supplemented vs supplemented calves; L and Q = linear and quadratic effects of the supplement RUP levels.

Table 4

Nitrogen compounds flow and serum metabolites of suckling beef calves fed supplements with increasing rumen undegradable protein (RUP) levels

Item ¹	Control	Supplement RUP level ²			SEM	<i>P</i> -value ³		
		Low	Medium	High		C vs S	L	Q
MICN, g/day	54.5	65.0	68.5	62.0	4.47	0.042	0.593	0.310
RMICN, g/g	0.69	0.48	0.54	0.45	0.044	<0.001	0.558	0.150
EMP	150.8	130.6	153.0	111.8	13.18	0.208	0.268	0.034
SUN, mg/dL	10.81	18.02	17.34	13.50	1.256	<0.001	0.006	0.272
UNE, g/day	26.06	62.80	59.57	44.41	3.554	<0.001	0.002	0.228
Glucose, mg/dL	52.27	70.27	61.22	63.12	4.791	0.031	0.246	0.304

¹ MICN, microbial nitrogen production; RMICN, relative MICN; EMP, efficiency of microbial protein production (g microbial CP/ kg digested OM intake); SUN, serum urea-N; UNE, urinary urea-N excretion.

²Supplement RUP levels (low = 94, medium = 132, High = 170 g RUP/kg DM).

³Contrasts: C vs. S = Non-supplemented vs supplemented calves; L and Q = linear and quadratic effects of the supplement RUP levels.

Table 5

Relative mRNA expression in liver and serum metabolites of suckling beef calves fed supplements with increasing rumen undegradable protein (RUP) levels

Item ¹	Control	Supplement RUP level ²			SEM	P-value ³		
		Low	Medium	High		C vs S	L	Q
<i>Relative mRNA expression</i>								
CPS	2.94	5.48	4.09	2.61	0.818	0.269	0.034	0.969
AS	2.20	4.17	3.64	2.79	0.535	0.049	0.088	0.830
PEPCK	3.33	3.39	4.28	2.93	1.038	0.871	0.769	0.402
<i>Serum metabolites</i>								
SUN, mg/dL	14.07	23.38	20.98	20.79	1.047	<0.001	0.089	0.410
Glucose, mg/dL	82.62	89.49	92.39	91.34	3.072	0.035	0.246	0.304
Insulin, pg/mL	34.06	170.4	159.3	175.5	46.7	0.028	0.938	0.812
T3, ng/mL	3.13	4.27	3.59	3.65	0.267	0.056	0.123	0.277
T4, ng/mL	155.1	174.3	166	187.2	13.546	0.235	0.528	0.395

¹Carbamoyl phosphate synthetase (CPS), Argininosuccinate synthetase (AS), phosphoenolpyruvate carboxykinase (PEPCK).

²Supplement RUP levels (low = 94, medium = 132, High = 170 g RUP/kg DM)

³Contrasts: C vs. S = Non-supplemented vs supplemented calves; L and Q = linear and quadratic effects of the supplement RUP levels.

Table 6.

Performance and carcass ultrasound traits of suckling beef calves fed supplements with increasing rumen undegradable protein (RUP) levels and performance of their dams

Item ¹	Control	Supplement RUP level ²			SEM	P-value ³		
		Low	Medium	High		C vs S	L	Q
<i>Calves</i>								
ADG, kg/d	0.793	1.011	0.985	0.994	0.0284	<0.001	0.633	0.565
Final BW, kg	241	272	268	269	4.0	<0.001	0.642	0.589
REA, cm ²	40.15	49.56	48.08	46.64	1.318	<0.001	0.127	0.993
SFT, mm	1.51	1.88	2.14	2.09	0.212	0.022	0.479	0.569
P8FT, mm	1.99	2.59	2.82	2.81	0.219	0.010	0.482	0.675
<i>Cows</i>								
ADG, kd/d	0.240	0.254	0.320	0.292	0.0364	0.296	0.480	0.297
Final BW, kg	491	493	502	498	5.1	0.301	0.485	0.306
FMY, kg/d	6.58	6.63	5.83	6.98	0.545	0.880	0.660	0.155

¹ADG, average daily gain; REA, rib eye area, SFT, subcutaneous fat thickness, P8FT, P8 fat thickness, FMY, fat-corrected milk yield

²Supplement RUP levels (low = 94, medium = 132, High = 170 g RUP/kg DM)

³Contrasts: C vs. S = Non-supplemented vs supplemented calves; L and Q = linear and quadratic effects of the supplement RUP levels.

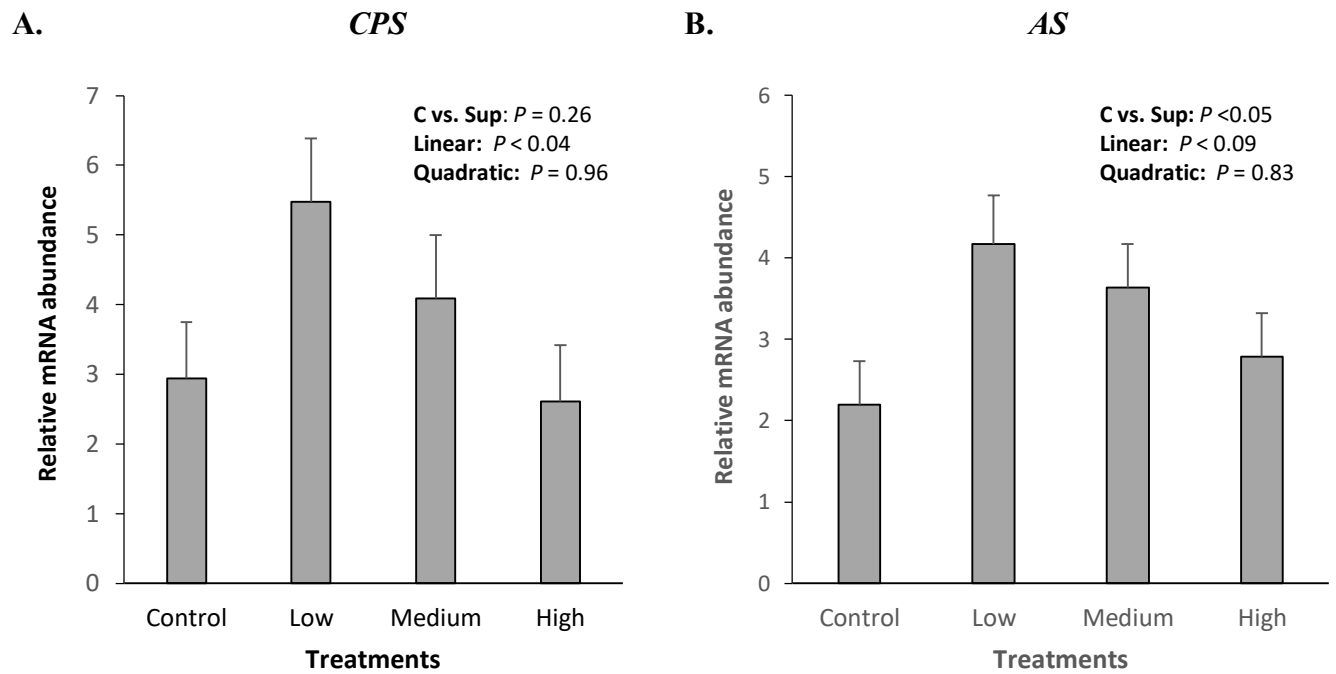


Fig 1. Relative mRNA expression of genes associated with liver ureagenesis A) *CPS*, Carbamoyl phosphate synthetase; B) *AS*, Argininosuccinate synthetase of suckling beef calves fed supplements with increasing RUP levels (low = 94, medium = 132, High = 170 g RUP/kg DM)

CHAPTER 2

A meta-analysis of the effects of creep-feeding supplementation on performance and nutritional characteristics by beef calves grazing on tropical pastures

ABSTRACT. The objective of this study was to evaluate effects of creep-feeding supplementation on growth performance and efficiency, feed intake and digestibility by Nellore calves grazing tropical pastures, using a meta-analytical approach. The dataset were collected from 18 studies, which were carried out between 2007 and 2017, totaling 75 treatment means that represented 832 cow-calf pairs. All studies had a control treatment with no creep-feeding supplementation (**non creep-fed calves**), and a wide variety of creep-feeding treatments (**creep-fed calves**), that were evaluated herein as daily amount of supplement intake (SUP, g/kg BW, DM basis) and supplemental crude protein intake (SCPI, g/kg BW); whereas dependent variables were evaluated as differential values relative to non creep-fed calves. Forage DM intake (g/kg BW) decreased linearly as SUP increased, whereas milk DM intake (g/kg BW) were not affected, though total DM intake increased linearly. The fibre digestibility responded quadratically to SUP and SCPI; however, DM digestibility and TDN intake increased linearly as SUP increased. The supplemental weight gain (SWG) responded quadratically as SUP ($P < 0.01$) and SCPI ($P < 0.01$) increased, and there was an influence of calf sex. The maximum responses were estimated at 7.6 g/kg BW (0.2 kg/d) for bull calves, and at 5.5 g/kg BW (0.1 kg/d) for heifer calves. The calculated supplement crude protein content for maximal responses were 225 and 224 g CP/kg DM for bull and heifer calves respectively. Likewise, supplemental feed efficiency responded quadratically to SUP and SCPI levels. We conclude that creep-feeding improves preweaning weight gain, in which calves responds differently according to sex. Nonetheless, the best results in supplemental weight gain is achieved with moderate amounts of protein supplements.

1. Introduction

Increasing calves' weaning weights might improve cow-calf operation economics. Additionally, by enhancing calves development during suckling phase, the time to slaughter and time to heifers' puberty attainment could be reduced. In tropical conditions, after three mo of age, milk is not sufficient to meet calf requirements to support potential growth (Costa e Silva et al., 2015, 2016). Thereby, calves diets is increasingly relying on pasture. However, concurrently with the post-peak, declining locational curve (Costa e Silva et al., 2015), there is a decrease in pasture quality and availability due to rainy to dry transition season in most production systems in Brazil, while calf protein and energy requirements increases as growing progresses (Fonseca et al., 2012). Consequently, calf preweaning performance is commonly not optimized. Therefore, creep-feeding has been used to provide supplemental nutrients, and has effectively increased weight gain in the tropics (Valente et al., 2012, 2014; Lopes et al., 2014, 2017), as well as in many others different regions (Hennessy et al., 2001; Lardy and Maddock, 2007, Viñoles et al., 2013, Moriel et al., 2017). However, the results have been variables among years (Lancaster et al., 2007; Moriel and Arthington, 2013). The variability in these results could be associated to between-years differences in forage quality and quantity, and within animals (Adams et al., 2000).

The rapid growth rate of young calves (Owens et al., 1993), suggests that supplementing cattle at this phase could be a very efficient management. Although the cost-effectiveness of a creep-feeding program depends on supplement cost, calf price, and eventually the supplemental feed efficiency (additional gain relative to non-creep fed calves per unit of supplement consumed, Aguiar et al., 2015). Besides calf genetics, supplemental feed efficiency could be related to amount and composition of the supplement used, and its effects on intake and digestion of the basal diet (i.e. forage and milk). Creep-feeding used to be provided free choice, but this has been associated

with decreased feed efficiency (Cremin et al., 1991; Faulkner et al., 1994). Higher levels of creep feeding have decreased fibre digestion (Cremin et al., 1991; Lopes et al., 2017), and had negative effects on calf performance (Lopes et al., 2017).

Protein supplements have been indicated to adequate diets and improve performance of growing beef cattle consuming either low to high-quality tropical grasses (Detmann et al. 2014a). During suckling phase, supplements with low protein-high energy content have decreased fibre digestion (Valente et al., 2014), whereas high protein supplements have decreased forage intake and had negative effects on performance (Lopes et al., 2014). Nonetheless, these supplementation effects have not been consistently reported, which could support nutritionists to design effective creep-feeding programs. Thus, our objective was to evaluate the effects of supplement amount and composition on supplemental weight gain, supplemental feed efficiency, feed intake and digestibility by Nelore calves during suckling phase, as well as the influence of calf sex on these variables, using a meta-analytical approach.

2. Material and methods

2.1. Data acquisition and experimental procedures

The dataset were collected from 18 studies carried out in Brazil, which were conducted between 2007 and 2017, totaling 75 treatment means that represented 832 cow-calf pairs, in which 401 were bull calves and 431 were heifer calves (Appendix).

All experiments were conducted with Nelore cattle grazing *Brachiaria* sp. under continuous stocking rate, generally between February and June of each year, which corresponded the rainy-to-dry transition seasons. The experiments were carried out according to completely randomized designs. The pastures were divided into different paddocks provided with covered troughs and drinkers. Next to dams' troughs, there were troughs with access restricted to the calves (creep feeders), in which the different treatments were provided. All studies had a control treatment with

no creep-feeding supplementation (**non creep-fed calves**), and a wide variety of creep-feeding treatments using different concentrates amounts and/or compositions (**creep-fed calves**). All animals (cows and calves) were provided with minerals *ad libitum*. Calves were supplemented from approximately 3 to 8 mo of age, when they were weaned.

For weight gain evaluations, calves were weighed at the beginning and end of the experimental period after 14-h fasting. Due to the heterogeneity among animals, locals, years, etc., the values of preweaning average daily gain (ADG, kg/d) were expressed as supplemental weight gain (SWG, kg/d), calculated as (ADG of creep-fed calves – ADG of non creep-fed calves) for each experiment. To decrease the influence of the different animal weights, the values of the supplement intake were expressed as a fraction of BW according to Detmann et al. (2014a):

$$ABW = iBW + \left(\frac{DIE}{2} \times ADG \right) \quad (1)$$

$$SUP = \frac{SUPM}{ABW} \times 1000 \quad (2)$$

$$SCPI = \frac{SUPM \times SCP}{ABW} \quad (3)$$

where ABW is the average BW of the animal (kg), *i*BW is the initial BW of the calf (kg), DIE is the number of days in the experiment, SUP is the daily amount of the supplement intake by calves (g/kg BW), SUPM is the absolute mass of supplement consumed by calves (kg/d, DM basis), SCPI is the supplemental crude protein intake (g/kg BW, DM basis), and SCP is the supplement crude protein content (g/kg DM).

The supplemental feed efficiency (SFE) was calculated as (SWG, supplemental weight gain, kg/d per SUP, supplement intake, g/kg BW) (Faulkner et al. 1994) for each experiment. The data used to analyze calves' performance are presented in Table 1.

To estimate feed intake and digestibility by the calves, a digestion trial was performed at the middle phase of each experiment following similar procedures described by Lopes et al. (2014). The faecal excretion was estimated using chromium oxide (Cr_2O_3) as an external marker. The forage intake was estimated using indigestible neutral detergent fibre as an internal marker, while supplement intake was estimated using titanium dioxide (TiO_2) as an external marker. The forage sampling was performed using hand-plucked method.

The milk intake by the calves was assumed to be equal to dams' milk yield, which was estimated using oxytocin and machine milking method, performed at three different periods throughout each experiment, following similar procedures described by Lopes et al. (2016). Milk samples were analyzed for protein, fat, lactose, and total solids content by infrared spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark). Then, average milk yield was calculated and corrected to 4% of fat (fat-corrected milk yield, FMY, kg/d; NRC 2001).

The following variables were considered to evaluate nutritional characteristics, analyzed as differential values relative to non creep-fed calves due to reasons abovementioned, according to SUP or SCPI: dry matter (DM) intake, forage DM intake, total digestible nutrients (TDN) intake and milk DM intake (expressed as a g/kg BW); dams' fat-corrected milk yield (expressed as kg/d), neutral detergent fibre (NDF) and dry matter (DM) digestibility (expressed as g/kg) (Table 2). Not all of the informations were reported in every experiment, which resulted in some differences among variables regarding the number of observations (treatment means).

2.2. Statistical Analysis

The data were analyzed by meta-analysis techniques (St-Pierre, 2001), using the mixed procedures of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The random effect of the

different subjects (experiments) was considered in the regression parameters. As all the variables were analyzed as differential values relative to control treatment, the intercept was taken to zero in the equations. The influence of calf sex was evaluated as a dummy variable. The adequacy of the models and the best covariance structures were evaluated using the corrected Akaike's information criterion. All variance components were estimated using the restricted maximum likelihood method, and the statistical evaluations were considered significant at $P < 0.05$.

3. Results

3.1. Nutritional characteristics

There was no influence ($P > 0.05$) of calf sex on feed intake and digestibility variables. The forage DM intake (g/kg BW) decreased linearly as daily amount of supplement intake (SUP, g/kg BW; $P < 0.02$) and supplemental crude protein intake (SCPI, g/kg BW; $P < 0.02$) increased (Table 3). The SUP and SCPI levels had no effect ($P > 0.05$) on dams' fat-corrected milk yield and eventually no effect ($P > 0.05$) on calves' milk DM intake (g/kg BW), whereas total DM intake (g/kg BW) increased linearly ($P < 0.01$) as both SUP and SCPI increased. Forage DM intake decreased by 0.43 g/kg BW per each g/kg BW of supplement DM intake (Table 3; Fig. 1a).

The neutral detergent fibre digestibility (g/kg) responded quadratically to increasing levels of SUP ($P < 0.02$) and SCPI ($P < 0.02$). However, total DM digestibility (g/kg) increased linearly ($P < 0.01$). Similarly, the total digestible nutrients intake (g/kg BW) increased linearly as SUP increased (Table 3; Fig. 1b); however, there was a quadratic response ($P < 0.05$) to increasing levels of SCPI (Table 3).

3.2. Supplemental weight gain and supplemental feed efficiency

The supplemental weight gain (SWG, kg/d) presented a quadratic pattern ($P < 0.01$) according to variations in daily amount of supplement intake (SUP, g/kg BW), and there was an influence of calf sex ($P < 0.01$), in which maximal responses were estimated at 7.6 g/kg BW for bull calves (0.20 kg/d), and at 5.5 g/kg BW for heifer calves (0.10 kg/d, Table 4; Fig.2). Likewise, SWG responded quadratically to supplemental crude protein intake (SCPI), in which maximum responses were estimated at 1.7 g/kg BW for bull calves, and at 1.2 g/kg BW for heifer calves (Table 4). Therefore, the calculated supplement crude protein content for maximal responses were 225 and 224 g CP/kg DM for bull and heifer calves respectively.

The supplemental feed efficiency (SFE) presented a quadratic response as both SUP ($P < 0.01$) and SCPI ($P < 0.01$) increased (Table 4). For SUP, maximal responses were estimated at 4.25 g/kg BW for bull calves ($0.026 \text{ kg}\cdot\text{d}^{-1}/\text{g}\cdot\text{kg BW}^{-1}$), and at 3.34 g/kg BW for heifer calves ($0.019 \text{ kg}\cdot\text{d}^{-1}/\text{g}\cdot\text{kg BW}^{-1}$). For SCPI, maximal responses were estimated at 1.17 g/kg BW for bull calves and 0.93 g/kg of BW for heifer calves. Therefore, the calculated supplement crude protein content for maximal responses were 275 and 278 g CP/kg BW for bull and heifer calves respectively. The estimated SWG for maximal SFE were 0.160 and 0.087 kg/d for bull and heifers calves respectively.

The forage availability (FAV) and forage crude protein (FCP) were not ($P > 0.05$) associated with performance variables.

4. Discussion

Our results are similar to previous studies (Cremin et al., 1991; Lardy et al., 2001; Lopes et al., 2014) that reported that creep-fed calves had lower forage intake compared with non creep-fed calves, while milk intake were similar and total DM intake was greater. Moreover, consistent with our results, forage intake decreased as supplementation levels increased in calves consuming either

tropical grasses (Lopes et al., 2017; Almeida et al., 2018) or cool-season grasses (Cremin et al., 1991; Faulkner et al., 1994).

There are few studies that investigated ruminal metabolism in creep-fed calves, especially in tropical conditions. Creep-feeding increased ruminal VFA concentrations and decreased acetate:propionate ratio as a result of greater intake of highly fermentable carbohydrates (Gelvin et al., 2004), and increased N-NH₃ ruminal concentration (Cremin et al., 1991). This suggests that the substitution effect could be related to metabolic feedbacks of supplementation (Allen, 2014; Detmann et al., 2014b), which in turn affects calves' feeding behavior. Indeed, several studies reported that creep-fed calves spent less time grazing but similar time suckling compared to non creep-fed calves (Valente et al., 2013; Viñoles et al., 2013; Martins et al., 2017; Lopes et al., 2017). Moreover, grazing time decreased linearly as supplement levels increased (Lopes et al., 2017). This supports our results, indicating that calves partially replace forage, but do not substitute milk by concentrate feeds.

The current study confirmed previous reports by Lopes et al. (2016), that by using a meta-analytical approach demonstrated that dams' fat-corrected milk yield was similar when their calves were either creep-fed or not, also no influence of calf sex were reported. We now have demonstrated that FMY was not affected by the increasing levels of either SUP or SCPI. Thus, the dams' milk yield would not be a factor influencing calves' performance and nutritional characteristics, therefore the differences between creep and non creep-fed calves would be only due to supplementation.

The fibre digestibility increased quadratically as SUP and SCPI increased. The increase in fibre digestibility at intermediate levels may be due to adequate provision of protein and energy required for rumen microbial growth (Detmann et. al, 2014a). This would increase ruminal

microbial production (Lopes et al., 2014); leading to increases in rates of fibre degradation (Detmann et al., 2009). Conversely, negative associative effects of supplementation on fibre digestion have been reported when suckling calves consuming tropical grasses are supplemented with low protein:high carbohydrates supplements (i.e. energy supplements) (Valente et al., 2014) or fed high amounts of supplements (Lopes et al., 2017). In agreement with our results, in beef calves consuming high quality forage, decreases in ruminal fibre digestion and total tract NDF digestion caused by unlimited creep feeding were partially avoided by limiting creep feed intake (Cremin et al., 1991). Additionally, the negative effect of supplementation on fibre digestibility were overcome when supplement protein concentration was increased to moderate levels (Faulkner et al., 1994).

Pasture is the major source of fibre in calves' diet, as the NDF content in concentrate supplements is low. In the present study, the DM intake and DM digestibility increased linearly as supplement intake increased, thereby the TDN intake presented similar pattern. This effect is a result of greater digestibility of concentrate feeds compared to that of the forage, which was partially replaced assuming lesser proportion in calves' diets. However higher levels of SCPI had a quadratic effect on TDN intake, likely due to negative effects on forage intake and fibre digestibility reported in the current study.

The increasing levels of supplement were followed by an increase in the available energy for growth and maintenance, as evidenced by the linear increase in TDN intake. Therefore, the positive responses of supplementation on SWG could be attributed to a better diet adequacy regarding protein and energy supply and calves' nutrient requirements. However, calves' growth rate increased quadratically (Table 4; Fig. 2) which can be explained by the quadratic pattern of supplemental feed efficiency responses (Table 4). In agreement with our results Lana et al., (2005)

reported a curvilinear response in weight gain as a function of concentrate supply in growing cattle grazing tropical pasture.

The decrease in efficiency within higher SUP levels may be related to differences in gain composition (Asher et al., 2018), in which fat deposition rates increases at expense of muscle deposition rates in response to increasing energy content of the diet. Gerrits et al. (1996, 1997) demonstrated that when fat deposition rate increases whereas protein deposition rate decreases, the rate of live weight gain decreases. It has been pointed out that cattle sex influences growth of body tissues, affecting carcass composition and feed efficiency (Berg and Butterfield, 1976). In fact, Silva et al., (2017) reported that heifer calves supplementation at levels of 0, 3 and 6 g/kg BW (supplement CP, 298 g/kg) increased backfat thickness (BFT, 0.51, 1.48 and 2.06 mm) while no significant difference were reported on ribeye area (REA, ~38.2 cm²) at weaning, whereas Martins (2017) reported that both BFT and REA increased when bull calves were creep-fed. However, it is important to mention that reserves of body fat may be related to energy status and could cause long-term positive effects on reproductive responses in heifers (Hall et al., 1995).

Consistent with our results Lopes et al. (2017), creep-feeding Nellore bull calves at levels of 0, 3, 6 and 9 g/kg BW (supplement CP, 276 g/kg DM) when grazing tropical pasture, reported that the ADG was greater for the moderate level compared to the highest level (0.72, 0.80, 0.92, 0.89 kg/d respectively). The maximal response in ADG resulted in calves 29 kg heavier than control (246.4 vs 275.4 kg). In the present study, considering the maximal SWG of 0.2 and 0.1 kg/d for bull and heifer calves respectively, and a supplementation period from 3 to 8 mo of age (~150 days), creep-fed bull calves would be 30 kg heavier, and creep-fed heifer calves, 15 kg heavier than non creep-fed calves at weaning.

The quadratic response in SFE across the increasing SCPI levels can be associated with protein excess within higher levels. The surplus of protein has been related with increases in energy cost (Jennings et al., 2018), and decreases in N use efficiency (Detmann et al., 2014a), likely due to metabolism of increasing dietary protein losses, which leads to increased urea N excretion in urine (Lopes et al., 2014, Jennings et al. 2018), resulting in deleterious effects on performance. Indeed, Lopes et al. (2014) provided similar amounts of supplement (5 g/kg BW) with increasing CP content (80, 190, 300 and 410 g CP/kg DM) to suckling calves grazing tropical pasture and reported a quadratic response on ADG and weaning weight.

Considering the average BW (ABW) observed in the current study of 179 kg and 165 kg for creep-fed bull and heifer calves respectively, and the estimated SUP for maximal SWG responses, the calculated SFE was 0.15 and 0.11 kg of added gain per kg of supplement DM intake (kg/kg). Whereas, when estimating SUP for maximal values of SFE, the values were 0.21 and 0.16 kg/kg. Gelvin et al. (2004) supplementing steers calves at amounts of 3.03 kg (supplement CP, 19%) or 1.86 kg (supplement CP, 19%), reported a SFE of 0.10 and 0.13 kg/kg respectively. Sexten et al., (2004), provided supplement *ad libitum* with different protein concentration (14 and 18%) for heifer calves and reported a SFE of 0.10 and 0.13 kg/kg respectively. It is noteworthy to mention that when reducing SUP in order to maximize SFE, the reduced growth rate should be considered, as well as the specific targets of each operation (e.g. gain per area, kg of weaned calf per cow, first calving age, time to slaughter, etc.). In addition, the fraction of SCPI in relation to SUP increased; thus, the economic return by using different supplement amounts and crude protein concentration should also be evaluated.

In the present study, neither forage availability (FAV) nor forage crude protein content (FCP) were associated with performance variables. In study by Detmann et al. (2014a) with growing beef

cattle consuming tropical grasses, no influence of FAV were reported as well, though the response in SWG to SCPI was inversely proportional to the FCP (i.e. animals responded more intensively to supplemental protein as forage CP declined), which has biological consistency. However, differently of our study, the dataset from Detmann et al., (2014a) included low to high quality forages, representing all seasons of the year, thereby forage CP content ranged from 35 to 210 g/kg DM (mean, 86 ± 36.8). In the present study, forage CP content ranged from 71 to 139 g/kg DM (mean, 98 ± 21.4). Additionally, the intake of milk, which is an important source of metabolizable protein (Lardy et al., 2001) could have reduced the impact of pasture deficiencies.

5. Conclusions

The increasing supplementation levels (SUP) decreases forage intake, but has no effect on milk intake, while total DM intake increases. Extreme levels of SUP and supplemental crude protein intake (SCPI) have negative effects on fibre digestibility. However, the DM digestibility increases as supplement amount increases, leading to increasing total digestible nutrients intake, thereby improving calves' performance, in which responses are influenced by calf sex. Moreover, the supplemental weight gain (SWG) and supplemental feed efficiency increases quadratically as both SUP and SCPI increases. Our results indicates that protein supplements provides best responses in performance and nutrients utilization. The maximum responses in supplemental weight gain is achieved when bull calves is creep-fed at amount of 7.6 g/kg BW containing 225 g CP/kg DM and heifer calves at amounts of 5.5 g/kg BW containing 224 g CP/kg DM.

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Table 1

Statistical description of the dataset used for the analysis of calves' performance.

Item ^a	Statistics				
	Mean	Minimum	Maximum	<i>s</i>	<i>n</i>
Overall dataset					
SFE	0.032	0.005	0.112	0.0183	57
SWG	0.123	0.015	0.347	0.0704	57
ADG	0.747	0.515	1.012	0.1137	75
SUP	4.1	0	8.1	1.84	75
SCP	255	80	553	74.2	57
SCPI	1.03	0	2.03	0.516	75
FAV	4.1	2.0	5.8	0.86	67
FCP	98	71	139	21.4	67
Bull calves					
SFE	0.037	0.009	0.112	0.0185	32
SWG	0.160	0.046	0.347	0.0717	32
ADG	0.800	0.571	1.012	0.1148	41
SUP	4.9	0	8.1	1.85	41
SCP	255	80	553	75.4	32
SCPI	1.22	0	2.03	0.521	41
FAV	4.4	3.7	5.8	0.86	37
FCP	103	71	139	21.4	37
Heifer calves					
SFE	0.025	0.005	0.050	0.185	25
SWG	0.075	0.015	0.144	0.0714	25
ADG	0.684	0.515	0.880	0.1155	34
SUP	3.1	0	5.4	1.84	34
SCP	256	80	400	72.5	25
SCPI	0.79	0	1.62	0.505	34
FAV	3.8	2.0	5.4	0.89	30
FCP	92	71	126	19.3	30

^aSFE, supplemental feed efficiency (kg/d)/(g/kg BW); SWG, supplemental weight gain (kg/d); ADG, average daily gain (kg/d); SUP, daily amount of supplement intake (g/kg BW); SCP, supplement crude protein (g/kg DM); SCPI, supplemental crude protein intake (g/kg BW); FAV, forage availability (ton DM/ha); FCP, forage crude protein content (g/kg DM).

Table 2

Statistical description of the dataset used for the analysis of calves' nutritional characteristics, and dams' milk production.

Item ^a	Statistics				
	Mean	Minimum	Maximum	<i>s</i>	<i>n</i>
DDMI	2.16	-1.78	8.00	2.518	49
DMI	19.43	13.20	25.90	2.565	63
DFDMI	-1.75	-6.28	3.04	2.387	49
FDMI	11.82	8.13	22.81	2.901	63
DMDMI	-0.14	-1.14	0.72	0.485	57
MDMI	4.6	2.8	6.1	0.80	75
DDMD	43	-6	191	44.4	47
DMD	682	492	770	52.3	60
DNDFD	26	-116	152	45.7	47
NDFD	658	498	755	63.7	60
DTDNI	2.76	-0.56	8.04	2.215	47
TDNI	15.28	9.06	21.02	2.403	60
DFMY	-0.03	-1.71	1.37	0.740	57
FMY	6.53	3.37	8.79	1.176	75

^a Differential (differential value relative to non creep-fed calves). DDMI, differential DM intake (g/kg BW); DMI, DM intake (g/kg BW); DFDMI, differential forage DM intake (g/kg BW); FDMI, forage DM intake (g/kg BW); DMDMI, differential milk DM intake (g/kg BW); MDMI, milk DM intake (g/kg BW); DDMD, differential DM digestibility (g/kg); DMD, DM digestibility (g/kg); DNDFD, differential neutral detergent fibre digestibility (g/kg); NDFD, NDF digestibility (g/kg); DTDNI, differential total digestible nutrients intake (g/kg BW); TDNI, TDN intake (g/kg BW); DFMY, differential fat-corrected milk yield (kg/d); FMY, fat-corrected milk yield (kg/d).

Table 3

Summary of the linear models for describing the pattern of feed intake and digestibility in function of intakes of amount of supplement or supplemental crude protein by suckling beef calves.

<i>Y</i> ^a	<i>X</i> ^a	Linear term			Quadratic term			Mean value ^c			RSD ^d
		Estimate	s.e. ^b	<i>P</i> value	Estimate	s.e. ^b	<i>P</i> value	Estimate	s.e. ^b	<i>P</i> value	
DDMI	SUP	0.5031	0.1364	0.002	-	-	-	-	-	-	3.311
DDMI	SCPI	2.0446	0.6006	0.004	-	-	-	-	-	-	3.026
DFDMI	SUP	-0.4277	0.1458	0.011	-	-	-	-	-	-	3.316
DFDMI	SCPI	-1.4569	0.5250	0.015	-	-	-	-	-	-	4.884
DNDFD	SUP	16.6029	4.8807	0.005	-2.1663	0.8667	0.017	-	-	-	86.266
DNDFD	SCPI	60.845	17.5971	0.004	-27.9362	11.2145	0.018	-	-	-	85.923
DDMD	SUP	10.2380	1.8778	<0.001	-	-	-	-	-	-	64.545
DDMD	SCPI	39.0403	6.9842	<0.001	-	-	-	-	-	-	65.009
DTDNI	SUP	0.5992	0.1046	<0.001	-	-	-	-	-	-	3.054
DTDNI	SCPI	3.6410	0.7975	<0.001	-1.0244	0.4830	0.041	-	-	-	3.368
DMDMI	-	-	-	-	-	-	-	-0.1502	0.1009	0.154	1.008
DMY	-	-	-	-	-	-	-	-0.05232	0.1467	0.725	1.676

^a Differential (differential value relative to non creep-fed calves). DDMI, differential DM intake (g/kg BW); DFDMI, differential forage DM intake (g/kg BW); DMDMI, differential milk DM intake (g/kg BW); DDMD, differential DM digestibility (g/kg); DMD, DM digestibility (g/kg); DNDFD, differential neutral detergent fibre digestibility (g/kg); DTDNI, differential total digestible nutrients intake (g/kg BW); DFMY, differential fat-corrected milk yield (kg/d).

^b s.e., standard error of the estimate.

^c Mean values adjusted for random study effects.

^d RSD, residual standard deviation of the relationship.

Table 4

Summary of the linear models for describing the pattern of supplemental weight gain and supplemental feed efficiency according to the intakes of supplement or supplemental crude protein intake.

Item ^a	Estimate	Standard error	<i>P</i> value
SWG <i>f</i> (SUP)			
SUP	0.03752	0.005933	<0.001
SUP-squared	-0.00342	0.001166	0.005
SUP × sex ^b	0.01464	0.005225	0.008
RSD ^c	0.124		
SWG <i>f</i> (SCPI)			
SCPI	0.1588	0.02137	<0.001
SCPI-squared	-0.0646	0.01500	<0.001
SCPI × sex	0.0634	0.01814	<0.001
RSD	0.132		
SFE <i>f</i> (SUP)			
SUP	0.01705	0.002150	<0.001
SUP-squared	-0.00255	0.000440	<0.001
SUP × sex	0.004636	0.001732	0.011
RSD	0.056		
SFE <i>f</i> (SCPI)			
SCPI	0.06335	0.008057	<0.001
SCPI-squared	-0.03394	0.005691	<0.001
SCPI × sex	0.01593	0.006669	0.022
RSD	0.051		

^a SWG, supplemental weight gain relative to non creep-fed calves (kg/d); SUP, daily amount of supplement per calf (g/kg of body weight, DM basis); SCPI, supplemental crude protein (g/kg of body weight, DM basis); SFE, supplemental feed efficiency (SWG, kg per day/SUP, g/kg of body weight).

^b Sex, bull calves = 1; heifer calves = 0.

^c RSD, residual standard deviation of the relationship.

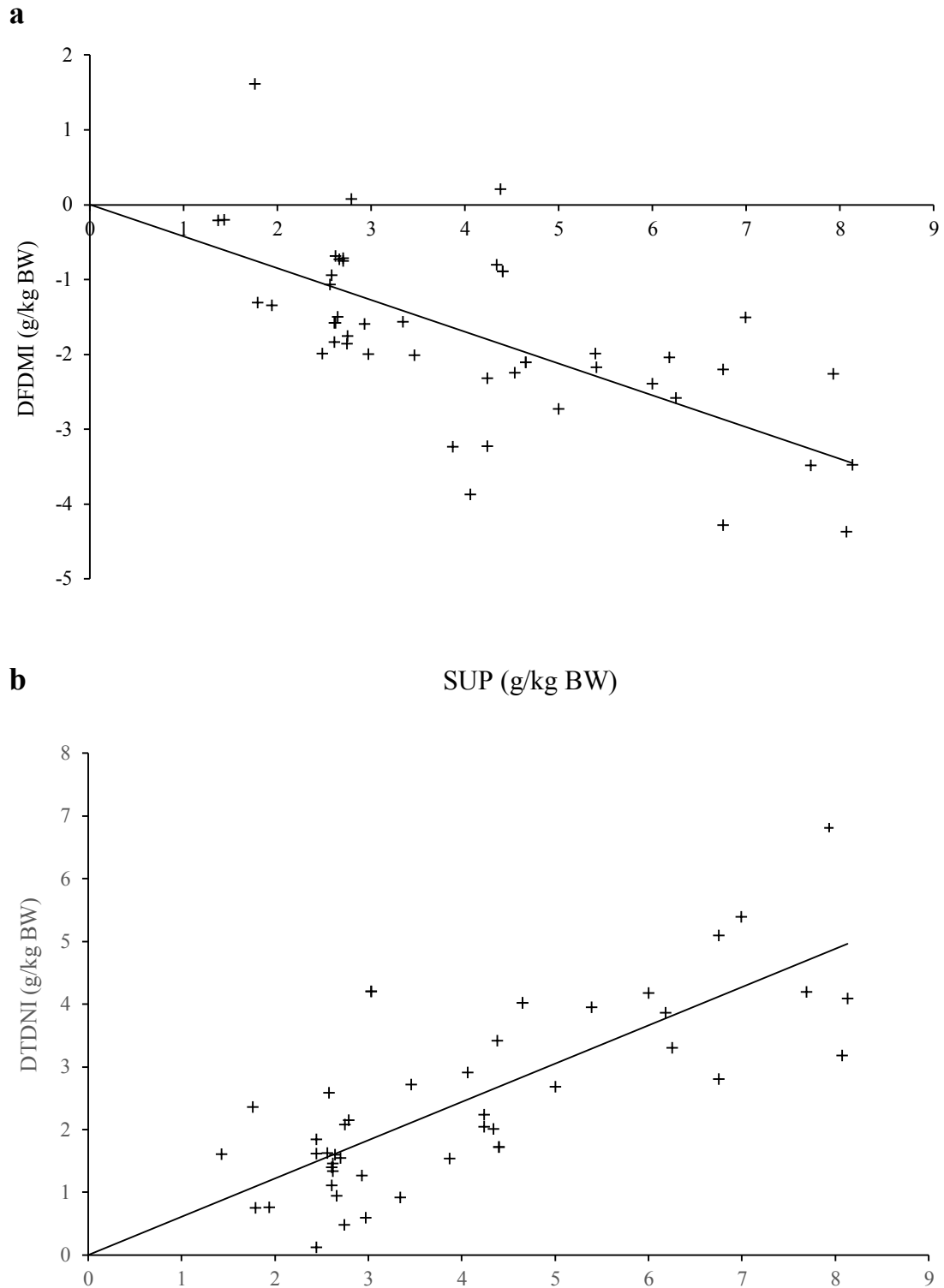


Fig. 1 - Relationship between daily amount of supplement (SUP) and differential forage DM intake relative to non creep-fed calves (DFDMI, a); and differential total digestible nutrients intake relative to non creep-fed calves (DTDNI, b). The data points were adjusted for random study effects (see details of the equations in Table 3).

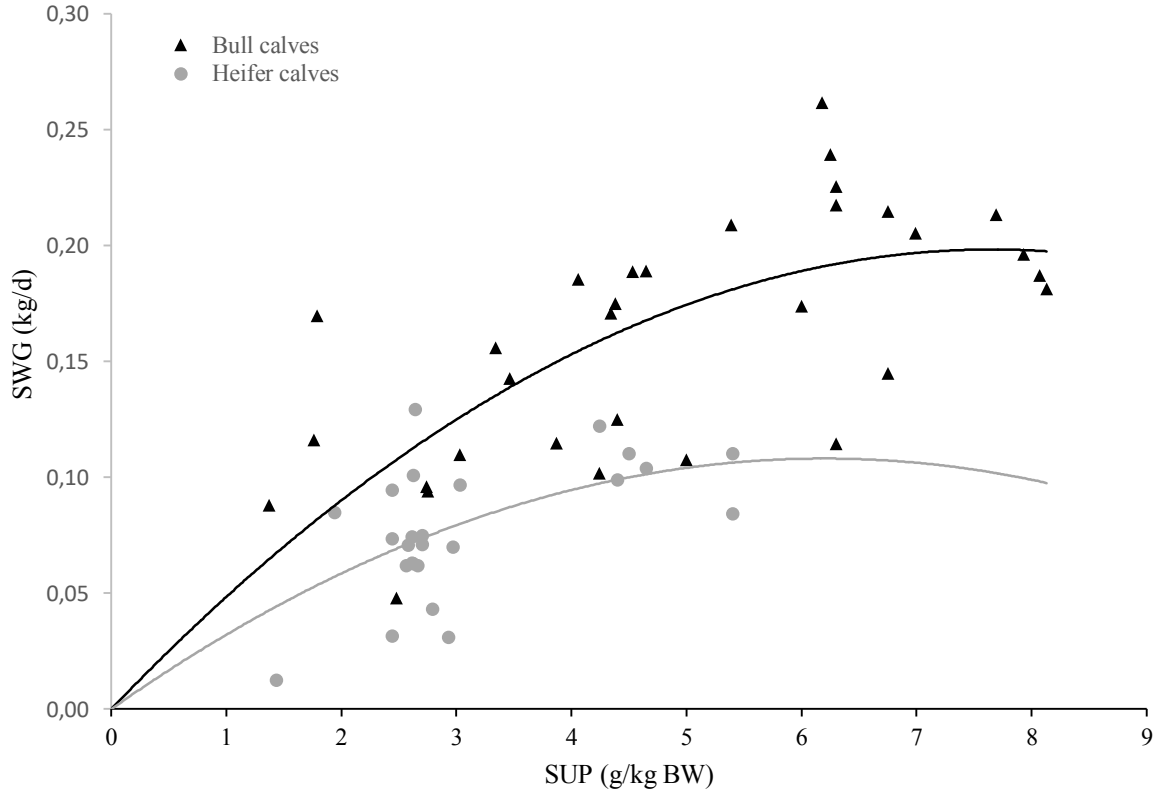


Fig. 2 - Relationship between daily amount of supplement (SUP) and supplemental weight gain relative to non-creep-fed calves (SWG) according to calf sex. The data points were adjusted for random study effects (see details of the equations in Table 4).

APPENDIX

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