JÚLIA TRAVASSOS DA SILVA

PARTIAL AND TOTAL REPLACEMENT OF SOYBEAN MEAL WITH UREA IN FINISHING DIET FOR RED ANGUS X NELLORE GROWING BULLS: EFFECTS ON THE INGESTIVE, DIGESTIVE, RUMINAL PARAMETERS, NITROGEN BALANCE, ANIMAL PERFORMANCE, AND CARCASS CHARACTERISTICS

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

Adviser: Sebastião de Campos Valadares Filho

VIÇOSA - MINAS GERAIS
2024

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

S586p
2024

Silva, Júlia Travassos da, 1995-

Partial and total replacement of soybean meal with urea in finishing diet for red angus x nellore growing bulls: effects on the ingestive, digestive, ruminal parameters, nitrogen balance, animal performance, and carcass characteristics / Júlia Travassos da Silva. - Viçosa, MG, 2024.

1 tese eletrônica (66 f.): il.

Texto em inglês

.

Orientador: Sebastião de Campos Valadares Filho.

Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2024.

Inclui bibliografia.

DOI: https://doi.org/10.47328/ufvbbt.2024.708

Modo de acesso: World Wide Web.

1. Bovinos - Alimentação e rações. 2. Bovinos - Registros de desempenho. 3. Uréia como ração. 4. Farelo. I. Valadares Filho, Sebastião de Campos, 1955-. II. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.2085

Bibliotecário(a) responsável: Euzébio Luiz Pinto CRB-6/3317

JÚLIA TRAVASSOS DA SILVA

PARTIAL AND TOTAL REPLACEMENT OF SOYBEAN MEAL WITH UREA IN FINISHING DIET FOR RED ANGUS X NELLORE GROWING BULLS: EFFECTS ON THE INGESTIVE, DIGESTIVE, RUMINAL PARAMETERS, NITROGEN BALANCE, ANIMAL PERFORMANCE, AND CARCASS CHARACTERISTICS

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

APPROVED: July 24, 2024

Assent:

Documento assinado digitalmente
gov.br
JULIA TRAVASSOS DA SILVA
Data: 24/10/2024 13:19:16-0300
Verifique em https://validar.iti.gov.br

_____

Júlia Travassos da Silva
Author

Documento assinado digitalmente
gov.br
SEBASTIAO DE CAMPOS VALADARES FILHO
Data: 24/10/2024 14:36:44-0300
Verifique em https://validar.iti.gov.br

_____

Sebastião de Campos Valadares Filho
Adviser

ACKNOWLEDGEMENTS

This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES), the National Council for Scientific and Technological Development (CNPq), INCT Ciência Animal, and Cargill Nutrição Animal, Itapira, SP, Brazil. I am profoundly grateful to the Universidade Federal de Viçosa, particularly the Animal Science Department, for nurturing my passion for research and providing the foundation that guided me into ruminant nutrition. UFV has been instrumental in my academic and professional development, and the 11 years I spent at the university were undoubtedly some of the best and most formative years of my life. To me, UFV will always be the most beautiful and inspiring university in Brazil.

I also express my deepest gratitude to the Animal Science Department at North Dakota State University (NDSU) for warmly welcoming me during my sandwich PhD period. Collaborating and learning within such a dynamic academic environment was invaluable.

Completing this work would not have been possible without the support of many people. While it is difficult to name everyone, I would like to acknowledge a few who played particularly significant roles. I apologize if I have unintentionally left anyone out.

First and foremost, I thank God and my guardian angel, whose guidance and protection sustained me through these long and challenging four years. To my parents, Mônica and Ailton, your unwavering love, sacrifices, and belief in my dreams have been my constant strength. To my siblings, Aline and Filipe, and my beloved niece and nephew, Maria Alice and Rodrigo, thank you for being my anchors and endless supporters. I dedicate this achievement to all of you—my success is as much yours as it is mine.

I owe immense gratitude to my advisor, Tião, for his friendship, mentorship, and continuous encouragement throughout this journey. Your guidance has been essential to my success. I also extend my heartfelt thanks to the professors who have supported me and shared

their wisdom over the past 11 years at UFV. Each of you has left a lasting impact, and I will be forever grateful.

I want to give a special thanks to Pauliane, who had been by my side throughout the highs and lows of this postgraduate journey. Our shared experiences over these five years have meant more to me than words can express, and I am deeply grateful for your companionship and unwavering support.

I also express my deep appreciation to the dedicated staff members who contributed to the success of my research projects. Without your help, none of this would have been possible. I sincerely thank Pinguim, Geleia, Monteiro, Edson, Jojo, and Fernanda. A special acknowledgment to my dear friend Mauro (in memoriam), whose tireless efforts and generosity will never be forgotten. Your legacy remains with me, and I will always carry your memory in my heart.

To the students from LabNUR, both undergraduate and postgraduate, thank you for your invaluable contributions. Special recognition goes to my interns—Leandro, Eduardo, Renata, Alex, Érica, João, Douglas, Nathalia, Bruna, Carolina, Nathalia S., and Luan—and to my scholarship students—Gillyard, Fernando, Germano, Júlia, Gabriela, Yuri, Lívia, and Mariana. Your hard work, dedication, and resilience during the most demanding phases of this project were crucial. Thank you for the shared laughter and celebrations and for enduring my stress during the more challenging moments of the experiments. I would also like to acknowledge the animals used in this research profoundly. Their lives were treated with the utmost respect, and their contribution was fundamental to the success of this study.

Lastly, to everyone who has been part of my journey at UFV and throughout my doctoral studies, know that this achievement is not mine alone—it belongs to all of us. Your support, guidance, and presence have made this possible.

Thank you from the bottom of my heart!

ABSTRACT

SILVA, Julia Travassos da, D.Sc., Universidade Federal de Viçosa, July, 2024. **Partial and Total Replacement of Soybean Meal with Urea in Finishing Diet for Red Angus X Nellore Growing Bulls: Effects on the Ingestive, Digestive, Ruminal Parameters, Nitrogen Balance, Animal Performance, and Carcass Characteristics.** Adviser: Sebastião de Campos Valadares Filho.

The objectives of this study were 1) to evaluate the effects of SBM replacement on an equal N basis by increasing urea level on nutrients intake, water intake, digestibility, N balance, animal performance, and carcass characteristics in Red Angus x Nellore crossbred bulls fed finishing diets; 2) to evaluate the effects of partial or total replacement of SBM with urea on an equal N basis on intake and total and partial digestibility of nutrients, ruminal pH, ruminal ammonia nitrogen (NH3-N) and volatile fatty acids (VFAs) concentration, N balance, and the efficiency of microbial protein synthesis in Red Angus x Nellore crossbred bulls fed finishing diets.Two experiments were conducted. In the first experiment, forty-one Red Angus x Nellore bulls (37 8.6 ± 5.8 kg, 10 ± 1 months) were divided into five groups. A baseline group (n = 5) was slaug htered to estimate initial carcass weight, and the remaining animals were randomly assigned to four groups (n = 9 per treatment) and fed for 84 days. The treatments consisted of a control die t (no urea) and three levels of urea (10.0, 14.5, and 19.0 g/kg DM) replacing SBM on an equal N basis. No differences ($P > 0.05$) were observed in nutrient intake, digestibility, or nitrogen b alance across treatments. Urea inclusion reduced rumen undegradable protein intake ($P < 0.00$ 1), but performance and carcass characteristics were not affected ($P > 0.05$). The second exper iment used four rumen- and ileum-cannulated bulls in a 4 × 4 Latin square design. Urea inclus ion did not affect ($P > 0.05$) nutrient digestibility, ruminal pH, or volatile fatty acid concentrati ons. Ammonia nitrogen was influenced by treatment and time post-feeding ($P < 0.05$). Microb ial protein synthesis and efficiency were also unaffected ($P > 0.05$). These findings suggest tha t replacing SBM with up to 19.0 g/kg DM of urea can be done without adverse effects on intak e, performance, or ruminal function in bulls fed corn-based diets.

Keywords: Nutrient digestibility; Nitrogen balance; Microbial protein synthesis

RESUMO

SILVA, Julia Travassos da, D.Sc., Universidade Federal de Viçosa, Julho, 2024. **Partial and Total Replacement of Soybean Meal with Urea in Finishing Diet for Red Angus X Nellore Growing Bulls: Effects on the Ingestive, Digestive, Ruminal Parameters, Nitrogen Balance, Animal Performance, and Carcass Characteristics.** Orientador: Sebastião de Campos Valadares Filho.

Os objetivos foram avaliar os efeitos da substituição do farelo de soja (FS) com base equivalente de nitrogênio (N) por níveis crescentes de ureia: 1) sobre o consumo de nutrientes e água, digestibilidade, balanço de N, desempenho animal e características de carcaça; 2) sobre o consumo e a digestibilidade total e parcial de nutrientes, pH ruminal, concentração de nitrogênio amoniacal (NH3-N) ruminal e ácidos graxos voláteis (AGVs), balanço de N e a eficiência da síntese de proteína microbiana (PBMic) em bovinos cruzados alimentados com dietas de terminação. No primeiro experimento, 41 bovinos Red Angus x Nelore (378,6 ± 5,8 kg, 10 ± 1 meses) foram divididos em cinco grupos. Um grupo de referência (n = 5) foi abatido para estimar o peso inicial da carcaça, e os animais restantes foram aleatoriamente alocados em quatro grupos (n = 9 por tratamento) e alimentados por 84 dias. Os tratamentos consistiram em uma dieta controle (sem ureia) e três níveis de inclusão de ureia (10,0, 14,5 e 19,0 g/kg MS) substituindo FS em base equivalente de N. Não foram observadas diferenças ($P > 0,05$) no consumo de nutrientes, digestibilidade ou balanço de N entre os tratamentos. A inclusão de ureia reduziu o consumo de PNDR ($P < 0,001$), mas o desempenho e as características de carcaça não foram afetados ($P > 0,05$). O segundo experimento utilizou quatro bovinos canulados no rúmen e íleo em um delineamento quadrado latino 4 × 4. A inclusão de ureia não afetou ($P > 0,05$) a digestibilidade de nutrientes, o pH ruminal ou as concentrações de AGVs. O NH3-N foi influenciado pelo tratamento e pelo tempo pós-alimentação ($P < 0,05$). A síntese e eficiência de PBMic também não foram afetadas ($P > 0,05$). A substituição do FS por até 19,0 g/kg MS de ureia pode ser realizada sem efeitos adversos sobre o consumo, desempenho ou função ruminal em bovinos cruzados alimentados com dietas de terminação.

Palavras-chave: Digestibilidade; Balanço de nitrogênio; Síntese de proteína microbiana

SUMMARY

INTRODUCTION

Over the last decade, the recommended inclusion level of concentrate in Brazilian feedlots has increased to 71-90% per kg of diet dry matter (DM; Silvestre and Millen, 2021). Currently, most of these feeding systems use diets containing high amounts of grain and soybean meal (SBM) as a source of crude protein (CP). In fact, in Brazil, SBM is the primary source of plant-based protein and is used by 55.6% of Brazilian nutritionists (Silvestre and Millen, 2021). However, SBM is an expensive ingredient, and due to its high cost, as well as the scarcity of protein feedstuffs in many regions of the world, beef producers have been actively exploring alternative protein sources that could reduce feedlot production costs (Benedeti et al., 2014; Pereira et al., 2020).

A widely studied dietary alternative is the partial or total replacement of true dietary protein with non-protein nitrogen (NPN) sources, which can provide nitrogen (N) for ruminal microbial protein synthesis (MPS; Storm and Ørskov, 1983) and consequently reduce feeding costs (Rozanski et al., 2019; Liang et al., 2020). Urea is the most commonly used NPN source in livestock systems due to its low cost per unit of N, ease of obtainability, and high N content compared with other true protein sources, such as SBM. In Brazil, urea is commonly used as a N source in feedlot diets (Pinto and Millen, 2019; Silvestre and Millen, 2021). Besides that, based on early reviews of the literature, it was proposed that supplemental urea can be effectively utilized when dietary inclusion is limited to one-third of the total N or 10.0g/kg of dietary DM (Chalupa, 1968; Reid, 1953). In contrast, other studies (Zinn et al., 1994; Rennó et al., 2005; Magalhães et al., 2006) have demonstrated that intake and ruminal fermentation were not affected when high urea levels (19.5/g/kg of dietary DM) were added in the diet or when SBM was replaced with urea.

Besides that, 97.2% of Brazilian nutritionists recommend ground corn as the primary grain source in finishing diets (Silvestre and Millen, 2021). However, corn-based diets may not

supply adequate amounts of rumen degradable protein (RDP) because corn CP is approximately 61% rumen undegradable protein (RUP; NRC, 2001). Consequently, the combination of a corn-based diet with a highly rumen-degradable N source, like urea, provides greater synchronism in the degradation of carbohydrates and N (Sniffen and Robinson, 1987). Ensuring the simultaneous availability of N and energy in the rumen is essential for enhancing microbial growth rates and nutrient utilization efficiency (Helmer and Bartley, 1971). However, few experiments in the last decade have been designed to evaluate the effects of total replacement of SBM with urea in high-concentrate diets for beef cattle using urea inclusion levels above 10.0 g/kg of dietary DM. Utilization of the correct inclusion levels of dietary urea required for optimum N use by ruminal microbes would allow adequate ruminal fermentation, thereby reducing feed costs. Thus, we hypothesized that 19.0 g/kg DM of urea inclusion can totally replace SBM in a corn-based diet without influencing on the ingestive, digestive, ruminal parameters, n balance, animal performance, and carcass characteristics in crossbred finishing bulls.

Thus the objectives of this study were 1) to evaluate the effects of SBM replacement on an equal N basis by increasing urea level on nutrients intake, water intake, digestibility, N balance, animal performance, and carcass characteristics in Red Angus x Nellore crossbred bulls fed finishing diets; 2) to evaluate the effects of partial or total replacement of SBM with urea on an equal N basis on intake and total and partial digestibility of nutrients, ruminal pH, ruminal ammonia nitrogen (NH3-N) and volatile fatty acids (VFAs) concentration, N balance, and the efficiency of microbial protein synthesis in Red Angus x Nellore crossbred bulls fed finishing diets.

REFERENCES

Benedeti, P.D.B., Paulino, P.V.R., Marcondes, M.I., Valadares Filho, S.C., Martins, T.S., Lisboa, E.F., Silva, L.H.P., Teixeira, C.R.V., Duarte, M.S., 2014. Soybean meal replaced by slow release urea in finishing diets for beef cattle. Livest. Sci. 165, 51–60. https://doi.org/10.1016/j.livsci.2014.04.027

Chalupa, W., 1968. No TitleProblems in feeding urea to ruminants. J. Anim. Sci. 27, 207–219.

Helmer, L.G., Bartley, E.E., 1971. Progress in the Utilization of Urea as a Protein Replacer for Ruminants. A Review. J. Dairy Sci. 54, 25–51. https://doi.org/10.3168/jds.S0022-0302(71)85776-4

Liang, H., Zhao, E., Feng, C., Wang, J., Xu, L., Li, Z., Yang, S., Ge, Y., Li, L., Qu, M., 2020. Effects of slow-release urea on in vitro rumen fermentation parameters, growth performance, nutrient digestibility and serum metabolites of beef cattle. Semin. Agrar. 41, 1399–1414. https://doi.org/10.5433/1679-0359.2020v41n4p1399

Magalhães, K.A., Valadares Filho, S.C., Paulino, P.V.R., Paulino, M.F., Valadares, R.F.D., 2006. Performance, digestibility and carcass characteristics of feedlot dairy steers fed diets with different urea levels. Arq. Bras. Med. Vet. e Zootec. 58, 860–867. https://doi.org/10.1590/S0102-09352006000500023

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7.rev.ed., Washinton, D.C.

Pereira, G.F.C., Carvalho, F.F.R. De, Souza, E.J.O. De, Medeiros, A.N. De, Maciel, M.D.V., Ramos, A.O., Branco, G.L.C., Neves, R.D.S., 2020. Urea can replace soybean meal in sheep diet without alter intake, digestibility and ruminal parameters. Artic. Int. J. Dev. Res. 10, 42092–42097. https://doi.org/10.37118/ijdr.20338.11.2020

Pinto, A.C.J., Millen, D.D., 2019. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: The 2016 Brazilian survey. Can. J. Anim. Sci. 99, 392–407. https://doi.org/10.1139/cjas-2018-0031

Reid, J.T., 1953. Urea as a Protein Replacement for Ruminants: A Review. J. Dairy Sci. 36, 955–996. https://doi.org/10.3168/jds.S0022-0302(53)91586-0

Rennó, L.N., De Campos Valadares Filho, S., Valadares, R.F.D., Cecon, P.R., Backes, A.A., Rennó, F.P., Alves, D.D., Silva, P.A., 2005. Effects of feeding dietary urea levels on intake and total digestibility for steers of four genetic groups. Rev. Bras. Zootec. 34, 1775–1785. https://doi.org/10.1590/s1516-35982005000500039

Rozanski, S., Vivian, D.R., Kowalski, L.H., Rochadelli, R., de Freitas, J.A., Garcez Neto, A.F., Fernandes, S.R., 2019. The economic viability of the inclusion of urea in the ration for feedlot lambs. Rev. Bras. Saude e Prod. Anim. 20, 1–12. https://doi.org/10.1590/S1519-9940200262019

Silvestre, A.M., Millen, D.D., 2021. The 2019 Brazilian Survey On Nutritional Practices Provided By Feedlot Cattle Consulting Nutritionists. Rev. Bras. Zootec. 50, 1–25. https://doi.org/10.37496/RBZ5020200189.

Sniffen, C.J., Robinson, P.H., 1987. Microbial Growth and Flow as Influenced by Dietary Manipulations. J. Dairy Sci. 70, 425–441. https://doi.org/10.3168/jds.S0022-0302(87)80027-9

Storm, E., Ørskov, E.R., 1983. The nutritive value of rumen micro-organisms in ruminants. Br. J. Nutr. 50, 463–470. https://doi.org/10.1079/BJN19830114

Valadares Filho, S. de C., Menezes, A. C. B., Benedeti, P. D. B., Lopes, S. A., Paulino, M. F., & Fonseca e Silva, F. (2018). *7th INTERNATIONAL SYMPOSIUM OF BEEF CATTLE PRODUCTION* (S. de C. Valadares Filho, M. F. Paulino, M. L. Chizzotti, D. Zanetti, L. H. P. Silva, M. V. C. Pacheco, F. H. Moura, M. R. Manso, J. M. V. Pereira, T. R. Moreira, R. O.

Souza, E. G. Mafort, S. S. Moreira, V. C. Morais, & N. G. Leite, Eds.). Scienza. https://doi.org/10.26626/978-85-8179-152-4/pal

Zinn, R.A., Borquez, J.L., Plascencia, A., Essig, H.W., Johnson, Z., Luther, L.W., 1994. Influence of Levels of Supplemental Urea on Characteristics of Digestion and Growth Performance of Feedlot Steers Fed a Fat-Supplemented High-Energy Finishing Diet. Prof. Anim. Sci. 10, 5–10. https://doi.org/10.15232/S1080-7446(15)31927-6

CHAPTER 1

Can Urea Totally Replace Soybean Meal in Finishing Diet for Crossbred Bulls?[1]

Corresponding author: julia.travassos@ufv.br ; Postal address: Av. P. H. Rolfs, sn, campus UFV, Departamento de Zootecnia, Viçosa-MG, Brazil, 36570-900 study was made possible by grants from CNPq-INCT/Ciência Animal, FAPEMIG and Capes.

ABSTRACT

The objective of the present study was to evaluate the effects of soybean meal (SBM) replacement on an equal nitrogen (N) basis by increasing urea level on nutrients intake and digestibility, N balance, animal performance, and carcass characteristics in crossbred bulls fed finishing diets. Forty-one Red Angus x Nellore growing bulls (initial BW of 378.6 ± 5.8 kg; age of 10± 1 mo) were randomized into 5 groups. A baseline group (n = 5) were slaughtered at the beginning of the experiment to estimate the initial carcass weight, and the remaining animals were randomly allocated to four experimental group (n = 9 bulls per treatment) and fed for 84d. The diets consisted of a Control (CTL) treatment consisting of diet without urea inclusion, and three levels of urea inclusion (10.0, 14.5 and 19.0 g/kg DM of a urea mixture urea with ammonium sulphate at the ratio of 9:1) replacing SBM on an equal N basis. The forage:concentrate ratio was 20:80 on a dry matter (DM) basis with corn silage as the forage. The diets were formulated to be isonitrogenous and supplying 125 g crude protein/kg diet DM. Voluntary intake, and total apparent digestibility of nutrients were not influenced ($P > 0.05$) by treatments. Increasing replacement of SBM with urea linearly decreased rumen undegradable protein intake ($P < 0.001$). The diet without urea inclusion has lower rumen degradable protein intake than diet with urea inclusion ($P = 0.017$). Increasing replacement of SBM with urea did not affect ($P > 0.05$) N balance, microbial protein synthesis and efficiency, plasma glucose and blood urea N concentration. Increasing replacement of SBM with urea did not affect ($P > 0.05$) performance and carcass characteristics in crossbred bulls fed finishing diets. These data suggest that 19.0 g/kg DM of urea inclusion can totally replace soybean meal on an equal N basis without adverse effects on nutrient intake and growth performance in crossbred bulls fed a ground corn-based diet.

Keywords: non-protein nitrogen, protein, microbial protein synthesis.

INTRODUCTION

Over the last decade, the recommended inclusion level of concentrate in Brazilian feedlots has increased to 71-90% per kg of diet dry matter (DM; Silvestre and Millen, 2021). Currently, most of these feeding systems use diets containing high amounts of grain and soybean meal (SBM) as a source of crude protein (CP). In fact, in Brazil SBM is the primary source of plant-based protein and it isused by 55.6% of Brazilian nutritionists in feedlots diets formulations (Silvestre and Millen, 2021). However, due to the high cost of SBM, as well as the scarcity of protein feedstuffs in many regions of the world, beef producers have been actively exploring alternative protein sources that could reduce feedlot production costs.

A dietary alternative widely studied is the partial or total replacement of true dietary protein with non-protein nitrogen (NPN) sources, which can provide nitrogen (N) for ruminal microbial protein synthesis (MPS; Storm and Ørskov, 1983) and consequently reduce feeding costs (Rozanski et al., 2019). Urea is a commonly used NPN source in livestock systems due to its low cost per unit of N, ease of obtainability, and high N content compared with other true protein sources, such as SBM. In Brazil, urea is commonly used as a N source in feedlot diets (Pinto and Millen, 2019; Silvestre and Millen, 2021). On average, the urea concentration in finishing diets recommended by Brazilian nutritionists is 10.0 g/kg of dietary dry matter (Silvestre and Millen, 2021). Moreover, the contradictory data on completely replacing SBM with urea highlight the need for further research on the impacts of using urea levels above 10.0 g/kg of DM in feedlot diets.

Besides, 97.2% of Brazilian nutritionists recommend ground corn as the primary grain energy in finishing diets (Silvestre and Millen, 2021). However, corn-based diets may not supply adequate amounts of rumen degradable protein (RDP) because corn CP is approximately 61% of rumen undegradable protein (RUP; NRC, 2001). Consequently, the combination of a corn-based diet with a highly rumen-degradable N source, as urea, could provide greater

synchronism in the degradation of carbohydrates and N. We hypothesized that 19.0 g/kg DM of urea inclusion can totally replace SBM in a corn-based diet without influence on nutrient intake, digestibility and growth performance in crossbred finishing bulls. The objective of the present study was to evaluate the effects of SBM replacement on an equal N basis by increasing urea level on nutrients intake, water intake, digestibility, N balance, animal performance, and carcass characteristics in Red Angus x Nellore crossbred bulls fed finishing diets.

MATERIALS AND METHODS

The study was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. All procedures were previously approved by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (Protocol CEUAP-UFV 023/2021).

Animals, experimental design, and diets

Forty-one Red Angus x Nellore growing bulls (initial BW of 378.6 ± 5.8 kg; age of 10± 1 mo) were used. The bulls were adapted to the finishing diet for a 9-day step-up protocol (Perdigão et al., 2018). At the end of this adaptation period, all growing bulls were weighed after a 16-h solid fasting period and randomized into 5 groups. A baseline group (n = 5) were slaughtered at the beginning of the experiment to estimate the initial carcass weight. And the remaining animals (i.e., growth performance; n = 36) were randomly allocated to four experimental group (n = 9 bulls per treatment) and fed for 84d. The diets included a Control (CTL) treatment consisting of a traditional diet without urea inclusion, and three levels of urea inclusion (10.0, 14.5 and 19.0 g/kg DM of a urea mixture urea with ammonium sulphate at the ratio of 9:1) replacing SBM on an equal N basis. The forage:concentrate ratio was 20:80 on a dry matter (DM) basis with corn silage as the forage. The diets were formulated according to the recommendations of BR-CORTE (Valadares Filho et al., 2016) to be isonitrogenous and

supplying 125 g CP/kg diet DM. The chemical composition of the diets is presented in Table 1. The RDP was calculated using the feed composition values typical of Brazil, with ground corn (GC), corn silage (CS), and soybean meal (SBM), as described by Valadares Filho et al. (2018). The calculation considered the rapidly degradable fraction (a), the potentially degradable fraction (b), and the degradation rate of fraction b (kd), assuming a passage rate (Kp) of 0.05 for all ingredients.

Measurements and sampling procedures

Each treatment was group-housed in a feedlot pen (48.0 m2) containing electronic feeders (Model AF-1000 Master; Intergado, Contagem, MG, Brazil) and waterers (ModelWD-1000 Master; Intergado, Contagem, MG, Brazil) that allow for measurement of individual feed and water intake. Before the experiment, each bull was fitted with an ear tag (left ear) containing a unique radio frequency transponder (FDX-ISO 11784/11785; Allflex, Joinville, Santa Catarina, Brazil; Chizzotti et al., 2015). The total mixed rations were provided twice a day, at 0700 and 1600 h. Feed delivery was adjusted daily to maintain the orts within 2 to 5% of the amount offered (as-fed basis). The corn silage was sampled daily and pooled weekly, oven-dried at 55°C for 72 h and ground in 1mm a knife mill (Willye mill, model TE-680, TECNAL, Brazil). Samples of each one of the concentrate ingredients were collected directly at the feed mill, and stored in a freezer at –20°C.

To evaluate apparent total-tract nutrient digestibility, microbial synthesis and efficiency, spot fecal and urine samples were collected from each bull from days 49 to 53. Fecal samples were collected during five consecutive days at 1800 h on day 1, at 1500 h on day 2, at 1200 and on day 3, at 0900 h on day 4, and at 0600 h on day 5. The samples were immediately placed in a forced ventilation oven at 55°C for 72 h. Subsequently, they were ground using a 2 and 1 - mm knife mill (Willye mill, model TE-680, TECNAL, Brazil). A composite sample (on an equal DM weight basis) from each animal was created and indigestible neutral detergent fiber

(iNDF) was used as a marker to estimate fecal DM excretion for each bull (Menezes et al., 2019; Alhadas et al., 2022).

Spot urine samples were collected through spontaneous urination or by gently stimulating for measurement of purine derivatives. Urine samples were collected at 12:00 on d 51 and at 18:00 on d 53. To preserve nitrogenous compounds, 10 ml of urine was diluted in 40 ml of 0.036 N sulfuric acid solution (Chen and Gomes, 1992) and samples were stored in a freezer at -20°C until further laboratory analyses.

Blood samples were collected on day 53 prior to morning feed delivery via jugular vein puncture and evacuated tubes containing a clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil) for blood urea N (BUN) concentration. A collection tube containing EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to obtain plasma for glucose concentration. For both serum and plasma, samples were centrifuged at 2200×g for 20 min after collection. Serum and plasma were immediately frozen at – 40 °C until analyzed.

Bulls were weighed at the beginning and end of the test period after 16-h period of feed removal to measure initial and final shrunk body weight (SBW) and average daily gain (ADG). The animal slaughter was performed using a captive bolt followed by bleeding. All organs and viscera were removed, washed, and weighed to obtain empty body weight (EBW). Subsequently, cleaned organs and viscera were ground in an industrial cutter to obtain a homogeneous sample, which, together with the samples of the limbs, head, leather, and blood, was used to estimate the chemical composition of the non-carcass components. All samples were collected in aluminum trays, weighed, and lyophilized. After removing the non-carcass components, the carcass of each bull was separated into two halves, weighed (hot carcass weight; HCW) and dressing to evaluate the hot carcass yield (HCY) and cooled at 4°C for 24 h. After this time, carcasses were weighed (cold carcass weight; CCW) to evaluate the cold

carcass yield (CCY). The carcass length (CL) was measured as the distance from the cranial edge of the ischiopubic symphysis to the medial cranial edge of the first rib. Subcutaneous fat thickness (backfat thickness; BFT) was measured using a digital caliper between the 11th and 12th rib cut on the left half-carcass. To calculate N retention in EBW, the left half-carcass of all animals was dissected into fat, muscle, and bone. These carcass components were then ground and sampled in aluminum trays for lyophilization. The carcass weight at the beginning of the finishing phase was estimated by multiplying the fasting body weight of each remaining bull by the average of baseline's animals' carcass dressing percentage. Therefore, hot carcass gain (HCG) and cold carcass gain (CCG) was calculated as the difference between the final and initial carcass weight divided by 84 d.

Laboratory analyses and calculations

Samples of feedstuffs, feces, carcass and non-carcass were analyzed for DM (AOAC, 2012; method 934.01) organic matter (OM; AOAC, 2012; method 930.05), CP (AOAC, 2012; 981.10 method), and ether extract (EE; AOAC, 2005; method 2003.05). The analysis of neutral detergent fiber (NDF) was performed according to the techniques described by Mertens (2002), without the addition of sodium sulfite, but with the addition of thermostable alpha-amylase to the detergent. The NDF concentration was corrected for residual ash and protein (apNDF). Estimations of neutral detergent insoluble nitrogen followed the technique described by Licitra et al. (1996). Indigestible NDF (iNDF) of fecal samples and feeds was determined according to Casali et al. (2008). The starch content of feed and fecal samples was quantified according to Silva et al. (2019). Non-fiber carbohydrates (NFC) were calculated according to the following equation (Detmann and Valadares Filho, 2010): NFC (% DM) = 100 - [%CP - (%CP derived from urea +% urea) + % apNDF +% EE + %ash].

The internal iNDF marker was used to estimate fecal DM excretion per animal per day. The calculation was performed by dividing iNDF intake by the fecal iNDF concentration. The

digestibility coefficient of each nutrient was estimated based on the total intake of each nutrient. The contents of total digestible nutrients (TDN) were estimated through the sum of the digestible nutrients, where: TDN = digestible CP+2.25 × digestible EE + digestible NDF + digestible NFC (NRC, 2001).

Urine samples were analyzed for creatinine, uric acid, and allantoin. The analyses of uric acid and creatinine were performed by using an automatic biochemical analyzer (Mindray/model BS200E, Shenzhen, China), whereas the allantoin analysis was performed according to the colorimetric method described by Chen and Gomes (1992). Uric acid and creatinine in urine were analyzed with kits Bioclin® (K0139 and K222, Belo Horizonte, Brazil). The daily excretion of creatinine (EC) was calculated based on the equation described by Costa e Silva et al. (2012), as following equation: EC (g/d) = 0.0345×BW ^ 0.9491 ×1.000, where BW = body weight. Then, the daily urinary volume was estimated from the ratio between the estimated EC and creatinine concentrations in the spot urine sample (Da Silva et al., 2001). Microbial efficiency was estimated as described by (Barbosa et al., 2011) using estimated daily purine derivative (sum of allantoin and uric acid) excretion in urine. Microbial efficiency was expressed in grams of microbial protein synthesized (MPS) per kilogram of TDN intake (g MPS/kg TDN).

The components of N balance were determined as described in Menezes et al. (2019), using the following equation and assuming urinary N excretion =N intake- (fecal N excretion + N retention in empty body weight). The N retained we obtained through comparative slaughter, whose procedures we described before.

Concentrations of blood urea (K056), and glucose (K082) in plasma were determined using Bioclin® kits (Belo Horizonte, Brazil) through an automatic biochemical analyzer (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Statistical analysis

The data were analyzed as a completely randomized design using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), where individual animals was considered as the experimental unit and treatment was included as a fixed effect in the model.

Contrast was used to compare urea levels group mean with the mean of the control group (characterized by a traditional SBM diet without urea). Comparisons between urea levels replacing SBM in the diets were conducted by the decomposition of sum of squares in orthogonal contrasts to linear and quadratic effects. The contrasts evaluated were CTL vs. Urea – Control versus urea inclusion levels, L – Linear effect across urea inclusion levels, and Q – Quadratic effect across urea inclusion levels. Differences were considered significant at $P \leq 0.05$.

RESULTS

Voluntary intake and Digestibility

Voluntary intake and total apparent digestibility of nutrients were not influenced ($P > 0.05$) by increasing replacement of SBM with urea (Table 2). Water intake was not affected ($P > 0.05$) by increasing urea level replacing totally SBM.

Nitrogen Balance, Microbial Protein Synthesis and Blood Metabolites

Increasing replacement of SBM with urea linearly decreased RUP intake ($P < 0.001$; Table 3). Additionally, as expected, the diet without urea inclusion has lower RDP intake than diet with urea inclusion ($P = 0.017$). However, increasing replacement of SBM with urea did not affect ($P > 0.05$) retained N, and MPS. The partial efficiency of ruminal N utilization (g MPS/kg TDN intake) was not ($P > 0.05$) influenced by treatment. Urinary and Fecal N excretion was not affected ($P > 0.05$) in response to increasing urea level replacing SBM. Increasing replacement of SBM protein with urea did not affect ($P > 0.05$) plasma glucose and BUN concentration (Table 3).

Animal Performance and Carcass Characteristics

Animal performance and carcass characteristics are presented in Table 4. Increasing replacement of SBM with urea did not affect ($P > 0.05$) initial BW, final BW, ADG, and gain-to-feed ratio (G:F). Also, no treatment differences were observed ($P > 0.05$) for backfat thickness and carcass weight, gain, yield, and length. The diet cost per day (US$/d) and per ADG decreased in response to increasing urea level replacing SBM. Thus, the total feeding cost (US$/bull) during the finishing period (84 d) also decreased.

DISCUSSION

In the current experiment, nutrient and water intake was not influenced by increasing urea inclusion replacing totally SBM on an equal N basis in crossbred bulls fed finishing diets. This lack of effect can be attributed to the absence of urea toxicity. The toxic dose of urea for cattle is approximately 0.5 g/kg of body weight (Huber and Kung, 1981). Considering that the average body weight of the animals in this study was 447 kg, the level required to induce intoxication would be approximately 224 g/d of urea-N intake. However, the animals on the highest urea inclusion diet (19.0 g/kg DM) consumed only 152 g/d of urea-N intake, clearly indicating no risk of intoxication. Besides that, no difference in DMI can be partly attributed to the similarity between the chemical composition of the experimental diets, since they were formulated to be isonitrogenous and to meet the nutritional requirements of a growing crossbred bull according to the recommendations of BR-CORTE (Valadares Filho et al., 2016). The results obtained in this study are similar to the results of Magalhães et al. (2006), who evaluated the total replacement of SBM with urea up to 19.5 g/kg of DM in feedlot dairy steers and reported no effect on DMI. Similarly, Rennó et al. (2005), observed no changes in nutrient intake in Holstein, ½ Holstein-Guzera, and ½ Holstein-Gir steers fed diets with four dietary urea levels (0, 0.65, 1.3, and 1.95%, DM basis). Additionally, according to several studies (Cardot et al., 2008; Kramer et al., 2009; Zanetti et al., 2019), there is a positive correlation

between water intake (WI) and dry matter intake (DMI). Therefore, since DMI remained unaffected by the total replacement of SBM with urea in the present study, it follows that WI was likewise unaffected.

Increasing replacement of SBM with urea did not influence nutrient digestibility. According to Russell et al. (1992), amylolytic bacteria derive up to 66% of their proteins from peptides or amino acids, and the remainder from ammonia N. This may indicate that a corn-based diet supplemented with a true protein source, such as SBM, supports ruminal microbial balance without causing disturbances in nutrient digestibility. Similar results were observed in beef cattle by Rennó et al. (2005), except for that NFC digestibility decreased linearly with increasing replacement of SBM with urea. Additionally, Corte et al. (2018) evaluating the effects of substituting ~50% of the soybeans in the diet of finishing Nellore steers with urea reported no differences in total digestibility of nutrients. Similarly, Spanghero et al. (2017) observed no changes in nutrient digestibility in finishing Italian Simmental bulls fed corn-based diets with SBM partly replaced by urea. However, Knaus et al. (2001) reported that SBM replacement with urea decreased the digestibility of DM, OM, and NFC. On the other hand, Magalhães et al. (2006) reported that replacement of up to one-third of the SBM with urea in confined dairy-cross steers resulted an increase in nutrient digestibility. Thus, there is inconsistency in the effects of the use of urea inclusion on nutrient digestibility in cattle fed finishing diets.

The diets were formulated to be isonitrogenous and DM intake, and thus N intake was not influenced by urea inclusion. Nitrogen intake has the greatest influence on urinary N excretion, including dietary RDP supply relative to the requirement (Holder et al., 2015). Urea is considered 100% RDP, whereas SBM contains both RDP and RUP (NRC, 2001). Urea is rapidly hydrolyzed in the rumen, resulting in the release of ammonia at a faster rate than SBM (Nichols et al., 2022). Ammonia in the rumen is utilized by microorganisms for MPS when

carbon skeletons from carbohydrates are available (Bach et al., 2005). If there is insufficient fermentable energy (carbon sources), ammonia is absorbed by the rumen wall and carried out to blood coming at the liver, where is converted to urea through the urea cycle, and predominately excreted in urine (Van Soest, 1994). Thus, the BUN is a reliable marker for the quantity of unused N in the rumen (Rennó et al., 2000). In our study, N, NFC, starch and TDN intake was not influenced by treatment, resulting in similar carbohydrate concentrations and fermentation in the rumen, and consequently having minimal effects on MPS. As a result, increasing SBM replacement with urea did not result in differences in urinary N excretion. Furthermore, animals showed a similar BUN, corroborating with Gardinal et al. (2017), who also found no difference in urea inclusion in the diet for BUN. Also, this data is in accordance with Harmeyer and Martens (1980), who noted that the BUN primarily influences the amount of N excreted in urine.

N retained and fecal N was not affected by increasing replacement of SBM with urea. Knaus et al. (2001) observed a similar result for N retained in steers fed a high-concentrate diet and, suggested that the use of urea as the only supplementary source of N can be efficiently used to meet the requirement of metabolizable protein and amino acids, in high concentrate diets, which agrees with the findings of the current study. The lack of difference in feed intake and ruminal MPS, suggests that the ground corn used in this experiment provided a ruminal environment that allowed utilization of N from urea hydrolysis. So, although urea hydrolysis in the rumen is rapid, the necessary energy input was guaranteed. Besides that, Maeng et al. (1976) suggested using an in vitro system, the optimal ratio for maximal microbial growth was 75% urea-N and 25% amino acid-N. According to Maeng et al. (1976), maximal microbial growth occurs when both ammonia-N and amino acid-N are available for cell protein synthesis, and experiments where the growth media consisted of either 100% urea or 100% amino acid failed to achieve maximum microbial growth. These findings align well with the data obtained in our

experiment, where diets containing urea (10.0, 14.5, and 19.0 g/kg DM), resulting in approximately 22%, 32%, and 42% urea- N, respectively.

The lack of effects on growth performance with increasing urea level replacing SBM in the diet may not be surprising since 60-90% of differences in animal performance are a consequence of differences in DM intake (Mertens, 1994). Similar to the present study, Spanghero et al. (2017) evaluated the effect of partial replacement of SBM with urea in a corn-based diet and observed no effect on ADG in finishing Italian Simmental bulls. Magalhães et al. (2006) reported that increasing SBM replacement for urea in the diet of steers did not influence final BW and G:F, but steers fed diets with urea had greater ADG. In contrast to our results, some studies have reported contrasting results regarding the effects of urea supplementation on ADG and G:F in feedlot cattle, with or without a simultaneous effect on feed intake when similar or greater urea inclusion levels to those used in this study (Pires et al., 2008; Fernandes et al., 2009; Porsch et al., 2018).

Carcass yield characteristics have become more important in recent years because marketing has shifted from a live weight to carcass weight (mainly HCW) basis (Menezes et al., 2005). Therefore, carcass weight is one of the most important factors influencing profitability of producers (Coyne et al., 2019). In the current study, carcass characteristics were not altered by urea inclusion. Similar results were reported by Magalhães et al. (2006), who observed no differences in carcass length and backfat thickness in steers that were fed with increasing urea inclusion. Dos Santos Cardoso et al. (2019), also found no difference in carcass weight and yield when SBM was replaced with urea in Charolais x Nellore crossbred steer diets. Thus, as the cost of SBM is high on an equal N basis relative urea, substituting SBM with urea result in reduced feed costs without altering carcass characteristics. Therefore, increasing urea inclusion totally replacing SBM a high-concentrate diet for crossbred Red Angus x Nellore

bulls can be a cost-effective alternative. This approach should reduce feed input costs, and thus improve profitability for the producer.

CONCLUSION

These data suggest that 19.0 g/kg DM of urea inclusion can totally replace soybean meal on an equal N basis without adverse effects on nutrient intake and growth performance in crossbred bulls fed a ground corn-based diet.

REFERENCES

Alhadas, H.M., Valadares Filho, S.C., Tedeschi, L.O., Vilela, R.S.R., Souza, G.A.P., Lage, B.C., Silva, B.C., Rennó, L.N., Paulino, M.F., 2022. Effect of inclusion levels of low-fat dried distillers grains in finishing diets on protein and energy intake and retention and estimation of protein and energy requirements of young Nellore bulls fed with high concentrate diets. Anim. Sci. J. 93, 1–11. https://doi.org/10.1111/asj.13678

AOAC, 2012. Association of Official Analysis Chemists International, Official Methods of Analysis of AOAC International, 19th ed. Gaithersburg, Maryland.

AOAC, 2005. Association of Official Analysis Chemists International, Official Methods of Analysis of AOAC International, 18th ed. ed. Gaithersburg, Maryland.

Bach, A., Calsamiglia, S., Stern, M.D., 2005. Nitrogen Metabolism in the Rumen. J. Dairy Sci. 88, E9–E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Barbosa, A.M., Valadares, R.F.D., Valadares Filho, S.C., Pina, D.S., Detmann, E., Leão, M.I., 2011. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle1. J. Anim. Sci. 89, 510–519. https://doi.org/10.2527/jas.2009-2366

Cardot, V., Le Roux, Y., Jurjanz, S., 2008. Drinking Behavior of Lactating Dairy Cows and Prediction of Their Water Intake. J. Dairy Sci. 91, 2257–2264. https://doi.org/10.3168/jds.2007-0204

Casali, A.O., Detmann, E., Valadares Filho, S. de C., Pereira, J.C., Henriques, L.T., Freitas, S.G. de, Paulino, M.F., 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. Rev. Bras. Zootec. 37, 335–342. https://doi.org/10.1590/S1516-35982008000200021

Chen, X.B., Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives -an overview of the technical details. 20.

Chizzotti, M.L., Machado, F.S., Valente, E.E.L., Pereira, L.G.R., Campos, M.M., Tomich, T.R., Coelho, S.G., Ribas, M.N., 2015. Technical note: Validation of a system for monitoring individual feeding behavior and individual feed intake in dairy cattle. J. Dairy Sci. 98, 3438–3442. https://doi.org/10.3168/jds.2014-8925

Corte, R.R., Brito, F.O., Leme, P.R., Pereira, A.S.C., Freitas, J.E., Rennó, F.P., Silva, S.L., Tedeschi, L.O., Nogueira Filho, J.C.M., 2018. The effects of partial substitution of soybean with urea or slow-release urea on finishing performance, meat quality, and digestion parameters of Nellore steers. Anim. Prod. Sci. 58, 2242–2248. https://doi.org/10.1071/AN16609

Costa e Silva, L.F., de Campos Valadares Filho, S., Chizzotti, M.L., Rotta, P.P., Prados, L.F., Valadares, R.F.D., Zanetti, D., da Silva Braga, J.M., 2012. Creatinine excretion and relationship with body weight of Nellore cattle. Rev. Bras. Zootec. 41, 807–810. https://doi.org/10.1590/S1516-35982012000300046

Coyne, J.M., Evans, R.D., Berry, D.P., 2019. Dressing percentage and the differential between live weight and carcass weight in cattle are influenced by both genetic and non-genetic factors1. J. Anim. Sci. 97, 1501–1512. https://doi.org/10.1093/jas/skz056

Da Silva, R.M.N., Valadares, R.F.D., Valadares Filho, S.D.C., Cecon, P.R., Rennó, L.N., Da Silva, J.M., 2001. Uréia para Vacas em Lactação. 2. Estimativas do Volume Urinário, da Produção Microbiana e da Excreção de Uréia. Rev. Bras. Zootec. 30, 1948–1957. https://doi.org/10.1590/s1516-35982001000700035

Detmann, E., Valadares Filho, S.C., 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. Arq. Bras. Med. Veterinária e Zootec. 62, 980–984. https://doi.org/10.1590/S0102-09352010000400030

Dos Santos Cardoso, G., Da Silva Rodrigues, L., Machado, D.S., Domingues, C.C., Da Silva, M.B., Cattelam, P.M.M., Adams, S.M., Borchate, D., Brondani, I.L., Filho, D.C.A., 2019. Effect of replacement of soybean meal with protected or common urea on the carcass and meat characteristics of confined steers. Semin. Agrar. 40, 353–364. https://doi.org/10.5433/1679-0359.2019v40n1p353

Fernandes, J.J.R., Pires, A.V., Oliveira Jr., R.C.., Santos, F.A.P., Susin, I., Carvalho, E.R., 2009. Farelo de soja em substituição à ureia em dietas para bovinos de corte em crescimento. Ciência Anim. Bras. 3, 373–378.

Gardinal, R., Calomeni, G.D., Cônsolo, N.R.B., Takiya, C.S., Freitas, J.E., Gandra, J.R., Vendramini, T.H.A., Souza, H.N., Rennó, F.P., 2017. Influence of polymer-coated slow-release urea on total tract apparent digestibility, ruminal fermentation and performance of Nellore steers. Asian-Australasian J. Anim. Sci. 30, 34–41. https://doi.org/10.5713/ajas.16.0058

Harmeyer, J., Martens, H., 1980. Aspects of Urea Metabolism in Ruminants with Reference to the Goat. J. Dairy Sci. 63, 1707–1728. https://doi.org/10.3168/jds.S0022-0302(80)83132-8

Holder, V.B., Tricarico, J.M., Kim, D.H., Kristensen, N.B., Harmon, D.L., 2015. The effects of degradable nitrogen level and slow release urea on nitrogen balance and urea kinetics in Holstein steers. Anim. Feed Sci. Technol. 200, 57–65. https://doi.org/10.1016/j.anifeedsci.2014.12.009

Huber, J.T., Kung, L., 1981. Protein and Nonprotein Nitrogen Utilization in Dairy Cattle. J. Dairy Sci. 64, 1170–1195. https://doi.org/10.3168/jds.S0022-0302(81)82695-1

Knaus, W.F., Beermann, D.H., Guiroy, P.J., Boehm, M.L., Fox, D.G., 2001. Optimization of rate and efficiency of dietary nitrogen utilization through the use of animal by-products and(or) urea and their effects on nutrient digestion in Holstein steers. J. Anim. Sci. 79, 753. https://doi.org/10.2527/2001.793753x

Kramer, E., Stamer, E., Spilke, J., Thaller, G., Krieter, J., 2009. Analysis of water intake and dry matter intake using different lactation curve models. J. Dairy Sci. 92, 4072–4081. https://doi.org/10.3168/jds.2008-1957

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. https://doi.org/10.1016/0377-8401(95)00837-3

Maeng, W.J., Van Nevel, C.J., Baldwin, R.L., Morris, J.G., 1976. Rumen Microbial Growth Rates and Yields: Effect of Amino Acids and Protein. J. Dairy Sci. 59, 68–79. https://doi.org/10.3168/jds.S0022-0302(76)84157-4

Magalhães, K.A., Valadares Filho, S.C., Paulino, P.V.R., Paulino, M.F., Valadares, R.F.D., 2006. Performance, digestibility and carcass characteristics of feedlot dairy steers fed diets with different urea levels. Arq. Bras. Med. Vet. e Zootec. 58, 860–867. https://doi.org/10.1590/S0102-09352006000500023

Menezes, A.C.B., Valadares Filho, S.C., Costa e Silva, L.F., Pacheco, M.V.C., Pereira, J.M.V., Rotta, P.P., Zanetti, D., Detmann, E., Silva, F.A.S., Godoi, L.A., Rennó, L.N., 2016. Does a reduction in dietary crude protein content affect performance, nutrient requirements, nitrogen losses, and methane emissions in finishing Nellore bulls? Agric. Ecosyst. Environ. 223, 239–249. https://doi.org/10.1016/j.agee.2016.03.015

Menezes, A.C.B., Valadares Filho, S.C., Pacheco, M.V.C., Pucetti, P., Silva, B.C., Zanetti, D., Paulino, M.F., Silva, F.F., Neville, T.L., Caton, J.S., 2019. Oscillating and static dietary crude protein supply. I. Impacts on intake, digestibility, performance, and nitrogen balance in young Nellore bulls. Transl. Anim. Sci. 3, 1205–1215. https://doi.org/10.1093/tas/txz138

Menezes, L.F.G. de, Restle, J., Brondani, I.L., Alves Filho, D.C., Kuss, F., Silveira, M.F. da, Amaral, G.A. do, 2005. Características da carcaça de novilhos de gerações avançadas do cruzamento alternado entre as raças Charolês e Nelore, terminados em confinamento. Rev. Bras. Zootec. 34, 934–945. https://doi.org/10.1590/S1516-35982005000300027

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217–40.

Mertens, D.R., 1994. Regulation of forage intake. Fahey Jr., G.C., Ed., Forage Qual. Eval. Util. Am. Soc. Agron. Madison 450–493.

Nichols, K., de Carvalho, I.P.C., Rauch, R., Martín-Tereso, J., 2022. Review: Unlocking the limitations of urea supply in ruminant diets by considering the natural mechanism of endogenous urea secretion. Animal 16, 100537. https://doi.org/10.1016/j.animal.2022.100537

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7.rev.ed., Washinton, D.C.

Perdigão, A., Millen, D.D., Brichi, A.L.C., Vicari, D.V.F., Franzói, M.C.S., Barducci, R.S., Martins, C.L., Estevam, D.D., Cesar, M.T., Arrigoni, M.D.B., 2018. Effects of restricted vs. step up dietary adaptation for 6 or 9 days on feedlot performance, feeding behaviour, ruminal and blood variables of Nellore cattle. J. Anim. Physiol. Anim. Nutr. (Berl). 102, 224–234. https://doi.org/10.1111/jpn.12681

Pinto, A.C.J., Millen, D.D., 2019. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: The 2016 Brazilian survey. Can. J. Anim. Sci. 99, 392–407. https://doi.org/10.1139/cjas-2018-0031

Pires, A.V., Oliveira Junior, R.C., Susin, I., Fernandes, J.J.R., Morais, J.B., Mendes, C.Q., 2008. Fontes nitrogenadas em rações contendo bagaço de cana-de-açúcar hidrolizado no desempenho de bovinos confinados em terminação. Arq. Bras. Med. Veterinária e Zootec. 60, 163–168. https://doi.org/10.1590/S0102-09352008000100023

Porsch, R.V., Machado, D.S., Brondani, I.L., Cocco, J.M., Filho, D.C.A., de Oliveira, L.M., 2018. Nitrogen sources associated with different physical forms of corn grain in the diet for steers in feedlot. Acta Sci. - Anim. Sci. 40, 1–8. https://doi.org/10.4025/actascianimsci.v40i1.42541

Rennó, L.N., De Campos Valadares Filho, S., Valadares, R.F.D., Cecon, P.R., Backes, A.A., Rennó, F.P., Alves, D.D., Silva, P.A., 2005. Effects of feeding dietary urea levels on intake and total digestibility for steers of four genetic groups. Rev. Bras. Zootec. 34, 1775–1785. https://doi.org/10.1590/s1516-35982005000500039

Rennó, L.N., Valadares, R.F.D., Valadares Filho, S. de C., Leão, M.I., Silva, J.F.C. da, Cecon, P.R., Gonçalves, L.C., Dias, H.L.C., Linhares, R.S., 2000. Concentração plasmática de uréia e excreções de uréia e creatinina em novilhos. Rev. Bras. Zootec. 29, 1235–1243. https://doi.org/10.1590/S1516-35982000000400038

Rozanski, S., Vivian, D.R., Kowalski, L.H., Rochadelli, R., de Freitas, J.A., Garcez Neto, A.F., Fernandes, S.R., 2019. The economic viability of the inclusion of urea in the ration for feedlot lambs. Rev. Bras. Saude e Prod. Anim. 20, 1–12. https://doi.org/10.1590/S1519-9940200262019

Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J., Sniffen, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70, 3551–3561. https://doi.org/10.2527/1992.70113551x

Silva, B. de C., Godoi, L.A., Valadares Filho, S. de C., Zanetti, D., Benedeti, P.D.B., Detmann, E., 2019. A suitable enzymatic method for starch quantification in different organic matrices. MethodsX 6, 2322–2328. https://doi.org/10.1016/j.mex.2019.09.040

Silvestre, A.M., Millen, D.D., 2021. The 2019 Brazilian Survey On Nutritional Practices Provided By Feedlot Cattle Consulting Nutritionists. Rev. Bras. Zootec. 50, 1–25. https://doi.org/10.37496/RBZ5020200189

Spanghero, M., Mason, F., Zanfi, C., Nikulina, A., 2017. Effect of diets differing in protein concentration (low vs medium) and nitrogen source (urea vs soybean meal) on in vitro rumen fermentation and on performance of finishing Italian Simmental bulls. Livest. Sci. 196, 14–21. https://doi.org/10.1016/j.livsci.2016.12.004

Storm, E., Ørskov, E.R., 1983. The nutritive value of rumen micro-organisms in ruminants. Br. J. Nutr. 50, 463–470. https://doi.org/10.1079/BJN19830114

Tedeschi, L.O., Baker, M.J., Ketchen, D.J., Fox, D.G., 2002. Performance of growing and finishing cattle supplemented with a slow-release urea product and urea. Can. J. Anim. Sci. 82, 567–573. https://doi.org/10.4141/A02-018

Valadares Filho, S. de C., Lopes, S.A., Silva, B. de C., Chizzotti, M.L., Bissaro, L.Z., 2018. Tabelas Brasileiras de Composição de Alimentos para Ruminantes. [WWW Document]. URL www.cqbal.com.br (accessed 6.5.23).

Valadares Filho, S. de C., Silva, L.F.C. e, Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, in: Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE. Editora Federal de Viçosa. https://doi.org/10.5935/978-85-8179-111-1.2016B002

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. Cornell university press.

Zanetti, D., Prados, L.F., Menezes, A.C.B., Silva, B.C., Pacheco, M.V.C., Silva, F.A.S., Costa E Silva, L.F., Detmann, E., Engle, T.E., Valadares Filho, S.C., 2019. Prediction of water intake to Bos indicus beef cattle raised under tropical conditions. J. Anim. Sci. 97, 1364–1374. https://doi.org/10.1093/jas/skz003

TABLES:

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

Item	CTL	Urea levels (g/kg DM)		
		10.0	14.5	19.0
Proportion, %				
Corn Silage	20.00	20.00	20.00	20.00
Ground corn	64.87	70.30	72.80	75.00
Soybean meal	12.00	5.57	2.67	-
Urea/A.S[1]	-	1.00	1.45	1.90
Mineral mix[2]	3.00	3.00	3.00	3.00
Virginiamycin	0.13	0.13	0.13	0.13
Chemical composition[3]				
DM, g/kg as-fed	605.6	605.6	605.7	605.7
OM, g/kg DM	943.5	947.1	948.8	950.3
apNDF, g/kg DM	181.0	179.4	178.7	178.0
CP, g/kg DM	126.2	125.7	125.5	126.3
RDP, g/kg CP	589.4	651.5	679.7	708.0
RUP, g/kg CP	410.6	348.5	320.3	292.0
EE, g/kg DM	24.2	25.4	25.9	26.4
Starch, g/kg DM	568.0	607.5	625.4	641.5
NFC, g/kg DM	612.1	634.1	644.0	652.9

[1] Urea/A.S, urea plus ammonium sulfate (9:1). [2] Probeef® Confinamento Performa (Cargill Nutrição Animal, Itapira, SP, Brazil). Assurance levels per kilogram of product: 242- 300.0 g of Ca; 11.10 mg of Co (Min); 556.0 mg of Cu (Min); 24.5 g of S (Min); 370.0 mg of Fe (Min); 13.50 g of P (Min); 27.7 mg of I (Min); 19.0 g of Mg (Min); 1668.0 mg of Mn (Min); 7.4 mg of Se (Min); 81.5 g of Na (Min); 2223.0 mg of Zn (Min); 92550.0 UI of vitamin A (Min); 14780.0 UI of vitamin D3 (Min); 136.0 UI of vitamin E (Min); and 928.0 mg of monensin. [3] DM, dry matter; OM, organic matter; apNDF, neutral detergent fiber corrected for residual ash and residual nitrogenous compounds; CP, crude protein; RDP, rumen degradable protein; RUP, rumen undegradable protein; EE, ether extract; NFC, non-fiber carbohydrates.

Table 2. Effect of soybean meal replacement by urea in finishing diet on water intake, voluntary intake and total apparent digestibility of nutrients in crossbred bulls.

Item[1]	CTL	Urea levels (%)			SEM[2]	CTL x Urea[3]	Urea Levels[4]	
		1	1.45	1.9			L	Q
Intake, kg/d								
DM	8.67	8.76	8.21	8.02	0.28	0.306	0.069	0.614
OM	8.18	8.3	7.79	7.62	0.27	0.377	0.080	0.612
CP	1.09	1.1	1.03	1.01	0.04	0.265	0.086	0.548
apNDF	1.57	1.57	1.47	1.43	0.05	0.180	0.051	0.615
Starch	4.92	5.32	5.14	5.14	0.17	0.170	0.469	0.656
NFC	5.31	5.55	5.29	5.24	0.18	0.792	0.216	0.632
TDN	6.24	6.4	6.11	6.04	0.21	0.817	0.222	0.677
Water	28.14	24.5	25.72	24.43	1.68	0.086	0.975	0.540
Intake, kg/kg BW								
DM	1.91	1.94	1.84	1.83	0.04	0.431	0.059	0.342
Apparent digestibility, g/kg								
DM	724	708	713	711	8.80	0.208	0.784	0.720
OM	741	727	731	728	8.20	0.187	0.904	0.675
CP	687	669	674	793	12.10	0.552	0.180	0.643
apNDF	349	339	323	319	19.30	0.336	0.484	0.788
Starch	979	970	975	968	4.70	0.142	0.773	0.278
NFC	870	856	864	856	7.10	0.190	0.971	0.396

[1] DM, dry matter; OM, organic matter; CP, crude protein; apNDF, neutral detergent fiber corrected for residual ash and residual nitrogenous compounds; NFC, non-fiber carbohydrates; TDN, total digestible nutrients. [2]SEM=standard error mean. [3]CTL vs. Urea – Control versus urea inclusion levels.[4] L – Linear effect across urea inclusion levels and Q – Quadratic effect across urea inclusion levels.

Table 3. Effect of increasing soybean meal replacement with urea in a finishing diet on nitrogen balance, microbial protein synthesis and blood parameters of crossbred bulls.

Item[1]	CTL	Urea levels (%)			SEM[2]	CTL x Urea[3]	Urea Levels[4]	
		1	1.45	1.9			L	Q
N intake, g/d	175	176	165	162	5.6	0.273	0.084	0.552
Urea-N intake, g/d	-	88	119	152	3.5	<.001	<.001	0.838
Fecal N, g/d	55.1	58.1	54.2	49.7	3	0.753	0.054	0.942
Urinary N, g/d	73.4	73.8	66.5	68.4	3.2	0.312	0.246	0.245
Retained N, g/d	46.6	44.4	44.4	43.8	1.8	0.26	0.833	0.915
Retained N/ N intake	26.7	25.3	26.9	27	0.9	0.787	0.179	0.475
RDP, g/d	645	718	701	717	23.1	0.017	0.981	0.563
RUP, g/d	449	384	330	296	12.3	<.001	<.001	0.535
MPS, g/d	638	689	675	667	45.9	0.476	0.759	0.958
Glucose, mg/dL	70.5	67.7	70.8	65.3	3.8	0.562	0.654	0.368
Urea, mg/dL	10.2	10.7	10.7	11.4	0.7	0.388	0.498	0.680

[1]RDP, Rumen degradable protein; RUP, Rumen undegradable protein; MPS, Microbial protein synthesis; TDN, Total digestible nutrients; BUN, Blood urea nitrogen. [2]SEM=standard error mean. [3]CTL vs. Urea – Control versus urea inclusion levels.[4] L – Linear effect across urea inclusion levels and Q – Quadratic effect across urea inclusion levels.

Table 4. Effect of soybean meal replacement by urea in finishing diet on diet cost, animal performance, and carcass characteristics of crossbred bulls.

Item[1]	CTL	Urea levels (%)			SEM[2]	CTL x Urea[3]	Urea Levels[4]	
		1	1.45	1.9			L	Q
Performance								
Initial BW, kg	380	381	381	373	12.2	0.919	0.637	0.808
Final BW, kg	528	521	511	504	13.8	0.326	0.385	0.955
ADG, kg	1.76	1.66	1.56	1.56	0.07	0.055	0.314	0.575
G:F	0.20	0.19	0.19	0.20	0.01	0.183	0.764	0.604
Carcass characteristics								
HCW, kg	316	314	306	301	8.64	0.356	0.299	0.851
CCW, kg	312	310	303	297	8.5	0.393	0.283	0.940
HCG, kg/d	1.17	1.13	1.05	1.04	0.04	0.072	0.129	0.493
CCG, kg/d	1.15	1.12	1.04	1.02	0.04	0.089	0.123	0.510
HCY, %	59.9	60.3	59.8	59.8	0.41	0.940	0.380	0.570
CCY, %	59.1	59.5	59.2	59	0.39	0.743	0.289	0.920
CL, cm	130	128	128	128	1.39	0.184	0.538	0.747
BFT, mm	6.16	6.34	6.88	6.61	0.76	0.617	0.800	0.669
Diet Cost[5]								
US$/kg DM	0.26	0.24	0.23	0.22	-	-	-	-
US$/d	2.25	2.10	1.89	1.76	-	-	-	-
US$/ADG	1.28	1.26	1.21	1.13	-	-	-	-
Total Cost, US$	189.4	176.6	158.6	148.2	-	-	-	-

[1]BW, body weight; ADG, average daily gain; G:F, gain-to-feed ratio; HCW, hot carcass weight; CCW, cold carcass weight; HCG, hot carcass gain; CCG, cold carcass gain; HCY, hot carcass yield; CCY, cold carcass yield; CL, carcass length; BFT, backfat thickness. [2]SEM=standard error mean. [3]CTL vs. Urea – Control versus urea inclusion levels.[4] L – Linear effect across urea inclusion levels and Q – Quadratic effect across urea inclusion levels.[5]Ingredients prices are based on February/2024 values in Campo Grande, Mato Grosso do Sul, Brazil. The cost per kg of DM for each ingredient is as follows: Corn silage (U$0.14), Ground corn (U$0.19), Soybean meal (U$0.52), Urea (U$0.53), Virginiamycin (U$10.08), Mineral mix (U$0.80).

CHAPTER 2

Partial and Total Replacement of Soybean Meal with Urea in Finishing Diets for Red Angus X Nellore Growing Bulls: Ingestive, Digestive, and Ruminal Parameters[1]

Corresponding author: julia.travassos@ufv.br ; Postal address: Av. P. H. Rolfs, sn, campus UFV, Departamento de Zootecnia, Viçosa-MG, Brazil, 36570-900 study was made possible by grants from CNPq-INCT/Ciência Animal, FAPEMIG and Capes.

ABSTRACT

Four rumen- and ileum-cannulated Red Angus X Nellore bulls were used in a 4 × 4 Latin square design. The diets included a Control treatment consisting of a diet without urea inclusion, and three levels of urea inclusion (10.0, 14.5 and 19.0 g/kg DM of a urea mixture urea with ammonium sulphate at the ratio of 9:1) replacing SBM on an equal N basis. The forage:concentrate ratio was 20:80 on a dry matter (DM) basis with corn silage as the forage. The diets were formulated to be isonitrogenous and supplying 125 g crude protein/kg diet DM. There were no differences ($P > 0.05$) for DM (kg/d or g/kg of BW) and nutrient daily intake when SBM was total replaced by urea. The rumen, small intestine, hind gut, and apparent total-tract digestibility of nutrients were not influenced ($P > 0.05$) by urea inclusion. Ruminal NH3 concentrations was affected by treatment ($P < 0.05$), and by time after feeding ($P < 0.05$). There were no significant differences in ruminal pH ($P > 0.05$) with SBM replacement by urea. The urea inclusion did not influence the total and individual VFA concentration or acetate:propionate ratio ($P > 0.05$). Increasing replacement of SBM with urea did not affect ($P > 0.05$) N intake, N fecal, N urinary, blood urea N, and N retained. Additionally, the MPS and efficiency of MPS based on total digestible nutrients intake (gMPS/kg TDNI) or digestible organic matter intake (gMPS/kg DOMI) were not ($P > 0.05$) influenced by increasing urea level replacing SBM. In conclusion, 19.0 g/kg DM of urea can totally replace soybean meal on an equal N basis without influencing intake, digestibility of nutrients, and ruminal parameters in crossbred fed a corn-based diet.

Keywords: Microbial protein synthesis, Total digestible nutrients, non-protein nitrogen

INTRODUCTION

Soybean meal (SBM), although a high-quality vegetable protein source is expensive ingredients in diets for beef cattle (Benedeti et al., 2014). Therefore, partial or total substitution of a true protein ingredients, such as SBM, with a non-protein nitrogen (NPN) source can significantly reduce feeding cost (Pereira et al., 2020). Thus, NPN sources have been used as an alternative to meet beef cattle protein requirements to decrease the cost of feeding feedlot cattle (Liang et al., 2020).

Urea is the most used NPN as a nitrogen (N) source to meet microbial requirements, due to availability and low cost. Urea is rapidly hydrolyzed to ammonia (NH3) in the rumen (Helmer and Bartley, 1971). Therefore, in order to enhance microbial growth rates and efficiency of nutrient utilization, N and energy must be simultaneously available in the rumen (Sniffen and Robinson, 1987).

To overcome this problem, the supply of finishing diets rich in rapid rumen fermentable carbohydrate is essential for the use of urea, as it will be the necessary input of carbon skeletons, maximizing the use of N. Besides that, based on early reviews of the literature, it was proposed that supplemental urea can be effectively utilized when dietary inclusion is limited to one-third of the total N or 10.0g/kg of dietary DM (Chalupa, 1968; Reid, 1953). In contrast, other studies (Zinn et al., 1994; Rennó et al., 2005; Magalhães et al., 2006) have demonstrated that intake and ruminal fermentation were not affected when high urea levels (19.5 g/kg of dietary DM) were added in the diet or when SBM was replaced with urea in high forage diet. However, few experiments in the last decade have been designed to evaluate the effects of total replacement of SBM with urea in high-concentrate diets for beef cattle using urea inclusion levels above 10.0 g/kg of dietary DM. Utilization of the correct inclusion levels of dietary urea required for optimum N use by ruminal microbes would allow adequate ruminal fermentation, thereby reducing feed

costs. Thus, we hypothesized that 19.0 g/kg DM of urea inclusion can totally replace SBM in a corn-based diet without influencing intake, digestibility of nutrients and ruminal parameters in crossbred finishing bulls. The objective of the present study was to evaluate the effects of partial or total replacement of SBM with urea on an equal N basis on intake and total and partial digestibility of nutrients, ruminal pH, ruminal ammonia nitrogen (NH3-N) and volatile fatty acids (VFAs) concentration, N balance, and the efficiency of microbial protein synthesis in Red Angus x Nellore crossbred bulls fed finishing diets.

MATERIALS AND METHODS

This study was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. All procedures were previously approved by the Animal Ethics and Welfare Committee of the Universidade Federal de Viçosa (#023/2021).

Animals, experimental design, facilities, and diets

Four rumen- and ileum-cannulated Red Angus X Nellore bulls (body weight = 292 ± 14 kg; age = 8 ± 1.0 mo) were used in a 4 × 4 Latin square design. The diets included a Control treatment consisting of a traditional diet without urea inclusion, and three levels of urea inclusion (10.0, 14.5 and 19.0 g/kg DM of a urea mixture urea with ammonium sulphate at the ratio of 9:1) replacing SBM on an equal N basis. The forage:concentrate ratio was 20:80 on a dry matter (DM) basis with corn silage as the forage. The diets were formulated according to the recommendations of BR-CORTE (Valadares Filho et al., 2016) to be isonitrogenous and supplying 125 g CP/kg diet DM. The chemical composition of the diets is presented in Table 1. The RDP was calculated using the feed composition values typical of Brazil, with ground corn (GC), corn silage (CS), and soybean meal (SBM), as described by Valadares Filho et al. (2018). The calculation considered the rapidly degradable fraction (a), the potentially degradable fraction (b), and

the degradation rate of fraction b (kd), assuming a passage rate (Kp) of 0.05 for all ingredients.

Prior to the start of the experiment, bulls underwent a 30-d adaptation period to experimental facilities and conditions. The animals were identified, weighed, treated against endo and ectoparasites, and housed in a tie-stall barn with a concrete floor, and had free access to feed and water throughout the experiment. Each experimental period lasted 21 days, with 14 days of adaptation to the diets (Machado et al., 2016), and 7 days for data collection. The total mixed rations (TMR) were provided twice a day, at 0700 and 1600 h. Feed delivery was adjusted daily to maintain orts at approximately 50 g/kg of the amount offered on an as-fed basis.

Intake, total tract digestibility, and microbial efficiency

Feeds offered and orts from each animal were weighed daily during the collection period (from days 15–21). All diet ingredients and refusals were sampled daily and stored at –20˚C. At the end of each collection period, all samples were dried in a ventilated oven (55 ˚C) for 72 h and ground using a knife mill (model TE-680, Tecnal, Piracicaba, São Paulo, Brazil) with a 1-mm sieve. Daily samples of each diet ingredient were equally composited (DM basis) for each period. Also, daily refusal samples were composited for each animal within a period. The proportion of daily refusal in the composite sample was based on the amount of daily refusal by the total amount of refusal samples during the collection period, on a DM basis. The ingredient samples were analyzed individually and used to calculate dietary composition.

Daily DM intake was calculated by subtracting feed refusal DM from DM offered during the collection period. To estimate nutrient digestibility, total feces were collected from each bull over four consecutive days, from days 15–18 of each experimental period. After 24 h of collection, the total fecal output was weighed, mixed, and approximately

250 g were oven-dried at 55 ◦ C for 72 h. Fecal samples then were ground using a knife mill (model TE-680, Tecnal, Piracicaba, São Paulo, Brazil) with a 1-mm sieve. Based on fecal DM production of each day of the 4-d fecal collection period, a composite sample was constituted for each animal per experimental period.

Total urine output was collected from days 15–18 (over a 24 h period) using a collection funnel connected to tubing and a 20-L container with 200 mL of 50 % sulfuric acid to prevent N volatilization. Total urine volume was measured daily using a 2-L volumetric cylinder, mixed manually, subsampled at a constant percentage per day per animal, and stored frozen at 20 ˚C.

Marker infusion, Partial Digestibility estimation and Ruminal Parameters

Collections of omasal digesta were performed from days 19–21 of each experimental period to estimate ruminal digestibility and ruminal flow. Simultaneously, ileal digesta were also collected and underwent the same procedures as the omasal digesta. The digesta flow was estimated using a double marker (Rotta et al., 2014) approach, with Co-EDTA associated with liquid and small particles, and iNDF associated with large particles, as described by Valadares Filho et al. (2011). From days 16–21 of each experimental period, a solution (5 g/d of Co-EDTA was continuously infused into the rumen of each bull via a peristaltic pump (model BP-600.4, Colombo, Parana, Brazil) and tubing connected to the ruminal fistula at a rate of 115 mL/h. A total of eight omasal and ileal samples per animal were collected over a 9-h interval for three days: day 1 at 0800 h and 1700 h; day 2 at 0200 h, 1100 h, and 2000 h; day 3 at 0500 h, 1400 h and 2300 h, resulting in a 24-h collection with 3-h intervals. Omasal digesta samples were collected as described by Huhtanen et al. (1997) and adapted by Leão et al. (2004) At each collection time, 200 mL of omasal and ileal digesta sample were filtered (porosity of 100 μ m, 44 % of the surface, Sefar Nitex 100/44, Sefar, Thal, Switzerland). Solid and

liquid phases were individually sampled and lyophilized (model LP510, Liobras, São Carlos, São Paulo, Brazil). The solid phase samples were combined for each bull, weighed, and ground using a knife mill with a 1-mm sieve. The same process was used for samples from the liquid phase. The reconstituted omasal and ileal digesta was considered the sum of both solid and liquid phases (DM basis).

Samples of ruminal contents (approximately 250 mL per collection time) were manually collected from the cranial, ventral, and caudal areas of the rumen, at the same times previously described for omasal collections. Samples were filtered through a 100 nylon filter (Sefar Nitex; Sefar, Thal, Switzerland; porosity of 100 μm), and the pH of the ruminal fluid was measured using a digital potentiometer (model K39–1014B, Kasvi, São José dos Pinhais, Paraná, Brazil). A 50-mL aliquot of ruminal fluid from each sample was stored at 80 (°C) for further analysis of VFA and NH3-N.

Blood Sample

Blood samples were obtained on day 21 prior to morning feed delivery. All samples were collected by jugular vein puncture, using vacuum tubes with a clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil) to quantity blood urea. A tube with EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to quantify the plasma glucose concentration. After collection, samples were centrifuged at 2200×g for 20 min. Serum and plasma were immediately frozen at – 40 °C until analyzed.

Laboratory analysis and calculations

Samples of feedstuffs, orts, feces, omasal digesta, and ileal digesta were analyzed for DM (method 934.01), N (method 981.10), and ash (method 930.05) according to AOAC (2012) and EE (method 2003.05) according to AOAC (2005) after acid hydrolysis with HCl. Organic matter (OM) was considered the difference between DM and ash.

Crude protein (CP) concentration was calculated by multiplying the total N concentration by 6.25. Neutral detergent fiber concentration was determined with the addition of thermostable α-amylase (Ankom Tech. Corp., Fairport, NY, USA), without the addition of sodium sulphite. The residue was corrected for ash (Mertens, 2002) and residual N (Licitra et al., 1996; apNDF). The iNDF concentration was determined according to Valente et al. (2011), through ruminal incubation of samples for 288-h. The starch concentration was quantified according to Silva et al. (2019). Samples of omasal and ileal digesta were analyzed for cobalt by atomic absorption spectrophotometry (Spctr AA-800; Varian spectrometer, Harbor City, CA, USA) according to Kimura and Miller (1957). Non-fiber carbohydrates (NFC) were calculated according to the following equation (Detmann and Valadares Filho, 2010):

NFC (% DM) = 100 - [%CP - (%CP derived from urea +% urea) + % apNDF +% EE + %ash],

The omasal and ileal digesta flow was estimated via the digesta reconstitution technique (Faichney, 1975) using the double marker system. Cobalt was used as marker of liquid added to small particles phase, and iNDF as marker of large particles phase. The reconstitution factor was calculated based on concentrations of the markers in the different phases of the digesta (France and Siddons, 1986). The nutrient flow (g/d) was obtained by multiplying nutrient concentration in the true digesta (g/kg DM) by the true DM flow.

The total-tract apparent digestibility was calculated as: Digestibility (%) = {(x – y)/x} × 100, where x (kg/d) and y (kg/d) are the intake and the output in feces of each nutrient, respectively. Ruminal digestibility coefficients were estimated by measuring the difference between nutrient intake and omasal flow. Small intestine digestibility coefficients were estimated by measuring the difference between omasal flow and ileum

flow. Hind gut digestibility coefficients were estimated by measuring the difference between large intestine nutrient flow and fecal output. The contents of total digestible nutrients (TDN) were estimated through the sum of the digestible nutrients, where: TDN = digestible CP+2.25 × digestible EE + digestible NDF + digestible NFC (NRC, 2001).

For VFA analysis (lactate, acetate, butyrate, and propionate), rumen fluid samples were centrifuged (12,000 × g, 10 min, 4°C), and supernatants were treated as described by Siegfried et al. (1984). Ruminal VFA were analyzed via high performance liquid chromatography (HPLC) using a Dionex Ultimate 3000 Dual detector HPLC (Dionex Corporation, Sunnyvale, CA, USA) coupled to a refractive index Shodex RI-101 maintained at 40°C using a ion exchange column Phenomenex Rezex ROA, 300 × 7.8 mm maintained at 45°C. Mobile phase was prepared with 5 mmol/l sulfuric acid (H2SO4) and a flow of 0.7 ml/min. Concentration of NH3-N in ruminal and omasal fluids was obtained according to the technique described by Okuda et al. (1965).

Urine samples were analyzed for uric acid, and allantoin. The analyses of uric acid and creatinine were performed by using an automatic biochemical analyzer (Mindray/model BS200E, Shenzhen, China), whereas the allantoin analysis was performed according to the colorimetric method described by Chen and Gomes (1992). Microbial synthesis was estimated as described by (Barbosa et al., 2011) using estimated daily purine derivative (sum of allantoin and uric acid) excretion in urine. Microbial efficiency was expressed in grams of microbial protein synthesized (MPS) per kilogram of TDN intake (g MPS/kg TDNI) and per kilogram of digestible organic matter intake (kgDOMI).

Concentrations of blood urea (K056), and glucose (K082) in plasma were determined using Bioclin® kits (Belo Horizonte, Brazil) through an automatic

biochemical analyzer (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Statistical analysis

The data were analyzed as a 4 ×4 Latin square design with PROC MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) using the following statistical model: Yijk =μ +Di +aj +pk +eijk where: Yijk =dependent variable, μ =general mean; Di =fixed effect of urea level i; aj =random effect of animal j; pk =random effect of experimental period k (random); and eijk =random error taken as normal and independently distributed (NID) (0, σ2e). Contrast was used to compare urea levels group mean with the mean of the control group (characterized by a traditional SBM diet without urea). Comparisons between urea levels replacing SBM in the diets were conducted by the decomposition of sum of squares in orthogonal contrasts to linear and quadratic effects. The contrasts evaluated were CTL vs. Urea – Control versus urea inclusion levels, L – Linear effect across urea inclusion levels, and Q – Quadratic effect across urea inclusion levels. Differences were considered significant at $P \leq 0.05$.

Data of ruminal pH and NH3-N were analyzed as repeated measures using the PROC MIXED procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) using the REPEATED statement. The model tested was similar to that described above, including time as a fixed effect and all interactions. The unstructured covariance structure was used for random factors. The best structure of the (co)variance matrix was chosen based on Akaike's information criterion with correction. Differences were considered significant at $P \leq 0.05$.

RESULTS

There were no differences ($P > 0.05$) for DM (kg/d or g/kg of BW) and nutrient daily intake when SBM was total replaced by urea inclusion in the diet (Table 2). The rumen, small intestine, hind gut, and apparent total-tract digestibility of nutrients were not influenced ($P > 0.05$) by urea inclusion (Table 3).

Ruminal NH3 concentrations was affected by treatment ($P < 0.05$), and by time after feeding ($P < 0.05$), which generally increased after feeding, reaching peak concentration 1 h after feeding in all groups of animals (Figure 1). There were no significant differences in ruminal pH ($P > 0.05$) with SBM replacement by urea inclusion in the diets (Figure 2).

The urea inclusion replacing parcial or total SBM in the diets did not influence the total and individual VFA concentration ($P > 0.05$) or acetate:propionate ratio ($P > 0.05$; Table 4).

Increasing replacement of SBM with urea did not affect ($P > 0.05$) N intake, N fecal, N urinary, BUN, N retained (Table 9). Additionally, the MPS and efficiency of microbial protein synthesis based on TDN (gMPS/kg TDNI) or DOM (gMPS/kg DOMI) were not ($P > 0.05$) influenced by increasing urea level replacing SBM (Table 5).

DISCUSSION

In corn-based diets, amylolytic bacteria are the predominant microbial population (Nagaraja, 2016). Russell et al. (1983) reported that microorganisms that ferment non-fiber carbohydrates (NFC) derive up to 66% of their protein from peptides or amino acids, with the remainder derived from ammonia nitrogen (N). This implies that, although urea is rapidly hydrolyzed to NH3 and SBM is degraded to peptides and AA and eventually deaminated into NH3 in the rumen (Bach et al., 2005), they may be similar as N sources

in high-concentrate diets. Therefore, in this experiment, all diets probably provided the same synchrony in the availability of N and energy within the rumen. This may explain the lack of significant differences in terms of nutrient intake and digestibility when SBM is totally replaced by urea on an equal N basis in crossbred bulls fed corn-based diets. Similar to that found by Magalhães et al. (2006) and Rennó et al. (2005), the authors did not verify difference in dry matter intake when they evaluated the total replacement of SBM with urea up to 19.5 g/kg of DM. Additionally, the results obtained in this study are similar to the results of Pina et al. (2009) who evaluated the increase RDP level using urea in beef heifers and reported no effect on ruminal, small, large intestines and total tract apparent digestibility of DM, OM, and NFC.

Ammonia plays a vital role as a N source for MPS and growth in the rumen (Abdoun et al., 2006). Although Detmann et al. (2009) determined that 8 mg/dL of NH3 is the minimum required to support ruminal microbial growth, the need for NH3 increases with the degradability of the feed material. In the present study, ruminal NH3 concentrations averaged 9.90, 11.31, 13.45, 14.82 mg/dL, for control, 10.0, 14.5 and 19.0 g/kg DM of urea inclusion, respectively, significantly exceeding the minimum concentration level of 8 mg/dL required to support ruminal microbial growth. Thus, all bulls had sufficient ammonia-N available for the ruminal microorganisms. However, according to Lewis (1960) ruminants can experience ammonia toxicity when the ruminal NH3 concentration exceeds 140 mg/dL. The ruminal NH3 concentration is influenced by both the N content of the diet and the influx of urea into the rumen through the urea circulation. According to Patra and Aschenbach (2018), rumen NH3 concentration serves as the primary negative regulator of urea influx. Urea, which is rapidly hydrolyzed to ammonia by ruminal ureolytic bacteria, is completely degradable in the rumen (Bach et al., 2005). Additionally, the level of urea inclusion to diets often surpasses the ruminal influx of urea.

Consequently, rumen NH3 concentration increases linearly with SBM replacement by urea inclusion in the diets, supporting the findings of some studies (Chizzotti et al., 2007; Paixão et al., 2006; Spanghero et al., 2017), which showed that NH3 accumulation positively correlates with the amount of NPN. Also, when ruminal ammonia levels increase, it signifies that enough protein has been degraded to meet growth microbial requirements (Gustafsson and Palmquist, 1993). However, the rumen NH3 concentration observed in bulls fed 19.0 g/kg DM of urea replacing totally the SBM was significantly below the toxic level reported by Lewis et al. (1960). Besides that, Maeng et al. (1976) proposed that maximal microbial growth is achieved when both NH3-N and amino acid-N are present for cell protein synthesis. According to their research, the optimal ratio for maximal microbial growth is 75% urea-N and 25% amino acid-N in an in vitro system. Experiments using either 100% urea or 100% amino acid media did not reach maximum microbial growth. These findings are consistent with our experiment's data, where diets containing urea (10.0, 14.5, and 19.0 g/kg DM) correspond to approximately 22%, 32%, and 42% urea-N, respectively.

The fluctuations in ruminal pH depend on the supply of ammonia derived from nitrogen sources, and on ammonia intake and energy available in the rumen (Van Soest, 1994). Given that the carbohydrate composition was consistent across all diets, it can be assumed that the availability of carbohydrates to ruminal microorganisms was similar among all diets. Therefore, the changes in pH were primarily dependent on the N sources. Ruminal urea degradation results in the ionization of ammonia molecules and the removal of free hydrogen ions from the solution, which in turn leads to a fast accumulation of ammonia in the rumen and an increase in the ruminal pH (Kertz, 2010). However, even though the peaks in ruminal pH followed the same pattern as the peaks in ruminal NH3 at the same times, the ruminal pH values of all the treatments were similar and within the

normal physiological range. According to, Nagaraja and Titgemeyer (2007), in beef cattle fed high-grain diets, ruminal pH can range from 6.5 to 5.6, with average pH typically around 5.8 to 6.2, but it can drop below 5.6 for a period during the feeding cycle. These results are in agreement with Zinn et al. (1994), who reported that pH was not affected by dietary urea level. Moreover, according to López et al. (1994) VFA concentrations can be influenced by factors such as pH. This fact might explain the absence of difference in the concentrations of acetate, butyrate, propionate, or total VFAs in the ruminal fluid for the different levels of urea inclusion replacing SBM in crossbred bulls fed corn-based diets. Also, a similar concentration of VFA, which is affected by the fermentation of carbohydrates (Heldt et al., 1999), indicates that total replacement of SBM by urea had no effects on ruminal fermentation.

Excess NH3 is absorbed predominately through the rumen wall and is transported to the liver, where it is detoxified and converted to the less toxic compound, urea (Hailemariam et al., 2021). Additionally, a higher ruminal pH facilitates the rapid transport of ammonia across the rumen epithelium, leading to an increase in BUN levels and potentially causing ammonia toxicity (Abdoun et al., 2006). Thus, the BUN is a reliable marker for the quantity of unused N in the rumen (Rennó et al., 2000). In our study, however, ruminal ammonia concentrations averaged over time in all treatments were much lower than the toxic levels, and the BUN level was within the normal range (BUN, 10 to 20 mg/dl) proposed by Kaneko et al. (2008). Furthermore, the finishing diet primarily composed of ground corn, the starch of which is highly fermentable in the rumen, met recommendations (Ensminger et al., 1990) for using urea in ruminants. Thus, 19.0 g/kg DM of urea inclusion coupled with a high level of concentrates on a corn-based diet appears to be safe and is unlikely to cause ammonia toxicity. Additionally, all animals

showed a similar BUN, corroborating with Gardinal et al. (2017), who also found no difference in urea inclusion in the diet for BUN.

According to Bach et al. (2005), in the rumen, microorganisms utilize ammonia for MPS when carbon skeletons from carbohydrates are available. If there is not enough fermentable energy (carbon sources) available, NH3 is absorbed by the rumen wall and transported to the blood, reaching the liver, where it is converted to urea through the urea cycle and predominantly excreted in urine (Van Soest, 1994). In our study, neither N, NFC, nor TDN intake was influenced by urea inclusion. As a result, there were similar concentrations of nitrogen and carbohydrates, which consequently did not affect ruminal fermentation, thus having minimal effects on MPS. As a result, increasing SBM replacement with urea did not result in differences in urinary N excretion. Besides that, increasing the replacement of SBM with urea did not affect N retained and fecal N. A similar was observed by Knaus et al. (2001) in steers fed a high-concentrate diet. So, this suggests that urea inclusion as the only supplementary source of N can be efficiently used to meet the requirement of metabolizable protein and amino acids, in finishing diets. Hence, the lack of difference in feed intake and ruminal MPS, implies that a corn-based diet provided a ruminal environment that allowed the utilization of N from urea hydrolysis. Therefore, although urea hydrolysis in the rumen is fast, the necessary energy input was guaranteed by high fermentable carbohydrates.

CONCLUSION

In conclusion, 19.0 g/kg DM of urea inclusion can totally replace soybean meal on an equal N basis without influencing intake, digestibility of nutrients, and ruminal parameters in crossbred fed a corn-based diet.

REFERENCES

Abdoun, K., Stumpff, F., Martens, H., 2006. Ammonia and urea transport across the rumen epithelium: a review. Anim. Heal. Res. Rev. 7, 43–59. https://doi.org/10.1017/S1466252307001156

AOAC, 2012. Association of Official Analysis Chemists International, Official Methods of Analysis of AOAC International, 19th ed. Gaithersburg, Maryland.

AOAC, 2005. Association of Official Analysis Chemists International, Official Methods of Analysis of AOAC International, 18th ed. ed. Gaithersburg, Maryland.

Bach, A., Calsamiglia, S., Stern, M.D., 2005. Nitrogen Metabolism in the Rumen. J. Dairy Sci. 88, E9–E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Barbosa, A.M., Valadares, R.F.D., Valadares Filho, S.C., Pina, D.S., Detmann, E., Leão, M.I., 2011. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle1. J. Anim. Sci. 89, 510–519. https://doi.org/10.2527/jas.2009-2366

Benedeti, P.D.B., Paulino, P.V.R., Marcondes, M.I., Valadares Filho, S.C., Martins, T.S., Lisboa, E.F., Silva, L.H.P., Teixeira, C.R.V., Duarte, M.S., 2014. Soybean meal replaced by slow release urea in finishing diets for beef cattle. Livest. Sci. 165, 51–60. https://doi.org/10.1016/j.livsci.2014.04.027

Chalupa, W., 1968. No TitleProblems in feeding urea to ruminants. J. Anim. Sci. 27, 207–219.

Chen, X.B., Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives -an overview of the technical details. 20.

Chizzotti, F.H.M., Pereira, O.G., Tedeschi, L.O., Valadares Filho, S.C., Chizzotti, M.L., Leao, M.I., Pereira, D.H., 2007. Effects of dietary nonprotein nitrogen on

performance, digestibility, ruminal characteristics, and microbial efficiency in crossbred steers. J. Anim. Sci. 86, 1173–1181. https://doi.org/10.2527/jas.2006-654

Detmann, E., Paulino, M.F., Mantovani, H.C., Filho, S. de C.V., Sampaio, C.B., de Souza, M.A., Lazzarini, Í., Detmann, K.S.C., 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis-Menten kinetics. Livest. Sci. 126, 136–146. https://doi.org/10.1016/j.livsci.2009.06.013

Detmann, E., Valadares Filho, S.C., 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. Arq. Bras. Med. Veterinária e Zootec. 62, 980–984. https://doi.org/10.1590/S0102-09352010000400030

Faichney, G.J., 1975. The use of markers to partition digestion within the gastrointestinal tract of ruminants. Dig. Metab. ruminant. Proc. Fourth Int. Symp. Rumin. Physiol. , Sydney, Aust.

France, J., Siddons, R.C., 1986. Determination of digesta flow by continuous market infusion. J. Theor. Biol. 121, 105–119. https://doi.org/10.1016/S0022-5193(86)80031-5

Gardinal, R., Calomeni, G.D., Cônsolo, N.R.B., Takiya, C.S., Freitas, J.E., Gandra, J.R., Vendramini, T.H.A., Souza, H.N., Rennó, F.P., 2017. Influence of polymer-coated slow-release urea on total tract apparent digestibility, ruminal fermentation and performance of Nellore steers. Asian-Australasian J. Anim. Sci. 30, 34–41. https://doi.org/10.5713/ajas.16.0058

Gustafsson, A.H., Palmquist, D.L., 1993. Diurnal Variation of Rumen Ammonia, Serum Urea, and Milk Urea in Dairy Cows at High and Low Yields. J. Dairy Sci. 76, 475–484. https://doi.org/10.3168/jds.S0022-0302(93)77368-3

Hailemariam, S., Zhao, S., He, Y., Wang, J., 2021. Urea transport and hydrolysis in the rumen: A review. Anim. Nutr. 7, 989–996. https://doi.org/10.1016/j.aninu.2021.07.002

Heldt, J.S., Cochran, R.C., Mathis, C.P., Woods, B.C., Olson, K.C., Titgemeyer, E.C., Nagaraja, T.G., Vanzant, E.S., Johnson, D.E., 1999. Effects of level and source of carbohydrate and level of degradable intake protein on intake and digestion of low-quality tallgrass-prairie hay by beef steers. J. Anim. Sci. 77, 2846. https://doi.org/10.2527/1999.77102846x

Helmer, L.G., Bartley, E.E., 1971. Progress in the Utilization of Urea as a Protein Replacer for Ruminants. A Review. J. Dairy Sci. 54, 25–51. https://doi.org/10.3168/jds.S0022-0302(71)85776-4

Huhtanen, P., Brotz, P.G., Satter, L.D., 1997. Omasal sampling technique for assessing fermentative digestion in the forestomach of dairy cows. J. Anim. Sci. 75, 1380. https://doi.org/10.2527/1997.7551380x

Kaneko, J.., Harvey, J.W., Bruss, M.L., 2008. Clinical Biochemistry of Domestic Animals. Elsevier. https://doi.org/10.1016/B978-0-12-370491-7.X0001-3

Kertz, A.F., 2010. Review: Urea Feeding to Dairy Cattle: A Historical Perspective and Review. Prof. Anim. Sci. 26, 257–272. https://doi.org/10.15232/S1080-7446(15)30593-3

Kimura, F.T., Miller, V.L., 1957. Chromic Oxide Measurement, Improved Determination of Chromic Oxide in Cow Feed and Feces. J. Agric. Food Chem. 5, 216–216. https://doi.org/10.1021/jf60073a008

Knaus, W.F., Beermann, D.H., Guiroy, P.J., Boehm, M.L., Fox, D.G., 2001. Optimization of rate and efficiency of dietary nitrogen utilization through the use of animal by-

products and(or) urea and their effects on nutrient digestion in Holstein steers. J. Anim. Sci. 79, 753. https://doi.org/10.2527/2001.793753x

Leão, M.I., Valadares Filho, S. de C., Rennó, L.N., Gonçalves, L.C., Cecon, P.R., Azevedo, J.A.G., Valadares, R.F.D., 2004. Consumos e digestibilidades totais e parciais de matéria seca, matéria orgânica, proteína bruta e extrato etéreo em novilhos submetidos a três níveis de ingestão e duas metodologias de coleta de digestas abomasal e omasal. Rev. Bras. Zootec. 33, 1604–1615. https://doi.org/10.1590/S1516-35982004000600028

Lewis, D., 1960. Ammonia toxicity in the ruminant. J. Agric. Sci. 55, 111–117. https://doi.org/10.1017/S0021859600021687

Liang, H., Zhao, E., Feng, C., Wang, J., Xu, L., Li, Z., Yang, S., Ge, Y., Li, L., Qu, M., 2020. Effects of slow-release urea on in vitro rumen fermentation parameters, growth performance, nutrient digestibility and serum metabolites of beef cattle. Semin. Agrar. 41, 1399–1414. https://doi.org/10.5433/1679-0359.2020v41n4p1399

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. https://doi.org/10.1016/0377-8401(95)00837-3

López, S., Hovell, F.D.D., MacLeod, N.A., 1994. Osmotic pressure, water kinetics and volatile fatty acid absorption in the rumen of sheep sustained by intragastric infusions. Br. J. Nutr. 71, 153–168. https://doi.org/10.1079/BJN19940123

Machado, M.G., Detmann, E., Mantovani, H.C., Valadares Filho, S.C., Bento, C.B.P., Marcondes, M.I., Assunção, A.S., 2016. Evaluation of the length of adaptation period for changeover and crossover nutritional experiments with cattle fed tropical

forage-based diets. Anim. Feed Sci. Technol. 222, 132–148. https://doi.org/10.1016/j.anifeedsci.2016.10.009

Magalhães, K.A., Valadares Filho, S.C., Paulino, P.V.R., Paulino, M.F., Valadares, R.F.D., 2006. Performance, digestibility and carcass characteristics of feedlot dairy steers fed diets with different urea levels. Arq. Bras. Med. Vet. e Zootec. 58, 860–867. https://doi.org/10.1590/S0102-09352006000500023

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217–40.

Nagaraja, T.G., 2016. Microbiology of the Rumen, in: Rumenology. Springer International Publishing, Cham, pp. 39–61. https://doi.org/10.1007/978-3-319-30533-2_2

Nagaraja, T.G., Titgemeyer, E.C., 2007. Ruminal Acidosis in Beef Cattle: The Current Microbiological and Nutritional Outlook. J. Dairy Sci. 90, E17–E38. https://doi.org/10.3168/jds.2006-478

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7.rev.ed., Washinton, D.C.

Okuda, H., Fujii, S., Kawashima, Y., 1965. Terms and conditions privacy policy A direct colorimetric determination of blood ammonia. Tokushima J. Exp. Med. 12, 11–23.

Paixão, M.L., Valadares Filho, S.C., Leão, M.I., Valadares, R.F.D., Paulino, M.F., Marcondes, M.I., Fonseca, M.A., Silva, P.A., Pina, D.D.S., 2006. Uréia em dietas para bovinos: Consumo, digestibilidade dos nutrientes, ganho de peso, características de carcaça e produção microbiana. Rev. Bras. Zootec. 35, 2451–2460. https://doi.org/10.1590/S1516-35982006000800035

Patra, A.K., Aschenbach, J.R., 2018. Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. J. Adv. Res. 13, 39–50. https://doi.org/10.1016/j.jare.2018.02.005

Pereira, G.F.C., Carvalho, F.F.R. De, Souza, E.J.O. De, Medeiros, A.N. De, Maciel, M.D.V., Ramos, A.O., Branco, G.L.C., Neves, R.D.S., 2020. Urea can replace soybean meal in sheep diet without alter intake, digestibility and ruminal parameters. Artic. Int. J. Dev. Res. 10, 42092–42097. https://doi.org/10.37118/ijdr.20338.11.2020

Pina, D.S., Valadares Filho, S.C., Tedeschi, L.O., Barbosa, A.M., Valadares, R.F.D., 2009. Influence of different levels of concentrate and ruminally undegraded protein on digestive variables in beef heifers1. J. Anim. Sci. 87, 1058–1067. https://doi.org/10.2527/jas.2008-1069

Reid, J.T., 1953. Urea as a Protein Replacement for Ruminants: A Review. J. Dairy Sci. 36, 955–996. https://doi.org/10.3168/jds.S0022-0302(53)91586-0

Rennó, L.N., De Campos Valadares Filho, S., Valadares, R.F.D., Cecon, P.R., Backes, A.A., Rennó, F.P., Alves, D.D., Silva, P.A., 2005. Effects of feeding dietary urea levels on intake and total digestibility for steers of four genetic groups. Rev. Bras. Zootec. 34, 1775–1785. https://doi.org/10.1590/s1516-35982005000500039

Rennó, L.N., Valadares, R.F.D., Valadares Filho, S. de C., Leão, M.I., Silva, J.F.C. da, Cecon, P.R., Gonçalves, L.C., Dias, H.L.C., Linhares, R.S., 2000. Concentração plasmática de uréia e excreções de uréia e creatinina em novilhos. Rev. Bras. Zootec. 29, 1235–1243. https://doi.org/10.1590/S1516-35982000000400038

Rotta, P.P., Valadares Filho, S.C., Detmann, E., Costa e Silva, L.F., Paulino, M.F., Marcondes, M.I., Lobo, A.A.G., Villadiego, F.A.C., 2014. Digesta sampling sites and marker methods for estimation of ruminal outflow in bulls fed different

proportions of corn silage or sugarcane1. J. Anim. Sci. 92, 2996–3006. https://doi.org/10.2527/jas.2013-7364

Russell, J.B., Sniffen, C.J., Van Soest, P.J., 1983. Effect of Carbohydrate Limitation on Degradation and Utilization of Casein by Mixed Rumen Bacteria. J. Dairy Sci. 66, 763–775. https://doi.org/10.3168/jds.S0022-0302(83)81856-6

Siegfried, V.R., Ruckemann, H., Stumpf, G., 1984. Method for the determination of organic acids in silage by high performance liquid chromatography. Landwirtsch. Forsch. 37, 298–304.

Silva, B. de C., Godoi, L.A., Valadares Filho, S. de C., Zanetti, D., Benedeti, P.D.B., Detmann, E., 2019. A suitable enzymatic method for starch quantification in different organic matrices. MethodsX 6, 2322–2328. https://doi.org/10.1016/j.mex.2019.09.040

Sniffen, C.J., Robinson, P.H., 1987. Microbial Growth and Flow as Influenced by Dietary Manipulations. J. Dairy Sci. 70, 425–441. https://doi.org/10.3168/jds.S0022-0302(87)80027-9

Spanghero, M., Mason, F., Zanfi, C., Nikulina, A., 2017. Effect of diets differing in protein concentration (low vs medium) and nitrogen source (urea vs soybean meal) on in vitro rumen fermentation and on performance of finishing Italian Simmental bulls. Livest. Sci. 196, 14–21. https://doi.org/10.1016/j.livsci.2016.12.004

Valadares Filho, S. de C., Lopes, S.A., Silva, B. de C., Chizzotti, M.L., Bissaro, L.Z., 2018. Tabelas Brasileiras de Composição de Alimentos para Ruminantes. [WWW Document]. URL www.cqbal.com.br (accessed 6.5.23).

Valadares Filho, S. de C., Rotta, P., Costa e Silva, L.., 2011. Técnicas de coleta duodenal, abomasal, omasal e reticular na avaliação do fluxo ruminal. III Simp. Int. Avanços em Tec. Pesqui. em. Nutr. çao De. Rumin. 14–45.

Valadares Filho, S. de C., Silva, L.F.C. e, Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, in: Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE. Editora Federal de Viçosa. https://doi.org/10.5935/978-85-8179-111-1.2016B002

Valente, T.N.P., Detmann, E., Valadares Filho, S. de C., Cunha, M. da, Queiroz, A.C. de, Sampaio, C.B., 2011. In situ estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. Rev. Bras. Zootec. 40, 666–675. https://doi.org/10.1590/S1516-35982011000300027

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. Cornell university press.

Zinn, R.A., Borquez, J.L., Plascencia, A., Essig, H.W., Johnson, Z., Luther, L.W., 1994. Influence of Levels of Supplemental Urea on Characteristics of Digestion and Growth Performance of Feedlot Steers Fed a Fat-Supplemented High-Energy Finishing Diet. Prof. Anim. Sci. 10, 5–10. https://doi.org/10.15232/S1080-7446(15)31927-6

TABLES:

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

Item[1]	CTL	Urea levels (g/kg DM)		
		10.0	14.5	19.0
Proportion, %				
Corn Silage	20.00	20.00	20.00	20.00
Ground corn	64.87	70.30	72.80	75.00
Soybean meal	12.00	5.57	2.67	0.00
Urea/A.S[1]	0.00	1.00	1.45	1.90
Mineral mix[2]	3.00	3.00	3.00	3.00
Virginiamycin	0.13	0.13	0.13	0.13
Chemical composition[3]				
DM, g/kg as-fed	613.8	613.8	613.8	613.9
OM, g/kg DM	944.2	947.9	949.5	951.0
apNDF, g/kg DM	177.6	176.0	175.3	174.7
CP, g/kg DM	126.5	126.1	125.9	126.6
RDP, g/kg CP	589.8	651.8	679.8	708.1
RUP, g/kg CP	410.2	348.2	320.2	291.9
EE, g/kg DM	22.6	23.8	24.3	24.8
NFC, g/kg DM	617.5	639.5	649.3	658.2

[1]Urea/A.S, urea and ammonium sulfate (9:1). [2]Probeef® Confinamento Performa (Cargill Nutrição Animal, Itapira, SP, Brazil). Assurance levels per kilogram of product: 242- 300.0 g of Ca; 11.10 mg of Co (Min); 556.0 mg of Cu (Min); 24.5 g of S (Min); 370.0 mg of Fe (Min); 13.50 g of P (Min); 27.7 mg of I (Min); 19.0 g of Mg (Min); 1668.0 mg of Mn (Min); 7.4 mg of Se (Min); 81.5 g of Na (Min); 2223.0 mg of Zn (Min); 92550.0 UI of vitamin A (Min); 14780.0 UI of vitamin D3 (Min); 136.0 UI of vitamin E (Min); and 928.0 mg of monensin. [3]DM, dry matter; OM, organic matter; apNDF, neutral detergent fiber corrected for residual ash and residual nitrogenous compounds; CP, crude protein; RDP, rumen degradable protein; RUP, rumen undegradable protein; EE, ether extract; NFC, non-fiber carbohydrates.

Table 2 - Effects of soybean meal replacement by urea in finishing diets on voluntary nutrients intake in crossbred bulls.

Items[1]	CTL	Urea levels (g/kg DM)			SEM[2]	CTL x Urea[3]	Urea Levels[4]	
		10.0	14.5	19.0			L	Q
Intake, kg/d								
DM	6.67	7.01	7.03	6.29	0.37	0.744	0.097	0.329
OM	6.32	6.65	6.68	5.99	0.35	0.675	0.107	0.323
apNDF	1.26	1.28	1.29	1.15	0.06	0.775	0.120	0.295
CP	0.85	0.90	0.89	0.81	0.05	0.651	0.119	0.423
EE	0.145	0.163	0.16	0.150	0.01	0.075	0.116	0.660
NFC	4.07	4.46	4.53	4.13	0.24	0.158	0.192	0.331
Intake, g/kg BW								
DM	2.05	2.05	2.03	1.94	0.06	0.543	0.234	0.615

[1]DM, dry matter; OM, organic matter; apNDF, neutral detergent fiber corrected for residual ash and residual nitrogenous compounds; CP, crude protein; NFC, non-fiber carbohydrates. [2]SEM=standard error mean.[3]CTL vs. Urea – Control versus urea inclusion levels.[4] L – Linear effect across urea inclusion levels and Q – Quadratic effect across urea inclusion levels.

Table 3 - Effects of soybean meal replacement by urea in finishing diet on ruminal, small intestine, hind gut, and apparent total-tract digestibility in crossbred bulls.

Items[1]	CTL	Urea levels (g/kg DM)			SEM[2]	CTL x Urea[3]	Urea Levels[4]	
		10.0	14.5	19.0			L	Q
Rumen digestibility, g/kg								
DM	435.1	442.3	438.1	461.8	30.69	0.375	0.304	0.971
OM	496.0	492.5	524.0	535.9	28.14	0.456	0.234	0.767
apNDF	341.8	305.1	325.2	319.8	39.67	0.565	0.777	0.799
CP	18.6	34.7	24.6	39.8	49.26	0.723	0.917	0.793
EE	-813.7	-807.6	-1004.4	-665.3	107.91	0.907	0.285	0.069
NFC	710.3	721.9	737.0	755.7	35.50	0.214	0.210	0.942
Small intestine digestibility, g/kg								
DM	289.6	276.6	279.4	241.0	21.27	0.381	0.289	0.507
OM	262.6	256.1	241.5	209.7	22.05	0.346	0.197	0.783
apNDF	147.3	143.1	144.4	149.1	70.00	0.979	0.944	0.983
CP	731.3	760.7	761.2	775.4	45.75	0.471	0.794	0.900
EE	1396.5	1294.0	1399.4	1174.8	93.22	0.222	0.254	0.128
NFC	135.1	123.1	105.7	100.9	17.83	0.116	0.220	0.708
Hind gut digestibility, g/kg								
DM	59.7	48.1	49.2	57.1	20.89	0.685	0.711	0.887
OM	38.4	32.5	48.0	46.7	21.52	0.829	0.530	0.706
apNDF	29.4	23.1	26.3	24.1	43.69	0.887	0.981	0.947
CP	-22.8	-38.0	-39.6	-39.0	9.35	0.161	0.936	0.924
EE	-27.1	44.8	78.1	21.7	69.59	0.380	0.816	0.642
NFC	58.2	47.1	57.6	45.1	24.79	0.787	0.957	0.745
Apparent total-tract digestibility, g/kg								
DM	784.3	766.9	788.5	777.1	16.09	0.659	0.591	0.382
OM	797.0	781.1	804.5	792.3	16.04	0.774	0.544	0.340
apNDF	518.5	471.3	490.5	493.0	23.39	0.260	0.528	0.797
CP	727.0	757.4	753.7	776.2	15.52	0.091	0.401	0.537
EE	555.8	531.3	456.2	531.2	65.07	0.510	0.999	0.399
NFC	903.6	892.2	923.9	901.7	17.31	0.857	0.550	0.124

[1]DM, dry matter; OM, organic matter; apNDF, neutral detergent fiber corrected for residual ash and residual nitrogenous compounds; CP, crude protein; NFC, non-fiber carbohydrates. [2]SEM=standard error mean.[3]CTL vs. Urea – Control versus urea inclusion levels.[4] L – Linear effect across urea inclusion levels and Q – Quadratic effect across urea inclusion levels.

Table 4- Effects of soybean meal replacement by urea in finishing diet on ruminal volatile fatty acids (VFAs) concentration in beef cattle.

Items	CTL	Urea levels (g/kg DM)			SEM[1]	CTL x Urea[2]	Urea Levels[3]	
		10.0	14.5	19.0			L	Q
Total VFA, mmol/L	79.8	73.2	71.2	64.4	4.05	0.057	0.137	0.647
Individual, mmol/100mmol								
Acetate	47.4	48.6	48.8	48.8	2.01	0.184	0.853	0.929
Propionate	41.8	42.7	41.1	43.3	2.24	0.578	0.64	0.202
Butyrate	10.8	8.7	10.1	7.9	0.98	0.105	0.519	0.169
Acetate:Propionate Ratio	1.1	1.2	1.2	1.1	0.11	0.479	0.753	0.278

[1]SEM=standard error mean.[2]CTL vs. Urea – Control versus urea inclusion levels.[3]L – Linear effect across urea inclusion levels and Q – Quadratic effect across urea inclusion levels.

Table 5 - Effects of soybean meal replacement by urea in finishing diet on Nitrogen (N) balance and microbial efficiency in beef cattle.

Items[1]	CTL	Urea levels (g/kg DM)			SEM[2]	CTL x Urea[3]	Urea Levels[4]	
		10.0	14.5	19.0			L	Q
N intake, g/d	135.8	143.1	143.1	129.5	8.04	0.664	0.121	0.392
Urea-N intake, g/d	-	31.2	45.5	53.1	2.21	<.001	<.001	0.185
N Fecal, g/d	32.6	37.3	35.6	28.8	2.86	0.684	0.069	0.510
N Urinary, g/d	45.0	47.0	49.0	47.0	3.12	0.133	0.997	0.331
Urinary urea N, g/d	24.5	24.3	30.8	33.2	2.81	0.040	0.008	0.396
Blood urea N, mg/dL	7.2	6.8	6.4	6.7	1.05	0.496	0.887	0.656
N Retained, g/d	58.2	58.8	57.8	53.6	4.67	0.747	0.342	0.753
TDN intake, kg/d	5.1	5.5	5.6	5.1	0.35	0.340	0.263	0.347
DOM intake, kg/d	5.0	5.2	5.3	4.8	0.34	0.827	0.145	0.307
MPS, g/day	642.1	607.4	586.7	551.2	30.82	0.097	0.176	0.841
gMPS/kg NDT intake	125.3	111.9	104.0	110.7	7.59	0.080	0.900	0.441
gMPS/lg DOM intake	127.9	117.3	109.4	118.9	8.04	0.173	0.875	0.398

[1]BUN, blood urea nitrogen; TDN, total digestible nutrients; DOM, digestible organic matter; MPS, microbial protein synthesized. [2]SEM=standard error mean.[3]CTL vs. Urea – Control versus urea inclusion levels.[4] L – Linear effect across urea inclusion levels and Q – Quadratic effect across urea inclusion levels.

FIGURES:

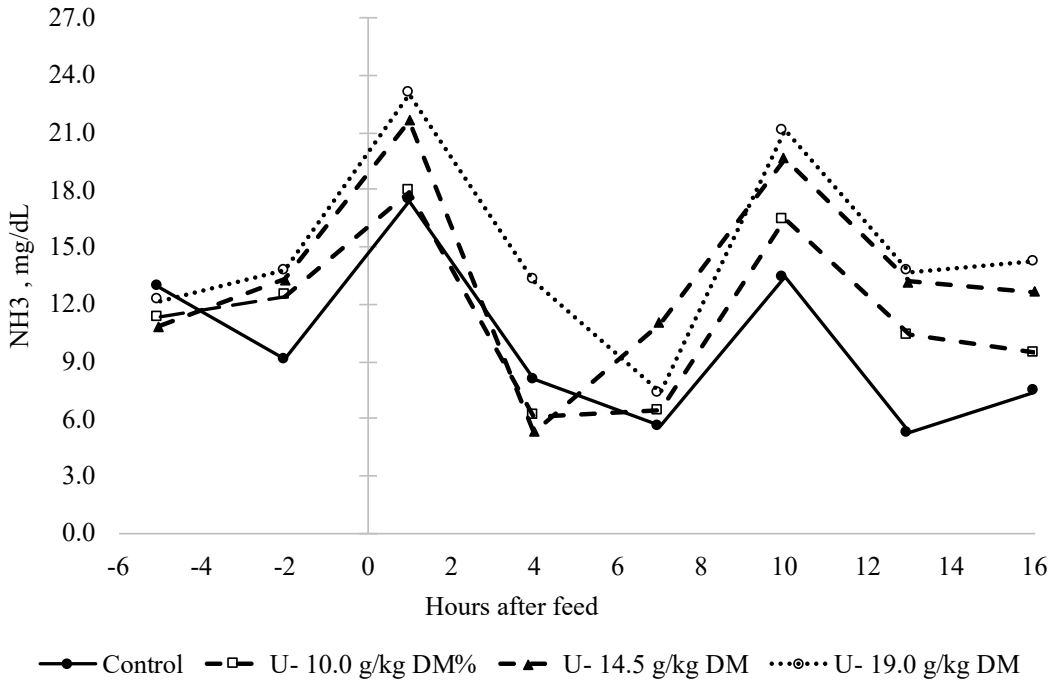

Fig. 1. Effects of soybean meal (SBM) replacement by urea in finishing diet on ruminal ammonia concentration. Averages per treatment (Control versus urea inclusion, P = 0.007; Linear effect in urea levels, P= 0.020; SEM =1.01) - Control =9.90, U-10.0g/kg DM =11.31, U-14.5g/kg DM =13.45, U-19.0g/kg DM =14.82. Averages per hour (P <0.001; SEM =1.32) - 2h =11.80, 5h =12.14, 8h =20.0, 11h = 8.19, 14h =7.61, 17h =17.65, 20h =10.64, 23h =10.95. Significative interaction Treatments * Sampling time was not found (P = 0.944).

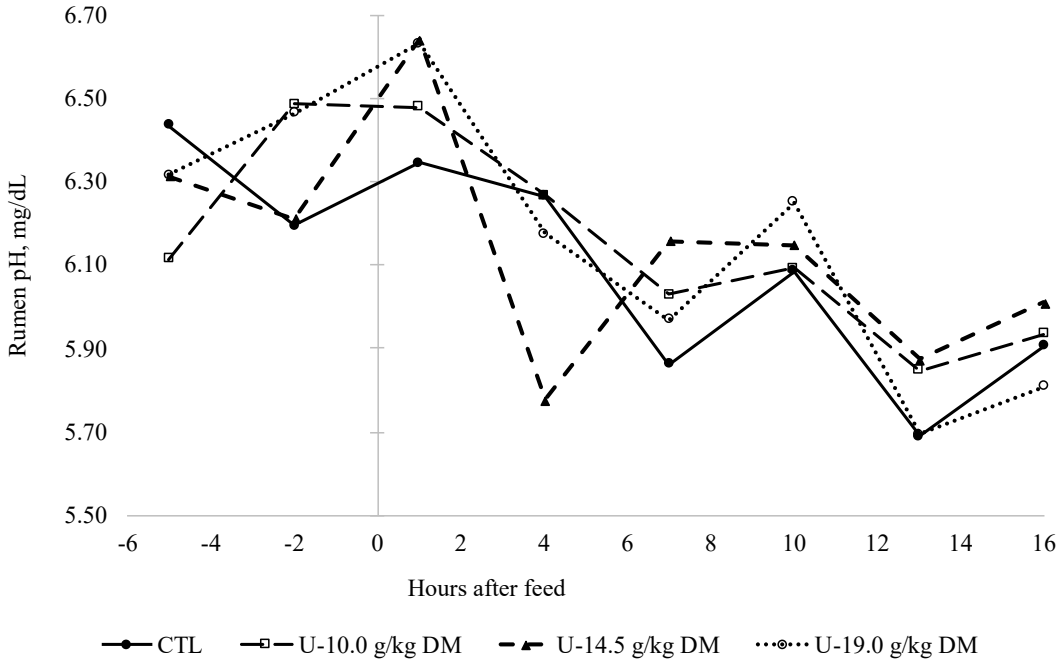

Fig. 2. Effects of soybean meal (SBM) replacement by urea in finishing diet on ruminal pH. Averages per treatment (P = 0.934; SEM = 0.07) - Control = 6.10, U-10.0g/kg DM =6.16, U-14.5g/kg DM =6.14, U-19.0g/kg DM =6.16. Averages per hour (P <0.001; SEM =0.07) - 2h =6.30, 5h =6.34, 8h = 6.52, 11h =6.12, 14h =6.0, 17h =6.14, 20h = 5.78, 23h = 5.92. Significative interaction treatments * Sampling time was not found (P= 0.875).