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UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CAMPUS BOTUCATU

CARACTERIZAÇÃO DA COMUNIDADE MICROBIANA E PADRÃO DA FERMENTAÇÃO RUMINAL DE BOVINOS ANGUS E NELLORE DURANTE A ADAPTAÇÃO A DIETAS COM ALTO TEOR DE ENERGIA COM DIFERENTES ESTRATÉGIAS NUTRICIONAIS

ANA CAROLINA JANSSEN PINTO

Tese apresentada ao Programa de Pós-Graduação em Zootecnia como parte das exigências para obtenção do título de Doutor em Zootecnia

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CHARACTERIZATION OF THE MICROBIAL COMMUNITY AND PATTERN OF RUMINAL FERMENTATION OF ANGUS AND NELLORE CATTLE DURING ADAPTATION TO DIETS WITH HIGH ENERGY CONTENT WITH DIFFERENT NUTRITIONAL STRATEGIES

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AUTHOR`S BIOGRAPHY

Ana Carolina Janssen Pinto was born in the city of São Paulo, SP, Brazil, on February 10, 1985. She attended elementary and high school at Colégio Visconde de Porto Seguro Unit I, in the city of São Paulo, concluding it in 2003. In August 2011, she began her degree in Animal Science at the Sao Paulo State University “Júlio de Mesquita Filho”, Dracena Campus, concluding in January 2016. During the undergraduate course, she received a scholarship from the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP, and she was a BEPE Research Internship Scholar at the University of Saskatchewan, Saskatoon, Canada. In March 2016, she started the Master's degree in Animal Science and Technology in the inter-unit program of the Ilha Solteira and Dracena campus of UNESP where she obtained the Master's degree in Animal Science and Technology in December 2017. During the Master's course, she received a scholarship from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). She was also a fellow of the BEPE research internship abroad at the University of Pennsylvania, State College, United States. In March 2018, she started her Doctoral course at the Faculty of Veterinary Medicine and Animal Science (Unesp Botucatu) and received a scholarship from the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP. She had a scholarship to the University of Wisconsin, but with the pandemic Covid-19, she could not complete the internship. She works in the area of Nutrition and the production of beef cattle.

DEDICATION

To my father and my best friend José Neudson (im), who even not present in this carnal world, has always guided me, enlightened me and wanted my steps. He has always gone out of his way for anything and has always been and will be the person who taught me what it is like to love the profession. Besides, he is the reason I open my eyes every morning.

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"Mas é preciso ter força, é preciso ter raça
É preciso ter gana sempre
Quem traz no corpo a marca, Maria, Maria
Mistura a dor e a alegria
Mas é preciso ter manha, é preciso ter graça
É preciso ter sonho sempre
Quem traz na pele essa marca possui
A estranha mania de ter fé na vida..."

Maria, maria
(Milton Nascimento)

RESUMO GERAL

RESUMO: O objetivo deste estudo foi avaliar o efeito de diferentes estratégias nutricionais durante o período de adaptação e terminação sobre a fermentação ruminal e comunidade microbiana ruminal de bovinos Angus e Nelore canulados confinados. Foram utilizados 4 bovinos da raça Nelore e 4 bovinos da raça Angus, castrados, com peso vivo inicial aproximado de 450 kg e canulados no rúmen, os quais foram divididos em dois quadrados latinos 4 x 4 contemporâneos, sendo que cada quadrado foi composto por animais de mesma raça. Os períodos foram divididos em: 14 dias de dieta de adaptação (5 dias de adaptação 1- 65% de concentrado, 4 dias de adaptação 2- 70% de concentrado e 5 dias de adaptação 3- 75% de concentrado) e 18 dias de dieta de terminação (80% de concentrado). As dietas fornecidas diferenciavam apenas no tocante ao processamento dos grãos de milho e pela presença ou não monensina sódica. Portanto, os tratamentos ocorreram da seguinte maneira: MF(+)- Milho moído fino + monensina (27ppm); MF(-)- Milho moído fino MU(+)- Milho grão úmido + monensina (27ppm) e MU(-)- Milho grão úmido. A duração deste estudo foi de 158 dias, sendo compostos por 4 períodos experimentais (32 dias cada período) e três intervalos de *washout* (7 dias) entre os períodos 1 e 2, 2 e 3 e 3 e 4. Os Angus tiveram maior IMS nos dias 12 e 16 quando comparado aos Nelores (P=0.02). Os Angus também tiveram o pH médio menor e maior temperatura ruminal que a raça Nelore. Os animais Nelore tiveram maior diversidade Shannon que os Angus (P=0.007). Além disso, a raça Nelore teve uma maior comunidade dos microrganismos *Butyrivibrio*, *Clostridiales* e *Prevotella*(P<0.01) que o Angus. Durante a transição da dieta de adaptação para a de terminação, os animais Angus tiveram um padrão da fermentação ruminal e a comunidade bacteriana diferentes dos animais da raça Nelore.

Palavras-chave: metabolismo, microrganismos, Angus, Nellore

ABSTRACT

The objective of this study was to evaluate the effect of different nutritional strategies during the adaptation and finishing period on ruminal fermentation patterns and microbial community of cannulated Angus and Nellore cattle. Four 30-mo-old Nellore and four 30-mo-old Angus steers were used, with an initial body weight of approximately 450 kg which was divided into two 4 x 4 Latin squares. Each square was composed of animals of the same breed. The periods were divided as follows: 14 days of adaptation diet (5 days of adaptation 1 - 65% concentrate, four adaptation days; 2 - 70% concentrate and five adaptation days; 3 - 75% concentrate) and 18 days of finishing diet (80% concentrate). The diets provided differed only concerning the corn grain processing method and the presence or absence of sodium monensin. The treatments were as follows: FG(+)- Finely ground corn + monensin (27ppm); FG(-)- Finely ground corn HM(+)- High moisture corn + monensin (27ppm) HM(-)-High moisture corn. The study lasted 158 days, in which animals were submitted to four experimental periods (32 days each one) and three washout intervals (7 days) between periods 1 and 2, 2 and 3, and 3 and 4. Angus had higher consumption on days 12 and 16 when compared to Nellore (P=0.02). Angus cattle the lowest pH and the higher ruminal temperatures. Nellore breed had higher Shannon diversity than(P=0.007). Nellore showed a higher community of Butyrivibrio, Clostridiales and Prevotella(P<0.01) than Angus. During the transition period ruminal fermentation pattern and bacteria community are different for Angus than Nellore cattle.

Keywords: metabolism, microorganism, Angus, Nellore

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SUMMARY

CHAPTER 1

GENERAL CONSIDERATIONS

Beef cattle is indeed one of the most significant activities of the Brazilian economy. According to the Brazilian Association of the Beef Importation Industry (ABIEC - "*Associação Brasileira das Indústria Importadoras de Carne" 2021)* in the year 2020, Brazil had a reduction in the gross domestic product (**GDP**) of R$ 7.4 million, which represents a drop of 4.1% compared to the previous year. However, the decrease in GDP increased the agriculture representation on the overall GDP of the country. The GPD of the agriculture sector increased from 8.4% to 10.0%, proving its importance for the Brazilian economy. Moreover, Brazil is one of the biggest beef exporters, exporting beef products to 157 countries and during the period previously mentioned, the beef products exportation increased by 8%.

Due to the market pressure, regarding both quantity and quality in beef products, the profile of cattle finished in the Brazilian feedlot has been changed in the last few years. According to Silvestre e Millen (2020), in a survey conducted in 2019, Brazilian beef cattle nutritionists reported that 85.0% of the feedlots Nellore cattle (zebu) and 52.0% finishing crossbreed cattle (zebu).

Several Brazilian researchers reported that taurine cattle have a greater performance when finished on a feedlot, compared to zebu cattle (CUCKI, 2006; CRUZ et al., 1995; LEME et al., 2000; LANNA et al., 1998). It could be justified by works of literature reports that show that zebu cattle are more susceptible to metabolic diseases than taurine cattle (PACHECO ET AL., 2012; MILLEN ET AL., 2015) such as acidosis, diarrhea, bloat, liver abscess and laminitis. Thus, a deeper understanding of the ruminal environment, and the characterization of the microbiota of these different genetic groups, seems to be significant to better nutritional planning for both transition and finishing phases to high-energy diets.

1. LITERATURE REVIEW

1.1 Genetic groups

Jorge (2013), when studying the origin and evolution of zebu and taurine cattle, reported that these different genetic groups had their origin in independent places and that different forms of creation and feeding made these differ in terms of their metabolic and nutritional evolution.

Nellore animals, *Bos taurus indicus*, are originally from India and were introduced to Brazil in the early 19th century, feed, for the most part, on forages with reduced digestibility, higher proportions of stem, and low nutritional value during the dry period (Characteristic of the tropical

Furthermore, Nellore cattle also presented an increase of ruminal communities, *Clostridiales* (Gram positive), B*utyrivibrio* (Gram positive), and *Provotella* (Gram negative) when compared to Angus, only *Succinivibrionaceae* (Gram negative) were higher in Angus animals, corroborating the higher DMI and consequently higher starch load to be fermented.

5. CONCLUSION

During the transition period ruminal fermentation pattern and bacteria community are different for Angus cattle. Angus breed appears to have a rumen more prepared for concentrated diets than the Nellore cattle, with a homogeneous bacteria community.

6. REFERENCES

Association of Official Analytical Chemists - AOAC. Official Methods of Analysis. 16. ed. AOAC, Washington, DC. 1995.

ANDRADE, B.G.N., BRESSANI, F.A., CUADRAT, R.R.C. The structure of microbial populations in Nelore GIT reveals inter-dependency of methanogens in feces and rumen. J Animal Sci Biotechnol 11, 6 2020.

CARVALHO, S.; RODRIGO, M. T.; BRANCO, R. H. et al. Comportamento ingestivo de cabras 5 Alpinas em lactação alimentadas com dietas contendo diferentes níveis de fibra em detergente neutro proveniente da forragem. **Revista Brasileira Zootecnia**. 35:562-568. 2006.

DEHORITY, B. A. **Classification and morphology of rumen protozoa**. Wooster: Ohio Agriculture Research and Development Center, 1977.

DILL-MCFARLAND, K. A.; BREAKER, J. D.; SUEN, G.. Microbial succession in the 10 gastrointestinal tract of dairy cows from 2 weeks to first lactation. **Scientific Reports**. 7:40864. 2017.

ERWIN, E.S.; MARCO, G.J.; EMERY, E.M. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. **Journal of Dairy Science**, v.44, n.9, p.1768-1771. 1961.

FOLDAGER, J. **Protein requirement and non-protein nitrogen for high producing cow in early lactation**. Tese (Doutorado em Ciência Animal) – Michigan State University, East Lasing, MI. 1977.

GALYEAN, M. L., D. G. WAGNER, and F. N. OWENS. 1979. Corn particle size and site and extent of digestion by steers. Journal of Animal.Science. 49:204. 1979

GOMES, R. C. et al. Leveduras vivas e monensina em dietas de alto concentrado para bovinos: parâmetros ruminais e degradabilidade" in situ". Revista Brasileira de Saúde e Produção Animal, v. 11, n. 1, 2010.

HARB, M. Y., V. S. Reynolds, and R. C. Campling.. Eating behavior, social dominance and voluntary intake of silage in groupfed milking cattle. Grass Forage Sci. 40:113–118. 1985.

HEINRICHS, J.; KONONOFF, P. In evaluating particle size of forages and TMRs using the Penn State Particle Size Separator. **Dairy Animal Science**. 1-14. 1996.

HENDERSON G, COX F, GANESH S, JONKER A, YOUNG W, ABECIA L. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci Rep. 2015; 5:14567

JORGE, W. A genômica bovina - origem e evolução de taurinos e zebuínos. **Veterinária e Zootecnia**, v. 20, n.2, p. 217-237, 2013.

KULASEK, G. A. A micromethod for determination of urea in plasma, whole blood cells using urease and phenol reagent. **Pol. Arch. Weter**. 15:801-810. 1972.

LITHERLAND, N.B. **Adequacy of processing adjustment factors (PAF's) and intake discounts for dairy cows**. Invited Presentation. Oklahoma Cattle Grain Processing Symposium. p. 110-116. 26, 2006

MEHRES, A. Z.; ØRSKOV, E. R. A study of the artificial fiber bag technique for determining the digestibility of feeds in the rumen. **Journal Agricultural Science**. 88:645-28 650. 1977.

NOCEK, J. E. 1987. Characterization of in situ dry matter and nitrogen of various corn grain forms. J. Dairy Sci. 70:2291.

NOCEK, J. E., and S. TAMMINGA. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74:3598. 1991

PERES ASSUMPÇÃO, A.H. The individual and associated use of monensin, tannins and calcium nitrate in the feeding of cattle promote a reduction in the emission of methane gas. Dissertação (Mestrado em Zootecnia) - Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Brasil, 2021.

PEZZATO, L.E. et al. Avaliação de dois métodos de determinação do coeficiente de digestibility aparente com a tilápia do Nilo (Oreochromis niloticus). Acta Scientiarium, Maringá, v. 24, n. 4, p.965-971. 2002.

PITTMAN. K. A and BRYANT, M. P. Peptides and other nitrogen sources for growth of Bacteroides ruminicola. f. Bacteriol., 88. 401-10. 1964.

POORE, M. H.; MOORE, J. A.; ECK, T. P. et al.. Effect of fiber source and ruminal starch degradability on site and extent of digestion in dairy cows. **Journal of Dairy Science**, 76:2244-2253. 1993. 14

ROBLES, V.; GONZÁLEZ, L. A.; FERRET, A. et al. 2007. Effects of feeding frequency on intake, ruminal fermentation, and feeding behavior in heifers fed high-concentrate diets. **Journal of Animal Science**, 85:2538-2547.

RUSSELL, J. B. Fermentation of peptides by Bacteroides ruminicola B\4. Appl. Environ. Microbiol., 45,1566-74. 1983

SAS. **SAS User's Guide: Statistics** (Version 5 Ed.). SAS Inst. Inc., Cary, NC, 2003.

VAN SOEST, P. J.; ROBERTSON, J. B.; LEWIS, B. A. Symposium: carbohydrate metodology, metabolism, and nutritional implications in dairy cattle. **Journal of Dairy Science**, 74(10):3. 1991.

WALLACE, R. J .. MCKAIN, N. and BRODERICK, G. A. Breakdown of different peptides by Prevotella (Bacteroides) ruminicola and mixed microorganisms from the sheep rumen. (1993). Curro Microbiol., 26, 333-6.