Universidade de São Paulo
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Effect of feed additives on performance of beef cattle finished in feedlot

Pedro Guerreiro

Dissertation presented to obtain the degree of Master in Science. Area: Animal Science and Pastures

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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RESUMO

Efeito de aditivos alimentares no desempenho de bovinos de corte terminados em confinamento

O objetivo deste estudo foi avaliar os efeitos de três aditivos alimentares – monensina sódica (MON; 31,3 mg / kg MS), monensina com adição do aditivo tamponante *Lithothamnium calcareum* (MON + LC; 31,3 mg / kg MS + 5g / kg MS) ou uma mistura de óleos essenciais (BEO; 0,3 g / kg MS) e suas interações com dois grupos raciais de bovinos de corte – Nelore (NEL) vs. cruzamento industrial (CROSS) – em uma dieta de terminação em confinamento. Noventa touros da raça Nelore (peso inicial = 394 ± 34 kg) e noventa touros cruzados (peso inicial = 406 ± 31 kg) foram alimentados por 112 dias com dieta basal contendo 8,5% de bagaço de cana, 42,2% de milho moído fino, 41,7% de polpa cítrica, 5% de farelo de soja, 1,3% de uréia, 0,35% de cloreto de sódio e 0,95% de núcleo mineral e vitamínico (na base de MS), variando apenas o tipo de aditivo adicionado à dieta. Os tratamentos foram NEL+MON; NEL+MON+LC; NEL+BEO; CROSS+MON; CROSS+MON+LC; CROSS+BEO. O delineamento estatístico foi o de blocos completos casualizados, com arranjo fatorial 2 x 3 (2 grupos raciais e 3 aditivos alimentares). Foram avaliados: consumo de matéria seca (CMS), ganho médio diário (GMD), eficiência alimentar (G:F), peso de carcaça quente (PCQ), rendimento de carcaça e as energias líquidas das dietas durante o período experimental. Os dados foram analisados usando o PROC MIXED do SAS. A baia foi considerada a unidade experimental. Não houve interações entre grupos raciais e aditivos alimentares (P> 0,05). Os touros CROSS apresentaram maior CMS (P <0,001), GMD (P <0,001), peso final (P <0,001) e PCQ (P <0,001) que os touros NEL. Os touros CROSS também foram mais eficientes na utilização de energia para mantença e ganho que os touros NEL (P <0,001). O CMS foi menor (P ≤ 0,05) para animais alimentados com MON+LC (8,127 kg) que animais alimentados com BEO (9,255 kg). O GMD (P = 0,177) e o peso corporal final (P = 0,238) não foram afetados pelos aditivos. Os aditivos não alteraram o PCQ (P = 0,252) nem o RC (P = 0,826). No entanto, a eficiência de utilização de alimentos foi maior (P = 0,048) para animais alimentados com MON (0,151) do que para animais alimentados com BEO (0,138). As energias líquidas de mantença e ganho foram maiores (P ≤ 0,05) para animais alimentados com MON (2,182 e 1,490 Mcal/kg) do que para animais alimentados com BEO (2,020 e 1,362 Mcal/kg). A relação de energia líquida observada:esperada também foi influenciada pelos aditivos (P ≤ 0,05). Os animais alimentados com MON apresentaram a energia líquida observada 32,3% e 43,2% maior do que a esperada para manutenção e ganho, respectivamente, enquanto que os animais alimentados com BEO apresentaram energia líquida 22,4% e 30,9% maior para manutenção e ganho, respectivamente. A combinação do aditivo tamponante de alga marinha com monensina não teve efeito positivo, nem sinergistico, quando comparado ao uso da monensina sozinha. A monensina foi mais eficiente que o BEO em eficiência alimentar e utilização de nutrientes.

Palavras-chave: Gado de corte, Tamponantes, Óleos essenciais, *Lithothamnium* sp, Monensina

ABSTRACT

Effect feed additives on performance of beef cattle finished in feedlot

The objective of this study was to evaluate the effects of three feed additives – sodium monensin (MON; 31.3 mg/kg DM), monensin plus the buffer additive *Lithothamnium calcareum* (MON+LC; 31.3 mg/kg DM + 5g/kg DM) or a blend of essential oils (BEO; 0.3 g/kg DM) and its interactions with two racial groups of beef cattle – Nellore (NEL) vs. Crossbreds (CROSS) – on a finishing feedlot diet. Ninety Nellore bulls (initial BW = 394 ± 34 kg) and ninety crossbreds (initial BW = 406 ± 31 kg), were fed for 112 days a basal diet containing 8.5% sugarcane bagasse, 42.2% of fine ground corn, 41.7% of citrus pulp, 5% of soybean meal, 1.3% of urea, 0.35% of sodium chloride and 0.95% of minerals and vitamins (DM basis), varying only the type of additive added to the diet. Treatments were NEL+MON; NEL+MON+LC; NEL+BEO; CROSS+MON; CROSS+MON+LC; CROSS+BEO. The statistical design was a randomized complete block with a 2 x 3 factorial arrangement (2 racial groups and 3 feed additives). It were evaluated: dry matter intake (DMI), average daily gain (ADG), feed efficiency (G:F), hot carcass weight (HCW), carcass dressing and observed NE of the diets during the experimental period. The data were analyzed using the PROC MIXED of SAS. Pen was considered the experimental unit. There were no interactions between racial groups and feed additives ($P > 0.05$). Crossbred bulls presented higher DMI ($P < 0.001$), ADG ($P < 0.001$) and final BW ($P < 0.001$) and HCW ($P < 0.001$) than Nellore bulls. Crossbreds were also more efficient in energy utilization for maintenance and gain than NEL ($P < 0.001$). Dry matter intake was lower ($P \leq 0.030$) for animals fed MON+LC (8.126 kg) than animals fed BEO (9.255 kg). Average daily gain ($P = 0.177$) and final body weight ($P = 0.238$) were not affected by treatments. Feed additives did not alter hot carcass weight ($P = 0.252$) and dressing ($P = 0.826$). However, feed efficiency was greater ($P \leq 0.05$) for animals fed MON (0.151) than for animals fed BEO (0.138). Net energies of maintenance and gain were higher ($P \leq 0.05$) for animals fed MON (2.182 and 1.490 Mcal/kg) than for animals fed BEO (2.020 and 1.362 Mcal/kg). Ratio of observed:expected NE concentrations were also influenced by feed additives ($P \leq 0.05$). Animals fed MON presented observed net energies values 32.3% and 43.2% higher than expected for maintenance and gain, respectively, whereas animals fed BEO presented observed net energies 22.4% and 30.9% higher for maintenance and gain, respectively. The combination of the seaweed buffer with monensin had no positive nor synergistic effects when compared to monensin alone. Monensin was more efficient than the commercial BEO in feed efficiency and nutrient utilization.

Keywords: Beef cattle, Buffers, Essential oils, *Lithothamnium* sp, Monensin

1. INTRODUCTION

Ruminants contain in their rumen a vast flora of microorganisms which they stabilish a simbiotic relationship and rely to digest energy and protein dietary sources. Although they rely on microorganisms to digest a great amount of ingredients, this simbiotic relationship is full of inefficiencies in which energy is lost through production of methane and protein is lost through ammonia excrection (Nagaraja et al., 1997). In addition to wasting nutrients and thus reducing potential production performance, these losses release pollutants to the environment (Tamminga, 1996). Thus currently, according to Goulart (2011) researchs in ruminant nutrition aim to understanding and improving effiency and productivity (animal performance) and sustainability (efficiency of N utilization and reduction of emission of CH_4).

Efficiency of utilization of energy and protein can be improved by selection of ruminal microbial population through optimization of diets by sincronizing supply of energy and protein (Hall and Huntington, 2008; Matthews et al., 2018), decreasing roughage:concentrate ratio of diets (Stock et al., 1990) or through the use of feed additives. Accordingly, Jouany and Morgavi (2007) defined ideal feed additives as ingredients that decrease methane production, increase propionate production along with the net energy balance of animals, improve nitrogen utilization efficiency and reduce the risk of metabolic disorders. Feed antibiotics, such as ionophores, are commonly included in beef cattle diets for those purposes (Benchaar et al., 2006; Benchaar et al., 2008).

Monensin is the most common feed additive for beef cattle finishing diets, known by its effects of improving animal performance, and acidosis prevention and, therefore serves as an unique comparison with other feed additives (Meyer et al., 2009). Monensin acts on gram-positive bacteria, thus changing fermentation routes towards more efficient ways, and by decreasing dry matter intake of animals and maintaining average daily gain, it also improves feed efficiency.

Some plant secondary metabolites, such as essential oils (EO) have shown antimicrobial and antisepctic properties (Calsamiglia et al., 2007) and a potential in mitigating methane emission from ruminants due to their effects on rumen microorganisms and fermentation process (Cobellis et al., 2016). However, due to widespread use of ionophores interest in the use of EO has been less than expected (Callaway et al., 2003). In 2006, due to the European Union banning the use of ionophores as growth promoters in animal production (European Commission, 2003), and a social concern that use of ionophores could lead to appearance of human harmful resistant bacteria (Russell and Houlihan, 2003) that interest in EO studies rose again (Duval et al., 2007). Consequently, information of essential oil on ruminant performance is still scarse.

Feed utilization efficiency (gain-to-feed ratio) can also be improved by increasing concentrate:forage ratio as in high concentrate diets the energy density is increased and thus animals consume higher amounts of energy in lower amounts of feed (Galyean and Defoor, 2003). Nevertheless, feeding cattle high concentrate diets increases the occurance of subacute ruminal acidosis (SARA) and in order to prevent the occurance of this metabolic disorder, buffers are routinely used for dairy cows (Calsamiglia et al., 2012). However, responses of beef cattle fed high concentrate diets to addition of buffers is controversial. Nevertheless, Enemark (2007) stated that a new buffer based on calcified seaweed is available. It claims to have more than twice the capacity of sodium bicarbonate to improve performance of dairy cows, however, at the time there were no studies to document the effects of this product.

Furthermore, Millen et al. (2015) comparing the effects of either monensin or polyclonal antibody preparation, as an ionophore substitute, on two biotypes (Nellore or Brangus) did not observe any interactions between biotype and feed additives on performance variables. However, it was reported that Nellore bulls presented higher rumenitis scores and greater DMI fluctuation than Brangus bulls.

Thus, it was hypothesized that the addition of the calcareous seaweed buffer to monensin could have a benefitial synergistic effect for beef cattle fed high concentrate diets and this benefitial effect would be greater for Nellore than for Crossed beef cattle. In addition to that, it was hyphotesized that a comercial blend of essential oils could impact beef cattle performance similarly to monensin. Therefore, based on the presented hypothesis, the objective of this study is to assess the potential benefits on feedlot finished beef cattle of a specific blend of essential oils and the association of seaweed buffer to monensin against the single use of monensin.

2. LITERATURE REVIEW

2.1. Composition of Brazilian livestock

The introduction of zebu cattle in Brazil, more specifically Nellore cattle, dates back to mid 19th century and it represented a huge mark in national beef cattle production (Fortes and Yassu, 2009). Introduction of Nellore cattle was crucial for increasing the national herd and making Brazil the country with the largest commercil herd in the world (ABIEC, 2019). The current brazilian beef cattle herd is estimated at 214.69 million animals (ABIEC, 2019) from which around 80% is zebu (Fortes and Yassu, 2009).

Nellore cattle was brought from India wherein it was used for milk production, however in Brazil it was bred and selected for meat production (Valadares-Filho and Chizzotti, 2010). Due to morphological characteristics, such as short and light-colored hair, dark hide, higher contact surface due to skin folds and intense sweating (Valadares-Filho and Chizzotti, 2010), Nellore cattle is more tolerant and addapted to tropical weather conditions and its introduction in Brazil was a success. For that reason it was also seen as a viable opportunity to adapt European breeds to the tropical climate through crossbreeding.

Despite the fact that most of the Brazilian herd is still zebu, with the increase in meat exportations (ABIEC, 2019) and the increase in domestic and global demand for higher-quality meat there has been an increase in crossbreds (*Bos taurus indicus* vs. *Bos taurus taurus*) in the national herd. British breeds are well known for its high marbled meat (Rotta et al., 2009), thus crossbreeding with zebu breeds can be beneficial to take advantage of a hybryd vigor effect on performance parameters as well as taking advantage of zebu cattle rusticity and British breeds' meat quality characteristics. For that reason several studies comparing *Bos taurus taurus*, *Bos taurus indicus* and the many breeds origined from their combinations (i.e. Braford, Canchim, Brangus, etc.) have been conducted aiming to a better understanding of energy requirements, performance and meat quality (Gregory et al., 1994; Putrino et al., 2006; Goulart et al., 2008; Rotta et al., 2009; Rubiano et al., 2009; Maggioni et al. 2010).

Nellore stands out in terms of acceptability across beef cattle producers and therefore most comparisons between *Bos taurus taurus* and *Bos taurus indicus* are made using Nellore as a representative of the second group. Crossbreds usually have higher rates of weight gain than zebu cattle (Putrino et al., 2006; Goulart et al., 2008) and the rate of gain increases as the participation of European breeds increases in the crossbreeding (Maggioni et al., 2010). The higher ADG observed in crossbreds usually comes at the expense of a higher feed intake (Goulart et al. 2008; Rubiano et al., 2009) and a worse feed conversion (Rubiano et al., 2009), in other words, crossbreds are less efficient in feed conversion than zebu cattle.

Goulart et al. (2008) compared steers from four genetic groups [Nellore (NE), ½ Aberdeen Angus + ½ Nellore (AN), ½ Canchim + ½ Nellore (CN) and ½ Simental + ½ Nellore (SN)] finished in feedlot regarding their performance and energy requirements. They observed that only AN crossbreds presented higher DMI compared to the other groups and ADG was higher for AN than NE and CN. However, they observed that NE presented higher dressing percentage and when comparing average daily carcass gain (ADGg), NE and AN presented same rates of carcass gain. The authors did not evaluate feed effiency nor feed conversion, however they observed that AN and NE presented the same rates of energy deposition due to lower requirements of NEg for NE than AN. Nellore steers presented lower requirements of energy for gain and higher requirements of protein for gain than AN.

Similarly, Rubiano et al. (2009) observed the same pattern as the previous authors. The authors compared steers from four genetic groups: Canchim , Nellore, ½ Canchim + ½ Nellore (CN) and ¾ Canchim + ¼ Nellore (TQ). The authors observed that Canchim, and TQ presented higher ADG than Nellore steers due to a higher DMI. That was probably due to an increase in European breed participation in the crossbreed. Feed conversion, however, was worse for Canchim than for Nellore.

Rotta et al. (2009) in a literature review observed that genetic groups that contain zebu present higher hot carcass dressing. However, the *Logissimus* muscle of genetic groups that contain zebu contributions present low marbling. Even though British breeds and crossbreds present higher requirements of energy for fat deposition (Goulart et al., 2008) they seem to have meat with higher marbling scores (Rotta et al., 2009).

Millen et al. (2015) compared the interactions of two breed types (Nellore vs Brangus bulls) and their interaction with either sodium monensin or a spray-dried multivalent polyclonal antibody preparation. The authors did not observe any interactions of breed and additive on any of the measured variables. Brangus bulls presented better feedlot performance and carcass traits than Nellore bulls. In addition, the authors observed that Nellore bulls presented grater fluctuation of daily DMI and higher rumenitis score than Brangus bulls. This last result is a possible indication that Nellore bulls are more susceptive to ruminal acidosis than Brangus bulls.

Thus, there is a continous interest in studying different genetic groups finished in feedlots due to the high energy content in the diets and its implications in performance and quality. There are some studies that report that different feed additives can influence differently fat deposition and meat quality (Jedlicka et al., 2009) and carcass yield (Purevjav et. 2013). There are however few studies that take into account iteractions between different genetic groups with different feed additives.

2.2. Use of feed additives in feedlots in Brazil

Within ten years, from the first national survey of feedlots nutritional strategies until present days some changes have been noticed. The average level of inclusion of roughage in feedlot finishing diets decreased from 28.8% (Millen et al., 2009) to 20.6%, however the most common value recommended by nutritionists is inclusion of 15% of roughage (Pinto and Millen, 2019), which makes this change in the diet profile even more expressive. The risk of acidosis and the inefficiencies of rumen microbes fermentation (Nagaraja et al., 1997) requires that strategies are adopted to improve efficiencies of energy and protein utilization in feedlot cattle. Feed additives, such as ionophores, have been used extensively for that purpose and according to Pinto and Millen (2019), 98.2% of interviewees informed they use some type of feed additive, of which 86.7% were ionophores and 13.3% combined ionophores and non ionophores antibiotics.

Even though the use of ionophores is proven to bring numerous benefits in performance and animal health (Page, 2003), the European Union, concerned with transmission of bacterial resistance to ionophores and, thus, to prevent the emergence of human pathogenic microorganisms resistant to conventional antibiotic, prohibited the use of these antibiotic feed additives as growth promoters in animal production (European Commission, 2003). Based on the same precautionary measure and an increasing social pressure against the use of these feed additives (Russell and Houlihan, 2003) it might be expected that Brazilian legislation becomes stricter regarding the use of antibiotics as growth promoters, as recently a proposal to ban the use of virginiamycin as a growth promoter has been established (DOU, 2018). Ionophores are still the most common antibiotic feed additive used in beef cattle diets, however the appeal to use alternative natural additives is becoming more frequent. Among the possibilities of natural antibiotic feed additives, essential oils take stand out.

2.3. Ionophores

Ionophores are a distinct group of antibiotics, produced mainly by bacteria of the genus *Streptomyces* spp. (Bergen and Bates, 1984), whose name derives from its mode of action. There are many different types of polyether ionophores that differ between molecular structure and physicochemical properties, most importantly the affinity for ions. Monensin is the most common ionophore feed additive used for beef cattle finishing diets, therefore it serves as a unique example and comparison with other antibiotic feed additives (Meyer et al., 2009). According to NASEM (2016), monensin is more effective than lasalocid to increase diet metabolizable energy.

2.3.1. Mode of action of monensin

Like many other ionophores, monensin affect predominantly gram-positive bacteria. The molecule acts as a cation carrier biding itself with an specific ion (hence its name, as ionophores from greek mean 'ion bearig'; Pressman, 1976; Page 2003.), forming a ionophore-cation complex that attaches to the cell wall of gram-positive organisms, dissolving in the lipid layer of the cell wall and finally transporting the cation into the cell of the gram-positive organism in exchange for a proton (Page, 2003).

Russell (1987) proposed a model to ilustrate the mode of action of monensin on *Streptococcus bovis* bacteria. When monensin attaches itself to a cation the complex becomes hydrophobic, allowing the molecule to penetrate the lipid bilayer of bacterial cells and act as an antiporter. Inicially, the concentration of K^+ is higher in the extracelullar medium than on the inside of the cell, but as the ionophore forms the complex monensin-cation it accelerates the efflux of K^+ and influx of H^+ causing a rapid decrease in pH. In order to maintaing normal pH levels the cell exports H^+ via proton bomb with energy expenditure. However this active mechanism of transport H^+ is not enough to fully reverse the decreased pH and, thus, the cell metabolism is directed towards expelling H^+ allowing influx of Na^+. In order to maintain ionic balance and pH the affected organisms expend energy, therefore it is uncapable of maintaining its regular hability to multiplicate leading to reduced proportion on the total number of microorganisms or even death.

2.3.2. Effects of monensin on beef cattle performance

Gram-negative microorganisms are little or not affected at all by ionophores as their cell membranes contain an external lipidic layer that prevents ionophores to penetrated it (Morais et al., 2006). As they are not affected, ionophores allow the manipulation of microbial population acting on gram-positive bacteria population and, consequently, shifting fermentation routes toward more efficient ways. Several authors reported that the use of monensin did not increase production of total volatile fatty acid (VFA), however shifted fermentation routes towards an increased production of propionated whereas acetate and lactate production are decreased (Raun et al., 1976; Potter et al, 1985; Russell and Strobel, 1989). Production of propionate instead of other VFAs leads to less heat production as there is no expenditure of H^+ on the process of glucose catabolism, therefore the efficiency of energy utilization is improved (Bergen and Bates, 1984).

The increased ruminal H^+ concentration resulted from glucose catabolism causes a decrease in ruminal pH which has to be expelled through production of methane by methanogic bacteria to maintain normal levels of pH and avoid metabolic disorders such as acidosis. Production of CH_4 is not only energetically costly, accounting to about 12% of fermentation energy losses (Johnson and Johnson, 1995), but also harmful to the environment (Tamminga, 1996). Hence, the use of monensin reduces substrate used by methanogenic bacteria (Tedeschi et al., 2003) reducing methane emissions up to 30% (Thornton and Owens, 1981; Russell and Strobel, 1989; Callaway et al., 2003; Page, 2003; Duffield et al., 2012a).

Propably because of increased propionate and consequent higher energy availabilty, monensin reduces intake in feedlot cattle fed high-concentrate diets. However, this particular ionophore has generally little or no effect at all on weight gain (Goordrich et al., 1984; Potter et al., 1985). As result of reduced feed consumption and unchanged average daily gain an improvement on feed utilization efficiency can be expected (Goordrich et al., 1984; Potter et al., 1985; Meyer et al. 2009). The increased feed efficiency with reduced intake can be explained by the shift in fermentation routes toward propionate production (Russell and Houlihan, 2003).

Not only monensin improves utilization of energy, but also improves nitrogen (N) utilization. Page (2003) reviewed literature and concluded that the use of monensin implied on less ruminal deamination of amino acids (AA), decreasing the concentration of ruminal NH_3 and increasing the flow of amino acid nitrogen (AA-N) to the small intestine. The results may be greater absorption of AA and less excretion of N. When consumption exceeds requirements for production, ruminal NH_3 is excrected which is energetically costly but also environmental harmful (Tamminga, 1979).

2.3.3. Restrictions to use of ionophores

Roussell and Houlihan (2003) pointed out that because the use of the ionophores-alternative antibiotics, avoparcin and vancomycin, in poultry and swine production and the occurrence of transmission of resistance to organisms harmful to humans, some people try to expand this analogy to the possibility that it may also occur with the use of ionophores. Although according to the literature reviewed by the authors this argument is not very well supported. Still, in 1999, Denmark prohibited use of ionophores as growth promoters in animal production soon followed by all countries in the European Union, in 2006, abiding to the banishment of these antibiotics as well (European Union, 2006). Hence, there is an increasing social and governmental pressure for the adoption of alternatives to ionopohores (Callaway et al., 2003). Due to the great variety and its many possible combinations, essential oils are presented as a potential alternative to ionophores.

2.4. Essential oils

Essential oil (EO) has its name dervided from "essence", as in odor, not because they are essential, as necessary, for plants. These oils are organic compounds synthesized as secondary metabolites and their essence or taste is a resource used by plants for its perpetuation, either for protection against predation, protection against environmental stress or to attract insects that aid in its reproduction (Jouany and Morgavi, 2007). Over the years these secondary metabolites have been thought to be anti-nutritional compounds (i.e. tanins), however many of them have antimicrobial activities against different microbes and, thus, may be used to manipulate rumen microbiome (Cobellis et al., 2016). These secondary metabolites can be grouped in three major groups: saponins, tanins and essential oil (Calsamiglia et al., 2007). Since European Union's ban of non-terapeutical use of antibiotics in animal production in 2006 the intereset in studies with essential oils has risen up again. Over the past decade authors have raised and compiled information regarding properties, mode of action and impacts on rumen microbiome of essential oils, their bioactive compounds (BC) or combination of different EO and/or BC (Greathed, 2003; Calsamigilia et al, 2007; Jouany and Morgavi, 2007; Castillejos et al., 2008; Benchaar et al., 2008; Hart et al., 2008; Benchaar and Greathed, 2011; Patra, 2011; Khiaosa-ard and Zebeli, 2013; Cobellis et al., 2016).

Contrary to what the name suggests, essential oils are not true oils (i.e. lipids; Benchaar et al., 2008) as they can contain different chemical groups, such as alcohols, aldehydes, ketones, esters, ethers and hydrocarbons (Benchaar et al., 2007). Essential oils are in fact a combination of different bioactive compounds. The most important BC can be categorized in two chemical groups: terpenes and phenylpropanoids. These two groups are result of different precursors of primary metabolism and are generated from two different metabolic routes (Calsamiglia et al., 2007). Terpenoids are the most common group of plant secondary metabolites, present in plants such as thyme, oregano and basil (Calsamiglia et al., 2007). Terpenes can be classified based on the number of 5-carbon-base (C_5) isoprene units (Cobellis et al., 2016) and the most important among them are monoterpenes (C_{10}), that constitute the majority of EO, and sesquiterpenes (C_{15}; Calsamiglia et al., 2007; Cobellis et al, 2016). Phenylpropaniods are aromatic compounds that consits of a C_6 aromatic ring with a C_3 branched chain (Hart et al., 2007). Though not as common as terpenes and most plants do not present high concentrations (Calsamiglia et al., 2007), some plants such as cinnamon, clove and anise contain high concentrations of these metabolites (Patra, 2011).

2.4.1. Mode of action of essential oils

Essential oils have many medicinal properties used for pharmaceutical purposes, however the most important, specially for the animal feed industry, are their anitimicrobial and antiseptic properties (Calsamiglia et al., 2007). Terpenes and phenylpropanoids are the main components of EO varying in proportion that can change due to morphophysiological factors, such as species, part of the plant used to extract or even stage of growth, environmental factors, such as soil, fertility, temperature, light exposure and water availability (Hart et al., 2008). The antimicrobial properties of EO are mainly due to terpenes and phenylpropanoids (Calsamiglia et al., 2007). However, these are major groups that comprise a great variety of different compounds, thus it is most probable that the antimicrobial effect is due to effect over a wide range of targets rather than a specific mechanism of action (Burt, 2004; Benchaar et al., 2008; Cobellis et al., 2016).

Burt (2004) has compiled different hypotesis for mode of action, however it is believed that the hydrophobic nature of EO grant them the hability to interact with bacterial cell membranes and mithocondria, causing changes in membranes structure increasing its permeability and, consequently, causing leakege of ions and other cell contents (Burt, 2004; Calsamiglia et al., 2007). Essential oil affect mostly gram-positive bacteria, however gram-negative bacteria are not immune to its effects. Gram-negative bacteria are indeed more resistant to influence of EO due to the extra membrane external to the cell wall, but they can be affected. Helander et al., (1998) showed that carvone, thymol, cinnamaldehyde and carvacrol (present in cumin, thyme, cinnamon and oregano, respectively) had inhibitory effect over gram-negative bacteria. Calsamiglia et al. (2007) compiled data from multiple essays and reported that gram-negative bacteria are susceptible to essential oil from dill, paprika, tea tree, oregano, thyme and ginger. The wide spectrum of action of essential oil against gram-positive bacteria, but also gram-negative bacteria, makes it difficult to predict effects on rumen fermentation and the magnitude of impact on it.

Different isolated bioactive compounds can be manipulated and combined with others to obtain different effects. When combined, EO and BC can have additive, antagonistic or synergistic effects (Burt, 2004).

2.4.2. Effects on ruminant performance

Most reviews are foccused on *in vitro* effects of essential oil on rumen fermentation (Castillejos et al., 2007; Calsamiglia et al., 2007; Benchaar et al., 2008). Still, data of *in vivo* effects of EO over ruminant performance is scarse. Acording to Calsamiglia et al. (2007) *in vitro* studies are important to determine antimicrobial effects of essential oils and blend of essential oils (BEO), however it may overestimate these properties since concentrations used in *in vitro* studies are generally much higher than the ones in the rumen when tested *in vivo*.

Castillejos et al. (2007) evaluated ten different EO in three different concentrations (5, 50 and 500 mg/l of culture fluid) on ruminal fermentation *in vitro*. Authors considered effects to be positive if total VFA and propionate production increased or C_2:C_3 ratio and NH_3 decreased. The same authors found that most doses of thyme, savory, oregano and clove oils increased total VFA concentrations while decreasing NH_3, with little or no effect on acetate:propionate ratio. Thus, it might be expected that under the same concentrations an improvement on performance could be expected. Busquet et al. (2005) showed cinnamaldehyde improved propionate while decreasing acetate concentration, thus reducing C_2:C_3 ratio.

According to Benchaar et al. (2008) and Patra (2011) in most studies with EO or BEO total VFA concentration remained unaltered rather than improved or even decreased in some cases. Supplementation with EO leading to unchanged concentrations of VFA can still be benefic if propionate concentration is increased or if acetate:propionate ratio, NH_3 concentration and CH_4 production decrease. Cobellis et al. (2016) complied several studies in which EO from garlic, clove, eucalyptus, oregano and peppermint inhibited methanogenic bacteria, thus probably mitigating CH_4 emissions. The same authors summarized a large number of experiments in which the concentration of rumen NH_3 was reduced, therefore showing the potential of EO to improve protein utilization.

Khiaosa-Ard and Zebeli (2013) did a meta-analysis only with data of *in vivo* studies evaluating use of EO for dairy cows, beef cattle and other small ruminants. These authors observed that overall the use of EO, BC or BEO did not alter total concentration of VFA, but increased molar concentration of propionate whereas decreased acetate's. However, the feed additive did not alter DMI, similarly to Calsamiglia et al. (2007), nor performance (milk yield), thus feed efficiency remained unaltered. Data of beef cattle was not sufficient for analysing average weight gain nor feed efficiency (Khiaosa-Ard and Zebeli, 2013). In spite of that, these authors concluded that beef cattle were more responsive to EO than dairy cattle or small ruminants, possibly due to synergistic effect with lower ruminal pH resulted from feeding high-concentrate diets.

Results of use of EO are dose dependent and the success rate of its use is linked to the determination of optimal dose (Calsamiglia et al., 2007; Khiaosa-Ard and Zebeli, 2013; Cobellis et al., 2016). Khiaosa-Ard and Zebeli (2013) also reported that maximum dosage of essential oil used for beef cattle was 0.25g/kg DM in ruminal fermentation trials whereas in performance trial maximum dose was 0.22g/kg DM. Authors also suggested that lower doses resulted in more pronounced effects on performance.

2.4.3. Characterization of the blend of essential oils (BIOPhytus®)

The essential oils that compose the commercial blend used in this trial are oils from copaiba tree (*Copaifera* spp.), castor bean (*Ricinus communis*) and cashew (*Anacardia occidentalis*) nuts. The compounds derived from copaiba tree and cashew nuts are terpenoids whereas the bioactive compounds from castor bean are consired fatty acids and not essential oil as they are primary metabolism generated compounds.

Cashew nut shell liquid (CNSL) contains a high proportion of phenolic compounds, being them cardanol, cardol, anacardic acid, and 6-methyl cardol (1.2, 11.3, 64.9, and 2.0% by weight, respectively; Lubi and Thachil, 2000). However, in commercial-grade cashew nut extracts, due to heating process the concentration of anacardic acid is fairly reduced (Himejima and Kubo, 1991; Lubi and Thachil, 2000). Cashew nut shell liquid has shown antibacterial, mainly against gram-positive bacteria (Himejima and Kubo, 1991), and antimicotic properties (Lubi and Thachil, 2000). Anacardic acid is a phenolic compound and its antimicrobial properties are stronger than of cardol and cardanol's, which are lipids (Himejima and Kubo, 1991; Branco et al., 2015). Watanabe et al. (2010) evaluating *in vitro* effects of CNSL showed that the raw CNSL, exctracted without heating process increased propionate production, decreased concentrations of acetate, and decreaseed emissions of CO_2 and CH_4. Branco et al. (2015) reported that the use of CNSL in lactating cows tended to decrease the amount of CH_4 when expressed over DMI. Authors attributed the lack of significant effect to the absence of anacardic acid, that was probably converted to cardanol during the roasting process.

The oil extracted from castor bean is a mixture of several chemical components, but its main bioactive compound is the ricinoleic acid, which is a fatty acid and not an essential oil (Vieira et al., 2001). Authors observed that ricinoleic acid possesses anti-inflammatory properties. Novak et al. (1960) reported that ricinoleic acid and derivatives of ricinoleic acid act against a large spectrum of microorganisms. Wallace et al. (2007) reported that ricinoleic acid (50 mg/l of culture medium) was highly toxic against the gram-positive bacteria *Butyrivibrio fibrisolvens*, *Clostridium proteoclasticum* and *Propionibacterium acnes*, since none of them were capable of growing in culture medium.

Oleoresin extract from Copaifera spp. trees are mixture of terpenes, mainly sesquiterpenes (Cascon and Gilbert, 2000), which present antibiotic activity (Calsamiglia et al., 2007). There are more than 20 species of Copaifera (Cascon and Gilbert, 2000), thus a great variation in composition of oleoresin extracted from these plants can be expected. More than sixty diterpenes carboxylic acids were indentified among extracts of these species, however the only common compound among them was the diterpen copalic acid, thereby being a biomarker to identify origin of authenticity of the extracted oil (Veiga et al., 1997). The authors also state that the antimicrobial activity of the copaiba extract can be atributed to caurenoic acid, also identified in several species. Copaiba oil has been described repeatedly as highly effective against gram-positive bacteria with moderate action against fungi and no action against gram-negative bacteria (Pacheco et al., 1997; Santos et al., 2008).

There is enough scientific evidence of antimicrobial activity of the different substances alone that compose BIOPhytus® BEO. However due to possible interactions of the different compounds with each other the effects of the commertial mixture on ruminant performance and metabolism have to be assessed.

2.5. Use of buffers in ruminants

2.5.1. Acidosis

Attempting to maximizing performance of ruminants and reduce operational costs, nutritionists decrease amounts of roughage fed to cattle, thus increasing energetic density of the diet (Pinto e Millen, 2019) Reducing the amount of roughage fed to cattle can be risky as roughage, more specifically its physically effective NDF (peNDF) content , is necessary to stimulate chewing activity, salivary secretion and rumen motility, all of which are essential to avoid metabolic disorders, such as acidosis, and preserve ruminant's sanity (Allen, 1997). For that purpose, over the years authors have studied and described the causes behind acidosis (Owens et al., 1998; Enemark, 2008; Calsamiglia et al., 2012; Humer et al. 2018) and strategies to prevent and treat it (Erdman, 1988; Staples and Lough, 1989; Hu and Murphy, 2005; Calsamiglia et al. 2012; Humer et al. 2018).

Increasing energetic density of the diet is generally done at the expense of roughage by increasing amounts of carbohydrate or fat in the diets. However, inclusion of fat is limited to about 7% of whole diet (NASEM, 2016), therefore increasing carbohydrates are the most expressive way to increase energy content of the diet. The products generated from carbohydrate fermentation (VFA) are, as the name suggests, acids and if its production rate exceeds rumen's uptake and buffering capacity, pH levels may drop below the threshold in which acidosis may occur. Acute or clinical acidosis which can be diagnosed by blood pH below 7.35 (Owens et al., 1998). On the other hand, subacute ruminal acidosis (SARA) or chronic acidosis does not have exclusive characteristics/symptoms, thus its definition can be controversial (Calsamiglia et al., 2012; Humer et al., 2018). The term SARA is commonly used to designate poor rumen health (Humer et al., 2018) and according to scientific matherial reviewed by the authors the risk of SARA occurrence increases when ruminal pH drops below 5.6 for more than 3 hours in the whole day (Plaizier et al., 2008) or below 5.8 for more than 5 to 6 hours a day (Zebeli et al., 2008).

Although SARA is more frequent in dairy herds due to frequent diet changes (Humer et al., 2018) to supply adequate amounts of nutrients in their different stages (early, mid or late lactation stages, dry stage, during pregnancy, etc.), beef cattle finished in feedlots, particularly when fed high-starch and low fiber diets, are also susceptible to this metabolic disorder (Hernández et al., 2014). Lack of clinical symptoms make the diagnose difficult, but there are signs of SARA's occurrence that can be observed. On dairy cows SARA can be manifest through depression on feed intake, milk yield and fat content, laminitis, loss of body score and alteration in faeces (Abdela, 2016), whereas in beef cattle the main signs are decrease in dry matter intake, chewing activity, weight loss, laminitis and diarrhea (Hernández et al., 2014).

Even though there are natural occurring mechanisms to avoid ruminal pH drops below acidosis threshold, such as rumen epithelium absorption of VFA, secretion of alkaline saliva, bicarbonate ions and VFA luminal exchange and consequential increase in buffering capacity in the rumen (Humer et al., 2018), they might not be enough to suppress the potential of acidosis caused by rapid rates of carbohydrates fermentation, especially in starch rich diets. Thus, there are some measures that can be used to reduce occurrence of SARA such as providing enough peFDN (Fox and Tedeschi, 2002), adequate adapting the animals to high-concentrate and high starch-content diets (Humer et al., 2018), ensuring mixture uniformity and lowering possibility of segregation of small particles when feeding animals with total mixed ration (Stone, 2004), controlling ruminal pH and fermentation process (Calsamiglia et al., 2012) through use of feed additives (Humer et al., 2018).

2.5.2. Effects of buffer on ruminants performance

Several reviews have been published to evaluate the effects of different buffer additives on dairy cattle performance (Erdman, 1988; Staples and Lough, 1989; Hu and Murphy, 2005), showing an increase on dry matter intake, milk production and fat content (Erdman, 1988; Staples and Lough, 1989). However, effects of buffers on beef cattle performance and ruminal pH are not consistent (Zinn and Borgues, 1993). Hunttington et al. (1977) noticed that buffers improved feedlot performance during the adaptation period, however the prolonged buffer supplementation did not sustain observed improvements or even had adverse effect impairing performance. The same results were observed by Dunn et al. (1979). Zinn (1991) observed that beef cattle fed diets with inclusion of 0.75% of sodium bicarbonate (DM basis) presented greater ADG (5.9%) and DMI (4.6%) than the control treatment. Roger and Davis (1982) observed that animals fed sodium bicarbonate had higher DMI than animals from control treatment, but increased production of acetate (C_2) and reduced propionate (C_3), thereby increasing C_2:C_3 ratio. Russell et al. (1980) noticed that supplementation of $NaHCO_3$ did not influence DMI, ADG and F:G when compared with non-supplemented animals and supplementation of limestone + $NaHCO_3$ reduced ADG.

The effect of buffers in the ruminal environment is ephemeral (Van Soest, 1994), which may explain the inconsistency of ruminal pH control and effects on animal performance. However, a new kind of buffer, based on calcified seaweed, thus a natural product, is being explored in ruminant nutrition. It claims to have more than twice the buffering capacity of $NaHCO_3$ on dairy cattle (Enemark, 2008).

2.5.3. Characterization of the buffer *Lithothamnium calcareum* (LithoNutri®) and effects on ruminant nutrition

Calcareous seaweed (*Lithothamnium calcareum*) is a renewable mineral resource mostly composed of calcium and magnesium carbonate ($CaCO_3$ and $MgCO_3$, respectively) as well as other micronutrients, such as Fe, Mn, B, Ni, Cu, Zn, Mo, Se e Sr (Dias, 2000). Calcareus algae possesses higher availability of micronutrients (Dias, 2000) due to higher solubility (Melo and Moura, 2009), thus being easily assimilated by plants and animals. LithoNutri® contains minimum values of 320 g of Ca, 30 g of Mg, 920 g of minerals per kilogram of product. The honeycomb structural arrangement of this seaweed allows the minerals to be released slowly in the rumen prolonging the time of buffering action when compared to sodium bicarbonate (Cruywagen et al., 2015).

Cruywagen et al. (2015) observed that Holstein cows fed *Lithothamnium calcareum* (0.4% of diet DM basis) presented higher milk yield, fat (yield and percentage of milk composition), protein yield, efficiency of feed utilization (milk/DMI) and less time with ruminal pH under 5.5, considered to be the threshold for SARA in high-yielding cows (Krauze and Oetzel, 2006) than cows fed diets with limestone (0.4% DM) or limestone + sodium bicarbonate (0.4% + 0.8% DM, respectively). DMI did not differ among treatments. Similarly, Montañez-Valdez et al. (2012) observed that the use of *Lithothamnium calcareum* resulted in higher pH than use of sodium bicarbonate or no buffer addition, however it did not improve nutrient digestibility.

Orsine et al. (1989) observed no differences in digestibility when comparing basal diet without buffer additive with supplementation with *Lithothamnium calcareum* (1000 mg/kg DM or 2000 mg/kg DM) or limestone (1000 mg/ kg DM). Carvalho et al. (2016) testing supplementation with limestone (7.1g/kg DM) or calcareous algae (7.4 g/kg DM), with or without addition of monensin (30 ppm) observed that limestone resulted in higher means of ruminal and blood pH, therefore being more efficient in pH control.

Comparin et al. (2013) testing performance of heifers in pastures consuming 1 kg DM/animal/day of protein-energy supplement and heifers in pastures consuming the same supplement at the amount 0.99 kg DM/animal day + 0.01 kg of *Lithothamnium calcareum* observed no differences in DMI, ADG, dressing or pH. Even though supplementation with calcareous algae reduced numerically ADG by 8.7%, the authors argue that the amount of *Lithothamnium calcareum* may not have been enough to provoke changes in performance and ruminal parameters.

Makkar et al. (2016) reviewed the use of different seaweed in animal nutrition and still data on its use with ruminants is scarse, hence the need for more studies regarding effects of the calcareus seaweed and its effects as buffer in pH control in dairy cows and beef cattle performance.

3. MATERIAL AND METHODS

The experiment was conducted at the Experimental Feedlot Cattle facilities of the Animal Science Department of the "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), in Piracicaba, State of São Paulo, Brazil. All procedures using animals followed the guidelines recommended by the Animal Care and Use Committee of the ESALQ/USP, protocol number 2017.5.1617.11.2all.

3.1. Animals and treatments

Ninety Nellore bulls [initial body weight (BW) = 393 ± 34 kg] and ninety crossbreds (Nellore x British and Nellore x Continental breeds) [initial body weight (BW) = 406 ± 31 kg] were used in a complete randomized block design experiment with factorial arrangement to evaluate the effects of three different feed additives [monensin (MON); monensin + *Lithothamnium calcareum* (MON+LC); a blend of essential oils (BEO)] and its interactions with two different racial groups [Nellore bulls (NEL) and Crossbred bulls (CROSS)]. At the beginning of the trial, animals were individually weighed after 16 hours of feed and water deprivation, ear tagged with individual identification, dewormed with 1 ml per 44 kg BW of albendazole sulfoxide (Agebendazol 15%, União Química S/A, São Paulo, SP, Brazil) and vaccinated against clostridial infection (Ourovac Poli-BT; Ourofino S/A, Cravinhos, SP, Brazil). Bulls were blocked according to genetic group and initial body weight in 6 blocks in which the 6 treatments were randomized totalizing 36 pens with 5 animals each. The pens (32 m^2) were partially roofed with concreted soil and every two pens shared a single water trough.

The ingredient and chemical composition of the diet are presented in Table 1 and the chemical compositions of the feed ingredients are presented in Table 2. The bulls (NEL or CROSS) in the control group (MON) were fed sodium monensin (Rumensin; Elanco Animal Health, Indiana, USA) as the only feed additive (31.3 mg/kg DM). The other treatments consisted of sodium monensin + *Lithothamnium calcareum* (MON+LC – 31.3 mg/kg DM + 5 g/kg DM, respectively) or a blend of essential oils (BEO – 0.3 g/kg DM). The blend of essential oils (BioPhytus; ProPhytus Agroindustrial, São José dos Campos, SP, Brazil) was composed of cardol, cardanol and anacardic acid from cashew nuts (*Anacardium occidentale*), ricinoleic acid from castor bean (*Ricinus communis* L.) and copaene from copaiba tree (*Copaifera spp.*). The calcareum marine algae was composed from *Lithothamnium calcarium* dried and milled into a floury texture (LithoNutri; OCEANA Minerals, Jundiaí, SP, Brazil).

Corn was ground to a mean particle size of 1.42 cm (Table 3) and particle size distribution was described according to Yu et al. (1998), using sieves with 6.0, 3.5, 2.0, 1.25 mm square pores (Produtest T Model; Telastem Peneiras para Analises Ltda., Sao Paulo, SP, Brazil). Sugarcane bagasse had a mean particle size of 2,74 mm (Table 4) and particle size was determined according to the Penn State Particle Size Separator method (Lammers et al., 1996) using sieves of 19, 8, and 4 mm. Sugarcane bagasse, ground corn and citrus pulp were weighed in the feed wagon's scale (Casale Rotormix 40, Casale Equipamentos, São Carlos, SP, Brazil), whereas soybean meal, urea, sodium chloride and the mineral mixture were weighed in a fixed scale (Weightech WT1000, Weightech Equipamentos de Pesagem, Florianopolis, SC, Brazil). Each treatment diet was mixed indiviadually in a feed wagon, the additives were added to the diet when mixing in the feed wagon and water was added in order to adjust DM of the total mixed ration (TMR) to 70%. The feed wagon was emptied and cleaned after each mixture to avoid contamination of treatments. The treatment diets were always mixed and provided to the bulls in the same order, starting with the diet from treatment MON, followed by treatment MON+LC and finalizing with the diet from treatment BEO.

The animals were adapted to the feedlot finishing diet during 21 days with a step-up diet (Table 5) initiating with 25% DM of sugarcane bagasse on the first week and lowering to 20% and 15% on the two following weeks, until the 22nd day when the final diet started. The feed bunk management was conducted aiming 3% of orts and the TMR was provided once daily at 0700 and the amount of feed by each pen was weighed using the feed wagon's scale. Orts were recovered twice a week, weighed, and samples were stored frozen under -18°C for posterior DM analyses and calculation of dry matter intake (DMI). Average daily gain (ADG) was calculated by dividing the difference between final and initial weight by the number of days of confinement and feed efficiency (G:F) was calculated as the ratio of ADG to DMI.

On the 112th day of the experiment, bulls were weighed without fasting discounting 4% of live full BW (NASEM, 2016) to calculate live shrunk weight and slaughtered at a commercial slaughterhouse. Hot carcasses were weighed and dressing percentage (DP) was calculated as the ratio between hot carcass weight (HCW) and final shrunk BW.

3.2. Feed analysis and calculations

Samples of sugarcane bagasse were collected every 3 days for periodic analysis of dry matter content and adjustment of its amount of inclusion in the diet.

Samples of each ingredient and of the TMR were collected every 15 days and stored at -18ºC. At the end of the experiment, the samples were thawed, composited for whole period dried in a forced-air oven at 55°C for 72 h, and ground through a 1 mm screen using a Wiley-type mill (MA-680; Marconi Ltda, Piracicaba, SP, Brazil). All samples were analyzed for DM (method 930.15; AOAC, 2000), ash (method 942.05; AOAC, 2000), ash-corrected neutral digestible fiber [(aNDF); Van Soest et al., 1991] using sodium sulfite and heat-stable α-amylase, acid detergent fiber [(ADF); Goering and Van Soest, 1970], and nitrogen [(N); Leco FP-528; Leco Corp., St Joseph, MI]. The crude protein (CP) content was calculated by multiplying nitrogen content by 6.25. Feed ingredients were analyzed for ether extract [(EE); method 920.85; AOAC, 1986].

The net energy observed for maintenance (NEm) and for gain (NEg) for each treatment were calculated according to equations proposed by Zinn and Shen (1998) using the mean values for shrunk BW (average weight from the whole experimental period), average DMI and average daily gain (ADG) of the bulls in each pen. The predicted NEm and NEm were calculated in two different ways. First, they were calculated with the TDN equations proposed by Weiss et al. (1992) and then converted to NE according to the equations proposed by NASEM (2016) with ionophore addition. The second method was using the tabular TDN values proposed by NASEM (2016) and then using these values to calculate NE values using the NASEM (2016) equations with addition of ionophore. The observed net energies for maintenance and gain were compared to the predicted NEm and NEg.

3.3. Statistical analyses

Tests for normality (Shapiro-Wilk and Kolmogrov-Smirnov) were performed before analyzing the data. Data from performance (initial and final BW, DMI, ADG based on measured or calculated shrunk weight and G:F) and carcass traits (HCW and dressing percentage) were analyzed using the PROC MIXED procedure of SAS software (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The statistical model included the fixed effect of treatment (breed, feed additive and breed x feed additive interaction) and the random effect of weight block. Results are reported as least-square means and significance was set at $P \leq 0.05$. Means were compared using Tukey test.

4. RESULTS

Cattle performance and carcass traits are presented in Table 7. The observed net energies for maintenance and gain as well as the observed:expected NE ratios are presented in Table 8. As no interactions were observed between breed type and feed additives results will be presented separately.

4.1. Breed type

As it was not possible to acquire crossbred and Nellore bulls with the same initial BW for the trial, crossbred bulls (406.39 kg) were heavier than Nellore bulls (393.83 kg) since the beginning of the experiment (P < 0.001). Final BW (P < 0.001), HCW (P < 0.001), DMI (P < 0.001) and ADG (P < 0.001) differed between breed type. Crossbred bulls presented higher final BW (BW (568.83 vs 525.94 kg) and higher HCW (319.83 vs 296.17 kg) than Nellore bulls respectively). Crossbreds also presented higher ADG than Nellore bulls (1.329 vs. 1.112 kg) as well as they presented higher DMI than Nellore (9.470 vs. 7.878 kg). Dressing percentage (P = 0.818) and feed efficiency (P = 0.097) were the same for crossbred bulls and Nellore bulls.

The observed NE for maintenance and gain were higher for crossbred bulls (P < 0.001; 2.220 and 1.527 Mcal/kg, respectively) than for Nellore (1.999 and 1.343 Mcal/kg, respectively). When TDN values calculated by equations from Weiss et al. (1992) were used to calculate expected diet NE, the observed:expected NE ratios both for maintenance and gain differed between breed (P < 0.001), being higher for crossbreds of fory Nellore (for maintenance and gain respectively, 1.345 and 1.469 Mcal/kg for crossbreds and 1.212 and 1.292 Mcal/kg for Nellore). When NASEM (2016) TDN tabular values were used to calculate expected NE of the diets, crossbreds presented higher ratio of observed:expected NE for maintenance (1.220 Mcal/kg) and gain (1.283 Mcal/kg) than Nellore (1.098 Mcal/kg for NEm and 1.129 Mcal/kg for NEg).

4.2. Feed additives

Dry matter intake was different among feed additives (*P* = 0.030). Daily dry matter intake was higher for animals fed diets containing BEO (9.255 kg) when compared to animals receiving MON+LC (8.127 kg). Dry matter intake of bulls fed MON (8.641 kg/d) was similar (*P* > 0.05) to DMI of bulls fed MON+LC and BEO. The ADG and final BW were not affected by feed additives (*P* > 0.05). Feed efficiency (G:F) was different between treatments (*P* = 0.048).

Animals fed diets with MON presented higher G:F (0.151), therefore being different than animals fed diets with BEO (0.138). However, G:F did not differ between animals fed MON and MON+LC (0.142) nor between animals fed BEO and MON+LC. Carcass traits were not affected by feed additives ($P > 0.05$).

The tested feed additives had different effects on observed dietary NEm ($P = 0.007$) and NEg ($P = 0.012$) concentrations as well as for observed:expected NEm ($P = 0.007$) and NEg ($P = 0.012$) ratios (Table 8). Animals fed diets with MON presented higher NEm (2.182 Mcal/kg) and NEg (1.454 Mcal/kg) than animals fed diets with BEO (2.020 Mcal/kg and 1.362 Mcal/kg, respectively). No differences were observed when comparing the observed NE concentrations for animals fed MON and MON+LC (2.125 Mcal/kg for maintenance and 1.454 Mcal/kg for gain) nor when comparing animals fed BEO and MON+LC. The same pattern was observed with the observed:expected NEm and NEg.

When TDN values calculated by equations from Weiss et al. (1992) were used to calculate expected diet NE, animals fed MON presented a higher observed:expected ratios for NEm (1.332 Mcal/kg) and NEg (1.432 Mcal/kg) when compared with animals fed BEO (1.224 Mcal/kg for NEm and 1.309 Mcal/kg for NEg). Treatment MON+LC resulted in intermediate values for observed:expected NE ratios so that the values did not differ statistically from the two extremes. When NASEM (2016) TDN tabular values were used to calculate expected diet NE values, MON resulted in higher ratio of observed:expected NE for maintenance (1.199 Mcal/kg) and gain (1.252 Mcal/kg) than BEO (1.110 Mcal/kg for NEm and 1.144 Mcal/kg for NEg).

5. DISCUSSION

As no interactions were observed between breed type and feed additives in the measured variables the results of breed type and feed additives will be discussed separately.

5.1. Breed type

The crossbred bulls started the trial 12.5 kg heavier than the Nellore bulls. By the end of the trial crossbred bulls presented a final body weight 42.89 kg higher than the Nellore bulls. The CROSS bulls had higher ADG than NEL, which explains the difference in final BW being larger than only the 12.5 kg of difference in initial BW. Several authors have reported greater ADG for crossbreds (*Bos taurus taurus* vs *Bos taurus indicus*) and european cattle (*Bos taurus taurus*) when compared with zebu cattle (Putrino et al., 2006; Goulart et al., 2008; Rubiano et al., 2009; Maggioni et al., 2010), similarly to what was observed in this trial.

The greater DMI of Crossbreds compared with NEL is in accordance with the literature that reports that *Bos taurus taurus* present higher DMI than *Bos taurus indicus* (Goulart et al., 2008; Rubiano et al., 2009). Nevertheless, feed efficiency did not differ between the two racial groups, therefore the difference observed in ADG is most likely a result of the higher DMI in CROSS animals. Similarly, Goulart et al. (2008) observed that Aberdeen Angus x Nellore crossbred steers presented higher final shrunk BW, ADG, DMI than Nellore purebred steers, however, feed efficiency was not analyzed.

Even though European breeds have higher NE requirements for maintenance and gain than zebu breeds (NASEM, 2016), in this trial even though breeds did not differ in feed efficiency, CROSS was more efficient in nutrient utilization than NEL as they presented higher observed dietary NE for maintenance and gain as well as higher observed:expected NE ratios, contrarily to what *Bos taurus inducus* and *Bos taurus taurus* comparative studies have shown (Putrino et al., 2006; Goulart et al., 2008).

Valadares-Filho & Chizzotti (2010) in a literature review compiled data to explain differences in energy requirements for maintenance between B. *taurus taurus* and B. *taurus indicus* and explained that bovines from these second group have lower total organ:live BW or liver:live BW ratio, which implies in a lower requirement of energy for maintenance for B. *taurus indicus* than for B. *taurus taurus*. Due to the lower total organ:live BW ratio, NEL should present a greater dressing percentage than CROSS in the present study. However, dressing percentage is highly affected by final BW and so, the greater final BW of CROSS may explain the same dressing percentage between both genetic groups.

Despite the lower energy requirement for maintenance for zebu cattle compared with B. *taurus taurus* as pointed by Valadares-Filho & Chizzotti (2010), CROSS cattle were more efficient energetically then NEL. The greater mature weight for CROSS compared with NEL may partially explain this result.

5.2. Feed additives

In In the present experiment, performance (DMI, ADG and G:F) and carcass traits (HCW and DP) of feedlot finished bulls fed a commercial blend of essential oils or a seaweed buffer + monensin were compared to bulls fed monensin. Monensin has been one of the most described and updated feed additives for ruminants (Goodrich et al., 1984; Potter et al., 1985; Tedeschi et al., 2003; Duffield et al., 2012) and its acceptance on feedlots protocols has been sustained throughout the years. Therefore, monensin serves as a unique comparison against other growth promoter feed additives.

The inclusion of the MON+LC resulted in a lower DMI, numerically lower than MON and statistically lower than BEO. Beauchemin and McGinn (2006) reported no differences on DMI when comparing the inclusion of 1 g/animal.d of a commercial blend of essential oils of thymol, eugenol, vanillin, guaiacol, and limonene (Crina® Ruminants) and animals fed no feed additives (control treatment). Chaves et al. (2008) reported that inclusion of carvacrol or cinnamaldehyde (0.2 g/kg DM) had no influence on DMI of lambs and so was the same as control treatment. Silva et al. (2014) testing inclusion of 3 g/animal.d of a commercial blend of essential oils of cashew nut and castor bean (Essential®) reported that DMI was the same between bulls receiving diets with Essential® and bulls fed diet without feed additive.

Benchaar et al. (2006) testing increasing doses (2 and 4 g/animal.d) of a commercial blend of essential oils of thymol, eugenol, vanillin and limonene (Vertan®) found no difference on DMI of BEO vs control, whilst monensin reduced DMI compared to control. Accordingly, Meyer et al. (2009) comparing inclusion of Crina® at 1 g/d (EOM), a blend of guaiacol, linalool and α-pinene (EXP) at 1g/d, Crina® + tylosin (EOM +T; 1g + 90 mg/d, respectively) and monensin + tylosin (MON+T; 300 mg +90 mg/d, respectively) reported that only MON+T had effect reducing DMI, whereas steers fed EOM, EXP and EOM+T had the same DMI as steers fed diet without feed additives. Similarly, Meschiatti et al. (2019) also reported that animals fed monensin presented lower DMI than bulls fed BEO (Crina®) during the feedlot period. Dry matter intake was reduced about 4% with the inclusion of monensin on diets, compared to the absence of feed additives (Tedeschi et al., 2003; Duffield et al, 2012a), whereas inclusion of EO has little impact on DMI and usually animals fed no feed additives or fed EO have similar feed intake (Calsamiglia et al., 2007; Khiaosa-Ard and Zebeli, 2013).

On the other hand, Ornaghi et al. (2017) testing two essential oils separately (cinnamaldehyde or clover oil) at two doses (3.5 or 7.0 g/d) reported positive linear response to inclusion of oils on DMI. These authors attribute the increased DMI to palatability effect of the essential oils. However, essential oils can reduce the feed intake as observed by Cardozo et al. (2006), that when testing a mixture of cinnamaldehyde plus eugenol (0.18 and 0.09 g/d, respectively) observed a reduction on DMI. This decreased DMI was attributed to a possible antinutritional effect, however actual cause was not concluded (Cardozo et al., 2006).

Feed intake of bulls fed MON was numerically higher (+6.3%) than of those fed MON+LC, but statistically the same. Therefore, there were no advantages or synergistic effects of combining MON and *Lithothamium calcareum*. Accordingly, Zinn and Borgues (1993) no interactions of monensin and sodium bicarbonate (BICARB) on DMI were observed, which was the same for steers fed no additive, monensin, sodium bicarbonate or monensin+sodium bicarbonate. Similarly, Adams et al. (1981) observed no differences on DMI for treatments with monensin or with sodium bicarbonate included at 2.5 or 5.0% of diet DM. Russell et al. (1980) testing inclusion of 0.9% of $NaHCO_3$ (DM basis), 1.8% of limestone (DM basis), the combination of both buffers (0.9% of $NaHCO_3$ + 1.8% of limestone) or no buffers (control diet) found no differences on DMI.

The use of limestone in high grain diets is a common practice for adjusting calcium supply to meet ruminant requirements (NASEM, 2016). Nevertheless, Clark et al. (1989) observed a decreased DMI of dairy cows when calcium carbonate ($CaCO_3$) was included both at 1.4% or 2.1% in the diet. Since $CaCO_3$ is the main constituent of *Lithothamnium calcareum* it might also be expected that the seaweed buffer could have negative influence on DMI. In spite of that, it has been reported that inclusion of limestone (Russell et al., 1980), calcium magnesium carbonate (Rauch et al., 2012) and calcareous seaweed buffers (Bernard et al., 2014; Cruywagen et al. 2015) does not change DMI from animals fed diets without feed additives. Therefore, the lower DMI observed for animals fed MON+LC may be a combination of possible adverse effect of seaweed buffer, as reported by Clark et al. (1989), but seems to be much more a result of the traditional and consistent effect of monensin reducing the feed intake (Goodrich et al. 1984; Tedeschi et al., 2003; Duffield et al., 2012a).

As the amount of Ca in seaweed buffer is minimum of 320 g/kg, the addition of seaweed buffer caused calcium:phosphorus ratio to change from 3.9:1 to at least 4.6:1 (Table 5). Although changes are not great, it has been reported in some experiments that the excess of Ca and/or increase in Ca:P ratios can impair ADG and total weight gain (Dowe et al., 1957), reduce DMI (Dowe et al., 1957) and decrease protein and energy digestibility (Golovos et al., 1957). No changes in DMI were observed between beef calves fed diets with calcium:phosphorus ratios of 1.3:1 and 4.3:1 (Dowe et al., 1957). However, these authors state that a critical Ca:P ratio may exist between 4.3:1 and 9.1:1. Which may lead to an explanation to why MON+LC emphasized the depression on DMI and reduced numerically ADG by 120 g when compared to MON.

In the present experiment, bulls fed MON+LC presented numerically the lowest ADG, followed by animals fed BEO and at last, by animals fed MON presenting numerically higher ADG value. However, even though DMI explains 60 to 90% of the variations in animal performance (NRC, 2001), the changes observed on DMI in this performance trial did not result in significant differences on ADG. Nevertheless, according to reviewed literature in high concentrate diets the use of monensin has minimal influences on weight gain, acting mainly reducing feed intake and thus improving feed efficiency (Goodrich et al., 1984; Potter et al., 1985; Tedeschi et al., 2003; Purevjav et al., 2013), similarly to what was found in this experiment.

Meschiatti et al. (2019) reported greater ADG of bulls fed Crina® plus α-amylase (BEO+AM) than bulls fed diet with monensin (MON). However, when compared with a control diet (no feed additives) no difference on ADG between animals fed diets with or without EO or BEO has been reported over the years (Benchaar et al., 2007; Yang et al., 2010; Purevjav et al., 2013; Silva et al., 2014; Cruz et al., 2016). In a recent meta-analysis from Khiaosa-Ard and Zebeli (2013) it was observed that supplementation of EO (pure bioactive compounds, individual essential oils or blend of essential oils) for dairy cows had no significant influence on milk production, fat content, nor feed efficiency. However, according to theses authors, regarding to the effects of EO supplementation on beef cattle performance, the amount of data for analysis was not enough for it to be assessed.

Regarding effects of buffers on performance of ruminants, a slight improvement on ADG or DMI might be observed during the period of adaptation to high-grain diets, however when analyzing the entire feedlot period, the positive effects of prolonged use of buffers did not sustain itself in some studies (Huntington et al., 1977; Dunn et al., 1979) or may even negatively impact the performance (Russell et al., 1980), which may elucidate the reason why animals fed MON+LC presented lower values of ADG. Differently, some authors have observed cases in which feed intake (Fulton et al., 1979) and ADG were improved with addition of buffers (Wise et al. 1965; Zinn, 1991; Lofgreen, 1976 apud Zinn and Borgues, 1993) with no improvements on feed efficiency.

Feed efficiency of animals fed MON was higher than of those fed BEO. In the reviewed papers wherein monensin and essential oils (EO or BEO) were compared and differed in G:F, they also differed in ADG or DMI (Jedlicka et al., 2009, Purevjav et al., 2013, Moura et al., 2017). Jedlicka et al. (2009) compared two levels of inclusion of Essential® (0.25 and 0.5 g/kg DM), the combination of the Essential® with monensin (0.25 g/kg DM and 223 mg/animal.day), monensin straight (223 mg/animal.day) or no feed additives. These authors reported that monensin resulted in better feed conversion (lower DM kg/kg ADG) than all other treatments but was only higher in ADG from the treatment with higher inclusion of essential oil (dose of 0.5 g/kg DM). Purevjav et al. (2013) testing the same blend of essential oils at level of 0.5 g/kg DM reported that animals receiving monensin had greater weight gain and greater feed utilization efficiency than those fed BEO. Meyer et al. (2009) comparing the commercial blend Crina® associated with tylosin (EOM+T) and monensin associated with tylosin (MON+T) found that even though the animals treated with MON+T had lower DMI than those of the control and EOM+T treatments, they did not differ in G:F. However, the authors attribute the improvement on feed efficiency observed for EOM+T to the presence of tylosin. Differently, Moura et al. (2017) testing copaiba oil (0.5 g/kg DM) observed a better performance in weight gain and higher feed efficiency of lambs supplemented with essential oil than those supplemented with monensin. Even though data of beef cattle performance was not enough to be analyzed in the authors meta-analysis (Khiaosa-Ard and Zebeli, 2013), the absence of differences in DMI, ADG and G:F between control treatments (no feed additive) and essential oils have been reported recurrently (Benchaar et al., 2006; Chaves et al., 2008; Yang et al., 2010; Gandra et al., 2012; Cruz et al., 2014; Moura et al., 2017; Ornaghi et al., 2017), contrary to what is generally the result of monensin on feed utilization efficiency of ruminants (Tedeschi et al., 2003; Duffield et al., 2012a; Duffield et al., 2012b).

In the present study, hot carcass weight and dressing percentage were the same among feed additive treatments. Similarly, Meschiatti et al. (2019) observed that BEO and MON resulted in bulls with the same HCW and dressing percentage, while the combination of BEO with amylase resulted in 12 kg greater HCW compared with monensin. Ornaghi et al. (2017) observed that clover oil (3.5 and 7.0 g/day), when compared to diet without additives, increased linearly bulls cold carcass weights (CCW) with no influence on dressing percentage. These authors also observed that cinnamon oil (3.5 and 7.0 g/day) improved CCW without changing DP. Purevjav et al. (2013) reported that even though HCW did not differ between cattle fed control diet

(CON), monensin or essential oils, MON decreased dressing percentage when compared to cattle from CON and high dose of essential oil.

In the present trial, the greater G:F presented by the animals fed MON when compared to the BEO, coupled with no differences observed on carcass traits, shows that the feed efficiency is not masked by visceral fat and visceral deposition, but that MON was indeed more efficient in converting feed into carcass, contrary to what was reported by Purevjav et al. (2013) wherein monensin lost its higher G:F due to lower dressing percentage.

Even though DMI did not differ statistically between MON and BEO, the numerically lower DMI of MON combined with the greater feed efficiency presented, could imply in a reduced cost during the total period of feedlot finishing. In addition, higher G:F implies in greater nutrient utilization, what was indeed observed in NE for maintenance and gain. Supplementation with MON resulted in higher NEm and NEg, as well as improved ratios of observed:expected NE for maintenance and for gain when compared with supplementation with BEO.

When TDN of ingredients calculated by equations of Weiss et al. (1992) were used to calculate expected NE for maintenance and gain, the observed NEm were higher than the predicted (+32.2%, +28.7% and +22.4% for MON, MON+LC and BEO respectively), as well as the NEg (+43.2%, +39.7% and +31.0% for MON, MON+LC and BEO, respectively). When diet expected NE values were calculated from NASEM (2016) TDN tabular values for each ingredient, the differences between observed and expected net energies was lower (+20.0%, +16.8%, +10.9% for NEm and +25.2%, +22.3%, +14.4% for NEg, for MON, MON+LC and BEO respectively). Therefore, equations proposed by Weiss et al. (1992) underestimated TDN values of ingredients resulting in lower diet NE for maintenance and for gain and, consequently, in greater differences between observed and expected NE. Nevertheless, regardless of the methodology employed to calculate TDN and subsequent net energies and ratios between observed:expected net energies, MON was always more efficient in nutrient utilization.

Bergen and Bates (1984) pointed out that monensin alters the route of ruminal fermentation for a higher production of propionate and lower production of acetate which would increase energy availability for the animal. Also, the use of this additive improves the retention of nitrogen (N) in the animal (Page, 2003). Thus, monensin increases the metabolizable energy (ME) value of the diet and as diet ME increases, more of the intake is available for production, which reduces the proportion of the diet used for maintenance (Tedeschi et al., 2003).

Khiaosa-Ard and Zebeli (2013) presented results in which shifts of fermentation caused by EO were similar to those provided by monensin, increasing propionate whilst reducing acetate, without changing total VFA production. These same authors also indicated the EO potential to mitigate methane emissions. All of these effects show a potential of EO to increase nutrient utilization efficiency. However, according to the authors, the effect on acetate, propionate and methane formation disappeared in terms of proportional changes relative to control. Additionally, authors found no beneficial effects of EO on protein utilization. In addition, data compiled from *in vitro* and *in vivo* studies of different essential oils and their combinations (Castillejos et al., 2008; Cobellis et al., 2016) show that effects on ruminal fermentation (shift in acetate:propionate proportion and retention of N) are type and dose dependent, which explains lack of consistency between results. In those studies when EO influenced positively on ruminal fermentation processes the impacts were not as great as those caused by monensin, which reflects in differences of diets ME, similar to what was observed when analizing observed:expected ratios of NE for maintenance and gain in the present trial.

In this meta-analysis conducted by Khiaosa-Ard and Zebeli (2013) authors stated that average inclusion of EO in *in vivo* experiments was 0.10 g/kg diet and the effect of EO on VFA composition was most pronounced in beef cattle with the maximum dose of 0.25 g/kg (DM basis) and increasing doses could lead to adverse effect. In the present study, the dose used of the BEO was 0.30 g/kg. Although each essential oil and blend of essential oils have its own optimum dosage, the average and maximum doses pointed by the authors could be an indication that the current dose used in the present experiment was too high. Regarding the results of combination of *Lithothamnium calcareum* and monensin, the reasons for lack of positive synergistic effect remain uncertain.

6. CONCLUSION

Crossbred cattle present greater potential for ADG, which results in greater final body weight, HCW and DMI than Nellore bulls.

Crossbred cattle are more efficient in energy utilization both for maintenance and gain than Nellore bulls.

The addition of the seaweed buffer *Lithothamnium calcareum* over monensin brings no advantages over the single use of monensin on the performance of feedlot cattle fed high concentrate diets.

The supplementation with BEO composed by castor beans, cashew nuts and copaiba at inclusion of 0.3 g/kg DM is not as efficient as monensin on feed efficiency and nutrient utilization of cattle fed finishing diets.

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LITERATURE CITED

Abdela, N. (2016). Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: A review of past and recent research at global prospective. Achievements in the life sciences, 10(2), 187-196. doi: 10.1016/j.als.2016.11.006

Adams, D. C., Galyean, M. L., Kiesling, H. E., Wallace, J. D. and Finkner, M. D. (1981). Influence of viable yeast culture, sodium bicarbonate and monensin on liquid dilution rate, rumen fermentation and feedlot performance of growing steers and digestibility in lambs. Journal of Animal Science, 53(3), 780-789. doi: 10.2527/jas1981.533780x.

Allen, M. S. (1997). Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. Journal of dairy science, 80(7), 1447-1462. doi: 10.3168/jds.S0022-0302(97)76074-0.

Associação Brasileira das Industrias Exportadoras de Carne, ABIEC (2019). Beef Report: Perfil da Pecuária no Brasil. Available at: (http://www.abiec.com.br/controle/uploads/arquivos/sumario2019portugues.pdf). Acessed [Oct 28, 2019].

Association of Official Analytical Chemists (AOAC). 1986. Official methods of analysis. 14th ed. AOAC, Arlington, VA.

Benchaar C., J. L. Duynisveld, and Charmley, E. (2006). Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Can J. Anim. Sci. 86:91-96.

Benchaar, C. and Greathead, H. (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed Science and Technology, 166, 338-355. doi: 10.1016/j.anifeedsci.2011.04.024.

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G. R., Colombatto, D., McAllister, T. A. and Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. Animal Feed Science and Technology, 145(1-4), 209-228. doi: 10.1016/j.anifeedsci.2007.04.014

Benchaar, C., Chaves, A. V., Fraser, G. R., Beauchemin, K. A. and McAllister, T. A. (2007). Effects of essential oils and their components on in vitro rumen microbial fermentation. Canadian journal of animal science, 87(3), 413-419. doi: 10.4141/CJAS07012.

Bergen, W. G. and Bates, D. B. (1984). Ionophores: their effect on production efficiency and mode of action. Journal of animal science, 58(6), 1465-1483. doi: 10.2527/jas1984.5861465x.

Branco, A.F.; Giallongo, F.; Frederick, T.; Weeks, H.; Oh, J. and Hristov, A.N. (2015). Effect of technical cashew nut shell liquid on rumen methane emission and lactation performance of dairy cows. J. Dairy Sci. 98, 1–11. doi: 10.3168/jds.2014-9015.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology, 94(3), 223-253. doi: 10.1016/j.ijfoodmicro.2004.03.022

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W. and Kamel, C. (2005). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. Journal of Dairy Science, 88(7), 2508-2516. doi: 10.3168/jds.S0022-0302(05)72928-3.

Callaway, T. R., Edrington, T. S., Rychlik, J. L., Genovese, K. J., Poole, T. L., Jung, Y.S., Bischoff, K. M., Anderson, R. C. and Nisbet, D. J. (2003) Ionophores: their use as ruminant growth promotants and impact on food safety. Curr Issues Intest Microbiol 4, 43–51.

Calsamiglia, S., Blanch, M., Ferret, A. and Moya, D. (2012). Is subacute ruminal acidosis a pH related problem? Causes and tools for its control. Animal feed science and technology, 172(1-2), 42-50. doi: 10.1016/j.anifeedsci.2011.12.007.

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, and Ferret, A. (2007). Essential oils as modifiers of rumen microbial fer- mentation. J. Dairy Sci. 90:2580–2595. doi: 10.3168/jds.2006-644.

Carvalho, R. F., Mazon, M. R., Silva, A. P. D. S., Oliveira, L. S., Zotti, C. A. and Leme, P. R. (2016). Use of calcareous algae and monensin in Nellore cattle subjected to an abrupt change in diet. Ciência Rural, 46(4), 713-718. doi: 10.1590/0103-8478cr20150278.

Cascon, V. and Gilbert, B. (2000). Characterization of the chemical composition of oleoresins of Copaifera guianensis Desf., Copaifera duckei Dwyer and Copaifera multijuga Hayne. Phytochemistry, 55(7), 773-778. doi: 10.1016/S0031-9422(00)00284-3.

Castillejos, L., Calsamiglia, S., Martin-Tereso, J. and Ter Wijlen, H. (2008). In vitro evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. Animal feed science and technology, 145:1-4, 259-270. doi: 10.1016/j.anifeedsci.2007.05.037

Chaves, A. V., K. Stanford, L. L. Gibson, T. A. McAllister, and Benchaar, C. 2008. Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and car- cass characteristics of growing lambs. Anim. Feed Sci. Technol. 145:396–408. doi: 10.1016/j.anifeedsci.2007.04.016

Cobellis, G., Trabalza-Marinucci, M. and Yu, Z. (2016). Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. Science of the Total Environment, 545, 556-568. doi: 10.1016/j.scitotenv.2015.12.103.

Comparin, M. A. S., Morais, M. D. G., Alves, F. V., Coutinho, M. A. D. S., Fernandes, H. J., Feijó, G. L. D., Oliveira, L. O. F. and Coelho, R. G. (2013). Performance, carcass and meat qualitative characteristics of Brangus heifers supplemented on pasture, getting different food additives. Revista Brasileira de Saúde e Produção Animal, 14(3), 574-586. doi: 10.1590/S1519-99402013000300015.

Cruywagen, C. W., Taylor, S., Beya, M. M. and Calitz, T. (2015). The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation. Journal of dairy science, 98(8), 5506-5514. doi: 10.3168/jds.2014-8875.

Cruz, O. T. B., Valero, M. V., Zawadzki, F., Rivaroli, D. C., Do Prado, R. M., Lima, B. S. and Do Prado, I. N. (2014). Effect of glycerine and essential oils (Anacardium occidentale and Ricinus communis) on animal performance, feed efficiency and carcass characteristics of crossbred bulls finished in a feedlot system. Italian Journal of Animal Science, 13(4), 3492. doi: 10.4081/ijas.2014.3492.

Diário Oficial da União, DOU. (2018). Portaria Nº 171, De 13 de Dezembro de 2018, do Ministério da Agricultura, Pecuária e Abastecimento, MAPA/Secretaria de Defesa Agropecuária, SDA. [website] (19/09/2018). Available at: (http://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/55878469/do1-2018-12-19-portaria-n-171-de-13-de-dezembro-de-2018-55878239). Acessed [Jul 10, 2019].

Dias, G. (2000). Marine bioclasts: calcareous algae. Revista Brasileira de Geofísica, 18(3), 307-318. doi: 10.1590/S0102-261X2000000300008.

Dowe, T. W., Matsushima, J. and Arthaud, V. H. (1957). The effects of adequate and excessive calcium when fed with adequate phosphorus in growing rations for beef calves. Journal of Animal Science, 16(4), 811-820. doi: 10.2527/jas1957.164811x.

Duffield, T. F., Merrill, J. K. and Bagg, R. N. (2012a). Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. Journal of Animal Science, 90(12), 4583-4592. doi: 10.2527/jas.2011-5018

Duffield, T. F., Rabiee, A. and Lean, I. J. (2012b). Overview of meta-analysis of monensin in dairy cattle. Veterinary Clinics: Food Animal Practice, 28(1), 107-119. doi: 10.1016/j.cvfa.2011.12.009

Dunn, B. H., Emerick, R. J. and Embry, L. B. (1979). Sodium bentonite and sodium bicarbonate in high-concentrate diets for lambs and steers. Journal of Animal Science, 48(4), 764-769. doi: 10.2527/jas1979.484764x.

Duval, S., McEwan, N., Graham, R., Wallace, R. and Newbold, C. (2007). Effect of a blend of essential oil compounds on the colonization of starch-rich substrates by bacteria in the rumen. Journal of Applied Microbiology, 103: 2132-2141. doi:10.1111/j.1365-2672.2007.03455.x

Enemark, J. M. (2008). The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. The Veterinary Journal, 176(1), 32-43. doi: 10.1016/j.tvjl.2007.12.021

Erdman, R. A. (1988). Dietary buffering requirements of the lactating dairy cow: A review. Journal of Dairy Science, 71(12), 3246-3266. doi: 10.3168/jds.S0022-0302(88)79930-0.

European Commission (2003) Regulation (EC) No 1831 ⁄ 2003 of the European Parliament and the Council of 22 September 2003 on additives for use in animal nutrition. Official Journal of the European Communities. L268, 29–43.

Fortes, G. and Yassu, F. (2008). O milagre do boi brasileiro. Instituto Brasileiro de Geografia e Estatística, IBGE, São Paulo.

Fox, D. G. and Tedeschi, L. O. (2002). Application of physically effective fiber in diets for feedlot cattle. In: Proceedings of the Plains Nutrition Conference (pp. 67-81).

Fulton, W. R., Klopfenstein, T. J. and Britton, R. A. (1979). Adaptation to high concentrate diets by beef cattle. II. Effect of ruminal ph alteration on rumen fermentation and voluntary intake of wheat diets. Journal of Animal Science, 49(3), 785-789. doi: 10.2527/jas1979.493785x.

Galyean, M. L. and Defoor, P. J. (2003). Effects of roughage source and level on intake by feedlot cattle. *Journal of Animal Science*, *81*(14_suppl_2), E8-E16. doi: 10.2527/2003.8114_suppl_2E8x

Gandra, J. R., Gil, P. N., Cônsolo, N. R. B., Gandra, E. R. S. and Gobesso, A. A. D. O. (2012). Addition of increasing doses of ricinoleic acid from castor oil (Ricinus communis L.) in diets of Nellore steers in feedlots. J Anim Feed Sci, 21, 566-576. doi: 10.22358/jafs/66131/2012

Goering, H. K. and Van Soest, P. J. (1970). Forage fiber analyses (apparatus, reagents, prcedures, and some applications). USDA Agr Handb.

Golovos, N. F., Keener, H. A. and Davis, H. A. (1958). Effect of pulverized limestone and dicalcium phosphate on the nutritive value of dairy cattle feed. Journal of Dairy Science, 41(5), 676-682. doi: 10.3168/jds.S0022-0302(58)90982-2.

Goodrich, R. D., J. E. Garrett, D. R. Gast, M. A. Kirick, D. A. Larson and Meiske, J. C. (1984). Influence of Monensin on the Performance of Cattle. Journal of animal science. 58:1484-98. doi: 10.2527/jas1984.5861484x

Goulart, R. C. D. (2011). Avaliação de antimicrobianos como promotores de crescimento via mistura mineral para bovinos de corte em pastejo (Doctoral dissertation, Universidade de São Paulo). doi: 10.11606/T.11.2011.tde-17032011-171637.

Goulart, R. S., Alencar, M. M. D., Pott, E. B., Cruz, G. M. D., Tullio, R. R., Alleoni, G. F. andLanna, D. P. D. (2008). Composição corporal e exigências líquidas de proteína e energia de bovinos de quatro grupos genéticos terminados em confinamento. Revista Brasileira de Zootecnia, 37(5), 926-935. doi: dx.doi.org/10.1590/S1516-35982008000500022.

Greathead, H. (2003). Plants and plant extracts for improving animal productivity. Proceedings of the Nutrition Society, 62(2), 279-290. doi: 10.1079/PNS2002197.

Gregory, K. E., Cundiff, L. V. and Koch, R. M. (1994). Breed effects, dietary energy density effects, and retained heterosis on different measures of gain efficiency in beef cattle. Journal of animal science, 72(5), 1138-1154. doi: doi.org/10.2527/1994.7251138x.

Hall, M.B. and Huntington, G.B. (2008) Nutrient synchrony: sound in theory, elusive in practice. Journal of Animal Science, 86: 287-292. doi: 10.2527/jas.2007-0516.

Hart, K. J., Yáñez-Ruiz, D. R., Duval, S. M., McEwan, N. R. and Newbold, C. J. (2008). Plant extracts to manipulate rumen fermentation. Animal Feed Science and Technology, 147(1-3), 8-35. doi: 10.1016/j.anifeedsci.2007.09.007.

Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., Gorris, L. G. M. and von Wright, A. (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of agricultural and food chemistry, 46(9), 3590-3595. doi: 10.1021/jf980154m.

Hernández, J., Benedito, J. L., Abuelo, A. and Castillo, C. (2014). Ruminal acidosis in feedlot: from aetiology to prevention. The Scientific World Journal, 2014. doi: 10.1155/2014/702572.

Himejima, M. and Kubo, I. (1991). Antibacterial agents from the cashew Anacardium occidentale (Anacardiaceae) nut shell oil. Journal of Agricultural and Food Chemistry, 39(2), 418-421. doi: 10.1021/jf00002a039.

Hu, W. and Murphy, M. R. (2005). Statistical evaluation of early-and mid-lactation dairy cow responses to dietary sodium bicarbonate addition. Animal feed science and technology, 119(1-2), 43-54. doi: 10.1016/j.anifeedsci.2004.12.005.

Humer, E., Petri, R. M., Aschenbach, J. R., Bradford, B. J., Penner, G. B., Tafaj, M., Südekum, K.H. and Zebeli, Q. (2018). Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. Journal of dairy science, 101(2), 872-888. doi: 10.3168/jds.2017-13191.

Huntington, G. B., Emerick, R. J. and Embry, L. B. (1977). Sodium bentonite or sodium bicarbonate as aids in feeding high-concentrate diets to lambs. Journal of animal science, 45(4), 804-811. doi: 10.2527/jas1977.454804x.

Jedlicka, M. E., Purevjav, T., Conover, A. J., Hoffman, M. P., Pusillo, G. and Torrent, J. (2009). Effects of Functional Oils and Monensin Alone or in Combination on Feedlot Cattle Growth and Carcass Composition (Progress Report). Animal Industry Report, 655(1), 46. doi: 10.31274/ans_air-180814-464

Johnson, K. A. and Johnson, D. E. (1995). Methane emissions from cattle. Journal of animal science, 73(8), 2483-2492. doi: 10.2527/1995.7382483x.

Jouany, J. and Morgavi, D. (2007). Use of 'natural' products as alternatives to antibiotic feed additives in ruminant production. Animal, 1(10), 1443-1466. doi:10.1017/S1751731107000742

Khiaosa-ard, R., and Zebeli, Q. (2013). Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants1. J. Anim. Sci. 91:1819-1830. doi: 10.2527/jas.2012-5691

Krause, K. M. and Oetzel, G. R. (2006). Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Animal feed science and technology, 126(3-4), 215-236. doi: 10.1016/j.anifeedsci.2005.08.004

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922–928. doi:10.3168/jds.S0022-0302(96)76442-1.

Lubi, M. C. and Thachil, E. T. (2000). Cashew nut shell liquid (CNSL) - a versatile monomer for polymer synthesis, Designed Monomers and Polymers, 3:2, 123-153. Doi: 10.1163/156855500300142834

Maggioni, D., de Araújo Marques, J., Rotta, P. P., Perotto, D., Ducatti, T., Visentainer, J. V. and do Prado, I. N. (2010). Animal performance and meat quality of crossbred young bulls. Livestock Science, 127(2-3), 176-182. doi: doi.org/10.1016/j.livsci.2009.09.006.

Makkar, H. P., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F. and Ankers, P. (2016). Seaweeds for livestock diets: a review. Animal Feed Science and Technology, 212, 1-17. doi: 10.1016/j.anifeedsci.2015.09.018

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W. and Cotter, P. D. (2019). The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. Gut microbes, 10(2), 115-132. doi: 10.1080/19490976.2018.1505176

Melo, T. V. and Moura, A. M. A. (2009). Utilização da farinha de algas calcáreas na alimentação animal. Archivos de Zootecnia, 58(2), 99-107.

Meschiatti, M. A., Gouvêa, V. N., Pellarin, L. A., Batalha, C. D., Biehl, M. V., Acedo, T. S., Dórea, J. R. R., Tamassia, L. F. M., Owens, F. N. and Santos, F. A. P. (2018). Feeding the combination of essential oils and exogenous α-amylase increases performance and carcass production of finishing beef cattle. Journal of Animal Science, 97(1), 456-471. doi: 10.1093/jas/sky415.

Meyer, N. F, G. E. Erickson, T. J. Klopfenstein, M. A. Greenquist, M. K. Luebbe, P. Williams, and Engstrom M. A. (2009). Effect of essential oils, tylosin and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation and digestibility. J Anim. Sci. 87:2346-2354. doi: 10.2527/jas.2008-1493.

Millen, D. D., Pacheco, R. D. L., Arrigoni, M. D. B., Galyean, M. L. and Vasconcelos, J. T. (2009). A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. Journal of animal science, 87(10), 3427-3439. doi: 10.2527/jas.2009-1880.

Millen, D. D., Pacheco, R. D. L., DiLorenzo, N., Martins, C. L., Marino, C. T., Bastos, J. P. S. T., Rodrigues, P. H. M. and Arrigoni, M. D. B. (2015). Effects of feeding a spray-dried multivalent polyclonal antibody preparation on feedlot performance, feeding behavior, carcass characteristics, rumenitis, and blood gas profile of Brangus and Nellore yearling bulls. Journal of animal science, 93(9), 4387-4400. doi: doi.org/10.2527/jas.2015-9227.

Montañez-Valdez, O. D., Pinos-Rodriguez, J. M., Rojo-Rubio, R., Salinas-Chavira, J., Martinez-Tinajero, J. J., Avellaneda-Cevallos, J. H. and Salem, A. Z. M. (2012). Effect of a calcified-seaweed extract as rumen buffer on ruminal disappearance and fermentation in steers. Available at: (http://hdl.handle.net/20.500.11799/65976).

Morais, J. D. S., Berchielli, T. T. and Reis, R. A. (2006). Aditivos. Nutrição de ruminantes, 1, 539-570.

Moura, L. V., Oliveira, E. R., Fernandes, A. R. M., Gabriel, A. M. A., Silva, L. H. X., Takiya, C. S., Cônsolo, N.R.B., Rodrigues, G.C.G., Lemos, T. and Gandra, J. R. (2017). Feed efficiency and carcass traits of feedlot lambs supplemented either monensin or increasing doses of copaiba (Copaifera spp.) essential oil. Animal Feed Science and Technology, 232, 110-118. doi: 10.1016/j.anifeedsci.2017.08.006.

Nagaraja T.G., Newbold C.J., van Nevel C.J. and Demeyer D.I. (1997) Manipulation of ruminal fermentation. In: Hobson P.N., Stewart C.S. (eds) The Rumen Microbial Ecosystem. Springer, Dordrecht. doi: 10.1007/978-94-009-1453-7_13.

National Academies of Sciences, Engineering, and Medicine, NASEM, 2016. Nutrient requirements of beef cattle: Eight Revised Edition. Washington, DC: The National Academies Press. doi.org/10.17226/19014.

Novak, A. F., Clark, G. C. and Dupuy, H. P. (1961). Antimicrobial activity of some ricinoleic acid oleic acid derivatives. Journal of the American Oil Chemists Society, 38(6), 321-324. doi: 10.1007/BF02638439.

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ornaghi, M. G., Passetti, R. A., Torrecilhas, J. A., Mottin, C., Vital, A. C. P., Guerrero, A., Sañudo, C., Campo, M.M. and Prado, I. N. (2017). Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. Animal feed science and technology, 234, 274-283. doi: 10.1016/j.anifeedsci.2017.10.008.

Orsine, G. F., Costa, C. D. P., Oliveira, B. D., Rodrigues, D. G. and Oliveira, C. R. (1989). Efeito da fonte de cálcio (calcário vs lithothamnium calcareum) na digestibilidade aparente do feno de capim brachiaria decumbens staph cv. basiliski. Available at: (https://www.revistas.ufg.br/pat/article/view/2566/2538).

Owens, F. N., Secrist, D. S., Hill, W. J. and Gill, D. R. (1998). Acidosis in cattle: a review. Journal of animal science, 76 (1), 275-286. Doi: https://doi.org/10.2527/1998.761275x

Pacheco, T. A. R. C., Barata, L. E. S. and Duarte, M. C. T. (1997). Antimicrobial activity of copaiba (Copaifera spp) balsams. Ciência e Cultura, 49(5/6), 339-344.

Page, S. W. (2003). The role of enteric antibiotics in livestock production. Canberra, Australia: Avcare Ltd.

Patra, A.K. (2011). Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. Asian Journal of Animal and Veterinary Advances 6 (5): 416-428. doi:10.3923:ajava.2011.416.428.

Pinto, A. C. and Millen, D. D. (2018). Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: the 2016 Brazilian survey. Canadian Journal of Animal Science, 99(2), 392-407. doi: 10.1139/cjas-2018-0031.

Plaizier, J. C., Krause, D. O., Gozho, G. N., and McBride, B. W. (2008). Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. The Veterinary Journal, 176(1), 21-31. doi: 10.1016/j.tvjl.2007.12.016

Potter, E. L., M. I. Wray, R. D. Muller, H. P. Grueter, J. McAskill, and Young, D. C. 1985. Effect of monensin and tylosin on average daily gain, feed efficiency and liver abscess incidence in feedlot cattle. J. Anim. Sci. 61:1058-1065. doi: 10.2527/jas1985.6151058x.

Pressman, B. C. (1976). Biological applications of ionophores. Annual review of biochemistry, 45(1), 501-530. doi: 10.1146/annurev.bi.45.070176.002441.

Purevjav, T., Hoffman, M. P., Ishdorj, A., Conover, A. J., Jedlicka, M. E., Prusa, K., Torrent, J. and Pusillo, G. M. (2013). Effects of functional oils and monensin on cattle finishing programs. The Professional Animal Scientist, 29(4), 426-434. doi: 10.15232/S1080-7446(15)30256-4.

Putrino, S. M. U., Leme, P. R., Alleoni, G. F., Lanna, D. P. D., Lima, C. G. D. and Grossklaus, C. S. E. (2006). Exigências líquidas de proteína e energia para ganho de peso de tourinhos Brangus e Nelore alimentados com dietas contendo diferentes proporções de concentrado. Revista Brasileira de Zootecnia. doi: doi.org/10.1590/S1516-35982006000100037.

Raun, A. P., Cooley, C. O., Potter, E. L., Rathmacher, R. P. and Richardson, L. F. (1976). Effect of monensin on feed efficiency of feedlot cattle. Journal of Animal Science, 43(3), 670-677. doi: 10.2527/jas1976.433670x.

Rogers, J. A. and Davis, C. L. (1982). Rumen volatile fatty acid production and nutrient utilization in steers fed a diet supplemented with sodium bicarbonate and monensin. Journal of Dairy Science, 65(6), 944-952. doi: 10.3168/jds.S0022-0302(82)82295-9.

Rotta, P. P., Prado, R. M. D., Prado, I. N. D., Valero, M. V., Visentaine, J. V. and Silva, R. R. (2009). The effects of genetic groups, nutrition, finishing systems and gender of Brazilian cattle on carcass characteristics and beef composition and appearance: a review. Asian-Australasian Journal of Animal Sciences, 22(12), 1718-1734. doi: doi.org/10.5713/ajas.2009.90071.

Rubiano, G. A. G., Arrigoni, M. D. B., Martins, C. L., Rodrigues, É., Gonçalves, H. C. and Angerami, C. N. (2009). Desempenho, características de carcaça e qualidade da carne de bovinos superprecoces das raças Canchim, Nelore e seus mestiços. Revista Brasileira de Zootecnia, 2490-2498. doi: doi.org/10.1590/S1516-35982009001200027.

Russell, J. B. (1987). A proposed mechanism of monensin action in inhibiting ruminant bacterial growth: Effects on ion flux and protonmotive force. Journal of Animal Science, 64(5), 1519-1525. doi: 10.2527/jas1987.6451519x.

Russell, J. B. and Houlihan, A. J. (2003). Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS microbiology reviews, 27(1), 65-74. doi: 10.1016/S0168-6445(03)00019-6.

Russell, J. B. and Strobel, H. J. (1989). Effect of ionophores on ruminal fermentation. Applied and environmental microbiology, 55(1), 1.

Russell, J. R., Young, A. W. and Jorgensen, N. A. (1980). Effect of sodium bicarbonate and limestone additions to high grain diets on feedlot performance and ruminal and fecal parameters in finishing steers. Journal of Animal Science, 51(4), 996-1002. doi: 10.2527/jas1980.514996x.

Samuelson, K. L., Hubbert, M. E., Galyean, M. L. and C.A. Löest. (2016). Nutritional recommendations of feedlot consulting nutritionists: the 2015 New Mexico State and Texas Tech University survey. Journal of animal science, 94(6), 2648-2663. doi: 10.2527/jas.2016-0282.

Santos, A. O. D., Ueda-Nakamura, T., Dias Filho, B. P., Veiga Junior, V. F., Pinto, A. C. and Nakamura, C. V. (2008). Antimicrobial activity of Brazilian copaiba oils obtained from different species of the Copaifera genus. Memórias do Instituto Oswaldo Cruz, 103(3), 277-281. doi:10.1590/S0074-02762008005000015.

Santos, F. A. P., da Silva Marques, R. and Dórea, J. R. R. (2016). Grain Processing for Beef Cattle. In Rumenology, Springer, Cham., 213-241. doi: 10.1007/978-3-319-30533-2_8.

SAS Institute. 2009. SAS/STAT® 9.2 User's Guide. 2ed. SAS Institute, Cary, NC, USA.

Staples, C. R. and Lough, D. S. (1989). Efficacy of supplemental dietary neutralizing agents for lactating dairy cows. A review. Animal Feed Science and Technology, 23(4), 277-303. doi: 10.1016/0377-8401(89)90050-3.

Stock, R. A., Sindt, M. H., Parrott, J. C. and Goedeken, F. K. (1990). Effects of grain type, roughage level and monensin level on finishing cattle performance. Journal of animal science, 68(10), 3441-3455. doi: 10.2527/1990.68103441x.

Stone, W. C. (2004). Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. Journal of Dairy Science, 87, E13-E26. doi: 10.3168/jds.S0022-0302(04)70057-0.

Tamminga, S. (1979). Protein degradation in the forestomachs of ruminants. Journal of Animal Science, 49(6), 1615-1630. doi: 10.2527/jas1979.4961615x.

Tamminga, S. (1996). A review on environmental impacts of nutritional strategies in ruminants. Journal of Animal Science, 74: 3112-3124. doi: 10.2527/1996.74123112x.

Tedeschi, L.O.; Fox, D.G. and Tylutki, T.P. (2003). Potential environmental benefits of ionophores in ruminant diets. J. Environ. Qual. 32:1591–1602. doi: 10.2134/jeq2003.1591.

Thornton, J. H. and Owens, F. N. (1981). Monensin supplementation and in vivo methane production by steers. Journal of Animal Science, 52(3), 628-634. doi: 10.2527/jas1981.523628x.

Valadares-Filho, S. C. and Chizzotti, M. L. (2010). Exigências nutricionais de bovinos de corte. In: Pires, A. V. (Ed.). (2010). Bovinocultura de corte. FEALQ (p.203-218).

Van Soest, P. J. (1994). Fiber. Nutritional ecology of the ruminant. Cornell university press, 140-155.

Van Soest, P. V., Robertson, J. B. and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science, 74(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2.

Veiga Jr, V. F., Patitucci, M. L. and Pinto, A. C. (1997). Controle de autenticidade de óleos de copaíba comerciais por cromatografia gasosa de alta resolução. Química Nova, 20(6), 612-615.

Vieira, C., Fetzer, S., Sauer, S. K., Evangelista, S., Averbeck, B., Kress, M., Reeh, P. W., Cirillo, R., Lippi, A., Maggi, C. A. and Manzini, S. (2001). Pro-and anti-inflammatory actions of ricinoleic acid: similarities and differences with capsaicin. Naunyn-Schmiedeberg's archives of pharmacology, 364(2), 87-95. doi: 10.1007/s002100100427.

Wallace, R. J., McKain, N., Shingfield, K. J. and Devillard, E. (2007). Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. Journal of lipid research, 48(10), 2247-2254. doi: 10.1194/jlr.M700271-JLR200.

Watanabe, Y., Suzuki, R., Koike, S., Nagashima, K., Mochizuki, M., Forster, R. J., & Kobayashi, Y. (2010). In vitro evaluation of cashew nut shell liquid as a methane-inhibiting and propionate-enhancing agent for ruminants. Journal of dairy science, 93(11), 5258-5267. doi: 10.3168/jds.2009-2754.

Weiss, W. P., H. R. Conrad, and Pierre R. S. 1992. A theoretically based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol. 39:95-119. doi: 10.1016/0377-8401(92)90034-4.

Wise, M. B., Blumer, T. N., Craig, H. B. and Barrick, E. R. (1965). Influence of rumen buffering agents and hay on performance and carcass characteristics of steers fed all-concentrate rations. Journal of Animal Science, 24(1), 83-88. doi: 10.2527/jas1965.24183x.

Yang, W. Z., Ametaj, B. N., Benchaar, C., He, M. L. and Beauchemin, K. A. (2010). Cinnamaldehyde in feedlot cattle diets: intake, growth performance, carcass characteristics, and blood metabolites. Journal of animal science, 88(3), 1082-1092. doi: doi.org/10.2527/jas.2008-1608

Yu, P., J. T. Huber, F. A. P. Santos, J. M. Simas, and Theurer C. B. (1998). Effects of ground, steam-flaked, and steam-rolled corn grains on performance of lactating cows. Journal of Dairy Science, 81(3), 777-783. doi: 10.3168/jds.S0022-0302(98)75634-6.

Zebeli, Q., Dijkstra, J., Tafaj, M., Steingass, H., Ametaj, B. N. and Drochner, W. (2008). Modeling the adequacy of dietary fiber in dairy cows based on the responses of ruminal pH and milk fat production to composition of the diet. Journal of dairy science, 91(5), 2046-2066. doi: 10.3168/jds.2007-0572.

Zinn, R. A. (1991). Comparative feeding value of steam-flaked corn and sorghum in finishing diets supplemented with or without sodium bicarbonate. Journal of animal science, 69(3), 905-916. doi: 10.2527/1991.693905x.

Zinn, R. A. and Borques, J. L. (1993). Influence of sodium bicarbonate and monensin on utilization of a fat-supplemented, high-energy growing-finishing diet by feedlot steers. Journal of animal science, 71(1), 18-25. doi: 10.2527/1993.71118x.

Zinn, R. A., and Shen, Y. (1998). An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. J. Anim. Sci. 76:1280–1289. doi:10.2527/1998.7651280x.

Zotti, C. A., Silva, A. P., Carvalho, R., Marino, C. T., Rodrigues, P. H. M., Silva, L. F. P., McAllistar, T.A. and Leme, P. R. (2017). Monensin and a blend of castor oil and cashew nut shell liquid used in a high-concentrate diet abruptly fed to Nellore cattle. Journal of animal science, 95(9), 4124-4138. doi: doi.org/10.2527/jas.2017.1580.

APPENDIX

Table 1. Ingredients (%DM unless specified otherwise) and chemical composition (%DM) of the experimental diets.

Item	
Sugarcane bagasse	8.50
Ground corn	42.20
Citrus pulp	41.70
Soybean meal	5.00
Urea	1.30
Minerals and vitamin supplement[1,2]	0.95
Sodium chloride	0.35
Net energy for maintenance, Mcal/kg[3]	1.65
Net energy for gain, Mcal/kg[3]	1.04
Crude protein, %DM	12.87
Ash, %DM	4.97
Ether extract, %DM	2.60
Neutral detergent fiber, %DM	23.45
Acid detergent fiber, %DM	19.86

[1] Mineral and vitamin supplement contained in treatments: MON = sodium monensin [3130 mg/kg dry matter (DM)]; MON+LC = sodium monensin + *Lithothamnium calcareum* (3130 mg/kg DM + 5 g/kg DM); BEO (0.3 g/kg DM). Sodium monensin (Rumensin) was obtained from Elanco Animal Health (Indiana, USA). *Lithothamnium calcareum* (LithoNutri®) was manufactured by OCEANA Minerals (Jundiaí, SP, Brazil). BEO (BIOPhytus®) was manufactured by ProPhytus Agroindustrial (São José dos Campos, SP, Brazil).

[2] Mineral and vitamin supplement was composed (DM basis) of 160 g/kg Ca, 131 g/kg P, 18 g/kg S, 82 mg/kg Co, 2283 mg/kg Cu, 15 mg/kg Cr, 2686 mg/kg Fe, 112 mg/kg I, 1940 mg/kg Mn, 22 mg/kg Se, 5417 mg/kg Zn, 1310 mg/kg F. Manufactured by DSM Nutritional Products, São Paulo, SP, Brazil.

[3] The net energy for maintenance and for gain were estimated with the equations proposed by NASEM (2016) with addition of ionophore from the sum of TDN values from each ingredient calculated using NRC (2001) according to the equations described by Weiss et al. (1992).

Table 2. Chemical composition (%DM) of the feed ingredients of experimental diet.

Item[1]	CP	Ash	EE	NDF	ADF	TDN[2]
Sugarcane bagasse	2.16	7.07	0.70	84.15	55.70	39.2
Ground corn	8.47	1.55	5.00	15.5	3.70	84.23
Citrus pulp	7.44	5.73	2.00	20.97	18.90	70.05
Soybean meal	51.17	7.07	2.20	26.35	11.16	71.57

[1] CP = crude protein; EE = ether extract; NDF = neutral detergent insoluble fiber; ADF = acid detergent insoluble fiber; TDN = total digestible nutrients.

[2] TDN values from each ingrediente were calculated using NRC (2001) according to the equations described by Weiss et al. (1992).

Table 3. Mean particle size and particle size distribution of the fine ground corn.

Item	
Retained/screen,%	
Sieve screen size,mm	
6.00	0.22
3.50	4.76
2.00	17.61
1.25	21.10
Pan	56.50
Mean particle size, mm[1,2]	1.42

[1] Mean particle size was calculated as the procedure described by Yu et al. (1998).

[2] Corn retained on the 6 mm screen was determined by measuring all the particles retained using a digital caliper. The residue retained in the bottom pan was assumed to have a mean particle size of 0.625 mm (Yu et al., 1998).

Table 4. Mean particle size and particle size distribution of the sugarcane bagasse.

Item	
Retained/screen,%	
Sieve screen size,mm	
19.0	0.15
8.00	2.86
4.00	23.45
Pan	73.55
Mean particle size, mm[1]	2.74

[1]Mean particle size was calculated according to the Penn State Particle Size Separator method as described by Lammers et al. (1996).

Table 5. Ingredients (%DM unless specified otherwise) of the diets in the step-up adaptation period (A1, A2, A3) and final diet.

Item	A1	A2	A3	Final
Sugarcane bagasse	25.00	20.00	15.00	8.50
Ground corn	33.20	35.70	38.70	42.20
Citrus pulp	33.20	35.70	38.70	41.70
Soybean meal	6.00	6.00	5.00	5.00
Urea	1.30	1.30	1.30	1.30
Minerals and vitamin supplement[1,2]	0.95	0.95	0.95	0.95
Sodium chloride	0.35	0.35	0.35	0.35

[1] Mineral and vitamin supplement contained in treatments: MON = sodium monensin [3130 mg/kg dry matter (DM)]; MON+LC = sodium monensin + *Lithothamnium calcareum* (3130 mg/kg DM + 5 g/kg DM); BEO (0.3 g/kg DM). Sodium monensin (Rumensin) was obtained from Elanco Animal Health (Indiana, USA). *Lithothamnium calcareum* (LithoNutri®) was manufactured by OCEANA Minerals (Jundiaí, SP, Brazil). BEO (BIOPhytus®) was manufactured by ProPhytus Agroindustrial (São José dos Campos, SP, Brazil).

[2] Mineral and vitamin supplement was composed (DM basis) of 160 g/kg Ca, 131 g/kg P, 18 g/kg S, 82 mg/kg Co, 2283 mg/kg Cu, 15 mg/kg Cr, 2686 mg/kg Fe, 112 mg/kg I, 1940 mg/kg Mn, 22 mg/kg Se, 5417 mg/kg Zn, 1310 mg/kg F. Manufactured by DSM Nutritional Products, São Paulo, SP, Brazil.

Table 6. Calcium (Ca) and Phosphorus (P) daily intake and Ca:P ratios in the experimental diets.

Item	MON	MON+ LC	BEO
DMI, kg/d	8,641	8,126	9,255
Dietary amount of Ca[1], g/d	84,54	79	90,54
Dietary amount of P[2], g/d	21,27	20	22,78
LitoNutri® amount of Ca[3], g/d	0	13	0
Ca:P ratio	3.9:1	4.6:1	3.9:1

[1,2] Calculated from concentrations of Ca and P in the feed ingredients as adopted by NASEM (2016) and concentrations in the mineral and vitamin supplement [composed (DM basis) of 160 g/kg Ca, 131 g/kg P, 18 g/kg S, 82 mg/kg Co, 2283 mg/kg Cu, 15 mg/kg Cr, 2686 mg/kg Fe, 112 mg/kg I, 1940 mg/kg Mn, 22 mg/kg Se, 5417 mg/kg Zn, 1310 mg/kg F. Manufactured by DSM Nutritional Products, São Paulo, SP, Brazil].

[3] Calculated according to the amount of *Lithothamnium calcareum* added in the diet (5 g/kg DM) and its Ca concentration (320 g Ca/kg). The *Lithothamnium calcareum* (LithoNutri®) was from OCEANA Minerals (Jundiaí, SP, Brazil).

Table 7. Effect of breed, feed additives and its interactions on performance and carcass characteristics of bulls finished on feedlot.

Item[3]	Breed[1]		Additive[2]			SEM	P-value		
	NEL	CROSS	MON	MON+LC	BEO		Breed	Additive	B x A
Animal performance									
Initial BW, kg	393.83	406.39	400.08	400.17	400.08	14.51	<0.001	0.996	0.996
Final BW, kg	525.94	568.83	552.67	540.33	549.17	11.71	<0.001	0.238	0.671
ADG, kg/d	1.122	1.329	1.309	1.189	1.275	0.042	<0.001	0.177	0.709
DMI, kg/d	7.878	9.470	8.641[ab]	8.127[b]	9.255[a]	0.292	<0.001	0.030	0.102
G:F	0.140	0.148	0.151[a]	0.142[ab]	0.138[b]	0.004	0.097	0.048	0.407
Carcass traits									
HCW, kg	296.17	319.83	311.17	303.25	309.59	7.280	<0.001	0.252	0.460
DP, %	56.3	56.2	56.3	56.1	56.3	0.004	0.818	0.826	0.219

[1] NEL = Nellore bulls; CROSS = Crossbred bulls.

[2] MON = sodium monensin [31.3 mg/kg dry matter (DM)]; MON+LC = sodium monensin (31.3 mg/kg DM) + *Lithothamnium calcareum* (5g/kg DM); BEO = blend of essential oils (0.3g/kg DM). Sodium monensin (Rumensin) was obtained from Elanco Animal Health (Indiana, USA). The *Lithothamnium calcareum* (LithoNutri®) was from OCEANA Minerals (Jundiaí, SP, Brazil). The blend of essential (BIOPhytus®) oils was from ProPhytus Agroindustrial (São José dos Campos, SP, Brazil).

[3] BW = body weight; ADG = average daily gain; DMI = dry matter intake; G:F = feed utilization efficiency; HCW = hot carcass weight, DP = dressing percentage

[a, b] Row means that do not have common superscript letter are different ($P \leq 0.05$).

Table 8. Effect of breed, feed additives and its interactions on observed dietary net energy concentration.

Item	Breed[1]		Additive[1]			SEM	P-Value		
	NEL	CROSS	MON	MON+LC	BEO		Breed	Additive	B X A
Observed NE[2]									
Maintenance	1.999	2.220	2.182[a]	2.125[ab]	2.020[b]	0.032	<0.001	0.007	0.068
Gain	1.343	1.527	1.490[a]	1.454[ab]	1.362[b]	0.027	<0.001	0.012	0.135
Observed:Expected NE ratios[3]									
Maintenance	1.212	1.345	1.323[a]	1.288[ab]	1.224[b]	0.019	<0.001	0.007	0.069
Gain	1.292	1.469	1.432[a]	1.398[ab]	1.309[b]	0.026	<0.001	0.012	0.136
Observed:Expected NE ratios[4]									
Maintenance	1.098	1.220	1.199[a]	1.168[ab]	1.110[b]	0.017	<0.001	0.007	0.069
Gain	1.129	1.283	1.252[a]	1.222[ab]	1.144[b]	0.023	<0.001	0.012	0.136

[1] NEL = Nellore bulls; CROSS = Crossbred bulls

[2] MON = sodium monensin [31.3 mg/kg dry matter (DM)]; MON+LC = sodium monensin (31.3 mg/kg DM) + *Lithothamnium calcareum* (5g/kg DM); BEO = blend of essential oils (0.3g/kg DM). Sodium monensin (Rumensin) was obtained from Elanco Animal Health (Indiana, USA). The *Lithothamnium calcareum* (LithoNutri®) was from OCEANA Minerals (Jundiaí, SP, Brazil). The blend of essential (BIOPhytus®) oils was from ProPhytus Agroindustrial (São José dos Campos, SP, Brazil).

[2] Calculated according to equations described by Zinn and Shen (1998).

[3] The expected net energies were calculated according to NASEM (2016), with addition of ionophore, based on the total digestible nutrient values of each ingredient (Weiss et al., 1992).

[4] The expected net energies were calculated according to NASEM (2016), with addition of ionophore, based on the NASEM (2016) tabular values of total digestible nutrient values of each ingredient.

[a, b] Row means that do not have common superscript letter are different ($P \leq 0.05$).