UNIVERSIDADE ESTADUAL PAULISTA - UNESP
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

PROTEIN METABOLISM AND UREA KINETIC IN FEEDLOT NELLORE STEERS FED WITH DIFFERENT PROTEIN SOURCES AND INCLUSION LEVELS

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VINÍCIUS CARNEIRO DE SOUZA - Born in the city of Uruaçu - GO, on October 8, 1990, son of Osvaldo Carneiro de Souza and Joana Darc Arruda de Souza. In August 2009, he joined the Animal Science course at the Federal Rural University of Rio de Janeiro, graduating in February 2014. In March 2014, he joined the Master in Animal Science course, in the area of nutrition and animal feeding, in the Faculty of Agricultural and Veterinary Sciences of the Sao Paulo State University - Jaboticabal Campus, under the guidance of Profª. Dr. Telma Teresinha Berchielli. In the period from October 2015 to January 2016 he performed his training internship abroad at The University of Queensland - Australia, under the guidance of Prof. Dennis Poppi. In February 29, 2016, he defended his thesis, having been considered approved by the examining committee. In March 2016, he joined the Doctoral of Animal Science course in the area of nutrition and animal feeding, in the Faculty of Agricultural and Veterinary Sciences of the Sao Paulo State University - Jaboticabal Campus, under the guidance of Profª. Dr. Telma Teresinha Berchielli. In the period from August 2019 to April 2020 he performed his training internship abroad at Virginia Tech – United States of America, under the guidance of Prof. Robin White. In April 6, 2020, he defended his dissertation, having been considered approved by the examining committee.

EPIGRAPH

“When you are experiencing difficulties, through the valleys of suffering, do not be afraid of pain, take the opportunity to write the most important chapters of your story.”
Augusto Cury

DEDICATION

This dissertation is dedicated to my family, friends, and professors, who provided me with their invaluable support during all my academic life.

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SUMMARY

METABOLISMO PROTEICO E CINÉTICA DA UREIA EM NOVILHOS NELORE CASTRADOS ALIMENTADOS COM DIFERENTES FONTES DE PROTEÍNA E NÍVEIS DE INCLUSÃO

RESUMO - O uso de fontes de proteína não degradável no rúmen (PNDR) em dietas de alta energia pode ser uma alternativa para aumentar a eficiência do uso de nitrogênio (ENU) em ruminantes. Neste estudo, tivemos dois objetivos principais: 1) compreender como o teor de proteína da dieta e a ingestão de proteína degradável no rúmen (PDR) podem afetar a reciclagem de uréia e sua utilização. 2) como o teor de proteína da dieta e o perfil de aminoácidos da proteína metabolizável (MP) podem afetar a eficiência do uso de aminoácidos (AA) em bovinos Nelore em confinamento recebendo dietas de alta densidade energética. Assim, avaliamos os efeitos de diferentes fontes e níveis proteína na dieta de bovinos Nelore confinados que recebendo dietas de alto concentrado. Tivemos duas hipóteses principais: 1) reduzir o teor de N na dieta associado às fontes de PNDR pode aumentar a ENU por uma redução na concentração de NH_3 ruminal, produção de uréia no fígado e excreção urinária de N, enquanto mantém o N disponível para a síntese de proteína microbiana (MICP) através da reciclagem de ureia. 2) existem diferenças na eficiência do uso de AA e a eficiência bruta de uso de AA é afetada por fatores dietéticos, como fontes e níveis de N. Essas hipóteses foram testadas utilizando seis novilhos Nelore, canulados no rúmen, duodeno e íleo com peso corporal inicial (PC) de 354 ± 11,8 kg e 18 meses de idade. Os animais foram distribuídos aleatoriamente para receber cada dieta por uma vez ao longo dos 6 períodos, em um quadrado latino 6 × 6. As dietas experimentais consistiram em 80% de concentrado e 20% de volumoso (base de MS), onde a cana-de-açúcar fresca foi usada como fonte de volumoso e os concentrados diferenciados em sua fonte de proteína. O farelo de glúten de milho (CGM) e os grãos secos de destilaria (DDG) foram utilizados como fonte de PNDR, com baixa e intermediária degradabilidade ruminal, respectivamente, e farelo de soja e ureia (SU) foram utilizados como fonte de PDR. Os tratamentos foram organizados em um fatorial A × B, onde o fator A consistiu em 3 fontes de proteína (FP; farelo de soja mais uréia, CGM e DDG) e o fator B consistiu em 2 níveis de proteína bruta (PB) na dieta (NP; 11 e 14%). Não houve interação entre FP e NP sobre o consumo e digestibilidade de nutrientes ($P > 0,05$). Os animais alimentados com dietas com nível de inclusão de 11% de PB tiveram maior ($P < 0,05$) ingestão de carboidratos não fibrosos (CNF) e tenderam ($P < 0,10$) a ter maior ingestão de MS (% do PC e kg / dia), matéria orgânica (MO) e nutrientes digestíveis totais (NDT). A ingestão de PDR foi maior ($P < 0,05$) e a PNDR menor ($P < 0,05$) quando os animais foram alimentados com as dietas SU. Os animais alimentados com as dietas contendo DDG tiveram maior ($P < 0,05$) consumo de FDN comparado às dietas SU ou CGM. Os animais alimentados com DDG tenderam ($P = 0,10$) a apresentar maior digestibilidade aparente do trato total da FDN comparado aos alimentados com as dietas SU. Os animais alimentados com as dietas SU tiveram maior ($P < 0,05$) concentração de amônia ruminal (NH_3-N) do que aqueles alimentados com CGM ou DDG. O fluxo de N microbiano e a eficiência de síntese de N microbiano não foram afetados pelo NP e FP. Os animais alimentados com as dietas SU tiveram menor ($P < 0,05$) ENU e maior taxa de entrada de ureia N (TEU). Além disso, o aumento do NP de 11 para 14% de PB tendeu ($P < 0,10$) a aumentar a TEU. Os animais alimentados com dietas SU tenderam ($P < 0,10$) a apresentar maior taxa de entrada de ureia no gastrointestinal (TEG) do que aqueles alimentados com dietas CGM ou DDG. Os animais alimentados com as dietas SU tiveram maior ($P < 0,05$) quantidade de ureia N retornando ao ciclo da ornitina (RCO) em comparação com os animais alimentados com as dietas

contendo CGM ou DDG. A quantidade de ureia reciclada utilizada para funções anabólicas (UUA) foi maior ($P < 0,05$) nos animais alimentados com 11% de PB, em comparação com aqueles alimentados com 14% de PB, quando predita pela equação do modelo de exigências nutricionais de gado de corte (BCNRM). Além disso, a UUA predita foi maior ($P < 0,05$) que a UUA mensurada. A quantidade de RCO expressa como proporção de TEU foi maior à medida que o NP diminuiu ($P < 0,05$). A ureia N excretada nas fezes (UFE) em proporção de TEG tendeu ($P < 0,10$) a ser maior nos animais alimentados com a fonte SU. A proporção da síntese de MICP (% do total de N microbiano) a partir da reciclagem da ureia foi maior ($P < 0,05$) nos animais alimentados com CGM em comparação com aqueles alimentados com as dietas SU e também pela redução do NP de 14 para 11% de PB. Quando a proporção da síntese de MICP a partir da reciclagem de ureia foi expressa como uma proporção de TEU e TEG, os animais alimentados com DDG tiveram o resultado mais alto. Os animais alimentados com as dietas contendo 11% de PB tiveram maior síntese de MICP a partir da reciclagem de ureia quando expressos como uma proporção de TEU ($P < 0,05$). Não houve interações entre FP e NP para o fluxo de qualquer AA avaliado ($P > 0,05$). O fluxo duodenal de AA essencial (AAE) e não essencial (AANE) não foram afetados ($P > 0,05$) pelas FP e NP. A concentração de prolina foi aumentada nas dietas SU, reduzida nas dietas CGM e não foi afetada nas dietas DDG pelo aumento no NP. Dietas contendo DDG e 11% de PB tenderam a ($P < 0,10$) a ter maior concentração de arginina e histidina nas bactérias do rúmen em comparação com as dietas SU ou GGM com 11% de PB; no entanto, não houve diferença entre as FP no nível de 14% de PB. A concentração de lisina nas bactérias do rúmen tendeu ($P < 0,10$) a ser maior à medida que o NP aumentou nas dietas CGM. A concentração de tirosina nas bactérias do rúmen tendeu ($P < 0,10$) a ser maior nos animais alimentados com as dietas contendo CGM em comparação aos animais alimentados com as dietas DDG. Além disso, o aumento do NP das dietas de 11 para 14% de PB tendeu ($P < 0,10$) a aumentar a concentração de tirosina na proteína microbiana. Os animais alimentados com DDG tenderam ($P < 0,10$) a ter maior fluxo de arginina, lisina e leucina de origem microbiana do que aqueles alimentados com as dietas contendo SU ou CGM. Os animais alimentados com DDG tiveram maior ($P < 0,05$) suprimento de AANE de origem microbiana do que aqueles alimentados com as dietas contendo SU, mas não diferiram dos animais alimentados com as dietas CGM. O suprimento de histidina e glutamato de origem microbiana foi maior ($P < 0,05$) pela inclusão de CGM ou DDG nas dietas em comparação com a fonte SU. Os animais alimentados com CGM ou DDG tenderam ($P < 0,10$) a apresentar maior fluxo de prolina e serina de origem microbiana do que aqueles alimentados com as dietas SU. Os animais alimentados com CGM ou DDG tenderam ($P < 0,10$) a ter maior fluxo de AAE, arginina, isoleucina e valina da fração PNDR. O suprimento de leucina da fração PNDR foi maior ($P < 0,05$) nos animais alimentados com CGM ou DDG em comparação com aqueles alimentados com as dietas SU. A concentração de histidina no plasma tendeu ($P < 0,10$) a ser maior nos animais alimentados com as dietas DDG. A concentração plasmática de leucina foi maior ($P < 0,05$) nos animais alimentados com CGM ou DDG em comparação com aqueles alimentados com as dietas SU. Os animais alimentados com as dietas contendo 14% de PB tiveram maior ($P < 0,05$) concentração de leucina em comparação com as dietas contendo 11% de PB. A concentração plasmática de fenilalanina foi maior ($P < 0,05$) nos animais alimentados com as dietas contendo CGM ou DDG em comparação com aqueles alimentados com as dietas SU. A concentração de valina plasmática foi maior ($P < 0,05$) nos animais alimentados com as dietas com um nível de PB de 14% em comparação com aqueles alimentados com as dietas contendo 11% de PB. A concentração plasmática de glutamina foi maior ($P < 0,05$) nos animais alimentados com as dietas SU em comparação com aqueles alimentados

com CGM ou DDG. A concentração plasmática de glicina foi maior ($P < 0,05$) nos animais alimentados com as dietas SU em comparação com aqueles alimentados com as dietas contendo CGM ou DDG. Houve interações, ou tendências para interações, entre FP e NP sobre a utilização bruta de AA de todos os AA avaliados, exceto para metionina e cistina. A utilização de arginina e histidina foi maior nos animais alimentados com as dietas contendo 11% de PB com DDG e 14% de PB com CGM. Os animais alimentados com a dieta contendo 11% de PB e DDG tiveram maior eficiência de uso de isoleucina, lisina, fenilalanina, treonina valina, alanina, ácido aspártico, glutamato, prolina, serina e tirosina do que outras dietas, exceto a dieta de 14% de PB com CGM que não diferiu. A utilização de metionina e cistina não foi afetada por FP ou NP ($P > 0,05$). A eficiência de uso de AA é afetada pelos níveis e fontes de proteína na dieta. Nossos resultados sugerem que é possível aumentar a suprimento de AA essencial usando CGM ou DDG (fontes RUP) na dieta em comparação ao farelo de soja mais ureia, especialmente em situações em que seja possível aumentar o fluxo de proteína microbiana. Os resultados deste estudo indicam que o nível de 11% de PB pode ser usada para bovinos Nelore em confinamento alimentados com dietas de alto concentrado, sem afetar negativamente a ingestão de nutrientes, a digestibilidade e a fermentação ruminal. Além disso, nas atuais condições experimentais, as fontes de PNDR testadas aumentaram acentuadamente a ENU, mantendo a síntese do MICP constante pelo estímulo do uso de uréia reciclada para o crescimento microbiano. Além disso, a eficiência bruta de uso dos AA é afetada pelos níveis e fontes de proteína na dieta.

Palavras-chave: Aminoácidos, Confinamento, Nelore, Reciclagem de ureia, Proteína degradável do rúmen

PROTEIN METABOLISM AND UREA KINETIC IN FEEDLOT NELLORE STEERS FED WITH DIFFERENT PROTEIN SOURCES AND INCLUSION LEVELS

ABSTRACT - The use of rumen undegradable protein (RUP) sources in high-energy diets may be an alternative to increase the nitrogen use efficiency (NUE) in ruminants. In this study we had two main objectives: 1) to understand how the protein content of the diet and the rumen degradable protein (RDP) intake can affect urea recycling and its utilization. 2) how the protein content of the diet and the amino acid profile of the metabolizable protein (MP) can affect the efficiency of the use of amino acids (AA) in feedlot Nellore cattle receiving high-energy density diets. Thus, we evaluated the effects of different sources and protein levels in the diet of feedlot Nellore cattle receiving high-concentrate diets. We had two major hypotheses: 1) reducing dietary N associated with RUP sources can increase NUE by reducing ruminal NH_3 concentration, urea production in the liver and urinary N excretion, while maintaining N available for microbial protein (MICP) synthesis through urea N recycling. 2) differences in AA use efficiency exists and the gross AA use efficiency is affected by dietary factors such as N sources and levels. These hypotheses were tested using six Nellore steers, cannulated in the rumen, duodenum and ileum with initial body weight (BW) of 354 ± 11.8 kg and 18 months of age. The animals were randomly assigned to receive each diet once over the 6 periods in a 6 × 6 Latin square design. Experimental diets consisted of 80% concentrate and 20% roughage (DM basis), where fresh chopped sugar cane was used as the roughage source and the concentrates differed in the protein source. Corn gluten meal (CGM) and dry distillers grains (DDG) were used as RUP sources, with low and intermediate ruminal degradability, respectively, and soybean meal and urea (SU) were used as RDP source. Treatments were arranged in a factorial A × B, where factor A consisted of 3 protein sources (PS; soybean meal plus urea, CGM and DDG) and factor B consisted of 2 dietary crude protein (CP) levels (PL; 11 and 14%). There was no interaction between PS and PL on nutrient intake and digestibility ($P > 0.05$). Animals fed diets with an inclusion level of 11% CP had greater ($P < 0.05$) non-fibrous carbohydrates (NFC) intake and tended ($P < 0.10$) to have greater intake of DM (% of BW and kg/day), organic matter (OM) and total digestible nutrients (TDN). Intake of RDP was greater ($P < 0.05$) and RUP intake was less ($P < 0.05$) when animals were fed SU diets. Animals fed DDG diets had greater ($P < 0.05$) NDF intake compared to SU or CGM diets. Animals fed DDG tended ($P = 0.10$) to have greater NDF apparent total-tract digestibility compared to those fed SU diets. Animals fed SU diets had a greater ($P < 0.05$) ruminal ammonia (NH_3-N) concentration than those fed with CGM or DDG diets. Microbial N flow and efficiency was not affected ($P > 0.05$) by PL and PS. Animals fed SU diets had lower ($P < 0.05$) NUE and greater urea entry rate (UER). In addition, increasing PL from 11 to 14% CP tended ($P < 0.10$) to lead to greater UER production. Animals fed SU diets tended ($P < 0.10$) to have greater gastrointestinal entry rate (GER) than those fed CGM or DDG diets. Animals fed SU diets had greater ($P < 0.05$) urea N returned to ornithine cycle (ROC) compared to those fed CGM or DDG. When predicted by the equation developed by the Beef Cattle Nutrient Requirements Model (BCNRM) the amount of urea used for anabolism (UUA) was greater ($P < 0.05$) in animals fed 11% CP diets compared to those fed diets containing 14% CP. In addition, the predicted UUA was greater ($P < 0.05$) than the measured UUA. The ROC expressed as a proportion of UER was greater for diets with 11% CP than for those with 14% CP ($P < 0.05$). The urea N excreted in feces (UFE) as a proportion of GER tended ($P < 0.10$) to be greater for SU than for DDG and CGM. The proportion of MICP synthesis (% of total microbial N) from urea recycling was greater ($P < 0.05$) for animals fed CGM compared to those

fed SU diets and also greater for diets with 11% CP than for those containing 14% CP. MICP synthesis from urea recycling expressed as a proportion of UER and GER, was greater for animals fed DDG. Animals fed diets containing 11% CP had higher MICP synthesis from urea recycling, when expressed as a proportion of UER, than did animals fed 14% CP diets ($P < 0.05$). There were no interactions between PS and PL for the flow of any AA evaluated ($P > 0.05$). The duodenal flow of essential (EAA) and non-essential AA (NEAA) was not affected ($P > 0.05$) by PS and PL. Proline concentration was increased in SU diets, reduced in CGM diets and not affected in DDG diets by the increase in PL. Diets containing DDG and 11% CP tended ($P < 0.10$) to have greater arginine and histidine concentration in rumen bacteria compared to SU and GGM diets with 11% CP; however, there was no difference between PS within the 14% CP level. Lysine concentration in rumen bacteria tended ($P < 0.10$) to be greater as the PL increased in the CGM diets. Tyrosine concentration in rumen bacteria tended ($P < 0.10$) to be greater in animals fed diets containing CGM compared to those fed DDG diets. Also, increasing PL in the diet from 11 to 14% CP tended ($P < 0.10$) to lead to higher concentrations of tyrosine in microbial protein. Animals fed DDG tended ($P < 0.10$) to have greater arginine, lysine and leucine supply from microbial protein than those fed diets containing SU or CGM. Animals fed DDG had greater ($P < 0.05$) NEAA supply from microbial protein flow than those fed diets containing SU, but they did not differ from animals fed CGM diets. Histidine and glutamate from microbial protein had a greater supply ($P < 0.05$) by the dietary inclusion of CGM or DDG compared to SU diets. Animals fed CGM or DDG tended ($P < 0.10$) to have greater proline and serine flow from microbial protein than those fed SU diets. Animals fed CGM or DDG tended ($P < 0.10$) to have greater EAA, arginine, isoleucine and valine supply from RUP fraction. The Leucine supply from RUP was greater ($P < 0.05$) in animals fed CGM or DDG compared to those fed SU diets. Plasma histidine concentration tended ($P < 0.10$) to be greater in animals fed DDG diets. Plasma leucine concentration was greater ($P < 0.05$) in animals fed CGM or DDG compared to those fed SU diets. Animals fed diets containing 14% CP had greater ($P < 0.05$) leucine concentration compared to the diets containing 11% CP. Plasma phenylalanine concentration was greater ($P < 0.05$) in animals fed diets containing CGM or DDG compared to those fed SU diets. Plasma valine concentration was greater ($P < 0.05$) in animals fed diets with a CP level of 14% compared to fed 11% CP diets. Plasma glutamine concentration was greater ($P < 0.05$) in animals fed SU diets compared to those fed CGM or DDG diets. Plasma glycine concentration was greater ($P < 0.05$) in animals fed SU diets compared to those fed CGM or DDG diets. There were interactions, or tendencies for interactions, between PS and PL for gross AA utilization of all AA evaluated, except methionine and cystine. Arginine and histidine utilization were greater in animals fed diets containing 11% CP with DDG and 14% CP with CGM. Animals fed the diet containing 11% CP and DDG showed greater isoleucine, lysine, phenylalanine, threonine, valine, alanine, aspartic, glutamate, proline, serine, and tyrosine use efficiency than other diets, except diet 14% CP with CGM which did not differ. Methionine and cystine utilization were not affected by PS or PL ($P > 0.05$). The AA use efficiency is affected by dietary protein levels and sources. Our results suggest that it is possible to increase the supply of essential AA using CGM or DDG (RUP sources) in the diet compared to soybean meal plus urea, especially in situations where it is possible to increase the microbial protein flow. Results from this study indicate that 11% of CP inclusion rate can be used for feedlot Nellore cattle fed high-concentrate diets without negatively affecting nutrient intake, digestibility and ruminal fermentation. Moreover, in the present experimental conditions, the tested RUP feed sources markedly increased NUE, while keeping the MICP synthesis constant by stimulating the use of recycled urea for microbial growth.

In addition, the gross AA use efficiency is affected by dietary protein levels and sources.

Keywords: Amino acids, Feedlot, Nellore, Urea recycling, Rumen degradable protein

GENERAL CONSIDERATIONS

Public concern about food product association with climate changes started to grow during the industrial revolution and since then it has grown significantly. Although efforts and strategies to mitigate environmental impact caused by food production system have been made, they are still not enough (Tedeschi et al., 2015).

Around the world, animal production systems are facing challenges related to profitability and environmental and social sustainability (FAO, 2016). Therefore, animal scientists are trying to find new strategies improve animal production in a sustainable manner. In order to provide high-quality protein food to a growing world population, which is estimated to be 9.55 billion people by 2050 (Godfray et al., 2010).

In this context, nitrogen (N) is an essential compound that has nutritional, environmental and economic importance to society due to its high cost when added in animal's diet and its potential as soil and water contaminating agent (Vasconcelos et al., 2007). When used in cattle's diets the use efficiency of N in cattle (NUE) can vary from 15-40% (Dijkstra et al., 2011). It is obtained by an equation that takes in consideration the relationship between the N retained in the animal's body and the amount of N ingested by the animal (Dijkstra et al., 2011). Therefore, more than 80% of the total N ingested can be excreted in inefficient feeding systems.

According to a survey conducted by Pinto and Millen (2019), in Brazil some cattle nutritionists formulate diets with high crude protein (CP) i.e. commonly adopt CP levels between 11.0% to 15.0%, with average values of 13.6% CP as an attempt to increase ruminal ammonia concentration and meet rumen degradable protein (RDP) requirements of the rumen microorganisms as the greater inclusion of concentrate feedstuffs. However, oftentimes it makes these diets susceptible to exceed the capacity of the animal to use the dietary N, which may result in negative consequences to the economy of the system and to the environment due to the increased cost with feed and N excretion in the feces and urine by the animals. Recently, some studies conducted in Brazil have demonstrated that excess of N added to ruminants' diets may not improve animal's performance (Menezes, et al., 2016; Amaral et al., 2018) instead, it can result in a surplus of N in the diet and consequently increases N excretion on the environment, which makes it a contaminating agent. Nitrogen that exceeds the animal uptake capacity is excreted by the animal in urine and feces and lost to the environment mainly in the form of urea (CH_4N_2O) and ammonia (NH_3). These two forms of N found in manure are the most available forms of N used by plants and they

are the most susceptible compounds to be lost into the environment (Powell et al., 2008). In the environment, by nitrification and denitrification processes these N fractions are converted to nitrous oxide (N_2O), nitric oxide (NO), dinitrogen (N_2), and nitrate (NO_3^-) (Oenema et al., 2007; Richardson et al. 2009). Bouwman et al. (1997) reported that NH_3 emissions are greater in ruminants than in other domestic animals, which increase the potential contribution of Brazilian's feedlots to the eutrophication and acidification of the ecosystems. Beef cattle can excrete approximately 60 to 80% of N in urine and 20 to 40% in feces, which depends on apparent total N digestibility, dietary CP content, and N intake (Dong et al., 2014). In addition, when dietary RDP is in excess of the amount required by ruminal microorganisms, the excess of ammonia N generated by protein degradation is absorbed, converted to urea in the liver, and excreted in the urine (Bach et al., 2005).

Nitrous oxide is an important greenhouse gas (GHG) due to its heating power, which is 298 times greater than carbon dioxide (CO_2). In 2012, it was created the Brazilian Plan for Mitigation and Adaptation to Climate Change to reduce GHG emissions in the range of 36.1 to 38.9% by 2020 (Brasil, 2012). Therefore, the development of mitigation strategies to reduce emissions of N_2O and NH_3 by production systems throughout the country became necessary. In addition, the eutrophication of water resources caused by N excreted into the environment by ruminant animals has been the subject of discussions in the scientific community on the last decades (Waldrip et al., 2015; Biagini and Lazzaroni, 2018; Kantera and Brownlie, 2019). However, the magnitude of this problem under Brazilian conditions is still unknown.

As mentioned previously, due to an increase in the world population the world meat and milk production is expected to double by 2050 compared to 2000 (Dijkstra et al., 2011). Although drastic reductions on N excretion are unlikely from a biological standpoint, it is estimated that the amount of N in manure per unit of milk and meat produced should be reduced by half only to avoid an increase in the pollution levels, which represents a great challenge for animal scientists in face of the low NUE values observed in ruminant production systems (Angelidis et al., 2019). Taken together, these data indicate that nutritional strategies able to mitigate excretion of excess dietary constituents, such as N, in manure of feedlot animals are required (Hünerberg et al., 2014). As such, one strategy involves the reduction of N excretion to the environment by reducing the amount of this nutrient fed to ruminant animals. Menezes et al. (2016) evaluated the performance of feedlot Nellore cattle fed increasing levels

of dietary CP (10, 12 and 14%) and reported a decrease in urinary N excretion and no differences in DMI and average daily gain (ADG) when animals were fed the 10% CP diet, suggesting that the use of 10% of CP diets for finishing Nellore cattle reduces their environmental impact. However, these authors used a low number of animals per treatment, which could compromise their results. In fact, Amaral et al. (2018) observed a reduction on ADG when fed Nellore cattle with a diet containing 10% CP compared to those fed 12 or 14% CP. Therefore, nutritional strategies that reduce the environmental impacts of feedlot cattle without compromise animal performance need to be developed.

Ruminal metabolism performed by microorganisms is known to be the most critical factor contributing to the lack of efficiency on the N use by ruminants (Tamminga, 1992). In addition, the manipulation of ruminal microbial activity is more practicable than the modification of other metabolic processes (Calsamiglia et al., 2010). Moreover, it has resulted in numerous studies conducted with the aim to optimize the ruminal fermentation by ruminal microorganisms and to increase the metabolizable protein (MP) flow to the small intestine (Brito et al., 2006; Broderick and Reynal, 2009). Therefore, the formulation of diets with balanced concentration of rumen degradable protein (RDP) and rumen undegradable protein (RUP), the control of ruminal protein degradation, the supply of ruminal fermentable carbohydrates (energy), and the modification of the AA profile reaching the small intestine, are some strategies that can be used improve NUE (Calsamiglia et al., 2010).

In the rumen, RDP can be degraded into NH_3 by the microorganisms, which is absorbed and converted to CH_4N_2O on the liver to detoxify the excess of N in the body (Huntington and Archebique, 1999). Moreover, it represents a challenge for the increase in NUE, since the excess of NH_3 will end up being excreted in the urine (Calsamiglia et al., 2010). Additionally, the ureagenesis process may require supply of amino acids (AA), which can have negative consequences on the protein deposition in the body (Awawdeh et al., 2005). Some evidences that support this are shown in studies where the relationship among urea synthesis, hepatic removal of NH_3 and AA availability were compared (Lobley et al., 1995). In cattle fed forage diets, a greater portal absorption and hepatic removal of NH_3 accompanied by extra hepatic AA uptake was reported (Huntington, 1989; Reynolds et al., 1991), which may contribute to the low NUE observed in ruminants. The relative contribution of ruminal NH_3-N and NH_3-N coming from AA catabolism to the total CH_4N_2O produced in the liver is dependent on the efficiency in which the dietary protein source meets the N required for microbial

protein (MICP) synthesis and for its post-absorptive functions (Arriola Apelo et al., 2014). In this context, protein feed sources with lower ruminal degradability are used as strategy to increase NUE and the essential AA reaching the small intestine and to decrease potential losses of N mainly in the urine.

In ruminants such as cattle and sheep, up to 40 to 80% of the urea synthesized in the liver can return to the gastrointestinal tract (GIT) via saliva or transepithelial absorption (Lapierre and Lobley, 2001). This N recycling process provides to the animal an enormous adaptive capacity to survive under adverse conditions such as when they are fed diets with low nitrogen content. The N-recycled can be used by ruminal microorganisms and incorporated into the MICP which, when digested and absorbed by the ruminant, can be used in metabolic processes such as anabolism (Brake et al., 2010). For instance, growing Holstein heifers fed diets with decreasing N content were reported to increase the urea recycling from 29.3 to 84.9 units (percentage of total urea production). Furthermore, microbial incorporation of the recycled urea-N was found to be increased from 1.48 to 35.4% (Marini and Van Amburgh, 2003).

Several factors affecting N recycling and use for anabolic purposes were reported in the literature. The main factors are dietary N level and N intake (Marini and Van Amburgh, 2003; Batista et al., 2017), oscillation of dietary N levels (Archibeque et al., 2007); starch processing method (Theurer et al., 2002), partial ruminal defaunation (Kiran and Mutsvangwa, 2010), DMI and energy intake (Sarraseca et al., 1998; Kennedy and Milligan, 1980), frequency of supplementation, and concentration of RDP in the diet (Wickersham et al., 2008; Rémond et al., 2009). In addition, factors such as ruminal NH_3-N concentration, ruminal bacterial urease activity, ruminally-fermentable carbohydrate concentration (RFC), ruminal concentrations of volatile fatty acids (VFA) and CO_2, and ruminal pH are also known to play a significant role in trans-epithelial transport of urea-N from the blood to the rumen (Kennedy and Milligan, 1980), which perhaps may affect the expression of the urea transporters genes (Simmons et al., 2009). Understanding the mechanisms in which these genes can effectively contribute to the N utilization has extensive implications on NUE and will contribute to improve NUE in ruminant production systems.

However, the inability to accurately determine the amount of N recycled to the rumen and its contribution to anabolic functions (*e.g.,* MICP synthesis) in different diet types and animal production scenarios compromise the incorporation of N recycled into feed models (Wickersham et al., 2009a). Under a reduction in N intake, ruminants

have mechanisms to reduce N lost in the urine and to increase the dietary N fraction that is recycled back to the rumen by renal urea reabsorption (Harmeyer and Martens, 1980). Therefore, to achieve this nutritional advantage of N recycling, the reduction of N intake is required. Additionally, MICP synthesis can be optimized by feeding the animals diets capable to meet their energy requirements for ruminal fermentation (Detmann et al., 2014). By doing so, the supply of MP and animal performance will be maintained, while intake and urine N excretion will be reduced, which will finally result in an increase of NUE in cattle. According to Brake et al. (2010), N recycling plays an important role in animals fed diets with low rumen degradability. An equation to estimate the N recycling in growing cattle consuming forage-based diets was reported by NASEM (2016). However, the application of this equation was not recommended for animals do not receiving forage-based diets. In the last edition of the Brazilian beef cattle ration formulation system, the authors removed the inefficiency factor of 1.10 (representing 10% of net N losses in the rumen) to estimate RDP requirements by recognizing that urea recycling provides a N source for MICP synthesis. Therefore, the requirements of RDP are equal to MCP (BR Corte, 2016). However, due to the lack of national data, there is no equation available to estimate urea recycling under Brazilian conditions. Moreover, to our knowledge, there is a lack in the literature on results regarding N recycling in high-concentrate diets, especially when they are combined with RUP feed sources.

Quantify the AA supply in ruminants is a big challenge for animal scientists around the world. It is due to the wide variation in RUP among feedstuff and to the interference of rumen microbiome in the AA profile reaching the small intestine (Titgemeyer, 2003). In non-ruminant nutrition, it is well established that AA supply and requirements must be expressed for individual AA instead of their aggregate form such as CP (Vieira et al., 2016). The benefits of this approach have positive economic and environmental impacts on animal production, and single AA adjustments have been used to improve animal performance and farm profitability in swine and poultry production systems (Lapierre et al., 2006). Formulating diets with decreased CP level is possible by adding specific AA if a supplemental source of the limiting AA is available commercially at competitive prices (Vieira et al., 2016). Although this practice is common for non-ruminant animals, for ruminants' nutritionists there are several challenges that prevent the immediate use of this ration formulation approach. The AA absorbed from the intestine do not match the dietary AA profile and this difference is due to ruminal fermentation (degradation of dietary proteins and ruminal MICP

synthesis), which makes the study of AA requirement in ruminants a difficult task (Titgemeyer, 2003).

According to the NASEM (2016), the essential AA content and the profile in the MP will determine its use efficiency by ruminants. Due to the complexity in manipulating the AA profile of MP and the variation on the ruminal MICP synthesis (Titgemeyer, 2003), the use of RUP sources on feedlot diets to increase intestinal supply of essential AA may be a strategy to increase NUE in ruminants (Scholljeger et al., 2005; Wickersham et al., 2009b). In cases where MICP is the primary source of MP for growing cattle, lysine is considered the second limiting AA (Richardson and Hatfield, 1978, Batista et al., 2016). However, in corn-based diets, which are considered to have high methionine content, lysine becomes the first limiting AA (Abe et al., 1997). Hussein et al. (2016) demonstrated that by supplying lysine for cattle that have a limitation of this AA in the MP, urinary N losses were reduced from 51.9 to 44.3 $g.day^{-1}$ and N retention was increased from 24.8 to 33.8 $g.day^{-1}$. The Nutrient Research Council (NRC) and The Cornell Net Carbohydrate and Protein System (CNCPS) consider that the utilization efficiency for different essential AAs are not affected by dietary factors, the body weight is the single factor to predict AA use efficiency for growth instead (Fox et al., 2004; NASEM, 2016). However, previous studies have observed individual variations in the efficiency of AAs use (Campbell et al., 1996, 1997; Awawdeh et al., 2005, 2006; Titgemeyer et al., 2012). Such variations are a result of the different oxidation rates (Heger and Frydrych, 1989) and metabolic processes that each AAs undergo during digestion and metabolism processes (Owens and Pettigrew, 1989). Therefore, the determination of the use efficiency of individual AAs is required for the adequate supply of limiting AAs to the animal in order to increase the NUE of beef cattle diets. More specifically, information about the supply of AA's derived from the RUP and MICP will allow formulation of diets in which AA's from RUP complement MICP to meet animal's requirements, while decreasing N intake and maintaining the animal performance (NASEM, 2016).

The nutritional system developed for *Bos indicus* animals known as BR-CORTE (Valadares Filho et al., 2016) and NASEM (2016) consider an intestinal digestibility of MICP around 80%, which was obtained by studies with sheep and bulls. Recently, MICP intestinal digestibility was assessed in Nellore cattle by using ^{15}N as a microbial marker and a constant value of 86% for true intestinal digestibility was reported (Mariz et al., 2018). However, it may be affected by the rumen microbiome, which can vary according to differences in dietary composition (Liu et al., 2017; Hulls et al., 2018).

Brazil significantly contributes to the world beef production and Nellore animals represent the major breed in the Brazilian cattle herd (Carvalho Filho et al., 2020). Therefore, studies evaluating the intestinal digestibility of the MICP are required to evaluate the effects of different types of production systems and diets on those animals. In addition, the understanding of the effects of dietary CP content and the MP AA profile on N recycling and AA efficiency utilization in feedlot Nellore cattle fed high-concentrate diets may provide relevant information, which can be used to maximize animal performance with simultaneous reduction of N concentration in ruminant's diets. Furtheremore, information on the N recycling to the GIT can be useful to obtain a more adequate estimation of the RDP requirements for the maximal MICP synthesis with lower N losses during ruminal fermentation (Wickersham et al., 2009a).

The incorporation of the results of N recycling into the equations to predict RDP requirements would improve the NUE in the rumen and allow the reduction of the dietary RDP content, which could ultimately result in economic and environmental benefits for feedlot Nellore cattle fed high-concentrate diets. Prates et al. (2017) reported that results regarding the N recycling in zebu animals are scarce, which justifies the conduction of studies evaluating the effects of diets containing decreasing N concentration and different protein sources on Nellore animals. Therefore, the understanding of the N recycling process in zebu cattle fed high-concentrate diets will allow nutritionists to decrease dietary N content in the feedlot phase without compromising animal performance. Moreover, exploring N recycling as an RDP source for MICP synthesis can be an important nutritional strategy that can ensure the supply of animal derived food to a growing world population and the decrease of the natural resources use by production animals, consequently reducing the public pressure for the reduction in the environmental impacts caused by animal production systems.

The inclusion of RUP sources in diets with low N content can be used as a strategy to supply essential AA to MP. However, the use of these feed sources may limit the availability of N to be used for the MICP synthesis in the rumen. Therefore, quantify the N reclycled to the rumen is required in order to evaluate whether or not this physiological process can compensate the reduction of dietary CP in the diet in zebu animals fed high-concentrate diets.

Thus, the hypotheses of the presente study were: 1) reducing dietary N associated with RUP sources can increase NUE by reducing ruminal NH_3 concentration, urea production in the liver and urinary N excretion, while maintaining N available for MICP synthesis through urea N recycling. 2) differences in AA use

efficiency exists and the gross AA use efficiency is affected by dietary factors such as N sources and levels. In addition, the understanding of N recycling process can help animal scientists to determine the RDP requirements of finishing beef cattle, which can increase profitability and environmental sustainability of animal production systems. Therefore, the objective of this study was to evaluate the effects of different CP levels and sources on urea kinetics and AA use efficiency in feedlot Nellore steers fed high-concentrate diets.

REFERENCES

Abe M, Iriki T, Funaba M (1997) Lysine deficiency in post-weaned calves fed corn and corn gluten meal diets. **Journal of Animal Science** 75:1974–1982.

Amaral P, Mariz L, Zanetti D, Prados L, Marcondes M, Santos S, Valadares Filho S (2018) Effect of dietary protein content on performance, feed efficiency and carcass traits of feedlot Nellore and Angus × Nellore cross cattle at different growth stages. **The Journal of Agricultural Science** 156:110-117.

Angelidis A, Crompton L, Misselbrook T, Yan T, Reynolds CK, Stergiadis S (2019) Evaluation and prediction of nitrogen use efficiency and outputs in faeces and urine in beef cattle. **Agriculture, Ecosystems and Environment** 280:1-15.

Arriola Apelo SI, Kanapp JR, Hanigan MD (2014) Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. **Journal of Dairy Science** 97:4000-4017.

Archibeque SL, Freetly HC, Ferrell CL (2007) Net portal and hepatic flux of nutrients in growing wethers fed high concentrate diets with oscillating protein concentrations. **Journal of Animal Science** 85:997-1005.

Awawdeh, MS, Titgemeyer EC, Mccuistion KC, Gnad DP (2005) Ruminal ammonia load affects leucine utilization by growing steers. **Journal of Animal Science** 83:2448-2454.

Awawdeh MS, Titgemeyer EC, Schroeder GF, Gnad DP (2006) Excess amino acid supply improves methionine and leucine utilization by growing steers. **Journal of Animal Science** 84:1801-1810.

Bach A, Calsamiglia S, Stern MD (2005) Nitrogen metabolism in the rumen. **Journal of Dairy Science** 88:9-21.

Batista ED, Detmann E, Valadares Filho SC, Titgemeyer EC, Valadares, RFD (2017) The effect of CP concentration in the diet on urea kinetics and microbial usage of recycled urea in cattle: a meta-analysis. **Animal** 11:1303-1311.

Batista ED, Hussein AH, Detmann E, Miesner MD, Titgemeyer EC (2016) Efficiency of lysine utilization by growing steers. **Journal of Animal Science** 94:648-655.

Biagini D, Lazzaroni C (2018) Eutrophication risk arising from intensive dairy cattle rearing systems and assessment of the potential effect of mitigation strategies. **Agriculture, Ecosystems & Environment** 266:76-83.

Bouwman AF, Lee DS, Asman WAH, Dentener, FJ, Van Der Hoek KW, Olivier JGJ (2017) A global high-resolution emission inventory for ammonia. **Global Biogeochemical Cycles** 11:561–587.

Brake DW, Titgemeyer EC, Jones ML, Anderson DE (2010) Effect Of Nitrogen Supplementation On Urea Kinetics And Microbial Use Of Recycled Urea In Steers Consuming Corn-Based Diets. **Journal of Animal Science** 88:2729-2740.

BRASIL 2012: Plano ABC (Agricultura de Baixa Emissão de Carbono). **Ministério da Agricultura, Pecuária e Abastecimento, Ministério do Desenvolvimento Agrário – MAPA/ACS:** Brasília/DF. 173 p. ISBN 978-85-7991-062-0. Cited 2019 Mar 30. Available from: <http://www.agricultura.gov.br/arq_editor/download.pdf>.

Brito AF, Broderick GA, Reynal SM (2006) Effects of different protein supplements on omasal nutrient flow and microbial protein Synthesis in lactating dairy cows. **Journal of Dairy Science** 90:1808-1841.

Broderick GA, Reynal SM (2009) Effect of source rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. **Journal of Dairy Science** 92:2822-2834.

Campbell CG, Titgemeyer EC, St-Jean G (1996) Efficiency of D- vs L-methionine utilization by growing steers. **Journal of Animal Science** 74:2482-2487.

Campbell CG, Titgemeyer EC, St-Jean G (1997) Sulfur amino acid utilization by growing steers. **Journal of Animal Science** 75:230-238.

Calsamiglia S, Ferret A, Reynolds CK, Reynolds CK, Kristensen NB, Van Vuuren, AM (2010) Strategies for optimizing nitrogen use by ruminants. **Animal** 4:1184-1196.

Carvalho Filho I, Marques DBD, Campos CF, Guimarães JD, Guimarães SEF, Lopes PS, Fonseca e Silva F, Veroneze R (2020) Genetic parameters for fertility traits in Nellore bulls. **Reproduction in Domestic Animals** 55:38-43.

CSIRO - Commonwealth Scientific and Industrial Research Organization. **Nutrient Requirements of Domesticated Ruminants.** Collingwood, VIC: Commonwealth Scientific and Industrial Research Organization, 2007. 270p.

Detmann E, Valente EEL, Batista ED, Huhtanen P (2014) An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. **Livestock Science** 162:141-153.

Dijkstra J, Oenema O, Bannink A (2011) Dietary strategies to reducing N excretion from cattle: implications for methane emissions. **Current Opinion in Environmental Sustainability** 3:414-422.

Dong RL, Zhao GY, Chai LL, Beauchemin KA (2014) Prediction of urinary and fecal nitrogen excretion by beef cattle. **Journal of animal Science** 92:4669-4681.

FAO (2016) Food and Agriculture Organization. In: Van Holsteijn FH, Vries M, Makkar HPS (Eds.) **Identification of indicators for evaluating of sustainable animal diets.** Animal Production and Health Working Paper, n.15. Rome, Italy, 35p.

Fox DG, Tedeschi LO, Tylutki TP, Russel JB, Van Amburgh ME, Chase LE, Pell AN, Overton TR (2004) The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. **Animal Feed Science and Technology** 112:29-78.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: The challenge of feeding 9 billion people. **Science** 327:812-818.

Harmeyer J, Martens H (1980) Aspects of urea metabolism in ruminants with reference to the goat. **Journal of Dairy Science** 63: 1707-1728.

Huws SA, Creevey CJ et al. (2018) Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future. **Frontiers in Microbiology** 9:2161, 2018.

Heger J, Frydrych Z (1989) Efficiency of utilization of amino acids. In: FRIEDMAN, M. (ed.). **Absorption and Utilization of Amino Acids.** CRC Press, Boca Raton, FL. p.31-56.

Hünerberg M, Little SM, Beauchemin KA, Mcginn SM, O'Connor D, Okine EK, Harstad OM, KRÖBEL R, Mcallister TA (2014) Feeding high concentrations of corn dried distiller's grains decreases methane, but increases nitrous oxide emissions from beef cattle production. **Agricultural Systems** 127:19-27.

Hussein AH, Batista ED, Miesner MD, Titgemeyer EC (2016) Effect of ruminal ammonia supply on lysine utilization by growing steers. **Journal of Animal Science** 94:656-664.

Huntington GB, Archibeque SL (1999) Practical aspects of urea and ammonia metabolism in ruminants. In: AMERICAN SOCIETY OF ANIMAL SCIENDE, Raleigh. **Proceedings...** Raleigh: American Society of Animal Science, p.1-11.

Huntington GB (1989) Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. **Canadian Journal of Animal Science** 69:215-223.

Kanter DR, Brownlie WJ (2019) Joint nitrogen and phosphorus management for sustainable development and climate goals. **Environmental Science & Policy** 92:1-8.

Kennedy PM, Milligan LP (1980) The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review**. Canadian Journal of Animal Science** 60:205-221.

Kiran D, Mutsvangwa T (2010) Effects of partial ruminal defaunation on urea-nitrogen recycling, nitrogen metabolism, and microbial nitrogen supply in growing lambs fed low or high dietary crude protein concentrations. **Journal of Animal Science** 88:1034-1047.

Lapierre H, Lobley GE (2001) Nitrogen Recycling in the Ruminant: A Review. **Journal of Dairy Science** 84:223-236.

Lapierre H, Pacheco D, Berthiaume R, Ouellet DR, Schwab CG, Dubreuil P, Holtrop G, Lobley GE (2006) What is true supply of amino acids for a dairy cow? **Journal of Dairy Science,** 89:1-14.

Liu K, Hao X, Li Y, Luo G, Zhang Y, Xin H (2017) The relationship between odd- and branched-chain fatty acids and microbial nucleic acid bases in rumen. **Asian-Australasian Journal of Animal Sciences** 30:1590-1597.

Lobley GE, Connell A, Lomax MA, Brown DS, Miller E, Calder AG, Farningham DAH (1995) Hepatic detoxification of ammonia in the ovine liver: Possible consequences for amino acid catabolism. **British Journal of Nutrition** 73:667-685.

Marini JC, Van Amburgh ME (2003) Nitrogen metabolism and recycling in Holstein heifers. **Journal of Animal Science** 81:545-552.

Mariz LDS, Paloma MA, Valadares Filho SC, Santos SA, Marcondes MI, Prados LF, Pacheco MVC, Zanetti D, Menezes GCC, Faciola AP (2018) Dietary protein reduction on microbial protein, amino acids digestibility, and body retention in beef cattle. I. Digestibility sites and ruminal synthesis estimated by purine bases and 15N as markers. **Journal of Animal Science** 96:2453-2467.

Menezes ACB, Valadares Filho SC, Costa E Silva LF, Pacheco MVC, Pereira JMV, Rotta PP, Zanetti D, Detmann E, Silva FAS, Godoi LA, Rennó LN (2016) Does a reduction in dietary crude protein content affect performance, nutrient requirements, nitrogen losses, and methane emissions in finishing Nellore bulls? **Agriculture, Ecosystems & Environment,** 223:239-249.

NASEM 2016: National Academies of Sciences, Engineering, and Medicine. Nutrient requirements of beef cattle. 8th ed. **Nutrient requirements of domestic animals.** National Academy Press, Washington, DC, 2016, 494p.

O'Connor JD, Sniffen CJ, Fox DG, Chalupa W (1993) A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. **Journal of Animal Science** 71:1298-1311.

Oenema O, Oudendag D, Velthof GL (2007) Nutrient losses from manure management in the European Union. **Livestock Science** 112:261-272.

Owens FN, Pettigrew JE (1989) Subdividing amino acid requirements into portions for maintenance and growth. In: FRIEDMAN, M. (Ed.). **Absorption and Utilization of Amino Acids.** CRC Press, Inc., Boca Raton, FL, p.15-30.

Pina DS, Valadares RFD, Valadares Filho SC, Chizzotti ML (2010) Degradação ruminal da proteína dos alimentos e síntese de proteína microbiana. In: Valadares Filho SC, Marcondes MI, Chizzotti ML, Paulino PVR (Eds.) **Exigências Nutricionais de Zebuínos Puros e Cruzados – BR CORTE.** Suprema Gráfica Ltda, Viçosa, MG, Brasil, 185p.

Pinto ACJ, Millen DD (2019) Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: The 2016 Brazilian survey. **Canadian Journal of Animal Science** 99: 392-407. doi:10.1139/cjas-2018-0031

Powell JM, Broderick GA, Misselbrook TA (2008) Seasonal diet affects ammonia emissions from tie-stall dairy barns. **Journal of Dairy Science** 91:857-869.

Prates LL, Valadares RFD, Valadares Filho SC, Detmann E, Oullet, DR, Batista ED, Zanetti D, Pacheco MVC, Silva BC (2017) Investigating the effects of sex of growing Nellore cattle and crude protein intake on the utilization of recycled N for microbial protein synthesis in the rumen by using intravenous $^{15}N^{15}N$-urea infusion. **Animal Feed Science and Technology** 231:119-130.

Rémond D, Bernard L, Savary-Auzeloux I, Noziere P (2009) Partitioning of nutrient net fluxes across the portal-drained viscera in sheep fed twice daily: effect of dietary protein degradability. **British Journal of Nutrition** 102:370-381.

Reynolds CK, Tyrrell HF, Reynolds PJ (1991) Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: net nutrient metabolism by visceral tissues. **The Journal of Nutrition** 121:1004-1015.

Richardson D, Felgate H, Watmough N, Thomson A, Baggs E (2009) Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle – could enzymic regulation hold the key? **Trends in Biotechnology,** 27:388-397.

Richardson CR, Hatfield EE (1978) The limiting amino acids in growing cattle. **Journal of Animal Science** 46:740-745.

Sarraseca A, Milne E, Metcalf M, Lobley G (1998) Urea recycling in sheep: Effects of intake. **British Journal of Nutrition** 79:79-88.

Scholljegerdes EJ, Weston TR, Ludden PA, Hess BW (2005) Supplementing a ruminally undegradable protein supplement to maintain essential amino acid supply to the small intestine when forage intake is restricted in beef cattle. **Journal of Animal Science** 85:2151-2161.

Simmons NI, Chaudhry AS, Graham C, Scriven ES, Thitlethwaite A, Smith CP, Stewart GS (2009) Dietary regulation of ruminal bovine UT-B urea transporter expression and localization. **Journal of Animal Science** 87:3288-3299.

Tamminga S (1992) Nutrition management of dairy cows as a contribution to pollution control. **Journal of Dairy Science,** v. 75, p. 345-357.

Tedeschi LO, Muir JP, Riley DG, Fox DG (2015) The role of ruminant animals in sustainable livestock intensification programs. **International Journal of Sustainable Development & World Ecology** 22:452-465.

Theurer CB, Huntington GB, Huber JT, Swingle RS, Moore JA (2002) Net absorption and utilization of nitrogenous compounds across ruminal, intestinal, and hepatic tissues of growing beef steers fed dry-rolled or steam-flaked sorghum grain. **Journal of Animal Science** 80:525-532.

Titgemeyer EC (2003) Amino acid utilization by growing and finishing ruminants. In: D'Mello JPF (Editor) **Amino acids in animal nutrition,** 2nd ed. CAB International, Wallingford, UK. p. 329-346.

Titgemeyer EC, Spivey KS, Parr SL, Brake DW, Jones Ml (2012) Relationship of whole body nitrogen utilization to urea kinetics in growing steers. **Journal of Animal Science** 90:3515-3526.

Valadares Filho SC, Costa E Silva LF, Gionbelli MP, Rotta PP, Marcondes MI, Chizotti ML, Prados LF (Eds.) (2016) BR-CORTE 3.0 Nutritional requirements, diet formulation and performance prediction of Zebu and Crossbred cattle. Viçosa: Suprema Gráfica Ltda. Available in: www.brcorte.com.br.

Vasconcelos JT, Tedeschi LO, Fox DG, Galyean Ml, Greene LW (2007) Review: Feeding nitrogen and phosphorus in beef cattle feedlot production to mitigate environmental impacts. **The Professional Animal Scientist** 23: 8-17.

Vieira SL, Stefanello C, Cemin, HS (2016) Lowering the dietary protein levels by the use of synthetic amino acids and the use of a mono component protease. **Animal Feed Science and Technology** 221:262-266.

Waldrip HM, Cole NA, Todd RW (2015) Review: Nitrogen sustainability and beef cattle feedyards: II. Ammonia emissions. **The Professional Animal Scientist** 31:395-411.

Wickersham TA; Titgemeyer EC; Cochran RC; Wickersham EE, Gnad DP (2008) Effect of rumen degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. **Journal of Animal Science** 86:3079–3088.

Wickersham TA, Titgemeyer EC, Cochran RC (2009a) Methodology for concurrent determination of urea kinetics and the capture of recycled urea nitrogen by ruminal microbes in cattle. **Animal** 3:372-379.

Wickersham TA, Titgemeyer EC, Cochran RC, Wickersham EE (2009b) Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. **British Journal of Nutrition** 101:225-232.

CHAPTER 1. EFFECTS OF PROTEIN SOURCES AND INCLUSION LEVELS ON NITROGEN METABOLISM AND UREA KINETICS OF FEEDLOT NELLORE STEERS FED HIGH-CONCENTRATE DIETS.

ABSTRACT - The objective of this study was to evaluate the effects of different protein sources and dietary inclusion levels on ruminal fermentation, urea kinetics and N excretion of feedlot Nellore steers fed high-concentrate diets. Six Nellore steers, cannulated in the rumen, duodenum and ileum with initial body weight (BW) of 354 ± 11.8 kg and 18 months of age were used. The animals were randomly assigned to receive each diet once over the 6 periods in a 6 × 6 Latin square design. Experimental diets consisted of 80% concentrate and 20% roughage (DM basis), where fresh chopped sugar cane was used as the roughage source and the concentrates differentiated on their protein source. Corn gluten meal (CGM) and dry distillers grains (DDG) were used as rumen undegradable protein (RUP) sources, with low and intermediate ruminal degradability, respectively, and soybean meal and urea (SU) were used as rumen degradable protein (RDP) sources. Treatments were arranged in a factorial A × B, where factor A consisted of 3 protein sources (PS; soybean meal plus urea, CGM and DDG) and factor B consisted of 2 dietary crude protein (CP) levels (PL; 11 and 14%). Jugular infusion of [^{15}N-^{15}N]-urea with measurement of enrichment in urine was used to evaluate urea kinetics. Ruminal fermentation, intestinal flow and N balance were evaluated to assess N metabolism. We hypothesized that reducing dietary N would increase nitrogen use efficiency (NUE) by reducing ruminal NH_3 concentration, urea production in the liver and urinary N excretion, while maintaining N available for microbial protein (MICP) synthesis through urea N recycling. We also hypothesized that providing RUP might improve protein deposition, while also providing N to ruminal microbes through recycling. There was no interaction between PS and PL on nutrient intake and digestibility ($P > 0.05$). Animals fed diets with 11% CP had greater ($P < 0.05$) non-fibrous carbohydrates (NFC) intake and tended ($P < 0.10$) to have greater intake of DM (% of BW and kg/day), organic matter (OM) and total digestible nutrients (TDN). Intake of RDP was greater ($P < 0.05$) and RUP intake was less ($P < 0.05$) when animals were fed SU diets. Animals fed DDG diets presented a greater ($P < 0.05$) NDF intake compared to SU or CGM diets. Animals fed DDG tended ($P = 0.10$) to have greater NDF apparent total-tract digestibility compared to those fed SU diets. Animals fed SU had a greater ($P < 0.05$) ruminal ammonia (NH_3-N) concentration than those fed with CGM or DDG. Microbial N flow and efficiency was not affected ($P > 0.05$) by PL and PS. Animals fed SU diets had lower ($P < 0.05$) nitrogen use efficiency (NUE) and greater urea entry rate (UER). In addition, increasing PL from 11 to 14% CP tended ($P < 0.10$) to lead to greater UER production. Animals fed SU diets tended ($P < 0.10$) to have greater gastrointestinal entry rate (GER) than those fed CGM or DDG diets. Animals fed SU diets had greater ($P < 0.05$) urea N returned to ornithine cycle (ROC) compared to those fed CGM or DDG. When predicted by the equation developed by the Beef Cattle Nutrient Requirements Model (BCNRM) the urea used for anabolism (UUA) was greater ($P < 0.05$) in animals fed 11% CP diets compared to those fed diets containing 14% CP. In addition, the predicted UUA was greater ($P < 0.05$) than the measured UUA. The ROC expressed as a proportion of UER was greater for diets with 11% CP than for those with 14% CP ($P < 0.05$). The urea N excreted in feces (UFE) as a proportion of GER tended ($P < 0.10$) to be greater for SU than for DDG and CGM. The proportion of MICP synthesis (% of total microbial N) from urea recycling was greater ($P < 0.05$) for animals fed CGM

compared to those fed SU diets and also greater for diets with 11% CP than those containing 14% CP. MICP synthesis from urea recycling, expressed as a proportion of UER and GER, was greater for animals fed DDG. Animals fed diets containing 11% CP had higher MICP synthesis from urea recycling when expressed as a proportion of UER, than did animals fed 14% CP diets ($P < 0.05$). Results from this study indicate that 11% of CP inclusion can be used for feedlot Nellore cattle fed high-concentrate diets without negatively affecting nutrient intake, digestibility and ruminal fermentation. Moreover, in the present experimental conditions, the tested RUP feed sources markedly increased NUE, while keeping the MICP synthesis constant by stimulating the use of recycled urea for microbial growth.

1. INTRODUCTION

Nowadays, modern intensive livestock systems are known to use high energy content and inclusion rates of crude protein (CP) to promote high animal performance (Ding et al. 2019). In Brazil and United States, diets with high concentrations of CP are used to meet rumen microbiome requirements (Samuelson et al., 2016; Pinto and Millen, 2019). However, in addition to its high cost, high protein diets have the potential to increase the excretion of nitrogen (N) that is not used by the rumen microorganism and by the animal's body into the environment.

Feedlot Nellore cattle can excrete approximately 85% of the consumed N in their feces and urine (Menezes et al., 2016). Therefore, nutritional strategies to reduce N losses in the manure should be evaluated and developed to improve the economic and environmental sustainability of feedlot systems. Improvement of N use efficiency (NUE) by the livestock animal can be used as a strategy to decrease N losses. It can be achieved by reducing the dietary N concentration. However, this decrease in N supply can reduce microbial protein (MICP) synthesis (Ipharraguerre and Clark, 2005).

An evolutionary advantage of ruminants is their capacity to recycle N (Reynolds and Kristensen, 2008). Under situations such as low N intake the percentage of this nutrient that is recycled back to the gastrointestinal tract (GIT) can be increased from 29 to approximately 83% of the total N intake (Marini and Van Amburgh, 2003). Considering that most animal nutritional models do not consider the recycled N (Prates et al., 2017) and that most Brazilian feedlot nutritionists (86.3%) formulate diets for rumen degradable protein (RDP) content (Pinto and Millen, 2019), it is not a surprise that RDP is being over-fed in most of the feedlot systems. In addition, Oliveira and Pinto (2019) reported in a survey that soybean meal is the most common protein source used in Brazilian feedlots, and often this source is fed in combination with urea which increases the RDP level in feedlot diets. In these types of diets, the excess of

RDP can increase ammonia (NH_3) concentration in the rumen, which in turn, can increase N losses from the rumen with high potential for excretion in urine (Calsamiglia et al., 2010).

The formulation of diets with increasing concentration of rumen undegradable protein (RUP) can be used as a strategy to reduce peptide degradation and amino acid (AA) deamination, thereby, decreasing ruminal NH_3 concentration, although with some negative effect on the supply of N to the rumen microbiome. However, the decrease of ruminal NH_3 concentration increases the amount of N recycled to the rumen, which can buffer the reduction in rumen N availability for MICP synthesis. In this sense the inclusion of corn gluten meal (CGM; high RUP content) or dry distillers grain (DDG; intermediate RUP) could be a strategy to reduce RDP content in feedlot diets. Therefore, we hypothesized that reducing dietary N associated with CGM or DDG (RUP sources) can increase NUE by reducing ruminal NH_3 concentration, urea production in the liver and urinary N excretion, while maintaining N available for MICP synthesis through urea N recycling. Therefore, the objective of this study was to evaluate the effect of protein sources with different RDP content and dietary inclusion levels on ruminal fermentation, urea kinetics and N excretion of feedlot Nellore steers fed high-concentrate diets.

2. MATERIAL AND METHODS

The study was conducted at São Paulo State University (UNESP), Jaboticabal, SP, Brazil. Animal care and handling was in accordance with the Brazilian College of Animal Experimentation Guidelines (COBEA) and were approved by the Ethics, Bioethics, and Animal Welfare Committee of UNESP, Jaboticabal, under Protocol 16.668/16.

2.1. Animals, Experimental Design, and Diets

Six Nellore steers, cannulated in the rumen, duodenum and ileum with initial body weight (BW) of 354 ± 11.8 kg and 18 months of age (average), were randomly assigned to receive each diet once over the 6 periods in a 6 × 6 Latin square design balanced for residual effects. The experiment lasted 132 days and had six experimental periods of 22 days each. Animals were adapted to diets for 14 days (Machado et al., 2016) and 8 days were used for sample collection (Figure 1). In

addition, animals were allocated in individual pens (12 m^2) with concrete floors, feed bunk and water fountain.

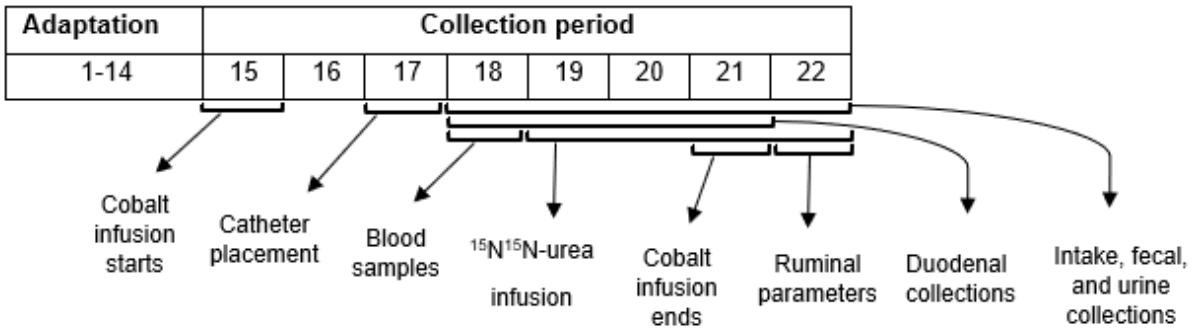

Figure 1. Collection protocol during each experimental period. Numbers from 1 to 22 represent the days of each period.

Before entering the feedlot, the animals were submitted to 16 hours total fasting, were treated to remove parasites (ivermectin, dosage of 200 µg/kg BW), and their initial BW were recorded. Before starting the study, the animals were fed four different adaptation diets (20, 40, 60, and 80% concentrate) with decreasing concentration of fiber (one week/diet). This practice was adopted with the aim of minimizing the deleterious effects of high-concentrate diets on ruminal physiology. At the end of the fourth week of adaptation, the animals were fed a diet containing 80% concentrate, which consisted of soybean meal (30%) and ground corn (70%).

Treatments were arranged in a 3 × 2 factorial, where factor A consisted of 3 protein sources (soybean meal plus urea, corn gluten and dry distillers grains) and factor B consisted of 2 dietary CP levels (11 and 14%). Therefore, the treatments were: 1) Soybean meal + urea (SU) at 14% CP: ground corn, soybean meal, urea, and fresh chopped sugar cane.

2) Corn gluten meal (CGM) at 14% CP: ground corn, corn gluten meal, and fresh chopped sugar cane.

3) Dried distillers grains (DDG) at 14% CP: ground corn, DDG, and fresh chopped sugar cane.

4) Soybean meal + urea (SU) at 11% CP: ground corn, soybean meal, urea, and fresh chopped sugar cane.

5) Corn gluten meal (CGM) at 11% CP: ground corn, corn gluten meal, and fresh chopped sugar cane.

6) Dried distillers grains (DDG) at 11% CP: ground corn, DDG, and fresh chopped sugar cane.

Experimental diets consisted of 80% concentrate and 20% forage (DM basis). Fresh chopped sugar cane was used as the roughage source and the concentrates differentiated on their protein source (soybean meal and urea, CGM, and DDG). Corn gluten meal and DDG were used as RUP sources, with low and intermediate ruminal degradability, respectively, and soybean meal and urea were used as RDP sources. Chemical composition of the ingredients and experimental diets are presented in Tables 1 and 2.

Table 1. Chemical composition of the ingredients.

	Ingredients[1]						
Item[2]	**Sugar cane**	**Ground corn**	**Soybean meal**	**DDG**	**CGM**	**Urea**	**Mineral mix[3]**
DM (%)	25.0	88.5	89.6	91.6	92.2	93.9	99.0
OM (%)	97.3	98.5	93.0	98.1	98.1	99.9	-
CP (%)	3.89	9.51	51.2	34.1	60.0	275.3	-
NDF (%)	48.6	15.7	23.1	55.9	36.9	-	-
EE (%)	1.20	5.25	2.18	6.77	2.87	-	-
Protein fractions (% CP basis)[4]							
RDP (%)	37.0	57.7	59.7	41.7	30.3	100.0	-
RUP (%)	63.0	42.3	40.3	58.3	69.7	-	-
A (%)	14.4	8.12	12.1	2.36	4.21	-	-
B1 (%)	19.2	8.83	1.90	3.57	4.20	-	-
B2 (%)	7.05	75.0	84.3	65.2	91.0	-	-
B3 (%)	8.33	2.12	0.39	7.36	0.32	-	-
C (%)	51.0	5.93	1.41	21.6	0.30	-	-

[1]DDG: Dried distillers grains, CGM: Corn gluten meal. [2]DM: Dry matter; OM, Organic matter, CP: crude protein, NDF: Neutral detergent fiber, EE: Ether extract. [3]Contained per kg of DM: 220 g Ca, 20 g P, 60 g Na, 25 g S, 10 g Mg, 100 mg Co, 500 mg Cu, 50 mg I, 1500 mg Zn, 9 mg Se, 1500 mg Mn, 100.000 IU vitamin A, 50 g sodium bicarbonate. [4]Rumen degradable protein (RDP) and rumen undegradable protein (RUP) content were estimated based on the protein fractions and the degradation rate of each fraction, considering a passage rate of 5%.h^{-1}. Protein fractions were determined based on the procedure standardized by Licitra et al. (1996).

Diets were formulated according to BR Corte (Valadares Filho et al., 2016) to meet an average daily gain (ADG) of 1.25 kg except the CP levels in 11% CP diets (requirements considered an intake (kg/day) of: DM = 8.15, TDN = 5.68, CP = 0.93 for diets with 13.7% CP. For the diets with 10.8% CP, DMI and TDN were maintained constant and the RDP and RUP concentration varied according to the tested protein

source. The roughage: concentrate ratio used was based on the survey of nutritional practices adopted by nutritionists in Brazilian feedlots (Oliveira and Millen, 2014). During the experiment, there was a variation in the chemical composition of the sugar cane, which resulted in change of the initial proposed diet formulation (Table 2).

Table 2. Ingredient and chemical composition of the experimental diets.

	Diets[1]					
	11% CP			14% CP		
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**
Ingredient (% of DM)						
Sugar cane	15.5	17.6	17.6	17.0	15.8	17.5
Ground corn	79.8	74.3	68.7	72.2	69.6	57.0
Soybean meal	1.11	-	-	7.12	-	-
Corn gluten meal	-	5.65	-	-	12.1	-
DDG	-	-	11.3	-	-	23.0
Urea	0.99	-	-	1.24	-	-
Mineral mix	2.57	2.47	2.47	2.51	2.52	2.46
Chemical composition						
Dry matter (%)	77.7	78.2	78.3	77.7	78.9	78.7
Organic matter (%)	95.7	95.8	95.8	95.4	95.7	95.8
Crude protein (%)	11.7	11.1	11.0	14.7	14.5	13.9
RDP (%, CP basis)[2]	66.6	48.0	50.9	67.1	43.1	47.7
RUP (%, CP basis)[3]	33.4	52.0	49.1	32.9	56.9	52.3
NDICP (%, CP basis)[4]	11.7	11.9	14.9	11.6	10.9	17.6
ADICP (%, CP basis)[5]	9.20	9.43	11.7	9.22	8.66	13.7
Neutral detergent fiber (%)	20.3	22.2	25.6	21.1	23.3	30.3
Non-fibrous carbohydrates (%)	59.2	58.3	54.7	55.4	53.5	46.9
Ether extract (%)	4.50	4.21	4.52	4.19	4.36	4.31
Total digestible nutrients (%)[6]	74.3	74.2	72.0	73.2	75.7	72.4

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); [2]Rumen degradable protein; [3]Rumen undegradable protein; [4]Neutral detergent insoluble crude protein; [5]Acid detergent insoluble crude protein; [6]TDN= CPD + 2.25 EED + NFCD + NDFD – 7 (Weiss et al., 1992). CPD = digestible crude protein, EED: digestible ether extract, NFCD: digestible non-fibrous carbohydrates, NDFD: digestible neutral detergent fiber.

Animals were fed twice daily at 0600 and 1600 h. Orts were collected before morning feeding and weights recorded; feeding rate was adjusted daily to yield orts of 5% of intake to avoid selection.

2.2. Intake and digestibility

Feed intake measurements and total feces collection were conducted during five consecutive days, from the 18th to the 22th day of each experimental period. Feces were collected soon after defecation, stored in a bucket, and kept under refrigeration (4ºC) to reduce microbial alteration of the fecal samples. After 24 hours, the feces were individually weighed and a sample of approximately 5-10% of the total weight was

collected. Total collections of feces from day 18 were used to determine background enrichments of ^{15}N, and those from day 22 were used to measure enrichments of ^{15}N for calculating urea kinetics, according to the sampling protocol validated by Wickersham et al. (2008). Samples from each day were stored separately at -20ºC for further analysis. In addition, during the five days of fecal collection, samples from the ingredients and orts of each animal were also collected and stored at -20ºC for further chemical composition analysis.

At the end of each experimental period, samples of feed, orts and feces proportionately on the basis of total fecal output or total orts were pooled, dried at 55°C for 72 h and were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1 and 2-mm screen. One-mm screen samples were analyzed for DM (INCT-CA, # G-003/1), and OM (INCT-CA, nº G-004/1) according to the standardized analytical procedures described by INCT (INCT-CA; Detmann et al., 2012). Total N was analyzed by the DUMAS method using the Leco-FP 528 LC equipment (2013 LECO Corporation, St. Joseph, MI, USA) according to the protocol described by Etheridge et al. (1998) and converted to CP using the factor of 6.25. The NDF was analyzed following the methodology described by Van Soest et al. (1991) and adapted for the ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY) using α-amylase and without sodium sulfite. Samples ground through a 2-mm screen were used for determination of indigestible NDF (iNDF) after in situ incubation for 288 h, as recommended by Valente et al. (2011).

2.3. Urinary parameters

Urine samples were obtained from the total collection, which was performed from the 18th to the 22th day of each experimental period. Total urine was collected using a funnel collector attached to the animal. The funnels were connected to polyethylene hoses that carried the urine to 20 L containers and were acidified with 250 mL of 20% (wt/wt) H_2SO_4 solution to prevent N loss by volatilization (Valadares et al., 1997). After 24 hours of collection, the total excreted volume was measured and homogenization was performed. Then, a 100-mL sample was collected, filtered through three layers of cheesecloth and subsequently stored at -20°C for further analysis. Total collections of urine from day 18 and day 22 were used to measure ^{15}N background and enrichment, respectively, for urea kinetics calculations (Wickersham et al., 2008). After 5 days of collection, urine samples were composited by animal and

period, and total N (DUMAS method) and NH_3-N (Broderick and Kang, 1980) were analyzed. In addition, a urine subsample (5 mL) was collected, and used to determine urea (Marsh et al., 1965) and creatinine concentrations (Chasson et al., 1961) with the use of LABTEST commercial kits.

2.4. Duodenal digesta flow

Markers were used for partial digestibility determinations, where duodenal and ileal DM flows were estimated using indigestible NDF (iNDF) as the internal and solid phase marker and Co-EDTA as the external and liquid phase marker (Úden et al. al., 1980). The Co-EDTA (5 g/animal/day or 0.8 g of Co/day) was continuously infused into the rumen from the 15th to the 21st day of each experimental period using a peristaltic pump (DOSATEC 2400, PROVITEC, São Paulo, SP, Brazil). Then, from the 18th to the 21st day of each experimental period, samples of duodenal digesta (800 mL) were collected at nine-hour intervals at 1030 and 1930 h on day 18, at 0430, 1330, and 2230 h on day 19, at 0730 and 1630 on day 20, and at 0130 h on day 21 (Allen and Linton, 2007). To perform duodenal sampling, plastic bags were held in place with an elastic band at the end of the duodenal cannula, allowing free digesta flow into the collection bags. Immediately after collection, duodenal samples were divided into two: one to estimate the nutrient flow (375 mL) and one for microbial isolation (425 mL). Samples used to estimate nutrient flow were frozen individually after each collection. At the end of each experimental period, samples were thawed and pooled by animal. Samples used for microbial isolation were kept under refrigeration (4 °C), pooled each 24 h and centrifuged. At the end of each sampling period, a subsample of the duodenal digesta used to estimate nutrient flow (2 L) was taken, filtered through a 100 µm nylon filter (44% surface pore area; Sefar Nitex 100/44, Sefar, Thal, Switzerland) for separation of liquid (LP) and solid (SP) phases. The remaining material (1 L) was processed as total or non-representative duodenal digesta. The LP and SP samples were weighed, frozen at -20°C, freeze-dried for 72 h, ground in a Willey mill (Thomas Scientific, Swedesboro, NJ) through 1- and 2-mm screens, and stored for further analysis.

Bacterial isolation of the duodenal digesta was carried out following methodology described by Reynal et al. (2005) with adaptations suggested by Krizsan et al. (2010) to estimate MICP flow. Digesta samples (850 mL) were filtered on a 100 µm nylon filter (44% surface pore area; Sefar Nitex 100/44, Sefar, Thal, Switzerland) and the retained material was washed with 800 mL of 0.9% (wt/vol) NaCl. The retained

material was then stored for further isolation of the particle associated bacteria (PAB). Then, the liquid associated bacteria (LAB) were isolated from the filtered liquid sample. After centrifugation (1,000 × g for 10 minutes at 5 °C) the pellet was stored for PAB isolation. The supernatant was centrifuged at 11,250 × g for 30 minutes at 5°C. Supernatant was discarded and 200 mL of McDougall's solution was added the pellet. Then, samples were centrifuged at 16,500 × g for 20 minutes at 5 °C and the resulting pellet composed of LAB was frozen (-80 °C), freeze-dried for 72 h and subsequently ground to through a 1-mm screen for further analysis. For PAB isolation, 700 mL of saline solution containing 1% Tween-80 (v/v) was added to the pots containing the pellet from the first centrifugation of LAB isolation and the solid phase retained in the nylon filter, homogenized for 30 seconds and stored in a refrigerator (4°C) overnight to detach the bacteria attached to particles. Subsequently, the samples were filtered through a nylon filter, the liquid obtained centrifuged (1,000 × g for 10 minutes at 5°C), the supernatant obtained centrifuged (11,250 × g; 30 minutes at 5°C); and to the pellet resulting from this centrifugation 200 mL of McDougall buffer solution was added prior to centrifugation again at 16,250 x g for 20 minutes at 5 °C. The pellet (BAP) obtained was frozen (-80°C) and lyophilized (72-h).

2.5. $^{15}N^{15}N$-urea infusion

On day 17 of each period a catheter was placed in the right jugular vein of all steers for $^{15}N^{15}N$-urea infusion (Cambridge Isotope Laboratories, Andover, MA, USA, 98% purity), which was used as an external marker for assessing urea kinetics and estimating duodenal MICP flow. The catheter (BD Angiotech®, 14-gauge, 133 mm; Becton Dickison, Sandy, Utah) was inserted into the vein by percutaneous venipuncture after skin disinfection using a 10% iodine solution. After catheter placement, 10 mL saline solution (0.9% NaCl and 10 IU heparin / mL) was infused every 6 h, to avoid patency loss, until 0600 h on day 19 at which time infusion of $^{15}N^{15}N$-urea started. Before the beginning of the marker infusion, 410 mL of a solution containing 2.6 g of $^{15}N^{15}N$-urea / L, required for 82 h of infusion/animal/period, were produced. The solution was prepared under sterile techniques in a laminar flow chamber, it was filtered on a bacteriological filter (0.22 µm; Millipore Corporation, Billeric, MA, USA) in a sterilized glass vessel, sealed with a sterile rubber septum and stored at 4 °C until the infusion. From the day 19 (0600 h) to 22 (1600 h), the $^{15}N^{15}N$-urea solution was continuously infused at a rate of 5.00 mL/h, allowing the delivery of

0.610 mmol N-urea/h through an infusion pump (BS-9000 Multi-Phaser, Braintree Scientific Inc., Braintree, MA). On day 22 of each experimental period, ruminal and duodenal digesta samples (200 mL/each) were collected at 0, 2, 4, 6, 8 and 10 hours after morning feeding (0600 h) for determinations of ^{15}N enrichment of MICP. The schedule used for sampling was conducted according to Titgemeyer et al. (2012) and corresponding to 72 to 82 h of infusion, a period in which the ^{15}N isotopic enrichment reaches a plateau (Wickersham et al., 2009a). Samples were frozen at -80°C and subsequently freeze-dried for 72 h.

Fecal samples from day 18 and 22, duodenal digesta from day 22, pooled ruminal bacteria from day 22 were analyzed for ^{15}N isotopic enrichment as described by Rotta et al. (2014). Briefly, ^{15}N atom excess was analyzed in an isotope ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany). Approximately 4 mg of sample was weighed and placed in 5 × 8 mm capsules. The ratio of stable N isotopes (^{15}N:^{14}N) was analyzed according to international standards as Δ / thousand and was converted to percentages of atoms in excess. Urinary urea and ammonia concentrations were quantified colorimetrically as described before. Measurement of [$^{15}N^{15}N$]-, [$^{14}N^{15}N$]-, and [$^{14}N^{14}N$]-urea enrichments in urinary urea was conducted on N2 samples produced from Hoffman degradation of urinary urea by using techniques similar to those described by Wickersham et al. (2009b), except 1) 250 µmol of urea was pipetted into a column; and 2) the procedures of column washing were conducted according to Archibeque et al. (2001). Samples were analyzed for the proportions of [$^{15}N^{15}N$]-, [$^{14}N^{15}N$]-, and [$^{14}N^{14}N$]-urea in urinary urea by IRMS (Thermo Finnigan Delta Plus, Thermo Electron Corporation, Waltham, MA, ^{15}N Analysis Laboratory, University of Illinois, Urbana, IL). Results were corrected for [$^{14}N^{15}N$]- N_2 produced by nonmonomolecular reactions (Lobley et al., 2000).

2.6. Ruminal parameters

On day 22 of each experimental period, ruminal fluid samples were collected at 0, 2, 4, 6, 8 and 10 h after morning feeding. Ruminal contents (500 mL) were manually collected through the cannula, filtered through two layers of cheesecloth and 50 mL of ruminal fluid was immediately used for pH determination (pHmeter Q400MT, Quimis, São Paulo, Brazil). Then, 40 mL was transferred to a plastic container and stored at -20 °C for further determination of NH_3-N concentration (Fenner, 1965). An 8 mL aliquot was combined with 2 mL of 25% (wt/vol) metaphosphoric acid and stored at -20 °C for

further determination of short chain fatty acids (SCFA) concentration (Leventini et al., 1990). The remaining fluid and solids were used to isolate rumen bacteria by centrifugation processes as described by Cecava et al. (1990).

2.7. Blood Parameters

On day 18 of each experimental period, blood samples (10 mL) were collected from the jugular vein of the animals through the catheter at 0, 6, 12 and 18 h after morning feeding. Then, blood samples were placed in tubes containing heparin (143 IU) and centrifuged (1000 × g for 20 minutes at 4°C) for plasma separation. After centrifugation, plasma was transferred to microtubes (Eppendorf) and frozen at -20°C until further analysis. Glucose, urea and creatinine concentrations were determined by commercial quantitative colorimetric kit (LABTEST, LABQTEST, São Paulo, Brazil) and spectrophotometer (Model Bio 2000, LABQTEST, São Paulo, Brazil). Insulin concentration was determined by ELISA (Multiscan MS; Labsystems, Vantaa, Finland) based on antibody–antigen interactions using commercial kits (Accubind; Monobind, Inc., Lake Forest, CA; code 2425-300).

2.8. Calculations

Digesta flow was calculated based on a single internal marker (iNDF) and the reconstitution technique was conducted according to Faichney (1975) by using a combination of 2 markers (Co-EDTA as the fluid phase marker and iNDF as the solid phase marker). The reconstitution factor was calculated based on the marker's concentrations in the different digesta phases (France and Siddons, 1986). The reconstitution factor of the solid phase was used to mathematically reconstruct the nutrient composition of the true duodenal digesta. Ruminal N balance was calculated as duodenal N flow minus N intake. Microbial protein flow was calculated by multiplying duodenal N flow by the ratio between duodenal and bacterial ^{15}N enrichment. The MICP derived from recycled urea N was calculated by multiplying MICP by the ratio of bacterial ^{15}N enrichment to ^{15}N enrichment of urinary urea (calculated as one-half the $^{14}N^{15}N$-urea enrichment plus the $^{15}N^{15}N$-urea enrichment). Urea kinetics were calculated according to the methods described by Lobley et al. (2000). The efficiency of N utilization by ruminal microorganisms (ENUR) was calculated by dividing the production of rumen microbial N (g/day) by the amount of available N as proposed by

Bach et al. (2005); available N compromised RDP intake (g N/day) plus the amount of urea recycled to GIT (GER, g/day).

2.9. Statistical analysis

Data were subjected to least squares ANOVA using the MIXED procedure of SAS (Statistical Analysis System, version 9.4 for Windows) as a 6 × 6 Latin square design (balanced for residual effects) with a 3 × 2 factorial arrangement of treatments. Factor A consisted of the three CP sources (PS; soybean meal plus urea, corn gluten meal and DDG) and factor B consisted of the two CP levels (PL; 11 and 14%) and. The model used was as follows:

$$Y_{ijkl} = \mu + S_i + L_j + S_i \times L_j + S_i \times L_j + A_k + P_l + \varepsilon_{ijkl}$$

where: Y_{ijkl} = dependent variable, μ = overall mean; S_i = fixed effect of protein source; L_j = fixed effect of protein level; $S_i \times L_j$ = interaction between protein source and level; A_k = random effect of animal; P_l = random effect of experimental period; and ε_{ijkl} = random error, assumption of normal distribution.

The results were evaluated for homoscedasticity of variances and normality of the data. In cases where the variances were identified as heterogeneous, the ANOVA was performed considering heterogenous variances by using the command REPEATED/GROUP. The averages of the treatments were estimated using the LSMEANS and compared using the Tukey test. Least squares means and SEM are reported for all data with a significance set at $P \leq 0.05$, and tendency at $0.05 < P \leq 0.10$. For the variables analyzed over time, repeated measurement was used and the fixed effect of time and its interaction with treatments (PL and PS) were added to the model. Covariance matrix used for ANOVA with repeated measures was that of corresponding to the lowest corrected Akaike Information Criterion (AICc). Outliers were removed when studentized residuals were > than 3 or < than -3.

3. RESULTS

There were no interactions ($P > 0.05$) between PS and PL for nutrient intakes and digestibilities (Table 3). Animals fed diets with CP inclusion level of 11% had greater ($P < 0.05$) NFC intakes than those fed diets with 14% CP (3.35 and 2.57 kg/day, respectively) and tended ($P < 0.10$) to have greater DM (%BW and kg/day), OM and TDN intake. Steers fed SU diets had greater ($P < 0.05$) RDP intake compared to those

fed CGM or DDG diets (0.50, 0.29 and 0.34 kg/day, respectively). Animals fed CGM or DDG had greater ($P < 0.05$) RUP intake compared to those fed SU diets (0.35, 0.35 and 0.24 kg/day, respectively). Animals fed DDG diets presented a greater ($P < 0.05$) NDF intake compared to those fed SU or CGM diets (1.53, 1.18 and 1.13 kg/day, respectively). In addition, animals fed DDG tended ($P = 0.10$) to have greater NDF apparent total-tract digestibility compared to those fed SU diets (54.1 and 42.4 %, respectively). Diets with CP inclusion level of 14% tended ($P < 0.10$) to lead to greater NFC total-tract digestibility than diets with 11% CP (96.0 and 94.6, respectively).

Table 3. Effects of protein sources and inclusion levels on intake and apparent digestibility of nutrients of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* - value		
	11% CP			14% CP			SEM	PS	PL	PS × PL
Item[2]	SU	CGM	DDG	SU	CGM	DDG				
DM intake, % BW	1.57	1.39	1.72	1.40	1.30	1.31	0.060	0.416	0.061	0.490
Intake, kg/day										
DM	5.96	5.25	6.32	5.41	4.63	4.78	0.26	0.416	0.078	0.644
OM	5.70	5.03	6.05	5.17	4.42	4.57	0.25	0.415	0.075	0.646
CP	0.70	0.57	0.70	0.79	0.68	0.64	0.03	0.284	0.430	0.488
RDP	0.47	0.28	0.36	0.53	0.29	0.31	0.02	0.001	0.714	0.475
RUP	0.23	0.30	0.34	0.26	0.39	0.35	0.02	0.012	0.139	0.507
NDF	1.20	1.15	1.61	1.16	1.10	1.44	0.07	0.023	0.459	0.858
NFC	3.51	3.09	3.46	2.98	2.44	2.28	0.16	0.304	0.008	0.557
EE	0.27	0.23	0.29	0.26	0.21	0.23	0.01	0.262	0.235	0.662
TDN	4.37	3.87	4.59	3.87	3.48	3.52	0.17	0.506	0.065	0.665
Apparent total-tract digestibility, %										
DM	79.1	80.2	79.2	79.3	79.7	78.2	0.66	0.769	0.754	0.936
OM	79.6	80.2	78.6	80.3	80.6	78.9	0.68	0.640	0.749	0.995
CP	70.2	68.0	66.7	73.1	72.1	69.9	1.05	0.450	0.132	0.972
NDF	42.1	49.8	53.7	42.8	50.2	54.6	2.20	0.100	0.876	0.999
NFC	94.2	96.1	93.6	95.6	96.1	96.2	0.38	0.282	0.059	0.275
EE	86.9	83.6	82.0	84.6	84.3	81.0	0.97	0.231	0.648	0.814
Ruminal digestibility, %										
DM	48.0	54.9	55.0	46.0	57.7	45.7	2.22	0.163	0.449	0.427
OM	54.1	60.8	58.7	54.6	62.8	51.6	2.10	0.246	0.689	0.593
CP[3]	-7.85	-13.3	-17.1	-9.97	-4.16	-33.2	5.91	0.237	0.733	0.490
NDF	38.8	40.1	53.0	43.9	49.2	42.3	3.14	0.618	0.677	0.193
NFC	68.6	76.1	70.0	78.8	71.9	73.7	2.28	0.924	0.502	0.489

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between PS and PL.[2]BW: body weight, DM: dry matter, OM: organic matter, CP: crude protein, RDP: rumen degradable protein, RUP: rumen undegradable protein, NDF: neutral detergent fiber, EE: ether extract, NFC: Non-fibrous Carbohydrates, TDN: total digestible nutrients. [3]Data from 1 animal from the treatment SU with 14% was removed due to problems not related to the treatments.

There were no interaction ($P > 0.05$) among protein level, source and time for ruminal pH and NH_3-N concentration (Table 4). Animals fed SU diets had a greater ($P < 0.05$) ruminal NH_3-N concentration than those fed CGM or DDG diets (20.6, 16.2 and 12.7 mg/dL, respectively). When analyzed over time, animals fed SU diets had a greater ($P < 0.05$) ruminal NH_3-N concentration than those fed CGM or DDG diets at 2 and 4 h after feeding. However, at 8 and 10 h after feeding SU treatment differed ($P < 0.05$) only from DDG treatment (Table 4 and Figure 1). Animals fed diets with CP inclusion level of 14% had greater ($P = 0.049$) ruminal NH_3-N concentration than those fed diets with 11% CP (18.3 and 14.7 mg/dL, respectively), but differed only at 6 h after feeding (Figure 2). Animals fed diets with 14% CP had greater ($P = 0.016$) ruminal pH compared to those fed 11% CP diets (6.80 and 6.43, respectively; Table 4 and Figure 3).

Protein levels and sources did not affect ($P > 0.05$) total SCFA concentrations (Table 4). However, there was a tendency ($P < 0.10$) for greater proportions of acetate in animals fed DDG diets compared CGM diets (64.2 and 59.3 % of total VFA, respectively). Isobutyrate proportion tended ($P < 0.10$) to be greater as dietary protein levels increased with 0.856 and 1.12% of total VFA for diets with 11 and 14% CP, respectively. In addition, diets containing CGM or DDG tended ($P < 0.10$) to lead to greater valerate proportion compared to SU diets (1.35, 1.45 and 1.03 % of total VFA, respectively).

Table 4. Effects of protein sources and inclusion levels on ruminal fermentation of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* – value			
	11% CP			14% CP			SEM	PS	PL	PL × PS	PL×PS×T
Item[2]	SU	CGM	DDG	SU	CGM	DDG					
pH	6.57	6.40	6.32	6.70	6.94	6.76	0.04	0.729	0.016	0.446	0.129
NH_3-N, mg/dL	18.4	13.6	12.0	22.7	18.8	13.4	0.57	0.007	0.049	0.634	0.377
Total SCFA, *mmol/L*	54.0	51.2	64.2	58.6	38.8	53.8	1.78	0.214	0.378	0.526	0.505
SCFA, % of total SCFA											
Acetate	62.7	58.2	63.4	62.4	60.3	65.0	0.42	0.080	0.508	0.823	0.798
Propionate	20.5	22.8	20.0	18.9	20.6	20.3	0.33	0.576	0.495	0.813	0.690
Butyrate	12.0	14.1	12.7	13.1	13.6	10.7	0.30	0.288	0.681	0.530	0.574
Isobutyrate	0.848	0.895	0.825	1.15	1.17	1.05	0.03	0.867	0.083	0.977	0.337
Valerate	0.917	1.63	1.61	1.14	1.07	1.30	0.04	0.085	0.158	0.106	0.231
Isovalerate	2.47	2.33	2.05	2.33	3.27	2.06	0.07	0.234	0.432	0.373	0.384
Acetate:propionate	3.21	2.78	3.20	3.42	3.35	3.18	0.07	0.781	0.397	0.712	0.599

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between PS and PL. PS **×** PL **×** T: interaction between PS, PL and time. [2]SCFA: short chain fatty acids.

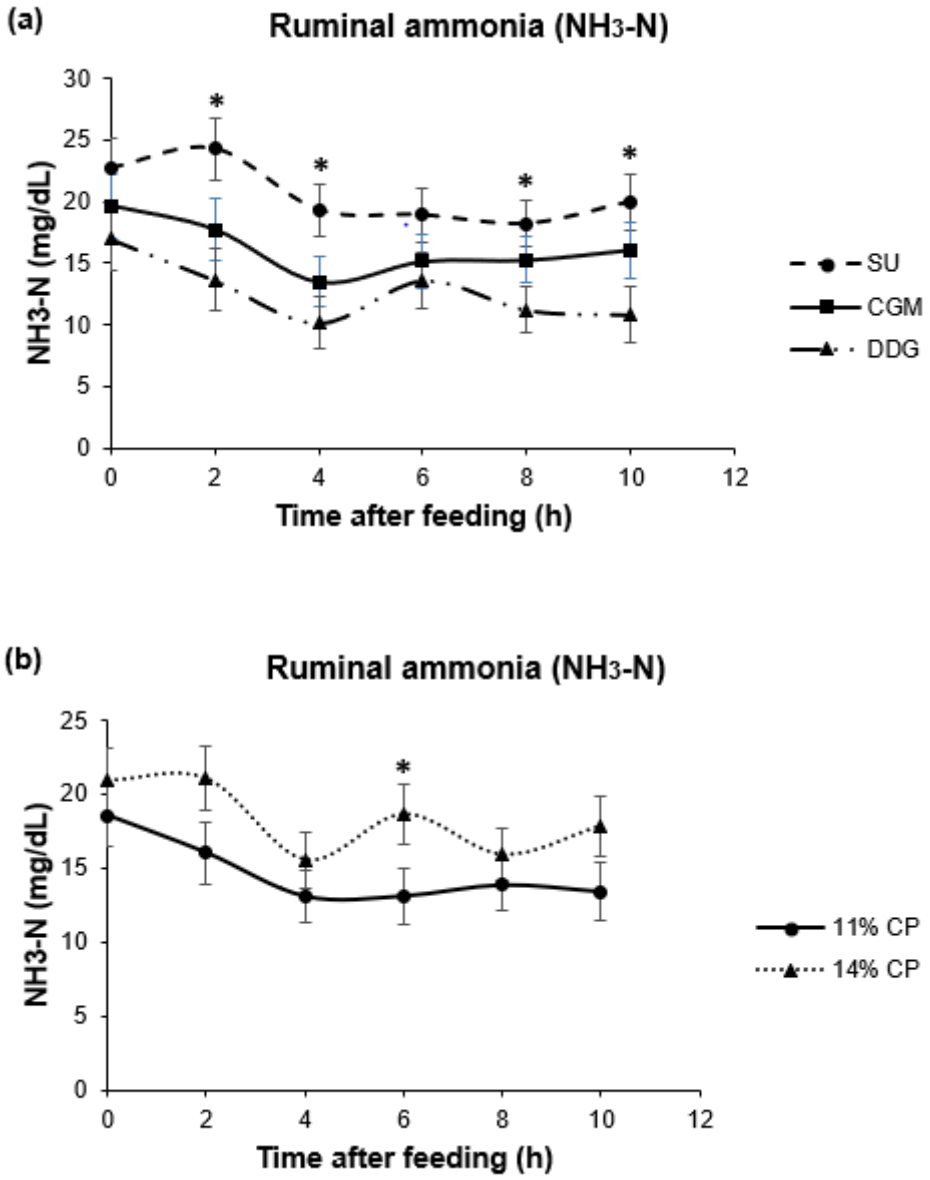

Figure 2. Effects of protein sources (a) and inclusion levels (b) on NH_3-N concentrations of feedlot Nellore cattle fed high-concentrate diets. Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); Time effect ($P < 0.0001$); Quadratic effect ($P = 0.005$). *Comparison of CP levels and sources within each time ($P < 0.05$).

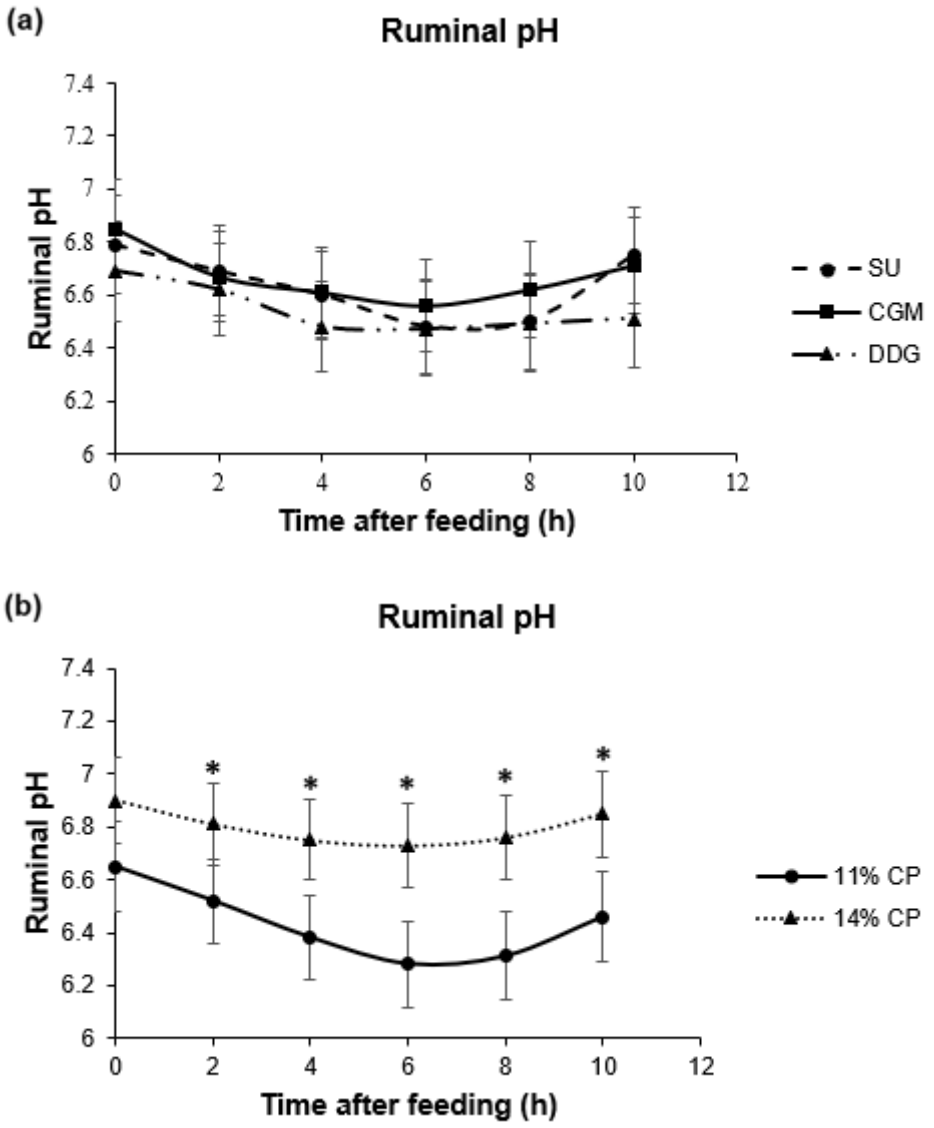

Figure 3. Effects of protein sources and inclusion levels on ruminal pH in feedlot Nellore cattle fed high-concentrate diets. Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); Protein level effect ($P = 0.016$); Time effect ($P < 0.0001$); Quadratic effect ($P < 0.0001$). *Comparison of CP levels within each time ($P < 0.05$).

Fecal N excretion (g/day) did not differ across treatments ($P > 0.05$, Table 5). However, when expressed as % of total N output, animals fed diets containing DDG had a greater ($P < 0.05$) fecal N excretion compared to those fed SU diets (54.6 and 42.1% of total N output, respectively), and a tendency ($P < 0.10$) for greater fecal N excretion was observed for 11% CP diets compared to the diets containing 14% CP (52.1 and 45.7% of total N output). Fecal NIDN was not affected by PS or PL ($P > 0.05$) when expressed as g N/day or % of fecal N. Fecal NDIN (g/day) had heterogeneous variance and CGM diets had a lower variance (95% confidence interval) compared to SU and DDG diets (7.54, 16.5 and 19.2,

respectively). In addition, diets with CP inclusion level of 14% had greater variance greater (95% confidence interval) than diets with 11% CP (19.1 and 9.84%, respectively). When expressed as % of total output diets containing 11% CP had greater fecal NIDN excretion compared to the diets containing 14% CP (13.9 and 10.7 % of total N output, respectively). NIDN expressed as % of total N output had heterogeneous variance and CGM diets had a lower variance (95% confidence interval) compared to SU and DDG diets (8.61, 17.6 and 29.1, respectively). In addition, diets with CP inclusion level of 14% had greater (95% confidence interval) variance than diets with 11% CP (22.0 and 11.9%, respectively). Fecal ADIN (g/day) tended to be greater ($P < 0.10$) in animals fed DDG diets compared to those fed SU and CGM diets (4.22, 3.33 and 3.01 g/day; Table 5). When expressed as % of fecal N, fecal ADIN was greater ($P < 0.05$) for animals fed DDG diets compared to those fed SU diets (12.6 and 10.2% of fecal N, respectively), and as % of total N output, fecal ADIN was greater ($P < 0.05$) for animals fed DDG diets compared to those SU and CGM diets (6.88, 4.42 and 5.18% of total N output, respectively; Table 5). There was an effect ($P < 0.001$) of PS and tendency ($P < 0.10$) of PL on urinary N excretion (g/day; Table 5). Animals fed SU diets had greater ($P < 0.05$) urinary N excretion compared to those fed CGM or DDG diets (46.8, 28.8 and 27.0 g/day, respectively). In addition, animals fed SU diets also had greater ($P < 0.05$) urinary N excretion expressed as a percentage of N intake (40.0, 30.1 and 27.3% of N intake, respectively) and % total N output (57.9, 50.0 and 45.4% of total N output, respectively) compared to those fed CGM or DDG diets. Diets with CP inclusion level of 14% tended ($P < 0.10$) to lead to greater urinary N excretion expressed as % of total N output compared to the diets containing 11% CP (54.2 and 47.9% of total N output, respectively). Urinary urea N excretion was greater ($P < 0.05$) for animal fed SU diets compared to those fed CGM or DDG diets (38.5, 22.4 and 18.3 g/day, respectively). In addition, diets with CP inclusion level of 14% also had greater ($P < 0.05$) urinary urea N excretion compared to the diets containing 11% CP (31.2 and 21.6 g/day, respectively). Urinary urea N excretion expressed as % of urine N was greater in SU and CGM diets compared to DDG diets (83.2, 78.5 and 69.2% of urine N, respectively). In addition, diets with CP inclusion level of 14% had greater urinary urea N excretion expressed as % of urine N than diets with 11% CP (82.2 and 71.7% of urine N, respectively). Nitrogen use efficiency

was decreased ($P < 0.05$) when animals were fed SU diets compared to those fed CGM or DDG diets (31.8, 40.3 and 41.2%, respectively; Table 5).

Duodenal N flow, ruminal N balance, microbial N flow and efficiency, and ENUR were not ($P > 0.05$) affected by treatments (Table 6).

Table 5. Effects of protein sources and inclusion levels on N intake, route, form of excretion, and retention of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* - value		
	11% CP			14%CP			SEM			
Item	SU	CGM	DDG	SU	CGM	DDG		PS	PL	PL × PS
N intake, g/day	112.0	91.9	111.3	127.0	109.0	102.5	5.16	0.283	0.430	0.487
N excretion										
Fecal N, g/day	33.7	30.3	37.0	33.5	30.7	30.0	1.95	0.752	0.561	0.680
Fecal N, % of N intake	29.8	32.0	33.3	26.9	27.9	30.1	1.05	0.450	0.132	0.972
Fecal N, % total N output	43.8	51.2	61.3	40.3	48.9	48.0	2.07	0.026	0.078	0.371
Fecal NDIN, g N/day[2]	9.40	7.38	10.3	9.03	6.15	6.73	0.70	0.218	0.137	0.468
Fecal NDIN, % fecal N	27.0	23.3	27.0	26.0	24.3	21.4	0.87	0.185	0.157	0.126
Fecal NDIN, % total N output	12.5	12.2	16.7	10.5	11.0	10.4	0.79	0.281	0.017	0.192
Fecal ADIN, g N/d[3]	3.63	3.19	4.40	3.04	2.83	4.04	0.21	0.071	0.293	0.963
Fecal ADIN, % fecal N	11.1	10.8	11.9	9.33	11.1	13.3	0.37	0.025	0.970	0.151
Fecal ADIN, % total N output	5.02	5.44	7.30	3.81	4.93	6.45	0.30	0.003	0.104	0.847
Urine N, g/day	41.8	27.2	22.3	51.8	30.4	31.7	2.45	<0.001	0.055	0.697
Urine N, % of N intake	39.3	30.5	21.2	40.7	29.6	33.4	2.00	0.011	0.191	0.219
Urine N, % total N output	56.2	48.8	38.7	59.7	51.1	52.0	2.07	0.026	0.078	0.371
Urinary urea N, g/day	30.5	19.4	14.9	46.4	25.4	21.7	2.43	<0.001	0.013	0.421
Urea N, % of urine N	75.2	71.6	68.3	91.2	85.3	70.2	2.53	0.010	0.004	0.197
Urinary ammonia N, g/day	3.90	2.21	1.68	3.49	3.56	3.51	0.35	0.424	0.200	0.399
Ammonia N, % of urine N	8.64	7.99	7.90	6.51	11.9	11.9	0.80	0.341	0.208	0.180
N retention, g/day	36.7	35.3	51.3	41.2	47.7	40.0	3.26	0.661	0.756	0.281
NUE, %[4]	31.4	37.5	45.7	32.2	43.1	36.6	1.78	0.030	0.751	0.129

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between PS and PL. [2]Neutral detergent insoluble nitrogen; [3]Acid detergent insoluble nitrogen; [4]Nitrogen use efficiency.

Table 6. Effects of protein sources and inclusion levels on N flow and microbial efficiency of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* - value		
	11% CP			14% CP			SEM	PS	PL	PL × PS
Item	SU	CGM	DDG	SU	CGM	DDG				
Duodenal flow, g/day										
Total N	129.9	103.4	133.5	142.9	135.4	134.1	8.75	0.442	0.542	0.416
Ruminal N balance	-13.2	-13.2	-18.5	-17.5	-9.47	-33.7	6.06	0.397	0.569	0.685
Microbial[2]	89.3	70.1	97.7	100.1	83.8	98.0	6.93	0.539	0.610	0.939
Microbial efficiency[3]	20.1	19.7	22.1	28.1	21.6	26.6	1.83	0.762	0.297	0.853
ENUR[4]	63.4	78.3	83.3	61.6	71.6	95.2	6.76	0.365	0.941	0.874

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried Distillers Grains (DDG); SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between PS and PL.[2]Microbial N flow was determined from ^{15}N-^{15}N urea marker. [3]Expressed as g N microbial/kg TDN. [4]ENUR: Efficiency of N utilization by ruminal microbes as proposed by Bach et al. (2005).

Urea-N entry rate was affected ($P < 0.05$) by PS and tended ($P < 0.10$) to be affected by PL (Table 7). Animals fed SU diets had greater ($P < 0.05$) UER compared to those fed CGM or DDG (107.4, 76.1 and 69.7 g/day, respectively). Also increasing PL from 11 to 14% CP tended ($P < 0.10$) to lead to greater UER (76.2 and 92.6 g/day, respectively). Animals fed SU diets tended ($P < 0.10$) to have greater gastrointestinal entry rate (GER) compared to those fed CGM or DDG diets (73.7, 55.6 and 52.4 g/day, respectively). Animals fed SU diets had greater ($P < 0.05$) urea returned to ornithine cycle (ROC) compared to those fed CGM or DDG diets (49.9, 36.6 and 33.6 g/day). The urea used for anabolism (UUA) was not affected by treatments ($P > 0.05$). When predicted by the equation developed by the Beef Cattle Nutrient Requirements Model (BCNRM) the UUA was greater ($P < 0.05$) in animals fed 11% CP diets compared to those fed diets containing 14% CP diets (25.9 and 20.3 g/day, respectively). In addition, the predicted UUA was greater ($P < 0.05$) than the measured UUA (23.2 and 12.0 g/day, respectively). The ROC expressed as a proportion of UER was greater ($P < 0.05$) with a CP inclusion level of 11% compared to the diets containing 14% CP (50.2 and 45.4%, respectively). The UUA as a proportion of GER was greater ($P < 0.05$) when animals were fed CGM or DDG diets compared to SU diets (23.0, 27.9 and 11.3%, respectively). The UFE as a proportion of GER tended ($P < 0.10$) to be greater in the animals fed SU diets compared to those fed CGM or DDG diets (15.0, 10.4 and 8.94%, respectively). The ruminal microbial capture of recycled N was affected ($P < 0.05$) by PS and PL (Table 7). The proportion MICP synthesis (% of total microbial N) from urea recycling was greater ($P < 0.05$) for animals fed CGM compared to those fed SU diets (18.5 and 12.3% of total microbial N, respectively). In addition, animals fed diets with CP inclusion level of 11% CP had greater MICP synthesis from urea recycling compared to the diets containing 14% CP (17.5 and 13.7%, respectively). When MICP synthesis from urea recycling was expressed as a proportion of UER and GER, animals fed DDG had the greater values (26.6 and 31.8%, respectively; $P < 0.05$). Animals fed diets with CP inclusion level of 11% CP had greater MICP synthesis from recycled urea when expressed as a proportion of UER compared to those fed diets containing 14% CP (21.2 and 14.4%, respectively; $P < 0.05$). Animals fed CGM or DDG sources and also those with lower CP inclusion level had greater bacterial ^{15}N enrichment ($P < 0.05$).

Table 7. Effects of protein sources and inclusion levels on urea kinetics and ruminal microbial capture of urea-N of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* - value		
	11% CP			14% CP			SEM	PS	PL	PL × PS
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**				
Urea kinetics, g of N/day										
Urea-N entry rate (UER)	96.8	68.2	63.5	118.1	84.1	75.8	5.24	0.007	0.066	0.904
Gastrointestinal entry rate (GER)	69.9	51.3	48.0	77.6	59.8	56.8	4.10	0.082	0.278	0.998
Returned to ornithine cycle (ROC)	48.9	34.1	30.7	51.0	39.1	36.4	2.55	0.022	0.332	0.933
Urea-N utilized for anabolism (UUA)	11.1	12.5	12.9	14.2	13.4	16.3	1.55	0.892	0.476	0.944
Predicted UUA[2,3]	26.3	22.2	29.2	23.2	19.6	18.1	1.44	0.425	0.045	0.354
Urea-N excreted in feces (UFE)	9.80	4.76	6.54	8.29	7.16	4.79	0.803	0.224	0.859	0.478
Fractional urea kinetics, %										
UUE[4]/UER (u)	30.1	25.8	20.1	33.9	29.6	27.3	1.56	0.124	0.122	0.865
GER/UER	69.9	74.2	79.9	66.2	70.4	72.7	1.56	0.124	0.122	0.865
ROC/UER (ρ)	50.0	49.6	50.9	43.9	46.5	45.8	0.844	0.736	0.007	0.687
ROC/GER (r)	72.5	66.8	63.7	66.4	66.4	63.9	1.34	0.231	0.408	0.546
UUA/GER (a)	12.9	23.7	28.1	9.75	22.3	27.8	1.89	0.003	0.585	0.934
UFE/GER (f)	14.7	9.50	8.86	15.4	11.3	9.01	1.12	0.093	0.679	0.943
Ruminal microbial capture of recycled N										
g of N/day	13.5	16.4	18.5	6.25	14.1	16.9	1.79	0.160	0.239	0.709
% of total microbial N	15.6	20.6	16.4	9.10	16.5	15.4	1.12	0.019	0.034	0.455
% of UER	14.9	16.1	32.7	6.35	16.5	20.4	1.97	0.001	0.018	0.141
% of GER	20.0	22.8	35.2	8.45	24.3	28.4	2.54	0.013	0.172	0.413
Bacterial ^{15}N enrichment[5]	0.035	0.065	0.054	0.016	0.042	0.050	0.004	<0.001	0.003	0.178

[a–b]Least squares means within the same row with different superscripts differ ($P \leq 0.05$). [1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between PS and PL.[2]UUA predicted by the mechanistic level of solution (MLS) of BCNRM (2016). [3]Measured UUA and predicted UUA differed ($P < 0.01$). [4]UUE: Urinary urea-N elimination. Data for UUE are presented in table 5. [5]Reported in atom percent excess.

Plasma glucose levels were affected ($P < 0.05$) by time and presented a quadratic behavior with significant increase 6 h after morning feeding in all treatments (Figure 1). Insulin concentration was not affected by time nor treatments ($P > 0.05$). Plasma urea-N (PUN) was affected ($P < 0.05$) by PS and PL. Diets with CP inclusion level of 11% led to lower ($P < 0.05$) PUN concentrations than diets with 14% CP (Table 8). In regards to protein sources, PUN concentrations were greater ($P < 0.05$) for SU diets compared to CGM and DDG diets. Plasma creatinine concentration was not affected ($P > 0.05$) by PS or PL. There was interaction between PS and PL for plasma urea N filtration ($P < 0.05$). Increasing protein level (14% CP) led to greater plasma ($P < 0.05$) urea N filtration in CGM and DDG diets, whereas SU diets were not affected ($P > 0.05$) by the increment in PL.

Fractional excretion of urea showed interaction between PS and PL ($P < 0.05$). When animals were fed a diet with an inclusion CP level of 11% the fractional excretion of urea was greater in the CGM diets. However, when animals were fed a diet with an inclusion CP level of 14%, the greatest fractional excretion of urea was observed in the SU diets compare with CGM or DDG diets. There was an interaction between PS and PL on plasma urea N reabsorption ($P < 0.05$). When animals were fed a diet with an inclusion CP level of 11% plasma urea N reabsorption was lower in the CGM diets. However, when animals were fed a diet with an inclusion CP level of 14%, the lowest plasma urea N reabsorption was observed in the SU diets compare with CGM or DDG diets. Renal Urea-N clearance did not differ ($P > 0.05$) across treatments.

Table 8. Effects of protein sources and inclusion levels on renal function and blood parameters of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]						SEM	*P - value*		
	11% CP			14% CP						
Item	SU	CGM	DDG	SU	CGM	DDG		PS	PL	PL × PS
Plasma glucose, mg/dL	80.7	85.2	83.0	81.6	81.2	83.9	0.95	0.553	0.694	0.491
Plasma insulin, µg/dL	5.12	3.30	2.29	2.46	3.70	2.84	0.25	0.494	0.487	0.206
Plasma urea, mg/dL	21.2	12.0	12.5	24.6	21.1	18.2	0.69	0.003	0.002	0.369
Plasma creatinine, mg/dL	1.50	1.44	1.32	1.43	1.30	1.40	0.02	0.232	0.407	0.274
Plasma urea N filtration, g/day	68.5a	28.4b	37.9b	53.7a	66.6a	55.6a	3.98	0.034	0.007	0.001
Fractional urea-N excretion, % of urea N filtered	44.3c	65.3a	44.9bc	59.1ab	41.0c	41.1c	2.60	0.161	0.301	0.005
Reabsorption of plasma urea-N, % of urea N filtered	55.7a	34.7c	55.1ab	40.9bc	59.0a	58.9a	2.60	0.161	0.301	0.005
Renal urea-N clearance L/day	301	384	278	334	273	249	15.9	0.222	0.263	0.185

[a-c]Least squares means within the same row with different superscripts differ ($P \leq 0.05$). [1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between PS and PL.

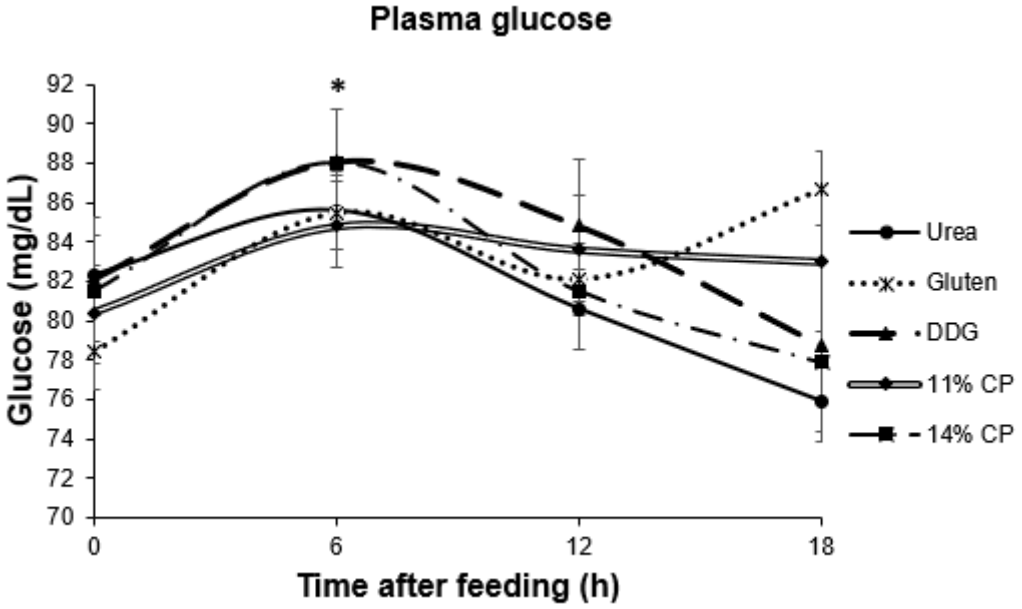

Figure 3. Effects of protein sources and inclusion levels on plasma glucose concentration (mg/dL) in Nellore steers receiving high-concentrate diets. *Time effect (P = 0.008). Quadratic effect (P = 0.024).

4. DISCUSSION

Results indicated that the decrease in CP inclusion level in diets containing RUP sources did not affect MIPC synthesis, however, they increased NUE. These findings can be explained by greater in the use of recycled urea for MICP synthesis in the treatments containing RUP sources (CGM and DDG) and by the reduction of urinary N excretion. In a similar study Brake et al. (2010) evaluated the effects of supplementing N as distillers dried grains with solubles (DDGS) or urea to steers consuming corn-based diets and observed no effects on MICP synthesis by the use of DDGS compared to urea. The DMI was similar across treatments, although animals fed diets containing 11% CP tended to have a greater DMI (% BW and kg/day), which resulted on similar N intake and higher NFC intake. Similar results were observed by Koenig and Beauchemin (2018) who evaluated the effects of inclusion of condensed tannin extract in a high protein finishing diet containing increasing levels of corn DG and observed a reduction of 15.2% in DMI when CP (%) was increased from 16.8% to 20.4% (DM basis). These results suggest that, at least within the limits of ruminal filling and physical impairment of the digestive passage rate (Mertens et al., 1994) or neuro-hormonal factors (Konturek et al., 2005), ruminants can adjust DMI in order to meet their N requirements.

A greater NDF intake for animals fed DDG was expected due to its chemical composition. In regards to the tendency for greater NFC total tract digestibility in the diets containing 14% CP, one of the possible explanations would be an increase in RUP flow to intestine as demonstrated by Brake and Swanson (2018), although the increments in RUP intake in the higher PL was only numeric. The negative ruminal CP digestibilities reflect urea recycling through saliva or ruminal wall (Yahaghi et al., 2013), which can result in a greater intestinal CP flow compared to the amount consumed by the animal.

The greatest NH_3-N concentrations for SU treatments were expected due to the high solubility of this N source in the rumen, which is consistent with those reported by Brake et al. (2010). Ammonia is the primary N source for ruminal MICP synthesis. The ammonia that is not incorporated into bacterial protein is absorbed across the ruminal epithelium and lower sections of the GIT. It enters the portal vein and is transported to the liver, where it is converted to urea. The synthesized urea can be eliminated from the body by excretion in urine or be recycled back to the gastrointestinal tract (Koenig and Beauchemin, 2018). Bach et al. (2005) demonstrated a negative and linear relationship between NH_3-N and NUE. In fact, the NUE was lowest for SU treatments.

Low rumen pH can depress fiber digestion and lead to metabolic disorders (Russell and Dombrowski, 1980; Plaizier et al., 2018). Although the diets had high amount of non-fibrous carbohydrates, which resulted in low ruminal pH over time, the differences among treatments were considered minor and within the ideal range for proper MICP synthesis (Russell and Wilson, 1996). We hypothesized that the low DMI from animals might also have contributed to the relatively high ruminal pH in this study. Furthermore, the total SCFA concentration and ruminal OM digestibility were not affected by treatments in the present study.

By reducing dietary CP level or by increasing rumen escape of CP, ruminal concentrations of branched-chain fatty acids (BCFA) may be reduced, which could result in a decrease in microbial growth and activity (Liu et al., 2009). The tendency for increasing the proportions of isobutyrate and valerate by diets with greater CP level and supplemented RUP sources, respectively, suggest a positive effect of these diets on microbial fermentation (Andries et al., 1987). However, in the present study, the MICP synthesis was not affected by treatments, which might suggest that the BCFA concentrations in the lower protein diets containing greater RUP were adequate to meet the needs of the ruminal microbial population.

In this study, urine tended to be the main route of N excretion for diets with the greater CP level, which is in agreement with Aarons et al. (2017) that showed that in situations of high N intake, urine is likely the major route for N excretion. The route of N excretion has environmental implications and highlights the importance for rational N use in feedlot diets to reduce environmental contamination caused by feedlot systems worldwide. In addition, N can be lost as gases such as ammonia (Hristov et al., 2011) and nitrous oxide (Luo et al., 2019), as well as leached as nitrate (Selbie et al., 2015). The use of CGM or DDG not only reduced the urinary N excretion by 38.5 and 42.3%, but also increased the proportion of the N excreted in feces by 15.8 and 22.9%, respectively. In addition, CGM and DDG increased the proportion of ADIN in feces. The N excreted in the urine is considerably more susceptible to gaseous losses than the N excreted in feces (Djikstra et al., 2013). The change of the route of excretion from labile urinary urea-N to stable bound forms in feces represent an opportunity to improve the stability of N in manure and reduce environmental issues associated with N excretion by ruminants.

The use of RUP sources not only increased the NUE but also decreased urinary N excretion without negatively affecting microbial N flow, which is an important strategy to reduce N in ruminant diets. According to Bach et al. (2005) when dietary RDP is in excess of the amount required by ruminal microorganisms, the protein is degraded to ammonia N, absorbed, metabolized to urea in the liver, and excreted in the urine. In this study, the use of urea + soybean meal as RDP source led to greater urinary N excretion than feeding diets containing RUP, indicating a concentration of ruminal NH_3-N that exceeded the capacity of the ruminal microorganism to incorporate it into MICP. In fact, animals fed RUP sources had lower NH_3-N concentration.

Although N retention was not affected in this study, NUE was increased by 26.7% in CGM and 29.6% in DDG treatments compared to SU. Our NUE were close to the range expected for ruminant diets (*i.e.,* 15-40%) as suggested by Dijkstra et al. (2011). Similar results for N retention were observed by other authors that used animals with similar BW and N intake (Brake et al. 2010; Brake et al. 2011). Considering the increasing production of ethanol from corn in Brazil (Eckert et al. 2018), DDG can be a strategic feedstuff to be used in Brazilian feedlot diets to increase NUE and thereafter reduce feeding costs.

Nitrogen recycling is one of the main priorities of N metabolism in ruminants, because it complements the RDP supply for rumen microbiome, especially in the situation of low N intake (Van Soest 1994; Batista et al. 2016), and it is considered a

survival strategy for ruminants. The N intake plays an important role in influencing the amount of urea produced in the liver as well as its fates (Wickersham et al. 2008; Wickersham et al. 2009a, b). However, in this study DMI was low and N intake was similar among treatments. Therefore, the effects observed on the urea kinetics might be attributed mainly to the N forms i.e. RDP or RUP, or to the mobilization of N from skeletal protein (Batista et al. 2016). Increasing N intake, mainly in the RDP form, consistently increases urea production in the liver (Marini and Van Amburgh, 2003; Wickersham et al. 2008). In this study, the higher RDP intake in SU diets resulted in a greater production of urea in the liver (UER) and also in the amount that was recycled back to the GIT (Reynolds and Kristensen, 2008). GER is affected by 2 key factors: the availability of urea to be recycled (UER) and the proportion of UER that gets recycled. When ruminal N is deficient (i.e., low ruminal ammonia concentrations), then we would expect GER/UER to be high. However, we typically have low UER in conditions when ruminal ammonia concentrations are low (i.e., there is usually a negative relationship between UER and GER/UER), and therefore we usually have a negative relationship between GER and the ruminal requirement for additional N. Therefore, due to the higher RDP intake in animals fed SU diets, the tendency of greater GER was already expected compared to those fed CGM or DDG.

In this study, UER:apparent digestible N was affected only by PS (P = 0.049; data not shown) and ranged from 98.1 ,110.9 and 132.5% in DDG, CGM and SU diets, respectively. The UER:apparent digestible N can be used to better understand the biological significance of UER. According to Marini and Van Amburgh (2003), this ratio can be affected by the N and energy content of the diet and the physiological state, and it is increased when animals are fed near maintenance requirements, as observed in our study. Lapierre and Lobley (2001) suggested that UER greater than digestible N intake might be explained by absorption of N compounds such as NH_3 or amino acids of endogenous origin; ROC would include most of the absorbed ammonia of endogenous origin as well as some of the absorbed amino acids of endogenous origin. In agreement with our findings, Batista et al. (2016) evaluated urea kinetics in heifers fed near to maintenance and attributed the large UER:digestible N in their study to protein mobilization from the body. The negative ruminal N balance in our study indicates that a significant amount urea was recycled to the rumen, because the duodenal N flow was higher than N intake (Detmann et al., 2014).

Largely, ROC is going to be recycled urea that is hydrolyzed to ammonia, with the ammonia absorbed from the gut (Reynolds and Kristensen, 2008). Thus, the

greater values for ROC observed in animals fed SU diets might be explained by: 1) the GER is in excess of the needs of the microbes, or 2) GER that is moving into the intestine and not into the rumen. In general, as we add more N to any given diet, ROC will increase as GER increases, although the relationship will not be 1:1. In addition, as PUN increases, we would expect more of GER to be transfer into the intestine rather than into the rumen. Unfortunately, the method developed by Lobley et al. (2000) does not allow separation of ruminal and intestinal recycling.

The UUA represent recycled urea-N that is used for anabolism (Lobley et al. 2000). In the mechanistic level of solution of BCNRM there is an equation that allows the user to estimate UUA (BCNRM, 2016). However, the committee recognized that this equation should be most applicable to growing cattle consuming forage-based diets. In this study, when we applied that equation to our data there was an overprediction of UUA, which demonstrates its inefficacy to estimate UUA for feedlot animals receiving high-concentrate diets. The lack of a relationship between UUA and ruminal microbial capture of recycled N (g/day) can be explained by the fact that these measures represent different aspects of utilization of recycled urea-N. UUA includes any portion of the labeled urea that does not get excreted either in feces or in urine as urea. Much of UUA would be urea-N retained in body proteins following its use for microbial protein synthesis, absorption by the animal, and deposition in body proteins. However, there are various other processes that could contribute to UAA (Wickersham et al., 2008). In contrast, ruminal microbial capture of recycled urea-N only represents the microbial utilization and is independent of whether the microbial protein is subsequently used by the host animal. Although UUA was not affected by treatments, UUA:GER was increased by the use of CGM or DDG, which suggests a higher utilization of recycled urea for anabolic functions. The UFE in our study (11.5 to 14.9% of GER) is in general agreement with other studies in the proportion of recycled urea-N that ultimately is excreted in feces (Marini and Van Amburgh, 2003; Wickersham et al., 2009b).

In this study, feeding diets with DDG led to a greater proportion of MICP synthesis derived from recycled than did feeding diets with SU. The slow rate of degradation of protein in RUP sources might increase the dependency of urea recycling to sustain MICP synthesis in the rumen (Bohnert et al., 2002; Recktenwald et al., 2014), which can explain the higher proportion of microbial protein from urea recycling in the treatments containing DDG compared to SU diets. In agreement with our findings, Brake et al. (2010) observed an increase in MICP synthesis derived from

urea recycling, when expressed as a proportion of total microbial N, for diets containing DDGS compared to those supplemented with urea. These findings could explain the absence of positive effects on animal performance by the addition of urea in feedlot diets containing DDG (Boyd et al., 2019). In addition, the greater bacterial ^{15}N enrichment in the treatments containing RUP sources and also for the lower CP level support our findings that ruminal bacteria are more dependent on N recycling when rumen protein availability is decreased by the reduction of PL in the diet or when PS is resistant to ruminal degradation (Brake et al. 2010).

In this study the greater PUN observed for steers fed SU diets compared to the steers fed diets containing RUP sources is positively associated with the higher RDP intake associated with that diet (Harmeyer and Martens, 1980). These findings are also in agreement with the higher plasma urea N filtration and UER observed in animals fed SU treatments. In addition, when fed a diet with 14% CP, animals fed SU treatments had higher fractional urea-N excretion and hence lower reabsorption of plasma urea-N, which highlights the benefits of increasing the RUP content in feedlot diets to avoid N losses from the rumen.

One important goal of feeding cattle is to maximize MICP synthesis and the amount of RDP that is used by ruminal bacteria (Bach et al., 2005), which can result in an improvement of N use efficiency. In the present study, reducing RDP in the diet not only improved the utilization of urea recycled by rumen microbes, but also decreased N losses. In addition, MICP synthesis was not affected by decreasing the RDP content in the diets (i.e. using RUP sources). Our efficiencies of N use by rumen microorganisms were not different among treatments. However, GER, which is included in the denominator of that calculation, includes not only the urea recycled to the rumen, but also that transferred to the post-ruminal portions of the GIT (Lobley et al., 2000). Therefore, an unknown portion of GER is not available for MICP synthesis, which can compromise this evaluation. In fact, Li et al. (2019) demonstrated based on model simulations, that feeding a diet with moderately low crude protein and high rumen-undegradable protein could increase apparent ruminal N efficiency by 20%. Hoover and Stokes (1991) reported a linear increase in ENU with up to 20% of dietary RDP (DM basis) in a continuous culture system as well as linear increase in ENU up to 12.9% of dietary RDP in lactating cows. Our results show that feeding moderately low CP diets associated with RUP supplementation may allow greater proportions of feed protein to escape the rumen, greater capture of recycled urea-N by rumen microbes, and reduced urinary N excretion. This nutritional strategy will enable feedlot

nutritionists to improve the efficiency of nutrient management in feedlots worldwide. Especially, in a scenario of increasing in the production of high protein by-product as dried distillers grains (DDG) that have become very attractive not only from the economic point of view but also with regard to environmental sustainability in feedlots as demonstrated here.

5. CONCLUSIONS

Nitrogen use efficiency is increased by the use of RUP sources, and this response is associated with lower ruminal NH_3 concentrations, reduced urea production in the liver, and reduced urinary N excretion. Our results suggest that urea recycling is able to buffer a reduction of dietary N levels to maintain MICP synthesis.

6. REFERENCES

Aarons SR, Gourley CJP, Powell, JM Hannah MC (2017) Estimating nitrogen excretion and deposition by lactating cows in grazed dairy systems. **Soil Research** 55:489-499.

Allen MS, Linton JAV (2007) In vivo methods to measure digestibility and digestion kinetics of feed fractions in the rumen. In: **Simpósio Internacional de Avanços em Técnicas de Pesquisa em Nutrição de Ruminantes.** Universidade de São Paulo, São Paulo, Brazil. p.72–89.

Andries JI, Buysse FX, De Brabander DL, Cottyn BG (1987) Isoacids in ruminant nutrition: Their role in ruminal and intermediary metabolism and possible influences on performances — A review. **Animal Feed Science and Technology** 18:169-180.

Archibeque SL, Burns JC, Huntington GB (2001) Urea flux in beef steers: Effects of forage species and nitrogen fertilization. **Journal of Animal Science** 79:1937-1943.

Bach A, Calsamiglia S, Stern MD (2005) Nitrogen metabolism in the rumen. **Journal of Dairy Science** 88:9-21.

Batista ED, Detmann, E, Titgemeyer EC, Valadares Filho, SC, Valadares RFD, Prates, LL, Rennó LN, Paulino M.F (2016) Effect of varying ruminally undegradable protein supplementation on forage digestion, nitrogen metabolism, and urea kinetics in Nellore cattle fed low-quality tropical forage. **Journal of Animal Science** 94:201-216.

Beef cattle Nutrient Requirements Model – BCNRM 2016: **Nutrient Requirements of Beef Cattle. Eighth Revised Edition.** Washington. D.C: The National Academies Press, 2016, 494p.

Bohnert DW, Schauer CS, Falck SJ, Delcurto T (2002) Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: II. Ruminal fermentation characteristics. **Journal of Animal Science** 80:2978-2988.

Boyd BM, MacDonald JC, Luebbe MK, Erickson GE (2019) Effect of adding urea to finishing diets containing two different inclusions of distillers grains on steer performance and carcass characteristics. **Nebraska Beef Cattle Reports** 1017. http://digitalcommons.unl.edu/animalscinbcr/1017

Brake DW, Swanson KC (2018) Effects of postruminal flows of protein and amino acids on small intestinal starch digestion in beef cattle. **Journal of Animal Science** 96:739-750.

Brake DW, Titgemeyer EC, Jones ML, Anderson DE (2010) Effect of nitrogen supplementation on urea kinetics and microbial use of recycled urea in steers consuming corn-based diets. **Journal of Animal Science** 88:2729-2740.

Brake DW, Titgemeyer EC, Jones Ml (2011) Effect of nitrogen supplementation and zilpaterol–HCl on urea kinetics in steers consuming corn-based diets. **Journal of Animal Physiology and Animal Nutrition** 95:409-416.

Broderick GA, Kang JH (1980) Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. **Journal of Dairy Science** 63:64-75.

Calsamiglia S, Ferret A, Reynolds CK, Kristensen NB, Van Vuuren AM (2010) Strategies for optimizing nitrogen use by ruminants. **Animal** 4:1184-1196.

Cecava MJ, Merchen NR, Gay LC, Berger LL (1990) Composition of ruminal bacteria harvest from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. **Journal of Dairy Science** 73:2480-2488.

Chasson Al, Grady HJ, Stanley MA (1961) Determination of creatinine by means of automatic chemical analysis. **American Journal of Clinical Pathology** 35:83-88.

Detmann E, Valente EEL, Batista ED, Huhtanen P (2014) An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. **Livestock Science** 162:141-153.

Detmann E, Souza MA, Valadares Filho SC, Queiroz AC, Berchielli TT, Saliba EOS, Cabral LS, Pina DS, Ladeira MM, Azevedo JAG (2012) **Métodos para análise de alimentos - Instituto Nacional de Ciência e Tecnologia em Ciência Animal.** 582 Suprema, Visconde do Rio Branco, Brazil.

Dijkstra J, Oenema O, Bannink A (2011) Dietary strategies to reducing N excretion from cattle: implications for methane emissions. **Current Opinion in Environmental Sustainability** 3:414-422.

Ding L, Shen Y, Wang, Y, Zhou G, Zhang X, Wang M, Loor J, Chen L, Zhang J (2019) Jugular arginine supplementation increases lactation performance and nitrogen utilization efficiency in lactating dairy cows. **Journal of Animal Science and Biotechnology** 10:1-10. doi: 10.1186/s40104-018-0311-8.

Eckert CT, Frigo EP, Albrecht LP, Albrecht AJP, Christ D, Santos WG, Berkembrock E, Egewarth VA (2018) Maize ethanol production in Brazil: Characteristics and perspectives. **Renewable and Sustainable Energy Reviews** 82:3907-3912.

Etheridge RD, Pesti GM, Foster EH (1998) A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. **Animal Feed Science and Technology** 73:21-28.

Fenner, H (1965) Method for determining total volatile bases in rumen fluid by steam distillation. **Journal of Dairy Science** 48:249–251.

Faichney GJ (1975) The use of markers to partition digestion within the gastrointestinal tract of ruminants. In: Digestion and metabolism in the ruminant. In: **Proceedings of fourth International Symposium on Ruminant Physiology,** Sydney, Australia, p. 277-291.

France J, Siddons RC (1986) Determination of digesta flow by continuous marker infusion. **Journal of Theoretical Biology** 136:485-486.

Harmeyer J, Martens H (1980) Aspects of urea metabolism in ruminants with reference to the goat. **Journal of Dairy Science** 63:1707-1728.

Hoover WH, Stokes SR (1991) Balancing carbohydrates and protein for optimum rumen microbial yield. **Journal of Dairy Science** 74:3630–3644.

Hristov AN, Hanigan M, Cole A, Todd R, Mcallister TA, Ndegwa PM, Rotz A (2011) Review: ammonia emissions from dairy farms and beef feedlots. **Canadian Journal of Animal Science** 91:1–35.

Hristov AN, Bannink A, Crompton LA, Huhtanen P, Kreuzer M, Mcgee M, Nozière P, Reynolds CK, Bayat AR, Yáñez-Ruiz DR, Dikkstra J, Kebreab E, Schwarm A, Shingfield KJ, Yu Z (2019) Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. **Journal Dairy Science** 102:5811-5852.

Ipharraguerre IR, Clark JH, Freeman DE (2005) Rumen fermentation and intestinal supply of nutrients in dairy cows fed rumen-protected soy products. **Journal of Dairy Science** 88:2879-2892.

Koenig KM, Beauchemin KA (2018) Effect of feeding condensed tannins in high protein finishing diets containing corn distillers grains on ruminal fermentation, nutrient digestibility, and route of nitrogen excretion in beef cattle. **Journal of Animal Science** 96:4398-4413.

Konturek PC, Konturek JW, Czesnikiewiczguzik M, Brzozowski T, Sito E, Konturek SJ (2005) Neuro-hormonal control of food intake: basic mechanisms and clinical implications. **Journal of Physiology and Pharmacology** 56:5-25.

Lapierre H, Lobley GE (2001) Nitrogen recycling in the ruminant: A review. **Journal of Dairy Science** 84:223-236.

Leventini MW, Hunt CW, Roffler RE, Casebolt DG (1990) Effect of dietary level of barley-based supplements and ruminal buffer on digestion and growth by beef cattle. **Journal Animal Science** 68:4334-4344.

Li MM, Titgemeyer EC, Hanigan MD (2019) A revised representation of urea and ammonia nitrogen recycling and use in the Molly cow model. **Journal of Dairy Science** 102:5109-5129.

Licitra G, Hernandez TM, Van Soest PJ (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. **Animal Feed Science and Technology** 57:347-358.

Liu Q, Wang C, Yang WZ, Zhang B, Yang XM. He DC, Huang YX (2009) Effects of isobutyrate on rumen fermentation, lactation performance and plasma characteristics in dairy cows. **Animal Feed Science and Technology** 154:58-67.

Lobley GE, Bremner DM, Zuur G (2000) Effects of diet quality on urea fates in sheep as assessed by refined, non-invase [$^{15}N^{15}N$] urea kinetics. **British Journal of Nutrition** 84:459-468.

Luo J, Saggar S, Van Der Weerden T, Klein C (2019) Quantification of nitrous oxide emissions and emission factors from beef and dairy cattle excreta deposited on grazed pastoral hill lands. **Agriculture, Ecosystems & Environment** 270-271:103-113.

Machado M, Detmann E, Mantovani HC, Valadares Filho SC, Bento CBP, Marcondes MI, Assunção AS (2016) Evaluation of the length of adaptation period for changeover and crossover nutritional experiments with cattle fed tropical forage-based diets. **Animal Feed Science and Technology** 222:132-148.

Marini JC, Van Amburgh ME (2003) Nitrogen metabolism and recycling in Holstein heifers. **Journal of Animal Science** 81:545-552.

Marsh WH, Fingerhut B, Miller H (1965) Automated and manual direct methods for determination of blood urea. **Clinical Chemistry** 11:624-627.

Menezes ACB, Valadares Filho SC, Costa E Silva LF, Pacheco MVC, Pereira JMV, Rotta PP, Zanetti D, Detmann E, Silva FAS, Godoi LA, Rennó LN (2016) Does a reduction in dietary crude protein content affect performance, nutrient requirements, nitrogen losses, and methane emissions in finishing Nellore bulls? **Agriculture, Ecosystems & Environment** 223:239-249.

Mertens DR (1994) Regulation of forage intake. In: Fahey JR, GC, Collins M, Mertens DR, Moser LE (Eds.) **Forage Quality. Evaluation and Utilization.** Madison: American Society of Agronomy. Crop Science of America. Soil Science of America, p.450-493.

NASEM 2016: National Academies of Sciences, Engineering, and Medicine. Nutrient requirements of beef cattle. 8th ed. **Nutrient requirements of domestic animals.** National Academy Press, Washington, DC, 2016, 494p.

Oliveira CA, Millen DD (2014) Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. **Animal Feed Science and Technology** 197:64-75.

Pinto ACJ, Millen DD (2019) Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: The 2016 Brazilian survey. **Canadian Journal of Animal Science** 99: 392-407. doi:10.1139/cjas-2018-0031

Plaizier J, Danesh Mesgaran M, Derakhshani H, Golder H, Khafipour E, Kleen J, Zebeli Q (2018) Review: Enhancing gastrointestinal health in dairy cows. **Animal** 12:399-418. doi:10.1017/S1751731118001921

Prates LL, Valadares RFD, Valadares Filho SC, Detmann E, Oullet DR, Batista ED, Zanetti D, Pacheco MVC, Silva BC (2017) Investigating the effects of sex of growing Nellore cattle and crude protein intake on the utilization of recycled N for microbial protein synthesis in the rumen by using intravenous $^{15}N^{15}N$-urea infusion **Animal Feed Science and Technology** 231:119-130.

Recktenwakd EB, Ross AD, Fessenden SW, Wall CJ, Van Amburgh ME (2014) Urea-N recycling in lactating dairy cows fed diets with 2 different levels of dietary crude protein and starch with or without monensin. **Journal of Dairy Science** 97:1611-1622.

Reynal, S.M.; Broderick, G.A.; Bearzi, C. Comparison of four markers for quantifying microbial protein flow from the rumen of lactating dairy cows. **Journal of Dairy Science** 88:4065-4082.

Reynolds CK, Kristensen NB (2008) Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. **Journal of Animal Science** 86:293-305.

Rotta PP, Valadares Filho SC, Costa E Silva LF, Detmann E, Villadiego FAC, Engle TE, Valadares RFD, Silva BC, Pacheco MVC, Zanetti D (2014) Comparison of purine bases and 15 N for quantifying microbial nitrogen yield using three marker systems and different sampling sites in zebu cross breed bulls. **Livestock Science** 167:144-153.

Russell JB, Dombrowski DB (1980) Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. **Applied and Environmental Microbiology** 39:604-610.

Russell JB, Wilson DB (1996) Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? **Journal of Dairy Science** 79:1503-1509.

Samuelson KL, Hubbert ME, Galyean ML, Löest CA (2016) Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico and Texas Tech University survey. **Journal of Animal Science** 94: 2648-2663.

Selbie DR, Buckthought LE, Shepherd MA (2015) Chapter four- the challenge of the urine patch for managing nitrogen in grazed pasture systems. **Advances in Agronomy** 129:229-292.

Titgemeyer EC, Spivey KS, Parr SL, Brake DW, Jones ML (2012) Relationship of whole body nitrogen utilization to urea kinetics in growing steers. **Journal of Animal Science** 90:3515–3526.

Udén P, Colucci PE, Van Soest PJ (1980) Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. **Journal of the Science Food and Agricultural**, 31:625-632.

Valadares RFD, Gonçalves LC, Valadares Filho SC, Coelho Da Silva JF (1997) Níveis de proteína em dietas de bovinos. 2. Consumo, digestibilidade e balanços de compostos nitrogenados. **Revista Brasileira de Zootecnia** 26:1259-1263.

Valadares Filho SC, Costa E Silva LF, Gionbelli MP, Rotta PP, Marcondes PP, Chizzotti ML, Prados LF (2016) **Exigências nutricionais de zebuínos puros e cruzados BR-CORTE**. Viçosa: DZO-UFV, p.163-220.

Valente TNP, Detmann E, Queiroz AC, Valadares Filho SC, Gomes DI, Figueiras JF (2011) Evaluation of ruminal degradation profiles of forages using bags made from different textiles. **Revista Brasileira de Zootecnia** 40:2565–2573. doi:10.1590/S1516-35982011001100039

Van Soest PJ (1994) Nutritional Ecology of the Ruminant. 2nd ed. Cornell Univ. Press, Ithaca, NY, 476p.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. **Journal of Dairy Science** 74:3583-3597.

Weiss WP, Conrad HR, Pierre NRS (1992) A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. **Animal Feed Science and Technology** 39:95-110.

Wickersham TA, Titgemeyer EC, Cochran RC (2009a) Methodology for concurrent determination of urea kinetics and the capture of recycled urea nitrogen by ruminal microbes in cattle. **Animal** 3:372-379.

Wickersham TA, Titgemeyer EC, Cochran RC, Wickersham EE, Gnad DP (2008) Effect of rumen degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. **Journal of Animal Science** 86:3079-3088.

Wickersham TA, Titgemeyer EC, Cochran RC, Wickersham EE (2009b) Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. **British Journal of Nutrition** 101:225-232.

Yahaghi M, Liang JB, Balcells J, Valizadeh R, Seradj AR, Alimon AR, Ho YW (2013) Effects of substituting barley with sorghum on starch digestion, rumen microbial yield and growth in Iranian Baluchi lambs fed high concentrate diets. **Animal Feed Science and Technology** 183:96-105.

CHAPTER 2. ASSESSING AMINO ACID UTILIZATION IN FEEDLOT NELLORE CATTLE FED HIGH-CONCENTRATE DIETS WITH DIFFERENT LEVELS AND NITROGEN SOURCES

ABSTRACT - The aim of this this study was to evaluate the availability and utilization of individual amino acids (AA) in Nellore cattle fed high-concentrate diets with different levels and sources of N. Six Nellore steers, cannulated in the rumen, duodenum and ileum with initial body weight (BW) of 354 ± 11.8 kg and 18 months of age (average), were randomly assigned to receive each diet once over the 6 periods in a 6 × 6 Latin square design balanced for residual effects. Treatments were arranged in a factorial 3 × 2, where factor A consisted of 3 protein sources (soybean meal plus urea (SU), corn gluten meal (CGM) and dry distillers grains (DDG)) and factor B consisted of 2 dietary crude protein (CP) levels (11 and 14%). Our hypothesis was that differences in AA use efficiency exists and that the gross AA use efficiency is affected by dietary factors such as N sources and levels. There were no interactions between PS and PL for the flow of any AA evaluated ($P > 0.05$). The duodenal flow of essential (EAA) and non-essential AA (NEAA) was not affected ($P > 0.05$) by PS and PL. Proline concentration was increased in SU diets, reduced in CGM diets and not affected in DDG diets by the increase in PL. Diets containing DDG and 11% CP tended ($P < 0.10$) to have greater arginine and histidine concentration in rumen bacteria compared to SU and GGM diets with 11% CP; however, there was no difference between PS within the 14% CP level. Lysine concentration in rumen bacteria tended ($P < 0.10$) to be greater as the PL increased in the CGM diets. Tyrosine concentration tended ($P < 0.10$) to be greater in the diets containing CGM compared to DDG diets. Also, increasing PL in the diet from 11 to 14% CP tended ($P < 0.10$) to lead to higher concentrations of tyrosine in microbial protein. Animals fed DDG tended ($P < 0.10$) to have greater arginine, lysine and leucine supply from microbial protein than those fed diets containing SU or CGM. Animals fed DDG had greater ($P < 0.05$) NEAA supply from microbial protein flow than those fed diets containing SU, but they did not differ from animals fed CGM diets. Histidine and glutamate from microbial protein had a greater supply ($P < 0.05$) by the dietary inclusion of CGM or DDG compared to SU diets. Animals fed CGM or DDG tended ($P < 0.10$) to have greater proline and serine flow from microbial protein than those fed SU diets. Animals fed CGM or DDG tended ($P < 0.10$) to have greater EAA, arginine, isoleucine and valine supply from RUP fraction. The Leucine supply from RUP was greater ($P < 0.05$) in animals fed CGM or DDG compared to those fed SU diets. Plasma histidine concentration tended ($P < 0.10$) to be greater in animals fed DDG diets. Plasma leucine concentration was greater ($P < 0.05$) in animals fed CGM or DDG compared to those fed SU diets. Animals fed diets containing 14% CP had greater ($P < 0.05$) leucine concentration compared to the diets containing 11% CP. Plasma phenylalanine concentration was greater ($P < 0.05$) in animals fed diets containing CGM or DDG compared to those fed SU diets. Plasma valine concentration was greater ($P < 0.05$) in animals fed diets with a CP level of 14% compared to fed 11% CP diets. Plasma glutamine concentration was greater ($P < 0.05$) in animals fed SU diets compared to those fed CGM or DDG diets. Plasma glycine concentration was greater ($P < 0.05$) in animals fed SU diets compared to those fed CGM or DDG diets. There were interactions, or tendencies for interactions, between PS and PL for gross AA utilization of all AA evaluated, except methionine and cystine. Arginine and histidine utilization were greater in animals fed diets containing 11% CP with DDG and 14% CP with CGM. Animals fed the diet containing 11% CP and DDG showed greater isoleucine, lysine, phenylalanine, threonine, valine, alanine, aspartic, glutamate, proline, serine, and tyrosine use efficiency than other diets, except diet 14% CP with

CGM which did not differ. Methionine and cystine utilization were not affected by PS or PL ($P > 0.05$). The AA use efficiency is affected by dietary protein levels and sources. Our results suggest that it is possible to increase the supply of essential AA using CGM or DDG (RUP sources) in the diet compared to soybean meal plus urea, especially in situations where it is possible to increase the microbial protein flow.

1. INTRODUCTION

Domestic ruminants are strategic and important suppliers of high-quality protein because of their ability to convert feed and by-products of little or no value into human food (Broderick, 2017). Unfortunately, this societal benefit comes at a cost, namely, excretion of various pollutants (Djikstra et al., 2011). In this context, beef cattle diets need to be developed and implemented with focus on more efficient transfer of dietary nitrogen (N) into meat. Increasing this transfer efficiency reduces N excretion in feces and urine, which are important environmental and economic N losses. However, improving the nitrogen use efficiency (NUE) in ruminant diets requires precise and accurate predictions of both protein (amino acid) supply and requirements.

Dried distillers grains (DDG) are an excellent source of RUP and energy that are commonly used in beef cattle diets (Council, 2012; Jolly-Breithaupt et al., 2018). Corn gluten meal (CGM) is a major byproduct of corn wet milling with a high content of CP and RUP, being used mainly as a protein supplement in animal feed (Hankis et al., 2005), however with an unbalanced AA profile (Zhuang et al., 2013). The AA profile supplied by the rumen undegradable protein (RUP) fraction in these sources can differ from that of the original feed (Harstad and Prestlokken, 2001). There is also a variability of RUP content in feedstuff and notable differences between feed, microbe, and RUP AA content and digestibilities (Titgemeyer, 2003). Determining the AA supply from the different RUP sources and also from microbial crude protein is a priority to understand how we can manipulate the diet to increase the supply of limiting AA to optimize animal performance. Unfortunately, data available about the supply of amino acids from RUP sources and rumen microbial protein in beef cattle are scarce.

The Brazilian beef cattle system (BR-Corte, Valadares Filho et al., 2016), the NASEM (Nasem, 2016), and the Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 2004) all provide estimates of NUE or individual AA use that can be leveraged to more precisely formulate low-N diets. However, the same efficiency for growth is applied to each essential AA, as well as to MP, and this efficiency is not affected by factors other than BW. However, research conducted with cattle demonstrated differences in the efficiency use among AA (e.g., methionine: Campbell

et al., 1996, 1997; Löest et al., 2002; leucine: Awawdeh et al., 2005; 2006; histidine: McCuistion et al., 2004; and lysine: Batista et al., 2016); changes in efficiencies associated with various factors such as differences in oxidation rates of individual AA (Lapierre et al., 2006); and differences in the roles of specific AA in processes other than protein synthesis (Owens and Pettigrew, 1989).

In order to develop better models, and specifically models for beef cattle, additional data is needed to relate dietary AA provision to AA utilization. Although there have been a number of studies advancing our ability to predict AA supplies and requirements in dairy cattle (White et al., 2016; Estes et al., 2018; Fleming et al., 2019a,b), minimal concurrent progress has been made in representing beef cattle diets. In the last two decades, the National Research Council (NRC) developed nutrition models for beef cattle to predict amino acid (AA) supplies and requirements to support precision feeding. However, the approach to modeling AA supplies and requirements was fairly simplistic and lacked coherence with known biological confounders, very likely because of the limited availability of data to derive more representative models.

To work toward filling this data gap, the objective of this study was to evaluate the availability and utilization of individual AA in Nellore cattle fed high-concentrate diets with different levels and sources of N. Our hypothesis was that differences in AA use efficiency exists and that AA use efficiency is affected by dietary factors such as N sources and levels.

2. MATERIAL AND METHODS

The study was conducted at São Paulo State University (UNESP), Jaboticabal, SP, Brazil. Animal care and handling was in accordance with the Brazilian College of Animal Experimentation Guidelines (COBEA) and were approved by the Ethics, Bioethics, and Animal Welfare Committee of UNESP, Jaboticabal, under Protocol 16.668/16.

2.1. Animals, Experimental Design, and Diets

Six Nellore steers, cannulated in the rumen, duodenum, and ileum with initial body weight (BW) of 354 ± 11.8 kg and 18 months of age (average), were randonmly assigned in a 6 × 6 Latin square (6 animals and 6 periods) design balanced for residual effects. The experiment lasted 156 days, six experimental periods of 26 days each.

Animals were adapted to diets for 14 days (Machado et al., 2016) and 8 days were used for sample collection (Figure 1). In addition, animals were allocated in individual pens (12 m^2) with concrete floors, feed bunk, and water fountain.

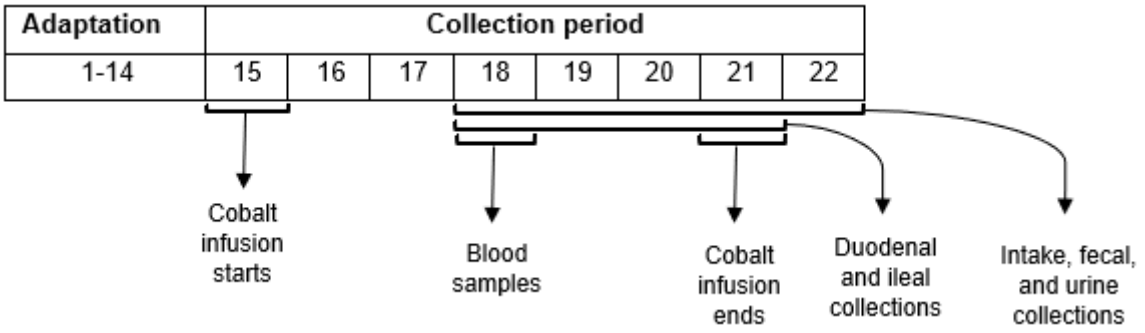

Figure 1. Collection protocol during each experimental period. Numbers from 1 to 22 represent the days of each period.

Before entering the feedlot, the animals were submitted to 16 hours total fasting, were treated to remove parasites (ivermectin, dosage of 200 µg/kg BW), and their initial BW were recorded. Before starting the study, the animals were fed four different adaptation diets (20, 40, 60, and 80% concentrate) with decreasing concentration of fiber (one week/diet). This practice was adopted with the aim of minimizing the deleterious effects of high-concentrate diets on ruminal physiology. At the end of the fourth week of adaptation, the animals were fed a diet containing 80% concentrate, which consisted of soybean meal (30%) and ground corn (70%).

Treatments were arranged in a factorial A × B, where factor A consisted of 2 dietary CP levels (11 and 14%) and factor B consisted of 3 protein sources (soybean meal plus urea, corn gluten, and dry distillers grains). Therefore, the treatments were:

1) Soybean meal + urea (SU) at 14% CP: ground corn, soybean meal, urea, and fresh chopped sugar cane.

2) Corn gluten meal (CGM) at 14% CP: ground corn, corn gluten meal, and fresh chopped sugar cane.

3) Dried distillers grains (DDG) at 14% CP: ground corn, DDG, and fresh chopped sugar cane.

4) Soybean meal + urea (SU) at 11% CP: ground corn, soybean meal, urea, and fresh chopped sugar cane.

5) Corn gluten meal (CGM) at 11% CP: ground corn, corn gluten meal, and fresh chopped sugar cane.

6) Dried distillers grains (DDG) at 11% CP: ground corn, DDG, and fresh chopped sugar cane.

Experimental diets consisted of 80% concentrate and 20% roughage (DM basis). Fresh chopped sugar cane was used as the roughage source and and the concentrates differentiated on their protein source (soybean meal and urea, corn gluten meal, and DDG). Corn gluten meal and DDG were used as rumen undegradable protein (RUP) sources, with low and intermediate ruminal degradability, respectively, and soybean meal and urea were used as RDP sources. The chemical composition of the ingredients and experimental diets is presented in Tables 1 and 2.

Table 1. Chemical composition of the ingredients.

Item[2]	Ingredients[1]						
	Sugar cane	Ground corn	Soybean meal	DDG	CGM	Urea	Mineral mix[3]
DM (%)	25.0	88.5	89.6	91.6	92.2	93.9	99.0
OM (%)	97.3	98.5	93.0	98.1	98.1	99.9	-
CP (%)	3.89	9.51	51.2	34.1	60.0	275.3	-
NDF (%)	48.6	22.1	29.9	55.9	36.9	-	-
EE (%)	1.20	5.25	2.18	6.77	2.87	-	-
Protein fractions (%CP basis)[4]							
RDP (%)	37.0	57.7	59.7	41.7	30.3	100	-
RUP (%)	63.0	42.3	40.3	58.3	69.7	-	-
Essential amino acids (EAA,% DM)							
Arginine	0.11	0.49	3.79	1.59	2.17	-	-
Histidine	0.04	0.26	1.29	1.00	1.17	-	-
Isoleucine	0.07	0.34	2.25	1.22	2.26	-	-
Leucine	0.15	1.23	4.06	4.67	10.12	-	-
Lysine	0.09	0.32	3.35	1.04	1.42	-	-
Methionine	0.01	0.14	0.50	0.59	1.30	-	-
Phenylalanine	0.08	0.46	2.62	1.75	3.67	-	-
Threonine	0.09	0.36	2.12	1.38	2.14	-	-
Valine	0.10	0.43	2.24	1.67	2.51	-	-
Non-essential amino acids (NEAA, % DM)							
Alanine	0.13	0.68	2.63	2.62	5.38	-	-
Aspartic	0.25	0.63	5.98	2.22	3.90	-	-
Cystine	0.02	0.12	0.74	0.48	0.74	-	-
Glutamic	0.20	1.76	8.28	6.26	13.23	-	-
Glycine	0.12	0.42	2.42	1.42	1.80	-	-
Proline	0.09	0.82	2.50	3.24	5.28	-	-
Serine	0.09	0.50	2.82	1.84	3.45	-	-
Tyrosine	0.04	0.33	1.67	1.31	2.75	-	-

[1]DDG: Dried distillers grains, CGM: Corn gluten meal. [2]DM: Dry matter; OM, Organic matter, CP: crude protein, NDF: Neutral detergent fiber, EE: Ether extract. [3]Provided per kg of DM: 220 g Ca, 20 g P, 60 g Na, 25 g S, 10 g Mg, 100 mg Co, 500 mg Cu, 50 mg I, 1500 mg Zn, 9 mg Se, 1500 mg Mn, 100.000 IU vitamin A, 50 g sodium bicarbonate. [4]Rumen degradable protein (RDP) and rumen undegradable protein (RUP) content were estimated based on the protein fractions and the degradation rate of each fraction, considering a passage rate of 5%.h^{-1}. Protein fractions were determined based on the procedure standardized by Licitra et al. (1996).

Diets were formulated according to BR-CORTE (Valadares Filho et al., 2016) to meet an average daily gain (ADG) of 1.25 kg (requirements considered an intake (kg/day) of: DM = 8.15, TDN = 5.68, CP = 0.93 for diets with 13.7% CP (Table 2). For the diets with 10.8% CP, DMI and TDN were maintained constant and the RDP and RUP concentration varied according to the tested protein source (Table 2). The forage: concentrate ratio used was based on the survey of nutritional practices adopted by nutritionists in Brazilian feedlots (Oliveira and Millen, 2014). Through the experiment, there was a variation in the chemical composition of the sugar cane, which resulted in change from the initial proposed diet formulation.

Table 2. Ingredient and chemical composition of the experimental diets.

	Diets[1]					
	11% CP			14% CP		
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**
Ingredient proportion (%)						
Sugar cane	15.5	17.6	17.6	17.0	15.8	17.5
Ground corn	79.8	74.3	68.7	72.2	69.6	57.0
Soybean meal	1.11	-	-	7.12	-	-
Corn gluten meal	-	5.65	-	-	12.1	-
DDG	-	-	11.3	-	-	23.0
Urea	0.99	-	-	1.24	-	-
Mineral mix[2]	2.57	2.47	2.47	2.51	2.52	2.46
Chemical composition						
Dry matter (%)	77.7	78.2	78.3	77.7	78.9	78.7
Organic matter (%)	95.7	95.8	95.8	95.4	95.7	95.8
Crude protein (%)	11.7	11.1	11.0	14.7	14.5	13.9
RDP (%, CP basis)[2]	66.6	48.0	50.9	67.1	43.1	47.7
RUP (%, CP basis)[3]	33.4	52.0	49.1	32.9	56.9	52.3
Neutral detergent fiber (%)	22.4	23.8	27.0	23.0	25.2	31.5
Ether extract (%)	4.50	4.21	4.52	4.19	4.36	4.31
Total digestible nutrients (%)[4]	74.3	74.2	72.0	73.2	75.7	72.4
Amino acids composition (% DM)						
Essential amino acids						
Arginine	0.45	0.50	0.53	0.64	0.62	0.66
Histidine	0.23	0.27	0.30	0.29	0.33	0.39
Isoleucine	0.31	0.39	0.38	0.42	0.52	0.49
Leucine	1.05	1.51	1.40	1.20	2.10	1.80
Lysine	0.30	0.33	0.35	0.48	0.41	0.43
Methionine	0.12	0.18	0.16	0.14	0.34	0.21
Phenylalanine	0.41	0.56	0.53	0.53	0.78	0.68
Threonine	0.33	0.40	0.42	0.43	0.52	0.54
Valine	0.38	0.48	0.50	0.49	0.62	0.65
Non-essential amino acids						
Alanine	0.59	0.83	0.78	0.70	1.14	1.01
Aspartic	0.61	0.73	0.73	0.92	0.95	0.91
Cystine	0.11	0.14	0.14	0.15	0.18	0.19
Glutamic	1.53	2.09	1.95	1.90	2.86	2.48
Glycine	0.38	0.43	0.47	0.49	0.53	0.59
Proline	0.70	0.93	0.95	0.79	1.23	1.23
Serine	0.44	0.58	0.56	0.57	0.78	0.72
Tyrosine	0.29	0.41	0.38	0.36	0.57	0.50

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG); [2]Contained per kg of DM: 220 g Ca, 20 g P, 60 g Na, 25 g S, 10 g Mg, 100 mg Co, 500 mg Cu, 50 mg I, 1500 mg Zn, 9 mg Se, 1500 mg Mn, 100.000 IU vitamin A, 50 g sodium bicarbonate. [2]Rumen degradable protein; [3]Rumen undegradable protein. RDP and RUP content were estimated based on the protein fractions (LICITRA et al. 1996) and the degradation rate of each fraction, considering a passage rate of 5%.h^{-1}. [4]Calculated based on the equation proposed by Weiss et al. (1992).

Animals were fed twice a daily at 0600 and 1600 h. Orts were collected before morning feeding with weights recorded, and feeding rate was adjusted daily to yield orts of 5% of intake.

2.2. Nitrogen balance and feed analysis

Feed intake measurements and total feces collection were conducted during five consecutive days, from the 18th to the 22th day of each experimental period. Feces were collected soon after defecation, stored in a bucket, and kept under refrigeration (4ºC) to avoid potential N loss by volatilization. After 24 hours, the feces were individually weighed and a sample of 5-10% of the total weight was stored at -20ºC for further analysis, during the five days. In addition, samples from the diets and orts of each animal were also collected and stored -20ºC for further chemical composition analysis.

At the end of each experimental period, samples of feed, orts and feces proportionately on the basis of total fecal output or total orts were pooled, dried at 55°C for 72 h and were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1- and 2-mm screen. Samples ground through a 1-mm screen were analyzed for DM (INCT-CA, # G-003/1) and OM (INCT-CA, nº G-004/1) according to the standardized analytical procedures described by INCT (INCT-CA; Detmann et al., 2012). Total N was analyzed by the DUMAS method using the Leco-FP 528 LC equipment (2013 LECO Corporation, St. Joseph, MI, USA) according to the protocol described by Etheridge et al. (1998) and converted to CP using the factor of 6.25. The NDF was analyzed following the methodology described by Van Soest et al. (1991) and adapted for the ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY) using α-amylase and without sodium sulfite. Samples ground through a 2-mm screen were used for determination of indigestible NDF (iNDF) after in situ incubation for 288 h, as recommended by Valente et al. (2011).

Urine samples were obtained from the total collection, which was performed from the 18th to the 22th day of each experimental period. Samples were collected through funnels equipped with tubes and a polyethylene container and were acidified with 250 mL of 20% (wt/wt) H_2SO_4 solution to prevent N loss by volatilization (Valadares et al., 1997). After 24 hours of collection, the total excreted volume was measured and homogenization was performed. Then, a 100-mL sample was collected, filtered through three layers of cheesecloth and subsequently stored at -20°C for further analysis. After 5 days of collection, urine samples were composited by animal and period, and total N (DUMAS method) was analyzed.

2.3. Intestinal flow determinations

Intestinal amino acid disappearance was determined through duodenal and ileal DM flows estimated using iNDF as the internal and solid phase marker and Co-EDTA as the external and liquid phase marker (Úden et al., 1980).

The Co-EDTA (5 g/animal/day or 0.8 g of Co/day) was continuously infused into the rumen from the 15th to the 21st day of each experimental period using a peristaltic pump (DOSATEC 2400, PROVITEC, São Paulo, SP, Brazil). Then, from the 18th to the 21st day of each experimental period, samples of duodenal digesta (800 mL) were collected at nine-hour intervals at 1030 and 1930 h on day 18, at 0430, 1330, and 2230 h on day 19, at 0730 and 1630 on day 20, and at 0130 h on day 21 (Allen and Linton, 2007). To perform duodenal sampling, plastic bags were held in place with an elastic band at the end of the duodenal cannula, allowing free digesta flow into the collection bags. Immediately after collection, duodenal samples were divided into two: one to estimate the digesta flow (375 mL) and one for microbial isolation (425 mL). Samples used to estimate nutrient flow were frozen individually after each collection. At the end of each experimental period, samples were thawed and pooled by animal. Samples used for microbial isolation were kept under refrigeration (4°C), pooled each 24 h and centrifuged. At the end of each sampling period, a subsample of the duodenal digesta flow (2 L) was taken, filtered through a 100 µm nylon filter (44% surface pore area; Sefar Nitex 100/44, Sefar, Thal, Switzerland) for separation of liquid (LP) and solid (SP) phases. The remaining material (1L) was processed as total or non-representative duodenal digesta. Samples of ileal digesta were divided into two: one used to estimate digesta flow and composition (100 mL) and one used for microbial isolation (150 mL). The LP and SP of duodenal samples and ileal digesta were weighed, frozen at -20°C, freeze-dried for 72 h, ground in a Willey mil (Thomas Scientific, Swedesboro, NJ) through 1- and 2-mm screens, and stored for further analysis.

Bacterial isolation of the duodenal and ileal digesta was carried out following methodology described by Reynal et al. (2005) with adaptations suggested by Krizsan et al. (2010) to estimate rumen microbial protein flow and digestibility. Pooled digesta samples (1 L) were filtered on a 100 µm nylon filter, (44% surface pore area; Sefar Nitex 100/44, Sefar, Thal, Switzerland) and the retained material was washed with 800 mL of 0.9% (wt/vol) NaCl. The retained material was then stored for further isolation of the particle associated bacteria (PAB). Then, the liquid associated bacteria (LAB) were

isolated from the filtered liquid sample. After centrifugation (1,000 × g for 10 minutes at 5°C) the pellet was stored for PAB isolation (described below). The supernatant was centrifuged at 11,250 × g for 30 minutes at 5°C. Supernatant was discarded and 200 mL of McDougall's solution was added to the pellet. Then, samples were centrifuged at 16,500 × g for 20 minutes at 5°C and the resulting pellet composed of LAB was frozen (-80°C), freeze-dried for 72 h and subsequently ground through a 1-mm screen for further analysis. For PAB isolation, 700 mL of saline solution containing 1% Tween-80 (v/v) was added to the pots containing the pellet from the first centrifugation of LAB isolation and the solid phase retained in the nylon filter, homogenized for 30 seconds and stored in a refrigerator (4°C) overnight to detach the bacteria attached to particles. Subsequently, the samples were filtered through a nylon filter, the liquid obtained centrifuged (1,000 × g for 10 minutes at 5ºC), the supernatant obtained centrifuged (11,250 × g; 30 minutes at 5ºC); and to the pellet resulting from this centrifugation added 200 mL of McDougall buffer solution which was centrifuged again at 16,250 x g for 20 minutes at 5°C. The pellet (PAB) obtained was be frozen (-80°C) and lyophilized (72-h). The purine bases were analyzed according to Ushida et al. (1985) with modifications suggested by Zinn and Owens (1986).

2.4. Blood parameters

At day 18 of each experimental period, blood samples (10mL) were collected from the jugular vein of the animals through the catheter at 6h after morning feeding. Then, blood samples were placed in tubes containing heparin (143 IU) and centrifuged (1000 × g for 20 minutes at 4°C) for plasma separation. After centrifugation, the plasma samples were transferred to microtubes (Eppendorf) and frozen at -20°C until the further analysis.

2.5. Amino acid analysis

The amino acid composition of the feed, duodenal (SP and NRD) and ileal digesta, duodenal microbial pellet (LAB and PAB), and blood samples were determined according to Hagen et al. (1993) by high performance liquid chromatograph (HPLC), using an analyzer of amino acids SPC 1000 adapted with a pre-column derivatization system with phenyl isothiocyanate (PITC), LUNA C18 100 Å 5u, 250x4.6mm 00G-4252-EQ silica reverse phase column and UV detection at 254 nm. Hydrolysis of the samples (200 mg) was performed in 9 mL 6 N HCl with 3%

(wt/vol) phenol in a vacuum sealed tube in a thermal reaction block for 24 h at 110°C. Then, an aliquot of the α-aminobutyric acid internal standard was added. The samples were then dried at 70 millitorr in cryogenic nitrogen trap system and neutralized with a 4:4:2 solution of 0.2 N sodium acetate trihydrate, methanol HPLC grade, and triethylamine. Then, the samples were dried again as previously described. In this step, PITC is added to derivatize the amino acids released by hydrolysis to form the PTC-amino acid. To the tube containing the derivatized amino acid crystals, 500 uL of mobile phase A is added as diluent. UV detection is performed at 254 nm after reverse phase chromatography (30 uL injection loop, pH 6.40, binary linear gradient with 1mL/min flow and column temperature 58°C), mobile phase A being a buffer 0.14 N sodium acetate, acetonitrile (240 mL/2000 mL 0.14 N sodium acetate) and triethylamine (1mL/2000 mL 0.14 N sodium acetate). Mobile phase B is a 6:4 solution of acetonitrile (HPLC grade) and milli-Q water. For AA analysis, plasma (200 µL) was mixed with 50 µL of a 0.1 N HCl solution containing alpha-aminobutyric acid as an internal standard and 250 µL of methanol 99%. Samples were vortexed for 10 seconds and centrifuged (13,000 × g for 10 min at 4°C) and dried in the vacuum station, up to 70 millitorr. Then, 20 µL of derivatizing solution containing PITC was added, vortexed for 10 seconds and rested for 20 minutes, and dried again. Then, 500 µL of diluent was added to derivatized amino acid crystals and left on ultrasound for 10 minutes, homogenized by vortexing for 15 seconds, and filtered through 0.45 µm Millex into the vial.

2.6. Calculations

Total digestible nutrients of the diets were calculated based on the following equation: TDN = CPD + 2.25 × EED + NFCD + NDFD – 7 (Weiss et al., 1992). Where: CPD = digestible crude protein, EED = digestible ether extract, NFCD = digestible non-fibrous carbohydrates, NDFD = digestible neutral detergent fiber.

Duodenal digesta flow was calculated based on a single internal marker (iNDF) and the reconstitution technique was conducted according to Faichney (1975) by using a combination of 2 markers (Co as the fluid phase marker and iNDF as the solid phase marker). The reconstitution factor was calculated based on the concentrations of the markers in the different digesta phases (France and Siddons, 1986). The reconstitution factors of the solid phase were used to mathematically reconstruct the composition of true duodenal digesta. The ileal flow was calculated based on a single internal marker,

by dividing iNDF intake by its concentration on ileal digesta. Microbial protein flow of LAB and PAB was calculated by dividing the flow of nucleic acid-N (g/day) by the ratio of nucleic acid-N/total N of each fraction of bacteria.

The duodenal and ileal flow (g/d) of each individual AA was calculated by multiplying the respective concentration (%) in the digesta sample by the DM flow from the respective sampling site. The duodenal flow (g/d) of each individual microbial AA was calculated by multiplying the respective concentration (%, CP basis) of each AA in the isolated bacteria (LAB and PAB) by microbial CP flow.

The AA deposition in body tissue was calculated as N retention × 6.25 × AA concentration in body protein (g AA/g protein). We used the data of AA body composition of Nellore cattle from comparative slaughter studies of Amaral (2016). The apparent intestinal digestibility of individual AA was calculated by difference between duodenal and ileal flows of AA, divided by duodenal AA flow. The intestinal supply of AA for maintenance and gain was considered as duodenal AA flow minus ileal AA flow. Gross efficiency of utilization (%) was calculated based on AA deposited in body tissue (g/day) divided by intestinal supply of AA (g/day).

2.7. Statistical analysis

Data were subjected to least squares ANOVA using the MIXED procedure of SAS (Statistical Analysis System, version 9.4 for Windows) as a 6 × 6 Latin square arrangement (balanced for residual effects) in a 2 × 3 factorial (two CP levels: 11 and 14% CP; and three CP sources: soybean meal plus urea, corn gluten, and DDG). The model used was:

$Y_{ijkl} = \mu + S_i + L_j + S_i \times L_j + Si \times L_j + A_k + P_l + \varepsilon_{ijkl}$

where: Y_{ijkl} = dependent variable, μ = overall mean; S_i = fixed effect of protein source; L_j = fixed effect of protein level; $S_i \times L_j$ = interaction between protein source and level; A_k = random effect of animal; P_l = random effect of experimental period; and ε_{ijkl} = random error, assumption of normal distribution.

The results were evaluated for the homoscedasticity of the variances and normality of the data. In cases where the variances were identified as heterogeneous the ANOVA was performed considering heterogenous variances by using the command REPEATED/GROUP. The treatment means were estimated using the LSMEANS and compared using the Tukey test. Least square means and SEM are reported for all data, with significance set at $P \leq 0.05$ and trends discussed at $0.05 < P$

≤ 0.10. Outliers were removed when studentized residuals were greater than 3 or less than -3.

3. RESULTS

There were no interactions between PS and PL for flow of any AA evaluated ($P > 0.05$). The duodenal flow of essential (EAA) and non-essential AA (NEAA) was not affected ($P > 0.05$) by PS and PL (Table 3).

Table 3. Effects of protein sources and inclusion levels on AA duodenal flow of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]						SEM	*P* - value		
	11% CP			14% CP						
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**		**PS**	**PL**	**PS × PL**
Duodenal flow (g/day)										
EAA	366.5	329.6	357.0	369.0	365.8	412.2	24.1	0.703	0.398	0.840
NEAA	286.4	259.2	277.6	297.0	286.1	315.4	18.8	0.759	0.382	0.926
Essential amino acids (EAA, g/day)										
Arginine	31.2	28.6	30.1	32.4	29.7	33.8	2.03	0.699	0.510	0.929
Histidine	15.3	13.7	15.7	16.0	14.6	17.0	1.08	0.515	0.549	0.984
Isoleucine	31.5	26.9	28.3	32.6	30.3	32.5	1.98	0.628	0.327	0.908
Leucine	60.7	60.5	64.6	60.2	70.9	74.6	4.50	0.562	0.338	0.770
Lysine	38.4	31.5	33.3	41.0	33.7	36.5	2.34	0.275	0.454	0.991
Methionine	11.6	10.2	12.3	12.2	12.4	13.0	0.962	0.756	0.431	0.889
Phenylalanine	30.9	29.3	30.9	32.7	32.6	36.3	2.17	0.799	0.299	0.904
Threonine	32.8	27.3	30.7	33.4	32.7	33.5	1.89	0.762	0.408	0.853
Valine	34.1	30.1	32.4	35.9	32.8	37.7	2.19	0.582	0.327	0.902
Non-essential amino acids (NEAA, g/day)										
Alanine	49.2	46.2	48.4	50.3	51.9	57.7	3.44	0.797	0.312	0.817
Aspartic	62.8	53.1	56.8	65.9	57.6	63.8	3.88	0.454	0.408	0.962
Cystine	11.6	8.78	12.2	11.5	12.5	11.7	0.782	0.639	0.388	0.287
Glutamate	99.8	96.0	101.1	101.8	108.3	119.8	7.16	0.738	0.323	0.824
Glycine	47.3	35.8	39.5	44.0	35.0	44.8	2.79	0.159	0.931	0.690
Proline	37.5	36.0	41.7	35.9	40.7	49.1	2.89	0.282	0.453	0.722
Serine	30.7	28.3	30.6	31.3	31.4	35.1	2.03	0.719	0.380	0.876
Tyrosine	27.6	24.7	26.7	28.1	28.3	29.9	1.74	0.842	0.369	0.880

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between dietary protein source and level of inclusion.

There was an interaction between PS and PL on the proline concentration ($P < 0.05$) and tendency of interaction on the arginine, histidine and lysine concentration in rumen microbial protein ($P < 0.10$). Proline concentration was increased in SU diets, reduced in CGM diets and not affected in DDG diets by the increase in PL. Diets containing DDG and 11% CP tended ($P < 0.10$) to have greater arginine and histidine concentration in rumen bacteria compared to SU and GGM diets with 11% CP; however, there was no difference between PS within the 14% CP level. Arginine concentration was greater ($P < 0.05$) in rumen bacteria from diets containing DDG compared to the diets containing SU and CGM (4.15, 3.93 and 3.91%, respectively; Table 4). Histidine concentration was greater ($P < 0.05$) in rumen bacteria from diets containing CGM or DDG compared to the diets containing SU (1.87, 1.94 and 1.71%, respectively). Lysine concentration in rumen bacteria tended ($P < 0.10$) to be greater as the PL increased in the CGM diets. Tyrosine concentration tended ($P < 0.10$) to be greater in the diets containing CGM compared to DDG diets (3.79, 3.68 and 3.64%, respectively). Also, increasing PL in the diet from 11 to 14% CP tended ($P < 0.10$) to lead to higher concentrations of tyrosine in microbial protein (3.65 and 3.76%, respectively).

Table 4. Effects of protein sources and inclusion levels on AA composition of rumen microbial protein of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]						SEM	*P* - value		
	11% CP			14% CP						
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**		**PS**	**PL**	**PS × PL**
Essential AA, % CP										
Arginine	3.84	3.82	4.22	4.02	3.99	4.08	0.044	0.014	0.279	0.093
Histidine	1.71	1.82	2.04	1.71	1.92	1.83	0.036	0.015	0.508	0.089
Isoleucine	4.46	4.16	4.54	4.49	4.36	4.36	0.069	0.296	0.897	0.443
Leucine	6.71	6.75	7.07	6.77	6.86	6.85	0.075	0.287	0.880	0.463
Lysine	5.61	5.02	5.53	5.21	5.62	5.35	0.084	0.796	0.979	0.050
Methionine	2.07	2.13	2.23	2.16	2.13	2.14	0.029	0.593	0.937	0.492
Phenylalanine	4.02	3.97	4.12	4.13	4.09	4.18	0.041	0.278	0.131	0.920
Threonine	4.23	4.04	4.42	4.25	4.23	4.24	0.045	0.116	0.905	0.138
Valine	4.65	4.43	4.79	4.67	4.56	4.60	0.073	0.342	0.909	0.534
Non-essential AA, % CP										
Alanine	5.95	6.13	6.50	6.30	6.34	6.25	0.067	0.279	0.396	0.132
Aspartic	8.42	8.28	8.46	8.11	8.34	8.79	0.183	0.648	0.944	0.742
Cystine	1.62	1.53	1.62	1.42	1.52	1.59	0.031	0.334	0.157	0.314
Glutamate	10.4	11.4	10.8	10.6	10.9	10.5	0.143	0.132	0.532	0.535
Glycine	5.30	4.96	5.31	5.26	5.13	5.10	0.056	0.210	0.802	0.378
Proline	3.36c	3.85a	3.64ab	3.59ab	3.45bc	3.53bc	0.067	0.233	0.282	0.025
Serine	3.66	3.75	3.93	3.72	3.78	3.77	0.041	0.149	0.740	0.315
Tyrosine	3.56	3.60	3.79	3.80	3.67	3.80	0.044	0.092	0.082	0.203

[a–b]Least squares means within the same row with different superscripts differ ($P \leq 0.05$). [1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between dietary protein source and level of inclusion.

There was no interaction between PS and PL on the supply of AA from microbial protein ($P > 0.05$; table 5). Microbial protein synthesis, proportion between LAB and PAB, and microbial AA digestibility were not affected by PL or PS ($P > 0.05$). Animals fed DDG tended ($P < 0.10$) to have greater EAA, arginine, lysine and leucine supply from microbial protein than those diets containing SU or CGM. Animals fed DDG had greater ($P < 0.05$) NEAA supply from microbial protein flow than those fed diets containing SU, but they did not differ from animals fed CGM diets (228.7, 197.2 and 221.7 g/day respectively). Histidine and glutamate from microbial protein had a greater supply ($P < 0.05$) by the dietary inclusion of CGM or DDG compared to SU diets. Animals fed CGM or DDG tended ($P < 0.10$) to have greater proline and serine flow from microbial protein than those fed SU diets.

Table 5. Effects of protein sources and inclusion levels on AA duodenal flow from microbial protein and its digestibility of feedlot Nellore steers fed high-concentrate diets.

Item[2]	Diets[1]						SEM	*P* - value		
	11% CP			14% CP				PS	PL	PS × PL
	SU	CGM	DDG	SU	CGM	DDG				
Nmic (g/day)	75.2	54.5	74.6	51.8	69.0	72.5	5.23	0.571	0.704	0.298
LAB proportion (% Nmic)	50.7	36.6	39.1	26.9	47.4	40.9	4.34	0.948	0.647	0.223
PAB proportion (% Nmic)	49.3	63.4	60.9	73.1	52.6	59.1	4.34	0.948	0.647	0.223
EAA flow (g/day)	176.5	170.2	219.2	169.8	202.0	181.5	10.5	0.108	0.675	0.174
NEAA (g/day)	201.9	209.6	248.1	192.5	233.8	209.3	14.7	0.049	0.395	0.181
Microbial AA digestibility (%)	84.4	77.9	77.1	76.2	86.6	83.1	1.61	0.590	0.347	0.567
Essential amino acids (g/day)										
Arginine	18.2	18.0	23.5	18.0	21.6	19.3	1.24	0.084	0.832	0.171
Histidine	8.30	8.94	11.4	8.31	10.7	9.10	0.667	0.010	0.653	0.116
Isoleucine	21.1	19.2	25.4	20.3	22.8	20.5	1.39	0.378	0.641	0.136
Leucine	31.6	31.1	38.9	29.7	35.3	33.2	2.09	0.074	0.515	0.147
Lysine	26.6	23.6	32.0	24.2	31.2	26.6	1.82	0.080	0.973	0.112
Methionine	9.91	10.9	12.7	9.87	12.0	10.3	0.674	0.112	0.436	0.143
Phenylalanine	18.9	18.2	23.6	18.1	21.2	19.8	1.25	0.121	0.664	0.150
Threonine	20.1	19.4	24.8	19.2	22.8	20.4	1.33	0.148	0.595	0.191
Valine	21.9	20.1	26.6	21.1	23.5	21.7	1.44	0.270	0.608	0.146
Non-essential amino acids (g/day)										
Alanine	29.5	30.2	36.5	28.4	34.3	29.6	2.00	0.153	0.464	0.195
Aspartic	40.1	41.3	48.1	36.9	45.7	40.3	2.92	0.192	0.390	0.218
Cystine	7.67	7.40	9.04	6.34	8.44	7.68	0.539	0.175	0.354	0.215
Glutamate	49.8	54.1	60.8	48.1	60.1	52.4	3.45	0.012	0.446	0.138
Glycine	25.2	24.5	30.4	24.0	28.7	25.4	1.63	0.123	0.605	0.154
Proline	15.7	16.7	19.4	14.6	16.8	16.7	0.978	0.064	0.207	0.506
Serine	17.2	18.1	21.6	16.0	20.1	18.1	1.12	0.055	0.355	0.145
Tyrosine	16.8	16.3	21.7	16.5	18.7	18.0	1.12	0.110	0.651	0.206

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between dietary protein source and level of inclusion. [2]Nmic: microbial nitrogen, LAB: liquid associated bacteria, PAB: particle associated bacteria.

There was no interaction between PS and PL for AA flow from RUP sources ($P > 0.05$; Table 6). Animals fed CGM or DDG tended ($P < 0.10$) to have greater EAA, arginine, isoleucine and valine supply from RUP fraction. The Leucine supply from RUP was greater ($P < 0.05$) in animals fed CGM or DDG compared to those fed SU diets (49.7, 56.8 and 27 g/day, respectively).

There was no interaction between PS and PL, nor effect of PS and PL for AA apparently absorbed from the small intestine (g/day; $P > 0.05$; Table 7).

Table 6. Effects of protein sources and inclusion levels on AA duodenal flow from RUP plus endogenous sources of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* - value		
	11% CP			14% CP			SEM			
Item	SU	CGM	DDG	SU	CGM	DDG		PS	PL	PS × PL
EAA flow (g/day)	109.9	184.3	200.3	106.6	176.4	196.6	15.0	0.067	0.860	0.997
NEAA (g/day)	184.6	249.4	140.5	238.6	226.4	264.8	20.6	0.738	0.193	0.330
Essential amino acids (g/day)										
Arginine	13.0	21.5	21.1	11.6	17.9	20.9	1.43	0.053	0.517	0.875
Histidine	7.00	9.27	8.88	6.50	7.60	9.67	0.788	0.405	0.759	0.804
Isoleucine	10.4	18.6	16.8	10.3	17.1	18.8	1.46	0.095	0.950	0.897
Leucine	29.1	44.6	60.9	24.9	54.7	52.8	4.13	0.025	0.916	0.580
Lysine	14.5	22.6	13.9	14.7	13.2	16.3	1.78	0.706	0.525	0.386
Methionine	2.48	4.45	7.45	2.09	5.56	6.72	0.847	0.131	0.999	0.891
Phenylalanine	12.0	17.3	11.5	18.7	18.3	20.6	1.96	0.862	0.155	0.661
Threonine	12.7	21.3	11.8	13.2	14.5	18.0	1.60	0.430	0.983	0.253
Valine	12.2	21.6	20.5	12.4	19.3	23.4	1.71	0.078	0.934	0.833
Non-essential amino acids (g/day)										
Alanine	19.8	36.0	14.8	31.3	29.2	34.9	3.04	0.451	0.154	0.162
Aspartic	27.2	36.6	22.3	39.4	28.8	31.2	3.38	0.688	0.522	0.456
Cystine	3.90	8.10	4.20	7.50	6.48	5.43	0.703	0.253	0.361	0.272
Glutamate	50.1	58.0	41.4	59.5	69.7	78.2	6.32	0.824	0.129	0.598
Glycine	42.0	29.7	28.1	35.6	30.9	40.0	2.75	0.309	0.624	0.285
Proline	21.8	23.3	20.7	21.7	29.5	35.8	2.52	0.575	0.178	0.486
Serine	13.5	21.2	9.70	19.4	18.3	21.6	1.85	0.596	0.162	0.250
Tyrosine	10.8	17.4	5.35	15.8	15.7	15.7	1.59	0.312	0.158	0.311

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between dietary protein source and level of inclusion.

Table 7. Effects of protein sources and inclusion levels on intestinal apparent absorption of amino acids of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]						SEM	*P* - value		
	11% CP			14% CP						
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**		**PS**	**PL**	**PS × PL**
Apparent absorption of EAA (g/day)										
EAA	267.3	242.4	274.2	260.6	284.2	267.3	16.1	0.972	0.747	0.750
Arginine	30.7	24.2	26.7	27.3	27.0	31.1	2.06	0.586	0.690	0.589
Histidine	14.3	10.8	12.8	12.6	12.4	15.0	1.04	0.374	0.655	0.558
Isoleucine	30.5	21.8	23.8	26.1	26.6	28.8	2.00	0.484	0.528	0.333
Leucine	58.2	50.9	55.6	48.3	63.8	66.7	4.42	0.654	0.495	0.356
Lysine	37.4	26.0	28.5	33.8	29.8	32.9	2.37	0.215	0.660	0.593
Methionine	10.8	8.18	10.5	9.83	11.1	12.4	0.963	0.620	0.411	0.585
Phenylalanine	29.9	24.2	26.4	26.3	28.9	32.2	2.16	0.767	0.483	0.469
Threonine	30.1	25.9	29.2	28.6	28.2	27.7	1.62	0.807	0.944	0.848
Valine	31.8	31.7	30.8	30.6	27.6	31.5	1.86	0.906	0.712	0.804
Apparent absorption of NEAA (g/day)										
NEAA	331.7	250.6	265.6	283.6	350.4	377.7	23.9	0.895	0.174	0.225
Alanine	44.8	42.8	47.3	42.7	51.3	49.2	3.02	0.777	0.611	0.737
Aspartic	61.6	44.4	49.3	55.7	52.5	58.7	3.87	0.340	0.496	0.504
Cystine	10.4	6.53	10.0	8.89	10.8	10.5	0.805	0.506	0.390	0.152
Glutamate	96.6	80.4	87.7	83.9	97.3	109.0	7.06	0.735	0.440	0.417
Glycine	43.3	29.5	33.2	36.0	30.1	40.4	2.82	0.175	0.964	0.427
Proline	35.0	29.3	34.7	27.7	34.8	42.4	2.76	0.359	0.654	0.365
Serine	28.2	23.0	25.7	24.7	27.4	31.1	2.02	0.670	0.498	0.470
Tyrosine	26.7	20.3	22.8	22.8	25.4	26.7	1.77	0.778	0.534	0.356

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS **×** PL: interaction between dietary protein source and level of inclusion.

There was interaction between PS and PL for intestinal apparent digestibility of most of AA evaluated ($P < 0.05$; Table 8). Increasing the PL from 11 to 14% CP in the SU diets reduced the digestibility of isoleucine, leucine, and tyrosine ($P < 0.05$). In contrast, the digestibility of leucine, methionine, aspartic acid, glutamate, serine, and tyrosine was improved by the increase in the PL in DDG diets ($P < 0.05$). Increasing the PL in SU diets tended to reduce the digestibility of lysine and valine ($P < 0.10$). There was a tendency ($P \leq 0.10$) of greater phenylalanine, threonine, alanine, proline, and glycine digestibility as the PL increased in the DDG diets. Increasing the PL in CGM diets improved cystine digestibility ($P < 0.05$).

Table 8. Effects of protein sources and inclusion levels on intestinal apparent digestibility of amino acids of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* - value		
	11% CP			14% CP						
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**	**SEM**	**PS**	**PL**	**PS × PL**
Essential AA, %										
Arginine	91.1	89.8	87.8	87.4	90.5	90.9	0.728	0.817	0.959	0.151
Histidine	87.1	84.2	82.0	82.4	85.6	87.4	0.992	0.996	0.701	0.143
Isoleucine	89.1a	85.7ab	82.9b	82.4b	87.6b	87.1ab	0.978	0.667	0.869	0.039
Leucine	89.1a	87.5ab	83.1b	83.0b	89.9a	89.4a	1.01	0.322	0.578	0.020
Lysine	90.0	87.8	85.1	85.2	88.0	88.6	0.814	0.786	0.806	0.075
Methionine	88.1a	83.9abc	81.9c	83.8ac	88.6a	89.6ab	1.28	0.971	0.121	0.031
Phenylalanine	89.1	86.5	84.4	86.4	88.9	88.5	0.684	0.474	0.247	0.063
Threonine	87.0	83.6	81.7	84.2	85.1	85.5	0.741	0.319	0.440	0.101
Valine	87.7	84.4	81.5	80.5	86.2	85.2	1.08	0.612	0.730	0.052
Non-essential AA, %										
Alanine	87.4	84.5	82.6	83.9	87.3	87.1	0.840	0.751	0.335	0.074
Aspartic	91.3a	87.4ab	83.9b	87.8a	90.2a	90.7a	0.819	0.220	0.073	0.007
Cystine	84.9ab	73.1b	77.7ab	78.1b	85.4a	83.4a	1.97	0.785	0.182	0.035
Glutamate	89.8a	87.2ab	83.4b	85.4ab	89.4a	90.4a	0.863	0.743	0.234	0.012
Glycine	89.2	86.8	84.2	86.6	86.8	88.2	0.635	0.351	0.616	0.050
Proline	86.5	81.9	80.2	83.3	85.4	86.9	1.16	0.735	0.159	0.065
Serine	86.9a	84.9ab	83.3b	84.9ab	87.4a	88.1a	0.663	0.905	0.083	0.049
Tyrosine	89.5a	85.9ac	82.3c	83.5bc	88.6ab	88.7ab	1.05	0.640	0.479	0.016

[a–c]Least squares means within the same row with different superscripts differ ($P \leq 0.05$). [1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between dietary protein source and level of inclusion.

Animals fed soybean plus urea tended ($P < 0.10$) to have greater arginine and lysine concentration in plasma (Table 9). Plasma histidine concentration tended ($P < 0.10$) to be greater in animals fed DDG diets. Plasma leucine concentration was greater ($P < 0.05$) in animals fed CGM or DDG compared to those fed SU diets (180.3, 175.3 and 142.3 µM, respectively). In addition, animals fed diets containing 14% CP had greater ($P < 0.05$) leucine concentration compared to the diets containing 11% CP (180.0 and 152.3 µM, respectively). Plasma phenylalanine concentration was greater ($P < 0.05$) in animals fed diets containing CGM or DDG compared to those fed SU diets (91.5, 90.3 and 82.9 µM, respectively). Plasma valine concentration was greater ($P < 0.05$) in animals fed diets with a CP level of 14% compared to fed 11% CP diets (198.3 and 170.3 µM, respectively). Plasma glutamine concentration was greater ($P < 0.05$) in animals fed SU diets compared to those fed CGM or DDG diets (189.6, 149.1 and 159.1 µM, respectively). Plasma glycine concentration was greater ($P < 0.05$) in animals fed SU diets compared to those fed CGM or DDG diets (167.5, 146.4 and 131.8 µM, respectively).

Table 9. Effects of protein sources and inclusion levels on plasma AA concentration of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]							*P* - value		
	11% CP			14% CP			SEM			
Item	SU	CGM	DDG	SU	CGM	DDG		PS	PL	PS × PL
Essential amino acids (µM)										
Arginine	104.7	79.6	77.4	90.3	82.3	85.4	3.13	0.057	0.835	0.290
Histidine	56.3	52.3	56.6	50.4	51.5	66.7	1.85	0.055	0.721	0.157
Isoleucine	79.8	72.5	76.2	78.4	82.8	83.7	2.48	0.934	0.301	0.625
Leucine	144.4	159.1	153.4	140.1	201.5	197.2	8.00	0.047	0.042	0.223
Lysine	71.1	50.4	50.2	55.4	49.0	52.7	2.76	0.087	0.339	0.327
Methionine	27.5	26.7	25.9	24.8	24.9	28.7	0.736	0.641	0.685	0.261
Phenylalanine	86.0	91.4	86.1	79.8	91.5	94.4	2.32	0.043	0.793	0.138
Threonine	72.3	70.6	66.4	61.3	69.3	82.3	3.56	0.654	0.856	0.280
Valine	171.7	160.8	178.3	181.1	220.5	193.4	6.55	0.547	0.022	0.150
Non-essential amino acids (µM)										
Alanine	173.1	162.2	168.8	167.4	155.3	178.3	4.34	0.221	0.890	0.595
Aspartic	38.2	37.6	35.6	36.1	38.1	35.8	0.683	0.206	0.623	0.498
Glutamate	142.0	133.1	154.9	146.3	140.9	142.6	6.86	0.394	0.992	0.483
Glutamine	189.1	161.2	153.6	190.0	137.0	150.4	6.96	0.024	0.442	0.608
Glycine	159.7	162.8	126.0	175.4	130.0	137.6	5.43	0.029	0.844	0.187
Proline	105.4	106.2	102.8	98.3	101.5	118.6	3.74	0.271	0.760	0.113
Serine	99.8	96.7	85.0	87.5	84.2	94.5	3.36	0.807	0.329	0.169
Tyrosine	63.9	65.7	61.3	65.3	72.0	75.1	2.48	0.725	0.144	0.565

[1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between dietary protein source and level of inclusion.

There were interactions, or tendencies for interactions, between PS and PL for gross AA utilization of all AA evaluated, except methionine and cystine (Table 10). Arginine and histidine utilization were greater in animals fed diets containing 11% CP with DDG and 14% CP with CGM. Animals fed the diet containing 11% CP and DDG showed greater isoleucine, lysine, phenylalanine, threonine, valine, alanine, aspartic, glutamate, proline, serine, and tyrosine use efficiency than other diets, except diet 14% CP with CGM which did not differ. The greatest glycine use efficiencies were observed in animals fed with 11% CP with DDG or 14% CP with CGM. Methionine and cystine utilization were not affected by PS or PL ($P > 0.05$).

Table 10. Effects of protein sources and inclusion levels on gross AA use efficiency of feedlot Nellore steers fed high-concentrate diets.

	Diets[1]						SEM	*P* - value		
	11% CP			14% CP						
Item	**SU**	**CGM**	**DDG**	**SU**	**CGM**	**DDG**		**PS**	**PL**	**PS × PL**
Essential amino acids, %										
Arginine	57.5b	52.1b	83.3a	45.4b	79.4a	51.8b	4.15	0.130	0.392	0.013
Histidine	42.6b	45.2b	61.4a	21.5c	58.3a	37.1bc	2.94	0.005	0.015	0.004
Isoleucine	28.6c	30.5bc	47.2a	26.1c	40.7ab	29.6bc	2.25	0.056	0.331	0.023
Leucine	27.7ab	26.1b	38.4a	25.3b	31.9ab	23.3b	1.80	0.536	0.230	0.059
Lysine	42.6c	42.5c	67.9a	34.9c	63.5ab	44.9bc	3.52	0.056	0.546	0.025
Methionine	42.3	48.1	59.4	34.5	53.0	39.9	2.89	0.152	0.176	0.193
Phenylalanine	27.2b	26.1b	39.0a	23.6b	34.1ab	23.9b	1.87	0.272	0.257	0.036
Threonine	28.5c	28.6bc	43.1a	24.2c	41.7ab	27.8c	2.27	0.116	0.540	0.030
Valine	31.6c	32.4bc	49.7a	28.9c	44.8ab	31.2bc	2.44	0.096	0.426	0.021
Non-essential amino acids, %										
Alanine	37.2bc	35.1bc	54.1a	32.8bc	46.4ab	32.1b	2.60	0.313	0.251	0.032
Aspartic	24.0bc	24.0bc	37.2a	20.2c	33.6ab	23.5bc	1.85	0.096	0.370	0.028
Cystine	22.0	30.1	27.8	16.9	25.0	20.4	1.97	0.305	0.179	0.962
Glutamate	25.2b	27.9b	42.5a	29.9b	36.4ab	24.9b	2.00	0.317	0.175	0.029
Glycine	67.6b	70.6b	108.9a	55.2b	112.4a	64.7b	5.96	0.035	0.551	0.008
Proline	53.3ab	52.1ab	70.8a	49.7ab	66.2ab	41.2b	3.62	0.639	0.349	0.065
Serine	33.9ab	31.5b	47.0a	28.3b	42.2ab	28.3b	2.30	0.337	0.247	0.034
Tyrosine	23.9b	23.5b	35.6a	20.5b	30.9ab	22.2b	1.70	0.179	0.278	0.041

[a–c]Least squares means within the same row with different superscripts differ ($P \leq 0.05$). [1]Soybean meal + urea (SU); Corn gluten meal (CGM); Dried distillers grains (DDG). SEM: Standard error of the mean. PS: Crude protein source (soybean meal, corn gluten meal, and dried distillers grains), PL: Crude protein level (11 and 14%), and PS × PL: interaction between dietary protein source and level of inclusion.

4. DISCUSSION

Schwab and Broderick (2017) have stated the importance of better understanding the effect of diet composition on the contribution of AA from rumen microbes for the development of models that address these changes to the AA supply of the animal. In this study, the dietary levels and source of protein affected the gross efficiency of AA use, which supports the importance of known individual variation of AA in future models to predict AA requirements in beef cattle instead of using a single factor for all AA. The similar duodenal supply of AA among diets may be related to the fact that steers tended to increase DMI in the lower CP level (unpublished data). In fact, the total AA supply by per unit of DMI was significatively greater for the diets containing 14% CP than for the 11% CP diets. Animals fed DDG had an increase in EAA supply from microbial protein, which highlights the potential of DDG to supply EAA in beef cattle.

Considering that MCP contributes more than 50% of duodenal CP flow (Clark et al., 1992), the AA supply from rumen microorganisms must be accurately represented to determine the duodenal flow of AA when a factorial approach is used to estimate AA duodenal flow (Sok et al., 2017). Our data indicate that bacterial AA contributed with approximately 63.4% of total AA reaching duodenum. In this study, the use of RUP sources tended to increase the supply of EAA from microbial protein with highlights for arginine, lysine, and leucine for diets containing DDG and histidine for diets containing either CGM or DDG. In addition, Sok et al. (2017) found higher concentrations of alanine, glycine, and threonine in FAB than in PAB, whereas concentrations of arginine, leucine, phenylalanine, and proline were lower in FAB than in PAB. However, microbial production and the proportion of FAB and LAB remained constant across treatments in this study which suggests a variation in terms of microbial AA composition across treatments. In fact, there was interaction or tendency of interaction between PS and PL on microbial AA composition in this study. In addition, arginine and histidine concentration significantly increased in rumen microbial protein by the use of DDG. In accordance with our findings, Clark et al. (1992) reported large differences in AA composition in a database of 441 bacterial samples from animals fed 61 dietary treatments in 35 experiments, expressed as grams of individual AA per 100 g of AA. Our results of microbial AA digestibility are in agreement with those found by Mariz et al. (2018), and also adopted by BR-CORTE (Valadares et al., 2016).

The use of RUP sources can be a strategy to alter postruminal supply of AA (Merchen and Titgemeyer, 1992). However, little is known about the AA content of RUP fraction (NASEM, 2016). In this study, we estimated the AA supply from RUP plus endogenous sources by subtracting the microbial AA supply from duodenal AA flow. Our results suggest opportunities to increase the flow of EAA (mainly arginine, leucine, isoleucine and valine) from RUP sources by the use of CGM or DDG in the diet. Recently, some AA has been associated to changes in phosphorylation of translation factors related to protein synthesis in the mammary tissue have been observed (Rius et al., 2010; Arriola Apelo et al., 2014). For instance, it was showed that Arginine, Leucine and isoleucine has a role activating mTOR pathway, which is related to the protein synthesis (Arriola Apelo et al., 2014; Sabatini, 2017; Liu et al., 2017). In line with these findings, Martineau et al. (2013) observed consistent and positive results in milk production and milk protein yield with use of canola meal replacing different protein sources in the diet, including soybean meal. These results were attributed to a more efficient N use in the diets with greater microbial protein synthesis and/or improved supply of essential AA when canola meal was fed (Maxin et al., 2013).

The amount of dietary protein that is converted to animal products (i.e. meat and milk) is influenced by the AA profile that reaches the small intestine and its digestibility (Mariz et al., 2018). The protein (AA) digestibility is not stable under different feedings conditions with variations in CP and energy intakes (Yang and Beauchemin, 2004; Huang et al., 2019). In addition, protein source and degradability of dietary CP may also cause variation among sources of common ingredients (Maxin et al., 2018). In this study, there was an interaction between PS and PL for apparent intestinal digestibility of some essential and non-essential AA. The higher leucine digestibility observed in the RUP sources (CGM or DDG) with 14% CP suggest a greater supply of this AA compared to the SU diets. Decreased mTORC1 activation due to Leu deficiency might lead to decreased mammary tissue anabolic activity and milk protein yield (Yoder et al, 2020). However, However, this theory of specific AA driving protein synthesis by mTORC1 regulation has not been fully elucidated in beef cattle.

Although the duodenal flow of AA was not affected by treatments, the differences observed in apparent intestinal digestibility may be related to the AA amount and profile reaching the duodenum (Hanigan et al., 1998; Mariz et al. 2018), and also to the RUP digestibility of the different protein sources used in this study as the microbial protein digestibility was similar across treatments. Determining the apparent AA digestibility for the diet or RUP in ruminants is technically difficult due

largely to errors of measurement associated with sample collection and animal variation (NRC, 2001).

Plasma AA are difficult to interpret because they are affected by several factors (Batista et al., 2016). There are two complementary aspects that need to be considered when interpreting net results of plasma AA concentrations when a significant increase or decrease is observed: one is to assume that an increase in the plasma concentration of an AA is due to positive effects on post ruminal protein supply and provision of digestible AA (Martineau et al., 2014). The second aspect is that a plasma AA concentration increases when the AA supply exceeds the protein synthetic capacity as dictated by the first limiting AA (Bergen, 1979). Otherwise, a decrease of plasma AA concentration may identify amino acids that become limiting with escape protein supplementation (Gibb et al., 1992). In comparison with the AA profile in the body tissues, the AA profile of microbial protein may be limiting in histidine, leucine and methionine (Sok et al., 2017; Mariz et al., 2018). Our findings suggest that DDG can be a good supplier of histidine by the increase of its concentration in the plasma. Also, leucine was increased by both CGM and DDG. Although the link is speculative, the use of the RUP sources (CGM or DDG) in this study could increase protein synthesis by a higher activation of mTOR pathway by histidine and leucine (Appuhamy et al., 2012; Arriola Apelo et al., 2014). Besides methionine and lysine, histidine has also been considered a potential limiting AA in dairy cows (Yoder et al., 2020). In fact, positive responses of supplementation of histidine on DMI, milk yield and milk protein yield in dairy cows fed a metabolizable protein-deficient diet (Giallongo et al., 2017; Zang et al., 2019). The increase in the plasma concentration of lysine and glycine in SU diets might be expected because soybean meal has a high content of these AA.

Most nutritional systems consider a sole factor for metabolizable protein use efficiency and are based solely on the equivalent shrunk BW of the animal (Mariz et al., 2018). However, the amount of AA flowing from rumen that is available for protein deposition is dependent on the intestinal flow, digestibility of the protein fractions in the small intestine, and maintenance requirements (NASEM, 2016). It has been suggested that not all the absorbed AA are converted into protein products (Titgemeyer, 2003). The inefficient use of absorbed AA is mainly due to oxidative losses related to these molecules (Lapierre et al., 2006), their use for urea synthesis (Awawdeh et al., 2004), and the limiting AA i.e. different AA profile in the metabolizable protein compared to animal requirement (NASEM, 2016). In fact, Batista et al. (2016) showed that lysine supplementation linearly increased N retention in growing steers fed diets deficient in

this AA. The combination of these factors clearly demonstrates the ineffectiveness of using a single factor for efficiency of protein (amino acids) use for gain. Therefore, it seems a reasonable goal that we use individual efficiency factors for each AA in ruminants. In this study, if we consider the average body weight (354 kg) of the animals used, the expected AA use efficiency predicted by Ainslie et al. (1993) would be 49%. Our data clearly demonstrate that we could overestimate the requirements for some AA and underestimate for other AA by using a single factor for AA use efficiency.

If all amino acids were used with the same efficiency (they are not), then we could determine which AA is most limiting by seeing which AA had the highest efficiency of use. The increase in the use of arginine, lysine and glycine agrees with their reduction in plasma and indicates a higher utilization of these AA in CGM and DDG diets. However, glycine is a non-essential AA, which we usually do not think about efficiencies of use because they are by definition produced by the body.

Additional in this study, we increased the PL from 11 to 14% CP by increasing the amount of each protein source in its respective diets. However, the increase in terms of AA profile was not equivalent. For example, by increasing PL in the SU diets the increase in arginine concentration in diets with 14% CP was of 30.1% compared to the diet with 11% CP while in the CGM and DDG treatments the increment was only of 18.7 and 19.7%, respectively (Table 2). This pattern may explain the interaction observed between protein level and protein source for AA use efficiency on the majority of AA evaluated and may be interpreted as an effect of the increase in the proportion of each source in the diet. In this situation, increase the amount of a protein source that is limiting in a specific AA due to a lower concentration or digestibility tended to increase its efficiency in order to compensate the reduction on its supply as can be observed for lysine utilization in the CGM diets in this study. The same argument can be applied for some essential AA that are not limiting in DDG, when the inclusion of this source is increased, they have a reduction in its efficiency (arginine, histidine, isoleucine, lysine, phenylalanine, threonine, valine). The values above 100% observed for glycine might reflect the endogenous synthesis to achieve the requirements for protein deposition (Titgemeyer, 2003). These values are feasible because glycine is a non-essential AA and is one of the most abundant AA in the body tissues *i.e*. about 12.4% of the CP (Amaral, 2016). Our findings not only suggest that we should use individual factors for the efficiency of use for each AA for estimate requirements, but also take in consideration the variation in AA use efficiency between protein sources.

5. CONCLUSIONS

The AA use efficiency is affected by dietary protein levels and sources. Future predictive models should take into consideration the variation across different diets and specific coefficient factors for individual AA digestibility and efficiency of use. Our results suggest that it is possible to increase the supply of essential AA using CGM or DDG (RUP sources) in the diet compared to soybean meal plus urea, especially in situations where it is possible to increase the microbial protein flow.

6. REFERENCES

Ainslie SJ, Fox DG, Perry TC, Ketchen DJ, Barry MC (1993) Predicting amino acid adequacy of diets to fed Holstein steers. **Journal of Animal Science** 71:1312-1319.

Allen MS, Linton JAV (2007) In vivo methods to measure digestibility and digestion kinetics of feed fractions in the rumen. In: **Simpósio Internacional de Avanços em Técnicas de Pesquisa em Nutrição de Ruminantes.** Universidade de São Paulo, São Paulo, Brazil. p.72–89.

Amaral, PM (2016) **Performance and amino acids requirements of Nellore and crossbred Angus×Nellore fed diets with different crude protein content during growing and finishing stages.** 100f. Tese (Doutorado em Zootecnia) – UFV, Viçosa.

Appuhamy JA, Knoebel NA, Nayananjalie WA, Escobar J, Hanigan MD (2012) Isoleucine and leucine independently regulate mTOR signaling and protein synthesis in MAC-T cells and bovine mammary tissue slices. **The Journal of Nutrition** 142:484-491.

Arriola Apelo SI, Singer LM, Lin XY, McGilliard ML, St-Pierre NR, Hanigan MD (2014) Isoleucine, leucine, methionine, and threonine effects on mammalian target of rapamycin signaling in mammary tissue. **Journal of Dairy Science** 97:1047–1056.

Awawdeh MS, Titgemeyer EC, McCuistion KC, GNAD DP (2005) Ruminal ammonia load affects leucine utilization by growing steers. **Journal of Animal Science** 83:2448-2454.

Awawdeh MS, Titgemeyer EC, Schroeder GF, Gnad DP (2006) Excess amino acid supply improves methionine and leucine utilization by growing steers. **Journal of Animal Science** 84:1801-1810.

Bergen WG (1979) Free amino acids in blood of ruminants-physiological and nutritional regulation. **Journal of Animal Science** 49:1577–1589.

Broderick GA (2017) Review: Optimizing ruminant conversion of feed protein to human food protein. **Animal** 12:1722-1734.

Batista ED, Hussein AH, Detmann E, Miesner MD, Titgemeyer EC (2016) Efficiency of lysine utilization by growing steers. **Journal of Animal Science** 94:648-655.

Campbell CG, Titgemeyer EC, St-Jean G (1996) Efficiency of D- vs L-methionine utilization by growing steers. **Journal of Animal Science** 74:2482-2487.

Campbell CG, Titgemeyer EC, St-Jean G (1997) Sulfur amino acid utilization by growing steers. **Journal of Animal Science** 75:230-238.

Clark JH, Klusmeyer TH, Cameron MR (1992) Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. **Journal of Dairy Science** 75:2304-2323.

COUNCIL 2012: A guide to distiller's dried grains with solubles (DDGS). USA, 2012, 406p. Available in: https://grains.org/wp-content/uploads/2018/01/Complete-2012-DDGS-Handbook.pdf

Detmann E, Souza MA, Valadares Filho SC, Queiroz AC, Berchielli TT, Saliba EOS, Cabral LS, Pina DS, Ladeira MM, Azevedo JAG (2012) **Métodos para análise de alimentos - Instituto Nacional de Ciência e Tecnologia em Ciência Animal.** 582 Suprema, Visconde do Rio Branco, Brazil.

Djikstra L, Oenema O, Bannink A (2011) Dietary strategies to reducing N excretion from cattle: implications for methane emissions. **Current Opinion in Enviromental Sustainability** 3:414-422.

Estes KA, White RR, Yoder PS, Pilonero T, Schramm H, Lapierre H, Hanigan MD (2018) An in vivo table isotope–based approach for assessment of absorbed amino acids from individual feed ingredients within complete diets. **Journal of Dairy Science** 101:7040-7060.

Etheridge RD, Pesti GM, Foster EH (1998) A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. **Animal Feed Science and Technology** 73:21-28.

Fleming AJ, Lapierre H, Martineau R, White RR, Hanigan MD (2019a) Modeling portal-drained viscera and liver fluxes of essential amino acids in dairy cows. **Journal of Dairy Science** 102:10964-10982.

Fleming AJ, Lapierre H, White RR, Tran H, Kononoff PJ, Martineau R, Weiss WP, Hanigan MD (2019b) Predictions of ruminal outflow of essential amino acids in dairy cattle. **Journal of Dairy Science** 102:10947-10963.

Faichney GJ (1975) The use of markers to partition digestion within the gastrointestinal tract of ruminants. In: Digestion and metabolism in the ruminant. In: Proceedings of fourth International Symposium on Ruminant Physiology, Sydney, Australia, p. 277-291.

Fox DG, Tedeschi LO, Tylutki TP, Russel JB, Van Amburgh ME, Chase LE, Pell AN, Overton TR (2004) The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. **Animal Feed Science and Technology** 112:29-78.

France J, Siddons RC (1986) Determination of digesta flow by continuous marker infusion. **Journal of Theoretical Biology** 136:485-486.

Giallongo F, Harper MT, Oh J, Parys C, Shinzato I, Hristov (2017) Histidine deficiency has a negative effect on lactational performance of dairy cows **Journal of Dairy Science** 100:2784-2800.

Gibb DJ, Klopfenstein TJ, Britton RA, Lewis AJ (1992) Plasma amino acid response to graded levels of escape protein. **Journal of Animal Science** 70:2885–2892.

Hagen SR, Augustin J, Grings E, Tassinari P (1993) Precolumn phenylisothiocyanate derivatization and liquid chromatography of free amino acids in biological samples. **Food Chemistry** 46:319-323.

Hanigan MD, Cant JP, Weakley DC, Beckett JL (1998) An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. **Journal Dairy Science** 81:3385-3401.

Hankins SL, Arseneau JD, Lemenager RP, Sutton AL (2005) Performance, carcass traits, and nutrient excretion of beef feedlot cattle fed a corn gluten feed diet. **The Professional Animal Scientist** 21:1-6.

Huang X, Estes KA, Yoder PS, Wang C, Jiang N, Pilonero T, Hanigan MD (2019) Assessing availability of amino acids from various feedstuffs in dairy cattle using a stable isotope-based approach. **Journal of Dairy Science** 102:10983-10996.

Jolly-Breithaupt ML, Nuttelman BL, Schneider CJ, Burken DB, Gramkow JL, Shreck AL, Macdonald JC, Klopfenstein TJ, Erickson GE (2018) Finishing performance and diet digestibility for feedlot steers fed corn distillers grains plus solubles and distillers solubles with and without oil extraction. **Journal of Animal Science** 96:1996-2011.

Krizsan SJ, Ahvenjärvi S, Volden H, Broderick GA (2010) Estimation of rumen outflow in dairy cows fed grass silage-based diets by use of reticular sampling as an alternative to sampling from the omasal canal. **Journal of Dairy Science** 9:1138-1147.

Lapierre H, Pacheco D, Berthiaume R, Ouellet DR, Schwab CG, Dubreuil P, Holtrop G, Lobley GE (2006) What is true supply of amino acids for a dairy cow? **Journal of Dairy Science** 89:1-14.

Licitra G, Hernandez TM, Van Soest PJ (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. **Animal Feed Science and Technology,** 57:347-358.

Liu GM, Hanigan MD, Lin XY, Zhao K, Jiang FG, White RR, Wang Y, Hu ZY, Wang ZH (2017) Methionine, leucine, isoleucine, or threonine effects on mammary cell signaling and pup growth in lactating mice. **Journal of Dairy Science** 100:4038-4050.

Löest CA, Titgemeyer EC, Lambert BD, Trater AM (2001) Branched-chain amino acids for growing cattle limited soybean hull-based diets. **Journal of Animal Science** 79:2747–2753.

Löest CA, Titgemeyer EC, St-Jean G, Van Metre DC, Smith JS (2002) Methionine as a methyl group donor in growing cattle. **Journal of Animal Science** 80:2197-2206.

Machado M, Detmann E, Mantovani HC, Valadares Filho SC, Bento CBP, Marcondes MI, Assunção AS (2016) Evaluation of the length of adaptation period for changeover and crossover nutritional experiments with cattle fed tropical forage-based diets. **Animal Feed Science and Technology** 222:132-148.

Mariz LDS, Amaral PM, Valadares Filho SC, Santos SA, Detmann E, Marcondes MI, Pereira JMV, Silva Júnior JM, Prados LF, Faciola AP (2018) Dietary protein reduction on microbial protein, amino acid digestibility, and body retention in beef cattle: II. Amino acid intestinal absorption and their efficiency for whole-body deposition. **Journal of Animal Science** 96:670-683.

Martineau R, Ouellet DR, Lapierre H (2013) Feeding canola meal to dairy cows: A meta-analysis on lactational responses. **Journal of Dairy Science** 96:1701–1714.

Martineau R, Ouellet DR, Lapierre H (2014) The effect of feeding canola meal on concentrations of plasma amino acids. **Journal of Dairy Science** 97:1603-1610.

Maxin G, Oullet DR, Lapierre H (2013) Effect of substitution of soybean meal by canola meal or distillers grains in dairy rations on amino acid and glucose availability. **Journal of Dairy Science** 96:7806-7817.

McCuistion KC, Titgemeyer EC, Awawdeh MS, Gnad DP (2004) Histidine utilization by growing steers is not negatively affected by increased supply of either ammonia or amino acids. **Journal of Animal Science** 82:759-769.

Merchen NR, Titgemeyer EC (1992) Manipulation of amino acid supply to the growing ruminant. **Journal of Animal Science,** 70: 3238–3247.

NASEM 2016: National Academies of Sciences, Engineering, and Medicine. Nutrient requirements of beef cattle. 8th ed. **Nutrient requirements of domestic animals.** National Academy Press, Washington, DC, 2016, 494p.

NRC 2001: **Nutrient Requirements of Dairy Cattle.** 7th rev. ed. National Academy Press, Washington, DC, 2001.

Oliveira CA, Millen DD (2014) Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. **Animal Feed Science and Technology** 197:64-75.

Owens FN, Pettigrew JE (1989) Subdividing amino acid requirements into portions for maintenance and growth. In: Friedman M (Ed.). **Absorption and Utilization of Amino Acids.** CRC Press, Inc., Boca Raton, FL. p.15-30.

Reynal SM, Broderick GA, Bearzi C (2005) Comparison of four markers for quantifying microbial protein flow from the rumen of lactating dairy cows. **Journal of Dairy Science** 88:4065-4082.

Rius AG, Appuhamy JA, Cyriac J, Kirovski D, Becvar O, Escobar J, McGilliard ML, Bequette BJ, Akers RM, Hanigan MD (2010) Regulation of protein synthesis in

mammary glands of lactating dairy cows by starch and amino acids. **Journal of Dairy Science** 93:3114–3127.

Sabatini DM (2017) Twenty-five years of mTOR: Uncovering the link from nutrients to growth. **Proceedings of the National Academy of Science** 114:11818-11825.

Schwab CG, Broderick GA (2017) A 100-Year Review: Protein and amino acid nutrition in dairy cows. **Journal of Dairy Science** 100:10094-10112.

Sok M, Oullet DR, Firkins JL, Pellerin D, Lapierre H (2017) Amino acid composition of rumen bacteria and protozoa in cattle. **Journal of Dairy Science** 100:5241-5249.

Titgemeyer EC (2003) Amino acid utilization by growing and finishing ruminants. In: D'Mello JPF (Editor) **Amino acids in animal nutrition,** 2nd ed. CAB International, Wallingford, UK. p. 329-346.

Udén P, Colucci PE, Van Soest PJ (1980) Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. **Journal of the Science Food and Agricultural**, 31:625-632.

Ushida K, Lassalas B, Jouany JP (1985) Determination of assay parameters for RNA analysis in bacterial and duodenal samples by spectrophotometry. Influence of sample treatment and preservation. **Reproduction Nutrition Développement,** 25:1037-1046.

Valadares RFD, Gonçalves LC, Valadares Filho SC, Coelho Da Silva JF (1997) Níveis de proteína em dietas de bovinos. 2. Consumo, digestibilidade e balanços de compostos nitrogenados. **Revista Brasileira de Zootecnia** 26:1259-1263.

Valadares Filho SC, Costa E Silva LF, Gionbelli MP, Rotta PP, Marcondes MI, Chizotti Ml, Prados LF (Eds.) (2016) BR-CORTE 3.0 Nutritional requirements, diet formulation and performance prediction of Zebu and Crossbred cattle. Viçosa: Suprema Gráfica Ltda. Available in: www.brcorte.com.br.

Valente TNP, Detmann E, Queiroz AC, Valadares Filho SC, Gomes DI, Figueiras JF (2011) Evaluation of ruminal degradation profiles of forages using bags made from different textiles. **Revista Brasileira de Zootecnia** 40:2565–2573. doi:10.1590/S1516-35982011001100039

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. **Journal of Dairy Science** 74:3583-3597.

Weiss, WP, Conrad HR, St Pierre NR (1992) A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. **Animal Feed Science and Technology,** 39:95-110.

White RR, Kononoff PJ, Firkins JL (2016) Technical Note: Methodological and feed factors affecting prediction of ruminal degradability and intestinal digestibility of essential amino acids. **Journal Dairy Science** 100:1946-1950.

Yang WZ, Beauchemin KA (2004) Grain processing, forage-to-concentrate ratio, and forage length effects on ruminal nitrogen degradation and flows of amino acids to the duodenum. **Journal of Dairy Science** 87:2578–2590.

Yoder PS, Huang X, Teixeira IA, Cant JP, Hanigan MD (2020) Effects of jugular infused methionine, lysine, and histidine as a group or leucine and isoleucine as a group on production and metabolism in lactating dairy cows. **Journal of Dairy Science** 103:2387-2404.

Zang Y, Silva LHP, Ghelichkhan M, Miura M, Whitehouse NL, Chizzotti ML, Brito AF (2019) Incremental amounts of rumen-protected histidine increase plasma and muscle histidine concentrations and milk protein yield in dairy cows fed a metabolizable protein-deficient diet. **Journal of Dairy Science** 102:4138-4154.

Zhuang H, Tang N, Yuan Y (2013) Purification and identification of antioxidant peptides from corn gluten meal. **Journal of Functional Foods** 5:1810–1821.

Zinn RA, Owens FN (1986) A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. **Canadian Journal of Animal Science,** 66:157-166.