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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

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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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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

climate) (EBERHARDT et al., 2009). They are resistant to endoparasites and ectoparasites, since their dark, thin and resistant skin prevents the action of sucking insects such as ticks, in addition, they also produce an oily secretion on the skin that has a repellent action (ARIEIRA et al., 2018). They have a dewlap that extends from under the lower jaw to the navel, which facilitates the exchange of heat with the environment.

Angus animals (Bos taurus taurus) are of European origin, used to temperate climates, feeding on forages with high digestibility and the presence of some grains. They have characteristics such as longevity, sexual precocity, allowing the slaughter of young animals with large body masses, and excellent carcass finishing and intramuscular fat. They are more demanding in handling, nutrition, health, and ambiance, but they are more precocious and are specialist breeds in meat production (BATISTELLI, 2009).

The breeds evolved separately, and during this period, cattle of Zebu breeds adapted to environments with hot and humid climates and fed on poor quality forage (TURNER, 1980). In addition, zebu animals have greater numbers of sweat glands, a digestive tract about 10% lower, and a slower metabolism (generating less metabolic heat) than Taureans (CARVALHO, 2021).

Zebu animals showed more efficiency than taurine animals when in diets with low nutritional quality (OLIVEIRA, 1991), whereas in diets containing a high concentration of concentrate or with high amounts of grains, taurine animals consume more food their maintenance requirements than zebu animals, and thus gain weight faster and more efficiently (KREHBIEL et al., 2000). This can also be proven by Cucki (2006), who compared animals with different proportions of the Bos indicus genotype and found greater daily weight gain (DWG), final weight, and better feed conversion for groups that had taurine genotype in their genetics, the which can be explained by a more intense selection for weight gain in European breeds (VAZ, 1999).

According to Putrino et al. (2007), Brangus cattle presented an increasing diet consumption, proportional to the increase of the concentrate of the same. On the other hand, Nellore animals showed a drop after reaching 60% of concentrate, raising the hypothesis that zebu cattle are less adapted to diets that are more energetic. Still on this type of diet, according to Moore et al. (1975) zebu animals showed lower digestibility than taurine animals, having higher amounts of fecal starch.

Bos taurus taurus animals have higher net maintenance energy (**NEm**), so there is a need to supply it via diet, and consequently, Angus animals consume more than Nellore animals

(WATANABE ET AL., 2016). which can change the behavior of the animals during the confinement period, which can change the rumination time, as well as the feeding and rumination rates of both dry matter (**DM**) and neutral detergent fiber (**NDF**).

It is known that Angus and Nellore crossbred animals meet market requirements regarding weight, carcass yield, and subcutaneous fat thickness, being ready for slaughter earlier, when compared to pure zebu (FAÇANHA et al., 2014). There are a lot of works in the literature that show this improvement in the animal crossed between Nellore and Angus, as well as work that shows the best performance of taurine about zebu when in confinement.

There are no studies in the literature that explain why Angus animals perform better than Nellore animals. Therefore, studying rumen metabolism, as well as characterizing the rumen microbiota of Angus and Nellore cattle during confinement with high-energy diets will help to identify possible differences between genetic groups, which can serve to design different nutritional strategies.

1.2 The ruminal environment and nutritional strategies

Cereal grains represent the main source of energy in feed for cattle finished in confinement (HUNTINGTON, 1997; OWENS et al., 1997; SANTOS et al., 2011). Thus, an increase in the inclusion of grains in finishing diets is observed. Pinto and Millen (2018), when surveying the evolution of feedlots in Brazil in 2015, reported that 33.3% of respondents use more than 66% of grains in finishing diets.

Corn is the most used grain in cattle diets (SILVESTRE and MILLEN, 2020). Corn is commonly processed in cattle rations to increase the use of starch, thus improving animal performance (CORRIGAN et al., 2009). Starch represents 60 to 70% of most cereal grains (ROONEY and PFLUGFELDER, 1986), and the corn grain has an average of 66.3% starch (VALADARES FILHO ET AL., 2002). According to Theurer (1992), starch is the main nutrient used to achieve high productivity for ruminants, so its use with maximum efficiency is essential.

The processing of corn grain promotes changes in the starch digestion site, in the total use of food and diet, in addition to providing substantial changes in the rumen environment. The digestibility of corn grain starch is limited by the protein matrix that encapsulates starch granules and the compact nature of the starch itself, particularly in the hard endosperm portion of the grain which prevents microbial colonization and delays penetration by amylolytic enzymes (MCALLISTER et al., 1990). Disruption of the protein matrix can increase the rate and extent of

starch digestion. Therefore, grain processing affects corn digestibility (OWENS, 2005). Starch availability can vary greatly depending on several factors such as the type of cereal grain, amylopectin and amylose content, the outer layer of the grain (pericarp), presence of a protein matrix coating the starch granule, and the method of processing this grain (ZEOULA and CALDAS NETO, 2001).

Depending on the type of corn processing, an increase in grain starch digestibility may occur, which may increase the total digestibility of the tract (RICHARD and HICKS, 2007). It may also improve the net energy available for gain (NRC, 2000) compared to beef consuming unprocessed grains. Brazilian nutritionists, aiming at the competing consumer market, are trying to make better use of corn, which can have better starch digestibility and consequent better animal performance.

Corn is made up of pericarp, endosperm, and germ. The pericarp is the outermost part of the grain, consisting of several layers with the function of protecting the inner layers (endosperm and germ), so high concentrations of lignin can be deposited in the pericarp (McALLISTER, 2006). The endosperm is made up of nutrients (in the insoluble form), such as starch, proteins, and lipids, thus having a nutritive function. The endosperm is divided into floury and vitreous, each with its specific physicochemical characteristics (Hosney, 1994). The germ is made up of the embryonic axis (germination).
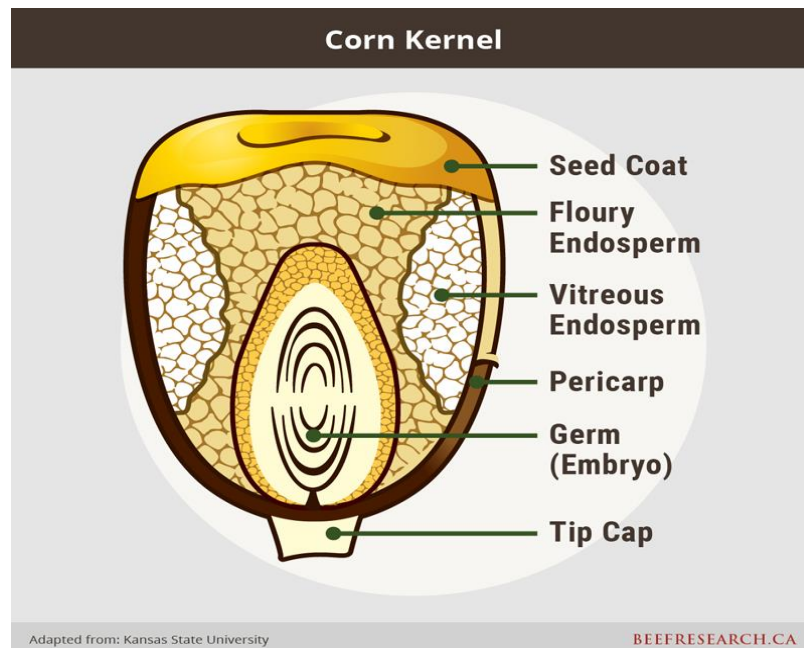
Figure 1. Description of the corn grain structure.

Source: Adapted from Kansas State University (website).

The corn kernel is composed of starch, proteins, lipids, and minerals, and their composition depends on the species and the part of the plant where it is found (ROONEY and PFLUGFELDER, 1986). Starch is a plant energy reserve polysaccharide consisting mainly of amylose and amylopectin, and the percentage varies with the botanical origin of the starch. In most species, starch is composed of 30% amylose and 70% amylopectin (WANG et al., 1998). Amylose is a long and relatively linear polymer formed by D-glucose molecules, with about 99% of the ∝-1-4 bonds. The amylose content tends to increase with the degree of maturity of the plant, having less digestibility. On the other hand, amylopectin is a larger molecule than amylose, more insoluble, formed by D-glucose molecules, with ∝-1-4 bonds, with ∝-1-6 branches (LEHNINGER, 1998). Starch is organized into granules, in which amylopectin and amylose are joined by hydrogen bonds (ROONEY and PFLUGFELDER, 1986).

There are five different types of corn grains: toothed, hard, floury, popcorn, and candy. The hard corn kernels (Flint type) have a higher proportion of vitreous endosperm, and the soft ones (Dent type) have a higher proportion of farinaceous. The starch granules of the vitreous endosperm form a compact and denser structure when compared to the floury endosperm (HUNTINGTON, 1997). In addition, in the farinaceous portion, the starch granules are more accessible to enzymatic attack.

Thus, as Flint corn is hard, it is known that the degradability and digestibility of starch in this type of grain are affected. It is worth mentioning that the degree of compaction of the starch granules directly influences its digestibility. With lower starch digestibility, there is less use of the food by the animal. Thus, this type of corn grain must be processed so that this starch is more available and can undergo the action of enzymes, with better use of it, improving the performance of the animals.

The digestion of starch is the initial step for it to be used as a source of energy by microorganisms and ruminants. It starts in the rumen, then in the small intestine, and, depending on whether it occurs, in the large intestine. It is noteworthy that, for ruminants, the main digestion of starch occurs in the rumen environment.

Starch digestibility can have two main limiting factors. One is the protein matrix that encapsulates the starch granules, and the other is the level of natural compaction of the starch itself,

which ends up preventing microbial colonization and delays the penetration of amylolytic enzymes (McALLISTER ET AL., 1990).

When the corn kernel (regardless of its processing) falls into the rumen, it will be degraded (hydrolysis) by bacteria (usually amylolytic) present in the rumen environment, and usually degraded to maltose and glucose. From this maltose and glucose, the bacteria will ferment these substrates, releasing short-chain fatty acids (**SCFA**) in the rumen that will be used by ruminants as a source of energy (ruminants are neoglycogenic).

Ruminal bacteria preferentially colonize starch granules that are most exposed within the protein matrix. As digestion proceeds, the starch is fully digested, and the protein matrix remains intact (SANTOS et al.; 2017). In addition to bacteria, protozoa also help in the fermentation of starch. They sequester starch granules and ferment, releasing maltose and glucose gradually.

Even though the rumen is the main place for starch digestion to occur, part of the corn grain that was not digested in the rumen can pass through the omasal reticulum orifice reaching the small intestine. In the intestine, this corn can undergo the action of pancreatic alpha-amylase (present in limited amounts in ruminants (LARSEN ET AL., 1956), and can be digested and used as energy by ruminants. However, the ruminant has a limitation of the alpha enzyme amylase and a limitation in glucose absorption capacity (HARMON, 2009).

If this corn grain is not digested in the small intestine (poor digestibility), it may undergo cecal fermentation (with microbial activity - which in ruminants is low). If it does not undergo this fermentation, the starch grain will come out in the feces. It is worth mentioning that the large intestine is the place that has the lowest digestibility of starch. It is of paramount importance that the corn grain is processed in feedlot finishing diets so that the starch present can be better used both by bacteria and by the animal itself, thus avoiding a waste of corn in the feces and possibly better performance of the animals.

Among the main corn processing methods, physical and chemical treatment is used, and the physical treatment does not change the chemical properties of the ingredient, aiming to reduce the particle size with the use of external forces, such as compression (MCKINNEY, 2006). The so-called chemical processes change the molecules that form the grain, such as starch granules and protein matrix, as well as their molecular organization, facilitating the enzymatic action on the glucose molecules present inside the starch granules (MOURÃO, 2012).

The most used processing in Brazilian feedlots is milling (SILVESTRE E MILLEN, 2020), where the grain is crushed and selected by sieves that will determine the desired particle size. The smaller the particle size, the better its homogeneity in the diet, however, as it has a greater surface for enzymatic action, the greater its fermentation, which increases the risk of metabolic disorders. It should also be noted that the higher the level of grinding the grain, the easier it will be to leave the rumen by the action of the passage rate, which may occur before being degraded (LUCCI, 2008).

Another corn grain processing that is widely used is high moisture grain silage, which is based on harvesting the grains about 3 to 4 weeks after maturation, with high moisture content, and ensiling them to promote anaerobic fermentation. Inside the silo, the humidity and temperature generated in the fermentation solubilize the nutrients and increase the susceptibility of the starch to enzymatic action. Such reactions facilitate the degradation and microbial use of the same in the rumen (JOBIM, 2003).

Knowing that Angus and Nellore cattle have differences in performance in confinement and that Angus animals are more efficient than Nellore animals when using high moisture grain in their diets, Nellore animals are expected to have a drop in consumption when compared to Angus animals since the energy input of high moisture grains is greater than that of the finely ground corn. In addition, a different fermentation pattern is expected between animals of the two breeds in response to better corn processing since Nellore cattle probably have their fermentation pattern and microbiota more affected than Angus animals, which are supposed to have a greater abundance of amylolytic bacteria based on higher consumption and better performance.

Another nutritional strategy widely used is ionophore additives in finishing diets. According to a survey carried out by Pinto and Millen (2018), 99.9% of the interviewed nutritionists' clients use some additive in their diet, with 86.7% of those interviewed recommending the use of ionophores. Monensin sodium (**MON**) is the most used among all ionophore additives used for beef cattle, classified as a polyether antibiotic (HIROHIKO ET AL., 1994).

MON acts on the permeability of membranes from Gram + bacteria, acting as a mobile carrier within them. It disrupts ion transport, making K+ efflux, by affinity, more favorable than Na+ efflux. K+ efflux results in H+ accumulation, and it decreases intracellular pH. In this way, the cell activates active transport mechanisms, resulting in excessive energy expenditure, causing bacteria to stop growing or die due to this energy expenditure (PACHECO, 2010). As they have

two membranes, one internal and one external, which protects the peptidoglycan layer, gram-negative bacteria are more resistant to monensin penetration, unlike gram-positive bacteria, which, because they have pores in their constitution, make the gram-positive bacteria permeable to the additive (MORAES ET AL., 2006).

The main effects of MON in the rumen are: increase in rumen propionate production by modifying fermentation patterns (PERRY ET AL, 1976); reduction of energy losses due to the methane production reduction (RUSSELL E 5 STROBEL, 1989); reduction of ruminal proteolysis (BERGEN AND BATES, 1984) and prevention of digestive disorders such as acidosis (OWENS ET AL, 1998). Taking into account that Angus and Nellore breeds have different rumen metabolism, it is expected that when manipulating rumen fermentation using sodium monensin as an additive, the effect of this manipulation will be greater in Nellore animals than in Angus animals, since supposedly Angus animals would already be more efficient in terms of ruminal fermentation.

Therefore, evaluating the difference in the variables of ruminal metabolism of Angus and Nellore cattle submitted to different nutritional strategies during the adaptation to diets with high levels of concentrate emerges with a new and broad field of research to be investigated. It can help clarify issues such as lower performance and a higher rate of rumenites in Nellore animals when they are fed high-energy diets. In addition, there are no different confinement management recommendations for Angus and Nellore cattle, which may lead to non-optimization of the potential of the two genetic groups.

2.   HYPOTHESIS AND OBJECTIVE

We hypothesized that the ruminal fermentation pattern, the ruminal microbiota, and the nutrient digestibility would be different between Angus and Nellore cattle when submitted to different nutrition strategies during both transition and finishing phase.

The objective of the project was to evaluate the effect of corn processing with or without the monensin sodium supplementation fed to Angus and Nellore cattle during the transition and finishing phase by evaluating ruminal pH, end products of ruminal fermentation, rumen microbiota profile, ruminal degradability, apparent total tract digestibility, as well as feeding behavior, and particle sorting.

The manuscript will be submitted to Frontiers in Microbiology (https://www.frontiersin.org/journals/microbiology).

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CHAPTER 2

"Characterization of ruminal fermentation pattern and ruminal microbial community of Angus and Nellore cattle during the feedlot. "

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: T1) Finely ground corn + monensin (27ppm); T2) Finely ground corn T3) High moisture corn + monensin (27ppm) and T4) 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.

1. INTRODUCTION

The profile of the cattle finished on Brazilian feedlots has been changing in the last years to achieve the market requirements. On the survey conducted by Pinto e Millen (2018) in 2015, among the feedlot served by the nutritionists consulted, 75.9% of them finish Nellore cattle (zebu), while only 39.8% finish crossbreed cattle (zebu × taurine). Jorge (2013) studied the origin and the evolution of the zebu and taurine cattle and reported that due to the geographical localization where these two genetic groups evolved, the raising system and the diet available made their metabolic and nutritional evolution differ from each other.

Several Brazilian authors have reported that confined taurine animals have better performance when compared to zebu (CUCKI, 2006; CRUZ et al., 1995; LEME et al., 2000; LANNA et al., 1998). Furthermore, Pacheco et al. (2012) and Millen et al. (2015) reported that Bos taurus indicus genotype cattle are more susceptible to metabolic problems than Bos taurus taurus genotype animals. Therefore, studying rumen metabolism, as well as characterizing the rumen microbiota of Angus and Nellore cattle during the adaptation phase and beginning of the finishing phase with high-energy diets can help to identify possible differences between genetic groups, which may serve to design different nutritional strategies.

2. MATERIAL AND METHODS

All procedures executed were in accordance with Sao Paulo State University Ethical Committee for Animal Research Guidelines (Protocol nº 0208/2018).

Animal and facilities

The study was conducted at the experimental feedlot at Sao Paulo State University (UNESP), Dracena campus, Brazil. Four Nellore (656 kg) and four Angus steers (512,5 kg) fitted with a ruminal cannula were used to conduct the present experiment.

Experimental design, treatments and management

Four Nellore and four Angus steers were fitted with ruminal cannulas submitted to two periods of feeding transition phase and high-energy feeding phase. Cattle were divided into two groups by genotype to form two 4 × 4 Latin squares. The cattle were kept on pasture before the beginning of the experimental period. Then they were brought to the feedlot and fed with diets that

differed, based on the grain processing and by the addition or not of monensin sodium. As it follows: T1) Finely ground corn + 27 ppm of monensin, T2) Finely ground corn + 0 ppm of monensin, T3) High-moisture corn + 27 ppm of monensin, and T4) High-moisture corn + 0 ppm of monensin, as described in Table 1.

A 3-phase step-up adaptation protocol was used in this experiment with diets containing 65% (Step 1), 70% (Step 2), and 75% (Step 3) of concentrate ingredients. The adaptation period lasted for 14 days, and diets were fed for 5, 4, and 5 days, respectively. The finishing diet contained 80% of concentrate ingredients and was fed for 18 days. Diets were formulated using the Large Ruminant Nutrition System (LRNS), based on the Cornell Net Carbohydrate and Protein System model computational engine (CNCPS, FOX, et al., 2004). Body weight was determined on days 1 and 32.

Steers were fed once daily at 0800h to reach ad libitum intake throughout the whole experimental period. Refusals were collected and weighted to recalculate the amount offered daily. Refusals were set up to be 3% of the daily amount offered. Dry matter intake (**DMI**) was determined by weighing the amount offered subtracted by the amount refused, and samples of both were submitted to dry matter determination. Daily, DMI was expressed both in kilograms and in the percentage of the body weight.

All the steers were assigned to all treatments in a sequence to balance the carry-over effect. Steers were also submitted to a 7-day wash-out period where they were fed with Tifton-hay and mineral mix ad libitum. The whole experimental period lasted for 158 days, with 14 days of the adaptation period, 18 days of the finishing period, and seven days of washout per period (excluding the last one).

Table 1: Experimental diets fed to Angus and Nellore steers fitted with a ruminal cannula during the experimental period.

Concentrate inclusion (%)	Experimental diets							
	Step 1		Step 2		Step 3		Finishing	
	65		70		75		80	
Item, %	FG[1]	HM[2]	FG	HM	FG	HM	FG	HM
Sugarcane bagasse	20.0	20.0	18.0	18.0	16.0	16.0	14.0	14.0
Tifton hay	15.0	15.0	12.0	12.0	9.0	9.0	6.0	6.0
Finely ground corn	40.0	0.0	45.0	0.0	50.0	0.0	55.0	0.0
High-moisture corn silage	0.0	40.0	0.0	45.0	0.0	50.0	0.0	55.0
Citrus pulp	7.10	7.10	7.60	7.60	8.1	8.1	8.6	8.6
Soybean meal	14.5	14.5	14.0	14.0	13.2	13.2	12.7	12.7
Mineral mix + urea[3]	3.4	3.4	3.4	3.4	3.7	3.7	3.7	3.7
Nutritional content								
Dry matter (**DM**)	77.0	70.0	77.0	70.0	77.0	69.0	78.0	69.0
Total digestible nutrients (**TDN**)	70.0	72.0	72.0	74.0	74.0	76.0	76.0	79.0
Crude Protein (**CP**)	16.1	16.1	15.5	15.5	15.1	15.1	14.5	14.5
Neutral detergent fiber (**NDF**)	34.8	33.5	31.7	30.2	28.6	26.9	25.4	23.4
peNDF[4]	27.0	27.0	24.0	23.0	23.0	20.0	17.0	16.0
Neg[5] (Mcal/kg DM)	1.03	1.07	1.09	1.13	1.13	1.19	1.19	1.27
Ca	0.57	0.57	0.55	0.54	0.53	0.52	0.51	0.50
P	0.31	0.30	0.31	0.30	0.32	0.31	0.31	0.30

[1] Finely ground corn; [2] High-moisture corn; [3] proportion of mineral mix and urea: Ca: 220g/kg, P: 20g/kg, Co: 24mg/kg, Cu: 400mg/kg, S: 25g/kg, F: 200mg/kg, I: 25mg/kg, Mg: 25g/kg, Se: 8mg/kg, Na: 86g/kg, Zn: 1800g/kg, Vit A: 100.000UI/kg, Vit. D: 12500UI/kg, Vit E: 500UI/kg; [4]physical effective neutral detergent fiber; [5] Net energy for gain.

At arrival, steers were submitted to a receiving protocol where they were vaccinated and dewormed (tetanus, bovine viral diarrhea virus, 7-way Clostridium sp.; Cattlemaster, Pfizer Animal Health, New York, NY). Steers were kept in individual pens (6 m × 4 m, with 6 m of bunk space) partially concreted near the bunk with constant provision of water.

Experimental Period

The collection schedule is represented on Figure 1, which shows the collection of all variables analyzed in the present study.

SCFA: Short-chain fatty acid; PCR: Polymerase chain reaction
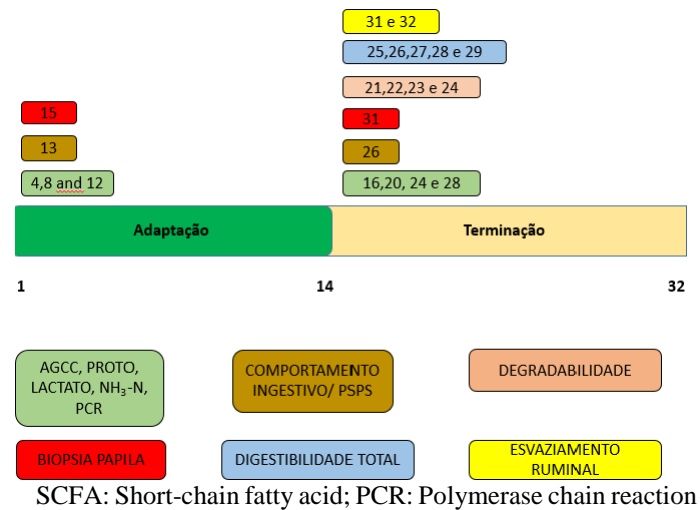
Figure 2: Collection schedule and activities for each experimental period, each color represents the indicated days on the experimental calendar

Feeding behavior and particle sorting

Feeding behavior was measured twice during each experimental period, on days 13 and 26, representing the adaptation and finishing period, respectively (Figure 1). Each feeding behavior evaluation was executed following the protocol established by Robles et al. (2007), where it was registered every 5 minutes for 24 hours. During the 24 hours, data were collected to determine time spent feeding, ruminating, and resting (in minutes), number of visits on the water trough, and number of meals per day (based on the number of visits to the bunk). Samples of feed offered and refused were collected on the feeding behavior observation days for each period, and the samples were submitted to chemical analysis of dry matter (AOAC, 1995) and NDF (VAN SOEST, 1991), used to determine the nutrients intake for each steer. As well as the DMI per meal, rumination and feeding rate of DM, and rumination and feeding rate of NDF were calculated as described by Carvalho et al. (2006).

On the days of each observation of ingestive behavior (13 and 26 of each experimental period; Figure 1) samples of the total diet were collected immediately after the supply, and of the leftovers from the 24 pens on the following day to determine the distribution of particles using a particle separator (Penn State Particle Separator, Nasco, Fort Atkinson, WI, USA) as described by Heinrichs and Kononoff (1996), to analyze the extent of selection, expressed as a preference index. The preference index was calculated for each portion retained on each sieve individually. The

fractions of natural matter from the samples retained on each sieve and in the solid box were then weighed to determine the particle size distribution of the sample. The selection index was calculated as the current intake / expected intake for each portion retained on the individual sieves, where the sieves diameters could be: 19mm (long particle), 8mm (medium particle), 1.18mm (short particle), and < 1.18 mm (fine particle). The expected intake was calculated as the particle size distribution of the total diet (based on natural matter) × the natural matter intake. Current intake was calculated as the amount of feed offered × the particle size distribution of the total diet − the number of leftovers × the particle size distribution of leftovers (%). Therefore, indices with values equal to 1 indicate an absence of selection, the indices that had a value < 1 indicate selection against, and the index > 1 a selection in favor according to each sieve evaluated (LEONARDI AND ARMENTANO, 2003).

Continuous measurement of ruminal pH

Ruminal pH was measured on days 4, 8, 12 (adaptation phase) 16, 20, 24, and 28 (finishing phase) of each of the four experimental periods. This measurement allows the calculation of variables: average pH, minimum pH, maximum pH, time in which the pH remained below 5.2; 5.6 and 6.2 in minutes and pH area below 5.2; 5.6 and 6.2 according to Bevans et al. (2005). Before and after placing the probes in the animals, they were calibrated in pH 7.0 and 4.0 solutions. Data calibration allows the calculation of a slope, and an intercept before and after the test to adjust the measured data.

Evaluation of ruminal fermentation products

Ruminal fluid samples were collected via cannula at 0, 4, 8, and 12 hours after the morning meal on days 4, 8, and 12 (adaptation phase) and 16, 20, 24, and 28 (termination phase) of each one of the four experimental periods. At each time, approximately 500 mL of rumen content were collected at different points in the rumen. The zero-hour sample was performed just before the feed (8:00). Soon after, the samples were prepared for later determination of the total concentration and molar proportion of SCFA, ammoniacal nitrogen (N-NH$_3$), and ruminal lactate concentration. For the determination of SCFA, a fraction of approximately 100 mL of rumen content was centrifuged at 3500 rpm for 15 minutes; 2 mL of the supernatant was placed in a corked test tube, conditioned with 0.4 mL of formic acid, and kept in a freezer (-20 °C) until analysis, which was performed

through gas chromatography as described by Erwin et al. (1961). For this evaluation, a gas chromatograph (Finnigan, model 9001) equipped with an Ohio Valley Megabore column, model OV-351, 1 Micron, 30 m long and 0.53 mm in diameter was used.

Fractions of 2 mL of rumen fluid were placed in test tubes containing 1 mL of 1N sulfuric acid solution and stored under refrigeration until the analysis by colorimetry to determine the concentration of ammoniacal nitrogen analyzed according to Kulasek (1972) and adapted by Foldager (1977). The total lactic acid concentration was also measured by the colorimetric technique, according to Pryce (1969).

Sequencing of ruminal bacterial communities

For genomic sequencing of rumen bacterial communities, rumen content (solid + liquid) was collected on days 4, 8, and 12 (adaptation phase) and 16, 20, 24, and 28 (termination phase). The rumen content samples were collected via cannula and stored in a -80°C freezer for future analysis. Total DNA was extracted by mechanical disruption and phenolic extraction protocol. They were diluted to 10ng DNA per µl to ensure at least 50ng per PCR reaction. Bacterial sequencing was performed at the "Department of Bacteriology" at the University of Wisconsin, Madison. The sequences of the bacterial communities obtained will be aligned against the reference database called SILVA 16S rDNA, according to Dill-McFarland et al. (2017). Moreover, relative abundances of bacterial communities of interest and those in larger numbers was also determined (Weimer et al., 2017).

Total and differential count of ruminal protozoa

For the differential count of ciliated rumen protozoa, the rumen content was manually collected by scanning the floor of this organ, and 10 mL of this material was stored in a flask containing 20 mL of 50% formaldehyde (v/v). The collections were carried out on days 4, 8, and 12 (adaptation phase) and 16, 20, 24, and 28 (finishing phase) at 12:00 pm, 4 hours after the morning treatment. The differential counts of the protozoa were determined using a reticle measuring 0.5 mm X 0.5 mm in area, with subdivisions of 25 squares. It was attached to the eyepiece of a microscope (Olympus model CH2) and with a counting chamber of "Sedgwick Rafter" with internal measurements of 50 mm X 20 mm X 1 mm (1 mL capacity), according to Dehority (1993).

Histology of ruminal papillae

For histological evaluation of the papillae, papillae were collected from the rumen wall on day 31 of each experimental period and stored in 70% alcohol until analyzed. The histological measurements, performed after mounting the slides, were as follows: height, width, and area of papillae, the thickness of the keratinized epithelium, and mitotic index. For the histological analysis, the Leica Qwin Image Analyzer was used through a Leica light electron microscope, with the images of each slice captured by a micro camera for further scanning at 5x or 10x magnification, depending on the size of the papilla. An increase of 400x was used to obtain the mitotic index (MI).

In-situ degradability

The in-situ degradability test was performed on days 21, 22, 23, and 24 of each experimental period. Degradability data were fitted using the Ørskov and McDonald (1979) model. Potential degradability (Dp) and effective degradability (De) were calculated using the formula of Ørskov et al. (1980).

DM and nutrients Digestibility

The in vivo digestibility of DM and its fractions (CP, NDF, ADF, and starch) were determined using the titanium dioxide marker. To this end, from days 20 to 29 of each experimental period, titanium dioxide (1 g/kg MS) was administered via ruminal cannula, and from days 20 to 24, the titanium provided was for adaptation. From days 25 to 29, samples of feces, rations, and leftovers were collected once a day. Composite samples were made and 200 g aliquots were taken from the collected samples, which were stored in a freezer until the determination of the titanium dioxide concentration. The apparent digestibility coefficients (ADC) were calculated based on the titanium dioxide (TiO_2) content of the feed, leftovers, and feces samples, according to Pezzato et al. (2002).

Statistical analysis

Tests for normality of residuals and heterogeneity of variances were performed before proceeding with the analysis of variance. The effect of treatments was considered to be fixed; however, when measurements were repeated over time, the effect of these measurements and their

interaction with treatments were considered random. Likewise, the effects of square, period (square), and animal (square) were considered random effects. Data from this study were analyzed by SAS PROC MIXED, considering the 5% significance level.

3. RESULTS

The data of the analyses carried out in the experiment will be presented below, as well as the figures referring to the variables in which there were interactions.

Feeding behavior and particle sorting

The data of feeding behavior and particles sorting are presented in tables 2 and 3, as well as the figures referring to the interactions.

Table 1. Feeding behavior and particle selectivity of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the transition's phases.

Variables	Angus				Nellore					P value			
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	SEM	Breed	Corn	Mon	Interaction
Feeding behavior													
Idleness time, min	871.2	806.2	830.0	766.2	933.7	813.7	856.5	856.5	54.70	0.27	0.39	0.08	-
Ruminanting time, min	267.5	307.5	317.5	388.7	240.0	336.2	306.2	331.2	46.42	0.59	0.13	0.07	-
Eating time, min	301.2	326.25	292.50	285.00	266.25	290.00	277.50	255.00	19.12	0.25	0.02	0.51	C*MON
Average time per meal, min	18.28	23.57	25.04	17.38	22.80	24.53	21.48	20.11	2.57	0.40	0.02	0.53	C*MON
Meals per day, n	16.50	14.25	15.00	17.00	12.25	12.50	13.25	13.25	1.40	0.10	0.21	1.00	-
Drinking fountain, n	9.75	12.25	12.00	15.50	9.50	7.75	9.00	6.25	1.80	0.02	0.30	0.65	B*MON*C
DMI, kg	13.96	17.58	13.90	15.16	12.56	14.56	12.29	11.83	1.34	0.22	0.05	0.02	-
DMI per meal, n	0.87	1.22	0.97	0.91	1.08	1.24	0.94	0.97	0.16	0.70	0.03	0.08	C*MON
DM FE[5], min/kg of DM	23.18	20.79	21.11	20.02	22.22	21.16	23.21	24.12	3.32	0.68	0.85	0.56	-
RE[6], min/kg da DM	20.26	21.82	22.84	26.53	18.94	23.18	25.43	28.70	4.46	0.81	0.02	0.11	-
NDF intake, kg	2.88	4.71	3.03	3.33	2.65	3.92	2.99	3.14	0.50	0.54	0.16	0.01	C*MON
NDF FE[5], min/kg of DM	109.8	74.43	101.2	90.64	107.2	77.29	147.7	100.8	26.54	0.89	0.57	0.03	-
NDF RE[6], min/kg of DM	87.83	75.17	109.59	125.72	94.70	88.01	143.79	113.68	24.29	0.55	0.02	0.58	-
Particle Sorting													
Long, (>19 mm)	0.79	0.99	1.05	1.02	1.05	0.96	1.03	1.00	0.06	0.47	0.20	0.88	-
Medium, (>8 mm)	0.97[b]	0.99[ab]	1.03[ab]	0.97[b]	1.05[a]	1.01[ab]	1.03[ab]	1.00[ab]	0.02	0.02	0.88	0.04	B*C*MON
Short, (>1.18 mm)	1.01	1.00	1.00	1.00	1.01	1.01	1.00	1.00	0.01	0.43	0.17	0.95	-
Fine, (<1.18 mm)	1.04	1.00	0.96	1.01	0.98	0.99	0.97	0.99	0.02	0.06	0.07	0.45	C*MON

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin; [5]Feeding efficiency; [6]Ruminating efficiency; [7]. Interactions: C*MON: corn * MON; B*C: breed*corn; B*MON: breed * MON; B*C*MON: breed*corn*MON.

According to the data presented in Table 2, during the adaptation phase, there was an interaction between corn and MON for the variable feeding time (minutes), where the animals receiving finely grind corn without MON stayed longer in feeding when compared to the animals that received high moisture corn without MON (Figure 2A) (P=0.01). Regarding the variable meantime at the meal, the animals that received finely grind corn without MON obtained better results when compared to the animals that received high moisture corn without MON (Figure 2B) (P=0.01).
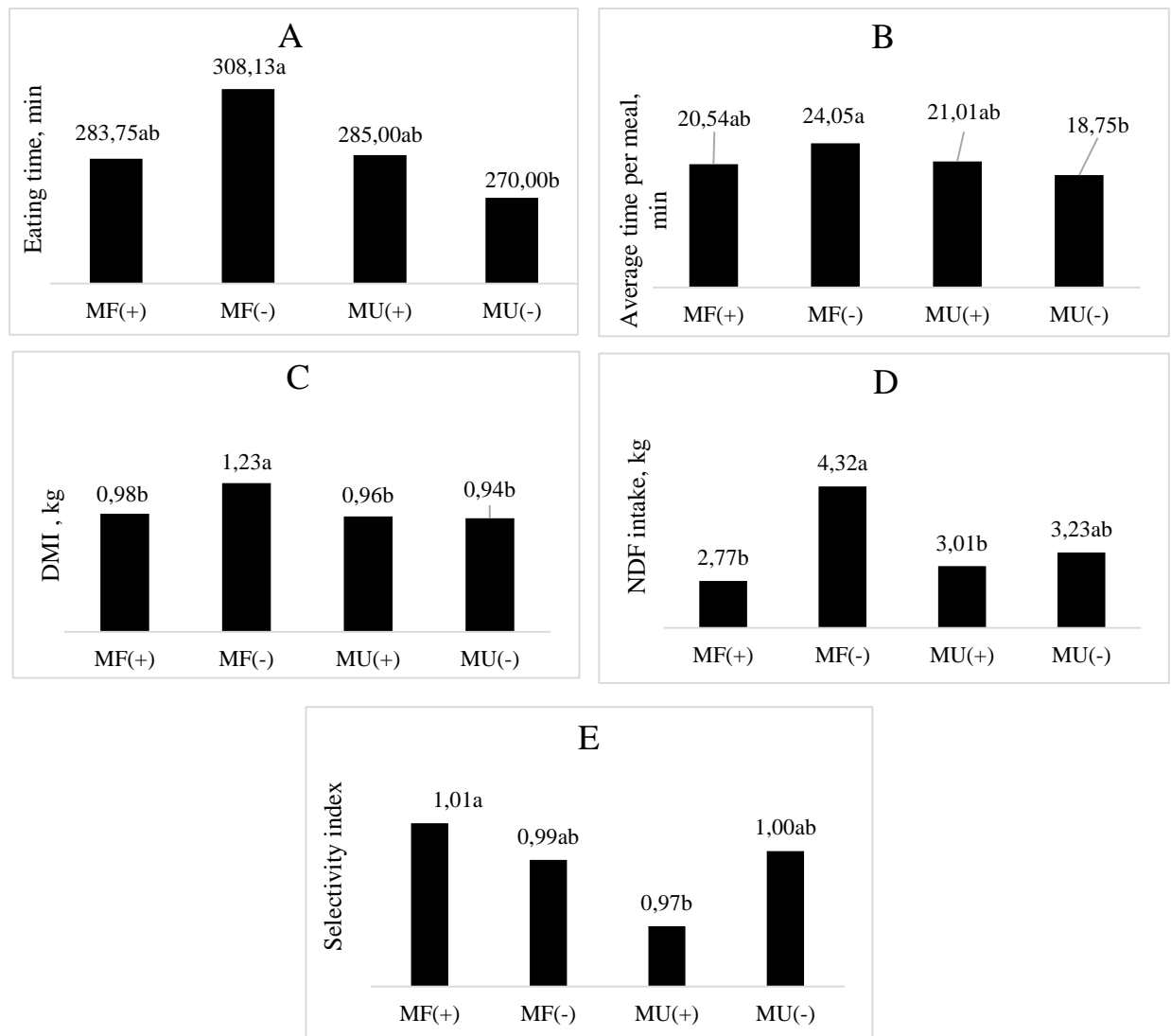
Figure 2. Interaction between corn processing type and monensin on eating time (A; SEM = 13.76), average time per meal (B; SEM = 2.08), DMI per meal (C; SEM = 0.13), NDF intake (D; SEM = 0.35) and fine particles selection (E; SEM = 0,01) during the transition phase of canulated Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin; MF(+): finely ground with MON; MF(-): finely ground without MON; MU(+): High-moisture with MON; MU(-): High-moisture without MON; [a,b] Different letters differ significantly among treatments.

When DMI was analyzed by the number of meals (Figure 2C), animals that received the finely grind corn diet without MON had higher DMI than the other treatments (P=0.05). There was also an interaction between corn and MON for the variable NDF consumption in kg (Figure 2D), whose treatment of finely grind corn without MON had higher NDF consumption in kg (P=0.03) compared

to animals that received MON regardless of the corn processing. Likewise, for the ssorting of medium particles, there was an interaction between corn processing, breed, and MON (P= 0.05); and the Angus receiving finely grind corn diet with MON sorted against medium particles when compared to the Nellore animals that received the same treatment (Table 2). Animals that received finely grind corn with MON sorted to fine MON particles (P=0.04) than animals receiving high moisture corn with MON (Figure 2E).

Regarding the number of visits to the drinking fountain (Figure 3), Angus animals fed with high moistures corn went to the drinking fountain more times than Nellore, regardless of the type of corn processing (P=0.03; Figure 3A). Likewise, Angus animals fed without MON went to the drinking fountain more often than Nellore regardless of the inclusion or not of MON (P=0.004; Figure 3B).
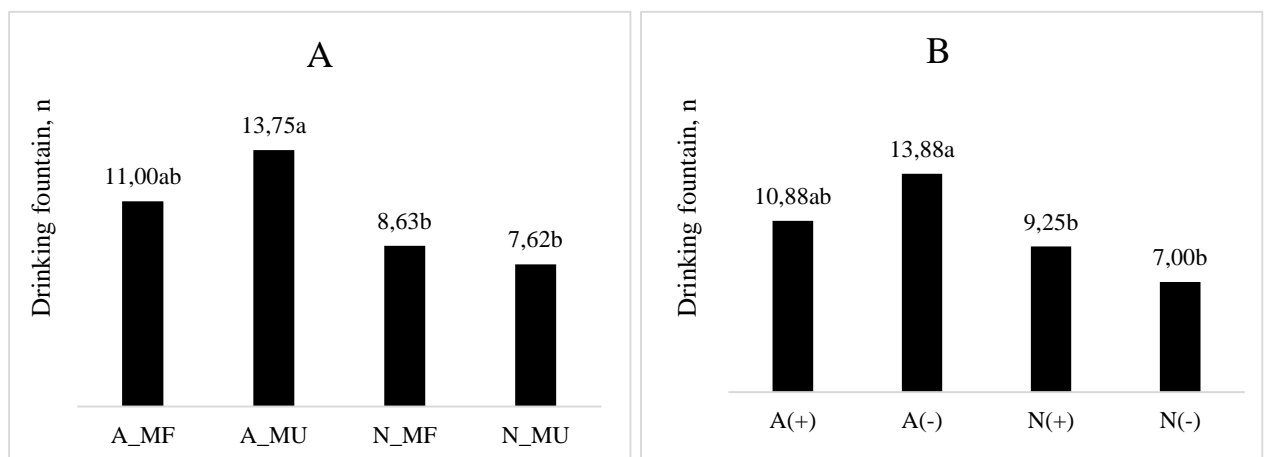
Figure 3. Treatments interaction between breed fator and corn processing type (A; SEM = 1.60) and effect of breed fator and monensin addition (B; SEM = 1.60) on goigns to drinkings fountains during the transition phase of Angus and Nellore bulls fed with with finely ground corn or high-moisture corn with addition or not of monensin; A_MF: Angus fed with finely ground corn; A_MU: Angus fed with high-moisture corn; N_MF: Nellore fed with finely ground corn; N_MU: Nellore fed with high-moisture corn. A(+): Angus supplemented with monensin; A(-): Angus without monensin supplementation; N(+): Nellore supplemented with monensin; N(-): Nellore without monensin supplementation .[a,b] different letters differ significantly among treatments.

Concerning DMI per kg, there was a significant difference between corn processing and the inclusion of MON. Cattle receiving finely grind corn treatment had greater DMI than those that received high moisture corn (Figure 4A) (P=0.05). Regarding the inclusion of MON, animals fed with MON had lower DMI per kg than the animals that did not receive MON in the diet (Figure 4B) (P=0.02).

Figure 4. Effect of the corn processing type (**A**; SEM = 1.26) and MON inclusion (**B**; SEM = 1.26) on the DMI (kg) of Angus and Nellore bulls fed with with finely ground corn or high-moisture corn with addition or not of monensin during the transition phase. MU: high-moisture corn; MF: finely ground corn. (-): Absence of monensin; (+): Presence of monensin.

Regarding the Rumination Efficiency of DM (Figure 5A), there was a significant difference for the processing of corn, and cattle receiving high moisture corn had greater rumination efficiency than animals with finely grind corn (P=0.02). Regarding NDF Feeding Efficiency (Figure 5B), animals that received MON in the diet had greater efficiency than animals that did not receive MON in it (P=0.03). There was an interaction between corn and MON for the variable NDF consumption in kg (Figure 5C), in which the finely grind corn treatment without MON obtained higher NDF consumption in kg (P=0.03) when compared to animals receiving MON regardless of processing of corn.
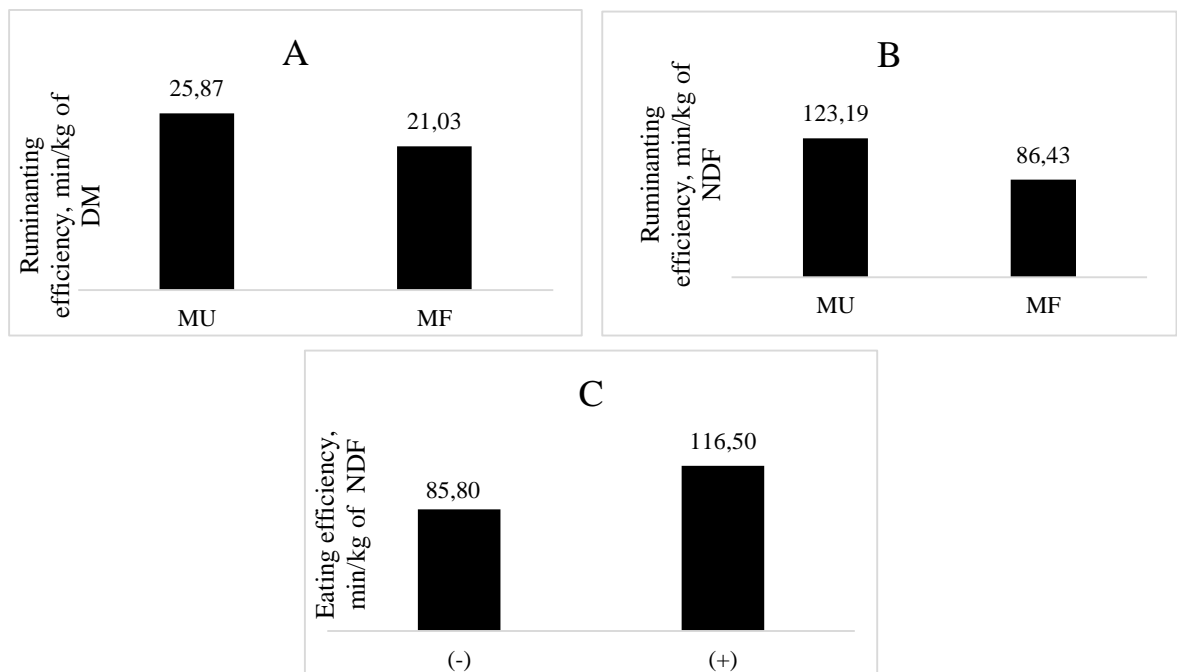
Figure 5. Effect of corn processing type on the DM ruminanting efficiency (**A**; SEM = 3.12), and NDF ruminating efficiency (**B**; SEM = 15.87) during the transition phase of Angus and Nellore bulls fed with different nutritional strategies. HM: high-moisture corn; FG: finely ground corn. Effect of monensins supplementation on NDF eating efficiency (**C**; SEM = 14.49), of of Angus and Nellore bulls fed with different nutritional strategies during the transition phase. MU: high-moisture corn; MF: finely ground corn; (-): Absence of monensin; (+): Presence of monensin.

Table 3 shows data on the feeding behavior and particle sorting of the animals in the finishing phase.

Table 3. Feeding behavior and particle selectivity of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the finishing phase.

Variables	Angus				Nellore				SEM	P value			
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Interaction
Feeding behavior													
Idleness time, min	857.5	890.0	826.3	841.3	888.8	885.0	882.5	905.0	38.23	0.32	0.41	0.41	-
Ruminanting time, min	335.0[ab]	280.0[b]	322.5[ab]	363.7[a]	300.0[ab]	305.0[ab]	313.7[ab]	288.7[b]	25.17	0.39	0.25	0.57	B*C*MON
Eating time, min	247.5	270.0	291.3	235.0	251.3	250.0	243.7	246.3	22.95	0.63	0.95	0.45	-
Avarage time per meal, min	17.74	19.94	17.52	15.83	18.38	21.73	18.60	18.09	2.07	0.58	0.02	0.29	C*MON
Meals per day, n	14.25	13.50	16.75	15.00	15.00	11.75	13.75	13.75	1.39	0.46	0.05	0.02	-
Drinking water, n	10.50	13.25	12.75	14.00	8.00	8.25	7.25	11.25	2.93	0.28	0.31	0.12	-
DMI, kg	13.63[bc]	17.87[a]	13.77[bc]	14.91[bc]	12.81[c]	15.12[ab]	10.15[d]	13.44[bc]	1.19	0.13	<0.01	<0.01	B*C*MON
DMI per meal, n	1.01	1.34	0.83	1.02	0.96	1.31	0.76	1.00	0.13	0.77	<0.0001	<0.0001	-
DM FE[5], min/kg of DM	19.53	15.34	21.28	16.04	19.95	16.65	25.07	18.31	1.95	0.43	0.01	<0.001	-
RE[6], min/kg da DM	26.94[ab]	15.60[d]	23.25[bc]	24.40[bc]	24.31[bc]	20.13[cd]	32.28[a]	21.56[bcd]	2.39	0.28	0.04	0.001	B*C*MON
NDF intake, kg	2.63	4.31	2.21	2.89	3.55	3.47	1.99	2.05	0.66	0.6	0.01	0.19	-
NDF FE[5], min/kg of DM	135.7	71.99	186.7	101.6	86.65	81.65	139.6	163.7	35.12	0.98	0.01	0.08	-
NDF RE[6], min/kg of DM	189.1	72.56	228.5	149.3	110.8	96.55	176.7	190.3	48.08	0.99	0.01	0.11	-
Particle Sorting													
Long, (>19 mm)	0.54	0.64	0.87	0.76	0.89	0.72	0.68	0.94	0.15	0.4	0.18	0.8	-
Medium, (>8 mm)	0.84[bc]	0.97[ab]	1.04[a]	0.97[ab]	1.00[a]	0.81[c]	1.06[a]	1.03[a]	0.05	0.69	0.23	0.22	B*C*MON
Short (>1.18 mm)	1.02	1.01	1.01	1.02	1.03	1.01	1.05	1.01	0.01	0.15	0.09	0.01	B*MON
Fine (<1.18 mm)	1.03	1.01	0.96	0.99	0.97	1.01	0.86	0.95	0.02	0.01	<0.001	0.06	-

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin; [5]Feeding efficiency; [6]Ruminating efficiency; [7]. Interactions: C*MON: corn * MON; B*C: breed*corn; B*MON: breed * MON; B*C*MON: breed*corn*MON.

In the finishing phase, cattle receiving high moisture corn in the diet had a higher number of meals than the animals receiving finely grind corn (P=0.05) (Figure 6A), the treatment with the highest number of meals was the high moisture corn treatment when compared to finely grind corn. Still, regarding the number of meals (Figure 6B), animals that received MON had a higher number of meals than animals that did not receive MON in the diet (P=0.02). There was an interaction between corn processing and the inclusion of MON, in which animals that received finely grind corn without monensin remained longer in the average meal time (min) when compared to the other treatments (P=0.02) (Figure 6C).
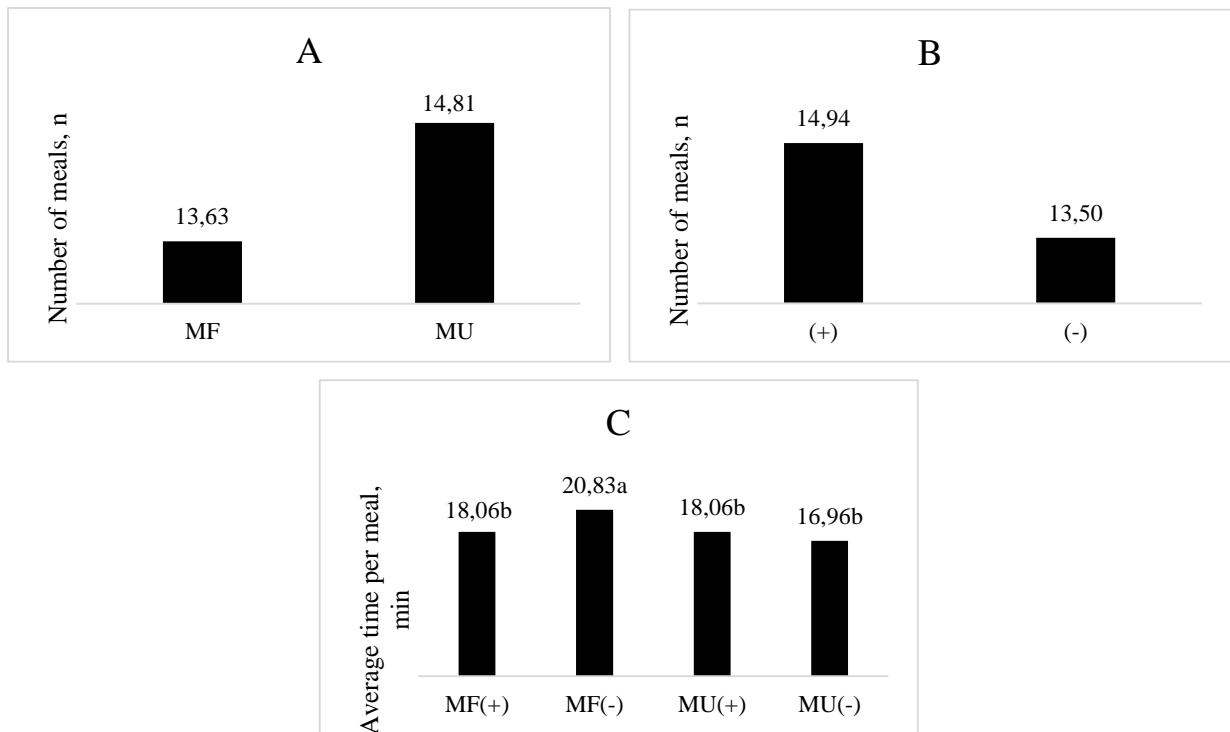
Figure 6: Effect of corn processing type (A; SEM = 0.90), and MON supplementation (B; SEM = 0.90) on the number of meals during the finishing phase of Angus and Nellore bulls fed with different nutritional strategies. MU: high-moisture corn; MF: finely ground corn. Interaction between treatments, corn factor and MON factor (C; SEM = 1.52) on the average time per meal during the finishing phase of Angus and Nellore bulls fed with different nutritional strategies. MF(+): finely ground with MON; MF(-): finely ground without MON; MU(+): High-moisture with MON; MU(-): High-moisture without MON; [a,b:] Different letters differ significantly among treatments.

Regarding rumination time (min), there was interaction concerning corn processing, breed, and MON (P=0.04); Angus breed receiving high moisture corn without MON stayed longer ruminating than Nellore receiving the same diet (Table 3).

The DMI per kg was higher (P=0.04) for Angus receveing high moisture corn with MON compared to Nellore receiving the same diet (Table 3). On the other hand, Nellore animals that received high moisture corn with MON had greater DM rumination efficiency (P=0.008) when compared to Angus animals with the same diet (Table 3).

There was a significant effect of corn processing for the variable NDF consumption in kg (P=0.01) (Figure 7C), for the NDF feeding efficiency (P=0.01) (Figure 7A), and the efficiency of NDF rumination (P=0.01) (Figure 7B). For NDF consumption, animals that consumed finely grind corn had higher NDF consumption than animals that consumed high moisture corn. As for the NDF feeding and rumination efficiency, cattle consuming high moisture corn had higher efficiency than those that consumed finely grind corn.
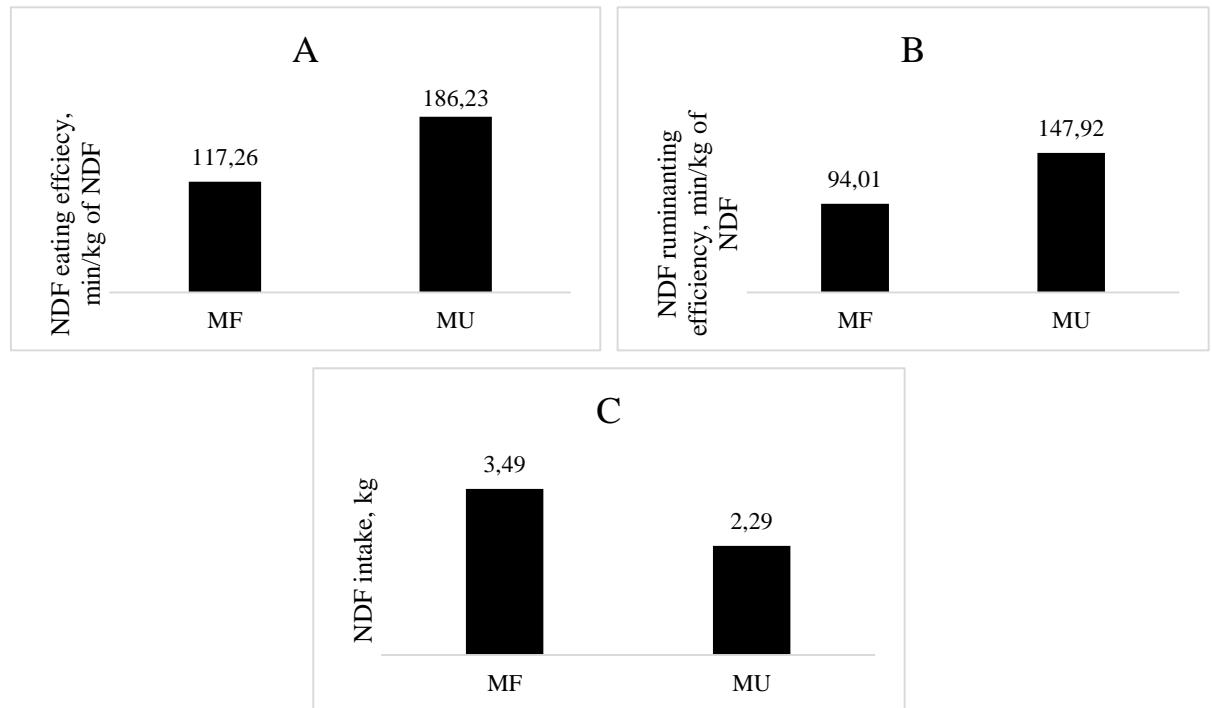
Figure 7. Effect of corn processing type on NDF eating efficiency (**A**; SEM = 17.66), NDF ruminanting efficiency (**B**; SEM = 25.17) and NDF intake (**C**; SEM = 0.40) during the finishing phase of Angus and Nellore canulated bulls fed with different nutritional strategies. Mu: high-moisture corn; MF: finely ground corn.

Angus breed consuming finely grind corn with MON sorted fewer medium particles (P=0.01) (stopped eating) when compared to Nellore receiving the same diet, which in this case did not sorted medium particles (neither against nor in favor, ate what was provided) (Table 3). On

the other hand, Angus that received finely grind corn without MON sorted more medium particles than Nellore consuming with the same diet (Table 3).

There was a significant difference between the breed for selection of fine particles (P=0.01) (Figure 8B); Angus animals sorted to fine particles than Nellore animals (which soted against fine particles, ate less than was provided). Still, concerning the sorting fine particles (Figure 8A), corn processing had a significant effect (P=<0.0001), where the animals consuming finely grind corn sorted to fine particles than animals consuming high moisture corn. The treatment that obtained the highest ssorted of fine particles was the finely corn treatment, compared to high moisture corn. Regarding the soerted of short particles (Figure 8C), there was an interaction between breed and inclusion of MON (P=0.02). Nelore fed with MON in their diet sorted to short particles than both Angus animals (with or without MON inclusion).

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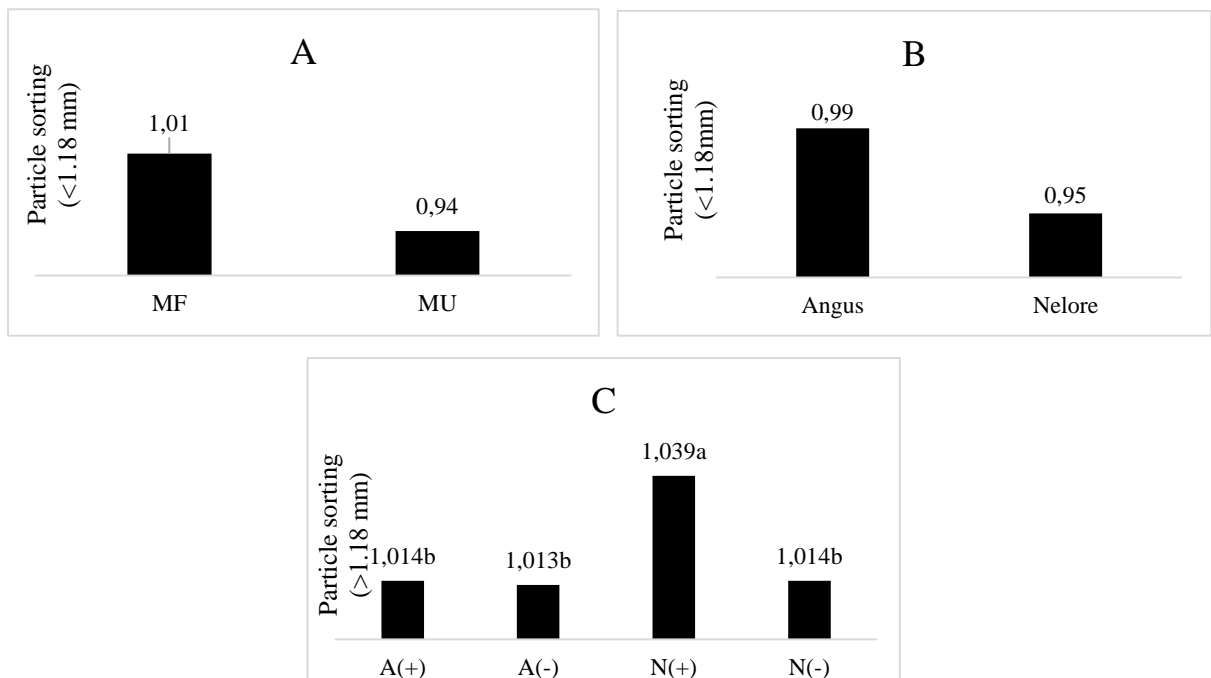
Figure 8: Effect of corn processing type (**A**; SEM = 0.006), breed (**B**; SEM = 0.006) and Mon supplementation (**C**; SEM = 0.006) on Particle sorting of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the finishing phase. MF: finely ground; MU: High-moisture; A(+): angus supplemented with Mon; A(-): angus non supplemented with Mon ; N(+): Nellore supplemented with Mon; N(-) Nellore non supplemented with Mon. [a,b]: different letters differ significantly among treatments.

Continuous measurement of pH

The results of the continuous measurement of pH are represented in table 4 and in the figures below.

Table 2. Dry matter intake, rumen pH, rumen temperature and oxi-redox potential of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin.

Variables	Angus				Nellore				SEM	P value			
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Interaction
DMI, kg	12.73	14.57	12.84	14.59	11.36	12.79	11.63	13.09	0.95	0.15	0.6	<0.01	MON*D; B*D
Rumen pH													
Medium	5.74	6.13	6.17	6.23	6.65	6.6	6.71	6.66	0.14	0.03	0.01	0.18	B*MON
Maximum	6.53	6.83	6.89	6.99	7.18	7.18	7.32	7.3	0.14	0.08	<0.01	0.1	-
Minimum	5.17	5.5	5.48	5.41	6.14	5.92	6.11	5.97	0.13	0.02	0.47	0.77	-
Time of pH, min/day													
<5.2	311.43	175.36	15.56	36.42	0.00	0.71	17.5	41.42	66.27	0.04	0.1	0.68	B*C
<5.6	538.57	294.29	184.93	230	49.81	7.85	78.92	123.57	74.41	0.03	0.07	0.18	B*C; B*D
<6.2	1089.64	660.36	749.94	719.64	382.65	276.43	318.93	347.5	117.2	0.03	0.17	0.01	C*MON; MON*D
Area, h.(pH/day)													
<5.2	118.73	74.77	2.44	4.56	0.21	0.05	1.93	12	116.9	0.23	0.03	0.68	B*C
<5.6	282.1	167.04	36.61	51.67	6.32	0.79	20.1	42.76	35.56	0.03	0.09	0.64	B*C
<6.2	768.93	448.94	310.27	330.96	120.35	66.4	136.02	175.74	101.5	0.05	0.01	0.09	B*C
Temperature	39.39	39.51	39.44	39.56	39.11	39.15	39.16	39.36	0.06	<0.01	0.02	<0.01	B*D
Oxi-redox potential	-419.19	-413.33	-389.45	-419.8	-331.02	-347.92	-348.62	-334.87	30.21	0.21	0.67	0.53	-

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin;[3] High-moisture corn with monensin;[4] High-moisture corn without monensin; Interactions: C*MON: corn * MON; B*C: breed*corn; B*MON: breed * MON; B*C*MON: breed*corn*MON

There was interaction for the DMI between days of collection and the breed, and also for the days of collection and the inclusion or not of the MON. About breed interaction, Angus had higher consumption on days 12 and 16 when compared to Nellore (Figure 9A) (P=0.02). And, regarding the inclusion or not of MON, the animals fed with MON in the diet had lower consumption on days 12, 16, 20, 24, and 28 of collection (P< 0.01) (Figure 9B).
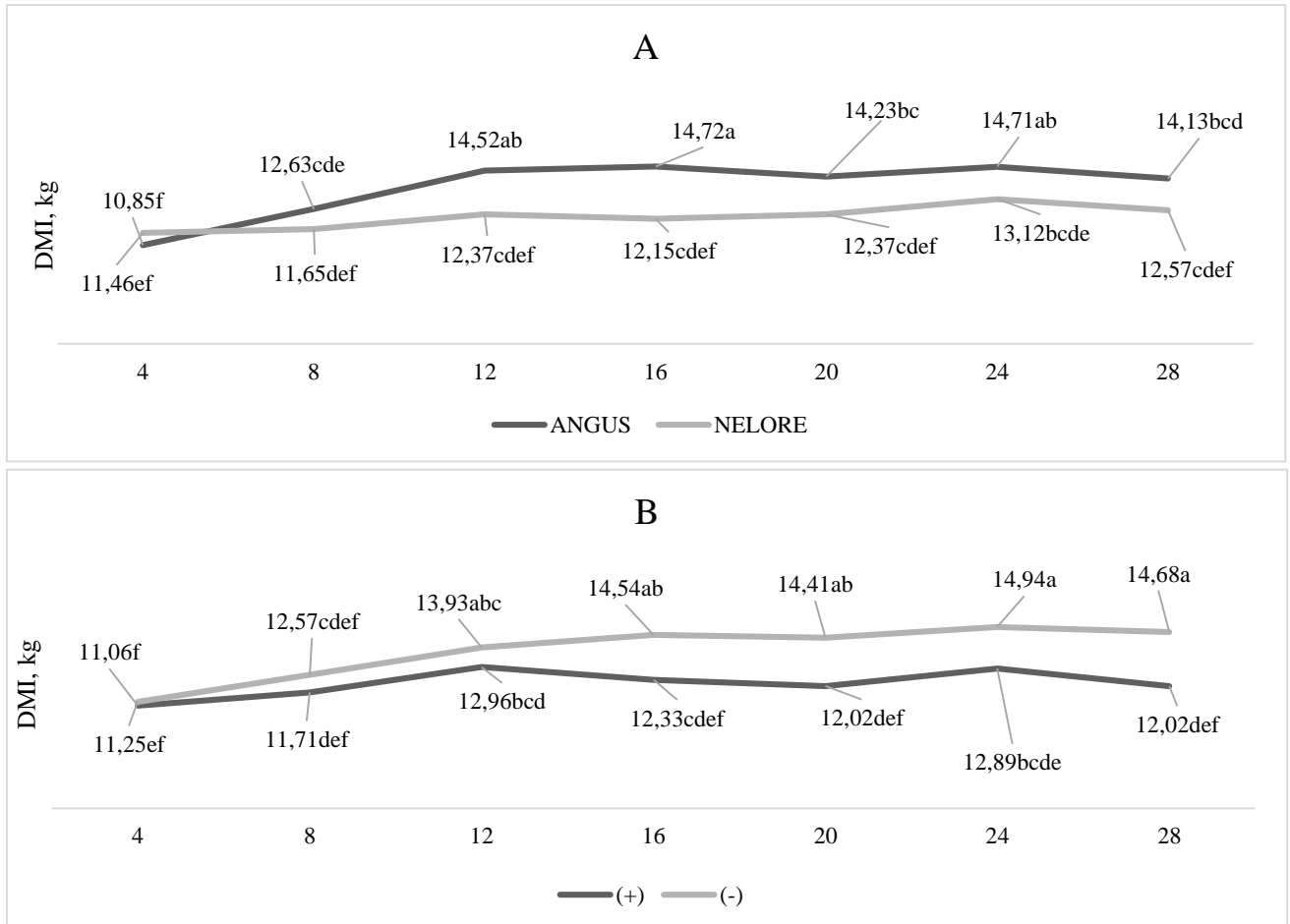
Figure 9. Interaction between breed and collection day (**A**; SEM = 1.06) and Mon suplementation (**B**; SEM = 13.72) on the DMI of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with additions of monensin or not. [a,b,c,d,e,f] Different letters differ significantly among treatments.

There was a breed effect for the minimum pH (P=0.02), where Angus cattle had a lower pH than Nellore (5.39 x 6.04; respectively) (SEM= 0.16). Angus receiving finely grind corn in the diet had a longer duration of pH below 5.2 and 5.6 compared to other cattle (P=0.04 and P<0.01).

There was an interaction between breed and type of corn processing for pH areas below 5.2; 5.6 and 6.2. Angus animals that received finely grind corn in the diet had greater area and in

the three analyzes when compared to the other animals (P=0.02; P=0.03 and P<0.01; respectively) (Figures 10C, 10B and 10A), similarly the duration of pH below 5.2 and 5.6 were longer in Angus animals with finely grind corn in the diet (Figures 10D and 10E). The average ruminal pH was lower for Angus receveing MON in the diet when compared to the other animals (P= 0.03) (Figure 10F).
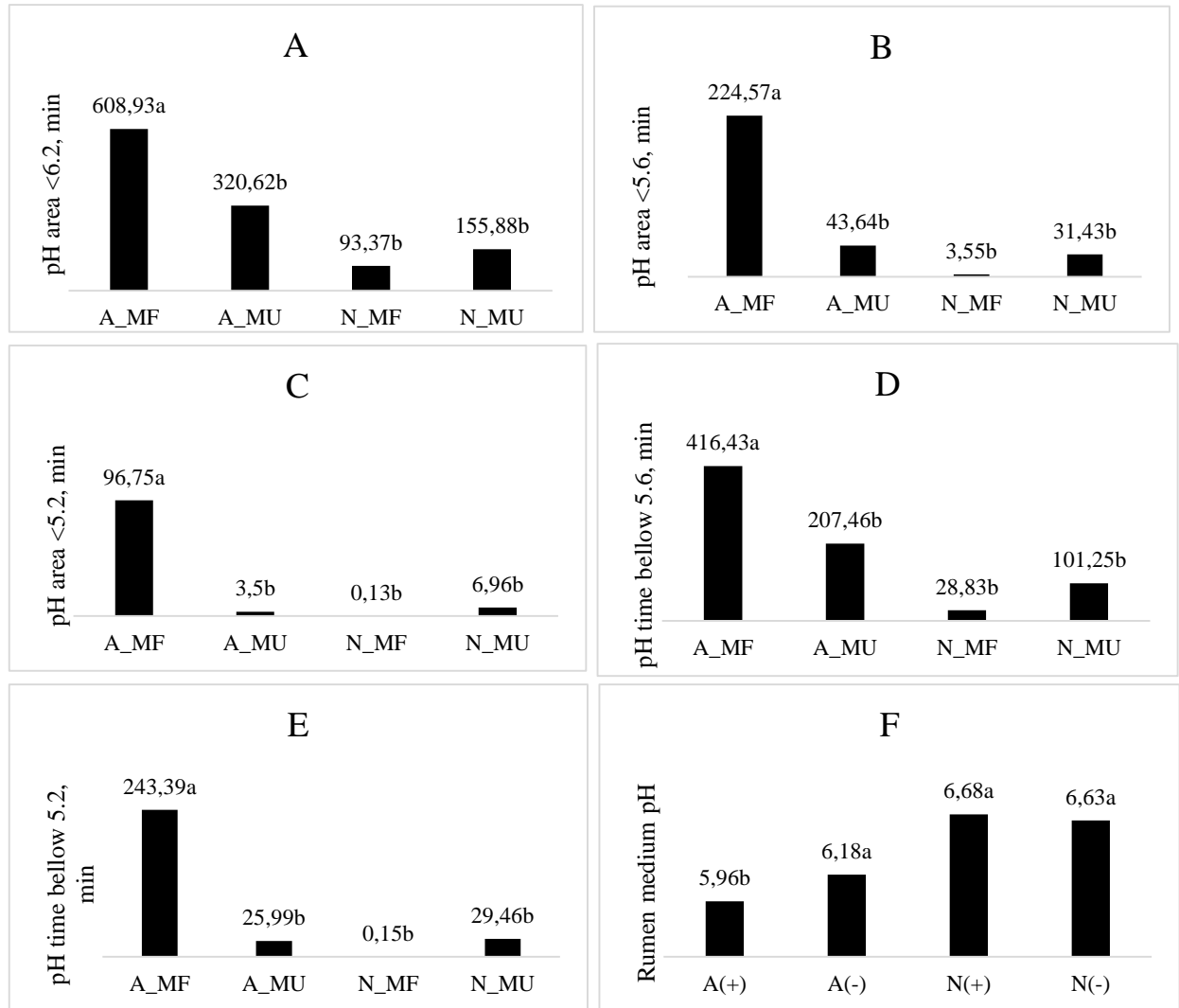
Figure 10. Interaction between breed and corn processing type on pH área bellow 6.2 (**A**; SEM = 122.41), pH area bellow 5.6 (**B**; SEM = 44.26), pH area bellow 5.2 (**C**; SEM = 28,94), time of pH bellow 5.6 (**D**; SEM = 85.92) and time of pH bellow 5.2 (**E**; SEM = 45.80) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin. Interaction between breed factor and Mon inclusion on medium pH (**F**; SEM = 0.18) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin. [a,b:] Mean with different letters differ significantly. A(-): Angus non supplemented with MON; A(+): Angus supplemented with MON; N(+): Nellore supplemented with MON; N(-): Nellore non supplemented with MON;

A_MF: Angus fed with finely ground corn; A_MU: Angus fed with high-moisture corn; N_MF: Nellore fed with finely ground corn; N_MU: Nellore fed with high-moisture corn.

The duration of pH below 5.6 was also affected by the interaction between breed and collection days (P<0.01), whereas Angus had a longer duration of pH below 5.6 on days 12, 20, 24, and 28 (Figure 11A). The ruminal temperature of Angus was higher than that of Nellore on days 16, 20, 24, and 28 (finishing phase) (P< 0.01) (Figure 11B).
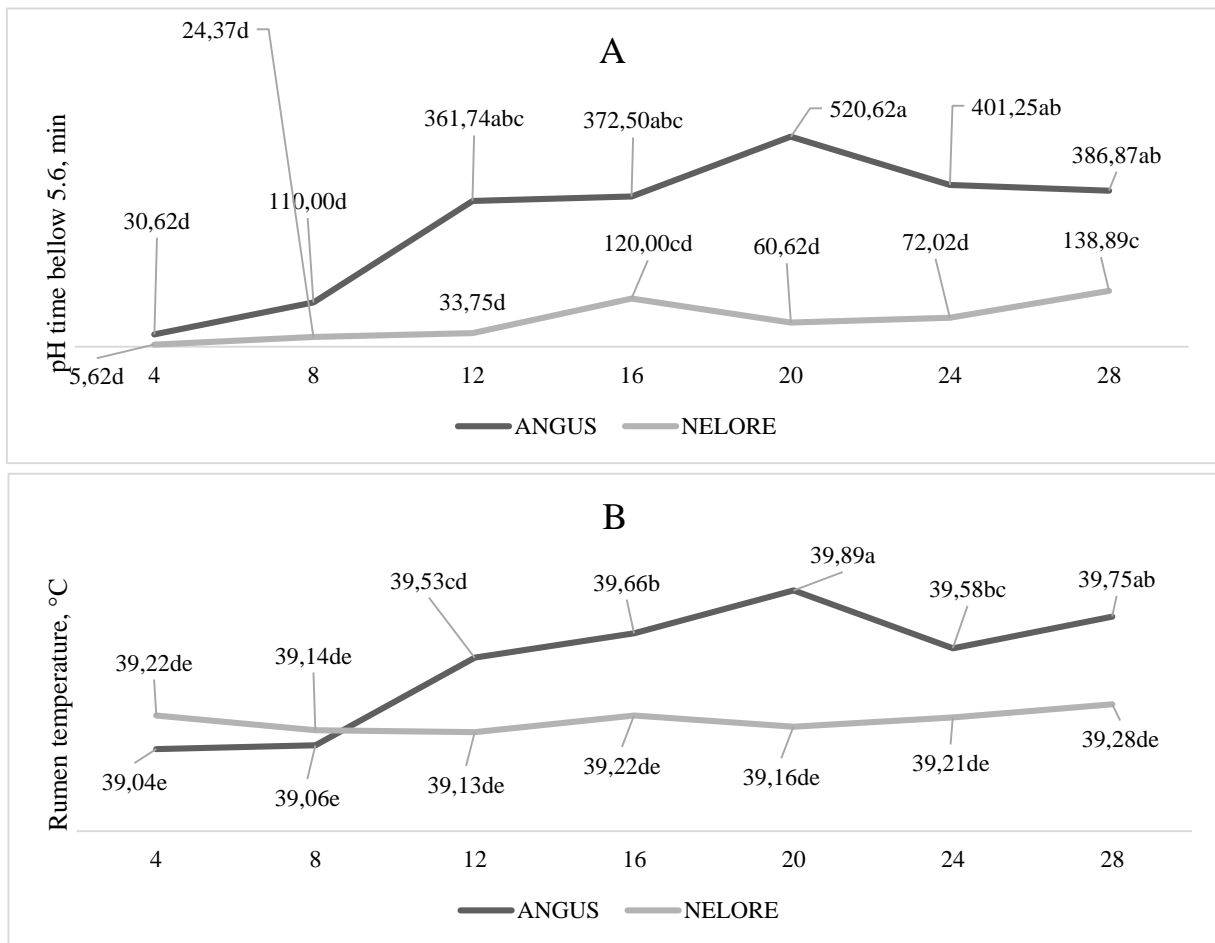
Figure 11: Interaction between breed fator and diet collection day factor on time of pH bellow 5.6 **A**; SEM = 98.40) and rumen temperature (**B**; SEM = 0.10) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin. [a,b,c,d:] mean with different letters differ significantly.

Fermentation products

The results of the concentration of the fermentation products are in Tables 5, 6, 7, 8, 9, 10 and 11; and, their interactions in the following figures.

Table 3. Products from rumen fermentation of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 4th experimental day.

Variables	Angus				Nellore				SEM	P value				
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Time	Interaction
Acetate, mol/100mol	65.83	63.3	67.89	66.92	72.51	74.00	75.76	77.83	3.68	0.07	0.06	0.99	<0.01	C*T; B*T
Propionate, mol/100mol	28.83	80.87	27.36	22.25	26.96	24.77	26.44	28.89	2.21	0.36	0.51	0.03	<0.01	B*MON; B*T;
Butyrate, mol/100mol	10.38	11.76	13.03	14.66	11.34	12.61	12.23	15.12	1.16	0.76	<0.01	<0.01	<0.01	B*MON*T
Total SCFA, mM	105.04	95.93	108.28	103.84	110.81	111.37	114.5	121.84	5.87	0.13	0.02	0.58	<0.01	B*MON; B*T;
Lactate, mM	0.043	0.039	0.038	0.04	0.035	0.03	0.03	0.032	0.002	0.03	0.15	0.18	<0.01	B*T
Ammonia, mM	17.97[ab]	14.33[b]	18.99[ab]	14.07[b]	23.58[a]	16.76[b]	16.06[b]	19.10[ab]	3.24	0.2	0.29	<0.01	0.02	B*C*MON;

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin; Interactions: C*MON: corn * MON; B*C: breed*corn; B*MON: breed * MON; B*C*MON: breed*corn*MON; C*T: corn*time; B*T: breed*time; MON*T: MON*time; ab: different letters differ statisticall

On day 4 (adaptation phase), there was an interaction between breed and time for acetate concentration (mM), where Nellore breed had higher acetate concentration 12 hours after feeding when compared to Angus s (Figure 12A). The concentration of ruminal lactate was influenced by the interaction of breed and time of collection after the feeding (P=0.03), Angus had a higher concentration of ruminal lactate than the Nellore before the and 12 hours after the feeding; even though it is not a concentration of concern, or that indicates acidosis (Figure 12C).

Nellore receiving finely grind corn with MON had a higher concentration of ruminal ammonia (P< 0.01) than Angus that did not receive MON, regardless of the type of corn processing (Table 5). In addition, there was an interaction between breed and time (Figure 12D) (P= 0.04). However, there was no difference between Angus and Nellore breeds, only between the hours within each breed.
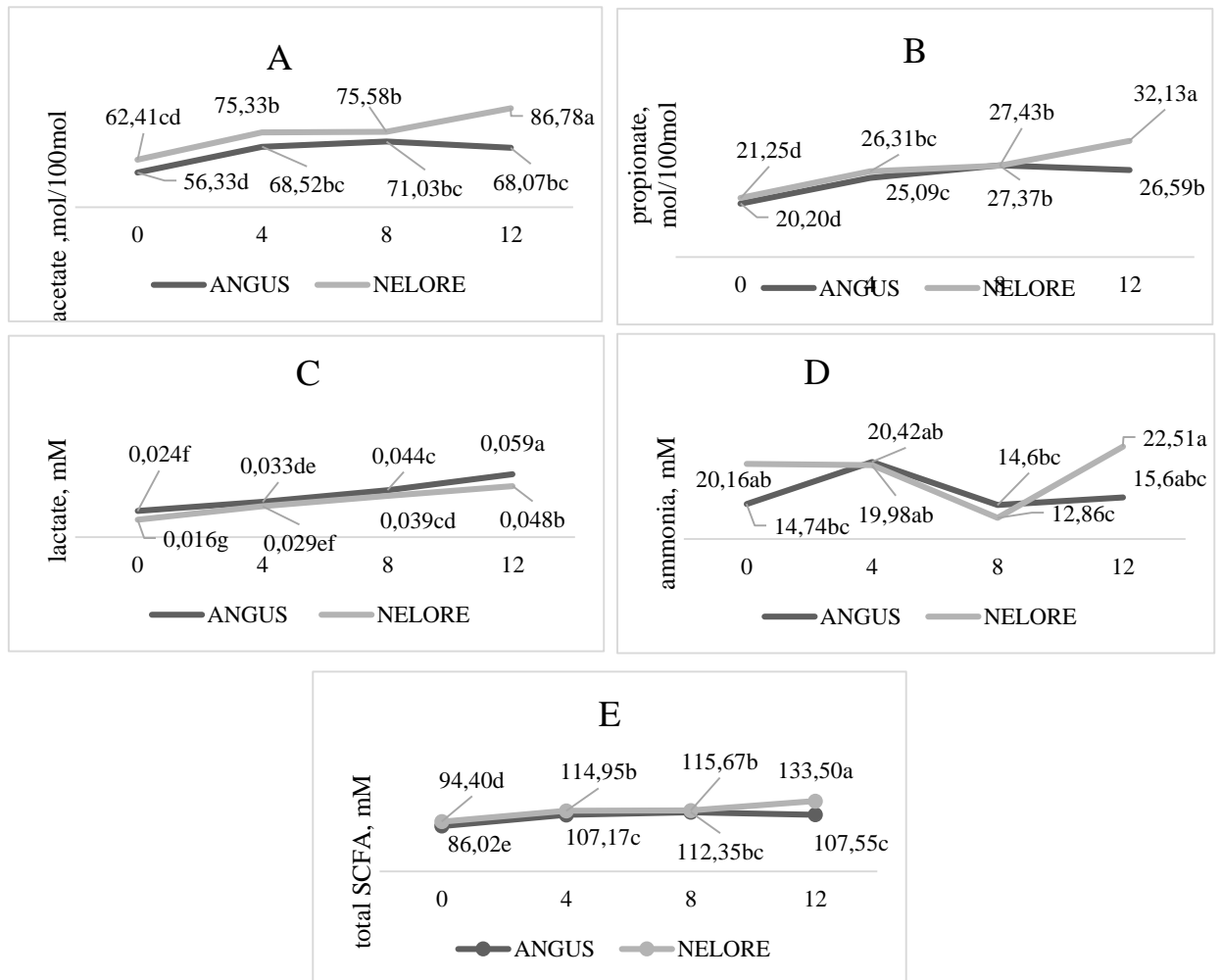
Figure 12. Interaction between breed fator and time after feeding on rumen acetate concentration (**A**; SEM = 3.00), propionate (**B**; SEM = 1.77), lactate (**C**; SEM = 0.002), ammonia (N-NH₃) (**D**; SEM =

3.30) and total SCFA (**E**; SEM = 5.61) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 4th experimental day. [a,b,c,d:] mean with different letters differ significantly.

Angus cattle that had MON included in the diet had a higher concentration of ruminal propionate (P= 0.02) than Nellore and Angus animals without the inclusion of MON (Figure 13A). In addition, Angus had a higher concentration of propionate than Nellore 12 hours after feeding(P<0.01) (Figure 12B). There was an interaction between breed, inclusion or not of MON, and hours after treatment (P= 0.02) on the concentration of ruminal butyrate (Figure 13C).

Evaluating the total concentration of SCFA, there was an interaction between breed and the inclusion or not of MON (P= 0.04), and Nellore cattle not consuming MON in the diet had a higher total concentration of SCFA than Angus also without the inclusion of MON (Figure 13B). There was also interaction for breed and time of collection after feeding (P< 0.01) (Figure 12E), and, the Nellore had a higher total concentration of SCFA than the Angus before the treatment and 4 and 12 hours after the feed.
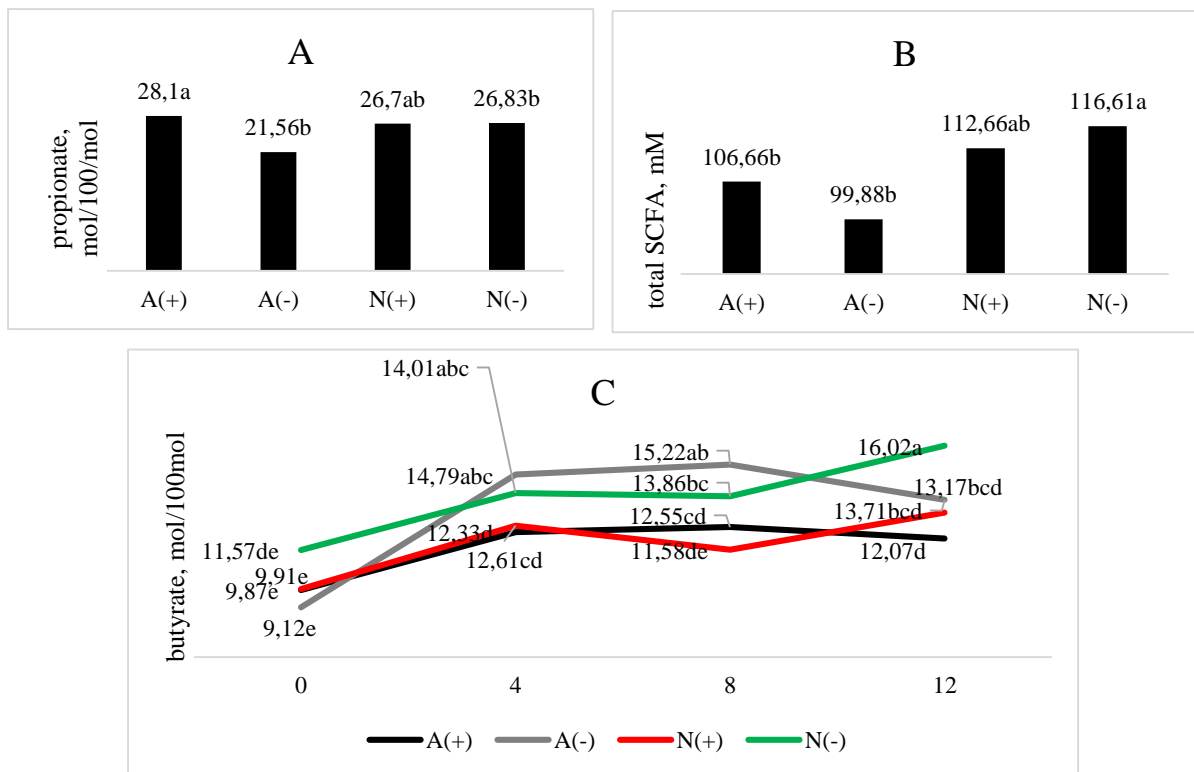
Figure 13: Interaction between breed factor and inclusion or not of MON on the propionate concentration (**A**; SEM = 1.77) and total SCFA (**B**; SEM = 5.32) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 4th experimental

day. Interaction between breed factor, inclusion or not f MON and times after feeding on the butyrate concentration (**C**; SEM = 1.05) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 4th experimental day. [a,b,c,d] mean with different letters differ significantly.. A(-): Angus non supplemented with MON; A(+): Angus supplemented with MON; N(+): Nellore supplemented with MON; N(-): Nellore non supplemented with MON**.**

Table 6. Products from rumen fermentation of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 8th experimental day.

Variables	Angus				Nellore				SEM	P value				
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Time	Interaction
Acetate, mol/100mol	62.31	71.04	66.55	68.3	73.21	74.56	75.2	76.1	3.88	0.04	0.45	0.07	0.10	B*T; C*T
Propionate, mol/100mol	27.94	21.4	28.23	20.92	24.8	21.52	26.17	23.7	1.93	0.72	0.45	<0.01	0.04	B*T
Butyrate, mol/100mol	13.01	13.78	14.04	17.47	12.53	14.33	14.66	14.52	1.7	0.74	0.06	0.12	0.35	B*T; C*T
Total SCFA, mM	103.26	106.22	108.8	106.75	110.53	110.41	116.04	114.33	6.53	0.29	0.15	0.93	0.06	B*T
Lactate, mM	0.04	0.046	0.044	0.044	0.035	0.035	0.035	0.036	0.002	0.02	0.79	0.16	<.0001	-
Ammonia, mM	21.61	17.69	17.23	15.92	23.02	19.92	22.31	19.75	2.24	0.12	0.08	0.01	<0.001	-

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin;[3] High-moisture corn with monensin;[4] High-moisture corn without monensin; Interactions: C*MON: corn * MON; B*C: breed*corn; B*MON: breed * MON; B*C*MON: breed*corn*MON; C*T: corn*time; B*T: breed*time; MON*T: MON*time.

On day 8 of the experiment, ruminal acetate concentration was higher 12 hours after feeding for Nellore comparing to Nellore s (P< 0.01) (Figure 14A). The concentration of propionate was also influenced by breed and time of collection (P<0.01), but there were no differences between breeds, only between the breed itself and the time of collection (Figure 14B). The same occurred with the concentration of ruminal butyrate, with no differences between breeds, only between collection hours (P<0.01) (Figure 14C). Angus animals had a higher concentration of ruminal lactate than Nellore on day 8 of the experiment (P= 0.02) (Figure 14D).
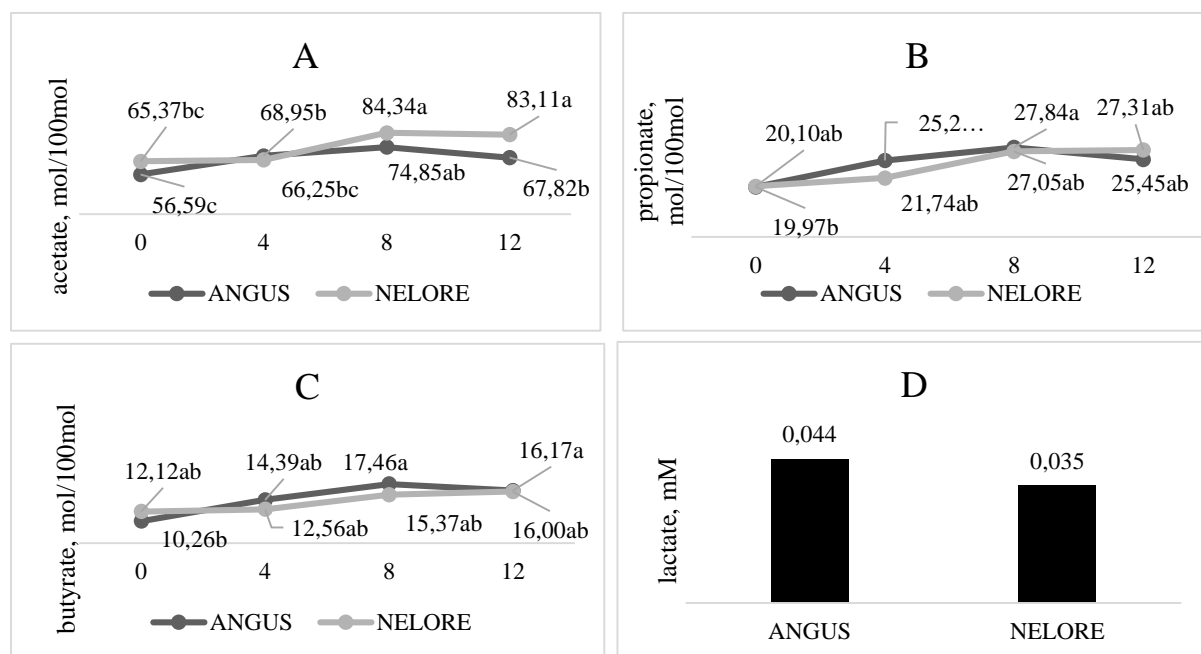
Figure 14. Interaction between breed factor and time after feeding on rumen acetate concentration (**A**; SEM = 5.79), proprionate (**B**; SEM = 2,10) and butyrate (**C**; SEM = 1.69) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 8th experimental day. Effect of breed factor on rumen lactate concentration (**D**; SEM = 0.002) on 8th experimental day. a,b,c: mean with different letters differ significantly.

Table 4: Products from rumen fermentation of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 12th experimental day.

Variables	Angus				Nellore				SEM	P value				
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Time	Interaction
Acetate, mol/100mol	66.63	74.67	70.27	70.62	72.48	74.86	67.00	80.45	4.34	0.51	0.98	0.03	<0.01	-
Propionate,	29.01	23.53	33.2	34.19	26.52	25.52	28.74	25.73	2.68	0.19	0.01	0.15	<0.01	B*C;MON*T
Butyrate, mol/100mol	13.34	16.24	16.28	16	13.38	15.07	13.53	18.82	1.38	0.82	0.1	0.02	<0.01	C*T;
Total SCFA, mM	110.4	114.4	119.7	120.8	112.4	115.5	109.3	125.0	6.48	0.9	0.2	0.17	<0.01	-
Lactate, mM	0.045	0.036	0.036	0.038	0.033	0.03	0.035	0.03	0.002	<.0001	0.04	0.05	<.0001	B*MON*T
Ammonia, mM	16.54	21.12	15.38	17.57	25.38	22.94	26.3	18.2	3.48	0.13	0.18	0.54	0.4466	B*MON

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin; Interactions: C*MON: corn * MON; B*C: breed*corn; B*MON: breed * MON; B*C*MON: breed*corn*MON; C*T: corn*time; B*T: breed*time; MON*T: MON*time.

There was an interaction between breed and corn processing on the ruminal concentration of propionate (P= 0.04) on day 12, and the Angus receiving high moisture corn had a higher concentration of ruminal propionate than Angus receiving finely grind corn (Figure 15A).

Ruminal lactate concentration was higher for Angus consuming MON before and 4 and 8 hours after feeding, compared to Nellore with or without MON (P=0.04) (Figure 15C). Twelve hours after feeding, Angus cattle with inclusion of MON also had a higher concentration of lactate than the Nellore without MON.

Nellore without the inclusion of MON in the diet had a lower concentration of NH_3 than the Angus without the inclusion of MON (P=0.01) (Figure 15B). Twelve hours after feeding, Angus with inclusion of MON also had a higher concentration of lactate than the Nellore without MON.
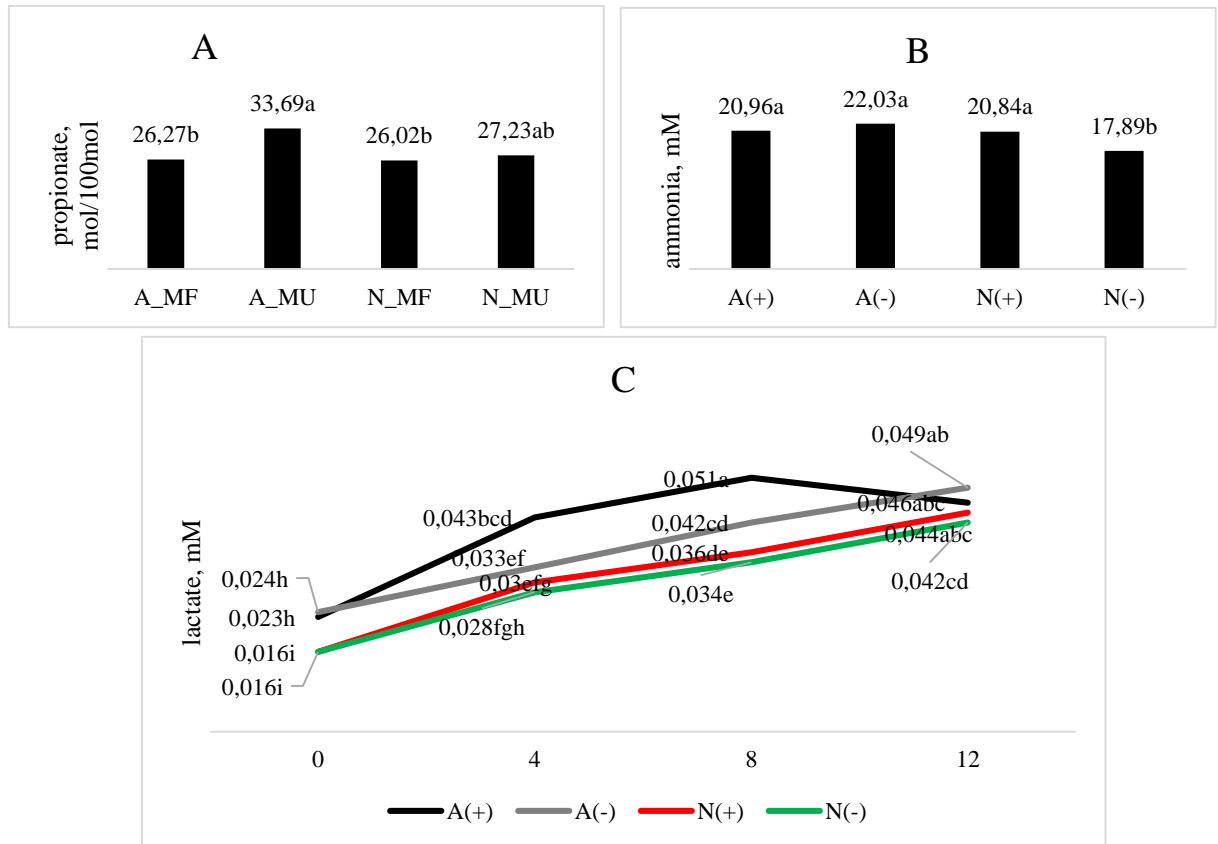
Figure 15: Effect of breed factor on rumen propionate concentration (**A**; SEM = 2.3) and ammonia (**B**; SEM = 4.49) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 12th experimental day. Interaction of breed factor, inclusion or not of MON and time after feeding on rumen lactate concentration (**C**; SEM = 1.72) on 12th experimental day. a, b, c, e, f, g, h, i: Mean with different letters differ significantly. A(-): Angus non suplemented MON; A(+): Angus suplemented with MON; N(+): Nellore suplemented with MON; N(-): Nellore non suplemented MON; A_MF: Angus fed with finely ground corn; A_MU: Angusfed with high-moisture corn; N_MF: Nellore fed with finely ground corn; N_MU: Nellore fed with high-moisture corn.

Table 5: Products from rumen fermentation of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 16th experimental day

Variables	Angus				Nellore				SEM	P value				
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Time	Interaction
Acetate, mol/100mol	65.64	74.48	66.92	67.39	73.4	81.78	75.42	83.63	3.24	0.01	0.8	0	<0.01	-
Propionate,	33.51[ab]	22.80[c]	31.79[ab]	36.13[a]	26.43[abc]	24.63[bc]	25.68[abc]	25.19[abc]	2.74	0.1	0.06	0.15	<0.01	-
Butyrate, mol/100mol	13.7	16.22	16.31	13.29	14.36	16.98	21.7	22.83	1.86	0.1	<0.01	0.34	<0.01	B*C; C*T
Total SCFA, mM	112.86	113.51	115.02	116.81	114.18	123.38	122.8	131.65	4.91	0.15	0.05	0.06	<0.01	-
Lactate, mM	0.046	0.043	0.048	0.045	0.04	0.036	0.036	0.037	0.004	0.008	0.671	0.027	<0.01	B*T
Ammonia, mM	19.99	20.76	19.71	16.01	26.9	22.26	29.07	22.89	4.12	0.09	0.81	0.15	0.06	-

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin; Interactions: B*C: breed*corn; C*T: corn*time; B*T: breed*time; abc: mean with different letters differ statistically.

On day 16 of the experiment, the concentration of ruminal acetate was influenced by the breed of the animals (P=0.01), Angus had a lower concentration than the Nellore (68.61x78.56; respectively- EPM= 2.46) (Table 8). On the same day, Nellore animals that receiving high moisture corn had a higher concentration of ruminal butyrate than the other animals (P< 0.01) (Figure 16A). Ruminal lactate concentration was also influenced by breed and time of collection; Angus had higher concentrations 8 and 12 hours after feeding (P<0.01) (Figure 16B).
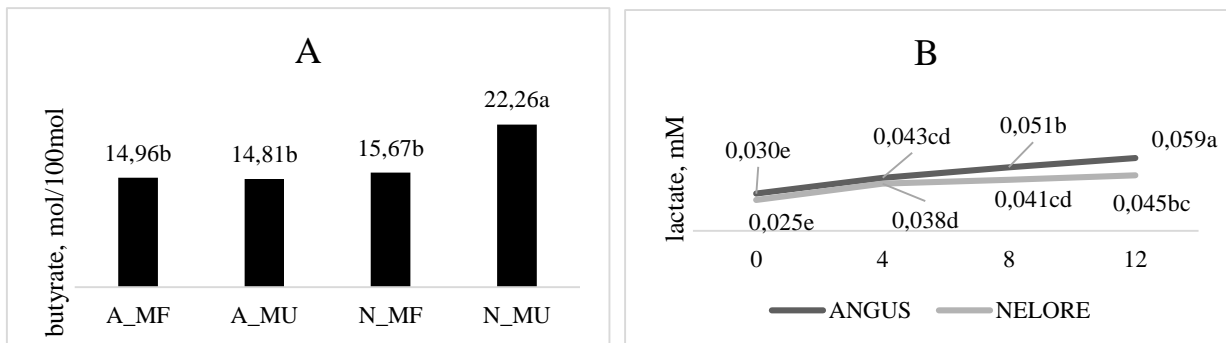
Figure 16: Interaction of breed factor and corn processing type on the butyrate concentration (**A**; SEM = 1.72) and breed factor and time after feeding on lactate concentration (B; SEM = 0.04) no dia 16 of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 16th experimental day. [a,b,c,d,e] Mean with different letters differ significantly. A_MF: Angus fed with finely ground corn; A_MU: Angus fed with high-moisture corn; N_MF: Nellore fed with finely ground corn; N_MU: Nellore fed with high-moisture corn.

Table 6: Products from rumen fermentation of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 20th experimental day.

Variables	Angus				Nellore				SEM	P value				
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Time	Interaction
Acetate, mol/100mol	62.82[c]	77.61[ab]	70.49[bc]	71.11[bc]	75.77[ab]	81.75[ab]	72.48[bc]	84.39[a]	3.17	0.03	0.94	<0.01	<0.01	C*T
Propionate, mol/100mol	31.36	25.51	32.34	37.91	27.93	25.35	27.81	27.36	2.89	0.05	0.09	0.7	<0.01	-
Butyrate, mol/100mol	11.56[c]	16.63[abc]	15.30[bc]	14.36[bc]	18.05[ab]	16.12[abc]	18.65[ab]	21.31[a]	1.65	0.04	0.08	0.23	<0.01	-
Total SCFA, mM	105.73	119.75	118.13	123.38	121.74	123.22	118.93	133.63	5.84	0.21	0.07	0.01	<0.01	-
Lactate, mM	0.041	0.037	0.038	0.04	0.034	0.035	0.031	0.033	0.003	0.038	0.317	0.95	0.002	B*T
Ammonia, mM	22.23	22.85	16.44	11.85	26.22	28.27	22.04	23.87	2.36	<0.01	<0.01	0.99	0.229	B*T

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin;[3] High-moisture corn with monensin;[4] High-moisture corn without monensin; Interactions: C*T: corn*time; B*T: breed*time; abc: different letters differ statistically

Breed effect was obtained for the ruminal concentration of acetate, propionate, and butyrate (Table 9). Angus had a higher concentration of propionate (31.78x27.37-EPM= 2.08), and lower concentrations of acetate (70.51x78.60-EPM=2.40) and butyrate (14.46x18, 53- SEM=1.12) than Nellore.

As for lactate concentration, there was an interaction between breed and time (P= 0.03) (Figure 17); where 8 and 12 hours after feeding, ruminal lactate concentration was observed in Angus. Differently from the lactate concentration, the NH_3 concentration was higher for Nellore cattle at 4, 8, and 12 hours after feeding (P= 0.04) (Figure 17B).
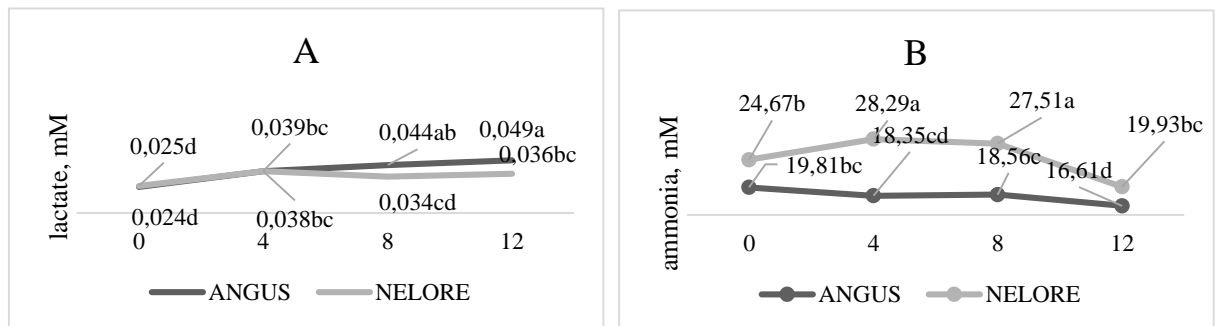
Figure 17: Interaction between breed factor and time after feeding on rumen lactate concentration (**A**; SEM = 0.04) and ammonia (**B**; SEM = 2.34) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 20th experimental day. [a,b,c,d] Mean with different letters differ significantly.

Table 7: Products from rumen fermentation of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 24th experimental day.

Variables	Angus				Nellore				SEM	P value				
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Time	Interaction
Acetate, mol/100mol	62.82[c]	77.61[ab]	70.49[bc]	71.11[bc]	71.12	78.56	70.31	78.25	3.01	0.2	0.25	<0.01	<0.01	C*T
Propionate, mol/100mol	31.36	25.51	32.34	37.91	30.28	26.34	25.23	26.31	2.46	0.28	0.44	0.21	<0.01	B*T
Butyrate, mol/100mol	11.56[c]	16.63[abc]	15.30[bc]	14.36[bc]	14.68	18.01	19.1	17.9	1.45	0.09	0.008	0.15	<0.01	C*T
Total SCFA, mM	105.73	119.75	118.13	123.38	116.09	122.91	114.64	122.46	4.85	0.37	0.2	0.08	<0.01	C*T
Lactate, mM	0.041	0.037	0.038	0.04	0.031	0.032	0.032	0.034	0.005	0.14	0.008	0.98	0.008	-
Ammonia, mM	22.23	22.85	16.44	11.85	28.73	30.91	25.02	24.22	3.27	0.09	0.004	0.29	0.85	C*T

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin; Interactions: C*T: corn*time; B*T: breed*time; abc: different letters differ statistically

On day 24 of the experiment, only the concentration of propionate interacted between breed and time (P= 0.04). Angus cattle had a higher concentration of ruminal propionate than Nellore cattle 4 hours after feeding (Figure 18).
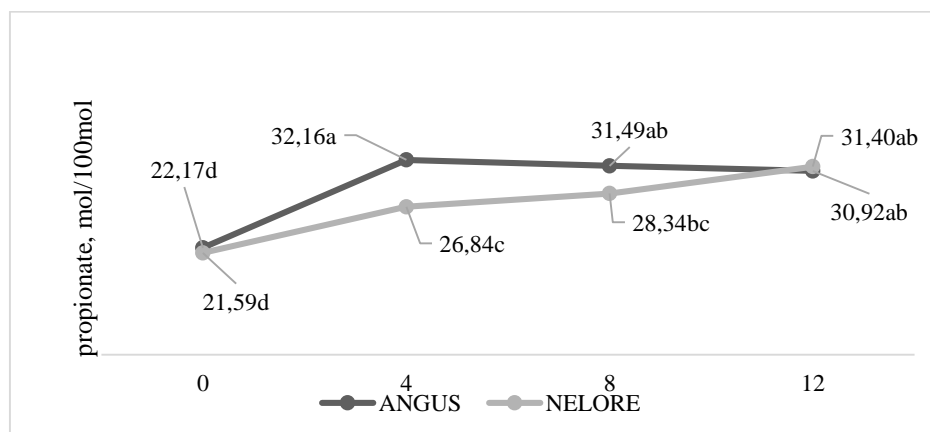
Figure 18: Interaction between breed factor and time after feeding on rumen propionate concentration of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensinon 24th experimental day. [a,b,c,d] Mean with different letters differ significantly (P= 0.04) (SEM=2.40).

Table 8: Products from rumen fermentation of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 28th experimental day.

Variables	Angus				Nellore				SEM	P value				
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON	HMA[4]		Breed	Corn	Mon	Time	Interaction
Acetate, mol/100mol	62.71	68.94	70.23	68.17	71.96	78.76	67.86	76.46	3.26	0.08	0.96	0.01	<0.01	B*T; B*C
Propionate, mol/100mol	39.94	26.05	28.38	29.97	29.20	26.60	25.90	22.43	2.41	0.26	0.38	0.16	<0.01	-
Butyrate, mol/100mol	13.54^d	14.80^d	18.51^{ab}	14.70^{cd}	15.23^{bc}	16.95^{bc}	15.08^{cd}	21.38^a	1.21	0.05	0.01	0.12	<0.01	B*C*MON; B*T;
Total SCFA, mM	106.19	109.79	117.12	112.85	116.39	122.3	108.83	120.27	5.51	0.4	0.69	0.14	<0.01	B*T; B*C
Lactate, mM	0.046	0.045	0.039	0.036	0.046	0.045	0.036	0.039	0.003	0.018	0.13	0.29	0.01	-
Ammonia, mM	19.69	21.73	19.84	15.6	27.27	24.91	22.81	19.24	2.98	0.04	<0.01	0.02	0.68	-

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin;[3] High-moisture corn with monensin;[4] High-moisture corn without monensin; Interactions: B*T: breed*time; B*C: breed*corn; B*C*MON: breed*corn*mon; abc: different letters differ statistically.

On the last day of collection of ruminal fermentation products (day 28), there was an interaction between breed and time (P< 0.01), Nellore cattle had a higher concentration of ruminal acetate before nt and 12 hours after feeding, compared to Angus cattle (Figure 19A).

The butyrate concentration was higher for Nellore compared to Angus cattle on the same diets (eg Nellore finely grind corn compared to Angus finely grind corn) (Table 11). There was also interaction for butyrate concentration, between the effects of breed and time (P= 0.01), with Nellore animals having a higher concentration than Angus animals 12 hours after feeding. (Figure 19B).

There were two interactions for the total SCFA concentration, being them between breed and time (P= 0.01), and breed and corn (P= 0.04). In the first interaction, Nellore had a higher total concentration of SCFA than Angus before and 12 hours after feeding. (Figure 19C).
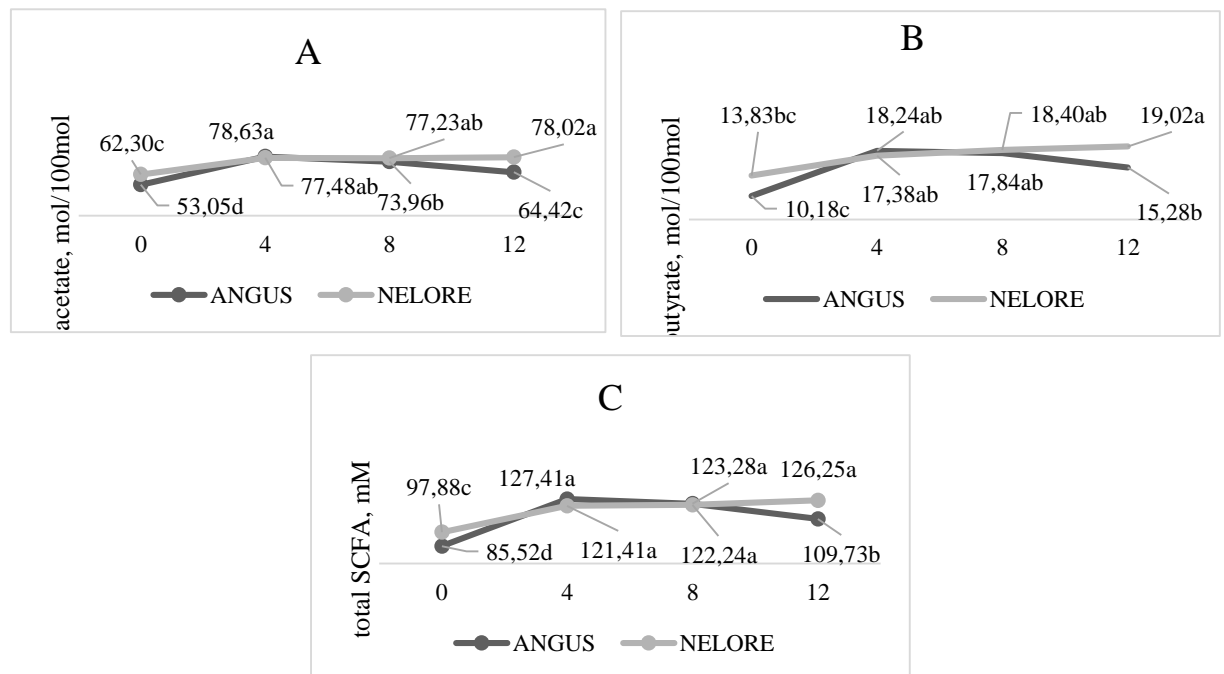
Figure 19: Interaction between breed factor and time after feeding on rumen acetate concentration (**A**; SEM = 3.37), butyrate (**B**; SEM = 0.81) and total SCFA (**C**; SEM = 5.25) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 28th experimental day. [a,b,c,d] Mean with different letters differ significantly.

There was also an interaction between breed and type of corn processing (P= 0.04), Nellore animals consuming finely grind corn had a higher concentration than Angus animals receiving the

same diet (Figure 20A). In the interaction between breed and corn, Nellore receiving finely grind corn had a higher concentration than Angus consuming the same corn processing. (Figure 20B).
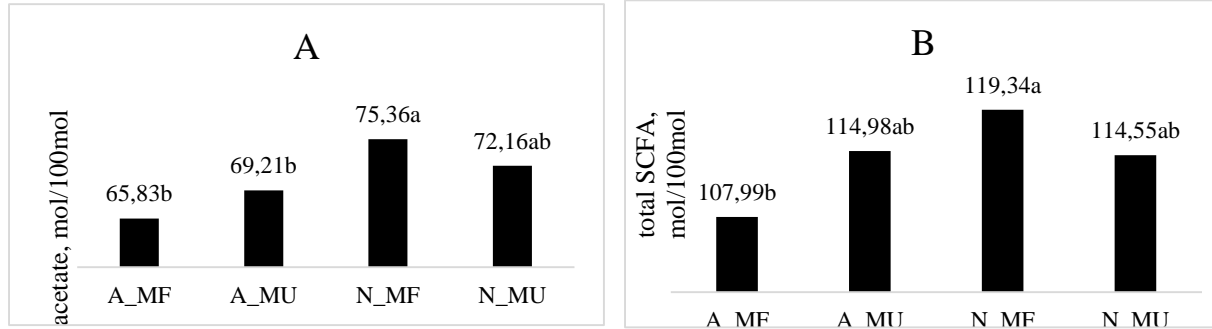
Figure 20: Interaction between breed factor and corn processing type on rumen acetate concentration (**A**; SEM = 3.09) and total SCFA (**B**; SEM = 4.79) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin on 28th experimental day. [a,b:] Mean with different letters differ significantly. A_MF: Angus fed with finely ground corn; A_MU: Angus fed with high-moisture corn; N_MF: Nellore fed with finely ground corn; N_MU: Nellore fed with high-moisture corn.

Ruminal degradability in situ

The results of ruminal degradability of Angus and Nellore cattle submitted to different nutritional strategies are represented in Table 12 and the figures below.

Tabela 9: Effective and potential rumen degradability of DM, Starch (ST), crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin.

Variables	Angus				Nellore				SEM	P value			
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Interaction
DM Effective Degradability													
2% of DM	69.57	69.50	69.22	71.19	71.76	69.68	70.39	69.66	1.29	0.67	0.98	0.63	B*MON
5% of DM	58.82	56.77	59.29	59.56	61.02	58.31	59.53	59.00	1.42	0.52	0.25	0.03	C*MON
8% of DM	51.68	48.97	52.47	52.00	53.76	50.97	52.28	51.80	1.44	0.49	0.16	0.01	B*C/C*MON
DM potential degradability	80.40	83.68	78.81	83.22	82.33	81.58	81.23	80.17	1.23	0.87	0.03	0.01	B*MON
ST Effective Degradability													
2% of ST	86.22	84.56	87.08	86.27	87.98	85.84	87.07	87.13	1.19	0.45	0.20	0.05	-
5% of ST	72.67	69.87	74.72	72.50	75.32	72.09	73.63	73.88	2.02	0.53	0.19	0.03	-
8% of ST	63.63	60.25	66.12	63.21	66.50	62.84	64.52	64.71	2.32	0.56	0.20	0.03	-
ST potential degradability	99.97	100.25	99.00	99.89	100.00	99.51	100.00	99.85	0.34	0.61	0.36	0.91	B*MON/B*C
CP Effective Degradability													
2% of CP	72.90	73.61	76.45	75.15	79.77	76.61	78.63	77.78	1.88	0.02	0.18	0.22	-
5% of CP	59.03	57.11	62.83	58.63	66.74	62.46	64.69	64.33	2.36	0.05	0.29	0.04	-
8% of CP	50.84	47.93	54.34	49.21	58.15	53.84	55.77	55.69	2.40	0.05	0.39	0.02	-
CP potential degradability	89.72	94.77	91.22	95.66	93.05	92.64	93.32	91.95	1.04	0.93	0.35	0.02	B*MON
ADF Effective Degradability													
2% of ADF	65.84	66.51	67.46	68.17	69.60	67.01	67.61	67.03	1.55	0.53	0.67	0.56	-
5% of ADF	54.94	53.74	57.06	56.88	58.79	55.39	56.45	56.00	1.59	0.46	0.27	0.11	B*C
8% of ADF	47.93	46.18	50.19	49.65	51.64	48.15	49.27	48.83	1.55	0.48	0.21	0.06	B*C
ADF potential degradability	77.26	81.44	78.02	80.14	80.52	79.72	79.34	78.40	1.67	0.84	0.38	0.20	B*MON
NDF Effective Degradability													
2% of ADF	34.32	34.26	29.04	36.47	35.68	30.11	32.72	30.75	2.77	0.61	0.29	0.97	B*MON/C*MON
5% of ADF	26.80	25.14	23.40	27.13	26.78	21.25	24.31	24.79	1.77	0.32	0.92	0.37	B*MON/C*MON
8% of ADF	23.69	21.84	20.94	23.49	23.18	17.91	21.06	22.35	1.51	0.25	0.69	0.28	C*MON
NDF potential degradability	54.38	63.82	41.82	59.98	56.92	58.89	56.36	44.52	7.81	0.93	0.02	0.15	B*MON

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin; Interactions: B*MON: breed*Mon; C*MON: corn*Mon; B*C: breed*corn; B*C*MON: breed*corn*mon.

When adopting a passage rate of 2% (Figure 21A), the Nellore receiving MON presented values of DM degradability higher than the Angus also receiving MON, values also higher than the Nellore animals that did not received MON (P = 0.02). Angus fed with MON had lower values of potential DM degradability than animals of the same breed without access to the additive, as well as Nellore animals that also received this ionophore (Figure 21B).

Considering the degradability of the NDF and the passage rates of 2% and 5%, the taurines that did not ingest MON had higher values of effective NDF degradability compared to the Nellore breed that also did not ingest this additive (Figure 21D and 21C). On the other hand, Angus animals that received MON in the diet had values of effective NDF degradability similar to Nellore animals. Angus animals that ingested MON had less potential NDF degradability than Angus that also had access to MON. There was no relationship between Nellore and Angus x Nellore breeds (Figure 21E).

For the potential degradability of ADF (Figure 21F), Angus animals without MON inclusion had higher degradation values when compared to other Angus animals that consumed MON. There was interaction for potential degradability of CP, of the animals that received MON in the diet, the Nellore had higher values of potential degradability of CP than the Angus animals. While among the animals that did not receive MON, the Angus had better results than the Nellore. (Figure 21G).
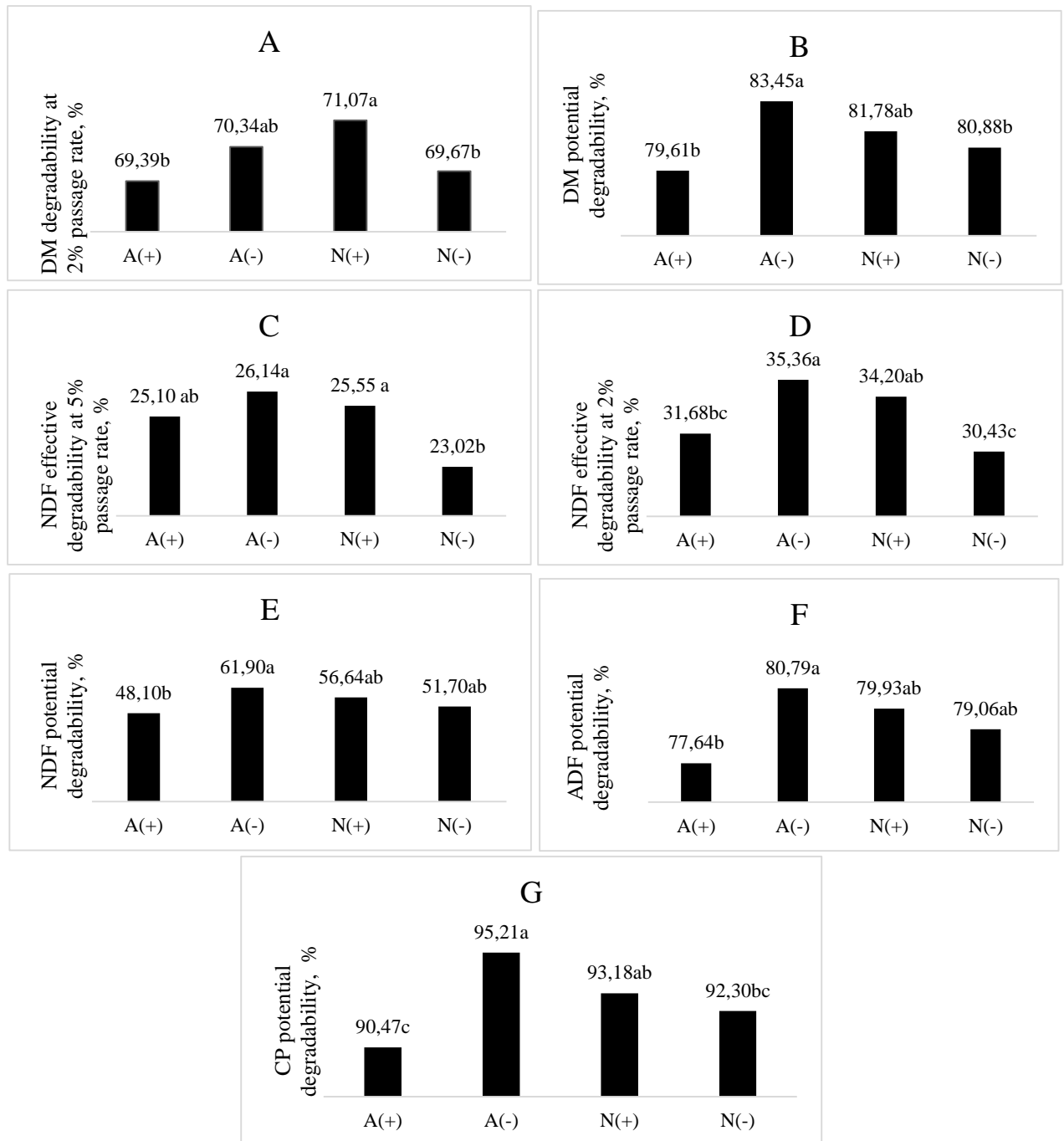
Figure 21*:* Interaction between breed factor and MON supplementation on DM degradability at 2% passage rate (**A**; SEM = 1.20), DM potential degradability (**B**; SEM = 1.12), NDF effective degradability at 5% passage rate (**C**; SEM = 1.56), NDF effective degradability at 2% passage rate (**D**; SEM = 2.49), NDF potential degradability (**E**; SEM = 7,19), ADF potential degradability (**F**; SEM = 1.44) and CP potential degradability (**G**; SEM = 0,91). a,b: Mean with different letters differ significantly. A(-): Angus non supplemented with Mon; A(+): Angus supplemented with Mon; N(+): Nellore supplemented with Mon; N(-): Nellore non supplemented with Mon.

When using a 5% passage rate (Figure 22A), the MON inclusion in the diets also increased the value of effective DM degradability. It is possible to notice a similarity between Figures 22B, 22C, and 22D, where adopting different passage rates of 2%, 5%, and 8% respectively, animals that received MON obtained higher values of effective starch degradability, regardless of breed or processing corn grain.
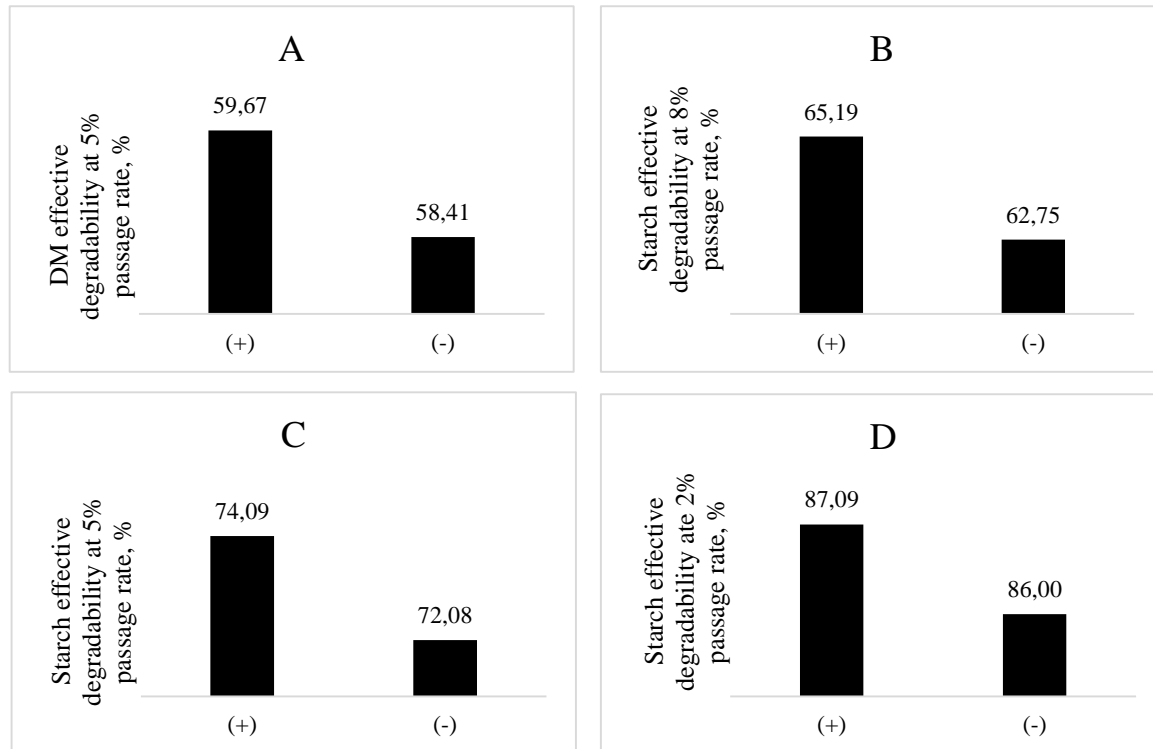
Figure 22: Effect of monensin factor on DM effective degradability at 5% passage rate (**A**; SEM = 1.13), Starch effective degradability at 8% passage rate (**B**; SEM = 1.70), Starch effective degradability at 5% passage rate (**C**; SEM = 1.48) and Starch effective degradability at 2% passage rate (**D**; SEM = 1,48).

Using 8% for the passage rate value (Figure 23A), Nellore that consumed finely grind corn had greater DM degradability than Angus consuming corn with the same processing. As for the high moisture corn, no difference was observed between the breeds.

For ADF degradability, when assuming values of 5% and 8% for the passage rate (Figure 23C and 23D), Angus consuming high moisture corn obtained better results in ADF degradation concerning animals of the same breed that consumed finely grind corn, also being lower when compared to Nellore animals also fed with finely grind corn.
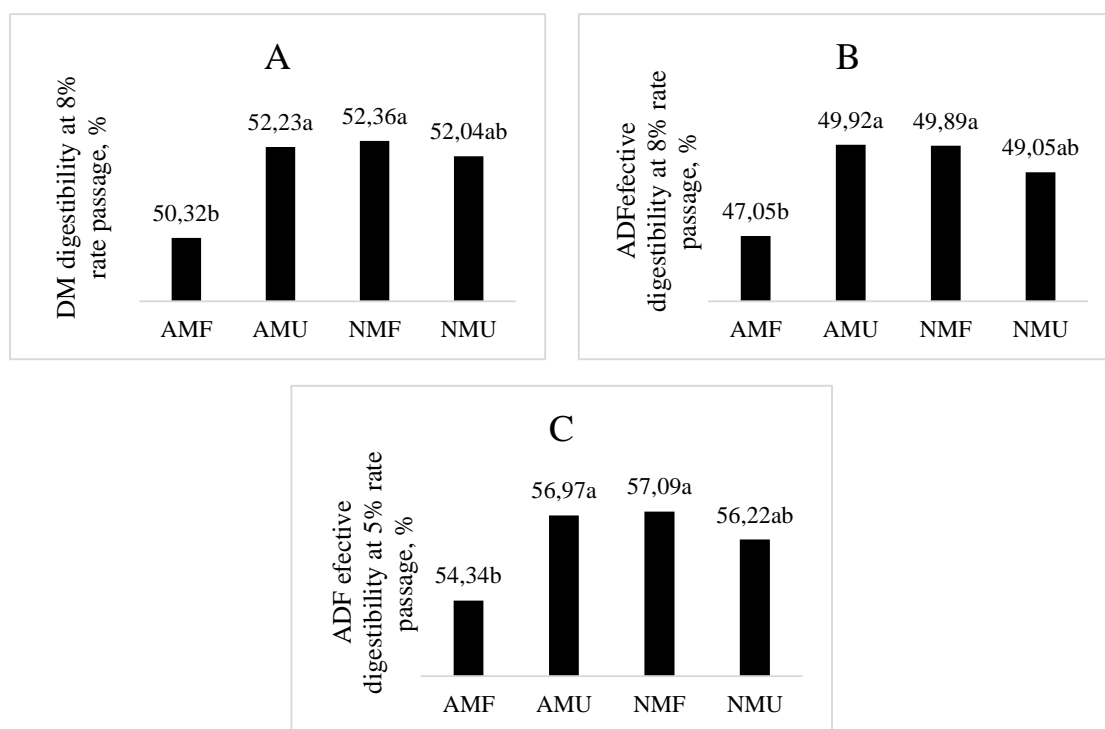
Figure 23: Interaction between breed factor and corn processing type on DM degradability at 8% passage rate (**A**; SEM = 1.33), ADF effective degradability at 8% passage rate (**B**; SEM = 1.34) and ADF effective degradability at 5% passage rate (**C**; SEM = 1.38). a,b: Mean with different letters differ significantly. A_MF: Angus fed with finely ground corn; A_MU: Angus fed with high-moisture corn; N_MF –Nellore fed with finely ground corn; N_MU: Nellore fed with high-moisture corn.

As for the potential degradability of DM, animals fed with fine corn degraded a higher percentage of DM than animals fed with high moisture corn silage (Figure 24A). It is observed that animals fed with finely grind corn had greater potential degradability of NDF compared to cattle fed with high moisture corn. (Figure 24B).
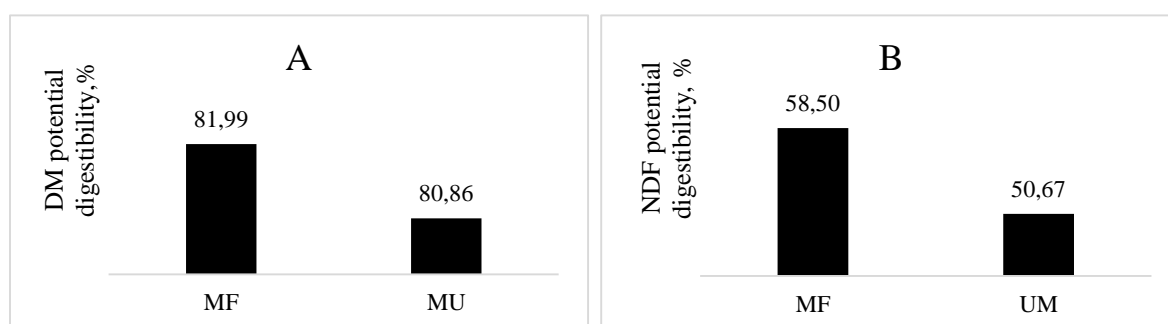
Figure 24. Effect of corn processing type on DM potential degradability (**A**; SEM = 0.94) and NDF degradability potential (**B**; SEM = 5,51). MF: final ground corn; MU: high-moisture corn.

According to Figures 25A, 25B and 25C, Nellore animals obtained values of effective CP degradation higher than Angus animals, regardless of the passage rate, 2%, 5% or 8%, respectively.
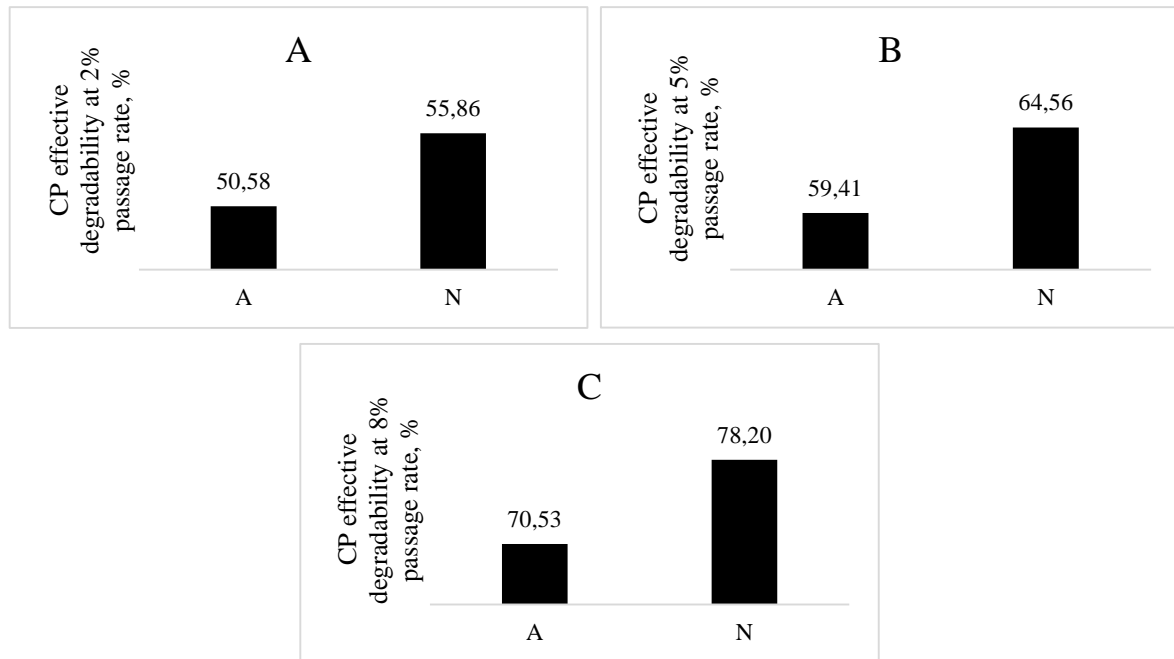
Figure 25: Effect of breed factor on CP degradability at 2% passage rate (**A**; SEM = 1.90) CP degradability at 5% passage rate (**B**; SEM = 1.86), CP degradability at 8% passage rate (**C**; SEM = 1.50). A- Angus; N- Nellore.

Total and apparent digestibility of nutrients

The effect of different nutritional strategies in the finishing phase on the total digestibility of nutrients in Angus and Nellore cattle are represented in Table 13 and in the figures below**.**

Table 10: Nutrient's digestibility of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the finishing phase.

Digestibility, %	Angus				Nellore				SEM	P value		
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	MON
Dry matter, %	80.89	79.71	82.96	82.87	78.16	81.04	81.29	82.74	1.47	0.48	0.01	0.42
Crude protein, %	84.08	84.08	85.54	84.35	83.54	82.61	85	84.22	1.8	0.55	0.29	0.52
Neutral detergent fiber	61.34	68.85	65.37	67.91	63.79	71.57	60.37	65.55	4.02	0.89	0.47	0.02
Acid detergent fiber	55.79	64.36	66.11	68.29	59.16	75.4	53.47	66.51	6.45	1	0.98	0.01
Starch	95.8	89.94	98.61	97.67	93.1	91.82	97.84	95.95	1.55	0.43	<0.001	0.02
Organic matter	85.57	82.47	87.02	87.31	81.7	82.34	86.34	86.2	1.02	0.05	<0.001	0.41

SEM: standard error of mean; [1]Finely groud corn with monensin; [2]Finely groud corn without monensin; [3] High-moisture corn with monensin; [4] High-moisture corn without monensin.

Animals fed with high moisture corn had higher DM digestibility (P=0.01) when compared to animals fed finely ground corn (Figure 26A). There was higher OM digestibility (P< 0.001) for high moisture corn when compared to cattle receveing finely ground corn (Figure 26B).

There was also a difference concerning the type of corn grain processing, and the treatment with high moisture corn had higher starch digestibility (P< 0.001) when compared to the treatments consisting of finely ground corn. (Figure 26C).
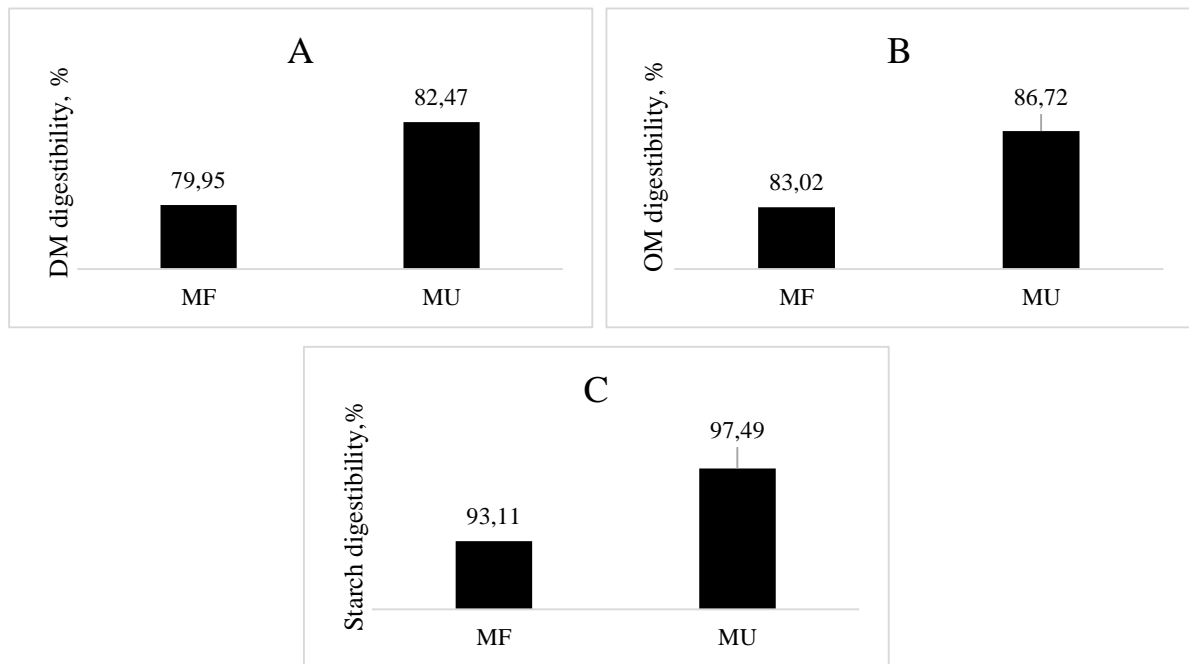
Figure 26: Effect of corn processing type on DM digestibility (**A**; SEM = 0.89), OM digestibility (**B**; SEM = 0.67) and Starch digestibility (**C**; SEM = 1,05) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the finishing phase. MF: finely ground corn; MU: high-moisture corn.

Treatment with inclusion of MON in the diet had a lower digestibility of NDF (P=0.02) when compared to the treatment that did not include MON (Figure 27A). The same occurred for ADF digestibility (P=0.01) (Figure 27B). Animals receiving the inclusion of MON showed a higher starch digestibility (P= 0.02) when compared to those that received treatment without the presence of the MON. (Figure 27C).
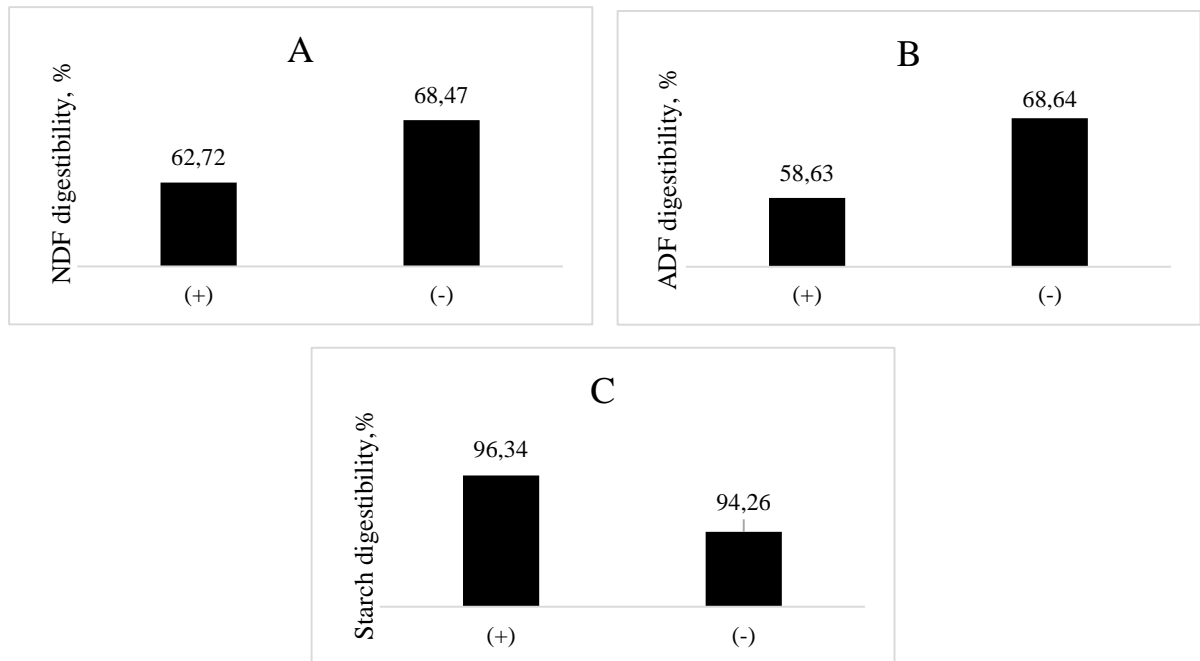
Figure 27: Effect of monensin factor on NDF digestibility (A; SEM = 2.54), ADF digestibility (B; SEM = 4.40) and Starch digestibility (C; SEM = 1.05) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the finishing phase. (+): presence of MON; (-): absence of MON.

OM digestibility was higher (P= 0.05) for Angus breed, thus, Nellore cattle showed a lower use of organic matter than Angus. (Figure 28).
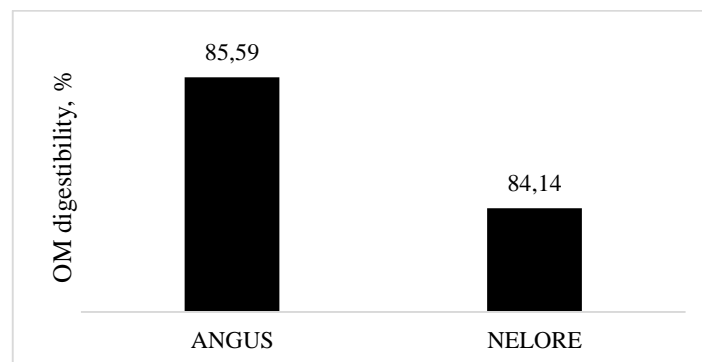
Figure 28: Effect of breed factor on OM digestibility (%) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the finishing phase (P= 0,05) (SEM = 0,68).

Total and differential count of protozoa

The results of the total and differential count of protozoa from Angus and Nellore cattle submitted to different nutritional strategies are shown in Table 14 and the figures below.

Table 11. Total and differential count of protozoa of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin

Variables	Angus				Nellore				SEM	P value			
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon	Interaction
Protozoa (x103 /ml)													
Isotricha	4.67	4.84	4.8	4.29	4.46	4.84	4.41	5.53	0.61	0.82	0.85	0.31	-
Dasytricha	26.5	25.97	28.33	25.54	24.86	26.83	23.27	27.31	2.7	0.72	0.96	0.67	-
Diplodiniinae	52.38	51.21	54.99	47.79	54.26	55.5	54.9	56.53	5.3	0.52	0.95	0.66	-
Entodinium	222.37	258.34	221.79	223.07	241.46	251.06	241.29	235.09	17.35	0.39	0.3	0.41	-
Total	305.96	340.37	309.9	300.69	325.03	338.23	323.87	324.39	20.9	0.36	0.39	0.51	-
Protozoa %													
Isoticha	1.44	1.39	1.42	1.33	1.31	1.36	1.3	1.65	1.4	0.97	0.53	0.41	-
Dasytricha	8.48	7.52	9.03	8.6	7.75	8.11	7.38	8.59	0.73	0.62	0.17	0.89	B*MON
Diplodiniinae	17.16	15.12	17.75	16.2	16.58	16.95	16.63	17.53	1.39	0.81	0.47	0.47	-
Entodinium	72.92	75.98	71.8	73.86	74.35	73.58	74.69	72.23	1.81	0.97	0.25	0.61	B*MON

SEM: standard error of mean; 1Finely groud corn with monensin; 2Finely groud corn without monensin;3 High-moisture corn with monensin;4 High-moisture corn without monensin; Interaction: B*MON: breed*mon.

Regarding the percentage of Dasytrichia, Nellore with absence of MON in the diet had a lower percentage than Nellore with MON inclusion (Figure 29A). For the genus *Entodinium*, there was a lower percentage in Angus receiving MON and did not differ from Nellore that did not receive (P=0.02) (Figure 29B).
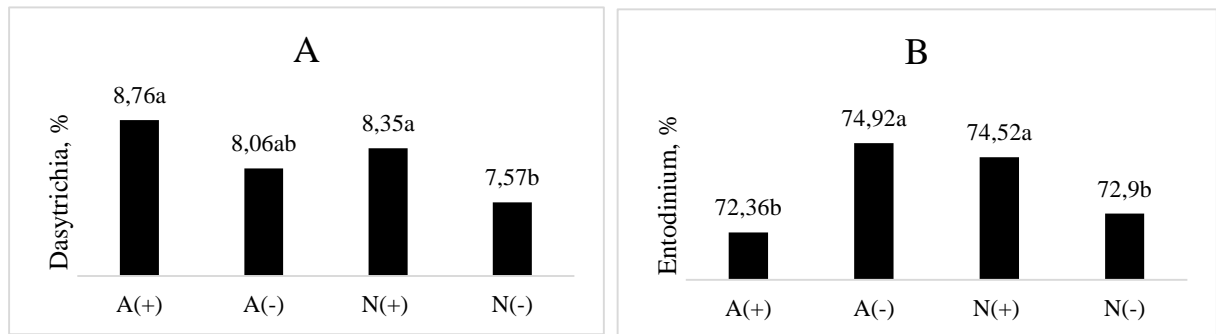

Figure 29: Interaction between breed factor and monensin factor on protozoa percentage *Dasytrichia* (A; SEM = 0.66) and *Entodinium* (B; SEM = 0.66) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin. [a,b] Mean with different letters differ significantly. A(-): Angus non supplemented with Mon; A(+): Angus supplemented with Mon; N(+): Nellore supplemented with Mon; N(-): Nellore non supplemented with Mon.

Genomic sequencing of the bacterial community

The sequencing data of the bacterial community of the rumen of the animals are presented in the following figures.

There was a difference in the bacterial community for the Shannon diversity index between rbreed (P=0.007), between corn processing (P=0.036), and for inclusion or not of MON (P=0.022) represented in figures 30A, 30B, and 30C respectively. For the Chao richness estimator, there was a difference between corn processing (P=0.036) and the inclusion of MON (P=0.001), figures 30E and 30F, but there was no difference between races (P=0.073) (Figure 30D).

A Bray-Curtis dissimilarity analysis was performed as shown in Figure 31, in which differences in bacterial communities were observed between races (P<0.01), corn processing type (P<0.01), and inclusion or not of MON in the diet (P<0.01), illustrated in figures 31A, 31B and 31C respectively. In addition, the seven main phyla found in the rumen of the animals are shown in Figure 32, comparing the breeds (Figure 32A), between the corn processing (Figure 32B), and between the inclusion or not of MON (Figure 32C).
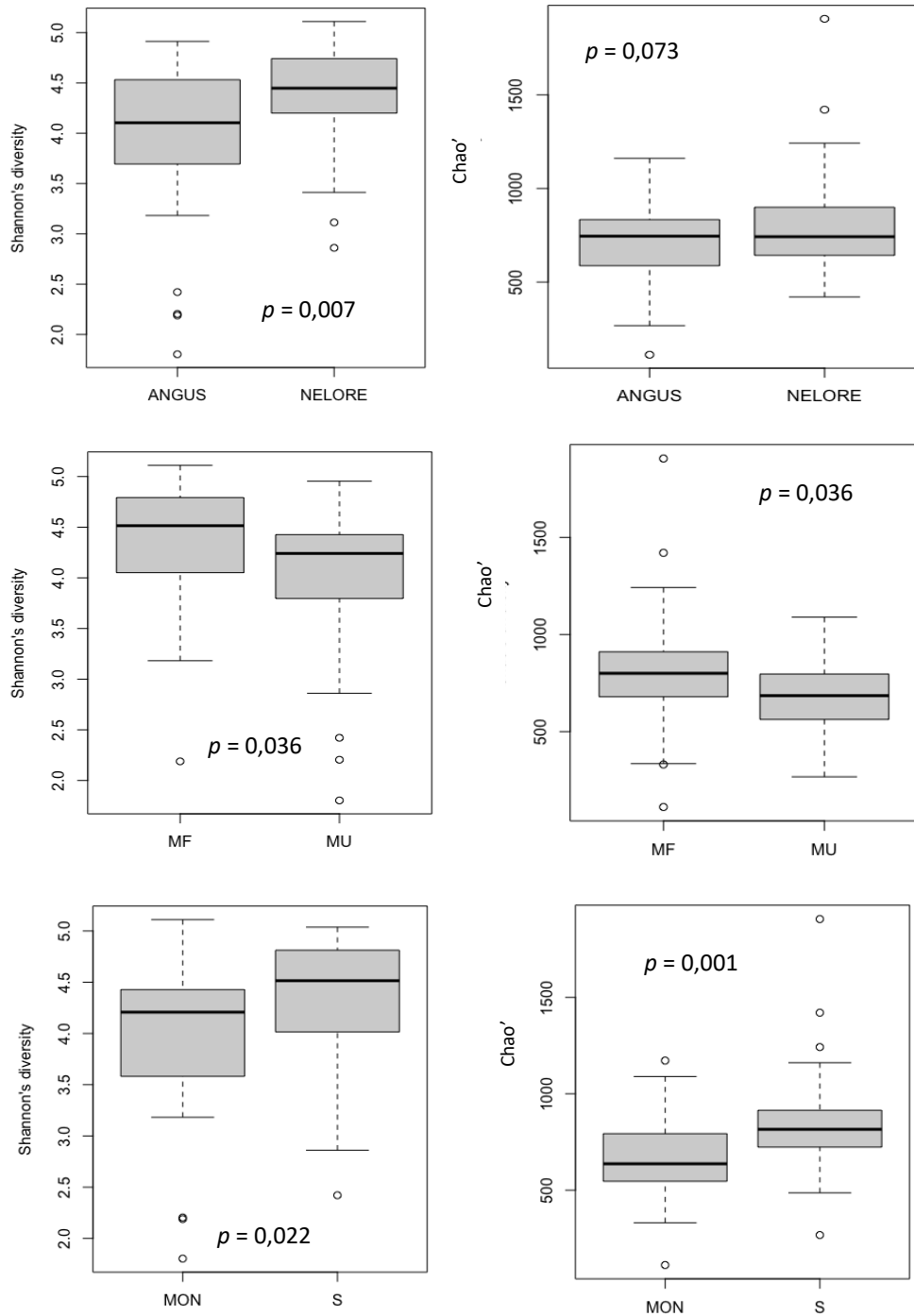
Figure 30. Shannon diversity index for breed factor (**A**), corn processing type factor (**B**) and MON factor (**C**). Chao's richness estimator for microbial communities in the rumen of Angus and Nellore cannulated (**D**), corn processing type factor (**E**) and MON factor (**F**).
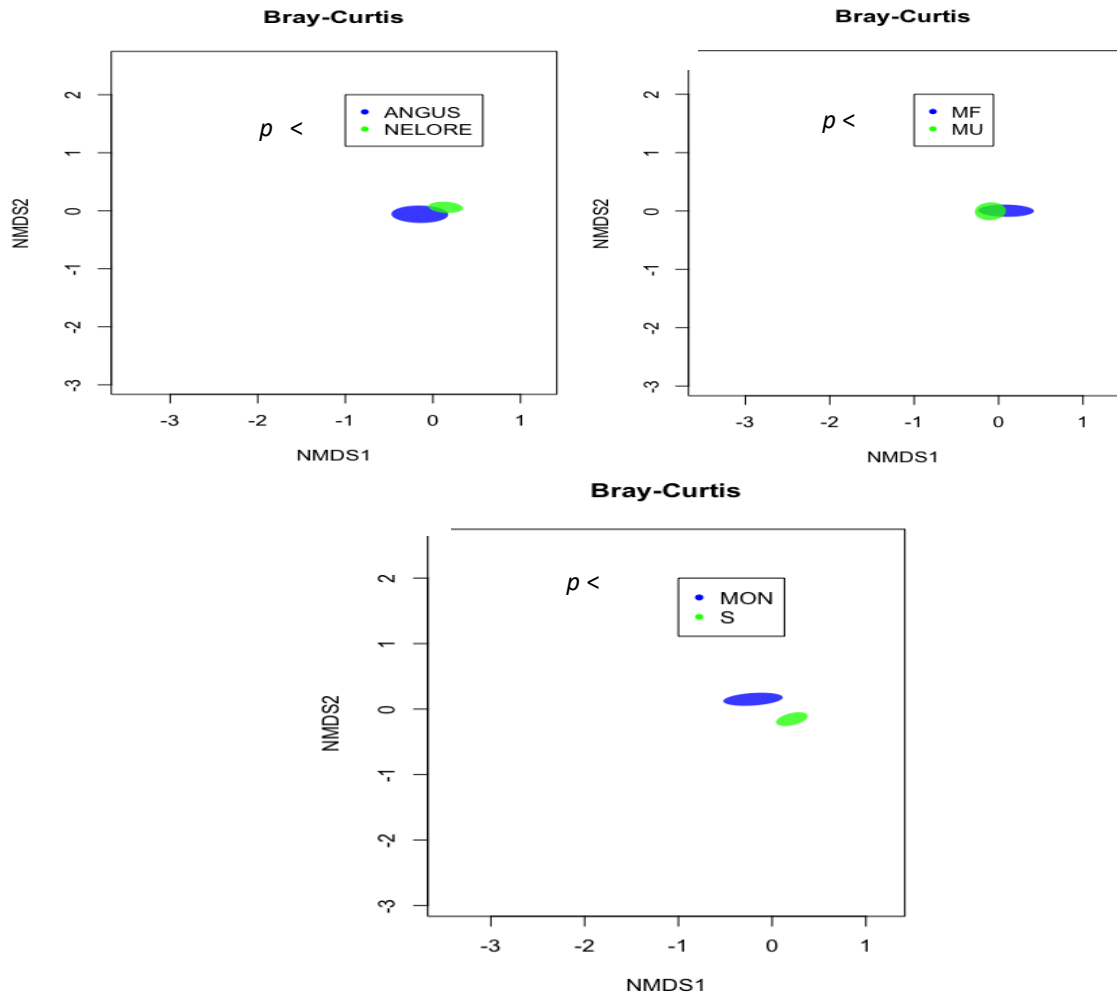
Figure 31: Non-metric multidimensional scaling (NMDS) representation of the Bray–Curtis dissimilarity metric for ruminal content of cannulated Angus and Nellore bulls (**A**), corn processing type (**B**) and inclusion or not of MON (**C**).
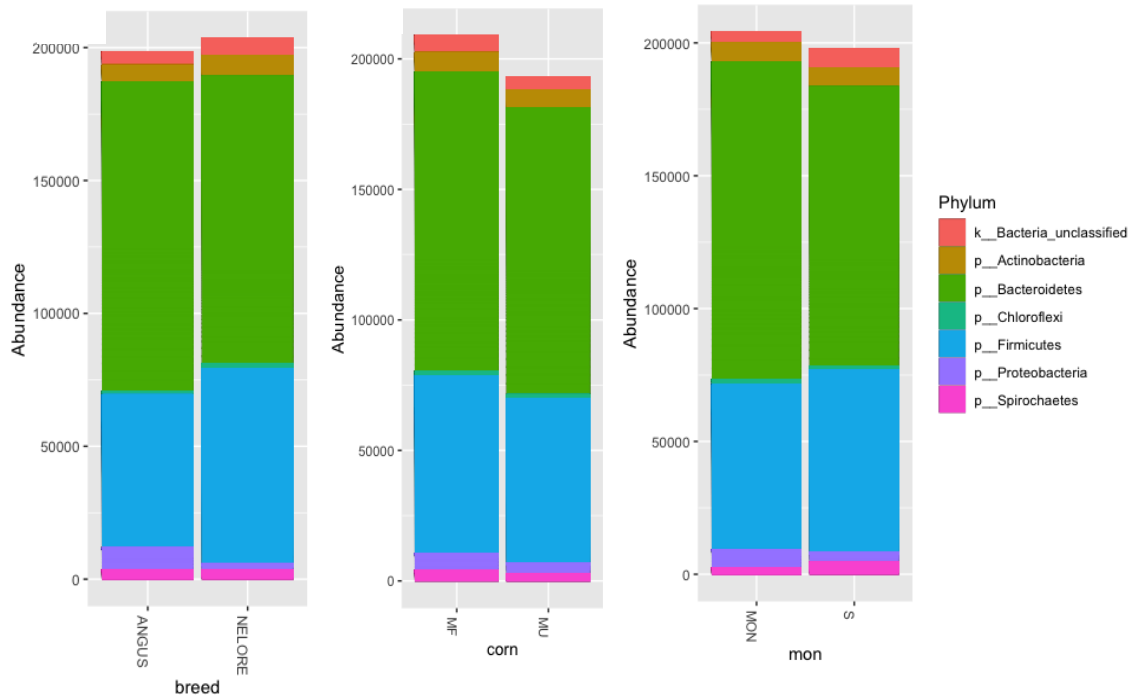
Figure 32. Relative abundance of the top seven phyla of ruminal bacterial communities observed in Angus and Nellore bulls (**A**), fed with different corn processing type (**B**) and supplemented or not with MON (**C**).

There was a significant difference between the Clostridiales community between the breeds (P<0.01), in which Nellore animals showed a higher community (Figure 33A). The same occurred for the communities of Butyrivibrio (Figure 33B) and Provotella (Figure 33D). However, the community of Succinivibrionaceae (Figure 33C) was smaller for Nellore animals compared to Angus. (P<0,01
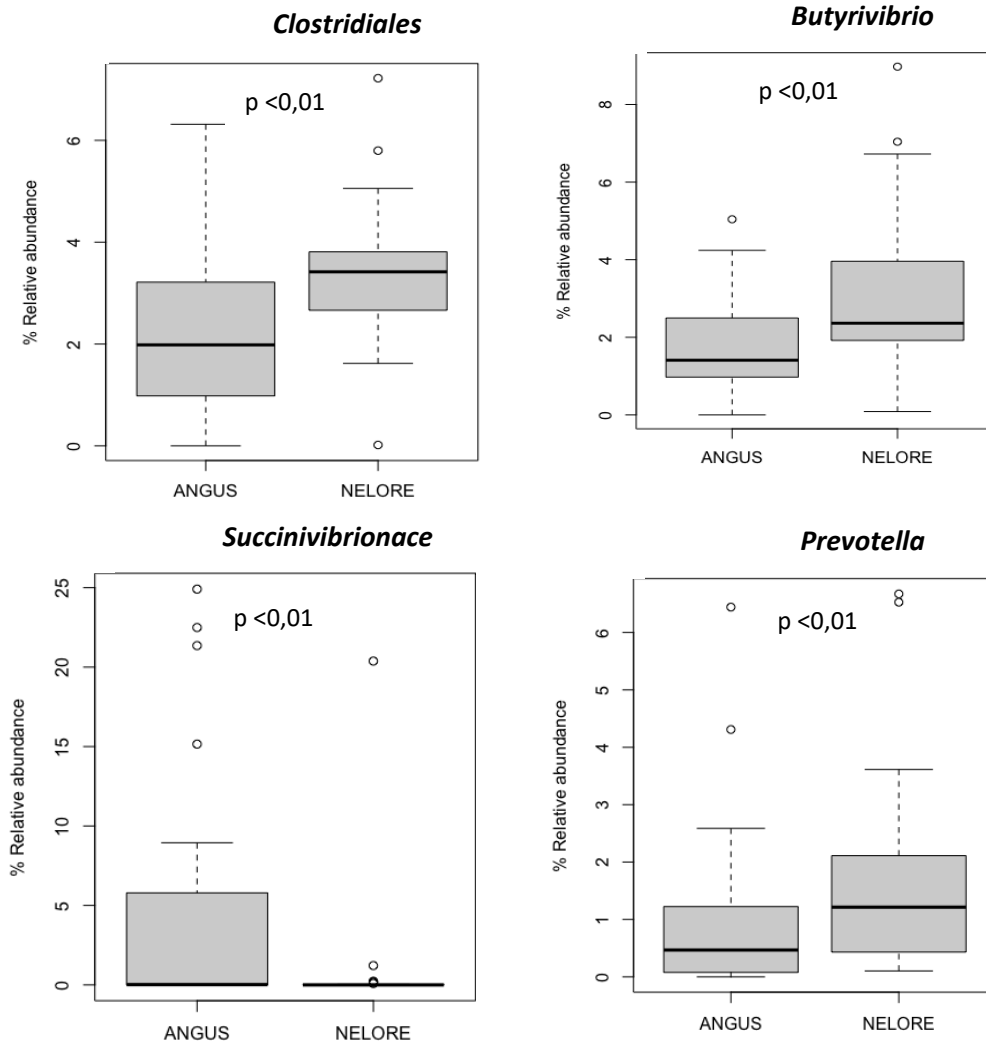
Figure 33. Relative abundance between *Clostridiales* (**A**), *Butyrivibrio* (**B**), *Succinivibrionaceae* (**C**) and *Provotella* (**D**) communities founded on rumen of Angus and Nellore canulated bulls submitted to different nutritional strategies.

There was a negative effect of the inclusion of MON (P<0.01) on the community of Butyrivibrio (Figure 34A), and Firmicutes (Figure 34B), however the community of Provotella increased with the inclusion of MON in the diet. (Figure 33C).
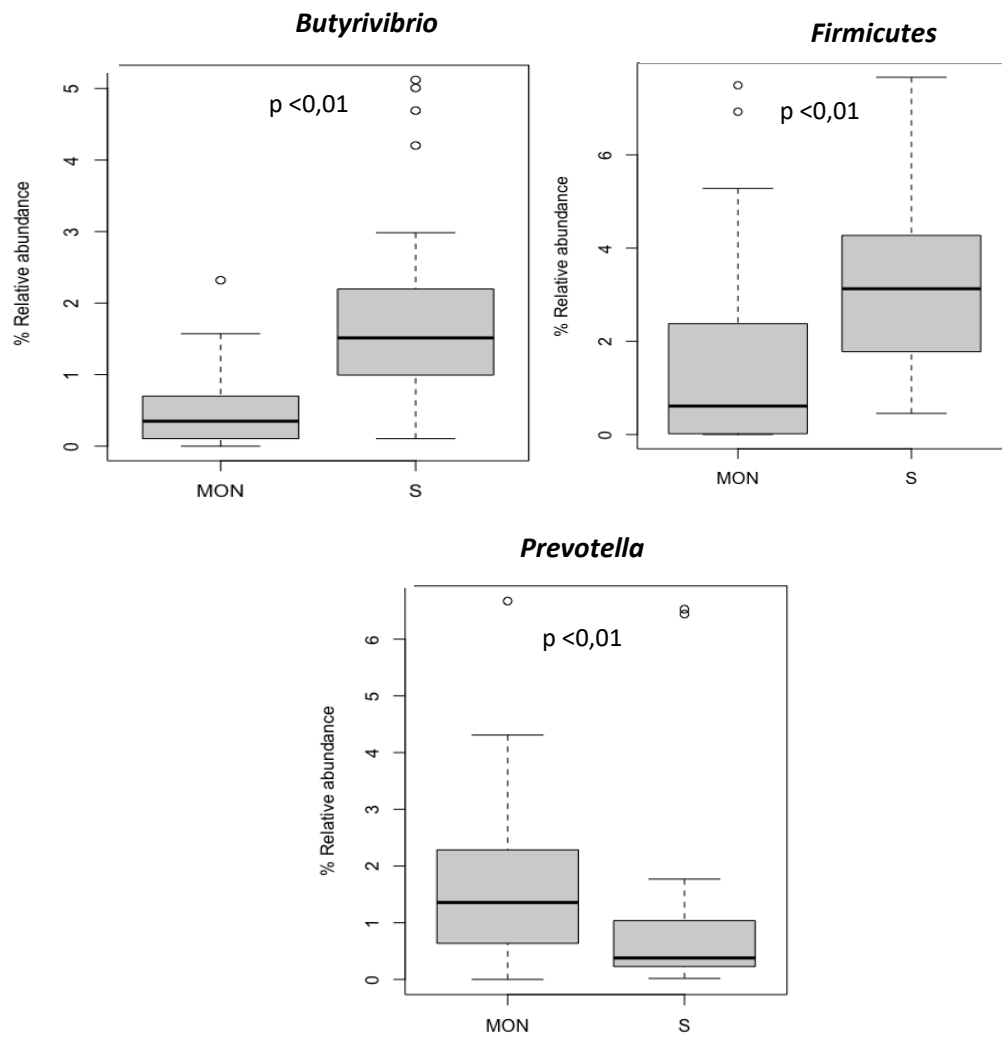
Figure 34. Relative abundance between *Butyrivibrio* (A), *Firmicutes* (B) e *Provotella* (C) communities present on Angus and Nellore canulated bulls supplemented with MON or not.

Rumen histology

Ruminal histology data are shown in Table 15 and in the graphs below.

Table 12. Rumen morfometrics of Angus and Nellore bulls fed with finely ground corn or high-moisture corn with addition or not of monensin during the finishing phase.

Miscrocopic variables	Angus				Nellore				SEM	P value		
	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]	FGMON[1]	FGA[2]	HMMON[3]	HMA[4]		Breed	Corn	Mon
Papillae surfacee area, mm^2	4.43	3.13	3.33	3.27	3.28	3.33	3.15	2.98	0.70	0.65	0.23	0.21
Papillae height, mm	13.21	10.13	9.27	9.11	11.14	9.99	10.07	10.19	1.23	0.94	0.03	0.09
Papillae width, mm	0.34	0.31	0.36	0.35	0.30	0.29	0.32	0.31	0.03	0.35	0.07	0.23
Papillae KLT, µm	15.45	15.88	15.76	15.64	13.72	15.58	13.67	14.81	0.76	0.04	0.74	0.15
Mitotic index, %	14.69	13.94	13.49	14.00	15.91	15.21	14.35	13.91	1.03	0.47	0.12	0.58

SEM: standard error of mean; 1Finely groud corn with monensin; 2Finely groud corn without monensin;3 High-moisture corn with monensin;4 High-moisture corn without monensin

There was no significant effect for the variables of papilla surface area, papilla width, and mitotic index. As for the variable papilla height, there was a significant effect of corn (P=0.03), where the animals consuming finely grind corn had a greater height of the papillae than the animals that ingested high moisture corn (Figure 35A). Another effect was found for the breed with keratin thickness, in which Angus showed higher thickness (P=0.04) compared to Nellore. (Figure 35B).
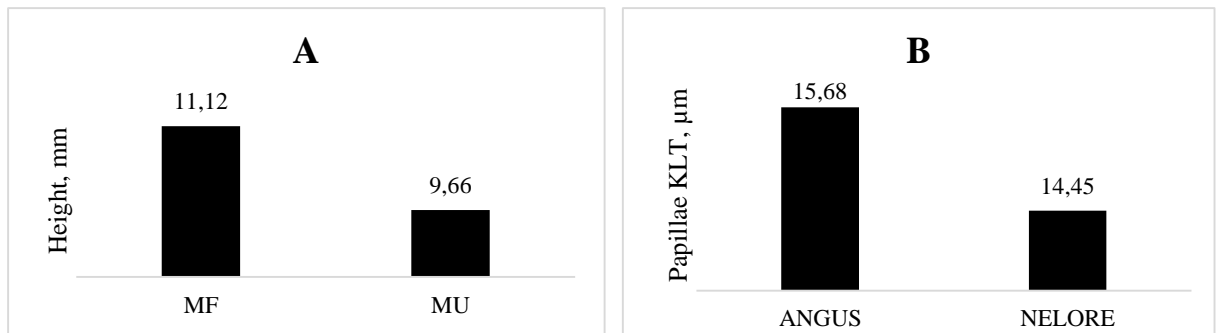
Figure 35*: Effect of corn processing type on rumen papillae height (mm) (**A**; SEM = 0.86) and breed on rumen papillae KLT (**B**; SEM = 0.39) of Angus and Nellore canulated bulls fed with finely ground corn or high-moisture corn with addition or not of monensin. MF: finely ground; MU: high moisture corn.

4.   DISCUSSION

Angus had higher DMI (%BW) (2.30% x 1.66%) and also a higher DMI (kg) (Day 12: 14.52 x 12.37kg and Day 16: 14.72 x 12.15kg) than Nellore cattle. Usually the DMI of Angus is higher than Nellore, as they a had greater requirement of maintence compared *to Bos indicus* (NRC, 2000). Millen et al., (2015) observed an increase of 7% in DMI of Brangus (⅝ Red Angus, ⅜ Nellore) compared to Nellore cattle. In the same way, Watanabe et al. (2021) comparing crossbred1/2 Angus x Nellore and Nellore cattle reported a greater DMI for crossbreed animals. Furthermore, this greater DMI on the 12th and 16th could indicate that Angus cattle adapts earlier than Nellore cattle to high-concentrate diet, however Watanabe et al. (2021) attempting to reduce adaptation length on 1/2 Angus x Nellore cattle compared to purebred Nellore, did not find any nutritional or physiological benefits when they shorten up the adaptation period from 14 to 9 days.

During the adaptation phase, the Angus fed with high moisture corn went to the drinking fountain trough more often than the Nellore, which may have occurred

because they ate more (numerically, although relevant, almost 1.50 kg more) than the Nellore animals of the same treatment (14.58 x 12.94; respectively), which may have had greater rumen fermentation than the Nellore animals. Postharvest processing of grains influences the site and extent of nutrient digestion (GALYEAN ET AL., 1979; NOCEK, 1987). Ruminal degradation of carbohydrate is greater for high moisture (HM) grain than for dry processed grain (NOCEK AND TAMMINGA, 1991). Angus sorted against medium particles than Nellore when both were fed with finely grind corn with MON. Although it doesn´t differ from the treatments, Angus eating finely grind corn with MON sorted against long particles severely than the others treatments.

On finishing phase, concerning rumination time (min), Angus fed high moisture corn with absence of MON spent longer ruminating when compared to Nellore from the same treatment. In addition, there was interaction about DM rumination efficiency, where Angus received high moisture corn with MON had a better efficiency, spending less time to ruminate the same amount of DM (kg) than Nellore of the same treatment. It can also be seen that for the selectivity of medium particles, the Angus and Nellore (both from the high moisture corn treatment with MON) had no difference in the sorted, so they sorted the same proportion of medium particles; however, the Angus breed managed to ruminate in less time, proving to be a more efficient animal in rumination. It is important to emphasize that the present study the cattle was allocated in individual pen, this change the cattle behavior against a group pen, increased competition in the feeders might disrupt the normal or preferred feeding patterns, limiting the ability of cattle to self-regulate their digestive function (HARB et al., 1985).

The greater DMI for Angus cattle probably lead to the minimal ruminal pH and the higher ruminal temperature. In addition, Angus cattle fed with finely ground corn had a greater area and longer duration of pH below 6.2, 5.6, and 5.2. A higher intake results in a greater amount of nutrients delivered into the rumen, which increases fermentation and consequently fermentation end-products as SCFA, resulting in a greater release of H+ into the ruminal environment, causing a reduction in the ruminal pH. Furthermore, the ruminal papillae of the aforementioned genetic group had thicker keratin, no changes on the absorptive capacity of the ruminal epithelium were

observed, this could evidence a higher fermentation and probably a consequence of the lowest pH. For medium pH Angus feed with MON had the lowest number than Angus and Nellore with or without MON, that may not make sense because MON has been extensively used to manipulate ruminal fermentation, but in this case may indicate a higher pH fluctuation on the day of this treatment.

The pH data corroborate the rumen fermentation products data, in which a higher concentration of acetate, butyrate, and NH_3 was observed for Nellore cattle, whereas the concentration of propionate was higher for Angus. This NH_3 concentration could be a consequence of the higher number of *Prevotella* bacteria present in the rumen, since these bacteria are also believed to play an important role in the degradation of proteins and in the uptake and fermentation of peptides (PITTMAN and BRYANT, 1964; RUSSELL, 1983; WALLACE ET AI., 1993).

Furthermore, fermentation products of rumen *Prevotella sp.* include acetate, succinate and propionate. Similarly, the butirate concentration could be a response of the highest number of *Butyrivibrio* bacteria in Nellore against Angus. A result from a recent study suggested that *Butyrivibrio* species can degraded a variety of substrates using glucose and H_2 for the formation of butyrate (PALEVICH et al., 2019). Peres Assumpcao (2021) founded similar results, were Nellore cows presented a higher population of *Butyrivibrio*, as well an increase on butyrate concentration. There was a higher concentration of lactate in Angus, but the values found do not give evidence of acidosis (not a normal concentration), it only shows that the Angus breed had a higher concentration than the Nellore.

The addition of MON in the diets of Nellore animals favored food degradation, being beneficial for both concentrated and forage parts. In Angus animals, the inclusion of monensin provided higher starch degradation but had negative effects on the degradability of other nutrients. It can be thought that Angus animals have a naturally prepared and efficient rumen for fermenting diets that contain high energy, while Nellore animals no longer have this prepared rumen, and can benefit from the use of ionophore additives.

For DM apparently degradation on 8% kp, the different corn processing type appear to be not different for Nellore bread, instead Angus breed, when eaten finely

grind corn decrease the DM degradability. In both breeds, corn processing didn´t differ the starch degradation, but the inclusion of MON supports its effective degradation, regardless of the kp (slow, medium or accelerated). Gomes (2010) also found improvements starch potential degradability, where, regardless of the kp, the rumen environment of animals exposed to MON is better for the starch degradation. Apparently the Nellore breed increase starch degradation than Angus when submitted a different corn process type.

Nellore cattle had a higher CP degradation than Angus cattle regardless of the kp (slow, medium or accelerated), and it may occur due Nellore cattle to present a greater count of *Prevotella* into the ruminal environment compared to Angus cattle, which as reported in the literature (add reference here), *Prevotella* is well known to hydrolyze protein. The MON inclusion clearly affects the potential degradability of CP of Angus cattle. When cattle were not supplemented with MON, Angus cattle presented highest potential degradability of CP than Nellore cattle, however when MON started to be supplemented, only Angus cattle had a significatively decrease in potential degradability of CP.

It is possible to observe a reduction in potential and effective NDF and ADF degradability of Angus receiving MON, which can be explained by the sensitivity of cellulolytic bacteria to the effects of ionospheres, in addition to having a lower pH, since the substrate intake was higher. The digestibility was different only for OM, being higher for Angus cattle when compared to Nellore. The digestibility of OM is extremely important because it will make energy available to the animal, positively influencing its performance.

Regarding the sequencing data, the Nellore breed had a higher Shannon diversity index compared to the Angus breed. Nellore is a beef breed adapted to tropical environments, and require an abundant and diverse ruminal microbiota in order to digest complex polysaccharides, such as cellulose (ANDRADE ET AL, 2020 and HENDERSON ET AL, 2015).

The Shannon diversity for Angus is lower from Nellore, that means Angus rumen is more homogenous, but there is a range among the Angus samples.

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.

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