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**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**

**Vinícius Carneiro de Souza**

Zootecnista

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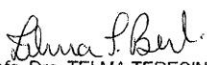


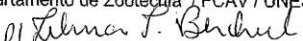
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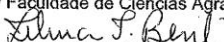
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## EPIGRAPH

“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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## **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 de proteína na dieta de bovinos Nelore confinados que recebem 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  $\text{NH}_3$  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  $354 \pm 11,8$  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  $6 \times 6$ . 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  $A \times B$ , 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 ( $\text{NH}_3\text{-N}$ ) 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 suprimimento 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  $\text{NH}_3$  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  $354 \pm 11.8$  kg and 18 months of age. The animals were randomly assigned to receive each diet once over the 6 periods in a  $6 \times 6$  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  $A \times B$ , 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 ( $\text{NH}_3\text{-N}$ ) 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





























## 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 ( $\text{NH}_3$ ) 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  $\text{NH}_3$  concentration, although with some negative effect on the supply of N to the rumen microbiome. However, the decrease of ruminal  $\text{NH}_3$  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  $\text{NH}_3$  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.

## 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.

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