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Effect of the addition of essential oils, enzymes, ionophores and combinations of feed additives in finishing cattle diets

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Thesis presented to obtain the degree of Doctor 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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SUMÁRIO

RESUMO

Efeito da adição de óleos essências, enzimas, ionóforos e combinações de aditivos em dietas para bovinos em terminação

Independente do tipo de dieta utilizada, a busca por aumento da digestão dos nutrientes tem sido alvo da maior parte das pesquisas realizadas no mundo. O objetivo dessa tese foi avaliar os efeitos da inclusão de óleos essenciais, enzimas, ionóforos e combinações de aditivos na performance animal, saude ruminal e digestibilidade aparente em bovinos de corte da raça Nelore confinados alimentados com dietas de terminação com alto concentrado. O estudo foi composto por dois experimentos de desempenho e dois de metabolismo. No Capitulo 1 dessa tese 2 experimentos foram conduzidos para avaliar o desempenho de bovinos terminados em confinamento recebendo dietas que continham óleos essências e enzimas exógenas. Os tratamentos em cada experimento consistiram em: MON (monensina sódica, 26 mg/Kg); BEO (blend de óleos essênciais – 90 mg/kg); BEO+MON; BEO+AM (blend de óleos essenciais mais α-amilase - 90 mg/kg + 560 mg/kg, respectivamente) e BEO+AM+PRO (óleos essenciais mais α-amilase mais protease (90 mg/kg + 560 mg/kg + 840 mg/kg, respectivamente). No Exp. 1 foram utilizados 300 animais Nelore distribuídos aleatoriamente em 60 baias. Comparado com MON, a combinação BEO+AM resultou em maior CMS, maior GPD e proporcionou 12 kg a mais de carcaça, sem alterar a eficiência alimentar. BEO+MON e BEO não proporcionaram melhora no desempenho, quando comparada com MON. No Exp. 2, 5 animais canulados no rúmen foram usados para avaliar, CMS, digestibilidade aparente dos nutrientes e parâmetros ruminais em um quadrado latino 5 x 5. BEO aumentou a digestibilidade no trato total da PB comparada com MON. Os consumos de todos nutrientes, com exceção de EE foram maiores para animais alimentados com BEO+AM, quando comparados com MON, sem diferenças na digestibilidade no trato total. Em resumo, BEO aumenta o CMS de animais confinados em relação a MON, sem alterar a eficiência alimentar. Um sinergismo entre BEO e AM foi observado, resultando em aumento da produção de carcaça. No Capitulo 2, avaliou-se o desempenho e respostas metabolicas de animais confinados recebendo dietas com alto teor de milho (82.5% da MS), processado em moido (1,66 mm; MG) e moido grosso (2,12 mm; CG), combinado com monensina (monensina sódica, 26 mg/Kg; MON) ou com blend de óleos essenciais mais α-amilase (90 mg/kg + 560 mg/kg, respectivamente; BEO+MON). No Exp. 1, 256 animais Nelore foram blocados em 48 baias, as quais foram distribuidas aleatoriamente a um arranajo fatorial 2 x 2 nos tratamentos por 99 dias. Animais alimentados com BEO+MON apresentaram maior CMS do que animais alimentados com MON. Foi observada interação entre processamento e aditivo para PCQ, na qual animal alimentados com BEO+MON e CG obtiveram 11 kg a mais. No Exp. 2, quatro animais canulados no rúmen foram assimilados as mesmos tratamentos do Exp. 1, em um quadrado latino 4 x 4. Efeito de processamento foi detectado no consumo de MS, PB, FDN, CNF, amido e NDT, os quais foram maiores para animais alimentados com milho CG do que MG. Efeito de aditivo foi observado na concentração de NH_3-N ruminal, a qual foi menor para animais alimentados com BEO+AM comparado a animais que receberam MON. Em suma, reduzir o tamanho de particula de 2,12 mm para 1,66 mm não melhorou o desempenho e os parametros de fermentação ruminal. A suplementação com BEO+AM resulta em maior produção de carcaça comparada com monensina, quando incluida em dietas com milho moido grosseiramente.

Palavras-chave: Milho; Aditivos; Confinamento; Amido

ABSTRACT

Effect of the addition of essential oils, enzymes, ionophores and combinations of feed additives in finishing cattle diets

Regardless of the type of diet used, the search for increased digestion of nutrients has been the target of most research worldwide. The objective of this thesis was to evaluate the effects of the inclusion of essential oils, enzymes, ionophores and combinations of additives in animal performance, ruminal health and apparent digestibility in Nellore cattle fed with high concentrate diets. The study was composed of two performance experiments and two of metabolism In Chapter 1 of this thesis 2 experiments were conducted to evaluate the performance responses of finishing feedlot cattle receiving diets containing essential oils and exogenous enzymes. The treatments in each experiment consisted of (dry matter basis): MON - sodium monensin (26 mg/kg); BEO - a blend of essential oils (90 mg/kg); BEO+MON - a blend of essential oils plus monensin (90 mg/kg + 26 mg/kg, respectively); BEO+AM - a blend of essential oils plus exogenous α-amylase (90 mg/kg + 560 mg/kg, respectively); and BEO+AM+PRO - a blend of essential oils plus exogenous α-amylase and exogenous protease (90 mg/kg + 560 mg/kg + 840, mg/kg respectively). Exp. 1 consisted of a 93-d finishing period using 300 Nellore bulls in a randomized complete block design. Compared with MON, the combination of BEO+AM resulted in greater dry matter intake, greater average daily gain, 12 kg heavier hot carcass weight, although feed efficiency was not significantly different between BEO+AM and MON. BEO+MON and BEO did not improve performance when compared to MON. In Exp. 2, five ruminally cannulated Nellore steers were used to evaluate intake, apparent total tract digestibility of nutrients, and ruminal parameters in a 5 × 5 Latin Square design. Intakes of all nutrients measured, except for EE were greater in animals fed BEO+AM when compared with MON, with no differences on total tract nutrient digestibilities between these two treatments. Feeding BEO increased the total tract digestibility of CP compared to MON. In summary, diets containing the BEO used herein enhanced dry matter intake of growing-finishing feedlot cattle compared with a basal diet containing MON without impair feed efficiency. A synergism between BEO and AM was detected, resulting in incresed carcass production. This study evaluated the growth performance and digestion responses of finishing feedlot beef cattle fed high-concentrate diets containing 82.5% flint corn (DM basis) ground to medium (1.66 mm; MG) or coarse particle sizes (2.12 mm; CG), added with monensin (26 mg/kg; DM basis; MON) or a blend of essential oils + exogenous α-amylase (90 mg/kg + 560 mg/kg commercial product, respectively, DM basis; BEO+AM). In Exp. 1, 256 Nellore bulls were blocked by initial body weight (360 ± 12 kg), assigned to 48 pens and pens within blocks were randomly assigned, in a 2 × 2 factorial arrangement, to treatments during 99 d. Feed additive effect was detected for DMI, which was greater for bulls fed BEO+AM vs. MON. The HCW was 11 kg heavier for bulls fed BEO+AM vs. MON in diets containing CG, but not MG particle size. In Exp. 2, four ruminally cannulated Nellore steers were offered the same treatments of Exp. 1, in a 4 × 4 Latin Square design, to evaluate intake, apparent total tract digestibility of diets and ruminal fermentation parameters. Effect of corn particle size was detected for intake of DM, CP, NDF, NFC, starch and TDN which were greater for steers fed CG than steers fed MG corn. Feed additive affected (P = 0.02) ruminal NH3-N concentration, which was less for steers fed BEO+AM compared to MON. In summary, reducing flint corn particle size from 2.12 to 1.66 mm in finishing diets failed to improve cattle growth performance, digestibility of most nutrients and ruminal fermentation characteristics. A blend of essential oils associated with exogenous α-amylase resulted in the heavier carcass weights compared to monensin supplementation when included in diets containing coarse ground corn.

Keywords: Corn; Degradability; Feed additives; Feedlot; Starch

1 INTRODUCTION

Regardless of the type of diet used, the search for increased digestion of nutrients has been the target of most research worldwide. Some nutrients such as fiber still significantly restrict the increase in animal performance, due to limitations in cell wall degradation in the rumen. However, components of the diet such as starch, which despite being almost fully digested in the total tract of ruminants, can increase animal performance when their digestion is increased.

Diets with high grain contents provide faster weight gain, better feed conversion, and better carcass characteristics, which can make the activity more profitable (PRESTON, 1998; SANTOS et al., 2004; NUNEZ, 2008). However, this type of diet can also provide risks to animals, such as the incidence of metabolic disorders, jeopardizing their performance. As an alternative to mitigate these risks and provide good results, feed additives can be added to ruminant diets. Among them, ionophores, non-ionophores, probiotics, essential oils and enzymes.

Monensin (MON) is the most commonly used feed additive in finishing diets for ruminants (Samuelson et al. 2016); it alters ruminal fermentation (Butaye et al., 2003) and improves feed efficiency [(G:F); Ellis et al., 2012]. Alternative feed compounds such as essential oils and their blends (BEO) have been evaluated as novel feed additives that could alter patterns of ruminal fermentation to enhance animal performance and might help allay some of the increasing public concern about antibiotic residues and antimicrobial resistance (Tassoul and Shaver, 2009; Khiaosa-ard and Zebeli, 2013).

Feed additives act in the modification of the ruminal microbial population by selection of gram-negative bacteria and inhibition of the gram-positive, creating as advantages the improvement of the energy and protein metabolism efficiency, as well as reduction in the incidence of digestive disorders (BERGEN and BATES, 1984).

The use of exogenous enzymes, i.e. not produced by the animal but added to the diet, had its studies started in the 60's, demonstrating potential for use in the diet of ruminants. In that decade, Clark et al. (1961) reported that supplementation with exogenous enzyme raised the average daily gain of the animals by about 20%.

Exogenous amylase (AM) has been proposed to improve animal performance by increasing nutrient utilization. Some studies with AM supplementation to lactating dairy cows have reported increased milk production, improved energy balance, enhanced conversion of feed to milk, and increased ruminal starch digestion (Tricarico et al. 2005; DeFrain et al.

2005; Gencoglu et al., 2010; Klingerman et al. 2009; Nozière et al. 2013; Andreazzi et al., 2018). Because the value of supplementing AM to diets for finishing beef cattle has not been studied extensively, it deserves further investigation (Tricarico et al. 2007; DiLorenzo et al. 2010).

An increase in the particle size when corn is coarsely ground or in the degree of grain vitreousness have been correlated with reduced starch availability both in the rumen (Philippeau and Michalet-Doreau, 1998; Correa et al. 2002) and total digestive tract (Corona et al. 2006). Therefore, greater benefit from AM supplementation may be expected from diets where corn grain is less extensively processed, such as coarsely ground corn, as well as in diets containing strains of dry rolled corn grain that are more vitreous, including flint corn hybrids as used extensively in South America (Correa et al. 2002; Gouvêa, et al. 2016; Marques et al., 2016).

Even though we consider the published literature reviews, which point to positive effects on the use of exogenous enzymes in the ruminant diet (Krause et al., 2003; Beauchemin et al., 2004; Beauchemin and Holtshausen, 2011; Tricarico et al., 2008), some authors (Meale et al., 2014, McAllister et al., 1999, Beauchemin et al., 1997) report inconsistencies in published results and attributed this inconsistency to variations related to the use of different diets with different nutritional compositions, enzyme application method, stability and enzyme activity.

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2 FEEDING THE COMBINATION OF ESSENTIAL OILS AND EXOGENOUS α-AMYLASE INCREASES PERFORMANCE AND CARCASS PRODUCTION OF FINISHING BEEF CATTLE[1]

Abstract

Two experiments were conducted to evaluate the performance responses of finishing feedlot cattle to dietary addition of essential oils and exogenous enzymes. The treatments in each experiment consisted of (dry matter basis): MON - sodium monensin (26 mg/kg); BEO - a blend of essential oils (90 mg/kg); BEO+MON - a blend of essential oils plus monensin (90 mg/kg + 26 mg/kg, respectively); BEO+AM - a blend of essential oils plus exogenous α-amylase (90 mg/kg + 560 mg/kg, respectively); and BEO+AM+PRO - a blend of essential oils plus exogenous α-amylase and exogenous protease (90 mg/kg + 560 mg/kg + 840, mg/kg respectively). Exp. 1 consisted of a 93-d finishing period using 300 Nellore bulls in a randomized complete block design. Animals fed BEO had higher dry matter intake ($P < 0.001$) but similar feed efficiency to animals fed MON ($P \geq 0.98$). Compared with MON, the combination of BEO+AM resulted in 810 g greater dry matter intake ($P = 0.001$), 190 g greater average daily gain ($P = 0.04$), 18 kg heavier final body weight ($P = 0.04$) and 12 kg heavier hot carcass weight ($P = 0.02$), although feed efficiency was not significantly different between BEO+AM and MON ($P = 0.89$). Combining BEO+MON tended to decrease hot carcass weight compared with BEO alone ($P = 0.08$) but not compared with MON ($P = 0.98$). Treatments did not impact observed dietary net energy values ($P \geq 0.74$) or the observed:expected net energy ratio ($P \geq 0.11$). In Exp. 2, five ruminally cannulated Nellore steers were used to evaluate intake, apparent total tract digestibility of nutrients, and ruminal parameters in a 5 × 5 Latin Square design. Feeding BEO increased the total tract digestibility of CP compared to MON ($P = 0.03$). Compared to MON, feeding the combination of BEO+MON increased the intake of CP ($P = 0.04$) and NDF ($P = 0.05$), with no effects on total tract digestibility of nutrients ($P \geq 0.56$), except for a tendency ($P = 0.09$) to increase CP digestibility. Intakes of all nutrients measured, except for EE ($P = 0.16$) were greater in animals fed BEO+AM when compared with MON ($P \leq 0.03$), with no differences on total tract nutrient digestibilities ($P \geq 0.11$) between these two treatments. In summary, diets containing the BEO used herein enhanced dry matter intake of growing-finishing feedlot cattle compared with a basal diet containing MON without impair feed efficiency. A synergism between BEO and AM was detected, further increasing cattle performance and carcass production compared to MON.

Keywords: Corn; Degradability; Feed additives; Feedlot; Starch

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2.1 Introduction

Monensin (**MON**) is the most commonly used feed additive in finishing diets for ruminants (Samuelson et al. 2016); it alters ruminal fermentation (Butaye et al., 2003) and improves feed efficiency [(**G:F)**; Ellis et al., 2012]. Alternative feed compounds such as essential oils and their blends (**BEO**) have been evaluated as novel feed additives that could alter patterns of ruminal fermentation to enhance animal performance and might help allay some of the increasing public concern about antibiotic residues and antimicrobial resistance (Tassoul and Shaver, 2009; Khiaosa-ard and Zebeli, 2013).

Exogenous amylase (**AM**) has been proposed to improve animal performance by increasing nutrient utilization. Some studies with AM supplementation to lactating dairy cows have reported increased milk production, improved energy balance, enhanced conversion of feed to milk, and increased ruminal starch digestion (Tricarico et al. 2005; DeFrain et al. 2005; Gencoglu et al., 2009; Klingerman et al. 2009; Nozière et al. 2013; Andreazzi et al., 2018). Because the value of supplementing AM to diets for finishing beef cattle has not been studied extensively, it deserves further investigation (Tricarico et al. 2007; DiLorenzo et al. 2010). An increase in the particle size when corn is coarsely ground or in the degree of grain vitreousness have been correlated with reduced starch availability both in the rumen (Philippeau and Michalet-Doreau, 1998; Correa et al. 2002) and total digestive tract (Corona et al. 2006). Therefore, greater benefit from AM supplementation may be expected from diets where corn grain is less extensively processed, such as coarsely ground corn, as well as in diets containing strains of dry rolled corn grain that are more vitreous, including flint corn hybrids as used extensively in South America (Correa et al. 2002; Gouvêa, et al. 2016; Marques et al., 2016).

Supplementing exogenous proteases (**PRO**) may also increase utilization of nutrients from finishing diets containing grains with more vitreous starch, given that the hygroscopic protein matrix of the vitreous corn endosperm prevents activity of digestive enzymes from rumen microbes, particularly in flint corn that has a higher proportion of vitreous starch than dent corn (McAllister and Ribeiro, 2013). Yet, information regarding the supplementation of finishing beef diets with various proteases is limited.

Based on the aforementioned information and rationale, it was hypothesized that BEO should be an alternative to MON in finishing feedlot diets. We also hypothesized that combining BEO with exogenous enzymes (AM or PRO) would further improve nutrient utilization and consequently the cattle performance. Therefore, two experiments were

conducted to examine performance and diet digestibility effects of including BEO, with or without addition of an exogenous AM or PRO, in finishing diets compared with inclusion of MON.

2.2 Materials and methods

Our studies were 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 **2015-29**.

2.2.1 Experiment 1 Animal Performance

Animals, Housing, and Experimental Procedures. Three hundred finishing Nellore bulls [initial body weight (**BW**) = 330 ± 33 kg] in a randomized complete block design experiment were used to evaluate the effects of selected feed additives and exogenous enzymes on animal performance and carcass characteristics.

At the start of the feeding trial, animals were weighed individually after 16 h of feed and water deprivation, identified with ears tags, vaccinated against clostridiosis (Sintoxan Polyvalente, Merial Saúde Animal Ltda, Paulínia, Brazil), and dewormed with 1 ml per 50 kg BW of 3.15% ivermectin (Ivomec Gold; Merial Brazil Saúde Animal Ltda). Bulls were blocked by initial BW into 10 weight blocks. Pens within each block were allocated randomly to 1 of 5 treatments (**MON, BEO, BEO+MON, BEO+AM and BEO+AM+PRO**). Animals were housed in 50 feedlot pens: 25 were partially roofed with concrete-floors (32 m^2) where each pen held 5 animals, 15 that had no roof but soil floor (84 m^2) where each pen held 7 animals, and 10 were partially roofed with soil floor (84 m^2) where each pen held 7 animals. Treatments were equally replicated within each pen type. All animals had free access to fresh water during the feeding experiment.

Treatments consisted of a basal diet fed as total mixed ration (**TMR**; Table 1) with addition of either a single feed additive or a combination of additives and enzymes on the diet dry matter (**DM**) basis. Animals in the control group (MON) were fed sodium monensin as their only feed additive (26 mg/kg DM; RUMENSIN, Elanco Animal Health, Indianapolis, IN).

The other treatments included: a blend of essential oils (BEO - 90 mg/kg DM); a blend of essential oils plus monensin (BEO+MON - 90 mg/kg DM and 26 mg/kg diet DM, respectively); a blend of the essential oils plus an exogenous α-amylase (BEO+AM – 90 mg/kg DM and 560 mg/kg diet DM, respectively); or a blend of essential oils plus an exogenous α-amylase and an exogenous protease (BEO+AM+PRO - 90 mg/kg DM, 560 mg/kg DM and 840 mg/kg diet DM, respectively). The blend of essential oils (CRINA RUMINANTS; DSM Nutritional Products, Basel, Switzerland) that was used contained thymol, eugenol, limonene and vanillin on an organic carrier (McIntoch et al., 2003). The exogenous enzymes produced by *Bacillus licheniformis* [α-amylase (RONOZYME RUMISTAR) and protease (RONOZYME PROACT)] were also provided by DSM Nutritional Products. RONOZYME RUMISTAR, a granular amylase formulation with an amylase activity of 600 Kilo Novo units (**KNU**) per g, was added to the appropriate TMR to achieve 336 KNU of amylase activity/kg of DM. One KNU is defined as the amount of enzyme that releases, in a two-step reaction, 6 μmol of p-nitrophenol per min from 1.86 mM 4.6-thylidene-G7-pnitrophenyl-maltoheptaoside at pH 7.0 and 37°C (Jung and Vogel, 2008). This dosage level was based on dairy trials that had demonstrated its efficacy (Klingerman et al., 2009; Gencoglu et al., 2010; Andreazzi et al., 2018). RONOZYME PROACT, a granular serine protease with a measured activity of 75,000 PRO/g, when added to the TMR provided 63,000 of protease activity/kg of DM. One PRO is defined as the amount of enzyme that releases 1 μmol of p-nitroaniline from 1 μM of substrate (Suc-Ala-Ala-Pro-Phe-p-nitroaniline) per min at pH 9.0 and 37°C (Guggenbuhl et al. 2015). Although the optimum dosage of RONOZYME PROACT has not been established for ruminants, the dosage used in this trial provided the same ratio of amylase to protease as has been used in broilers diets (Angel et al., 2011, Stefanello et al., 2015).

To provide the desired dietary concentrations of the feed additives and enzymes, these ingredients were incorporated into the mineral-vitamin supplement that was included as 3% of the dietary DM (Table 1). These mineral-vitamin supplements with the appropriate additives and enzymes were produced at a commercial feed mill following all the manufacturing standards for quality and guaranteed levels (DSM Nutritional Products Brazil S.A, Mairinque, SP, Brazil).

The total feeding period lasted 93-d. The initial 15-d of the trial consisted of an adaptation period to the diets, with bagasse replacing 25%, 20%, and 15% of cracked corn (DM basis) during sequential 5-d periods, respectively. From d 16 to 93, all animals received their final diet containing 8.5% sugarcane bagasse with 92.5% concentrate that was formulated to meet the nutrient requirements specified by NRC (1996) and to contain equal

concentrations of crude protein [(**CP**); Table 1]. Feed additives and exogenous enzymes were included in the TMR beginning on the first day of the experiment and throughout the entire feeding period. The flint corn grain was processed through a hammer mill (Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil) to achieve a mean particles size of 2.04 mm (Table 2) as assayed by the procedure of Yu et al. (1998), using sieves with 6.0, 3.5, 2.0, 1.25 mm square pores (Produtest T Model; Telastem Peneiras para Análises Ltda., São Paulo, SP, Brazil).

Table 1. Ingredient and chemical composition of diet (dry matter basis)

Item	
Ingredients, %	
Sugarcane bagasse	8.50
Ground flint corn	82.5
Soybean meal	5.00
Urea	1.00
Mineral and vitamin supplement[1, 2]	3.00
Chemical composition, %	
Dry matter, as fed	69.2
Crude protein	14.0
Neutral detergent fiber	19.6
Acid detergent fiber	8.16
Ether extract	3.18
Starch	55.0
Total digestible nutrients[3]	76.0
Net energy of maintenance[4], Mcal/kg	2.01
Net energy of gain[4], Mcal/kg	1.37

[1] Mineral and vitamin supplement containing dietary treatments: MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO = blend of essential oils (90 mg/kg DM); BEO+MON = blend of essential oils + monensin (90 and 26 mg/kg DM respectively); BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg/kg DM respectively); BEO+AM+PRO = blend of essential oils + exogenous α-amylase + exogenous protease (90, 560 and 840 mg/kg DM respectively).

[2] Mineral and vitamin supplement was composed (DM basis) of 140 g/kg Ca, 16 g/kg P, 36 g/kg S, 20 g/kg Mg, 34 g/kg K, 56 g/kg Na, 8 mg/kg Co, 540 mg/kg Cu, 6.7 mg/kg Cr, 27.5 mg/kg I, 1070 mg/kg Mn, 6.7 mg/kg Se, 2000 mg/kg Zn, 16,8000 IU/kg vitamin A, 17000 IU/kg vitamin D_3, 1740 IUI/kg vitamin E, 90 mg/kg biotin, 2.7 × 10^9 CFU/kg *Saccharomyces cerevisiae*. Manufactured by DSM Nutritional Products, São Paulo, Brazil.

[3] Total digestible nutrients (TDN) were estimated from equations described by Weiss et al. (1992) assuming processing adjustment factor of 1.00 for flint ground corn (NRC, 2001).

[4] Net energy of maintenance (NEm) and gain (NEg) were estimated with the equations proposed by NASCEM (2016; empirical model) with addition of ionophore.

Each treatment diet was mixed individually using a feed wagon (Siltomac S-2.3; Indústria de Implementos Agrícolas Siltomac Ltda., São Carlos, SP, Brazil), weighed into 50 kg nylon bags using a fixed scale (Weightech WT1000, Weightech Equipamentos de Pesagem, Florianópolis, SC, Brazil), and delivered manually to each pen once daily at 0800. The feed wagon was carefully emptied and cleaned after delivering each ration mixture to avoid cross-contamination between treatments. Feed bunks were evaluated visually each day and managed for a maximum of 3% orts. For diet DM adjustment, samples of sugarcane

bagasse were collected once each week and dried at 105ºC for 24-h. Orts were removed twice weekly, weighed, sampled and discarded. Feed and orts samples were dried at 105ºC for 24-h to determine DM and calculate DM intake. On d 27, animals were weighed without fasting with live full BW being discounted by 4% (NASCEM, 2016) to calculate shrunk weight. Shrunk BW (after 16 h of feed and water deprivation) was recorded again on d 93. Dry matter intake (**DMI**), average daily gain (**ADG**) and feed efficiency (**G:F**; calculated as the ratio of ADG to DMI) were calculated for each period evaluated.

Table 2. Corn grain particle size distribution

Pores in the sieve	% of total
> 6.0 mm	0.44
≤ 6.0 and > 3.5 mm	6.56
≤ 3.5 and > 2.0 mm	31.4
≤ 2.0 and > 1.25 mm	43.4
≤ 1.25 mm	18.2
Mean particle diameter of corn, mm[1]	2.04

[1] Corn retained on the 6 mm screen was determined in 20 randomly particles using a digital caliper. The residue retained in the bottom was assumed to have a mean particle size of 6.25 mm. Based on Yu et al. (1998).

Individual fecal grab samples were obtained from the rectum of each bull on d 70 of the trial and immediately frozen (-20°C) for further fecal starch analyses. Upon BW assessment on d 93, animals were transported (8.4 km) to a commercial packing plant (Friuna Alimentos Ltda, Piracicaba, Brazil). Hot carcasses weight (**HCW**) were collected following kidney and heart and pelvic fat removal. Dressing percent was calculated as the ratio of HCW to final shrunk BW. Subcutaneous fat thickness and longissimus muscle (**LM**) area were measured at the 12th rib from each carcass after a 24-h chill at 2°C, using a numbered grid and a digital caliper, respectively.

2.2.2 Feed Analysis and Calculations.

Samples of each ingredient were collected every 10 days and stored at -20°C. At the end of the trial, samples were thawed, composited for each trial 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, 1986), ash (method 942.05; AOAC, 1986), ether extract [(EE); method 920.85; AOAC, 1986], 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 CP content was calculated by multiplying nitrogen content by 6.25. Corn and fecal grab samples were analyzed for starch using a Total Starch K-TSTA KIT (Megazyme, Chicago IL, USA; method 996.11; AOAC, 1986 and method 76-13.01; AACC, 1976). The total digestible nutrients (TDN) values for each diet were estimated according to Weiss et al. (1992) using a processing factor of 1.00 for ground corn (NRC, 2001). Corn net energy content for maintenance (NEm) and gain (NEg) were estimated from the fecal starch concentration using the equation: NEm = 2.49 − 0.0127 × FS − 0.000292 × FS2, and NEg = 0.877 × NEm − 0.41 (Zinn et al., 2007), where FS is fecal starch expressed as a percentage of fecal DM. Total tract starch (TSD) digestion was calculated from fecal starch content according to Zinn et al. (2002). Net energy concentrations of each diet were calculated according to Zinn and Shen (1998) using mean values for shrunk BW, DMI, and ADG of the bulls in each pen. These calculated NE concentrations were compared with those predicted using the Weiss et al. (1992) equations for TDN that were converted to NE concentrations using equations from the NASCEM (2016) empirical model.

2.2.3 Experiment 2 Digestibility and Ruminal Fermentation

2.2.3.1 Animals, Housing, and Experimental Procedures.

Five ruminally cannulated Nellore steers (425 kg ± 55 kg) were assigned to the same diets and treatments described in Exp. 1 in a 5 x 5 Latin Square design, to evaluate intake, apparent total tract digestibility of nutrients, ruminal parameters, and rumen microbial protein synthesis. Animals were maintained in individual pens (32 m^2) with a solid roof and concrete floors and given free choice access to water during the experiment. Each period lasted 20-d, with 15-d for adaptation to diets and 5-d for sample and data collection. During the adaptation period, additional feed was provided once daily at 0800 in amounts that allowed 10% orts. During the collection period, to reduce the amount of orts, daily feed intake was restricted to 90% of the mean feed intake of that specific steer measured during the 5 last days of the adaptation period as described by Zinn (1990).

2.2.3.2 Sample Collection.

During the 5-d of collection, (d 16 to 20), total feces were collected to estimate fecal production. Animals were monitored every 3-h of each day to check if they had defecated. Feces were collected from the concrete floor, weighed, and sampled with samples frozen at -20ºC for later analysis of DM for calculating total fecal nutrient excretion. On d 18, d 19 and d 20 fecal samples also were collected manually directly from the rectum from each animal and frozen at -20ºC for analysis. These collections were staggered over sampling hours, in a manner that all collections represented one full day. More specifically, samples were taken at 1000 and 1600 on d 18, at 0600, 1400, and 1800 on d 19, and at 0800, 1200 and 2000 on d 20. These samples were composited by period and animal and analyzed for DM, CP, EE, aNDF, ADF, ash and fecal starch as described for Exp. 1. Non fibrous carbohydrate (**NFC**, %) was calculated as: NFC = 100 − (CP, % + aNDF, % + Ash, % + EE, %). Feed ingredients were collected every 10 days, as described for Exp. 1.

On d 16, approximately 50 mL of rumen fluid samples were obtained from each steer via the ruminal cannula at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h after feeding as described by Danes et al. (2013). Ruminal fluid samples were squeezed through 4 layers of cheesecloth with ruminal pH being measured immediately using a portable pH meter (Digimed Model DM22, Digicrom Analítica Ltda., São Paulo, SP, Brazil). Samples were preserved with 1 mL of 8.6 *M* H_2SO_4 solution and stored at -20°C. At the end of the experiment, ruminal fluid samples were thawed and centrifuged at 15.000 × *g* for 30 min at 4°C. The supernatant fluid was analyzed for volatile fatty acids (**VFA**) by gas-liquid chromatography (Palmquist and Conrad, 1971) and for ammonia nitrogen [(**NH_3-N)**; Chaney and Marbach, 1962]. Results from VFA, N-NH_3, and pH analyses were averaged across the 24-h collection, and used for statistical analyses. Spot samples of urine were spontaneously collected into sterile plastic cups on d 17 of each period, 4-h after the TMR was offered (Zanetti et al., 2017). When spontaneous urination didn't occur, animals were mild manually stimulated in the prepuce until urination. Urine was acidified [0.072 N H_2SO_4 at a ratio of 10 ml urine to 40 ml of acid; (Broderick et al., 2009)] and frozen at -20ºC for further analysis.

2.2.3.3 Microbial Protein Synthesis.

The concentrations of creatinine and uric acid in urine were determined using commercial kits (Bioclin - Belo Horizonte, MG, Brazil and CELM - Compania Equipadora de

Laboratórios Modernos, São Caetano do Sul, SP, Brazil) based on the enzymatic colorimetric method of kinetic endpoint, with readings from an automatic biochemistry analyzer (Automatic System of Biochemistry SBA-200 - CELM). Allantoin concentrations were determined by the method of Fujihara et al. (1987) as described by Chen and Gomes (1992). Total excretion of purine derivatives was determined as the sum of allantoin and uric acid excreted in the urine (mmol/d). The absorbed purines (**AP**, mmol/d) were calculated from excretion of purine derivatives (mmol/d) according to Verbic et al. (1990). Urine volume was estimated from the concentration of creatinine in the urine and its expected daily excretion per unit of body weight (Chizzotti et al., 2008).

Ruminal synthesis of nitrogenous compounds (**N mic**, g/d) was calculated based on the absorbed microbial purine (AP, mmol/d), according the equation proposed by Barbosa et al. (2011): N mic = (70 × AP) / (0.93 × 0.137 × 100), where 70 is the N content of purines (mg/mol of N), 0.137 is the ratio of purine N to total N in bacteria, and 0.93 is the assumed intestinal digestibility of microbial purines. Microbial nitrogen efficiency was calculated as the ratio of g microbial nitrogen to kg TDN ingested.

2.2.3.4 Total Digestible Nutrient Calculations

Six different TDN estimates for each of the five diets fed in the digestibility and performance trials were calculated to allow contrasts among TDN estimates from these various methods: 1) Classical: calculated on the basis of digested carbohydrate equivalent, assuming that CP has a value equal to that of carbohydrate: 2) Digested organic matter (**OM**): considering that 1 kg of TDN is obtained from 4.4 Mcal of OM digested; 3) Component kcal: assuming that digestible energy (**DE**) is considered to contain 9.37 kcal/g for digested fat, 5.63 kcal/g for digested protein, and 4.18 kcal/g for digested carbohydrates; 4) Ingredient composition: based on the equation of Weiss et al. (1992) using the analyzed ingredient composition; 5) NASCEM (2016): calculated based in the diet components from NASCEM (2016) for all ingredients except for sugarcane bagasse that was reported in BR-Corte (Valadares Filho et al., 2010); 6) Performance trial: based on NEm values calculated from DMI and ADG of bulls in the 93-d feeding trial based on animal performance (Zinn and Shen, 1998).

2.2.4 Statistical Analysis

In Exp. 1, performance data (DMI, initial BW, final BW, ADG based on measured or calculated shrunk weight, G:F and carcass traits) were analyzed using MIXED procedure of SAS software (SAS Inst. Inc., Cary, NC) as a randomized complete block design with pen as the experimental unit. The statistical model included the fixed effect of treatment and the random effect of weight block.

Data from Exp. 2 were also analyzed using the MIXED procedure of SAS. The statistical model used to analyze intake of nutrients, total tract apparent digestibility, ruminal fermentation parameters (pH, VFA, and NH_3-N), nitrogen metabolism and microbial protein synthesis included the fixed effect of treatment and the random effects of animal and period.

Data from all experiments are reported as least-square means. The Kenward-Roger approximation was used to determine the correct denominator degrees of freedom for testing fixed effects. When treatment effect was significant ($P \leq 0.05$) or tended ($P > 0.05$ and ≤ 0.10) to affect response variables, Tukey-Kramer was used to determine significant differences among means.

2.3 RESULTS

2.3.1 Animal Performance (Exp. 1)

Animal performance and carcass data are shown in Table 3. In all periods evaluated, animals fed BEO presented higher DMI (8.5 to 6.7%; $P \leq 0.001$) than MON, despite no differences in ADG ($P \geq 0.42$), G:F ($P \geq 0.97$) and HCW ($P = 0.26$) were observed between these two treatments. Feeding BEO+MON did not affect DMI ($P \geq 0.21$), ADG ($P \geq 0.91$), G:F ($P \geq 0.95$) and HCW ($P = 0.98$) compared to MON. When compared to BEO, feeding BEO+MON decreased DMI in each period ($P < 0.001$) and tended to decrease HCW ($P = 0.08$), nevertheless, no differences in ADG ($P \geq 0.32$) and in G:F ($P \geq 0.99$) were observed.

During the first 27-d of the feeding period, animals fed diets containing BEO+AM had 23% greater ADG ($P = 0.03$) than animals fed MON. This can be attributed at least partially, to 11.5% greater DMI ($P < 0.001$), whereas the G:F ratio was not different ($P = 0.66$) between these two treatments. The tendency ($P = 0.07$) for an increased BW (9 kg) at 27-d had increased to statistical significant 18 kg ($P = 0.04$) by the end of the 93-d trial between these two treatments.

Table 3. Effect of feed additives, exogenous enzymes and its combinations on performance and carcass characteristics of feedlot Nellore bulls (Exp. 1)

Item[2]	Treatments[1]					SEM	*P*-value
	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO		
Performance							
Initial BW, kg	331	331	331	331	331	10.9	-
Adj. BW d 27, kg	367^{ab}	373^{ab}	368^{ab}	376^{a}	365^{b}	10.6	0.005
Final BW, kg	476^{bc}	486^{ab}	474^{bc}	494^{a}	463^{c}	12.7	< 0.001
d 0 to 27							
DMI, kg	7.42^{b}	8.05^{a}	7.57^{b}	8.27^{a}	7.34^{b}	0.255	< 0.001
ADG, kg	1.35^{b}	1.54^{ab}	1.45^{ab}	1.66^{a}	1.26^{b}	0.084	0.002
G:F	0.183	0.192	0.193	0.201	0.173	0.012	0.30
d 28 to 93							
DMI, kg	9.12^{b}	9.73^{a}	8.94^{b}	9.96^{a}	8.91^{b}	0.280	< 0.001
ADG, kg	1.65^{ab}	1.72^{a}	1.60^{ab}	1.76^{a}	1.47^{b}	0.062	0.001
G:F	0.180	0.177	0.178	0.177	0.164	0.006	0.28
d 0 to 93							
DMI, kg	8.65^{c}	9.24^{b}	8.50^{c}	9.46^{a}	8.44^{c}	0.235	< 0.001
ADG, kg	1.57^{bc}	1.67^{ab}	1.54^{bc}	1.76^{a}	1.43^{c}	0.054	< 0.001
G:F	0.182^{ab}	0.182^{ab}	0.182^{ab}	0.186^{a}	0.169^{b}	0.006	0.04

Carcass characteristics							
HCW, kg	265^{bc}	272^{ab}	262^{bc}	277^{a}	257^{c}	8.00	< 0.001
Dressing, %	55.5	56.0	55.6	56.1	55.8	0.270	0.35
LM area, cm^2	67.9	68.0	68.1	69.6	63.1	1.75	0.08
12th-rib fat, mm	3.18	3.20	3.24	3.22	3.08	0.06	0.31

[1] MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO = blend of essential oils (90 mg/kg DM); BEO+MON = blend of essential oils + monensin (90 and 26 mg/kg DM respectively); BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg/kg DM respectively); BEO+AM+PRO = blend of essential oils + exogenous α-amylase + exogenous protease (90, 560 and 840 mg/kg DM respectively). Sodium monensin (RUMENSIN) was from Elanco Animal Health, Indianapolis, IN. The blend of essential oils (CRINA RUMINANTS), and the exogenous enzymes [α-amylase (RONOZYME RUMISTAR) and protease (RONOZYME PROACT)] were provided by DSM Nutritional Products, Basel, Switzerland.

[2] BW = body weight; Adj. BW = discounted by 4% from the full BW as ruminal fill; DMI = dry matter intake; ADG = average daily gain; G:F = feed efficiency; HCW = hot carcass weight; LM = longissimus muscle.

[abc] Row means that do not hae common superscript letter are different ($P < 0.05$)

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This BW difference can be ascribed to a continuation of an increased DMI (9.2%; $P < 0.001$) even though no significant differences in ADG (110 g/d; $P = 0.57$) and G:F ($P = 0.99$) between d 28 to 93 were observed. During the total feeding period (d 0 to 93) animals fed BEO+AM presented 9.3% higher DMI ($P < 0.001$), 12% higher ADG ($P = 0.04$), 12 kg higher HCW ($P = 0.02$) and the same feed efficiency ($P = 0.89$) compared to MON.

Compared to BEO, feeding BEO+AM tended to increase DMI from d 0 to 27 and from d 28 to 93 ($0.06 \leq P \leq 0.09$) and increased the DMI during the total feeding period (d 0 to 93; $P = 0.03$) despite no significant differences in ADG ($P \geq 0.68$), G:F ($P \geq 0.91$) or HCW ($P = 0.78$).

Compared to BEO+MON, feeding animals with BEO+AM resulted in greater DMI (9 to 11%; $P < 0.001$), greater ADG (14%; $P = 0.01$) in the 93-d feeding period and greater HCW (15 kg, $P = 0.002$) with no significant effect on G:F ($P = 0.93$).

Feeding BEO+AM+PRO decreased DMI (10 to 11%; $P < 0.001$) and ADG (16 to 24%; $P \leq 0.002$) in the various periods evaluated, but G:F was reduced only during the full trial (10%; $P = 0.02$) when compared with BEO+AM. Also, HCW was 20 kg lower ($P < 0.001$) for animals fed BEO+AM+PRO compared with BEO+AM.

Treatments tended to affect the LM area ($P = 0.08$). Animals fed BEO+AM+PRO had smaller LM area compared to BEO+AM ($P = 0.05$). Effects of treatments on dressing percentage and 12th-rib fat were not significant ($P \geq 0.31$).

Intakes of starch among diets paralleled intakes of DM as would be expected (Table 4). Animals fed BEO+AM had higher starch intake than other treatments ($P \leq 0.01$) whereas no differences were detected between animals fed BEO and BEO+AM ($P = 0.87$). Treatments tended ($P = 0.06$) to affect fecal starch concentration, TSD, and estimated net energy values of the corn grain based on fecal samples taken on d 70. Fecal starch concentration was 25.6% lower ($P = 0.04$) and total tract starch digestibility was 5.11% greater ($P = 0.04$) with BEO+AM than with MON. Estimated corn NE_m and corn NE_g also were 6.3 and 7.8% respectively greater ($P = 0.04$) with BEO+AM than with MON. Feeding animals with the combination of BEO+AM+PRO decreased starch intake ($P < 0.001$) but failed to decrease fecal starch ($P = 0.97$) or to increase total tract starch digestibility ($P = 0.98$) compared with animals fed BEO+AM.

Treatments did not impact observed NE values ($P \geq 0.74$; Table 5) or the observed:expected NE ratios ($P \geq 0.11$; Table 5) despite the greater ($P = 0.04$) corn energy values for BEO+AM compared with MON.

Table 4. Starch intake, fecal starch, total tract starch digestion and corn net energy estimates of feedlot Nellore bulls (Exp. 1)

Item	Treatments[1]					SEM	*P*-value
	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO		
Starch intake[2], kg	4.76^{bc}	5.08^{ab}	4.68^{c}	5.20^{a}	4.64^{c}	0.150	< 0.001
Fecal starch, %	21.5^{a}	18.1^{ab}	18.2^{ab}	16.0^{b}	17.1^{ab}	1.32	0.06
TSD[3], %	86.1^{b}	88.8^{ab}	88.8^{ab}	90.5^{a}	89.6^{ab}	1.04	0.06
Corn NE_m[4], Mcal/kg	2.08^{b}	2.16^{ab}	2.16^{ab}	2.21^{a}	2.18^{ab}	0.030	0.06
Corn NE_g[4], Mcal/kg	1.41^{b}	1.48^{ab}	1.48^{ab}	1.52^{a}	1.51^{ab}	0.027	0.06

[1] MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO = blend of essential oils (90 mg/kg DM); BEO+MON = blend of essential oils + monensin (90 and 26 mg/kg DM respectively); BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg/kg DM respectively); BEO+AM+PRO = blend of essential oils + exogenous α-amylase + exogenous protease (90, 560 and 840 mg/kg DM respectively). Sodium monensin (RUMENSIN 200) was from Elanco Animal Health, Indianapolis, IN. The blend of essential oils (CRINA RUMINANTS), and the exogenous enzymes [α-amylase (RONOZYME RUMISTAR) and protease (RONOZYME PROACT)] were provided by DSM Nutritional Products, Basel, Switzerland.

[2] Calculated using the starch content in the diet and dry matter intake data of each pen.

[3] TDS = total tract starch digestion estimated according Zinn et al. (2002).

[4] Estimated from the fecal starch concentration using the equation proposed by Zin et al. (2007).

[abc] Row means that do not have common superscript letter are different ($P < 0.05$).

Table 5. Effect of feed additives, exogenous enzymes and its combinations on observed dietary net energy concentration (Exp. 1)

Item	Treatments[1]					SEM	*P*-value
	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO		
Observed NE[2], Mcal/ kg							
Maintenance	2.00	2.00	2.01	2.02	1.97	0.025	0.74
Gain	1.34	1.35	1.35	1.36	1.32	0.022	0.74
Observed:Expected NE[3] ratio							
Maintenance	1.00	1.00	1.03	1.04	1.01	0.012	0.11
Gain	0.99	0.99	1.03	1.03	1.00	0.016	0.13

[1] MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO = blend of essential oils (90 mg/kg DM); BEO+MON = blend of essential oils + monensin (90 and 26 mg/kg DM respectively); BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg/kg DM respectively); BEO+AM+PRO = blend of essential oils + exogenous α-amylase + exogenous protease (90, 560 and 840 mg/kg DM respectively). Sodium monensin (RUMENSIN 200) was from Elanco Animal Health, Indianapolis, IN. The blend of essential oils (CRINA RUMINANTS), and the exogenous enzymes [α-amylase (RONOZYME RUMISTAR) and protease (RONOZYME PROACT)] were provided by DSM Nutritional Products, Basel, Switzerland.

[2] Calculated according Zinn and Shen (1998).

[3] Expected values were calculated using the NASCEM (2016), empirical model, based on the total digestible nutrients values (Weiss et al., 1992).

[abc] Row means that do not have common superscript letter are different ($P < 0.05$).

2.3.2 Digestibility and Ruminal Fermentation Responses (Exp. 2)

In the 5 × 5 Latin Square with 5 ruminally cannulated steers, no differences in the intake of nutrients were observed between steers fed BEO and MON ($P \geq 0.16$; Table 6). However, feeding BEO increased the total tract digestibility of CP compared to MON ($P = 0.03$). Compared to MON, feeding the combination of BEO+MON increased the intake of CP ($P = 0.04$) and NDF ($P = 0.05$), and tended to increase the intake of EE ($P = 0.07$) with no effects on total tract digestibility of nutrients ($P \geq 0.56$), except for a tendency ($P = 0.09$) to increase CP digestibility. However, compared to BEO, the combination of BEO+MON did not affect the intake and digestibility of nutrients ($P \geq 0.31$).

Intakes of all nutrients measured, except for EE ($P = 0.16$) were 22.9 to 36.5% greater in animals fed BEO+AM when compared with MON ($P \leq 0.03$), with no differences on total tract nutrient digestibilities ($P \geq 0.11$). Compared with BEO, the combination of BEO+AM increased intake of DM, NDF, NFC, starch and TDN ($P \leq 0.03$), with no differences on total tract nutrient digestibilities ($P \geq 0.86$).

Also, intakes of all nutrients measured, except for EE ($P = 0.36$) and CP ($P = 0.15$) were 14.5 to 23% lower in animals fed BEO+AM+PRO when compared with BEO+AM ($P \leq 0.03$), with no differences on total tract nutrient digestibilities between these two treatments ($P \geq 0.51$). Total tract apparent digestibility of CP was greater for cattle fed BEO+AM+PRO than for cattle fed MON ($P \leq 0.03$). Treatments tended to affect total tract apparent digestibility of NFC and starch ($P \geq 0.06$). Animals fed BEO+AM+PRO had greater total tract apparent digestibility of NFC ($P = 0.04$) and starch ($P = 0.05$) compared to MON. No other effects of treatments on nutrient digestibility were noted ($P \geq 0.27$).

Because nutrient digestibility can be altered by level of intake, the amount of each nutrient digested ($kg \cdot animal^{-1} \cdot d^{-1}$) also is presented in Table 6. The combination of BEO+MON resulted in greater amount of DM, CP and EE digested in the total tract ($P \leq 0.05$) and tended to increase the amount of NDF ($P = 0.07$) digested compared to MON fed alone. No differences ($P \geq 0.15$) in amount of nutrient digested were observed between BEO+MON and BEO. Cattle fed BEO had greater amount of digested protein than cattle fed MON ($P = 0.006$). Compared with MON, the combination of BEO+AM resulted in higher amounts of all nutrients digested ($P \leq 0.03$), except for EE ($P = 0.08$). Feeding BEO+AM increased the amount of NFC and starch digested ($P \leq 0.04$) compared to BEO and to BEO+AM+PRO.

Table 6. Effect of feed additives, exogenous enzymes and its combinations on nutrient intake and total apparent digestibility of finishing beef cattle.

Item[2]	Treatments[1]					SEM	*P*-value
	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO		
Intake, kg·animal^{-1}·d^{-1}							
DM	7.69^{b}	8.15^{b}	8.99^{ab}	9.77^{a}	8.05^{b}	0.69	0.008
CP	1.00^{b}	1.19^{ab}	1.26^{a}	1.36^{a}	1.18^{ab}	0.10	0.008
EE	0.25	0.27	0.30	0.29	0.26	0.02	0.05
NDF	1.47^{c}	1.59^{bc}	1.79^{ab}	2.00^{a}	1.54^{bc}	0.14	< 0.001
NFC	4.67^{b}	4.73^{b}	5.27^{ab}	5.74^{a}	4.75^{b}	0.41	0.01
Starch	4.57^{b}	4.84^{b}	5.34^{ab}	5.8^{a}	4.48^{b}	0.41	0.008
TDN	5.89^{b}	6.49^{b}	7.20^{ab}	7.73^{a}	6.61^{b}	0.57	0.004
Total apparent digestibility, %							
DM	73.5	77.6	77.0	75.6	79.6	1.90	0.27
CP	65.3^{b}	74.9^{a}	73.2^{ab}	72.8^{ab}	75.0^{a}	2.28	0.03
EE	75.5	79.8	82.1	80.6	83.5	3.36	0.61
NDF	56.7	62.1	61.2	57.4	61.6	4.80	0.74
NFC	84.9^{b}	88.6^{ab}	87.6^{ab}	88.2^{ab}	90.7^{a}	1.49	0.07
Starch	91.5^{b}	93.8^{ab}	92.9^{ab}	93.8^{ab}	95.5^{a}	1.12	0.06
TDN	76.1	80.1	79.7	78.8	82.1	1.95	0.28
Nutrient digested, kg·animal^{-1}·d^{-1}							
DM	5.66^{b}	6.27^{ab}	6.95^{a}	7.40^{a}	6.41^{ab}	5.66	0.007
CP	0.65^{b}	0.88^{a}	0.92^{a}	1.00^{a}	0.89^{a}	0.65	< 0.001
EE	0.19^{b}	0.21^{ab}	0.25^{a}	0.23^{ab}	0.21^{ab}	0.19	0.03
NDF	0.83^{b}	0.97^{ab}	1.11^{ab}	1.15^{a}	0.95^{ab}	0.83	0.03
NFC	3.99^{b}	4.16^{b}	4.61^{ab}	5.06^{a}	4.30^{b}	3.99	0.006
Starch	4.28^{b}	4.60^{b}	5.06^{ab}	5.55^{a}	4.63^{b}	4.28	0.006

[1] MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO = blend of essential oils (90 mg/kg DM); BEO+MON = blend of essential oils + monensin (90 and 26 mg/kg DM respectively); BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg/kg DM respectively); BEO+AM+PRO = blend of essential oils + exogenous α-amylase + exogenous protease (90, 560 and 840 mg/kg DM respectively). [abc] Row means that do not have common superscript letter are different ($P < 0.05$)

Treatments tended to affect the total VFA concentration in rumen ($P = 0.06$; Table 7). Feeding the combination of BEO+AM+PRO tended to decrease the total VFA concentration compared with BEO+MON ($P \leq 0.09$). Treatments affected the molar proportion of propionate ($P = 0.02$) and the acetate:propionate ratio ($P = 0.05$). Feeding BEO+AM+PRO resulted in lower molar proportions of propionate compared with BEO and BEO+AM ($P \leq 0.05$). The acetate:propionate ratio was higher ($P = 0.05$) or tended to be higher ($P = 0.08$) for animals fed the combination of BEO+AM+PRO compared to BEO+AM and BEO respectively. No other effects of treatments were observed in the pattern of ruminal fermentation products ($P \geq 0.14$).

Compared with MON, the combination of BEO+MON and BEO+AM increased the N intake ($P \leq 0.004$; Table 8), but no differences were observed between BEO+MON, BEO and BEO+AM+PRO for N intake ($P \geq 0.82$). Animals fed MON absorbed less N ($P \leq 0.04$) compared with the other treatments. No effects of treatments were observed for microbial nitrogen synthesis or microbial nitrogen efficiency ($P = 0.43$; Table 8).

Table 7. Effect of feed additives, exogenous enzymes or its combinations on ruminal fermentation characteristics of finishing beef cattle (Exp. 3)

Items[2]	Treatments[1]					SEM	*P*-value
	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO		
Total VFA concentration, m*M*	98.4	107	111	108	96.8	3.11	0.06
VFA proportion, mol/100 mol							
Acetate	50.1	52.9	57.0	55.3	51.2	1.65	0.15
Propionate	35.1^{ab}	39.6^{a}	36.3^{ab}	41.7^{a}	27.9^{b}	2.19	0.02
Butyrate	8.46	9.84	12.0	7.42	12.7	0.853	0.14
Isobutyrate	0.98	0.92	1.20	0.91	1.04	0.055	0.41
Valerate	1.31	1.79	1.34	1.54	1.33	0.087	0.29
Isovalerate	2.44	1.83	2.70	2.01	2.68	0.153	0.26
Acetate:Propionate	1.56^{ab}	1.52^{ab}	1.63^{ab}	1.46^{b}	2.20^{a}	0.107	0.05
Ruminal pH	5.79	5.82	5.76	5.96	5.87	0.06	0.85
Ruminal NH_3-N, mg/dL	16.5	14.4	20.7	12.4	14.8	6.48	0.27

[1] MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO = blend of essential oils (90 mg/kg DM); BEO+MON = blend of essential oils + monensin (90 and 26 mg/kg DM respectively); BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg/kg DM respectively); BEO+AM+PRO = blend of essential oils + exogenous α-amylase + exogenous protease (90, 560 and 840 mg/kg DM respectively). Sodium monensin (RUMENSIN 200) was from Elanco Animal Health, Indianapolis, IN. The blend of essential oils (CRINA RUMINANTS), and the exogenous enzymes [α-amylase (RONOZYME RUMISTAR) and protease (RONOZYME PROACT)] were provided by DSM Nutritional Products, Basel, Switzerland.

[2] VFA = volatile fatty acids; NH_3-N = ammonia nitrogen.

[abc] Row means that do not have common superscript letter are different ($P < 0.05$).

Compared with MON, the combination of BEO+MON and BEO+AM increased the N intake ($P \leq 0.004$; Table 8), but no differences were observed between BEO+MON, BEO and BEO+AM+PRO for N intake ($P \geq 0.82$). Animals fed MON absorbed less N ($P \leq 0.04$) compared with the other treatments. No effects of treatments were observed for microbial nitrogen synthesis or microbial nitrogen efficiency ($P = 0.43$; Table 8).

Table 8. Effect of feed additives, exogenous enzymes or its combinations on nitrogen metabolism and microbial protein synthesis of finishing beef cattle (Exp. 2)

Items	Treatments[1]					SEM	*P*-value
	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO		
Nitrogen intake, g/d	159[b]	190[ab]	201[a]	218[a]	189[ab]	7.83	0.007
Fecal excretion of nitrogen, g/d	55.6	48.7	53.2	58.5	46.9	2.43	0.41
Nitrogen absorbed, g/d	105[b]	141[a]	148[a]	159[a]	142[a]	12.9	< 0.001
Microbial nitrogen, g/d	106	99.0	144	139	150	32.8	0.43
Emic[2], g microbial nitrogen/kg TDN	13.6	15.6	19.4	18.0	21.3	3.41	0.43

[1] MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO = blend of essential oils (90 mg/kg DM); BEO+MON = blend of essential oils + monensin (90 and 26 mg/kg DM respectively); BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg/kg DM respectively); BEO+AM+PRO = blend of essential oils + exogenous α-amylase + exogenous protease (90, 560 and 840 mg/kg DM respectively). Sodium monensin (RUMENSIN 200) was from Elanco Animal Health, Indianapolis, IN. The blend of essential oils (CRINA RUMINANTS), and the exogenous enzymes [α-amylase (RONOZYME RUMISTAR) and protease (RONOZYME PROACT)] were provided by DSM Nutritional Products, Basel, Switzerland.
[2] Emic: microbial nitrogen efficiency; TDN = total digestible nutrients.
[abc] Row means that do not have common superscript letter are different ($P < 0.05$).

The TDN estimates for each of the five diets are presented in Fig. 1. Calculated by the classical method, TDN values were 74.7, 80.4, 79.4, 78.8, and 81.8%, respectively, for MON, BEO, BEO+MON, BEO+AM, and BEO+AM+PRO. Based on the digested OM, calculated TDN values were 72.1, 77.4, 76.5, 75.9, and 78.8%, respectively. Using component kcal for digestible energy, calculated TDN values uncorrected for catabolism of protein were 74.0, 79.9, 79.0, 78.3, and 81.4%, respectively. Based on analyzed ingredient composition and using the equation of Weiss et al. (1992), TDN values for all five diets were 76%. Similarly, TDN values calculated from NASCEM (2016) were equal at 79.9%. Finally, based on animal performance, TDN were 82.0, 82.0, 82.5, 82.7, and 81.1%, respectively.

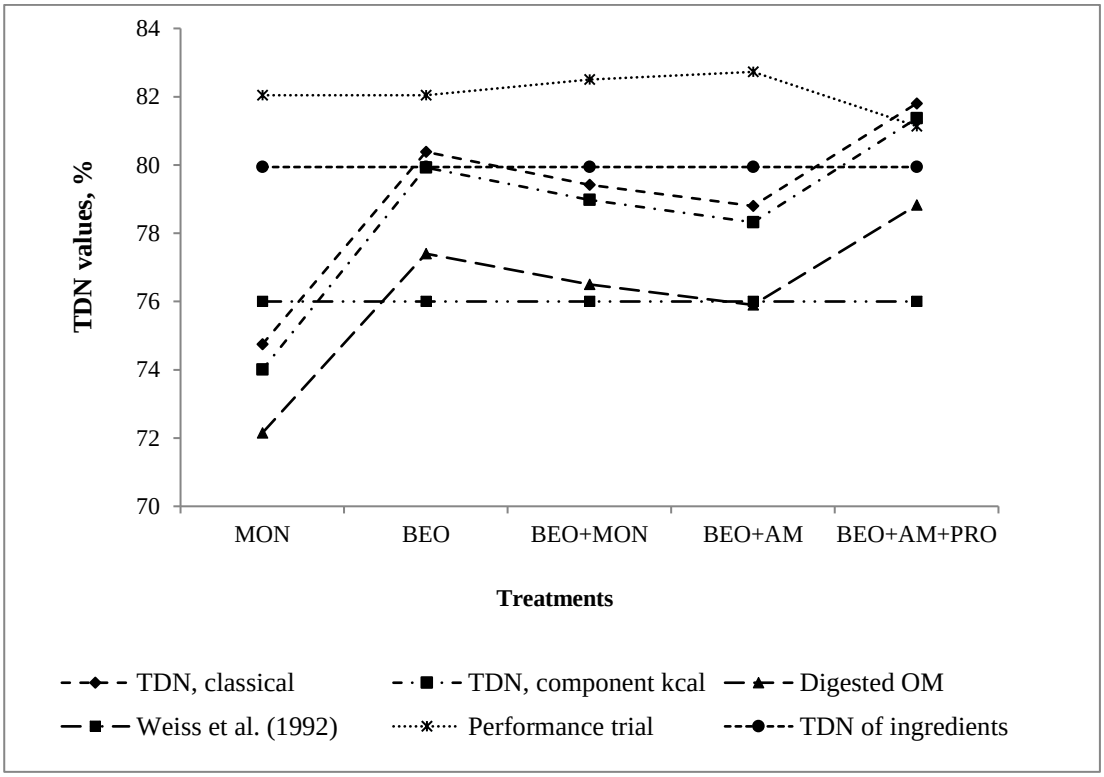

Figure 1. Diet total digestible nutrients (TDN, %) values from the digestion trial as calculated from component digestibility, from TDN content of digested components, from digested organic matter, from the equations of Weiss et al (1992), and from the performance trial based on net energy values.

2.4 DISCUSSION

Numerous nutritional strategies and various breed types have been employed in an attempted to increase productivity of growing-finishing beef cattle in feedlots (McPhee et al., 2006). By selection for an increased rate of gain, the duration of the feeding period can be shortened; productive efficiency also can be improved through the use of steroid-implants and ionophores (Wileman et al., 2009; Maxwell et al., 2015). The efficacy and the environmental benefits of growth-enhancing technologies have been demonstrated in feeding experiments with commercial cattle (Capper and Hayes, 2012). In contrast, productive efficiency of organic and natural beef production also has increased but at a slower rate (Wileman et al., 2009). Substitutes for ionophores and growth-enhancing hormones including essential oils and exogenous enzymes may have potential to increase beef production (Meyer et al, 2009; Nozière et al. 2013), but the amount of research information concerning the potential benefits of these novel technologies when compared with classical feed additives for feedlot cattle has been limited. Moreover, combinations of these technologies to assess synergisms and the overall effects of best nutritional protocols often have not been studied.

Essential oils are aromatic oily liquids extracted from plant material that possesses a wide range of antimicrobial activities (Burt, 2004; Benchaar et al., 2008; Kung et al., 2008). A mechanistic explanation for how essential oils affect ruminal fermentation through microorganism modification has not been clearly established (Meyer et al., 2009).

In our study, specific responses (DMI, ADG, G:F, digestibility, ruminal VFA) by feedlot cattle to a commercial mixture of specific essential oils fed with or without added commercial enzyme sources (α-amylase and protease) were compared with a diet that included a routinely fed ionophore, monensin. During all evaluated periods, cattle fed this specific BEO had greater DMI, but similar ADG and G:F when compared with cattle fed MON. Kung et al. (2008) fed dairy cows this same BEO and observed increases in both DMI (7.1%) and 3.5% fat corrected milk yield (7.6%) when compared with cows fed a TMR with no feed additives. According to those authors, the results were attributed to an impact of BEO that reduced *in vitro* molar proportion of acetate and increased the molar proportion of propionate. According to Li et al. (2013) this same BEO improved the *in vitro* fermentation pattern by increasing propionate concentration, reducing methane (CH_4) production and increasing fiber digestibility. In contrast, Benchaar et al. (2006; 2007) failed to detect any DMI increase from BEO supplementation of lactating dairy cows. Similarly, Meyer et al. (2009) did not observe an increase in DMI from feeding BEO to finishing beef cattle when compared with cattle fed no feed additive. Different from these studies, we contrasted the response to BEO against those obtained with feeding of monensin. Performance benefits from monensin by beef cattle fed high energy diets when compared with diets containing no monensin have been well documented in the literature (Goodrich et al., 1984, Schelling, 1984, Potter et al., 1985 and Duffield et al., 2012); therefore it seemed reasonable to feed monensin to our reference cattle group. In a meta-analysis conducted by Duffield et al. (2012) monensin decreased DMI an average of 3% but improved feed efficiency of finishing beef cattle by 2.5 to 3.5%. In our study, the greater DMI in animals fed BEO was accompanied by a numerical increase in ADG and for this reason no significant differences in G:F between BEO and MON were detected. Observed net energy concentrations between these two treatments confirm this assumption. When compared with MON, the BEO diet increased CP digestibility and the amount of CP digested but no changes on ruminal fermentation characteristics were observed. Increased VFA concentrations was observed when BEO was added to continuous-culture fermenters (Castillejos et al., 2005). The greater CP digestibility for animals fed BEO can be associated to increased nitrogen absorbed compared to MON in the current trial. In overall, through the higher DMI and minimal differences over nutrient digestibility resulting in similar

ruminal fermentation patterns, animals fed BEO were able to sustain the same performance when compared to animals fed MON.

No advantages or positive synergic effects were observed from the combination of BEO and MON. This may reflect the traditional and persistent effect of monensin to reduce DMI and ruminal fermentation pattern (Duffield et al., 2012).

Although few performance benefits from addition of AM to the BEO diet were detected, performance benefits and potential digestibility benefits frequently were superior for the combination of BEO+AM over the combination of BEO+MON. Compared with BEO+MON, the combination of BEO+AM resulted in a 9 to 11% greater DMI, 10 to 14% greater ADG, and 15 kg greater HCW; these responses were numerically similar to the advantages of BEO+AM over MON. Tricarico et al. (2014) reported that dietary α-amylase supplementation of diets fed to finishing beef cattle increased ADG and HCW by increasing DMI. According to these last authors, the increase in DMI from α-amylase supplementation was due to an altered pattern of ruminal fermentation with an increased molar proportion of butyrate and a reduced propionate proportions as well as a decrease in lactate production that ultimately increased fed intake. In our study, the molar proportion of butyrate was not affected by feeding amylase. Klingerman et al. (2009) also observed an increase in DMI by cows fed exogenous α-amylase, but no explanation for this increase in DMI was apparent.

An increased DMI has potential to decrease the length of the feeding period needed to reach a specific final BW. The increased carcass weight that we detected for animals fed BEO+AM compared to MON can be ascribed to greater intakes of DM and energy - especially starch. As compared with MON, animals fed BEO+AM increased the intake of starch (5.20 vs. 4.76 kg for BEO+AM and MON, respectively) as well as total tract starch digestibility (90.5 vs. 86.1%). Despite a 9.2% greater starch intake, cattle fed BEO+AM had fecal starch concentrations that were 25.6% lower (16% and 21.5% for BEO+AM and MON, respectively).

To examine how additives influenced energy intake and availability, digestion of DM and of various nutrients were calculated and expressed as kg/d. Based on nutrient intake and digestibility, animals fed BEO+AM digested a mean of 1.27 kg more starch compared with animals fed MON. Although differences in the total tract apparent starch digestibility were not significant, the superiority of animal performance and carcass weights observed in the Exp. 1 likely can be ascribed to this increase in the amount of starch digested. Site of this increase in starch digestion in this trial is uncertain.

When first-lactation cows were fed exogenous amylase, Nozière et al. (2014) detected no increase in total tract starch digestibility even though apparent ruminal digestibility was greater for cows fed exogenous amylase possibly due to reduced residual starch that can depress digestion of NDF; in contrast, amylase supplementation failed to increase total tract NDF digestion in our trial. Other authors (Klingerman et al., 2009; Gencoglu et al., 2010 and DiLorenzo et al., 2011) have reported that apparent total digestibility of NDF was increased when amylase was added in a diet. However, NDF digestibility of bagasse is notability low relative to other NDF sources (Almeida et al., 2018) and may be less responsive to ruminal changes. Tricarico et al. (2007) suggested that adding exogenous amylase to ruminant diets may increase cross-feeding wherein hydrolysis of starch to maltodextrins provides a substrate for both amylolytic and non-amylolytic bacteria. In our study sugar cane bagasse was used as a forage source at low level (8.5% DM basis). This low NDF content of our diet and the low digestibility of the NDF from bagasse due to its high degree of lignification might explain why NDF digestibility was not increased by amylase utilization in our trial. Alternatively, if amylase increased rate of ruminal starch digestion, the time that pH was depressed and NDF digestion was inhibited in either the rumen or the large intestine could be reduced allowing fiber-digesting microbes to be more active. Further studies with finishing beef cattle fed higher quality forages (e.g., corn silage) are needed to test and understand any cross-feeding mechanisms or pH responses of amylase supplementation on dietary fiber. Nevertheless, our data related to the total amount of nutrient digested (NDF and starch) indicate that utilization of the nutrients was enhanced when amylase was included in the diet. Increasing the amount of ruminally fermentable carbohydrates by the addiction of exogenous amylase also could reduce ruminal N-NH_3 by increasing the microbial protein synthesis, resulting in increased amount of nitrogen absorbed as observed in the present trial. According to Ramos et al. (2009) feedstuffs differing in their rates of ruminal digestion also differ in their ability to support microbial protein synthesis. It is probable that the combination of BEO+AM resulted in a better synchrony between protein and carbohydrate degradation, increasing the nitrogen absorbed even at a higher level of nitrogen intake. However, according Duval et al. (2007) the specific BEO used in the current trial failed to affect bacterial colonization of starch-rich substrates in the rumen.

Few research trials have been conducted to evaluate the effects of amylase supplementation on animal performance in finishing feedlot cattle. DiLorenzo et al. (2010) detected no significant responses in DMI, ADG and G:F from addition of exogenous amylase to the diet even through substantial numerical improvements in ADG (150 g/d) were apparent

with enzyme supplementation. Based on the POWER procedure (SAS Inst. Inc., Cary, NC), the sample size needed to detect a difference of 150 g ADG as being significant among 4 treatments (mean diff = 0.150; α = 0.05; power = 95; standard deviation = 0.14), the total sample size must be equal to or exceed 48 animals (12 experimental units per treatment). Those authors used 8 experimental units/treatments (total of 32 animals). Therefore, data variability within individual feedlot studies associated with a low number of pens per treatment may fail to detect a response in economically important production traits as being significant (Ballow et al., 2015). Differences among trials in availability of starch from the dietary grain (dent grain hybrids in most trials versus flint grain hybrids in our trial) also might alter the response to added amylase.

The initial concept behind using PRO to enhance starch utilization was to increase the degradation of the protein matrix encasing starch granules in the endosperm of flint corn. The PRO we employed (a serine protease preparation) is active on substrates like soybean meal, the primary RUP source in our diet. Presence of PRO may have increased ruminal degradation of soybean meal; this decrease in RUP supply would be expected to decrease DMI and performance. According to Beauchemin et al. (2002), enzymes differ widely in both specificity and activity. Hence, diet variability may alter the benefit from added enzymes and drastically complicate widespread applicability of results. Feedstuff specificity likely will continue to be a major bottleneck for formulating new ruminant feed enzyme products. Some suggestions that cleavage of structural barriers that potentially involve proteins of the cell wall can increase accessibility of substrates for ruminal microbes (Colombatto et al. 2003; Wallace and Kopecny, 1983) and thereby increase the potential for DM degradation were observed by Colombatto and Beauchemin (2009). The degree to which feed additives can serve as wetting agents and azeotropes to decrease *lag time* deserves further research attention.

Various methods to assess the energy availability of cattle diets currently are being used. Although GE of products is used as an index of energy content of retained energy, TDN has served as the classical basis for estimates of energy availability (DE, ME, or NE values) of feeds in published tables based on standard NASCEM (2016) equations. Even though, TDN is considered obsolete by some nutritionists enamored by the metric system and modern systems for feed analysis that have displaced crude fiber and nitrogen free-extract. Differences among these TDN estimates in the current study ranging from 3 to 10% of the mean can be attributed to failure to fully consider potential associative effects or impacts of additives, depressed digestibility partially compensated by a reduced methane loss associated with greater DMI, failure to appropriately account for added ME from fat versus fatty acids,

or to fully discount digested protein for the additional energy lost in urine that leads to overestimation of the energy value of high protein feedstuffs, and to inaccuracy of estimating empty body weight or predicting equivalent body weight within the NE equations. This discrepancy among diets in TDN values estimated from various procedures of 3 to 10% the mean, though similar in magnitude to ranges in TDN in NASCEM (2016) tables, are above and beyond the differences attributable to differences in nutrient composition associated with feed samples across the US. So that animal performance can be predicted more reliably, more complete definition and standardization of calculation methods used for predicting TDN are needed so that values match with realized NE values.

2.5 CONCLUSIONS

The specific BEO enhanced DMI without impair feed efficiency compared to a basal diet that included MON for finishing feedlot cattle. A synergism between BEO and AM further increased cattle performance and carcass production as compared to MON. Therefore, these two specific feed additives as evaluated in the current trial (BEO or BEO+AM), may be an alternative to replace MON in finishing feedlot diets. Further studies with PRO supplementation in finishing beef diets should focus on enzyme sources, doses and specificity to the substrate to increase potential degradation of DM from the specific feedstuffs being fed.

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3. THE COMBINATION OF ESSENTIAL OILS AND EXOGENOUS α-AMYLASE INTERACTS WITH CORN GRAIN PARTICLE SIZE TO INCREASE CARCASS WEIGHT OF FINISHING BEEF CATTLE

Abstract

This study evaluated the growth performance and digestion responses of finishing feedlot beef cattle fed high-concentrate diets containing 82.5% flint corn (DM basis) ground to medium (1.66 mm; **MG**) or coarse particle sizes (2.12 mm; **CG**), added with monensin (26 mg/kg; DM basis; **MON**) or a blend of essential oils + exogenous α-amylase (90 mg/kg + 560 mg/kg commercial product, respectively, DM basis; **BEO+AM**). In Exp. 1, 256 Nellore bulls were blocked by initial body weight (360 ± 12 kg), assigned to 48 pens and pens within blocks were randomly assigned, in a 2 × 2 factorial arrangement, to treatments during 99 d. Effect of corn particle size × feed additive was not detected ($P \geq 0.15$) for final BW, DMI, ADG and G:F. Feed additive effect was detected for DMI, which was greater for bulls fed BEO+AM vs. MON ($P = 0.001$). A tendency for corn particle size effect was detected for final BW and ADG ($P = 0.09$), which was greater for bulls fed CG than MG corn. A corn particle size × feed additive interaction was detected for HCW ($P = 0.01$). The HCW was 11 kg heavier for bulls fed BEO+AM vs. MON in diets containing CG ($P = 0.007$), but not MG particle size ($P = 0.42$). In Exp. 2, four ruminally cannulated Nellore steers were offered the same treatments of Exp. 1, in a 4 × 4 Latin Square design, to evaluate intake, apparent total tract digestibility of diets and ruminal fermentation parameters. Effect of corn particle size × feed additive was not significant ($P \geq 0.23$) for the intake of most nutrients. Effect of corn particle size was detected for intake of DM, CP, NDF, NFC, starch and TDN ($P \leq 0.009$), which were greater for steers fed CG than steers fed MG corn. Effects of corn particle size × feed additive was not detected ($P \geq 0.11$) for any ruminal fermentation characteristics evaluated. A tendency ($P = 0.08$) for corn particle size effect was detected for total VFA concentration in the rumen, which was greater for steers fed CG vs. MG corn. Feed additive affected ($P = 0.02$) ruminal NH_3-N concentration, which was less for steers fed BEO+AM compared to MON. In summary, reducing flint corn particle size from 2.12 to 1.66 mm in finishing diets failed to improve cattle growth performance, digestibility of most nutrients and ruminal fermentation characteristics. A blend of essential oils associated with exogenous α-amylase resulted in the heavier carcass weights compared to monensin supplementation when included in diets containing coarse ground corn.

Keywords: Alternative feed additive; Feedlot; Monensin; Starch digestion

3.1 Introduction

The impact of grain particle size on feedlot performance has been studied extensively in diets containing dent corn, and despite advantages to starch digestion and milk yield from feeding dairy cows with fine ground corn (Moe and Tyrrell, 1977; Mitzner et al., 1994; Knowlton et al., 1998; Firkins et al., 2001; Rémond et al., 2004) few studies reported positive

outcomes to fine grain grinding on growth performance of beef cattle fed high-concentrate diets (Galyean et al., 1979; Turgeon et al., 1983; Secrist et al., 1995; Swanson et al., 2014; Schwandt et al., 2016). Nevertheless, McAllister et al. (1993) and Ramos et al. (2009) reported that starch digestion *in vitro* and *in sacco* were greater for smaller (0.25 to 1.3 mm) compared to larger (2.00 to 3.00 mm) corn particle sizes. Flint is the primary corn type fed in feed yards in Brazil, with processing methods varying from fine grinding (Millen et al., 2009) to coarse cracking (Oliveira and Millen, 2014). However, few studies evaluated the impact of the processing of flint corn grain on nutrient digestibility and growth performance of finishing feedlot beef cattle (Carareto et al., 2011). Benefits to net energy content of the grain and cattle growth performance were greater after steam flaking flint corn than is generally reported in the published literature for flaking dent corn (Gouvêa et al., 2016; Marques et al., 2016). Hence, it was expected that reducing the grain particle size may increase starch digestion and growth performance of finishing beef cattle fed flint corn.

Rumen fermentation characteristics and growth performance of feedlot cattle also can be enhanced through feeding certain feed additives such as monensin **[(MON)**; Butaye et al., 2003; Duffield et al., 2012] and more recently trough feeding certain essential oils or their blends [(**BEO**); Khiaosa-ard et al., 2013; Khorrami et al., 2015). Also, adding exogenous α-amylase (**AM**) has been proposed to optimize starch utilization in feedlot diets but results are inconsistent (Tricarico et al., 2007; DiLorenzo et al., 2011). According Beauchemin et al. (2004) the lack of interaction of enzymes with the substrate can explain the lack of response in some *in vivo* studies in which exogenous enzymes were added in cattle diets. The combination of a specific BEO and exogenous AM resulted in greater average daily gain and carcass weight compared with MON primarily by increasing energy intake and the amount of nutrient digested in the total tract of finishing Nellore bulls (Meschiatti et al., 2018). The combination of these two additives could circumvent concerns regarding the use of antibiotics in commercial beef production (Jouany and Morgavi, 2007; de Souza et al., 2018) and should be further evaluated. We hypothesized that reducing the particle size of flint ground corn could improve starch digestion and growth performance of finishing beef cattle, and that the extent of the improvement on growth performance would be greater with the combination of BEO+AM due to greater interaction of exogenous enzyme with the substrate. Thus, the present study evaluated the growth performance and carcass characteristics of finishing Nellore bulls (Exp. 1) and nutrient digestibility and ruminal fermentation characteristics of Nellore steers (Exp. 2) fed flint corn ground to two different particle sizes (medium vs. coarse) added with monensin or the combination of essential oils and exogenous α-amylase.

3.2 MATERIALS AND METHODS

Animals were cared for according the guidelines recommended by the Animal Care and Use Committee of the "Luiz de Queiroz" College of Agriculture (**ESALQ**), University of São Paulo (**USP**) - protocol # **2015-29**.

3.2.1 Experiment 1. Growth Performance and Carcass Characteristics

Animals, Housing, and Experimental Procedures. At the start of the study, 256 Nellore bulls [(360 ± 12 kg initial body weight (**BW**)], sourced commercially, were weighed individually after 12 h of feed and water withdrawal, administrated vaccination against clostridiosis (3 ml s.c; Sintoxan Polyvalente, Merial Saúde Animal Ltda, Paulínia, Brazil), and dewormed (3.15% ivermectin at 1 ml/50 kg BW; Ivomec Gold; Merial Saúde Animal Ltda.). Thereafter, bulls were blocked by initial BW (12 weight blocks) and assigned to 48 pens (12 pens/treatment). Pens had different dimensions and characteristics so the number of bulls assigned to each pen varied as follow: 28 pens had concrete-floor and partial roof cover (32 m^2 and 5 bulls/pen; weight block 1 to 7), 12 pens had soil-floor and no roof cover (84 m^2 and 6 bulls/pen; weight block 8 to 10), and 8 pens had soil-floor and no roof cover [120 m^2; 4 pens with 5 bulls/pen (weight block 11) and 4 pens with 6 bulls/pen (weight block 12)]. All bulls had free choice access to fresh water during the entire study.

Treatments were randomly assigned to pens within each weight blocks, in a 2 × 2 factorial arrangement, consisted of two corn particle sizes [medium ground corn (1.66 mm; **MG**) or coarse ground corn (2.12 mm; **CG**)] added with sodium monensin [26 mg/kg of dry matter (**DM**); MON] or the combination of a blend of essential oils with an exogenous α-amylase (90 and 560 mg commercial product/kg DM, respectively; BEO+AM)].

The basal diet (Table 1) was formulated to meet the requirements of finishing Nellore bulls for 1.5 kg average daily gain as specified by NRC (1996). Sodium monensin (POULCOX) was obtained from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) containing thymol, eugenol, limonene and vanillin on an organic carrier (McIntoch et al., 2003) and the exogenous α-amylase produced by *Bacillus licheniformis* (RONOZYME RUMISTAR) were provided by DSM Nutritional Products, Basel, Switzerland. The dose level employed for BEO was based on previous studies (Kung Jr., 2008; Meyer et al., 2009). The exogenous enzyme α-amylase (RONOZYME RUMISTAR), a granular amylase formulation produced by *Bacillus licheniformis* with amylase activity of 600 Kilo Novo units

(**KNU**) per g was added to the appropriate total mixed rations (**TMR**) to achieve 336 KNU of amylase activity per kg of TMR DM (Meschiatti et al., 2018; DiLorenzo et al., 2011). One KNU is defined as the amount of enzyme that releases in a two-step reaction 6 μmol of p-nitrophenol per min from 1.86 mM 4.6-thylidene-G7-pnitrophenyl-maltoheptaoside at pH 7.0 and 37 °C (Meschiatti et al., 2018; DiLorenzo et al., 2011). The inclusion concentration of α-amylase was selected based on previous studies (Klingerman et al., 2009; Gencoglu et al., 2010; Andreazzi et al., 2018; Meschiatti et al., 2018).

Table 1. Ingredient and chemical composition of diet (Exp. 1 and 2)

Item	% DM
Ingredient	
Sugarcane bagasse	8.50
Flint ground corn[1]	82.5
Soybean meal	5.00
Urea	1.00
Mineral and vitamin supplement[2]	3.00
Analyzed composition	
Dry matter, % (as fed)	81.4
Crude protein, %	13.4
Neutral detergent fiber, %	16.1
Acid detergent fiber, %	8.12
Ether extract, %	3.93
Starch, %	54.5
TDN[3], %	81.5
NE_m[4], Mcal/kg	2.05
NE_g[4], Mcal/kg	1.39

[1] Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil) to medium ground (MG; 1.66 mm) or coarse ground (CG; 2.12 mm).
[2] Feed additives were incorporated into the mineral and vitamin supplement to reach the treatments: MON = sodium monensin [26 mg/kg dry matter (DM)]; BEO+AM = blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg DM respectively). Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous α-amylase (RONOZYME RUMISTAR) were provided by DSM Nutritional Products, Basel, Switzerland.
[2] Mineral and vitamin supplement was composed (DM basis) of 140 g/kg Ca, 16 g/kg P, 36 g/kg S, 20 g/kg Mg, 34 g/kg K, 56 g/kg Na, 8 mg/kg Co, 540 mg/kg Cu, 6.7 mg/kg Cr, 27.5 mg/kg I, 1.070 mg/kg Mn, 6.7 mg/kg Se, 2000 mg/kg Zn, 168000 IU/kg vitamin A, 17000 IU/kg vitamin D3, 1740 IUI/kg vitamin E, 90 mg/kg biotin, 2.7×10^9 CFU/kg *Saccharomyces cerevisiae*. Manufactured by DSM Nutritional Products, São Paulo, Brazil.
[3] Total digestible nutrients (TDN) were estimated from equations of Weiss et al. (1992) using the analyzed ingredient composition and assuming processing adjustment factor of 1.00 for flint ground corn (NRC, 2001).
[4] The net energy for maintenance (NE_m) and gain (NE_g) were estimated with the equations proposed by the NASEM (2016; empirical solution type) with addition of ionophore and using the TDN values from Weiss et al. (1992) obtained by NRC (2001).

The flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil) at a commercial feed mill (Coplacana, Cooperativa dos Plantadores de Cana do Estado de São Paulo, Piracicaba, SP, Brazil) and delivered every 7 d to the experimental feedlot. Corn samples were taken from every load (1 sample/load and 14 total samples) and particle distribution was analyzed 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 Análises Ltda., São Paulo, SP, Brazil). The residue retained in the bottom (< 1.25 mm) was assumed to represent a 0.625 mm square pores sieve. Average particle size, reported in terms of geometric mean of the diameter (d_{gw}), log-normal standard deviation (S_{gw}), surface area (cm^2/gram) and particles/gram, were calculated using equations of Baker and Herrman (2002) (Table 2 and Figure 1).

Table 2. Corn grain particle size distribution, % of total

Item	Corn particle size	
	MG	CG
Pores in the sieve		
> 6.0 mm	0.05	3.70
≤ 6.0 and > 3.5 mm	3.10	15.7
≤ 3.5 and > 2.0 mm	31.0	34.1
≤ 2.0 and >1.25 mm	40.6	27.6
≤ 1.25 mm	25.3	18.9
Geometric mean diameter[1] (d_{gw}), mm	1.66	2.12
Log-normal standard deviation[1] (S_{gw})	1.57	1.77
Surface area[1] (cm^2/gram)	30.4	25.3
Particles/gram	414	344

[1] Corn grain particle distribution was determined 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 Análises Ltda, São Paulo, SP, Brazil). Particles < 1.25 mm was assumed to represent a 0.625 mm square pores sieve. Geometric mean diameter (d_{gw}), log-normal standard deviation (S_{gw}), surface area (cm^2/gram) and particles/gram were calculated using equations of Baker and Herrman (2002).

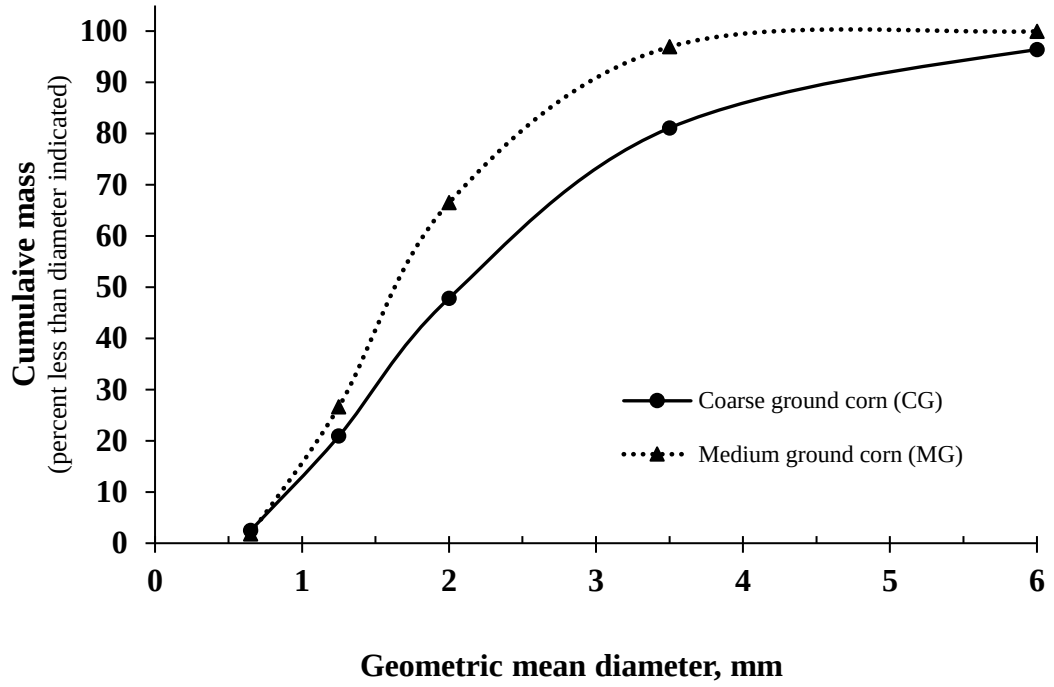

Figure 1. Cumulative mass distribution of ground corn grain. Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil). Corn grain particle distribution was determined according to Yu et al. (1998) using sieves with 6.0, 3.5, 2.0, 1.25 mm square pores (Produtest T Model; Telastem Peneiras para Análises Ltda, São Paulo, SP, Brazil). Particles < 1.25 mm was assumed to represent a 0.625 mm square pores sieve. Geometric mean of the diameter (d_{gw}) was calculated using equations of Baker and Herrman (2002).

Bulls were fed an adaptation TMR diet during the first 15 days of experiment and finishing TMR from d 16 to 99. During the adaptation period the dietary concentration of the sugarcane bagasse was reduced from 25 to 15% (5% units every 5 d; DM basis) and replaced with ground corn accordingly. From d 16 to 99, all bulls received the finishing diet [8.5% sugarcane bagasse plus 91.5% concentrate (DM basis; Table 1)]. Treatments were offered during the entire experiment (99 d).

Feed additives and enzymes were incorporated into the mineral-vitamin supplement that was included as 3% of the dietary DM (Table 1). These mineral-vitamin supplements with the appropriate additives and enzymes were produced at a commercial feed mill following all the manufacturing standards for quality and guaranteed levels (DSM Nutritional Products Brazil S.A, Mairinque, SP, Brazil).

Each treatment diet was mixed individually using a feed wagon (Siltomac S-2.3; Indústria de Implementos Agrícolas Siltomac Ltda., São Carlos, SP, Brazil) and delivered to each pen once daily at 0800. The feed wagon was carefully cleaned after delivering each ration to avoid cross-contamination. Feed bunks were evaluated visually each day and managed for a maximum of 3% orts that were removed weekly, weighed and sampled. Feed and orts samples of each pen were dried at 105ºC for 24 h to determine DM content and to calculate dry matter intake (**DMI**).

On d 70, individual fresh fecal samples were collected from the pen floor (3 randomly bulls/pen) before feeding. Approximately 50 g of feces were collected from the inside of each fecal pats, using sterile cups. Feces were pooled by pen and immediately frozen (-20°C) for further fecal starch analyses.

At the end of the 99 d feeding trial, bulls were weighed following 12 h of feed and water deprivation and transported (8.4 km; approximately 20 min.) in a commercial cattle truck (11 trucks with 22 bulls/truck and 1 truck with 14 bulls) to a commercial packing plant (Friuna Alimentos Ltda, Piracicaba, Brazil). Average daily gain (**ADG)** was calculated using the initial and final shrunk BW. Feed efficiency (**G:F**) was calculated as the ratio of ADG to DMI. After harvesting, hot carcasses weight (**HCW**) were collected and dressing percent was calculated (HCW to final shrunk BW ratio) Subcutaneous fat thickness and longissimus muscle (**LM**) area were measured at the 12^{th} rib after a 24 h chill (2°C), using a numbered grid and a digital caliper, respectively.

3.2.2 Feed Analysis and Calculations.

Samples of ingredient collected every 10 days were 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). Samples were analyzed for DM (method 930.15; AOAC, 1986), ash (method 942.05; AOAC, 1986), ether extract [(**EE**); method 920.85; AOAC, 1986], 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]. Corn and fecal samples were analyzed for starch using a Total Starch K-TSTA KIT (Megazyme, Chicago IL, USA; method 996.11; AOAC, 1986 and method 76-13.01; AACC, 1976). The total digestible nutrients (**TDN**) values for each diet were estimated according to Weiss et al. (1992) using a processing factor of 1.00 for ground corn (NRC, 2001). Corn net energy content for maintenance ($\mathbf{NE_m}$) and gain ($\mathbf{NE_g}$) were estimated from the fecal starch concentration (Zinn et al., 2007). Total

tract starch (**TSD**) digestion was calculated from fecal starch content according to Zinn et al. (2002). Net energy concentrations of each diet were calculated according to Zinn and Shen (1998) using mean values for shrunk BW, DMI, and ADG of the bulls in each pen. These calculated NE concentrations were compared with those predicted using the Weiss et al. (1992) equations for TDN that were converted to NE concentrations using equations from the NASEM (2016) empirical model. Specific details related to feeding procedures, sampling, laboratory analysis and calculations were described by Meschiatti et al. (2018).

3.2.3 Experiment 2. Digestibility and Ruminal Fermentation

Four ruminally cannulated Nellore steers (BW = 594 ± 22 kg) were used in a 4 × 4 Latin Square design to evaluate intake, apparent total tract digestibility of nutrients, ruminal parameters, and rumen microbial protein synthesis of diets used in the Exp. 1. Detailed information related to feeding procedures, sample collection, laboratory analysis and calculations for Exp. 2 were described by Meschiatti et al. (2018). Briefly, steers were housed in individual pens (32 m^2; concrete-floor and solid roof) and given free choice access to water during the experiment. Four consecutive periods were conducted, each consisting of 15 d for adaptation to diets and 5 d for sample collection. From d 16 to 20 of each period, steers were monitored every 3 h and total feces were collected from the concrete floor and analyzed for DM content to estimate total fecal production. On d 18 (at 8000, 1400, 2000 and 0200), d 19 (at 1000, 1600, 2200 and 0400) and d 20 (at 1200, 1800, 2400 and 0600) approximately 50 g of fecal samples also were collected directly from the rectum of each steer in a manner that all collections represented one full day. These last samples were composited by period and steer, and analyzed for DM, CP, EE, aNDF, ADF, ash and fecal starch as described for Exp. 1. Non fibrous carbohydrate (**NFC**, %) was calculated as: NFC = 100 − (CP, % + aNDF, % + Ash, % + EE, %). Total apparent digestibility was calculated using the equation: Nutrient digestibility, % = ([Nutrient intake, kg – Fecal nutrient output, kg] / Nutrient intake, kg) × 100.

On d 16, rumen fluid samples (50 ml) were obtained from each steer via ruminal cannula at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h after feeding as described by Danes et al. (2013). Ruminal fluid samples were squeezed through 4 layers of cheesecloth and the pH was immediately recorded using a portable pH meter (Digimed Model DM22, Digicrom Analítica Ltda., São Paulo, SP, Brazil). Sub-samples were latter analyzed for volatile fatty acids (**VFA**) according Palmquist and Conrad (1971) and ammonia nitrogen (**NH_3-N)**

according Chaney and Marbach (1962). Results from pH, VFA and N-NH_3, analyses were averaged across the 24 h collection, and used for statistical analyses.

Spot samples of urine were spontaneously collected into sterile plastic cups on d 17 of each period, 4 h after the TMR. The concentrations of creatinine and uric acid in urine were determined using commercial kits (Bioclin - Belo Horizonte, MG, Brazil and CELM - Compania Equipadora de Laboratórios Modernos, São Caetano do Sul, SP, Brazil) and readings were performed using automatic biochemistry analyzer (Automatic System of Biochemistry SBA-200 - CELM). Allantoin concentrations were determined as described by Chen and Gomes (1992). The absorbed purines were calculated from excretion of purine derivatives according to Verbic et al. (1990). Urine volume was estimated from the concentration of creatinine in the urine (Chizzotti et al., 2008) and ruminal synthesis of nitrogenous compounds (**N mic**, g/d) was calculated based on the absorbed microbial purine, according the equation proposed by Barbosa et al. (2011). Microbial nitrogen efficiency was calculated as the ratio of g microbial nitrogen to kg TDN ingested.

3.2.4 Statistical Analysis

Data in Exp. 1 were analyzed using the MIXED procedure of SAS (version 9.3, SAS Inst. Inc., Cary, NC) as a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Pen was the experimental unit and the Satterthwaite approximation method was used to determine the correct denominator degrees of freedom for the test of fixed effects. The statistical model included the fixed effects of corn particle size, feed additive and particle size × feed additive. Pen (corn particle size × feed additive), bull (pen), weight of block and pen type were considered random variables for IBW, FBW, ADG and carcass characteristics. Bull (pen) was not included as random effect for statistical analysis of intake, feed efficiency, fecal starch, TSD and corn and diets net energy concentrations.

All data in Exp. 2 were analyzed as a 4 × 4 Latin Square design with a 2 × 2 factorial arrangement of treatments, using the MIXED procedure of SAS (version 9.3, SAS Inst. Inc., Cary, NC). The statistical model included the fixed effect of corn particle size, feed additive and corn particle size × feed additive. The steers and periods of Latin square were included as random term. Steer within period was the experimental unit. Averages across the 24 h period for VFA, N-NH_3, and pH were used for statistical analysis to simplify data interpretation and reporting. The Kenward-Roger approximation was used to determine the denominator degrees of freedom that was used for testing fixed effects.

Results from Exp. 1 and 2 were all reported as least-squares means. When no significant interactions ($P > 0.05$) were detected, main effects of corn particle size and feed additives were examined. Differences between each factor were tested by *F*-test. When significant interactions ($P < 0.05$) were observed for a trait, means were separated using PDFF option. Differences were declared significant when $P \leq 0.05$, whereas trends were discussed when $0.05 < P \leq 0.10$.

3.3 RESULTS

3.3.1 Growth Performance and Carcass Characteristics (Exp. 1)

Effects of corn particle size × feed additive were not detected ($P \geq 0.15$) for final BW, DMI, ADG and G:F (Table 3). Feed additive effect was detected for DMI, which was 7.4% greater for bulls fed BEO+AM vs. MON ($P = 0.001$). A tendency for corn particle size effect was detected for final BW and ADG ($P = 0.09$), which was greater (9 kg and 100 g/d, respectively) for bulls fed CG than bulls fed MG corn. Neither corn particle size nor feed additive impacted feed efficiency ($P \geq 0.26$).

A corn particle size × feed additive interaction was detected for HCW ($P = 0.01$; Table 3). The HCW was 11 kg heavier for bulls fed BEO+AM vs. MON in diets containing CG ($P = 0.007$), but not MG particle size ($P = 0.42$; Fig. 2). Corn particle size effect was detected for LM area, which was 4.1% greater for bulls fed CG than MG corn ($P = 0.02$).

Table 3. Performance and carcass characteristics of Nellore bulls finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM)

Item[3]	Corn particle size[1]		Feed additive[2]		SEM	*P*-value		
	MG	CG	MON	BEO+AM		Corn particle size	Feed additive	Corn particle size × Feed additive
Growth performance								
Initial BW, kg	360	360	360	360	11.6	-	-	-
Final BW, kg	509	518	511	516	10.1	0.09	0.41	0.15
DMI, kg/d	8.90	9.14	8.70	9.34	0.17	0.21	0.001	0.21
ADG, kg	1.50	1.60	1.53	1.57	0.044	0.09	0.39	0.17
G:F	0.170	0.176	0.176	0.170	0.005	0.26	0.26	0.49
Carcass characteristics								
Hot carcass weight, kg	283	289	284	288	6.39	0.03	0.18	0.01
Dressing, %	55.6	55.9	55.6	55.9	0.319	0.47	0.53	0.22
LM area, cm^2	65.7	68.4	66.7	67.4	1.44	0.02	0.58	0.87
12th-rib fat, mm	3.91	4.00	3.82	4.10	0.174	0.69	0.27	0.67

[1] Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil). Geometric mean diameter (mm) was calculated using equations of Baker and Herrman (2002).

[2] Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[3] BW = body weight; DMI = dry matter intake; ADG = average daily gain; G:F = feed efficiency; LM = *longissimus* muscle.

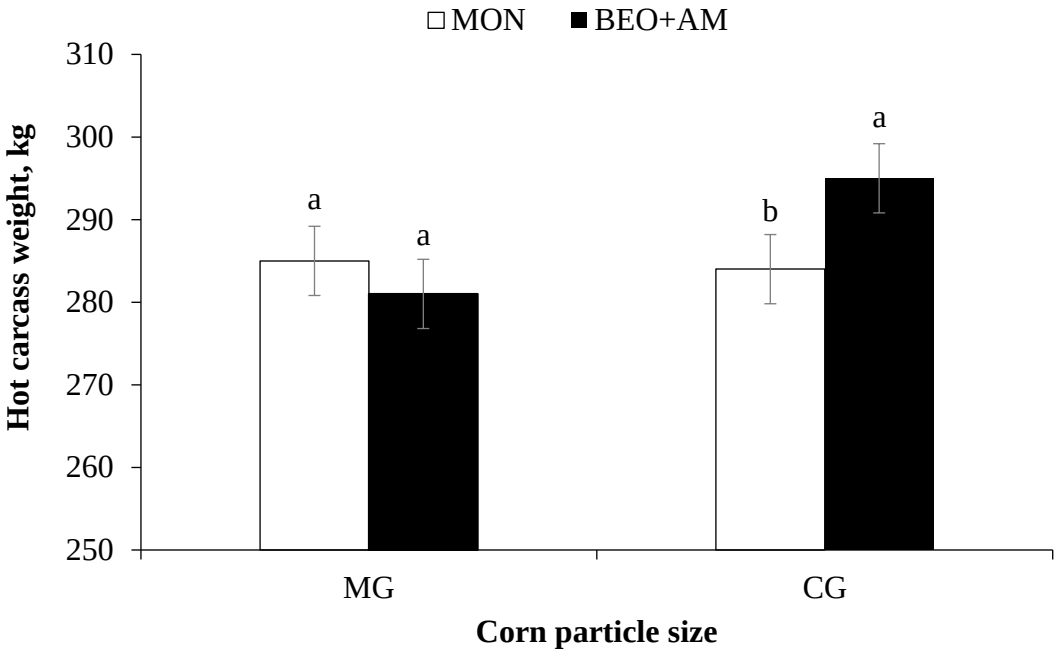

Figure 2. Hot carcass weight (kg) of Nellore bulls finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM) for 99 d (Exp. 1; Randomized complete block design; 12 pens/treatment; 5 or 6 bulls/pen). Effect of corn particle size × feed additive was detected for hot carcass weight (P = 0.01; SEM = 4.19). Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.
[ab] Within corn particle size, bars without a common superscript differ (P < 0.05).

No effects of corn particle size × feed additive or corn particle size ($P \geq 0.21$), but effects of feed additive ($P \leq 0.01$) were detected for starch intake, fecal starch, TSD, and corn net energy concentration (Table 4). Bulls fed the combination of BEO+AM had 7.4% greater starch intake than bulls fed MON (P = 0.001). Fecal starch concentrations averaged 24 % greater (P = 0.01) leading to lower total tract starch digestibility (3.4%; P = 0.01) for bulls fed BEO+AM compared to MON. Based on fecal starch alone, estimated corn NE_m and corn NE_g averaged 5.4% and 6.5% respectively greater (P = 0.01) for bulls fed MON than bulls fed BEO+AM.

Effects of corn particle size × feed additive, corn particle size and feed additive were not detected ($P \geq 0.12$) for observed dietary NE values estimated based on intake and growth performance (Table 5).

Table 4. Starch intake, fecal starch and total tract starch digestion of Nellore bulls finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM)

Item	Corn particle size[1]		Feed additive[2]		SEM	*P*-value		
	MG	CG	MON	BEO+AM		Corn particle size	Feed additive	Corn particle size × Feed additive
Starch intake,[3] kg·bull^{-1}·d^{-1}	4.85	4.98	4.74	5.09	0.093	0.21	0.001	0.21
Fecal starch, %	20.9	21.1	18.8	23.3	1.22	0.90	0.01	0.35
TSD, [4] %	86.9	86.8	88.3	85.4	0.79	0.90	0.01	0.35
Corn NE_m,[5] Mcal/kg	2.09	2.08	2.14	2.03	0.031	0.92	0.01	0.37
Corn NE_g,[5] Mcal/kg	1.42	1.42	1.47	1.38	0.027	0.91	0.01	0.37

[1] Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil). Geometric mean diameter (mm) was calculated using equations of Baker and Herrman (2002).

[2] Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[3] Calculated using the starch content in the diet and dry matter intake data of each pen.

[4] TDS = total tract starch digestion was estimated according to Zinn et al. (2002).

[5] Corn net energy for maintenance (NE_m) and gain (NE_g) were estimated according to Zinn et al. (2007).

Table 5. Observed dietary net energy (NE) concentration of Nellore bulls finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM)

Item	Corn particle size[1]		Feed additive[2]		SEM	*P*-value		
	MG	CG	MON	BEO+AM		Corn particle size	Feed additive	Corn particle size × Feed additive
Observed NE,[3] Mcal/kg								
Maintenance	2.02	2.05	2.06	2.01	0.023	0.34	0.12	0.53
Gain	1.36	1.39	1.40	1.35	0.020	0.34	0.12	0.53
Observed : Expected NE ratio[4]								
Maintenance	1.00	1.01	1.00	1.01	0.012	0.34	0.81	0.52
Gain	1.00	1.02	1.00	1.01	0.015	0.34	0.88	0.52

[1] Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil). Geometric mean diameter (mm) was calculated using equations of Baker and Herrman (2002).

[2] Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[3] Observed NE was calculated using cattle performance data based on the equation proposed by Zinn and Shen (1998).

[4] Expected values were calculated using the NASEM (2016) - empirical solution type - based on the TDN values (Weiss et al., 1992).

3.3.2 Nutrient Digestibility and Ruminal Fermentation Characteristics (Exp. 2)

Effect of corn particle size × feed additive was not significant ($P \geq 0.23$) for the intake of most nutrients, except for EE intake ($P = 0.07$; Table 6). Steers fed BEO+AM presented greater EE intake ($P = 0.002$) than steers fed MON in diets containing CG corn, but not MG corn ($P = 0.13$; Fig. 3).

Effect of corn particle size was detected for intake of DM, CP, NDF, NFC, starch and TDN ($P \leq 0.009$), which were greater for steers fed CG than steers fed MG corn. Feed additive affected intake of CP ($P = 0.04$) and tended to affect intakes of starch and TDN ($0.05 \leq P \leq 0.09$), and in both cases steers fed BEO+AM had greater intakes than MON.

Corn particle size × feed additive was not significant ($P \geq 0.17$) for the total apparent digestibility of most nutrients, except for EE ($P = 0.002$) and for a tendency for NFC and TDN apparent digestibility ($0.05 \leq P \leq 0.06$). Apparent total digestibility of EE was less for steers fed BEO+AM vs. MON in diets containing MG corn ($P = 0.01$) but was greater than MON in diets containing CG corn ($P = 0.02$; Figure 4). Total apparent digestibility of NFC ($P = 0.10$; Figure 5) and the TDN ($P = 0.06$; Figure 6) tended to be greater for MON vs. BEO+AM in diets containing MG corn but no differences between BEO+AM vs. MON were observed in diets containing CG corn ($P \geq 0.20$).

Effect of corn particle size was not detected for total apparent digestibility of all nutrients ($P \geq 0.33$; Table 6). Effect of feed additive was detected only for total apparent digestibility of NDF ($P = 0.02$), which was greater for steers fed MON than steers fed BEO+AM (Table 6).

Table 6. Nutrient intake and total apparent digestibility of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM)

Item[3]	Corn particle size[1]		Feed additive[2]		SEM	*P*-value		
	MG	CG	MON	BEO+AM		Corn particle size	Feed additive	Corn particle size × Feed additive
Intake, kg·steer^{-1}·d^{-1}								
DM	6.49	8.63	7.12	8.00	0.91	0.004	0.10	0.84
CP	0.91	1.16	0.95	1.11	0.12	0.006	0.04	0.27
EE	0.22	0.28	0.22	0.29	0.03	0.004	0.004	0.07
NDF	1.21	1.53	1.32	1.43	0.16	0.009	0.21	0.51
NFC	3.88	5.29	4.33	4.84	0.55	0.003	0.11	0.87
Starch	3.54	4.70	3.88	4.36	0.49	0.004	0.09	0.84
TDN	4.85	6.55	5.37	6.06	0.63	0.001	0.05	0.23
Total apparent digestibility, %								
DM	71.7	73.5	73.6	71.5	2.26	0.33	0.26	0.20
CP	67.7	68.8	69.4	66.8	2.27	0.60	0.16	0.24
EE	84.5	81.1	83.3	82.3	1.86	0.001	0.34	0.002
NDF	54.4	57.2	60.5	51.1	4.79	0.34	0.02	0.33
NFC	81.9	83.2	82.5	82.6	1.62	0.38	0.91	0.06
Starch	91.6	93.0	91.7	93.0	1.44	0.33	0.36	0.17
TDN, % MS	74.9	76.3	76.3	74.9	2.05	0.36	0.36	0.05

[1] Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil). Geometric mean diameter (mm) was calculated using equations of Baker and Herrman (2002).

[2] Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[3] DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber corrected by ash; NFD = non-fibrous carbohydrate; TDN = total digestible nutrients.

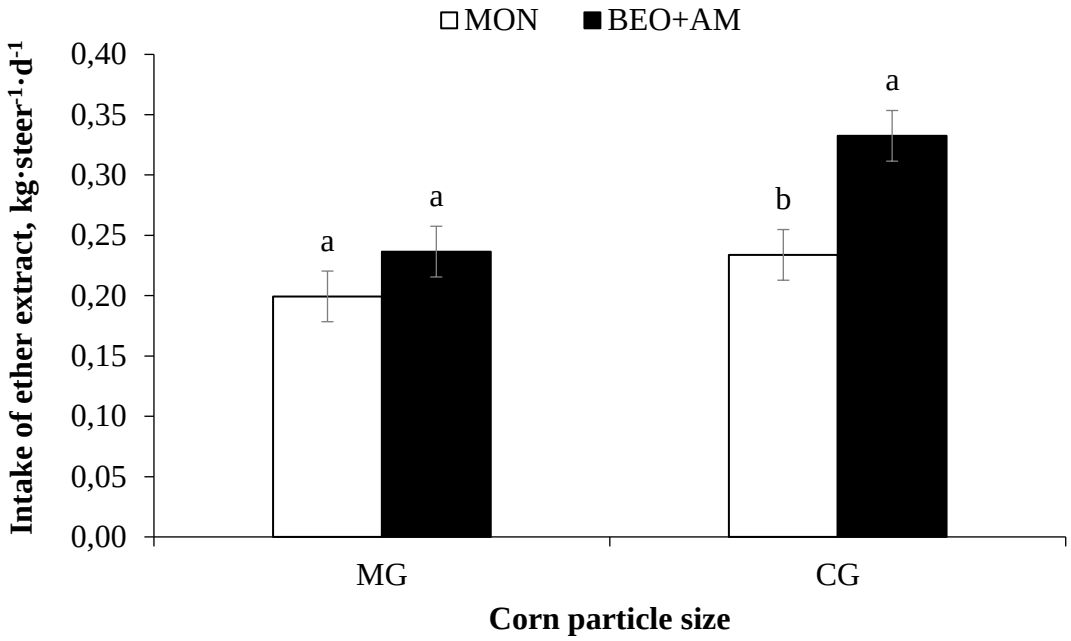

Figure 3. Intake of ether extract (kg·steer^{-1}·d^{-1}) of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM); Exp. 2: 4 × 4 Latin Square design. Effect of corn particle size × feed additive was detected for intake of ether extract (P = 0.07; SEM = 0.03). Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[ab] Within corn particle size, bars without a common superscript differ (P < 0.05).

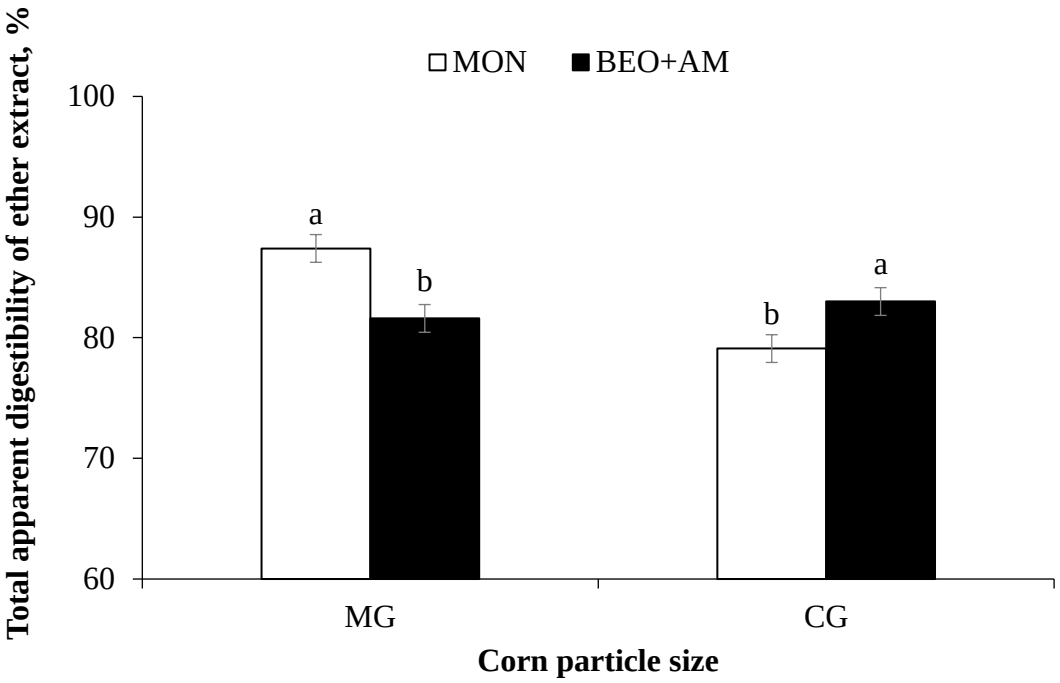

Figure 4. Total apparent digestibility of ether extract (%) of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM); Exp. 2: 4 × 4 Latin Square design. Effect of corn particle size × feed additive was detected for total apparent digestibility of ether extract (P = 0.002; SEM = 1.86). Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[ab] Within corn particle size, bars without a common superscript differ (P < 0.05).

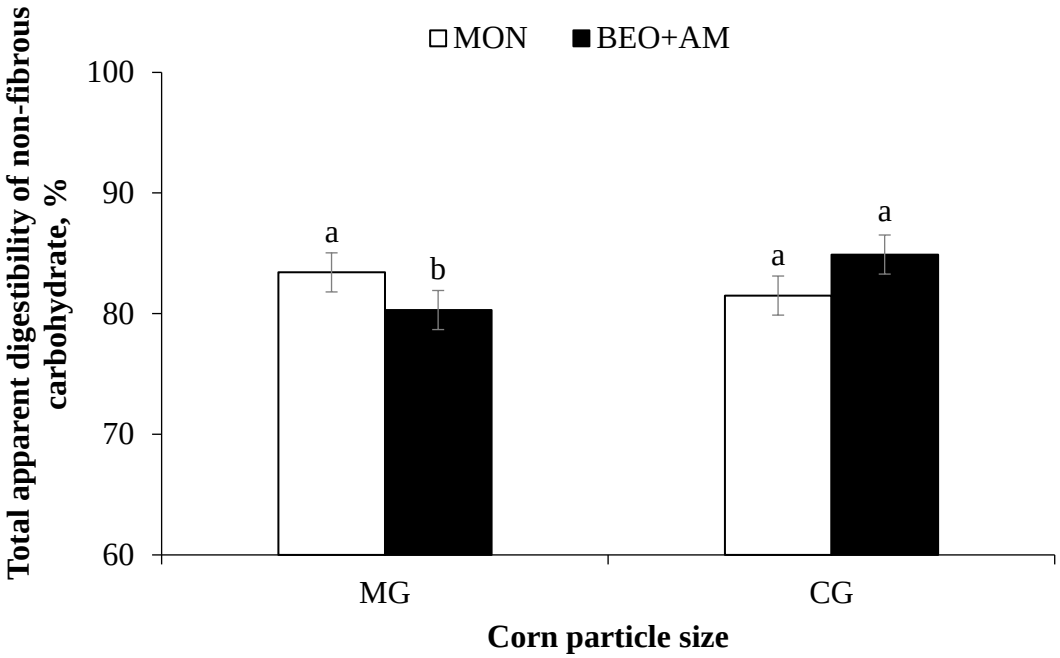

Figure 5. Total apparent digestibility of non-fibrous carbohydrate (%) of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM); Exp. 2: 4 × 4 Latin Square design. Effect of corn particle size × feed additive was detected for total apparent digestibility of non-fibrous carbohydrate ($P = 0.06$; SEM = 1.62). Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.
[ab] Within corn particle size, bars without a common superscript differ ($P \leq 0.10$).

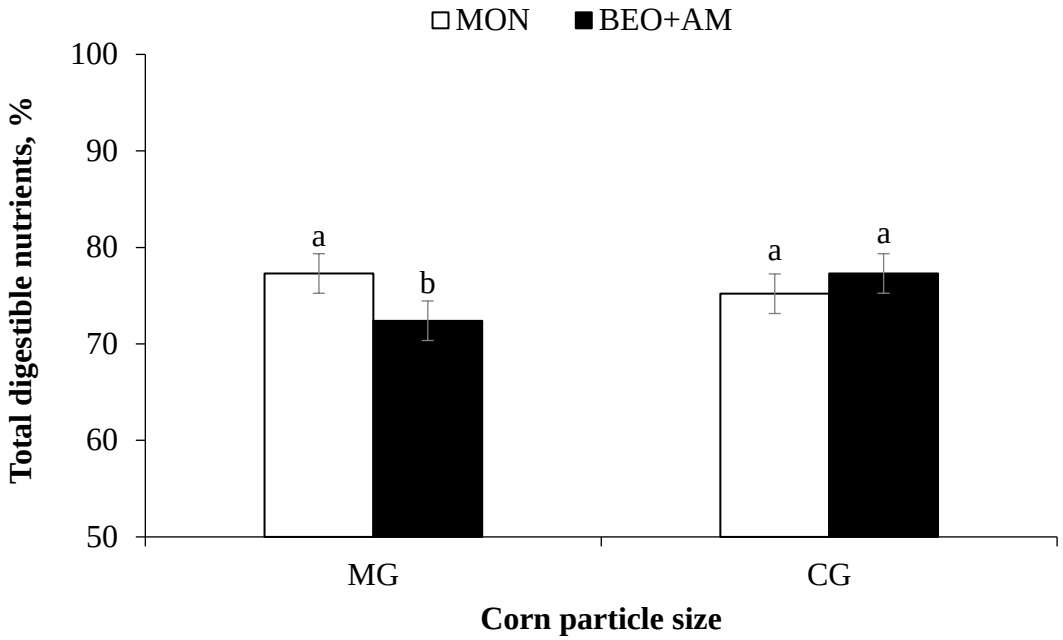

Figure 6. Total digestible nutrients (TDN; %) of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM); Exp. 2: 4 × 4 Latin Square design. Effect of corn particle size × feed additive was detected for total digestible nutrients (P = 0.05; SEM = 2.05). Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.
[ab] Within corn particle size, bars without a common superscript differ (P < 0.10).

Effects of corn particle size × feed additive was not detected ($P \geq 0.11$) for any ruminal fermentation characteristics evaluated (Table 7). A tendency (P = 0.08) for corn particle size effect was detected for total VFA concentration in the rumen, which was greater for steers fed CG vs. MG corn. Feed additive affected (P = 0.02) ruminal NH_3-N concentration, which was less for steers fed BEO+AM compared to MON.

A tendency for a corn particle size × feed additive effect ($P \geq 0.08$) was detected for nitrogen absorbed (Table 8). In diets containing CG corn, steers fed BEO+AM absorbed more nitrogen than steers fed MON (P = 0.01; Fig 7), but not in diets containing MG corn (P = 0.64).

Effect (P = 0.006) or a tendency (P = 0.07) for corn particle size effect were detected for nitrogen intake and fecal nitrogen excretion (Table 8), which were greater for steers fed CG than MG corn (28 and 25% respectively). Also, effects (P = 0.04) or a tendency (P = 0.09) for feed additive were detected for nitrogen intake and fecal nitrogen excretion (Table 8), which were greater for steers fed BEO+AM vs. MON (17 and 22% respectively).

Table 7. Ruminal fermentation characteristics of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM)

Item[3]	Corn particle size[1]		Feed additive[2]		SEM	*P*-value		
	MG	CG	MON	BEO+AM		Corn particle size	Feed additive	Corn particle size × Feed additive
Total VFA concentration, m*M*	97.3	110	100	107	6.23	0.08	0.33	0.86
VFA proportion, mol/100 mol								
Acetate	52.7	51.3	52.9	51.2	1.48	0.48	0.41	0.23
Propionate	28.8	32.0	28.2	32.5	2.91	0.46	0.32	0.42
Butyrate	12.1	11.4	12.7	10.9	1.54	0.77	0.42	0.82
Isobutyrate	1.30	1.14	1.27	1.17	0.104	0.30	0.49	0.91
Valerate	1.40	1.31	1.39	1.32	0.088	0.43	0.53	0.67
Isovalerate	3.66	2.87	3.56	2.97	0.553	0.33	0.47	0.56
Acetate:Propionate ratio	2.00	1.75	2.01	1.74	0.250	0.51	0.46	0.49
Ruminal pH	5.66	5.58	5.63	5.61	0.100	0.15	0.72	0.41
Ruminal NH_3-N, mg/dL	15.4	16.5	18.6	13.4	2.37	0.55	0.02	0.11

[1] Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil). Geometric mean diameter (mm) was calculated using equations of Baker and Herrman (2002).

[2] Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[3] VFA = volatile fatty acids; NH_3-N = ammonia nitrogen.

Table 8. Nitrogen metabolism and microbial protein synthesis of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM)

Item[3]	Corn particle size[1]		Feed additive[2]		SEM	*P*-value		
	MG	CG	MON	BEO+AM		Corn particle size	Feed additive	Corn particle size × Feed additive
Nitrogen intake, g·steer^{-1}·d^{-1}	145	185	152	178	17.5	0.006	0.04	0.27
Fecal nitrogen, g·steer^{-1}·d^{-1}	47.6	59.3	48.1	58.8	8.49	0.07	0.09	0.92
Nitrogen absorbed, g·steer^{-1}·d^{-1}	96.7	126	104	119	9.70	0.002	0.03	0.08
Microbial nitrogen, g·steer^{-1}·d^{-1}	150	164	158	156	29.1	0.60	0.93	0.46
Emic, g microbial nitrogen/kg TDN	33.0	25.6	32.4	26.2	4.88	0.19	0.26	0.27

[1] Flint corn grain was processed through a hammer mill (ML 100-A, Lucato, Indústria e Comercial Lucato, Limeira, SP, Brazil). Geometric mean diameter (mm) was calculated using equations of Baker and Herrman (2002).

[2] Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.

[3] Emic: microbial nitrogen efficiency. TDN = total digestible nutrients

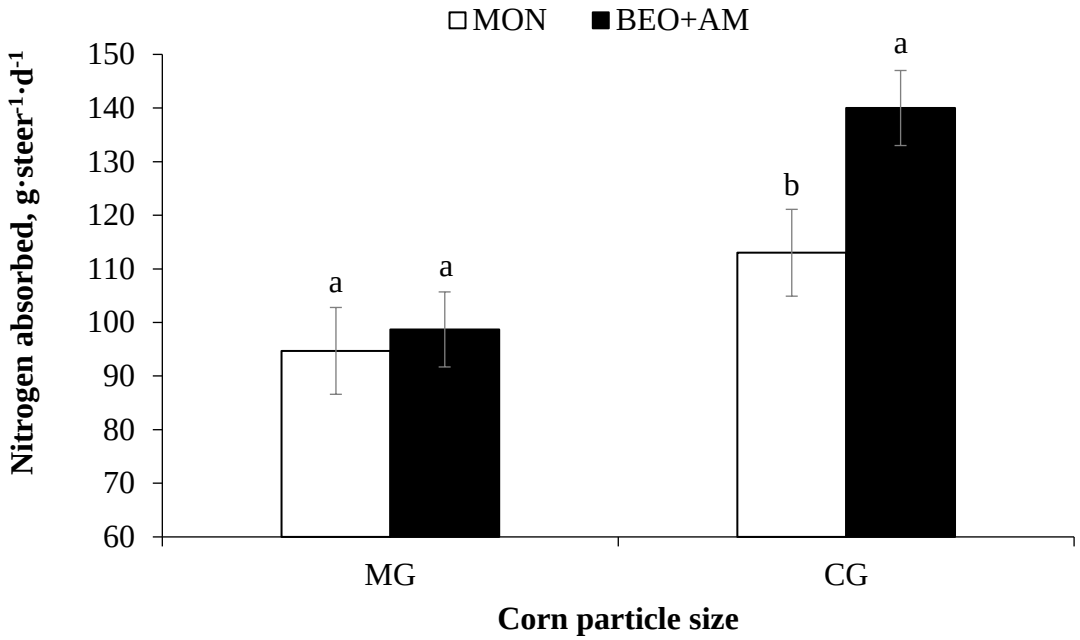

Figure 7. Nitrogen absorbed ($g \cdot steer^{-1} \cdot d^{-1}$) of Nellore steers finished in feedlot and provided, in a 2 × 2 factorial arrangement, diets containing flint corn ground to medium (1.66 mm; MG) or coarse (2.12 mm; CG) particle sizes, added with sodium monensin (26 mg/kg dry matter; MON) or a blend of essential oils + exogenous α-amylase (90 and 560 mg commercial product/kg dry matter respectively; BEO+AM); Exp. 2: 4 × 4 Latin Square design. Effect of corn particle size × feed additive was detected for nitrogen absorbed ($P = 0.08$; SEM = 9.70). Sodium monensin (POULCOX) was from Biovet Joint Stock Company, Sofia, Bulgaria. The blend of essential oils (CRINA RUMINANTS) and the exogenous enzyme [α-amylase (RONOZYME RUMISTAR)] were provided by DSM Nutritional Products, Basel, Switzerland.
[ab] Within corn particle size, bars without a common superscript differ ($P < 0.05$).

3.4 DISCUSSION

Effects of dent corn particle size on animal performance and nutrient digestibility have been investigated in previous trials (Galyean et al., 1979; Turgeon et al., 1983; Secrist et al., 1995; Secrist et al., 1996; Swanson et al., 2014; Lundy et al., 2015). In summary, among these published studies no significant differences in growth performance of finishing cattle were observed among the corn particles sizes (n = 6 studies; comparisons = 14; average particle size = 2.52 mm; maximum particle size = 7.94 mm; minimum particle size = 0.5 mm; particle size SEM = 0.52 mm) even though total tract starch digestion was greater with more finely ground dent corn grain in several of these studies. One possible reason for the lack of response may be because decreasing particle size in dent corn increases rate of ruminal

fermentation in the rumen and could increase the incidence of subacute acidosis (Owens et al., 1998). On the other hand, intestinal starch digestion may compensate for less extensive ruminal starch digestion when large particles sizes were fed (Owens et al., 1986; Huntington et al., 2006) and energy availability for animals from starch digested in the small intestine (estimated at 97%) is greater than starch fermented in the rumen (estimated at 80%) because losses of heat and methane are reduced (Huntington et al., 2006). Hence, even if total tract starch digestion is greater for small particles, differences in site of digestion may counterbalance this effect so that no detrimental effects in animal performance are observed with more coarsely ground grain.

Flint corn is more vitreous than dent corn. The starch from corn grain that is more vitreous is less rapidly digested in the rumen based on *in situ* measurements and less extensively digested in the total tract (Philippeau et al., 1999). However, compared with ground dent grain, ground flint grain yields more large particles, and large particles are less rapidly and extensively digested than small particles, so particle size differences may partly explain the *in situ* differences. Indeed, Ramos et al. (2012) illustrated that fine grinding can fully eliminate differences in *in situ* starch degradation associated with vitreousness. We hypothesized that grinding flint grain to a smaller particle size would increase nutrient digestion and consequently animal growth performance because finer grinding would increase the surface area that is exposed for microbial attack (Richards and Hicks, 2007). Reducing particle size from dry rolling (3.02 mm) to fine gridding (1.10 mm) flint corn increased feed efficiency by 12% in finishing cattle fed diets containing 12 or 20% sugarcane bagasse as forage source (Carareto et al., 2011). Increases in the observed NE_g value of flint corn also have been reported from more extensive corn processing methods such as steam flaking (Gouvêa et al., 2016; Marques et al., 2016). However, no advantages on animal performance were observed in the current study where corn particle size was reduced from 2.12 to 1.66 mm and the surface area increased from 344 to 414 particles/gram.

McAllister et al. (1993) reported that although grinding dry corn to a smaller particle size ruptured the endosperm cells, starch granules remained embedded within a protein matrix. This could explain the lack of effects, at least partially, associated to corn particle size over the animal performance observed in the current trial. On the other hand, these same authors observed that *in vitro* starch digestion was greater for smaller particles (0.25 to 0.89 mm) than larger particles (2.00 to 3.00 mm) suggesting that at a similar ruminal residence time, an increase in surface area should increase the extent of starch degradation. Most of the difference in particle size for MG and CG corn used in this trial came from changing corn

distribution between the sieve of 1.25 and 2.00 mm in MG corn to particles between 3.5 and 6.0 mm in the CG corn. Grain particle distribution between 2.0 and 3.5 mm was very similar in both MG and CG, and averaged 32.5%. Also particles sizes lower than 1.25 mm averaged 22% between the two particle sizes evaluated. So, in addition to the small difference between the two particles size evaluated in the current trial (1.66 vs. 2.12 mm), the range in particles size distribution and surface area (30.4 vs. 25.3 cm^2/gram) may have been too narrow to enhance microbial attachment and colonization in such a way as to affect the animal performance.

An interaction between flint corn grain processing and the amount of dietary NDF from roughage (**rNDF**) was reported by Caetano et al. (2015). The rNDF level for maximum DMI was 13.7% with high moisture flint corn (5.84 mm particle size) but 11.3% for flint ground corn (1.30 mm particle size) presumably due to the greater availability of starch and ME of diets (2.97 and 2.70 Mcal/kg for high moisture and ground corn respectively). Carareto et al. (2011) observed that observed NE_m and NE_g for animals fed flint ground corn (1.10 m particle size) was greater than animals fed dry rolled flint corn (3.10 mm particle size) but in that study no interaction between forage level (12 versus 20% of sugar cane bagasse) and grain particle size was observed. In our study, sugarcane bagasse was included at 8.5% (dry matter basis) or 6.9% rNDF. Relative to the Caetano et al. (2015) study, the amount of rNDF included in this trial may not have been sufficient to maximize DMI and growth performance and contributed to our failure to detect a benefit from the finer grind size.

Relative to feeding CG with MON, feeding CG with BEO+AM enhanced ADG and HCW despite having no effect on nutrient digestibility or on observed NE concentrations. This indicates that differences in DMI must be involved in the observed performance. Greater intakes of starch and TDN (that tended to increase the total VFA, to reduced ruminal ammonia nitrogen and increased the retained nitrogen) presumably can explain the increased carcass weight from this combination (MG+BEO+AM). This performance advantage for CG and BEO+AM appears related to greater DMI and ADG over the feeding trial. Similarly, Meschiatti et al. (2018) also observed 12 kg greater HCW for cattle fed the combination of BEO+AM as compared with cattle fed MON in diets containing flint corn with a 2.04 mm particle size. Consistent with our results, these authors observed that DMI was greater for animals fed BEO+AM vs. MON despite no differences in feed efficiency between these two feed additives.

Yang and Russell (1993) reported that when monensin is fed, acetate typically decreases or do not change, but propionate concentration increases; this lowers the

acetate:propionate ratio. In the current study neither total nor proportions of individual VFA concentration were significantly different between cattle given monensin and cattle given BEO+AM despite greater DMI of cattle fed the BEO+AM combination. If the reduced DMI with the MON diets reflects a satiety signal resulting from propionate based on its hepatic effects as proposed by Allen et al. (2009) or some other factor is uncertain. Nevertheless, the greater DMI of cattle fed the combination of BEO+AM appears to be the primary benefit from feeding this combination of additives. Meyer et al. (2009) using the same commercially available BEO as was used in our trial, reported that total VFA concentration were greater compared with monensin even though no differences were detected in the molar proportion of propionate agreeing with findings of our study.

According to Callison et al. (2001) ammonia nitrogen concentration in the rumen represents a net balance between ammonia production (degraded dietary protein), ammonia uptake by microbes (that varies with carbohydrate availability), ammonia absorption (which varies with ruminal pH) as well as recycling of urea to the rumen (via saliva and through the ruminal wall). In the current study, because RDP was similar between diets and pH was not affected by treatments and recycling should not have been altered, we speculate that the combination of BEO+AM, through increasing either DMI or ruminal starch digestibility was increasing the carbohydrate availability in the rumen for ruminal microbes to use for synthesis of microbial protein. However, no effects of feed additives were observed for apparent total tract digestibility of starch or other nutrients or on microbial yield. Consequently, benefits from the combination of BEO+AM may be related to the improved use of nutrients in the rumen. For example, Andreazzi et al. (2018) proposed that for lactating cows, the increased ruminal starch degradation induced by feeding exogenous α-amylase was reflected not by increased total tract starch digestibility, but instead to increased milk yield and reduced DMI, improving feed efficiency in dairy cows and that post-ruminal digestion of starch escaping ruminal digestion compensated the total starch digestion. Others have suggested that increased starch digestion associated with amylase supplementation instead increases total tract digestibility of NDF because less starch reaches the large intestine to decrease the pH and NDF fermentation at that site. Further information about the site and extent of starch and NDF digestion is needed to fully understand the reasons why the combination of BEO+AM increased the animal performance and carcass weight.

In overall, CG corn did not change efficiency of energy use even though it increased nutrient intake compared to MG corn. This conflicts with the concept that decreasing corn particle size should increase starch digestibility and thereby increase energy density of the diet

Owens et al. (1997) and thereby reduce DMI (Krehbiel et al., 2006). Instead, no increased in the total starch digestion nor in realized net energy values for flint corn were detected by grinding the grain to a smaller mean particle size.

The failures of fine grinding to increase total tract starch digestibility and increase NE value of these corn-rich diets conflict with the generally accepted dogma concerning starch digestion and utilization. *In vitro* and *in situ* measurements consistently indicate that rate and extent of ruminal digestion of starch is greater for smaller than larger corn grain particles. Increased rate of ruminal starch digestion should translate to increased extent of ruminal starch digestion if ruminal retention time remains unchanged. If ruminal starch digestion is increased, might expect total tract starch digestibility to increase unless starch digestion in the large intestine is sufficiently high. In a summary of 51 beef cattle diets from the published literature, the correlation between ruminal and total tract digestibility of starch was of only 0.22. (F. N. Owens, personal communication). Starch of a small particle size that is readily fermented in the rumen likely would be readily digested by enzymes in the small intestine of cattle.

Why an increase in ruminal starch digestion would not increase the realized NE value of the grain might be explained by the degree and relative energetic efficiency of compensatory starch digestion in the small intestine. The whole corn grain, the maximum particle size attainable for corn, often is fed to growing-finishing cattle and yields feed efficiency that with low roughage diets that rival or exceed that of rolled corn grain. Even though it seems intuitive that maximum ruminal digestion of starch would be preferable (for acetate and VFA yield and for microbial protein synthesis), methane and heat losses associated with ruminal digestion. Estimated energy recovery from starch that is digested in the rumen has ranged from 69 to 86% of that derived from starch digested in the small intestine Owens et al. (1986). These values were supported by calorimetric studies by McLeod et al. (2001) which indicated that energy from ruminally fermented starch was 74% that of starch digested in the small intestine. However, as clearly illustrated by Huntington et al. (2006), starch reaching the small intestine is not fully digested. Any starch that escapes digestion in the small intestine enters the large intestine where it again may be partially fermented. Energetic efficiency for starch fermented in the large intestine is lower than starch fermented within the rumen. Based on relative efficiencies of energy use of 80, 97, and 62% for starch digested in the rumen, small intestine, and large intestine as estimated by Huntington et al. (2006), the point and extent to which efficiency of use of energy from digested starch can be calculated as outlined by Owens et al. (2016).

Based on the study with 51 diets containing dry rolled corn grain fed to finishing beef cattle, ruminal starch digestion averaged 71.8 ± 12.9% of dietary starch (F. N. Owens, personal communication). If large intestinal starch fermentation remained constant at 40% of starch entering the large intestine and all starch not fermented in the rumen was digested in the small intestine, increasing ruminal starch digestion from 58.9% to 68.9% (1 standard deviation below the mean), energetic efficiency would be decreased by 2.3% based on 80% and 97% energetic efficiency of ruminally fermented versus small intestinally digested starch. This indicates that shifting site of digestion from the rumen to the small intestine can increase energetic efficiency if small intestinal starch digestion is sufficiently high. According to Harmon et al. (2004) the amount of starch digested in the rumen is a linear function of intake, but at high levels of DMI, factors other than intake can affect starch digestion, such as ruminal kinetics variables, chemical properties (pH, osmolarity), rates and extents of particle passage (Huntington et al., 2006).

3.5 CONCLUSIONS

In high concentrate diets containing flint corn grain, reducing particle size from 2.12 to 1.66 mm fails to increase growth performance, nutrient digestibility and ruminal fermentation characteristics of finishing beef cattle. The combination of a blend of essential oils associated with exogenous α-amylase enhance the intake of nutrients compared to monensin supplementation without affecting feed efficiency. The combination of a blend of essential oils with exogenous α-amylase results in the heavier carcass weights compared to monensin supplementation when included in diets containing coarse ground corn.

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4 CONCLUSIONS

Monensin can be replaced by the combination of a specific blend of essential oils with exogenous α-amylase resulting in increased carcass production when included in diets containing coarse ground corn, without altering feed efficiency.