Feeding the combination of essential oils and exogenous α-amylase increases performance and carcass production of finishing beef cattle[1]

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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 (DM 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 DMI ($P < 0.001$) 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 DMI ($P = 0.001$), 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 ether extract ($P = 0.16$) 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 DMI 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.

Key words: corn, degradability, feed additives, feedlot, starch

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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., 2010; Klingerman et al. 2009; Nozière et al. 2014; 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 in both 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.

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.

Experiment 1. Animal Performance

Animals, housing, and experimental procedures. Three hundred finishing Nellore bulls [initial 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 one of five 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 DM

Table 1. Ingredient and chemical composition of diet (DM 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, %	
DM, 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
NE_m [4], Mcal/kg	2.01
NE_g [4], Mcal/kg	1.37

[1]Mineral and vitamin supplement containing dietary treatments: MON = sodium monensin (26 mg/kg 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]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, 2,000 mg/kg Zn, 168,000 IU/kg vitamin A, 17,000 IU/kg vitamin D_3, 1,740 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]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]NE_m and NE_g were estimated with the equations proposed by NASCEM (2016; empirical model) with addition of ionophore.

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 gram, was added to the appropriate TMR to achieve 336 KNU of amylase activity per kilogram 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 per kilogram 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. 2012). 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 days 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 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, and 1.25 mm square pores (Produtest T Model; Telastem Peneiras para Análises Ltda., São Paulo, 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), 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 hours. 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 105 °C for 24 h. Orts were removed twice weekly, weighed, sampled, and discarded. Feed and orts samples were dried at 105 °C for 24 h to determine DM and calculate DMI. On day 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 day 93. DMI, ADG, and feed efficiency (G:F; calculated as the ratio of ADG to DMI) were calculated for each period evaluated.

Individual fecal grab samples were obtained from the rectum of each bull on day 70 of the trial and immediately frozen (−20 °C) for further fecal starch analyses. Upon BW assessment on day 93, animals were transported (8.4 km) to a commercial packing plant (Friuna Alimentos Ltda, Piracicaba, Brazil). Hot carcasses weight (**HCW**) was collected following kidney and heart and pelvic fat removal.

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 0.625 mm. Based on Yu et al. (1998).

Dressing percent was calculated as the ratio of HCW to final shrunk BW. Subcutaneous fat thickness and 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.

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, ADF (Goering and Van Soest, 1970), and 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; method 996.11 AOAC, 1986 and method 76-13.01 AACC, 1976). The TDNs 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 NE_m and NE_g were estimated from the fecal starch concentration using the equation: $NE_m = 2.49 - 0.0127 \times FS - 0.000292 \times FS^2$, and $NE_g = 0.877 \times NE_m - 0.41$ (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.

Experiment 2. Digestibility and Ruminal Fermentation

Animals, housing, and experimental procedures. Five ruminally cannulated Nellore steers (425 ± 55 kg) were assigned to the same diets and treatments described in Exp. 1 in a 5 × 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 m²) 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 hours 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).

Sample collection. During the 5 d of collection (days 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 −20 °C for later analysis of DM for calculating total fecal nutrient excretion. On days 18, 19, and 20 fecal samples also were collected manually directly from the rectum from each animal and frozen at −20 °C 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 hours on day 18, at 0600, 1400, and 1800 hours on day 19, and at 0800, 1200, and 2000 hours on day 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. Nonfibrous carbohydrate (**NFC**, %) was calculated as: NFC = 100 − (CP, % + aNDF, % + Ash, % + EE, %). Feed ingredients were collected every 10 days, as described for Exp. 1.

On day 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 four 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 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 day 17 of each period, 4 h after the TMR was offered (Zanetti et al., 2017). When spontaneous urination did not 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 −20 °C for further analysis.

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 BW (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 gram microbial nitrogen to kilogram TDN ingested.

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 OM: considering that 1 kg of TDN is obtained from 4.4 Mcal of OM digested. (3) Component kcal: assuming that 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).

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.

RESULTS

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. 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 days 28 and 93 were observed. During the total feeding period (days 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 days 0 to 27 and from days 28 to 93 ($0.06 \leq P \leq 0.09$) and increased the DMI during the total feeding period (days 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 day 70. Fecal starch concentration was

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

	Treatments[1]						
Item[2]	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO	SEM	*P*-value
Steers (pens)	60 (10)	60 (10)	60 (10)	60 (10)	60 (10)	—	—
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
Days 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
Days 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
Days 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

^{abc}Means that do not have common superscript letters are different ($P < 0.05$).

[1]MON = sodium monensin (26 mg/kg 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]Adj. BW = discounted by 4% from the full BW as ruminal fill;

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

	Treatments[1]						
Item	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO	SEM	*P*-value
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

^{abc}Means that do not have common superscript letters are different ($P < 0.05$).

[1]MON = sodium monensin (26 mg/kg 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]Calculated using the starch content in the diet and dry matter intake data of each pen.

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

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

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.

Digestibility and Ruminal Fermentation Responses (Exp. 2)

In the 5 × 5 latin square with five 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.

Treatments tended to affect the total VFA concentration in rumen ($P = 0.06$; Table 7). Feeding the combination of BEO+AM+PRO tended to

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

	Treatments[1]						
Item	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO	SEM	*P*-value
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

[abc]Means that do not have common superscript letters are different ($P < 0.05$).

[1]MON = sodium monensin (26 mg/kg 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]Calculated according to 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).

Table 6. Effect of feed additives, exogenous enzymes, and its combinations on nutrient intake and total apparent digestibility of finishing beef cattle (Exp. 2)

	Treatments[1]						
Item[2]	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO	SEM	*P*-value
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

^{abc}Row means that do not have common superscript letter are different ($P < 0.05$).

[1]MON = sodium monensin (26 mg/kg 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]EE = ether extract; NFC = nonfibrous carbohydrates.

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).

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 kilocalories 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.

Table 7. Effect of feed additives, exogenous enzymes, or its combinations on ruminal fermentation characteristics of finishing beef cattle (Exp. 2)

	Treatments[1]						
Items[2]	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO	SEM	*P*-value
Total VFA concentration, mM	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

[abc]Means that do not have common superscript letters are different ($P < 0.05$).

[1]MON = sodium monensin (26 mg/kg 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]NH_3-N = ammonia nitrogen.

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

	Treatments[1]						
Items	MON	BEO	BEO+MON	BEO+AM	BEO+AM+PRO	SEM	*P*-value
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

[abc]Means that do not have common superscript letters are different ($P < 0.05$).

[1]MON = sodium monensin (26 mg/kg 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]Emic = microbial nitrogen efficiency.

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. 2014), 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.,

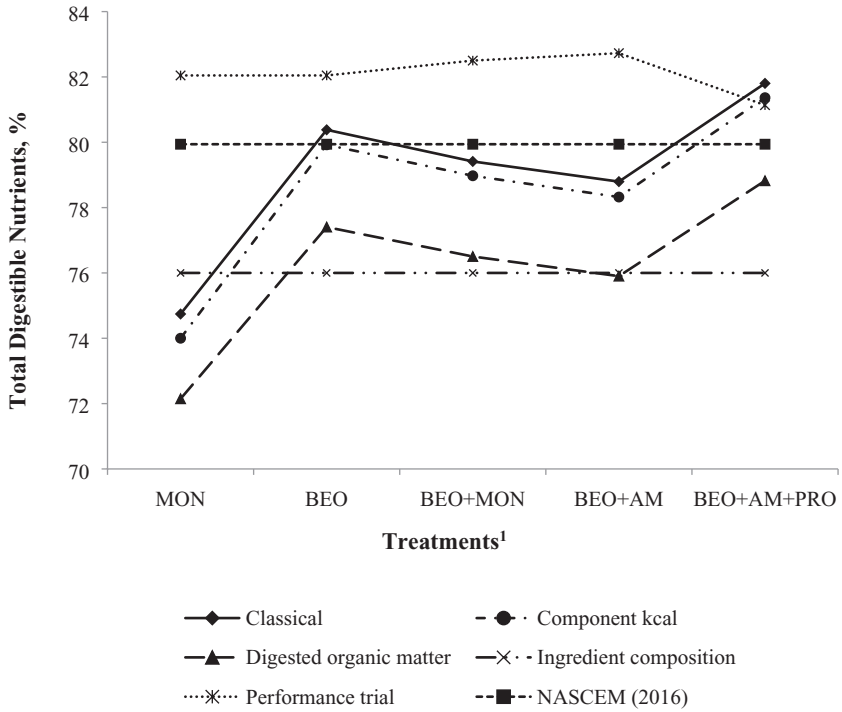

Figure 1. Diet total digestible nutrients (%) from various estimates methods: (1) Classical: calculated on the basis of digested carbohydrate equivalent, assuming that crude protein has a value equal to that of carbohydrate. (2) Digested organic matter: considering that 1 kg of TDN is obtained from 4.4 Mcal of organic matter digested. (3) Component kcal: assuming that digestible energy 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). (6) Performance trial: based on estimated net energy of maintenance values calculated from DMI and ADG of bulls in the 93 d feeding trial based on animal performance (Zinn and Shen, 1998). [1]MON = sodium monensin (26 mg/kg 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.

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, and 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; 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 were 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 kilogram per day. 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; 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 to 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 eight experimental units per 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 (Ballou 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. The 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. (2003), 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 (Wallace and Kopecny, 1983; Colombatto et al. 2003) 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 United States. 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.

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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