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Ruminants

Animal performance and nutrient digestibility of feedlot steers fed a diet supplemented with a mixture of direct-fed microbials and digestive enzymes

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ABSTRACT - The objective of this study was to evaluate the effect of a mixture of direct-fed microbials (DFM), yeast, and digestive enzymes on animal performance in feedlot cattle, as well as digestibility when finishing feedlot cattle. Thirty crossbreed (Charolais × Beefmaster) steers averaging 15 months old and 321.83±3.73 kg of initial body weight were used. Animals were randomly assigned to one of two treatment groups: control (basal diet) and a mixture of DFM, yeast, and digestive enzymes (basal diet + 30 g of the mixture). Each group contained fifteen experimental units. Animals were fed individually twice a day, and dry matter intake (DMI) was recorded daily. Body weights were recorded initially and subsequently at 28-day intervals for a total of 140 days to evaluate average daily gain (ADG). The gain:feed ratio (G:F) was also calculated per period. Dry matter digestibility, crude protein (CPD), and neutral detergent fiber (NDFD) were evaluated in the finishing phase. Ten steers per treatment were randomly selected for digestibility evaluations. Insoluble acid detergent fiber was used to calculate apparent digestibility. A completely randomized design with measurements repeated over time was used to evaluate animal performance, and a completely randomized design was used to evaluate apparent digestibility. No effect of treatment was observed for DMI. At the end of the trial, differences for ADG were found between treatments, with higher values in control than the treatment group. However, no effect for G:F was found. Dry matter digestibility, CPD, and NDFD were similar between treatments. The addition of the mixture of DFM, yeast, and digestive enzymes as a feed supplement in the diet of feedlot cattle does not improve animal performance and nutrient digestibility.

Key Words: enzymes, finishing, probiotics, *Saccharomyces cerevisiae*, yeast

Introduction

Feedlot cattle system is a common and important technique for meat production in Mexico. Feedlot activity is a major source of meat, which is considered a highly nutritious and valued food (Scollan et al., 2006), which are important characteristics for consumers and producers, respectively. Phelps et al. (2015) proposed that the goal of the beef industry is to produce a consistent, high-quality product as efficiently as possible. Different animal nutrition strategies, such as the use of different additives (Avendaño-Reyes et al., 2006; Bryant et al., 2010; Thompson et al., 2016), are practiced to increase efficiency in the feedlot.

Recently, concern about the use of antibiotics and other substances in animal feed has increased. For this reason, the use of direct-fed microbials (DFM) has been considered as a strategy for finishing feedlot cattle (Elam et al., 2003). Fuller (1989) defined probiotics as live supplements that benefit the host animal by improving its intestinal microbial balance. However, this definition does not consider the pre-existing ruminal microbial population. To address this deficiency, Kmet et al. (1993) defined ruminal probiotics as live cultures of microorganisms that are deliberately introduced into the rumen aiming at improving animal health or nutrition. The terms probiotics and DFM are often used interchangeably.

Probiotics are classified as viable microbial cultures, enzyme preparations, culture extracts, or combinations of the above (Yoon and Stern, 1995), and include both fungal and bacterial cultures (Krehbiel et al., 2003). Depending on the bacteria strain, they are classified as lactate acid-producing, lactate acid-utilizing, or other microorganisms (Seo et al., 2010).

When lactate acid-producing or lactate acid-utilizing bacteria are added to feedlot cattle diets, their use has been shown to improve feed efficiency (G:F) and daily

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gain (Galyean et al., 2000). It has been assumed that the presence of these strains of bacteria can encourage the adaptation of ruminal microorganisms to the presence of lactic acid, expediting its utilization (Yoon and Stern, 1995). Another theory holds that the production responses attributed to yeast are related to the stimulation of cellulolytic and lactate-utilizing bacteria; these responses include increased fiber digestion and microbial protein flow from rumen (Martin and Nisbet, 1992; Newbold et al., 1996).

Krehbiel et al. (2003) reported that the use of probiotics in animal feeding increases average daily gain (ADG) by 2.5 to 5% and G:F by 2% in feedlot cattle compared with a control group. Nonetheless, the results reported in the literature are inconsistent. Little information is available about the use of a mixture of DFM, yeast, and digestive enzymes on feedlot cattle. The objective of this study was to evaluate the effect of a mixture of DFM, yeast, and digestive enzymes on animal performance in feedlot cattle and on feed digestibility during the finishing process.

Material and Methods

All procedures involving animals were in accordance with both local official techniques for animal care (NOM-051-ZOO-1995: Humanitarian care of animals during mobilization; NOM-024-ZOO-1995: Animal health stipulations and characteristics during transportation) and the institutional code for Bioethics Regulation of Animal Welfare (case number: CFTZyE-ACTA-101/2015: ACUERDO 4.2).

The experiment was performed in Tepatitlán de Morelos, Jalisco, Mexico (20°47'46.1" N, 102°41'20.7" W, and 1,880 m altitude) from February to July, 2016. Thirty crossbreed (Charolais × Beefmaster) steers averaging 15 months old and 321.83±3.73 kg initial body weight (BW) were used. At the beginning of the experiment, all steers were identified, vaccinated for *Clostridium chauvoei*, *C. septicum*, *C. novyi*, *C. sordelli*, *C. perfringens*, *Pasteurella haemolytica*, and *P. multocida* A and D (Bacterina toxoide 8 vías; Laboratorios Pier S. A. de C. V.; Puebla, México), treated for internal and external parasites with ivermectin (Ivomec; Merial de México S. A. de C. V.; Querétaro, México), implanted (200 mg of trenbolone acetate and 28 mg estradiol benzoate; MaxiChoice 200; Lapisa S. A. de C. V.; Michoacán, México), and given vitamin A, D, and E supplements. Animals received a second vaccination and implant on day 84. Steers were kept outdoors in individual pens (13 m^2) and were randomly assigned to one of two treatments. Treatments consisted of control (basal diet; Table 1) and a mixture of DFM, yeast, and digestive enzymes (DFM; basal diet + 30 g $animal^{-1}$ day^{-1} of the additive) (Tables 1 and 2). Fifteen experimental units were considered for each treatment. Across the experiment, three diets were provided (Table 1). Animals were fed individually twice a day (07:00 and 17:00 h) with an adjustment based

Table 1 - Ingredients and calculated chemical composition (DM basis) of diets

Ingredient	Phase 1 (g kg^{-1} of DM)	Phase 2 (g kg^{-1} of DM)	Phase 3 (g kg^{-1} of DM)
Corn silage	263.4	175.0	-
Corn stover	200.0	158.5	253.1
Ground corn grain	356.8	476.3	675.0
Dry distillers grains	115.4	149.2	20.0
Soybean meal	50.0	30.0	30.0
Mineral premix[1]	9.4	6.0	5.0
Calcium carbonate	5.0	5.0	5.0
Magnesium oxide	-	-	5.0
Sodium bicarbonate	-	-	5.0
Sodium chloride	-	-	1.9
Calculated chemical composition			
DM (g kg^{-1} as fed)	581.0	657.8	876.1
NE_m (Mcal kg^{-1})	1.82	1.93	1.93
NE_g (Mcal kg^{-1})	1.19	1.28	1.28
CP (g kg^{-1} of DM)	120.0	120.0	100.5
Ca (g kg^{-1} of DM)	5.5	4.6	3.4
P (g kg^{-1} of DM)	3.2	3.1	2.8
K (g kg^{-1} of DM)	8.1	6.7	6.9
EE (g kg^{-1} of DM)	39.0	43.5	34.6
DE (Mcal kg^{-1})	3.30	3.46	3.43
ME (Mcal kg^{-1})	2.70	2.84	2.81
TDN (g kg^{-1} of DM)	729.0	760.1	743.0
NDF (g kg^{-1} of DM)	350.1	302.5	239.0

DM - dry matter; NE_m - net energy of maintenance; NE_g - net energy of gain; CP - crude protein; EE - ether extract; DE - digestible energy; ME - metabolizable energy; TDN - total digestible nutrients; NDF - neutral detergent fiber.
[1]Mineral premix: CP, 200 g kg^{-1}; Ca, 200 g kg^{-1}; P, 15 g kg^{-1}; K, 4 g kg^{-1}; Mg, 6.5 g kg^{-1}; Na, 50 g kg^{-1}; S, 0.9 g kg^{-1}; Cu, 11 mg kg^{-1}; Fe, 314 mg kg^{-1}; Mn, 14 mg kg^{-1}; Zn, 24 mg kg^{-1}; I, 0.08 mg kg^{-1}; Co, 0.5 mg kg^{-1}; Se, 0.2 mg kg^{-1}; vitamin A, 48 IU g^{-1}; vitamin D, 200 IU g^{-1}; vitamin E, 0.17 IU g^{-1}.

Table 2 - Composition of the mixture

Ingredient	Quantity[1]
Amylase (units)	3,000
Protease (units)	400
Cellulose (units)	160
Lipase (units)	120
Peptinase (units)	80
Lactase (units)	1.8
Lactobacillus acidophilus (cfu)	3.6×10^7
Bifidobacterium thermopilum (cfu)	3.6×10^7
Bifidobacterium longum (cfu)	3.6×10^7
Enterococcus faecium (cfu)	3.6×10^7
Saccharomyces cerevisiae (cfu)	8×10^6

cfu - colony-forming units.
[1]Quantity of direct-fed microbials, yeast, and digestive enzymes for each 30 g of product.

on refusal from 50 to 100 g kg^{-1} as fed. At the beginning of the experiment, animals underwent an adaptation period of 15 days to adapt to the diet. Diets were mixed daily and contained at least 120, 120, and 100.5 g kg^{-1} of crude protein (CP) and 1.19, 1.28, and 1.28 Mcal of net energy gain for each phase, respectively (NRC, 2000). Animals were allowed free access to water. In the last period (28 days), zilpaterol hydrochloride (Grofactor, Virbac México S. A. de C. V.; Jalisco, Mexico; 0.15 mg kg^{-1} of BW) and buffers (Table 1) were added to the control diet.

Body weights were recorded initially and subsequently at 28-day intervals for a total of 140 days to evaluate ADG. Dry matter intake (DMI) was recorded daily. Feed efficiency was calculated per period.

Feed and refusal samples were taken daily and composited for each period. Samples were ground in a Wiley mill (1-mm screen; Wiley mill model 4, Thomas Scientific, Swedesboro, NJ) and subjected to all or part of the following analysis: DM, organic matter, and CP (methods numbers 930.15, 942.15, and 990.02, respectively; AOAC, 2003). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined sequentially according to Van Soest et al. (1991) using an Ankom 200 fiber analyzer (Ankom Technology, Fairport, NY).

Digestibility of DM, CP, and NDF was evaluated. At the end of the performance trial, ten steers per treatment were randomly selected and kept outdoors in the pens used for the performance trial (13 m^2). The same schedule and feeding regime were followed. The digestibility trial consisted of three days of fecal sample collection. During collection, fecal samples were taken directly from the rectum four times daily as follows: day 1 – 08:00, 10:00, 12:00, and 14:00 h; day 2 – 16:00, 18:00, 20:00, and 22:00 h; and day 3 – 00:00, 02:00, 04:00, and 06:00 h (Castillo Rangel et al., 2017). Individual fecal samples weighed approximately 50 g (wet basis). Samples for each animal were composited for analysis and stored at −20° C.

Composited fecal samples were dried in a forced-air oven at 60 °C for five days. Feed, refusals, and fecal samples were ground in a Wiley mill (1-mm screen) and analyzed for DM, CP, ADF, and NDF as described previously. Feed and fecal samples were incubated in the rumen of cannulated heifers (Huhtanen et al., 1994). After incubation, bags were washed four times with cold water for 5 min and then dried (60 °C) for 24 h. The concentration of ADF remaining in the bag residue was determined to calculate the percentage of insoluble acid detergent fiber (IADF; Penning and Johnson, 1983).

Apparent DM digestibility was predicted using IADF according to the following formula (Schneider and Flatt, 1975):

$$DMD = 100 - \left[100 \times \left(\frac{\%\ IADF\ in\ feed}{\%\ IADF\ in\ feces}\right)\right]$$

Apparent digestibility of CP and NDF were calculated using the formula:

$$\text{Nutrient digestibility (ND)} = 100 - \left[100 \times \left(\frac{\%\ IADF\ in\ feed}{\%\ IADF\ in\ feces} \times \frac{\%\ of\ nutrient\ in\ feces}{\%\ of\ nutrient\ in\ feed}\right)\right]$$

Data for ADG, DMI, and G:F were analyzed via a completely randomized design, with measurements repeated over time using the MIXED procedure of SAS (Statistical Analysis System, version 9.1.3). Animals were the experimental units. When significant ($P<0.05$) F-statistics were noted, means were separated using least square differences method.

The mathematical model was:

$$Y_{ijk} = \mu + \tau_i + d_j + i_k(\tau_i) + \Theta_{ij} + e_{ijk},$$

in which Y_{ijk} = observed value of the variable that received the treatment; μ = overall mean; τ_i = effect of treatment; d_j = effect of the day of measurement; $i_k(\tau_i)$ = effect of animal within treatment; Θ_{ij} = effect of the interaction between treatment and day; and e_{ijk} = random error associated with each observation.

Data concerning the digestibility of DM, CP, and NDF were analyzed via a completely randomized design using the GLM procedure. Animals were the experimental units. When significant ($P<0.05$) F-statistics were noted, means were separated using least square differences method.

The mathematical model was:

$$Y_{ij} = \mu + \tau_i + e_{ij},$$

in which Y_{ij} = observed value of the variable that received the treatment; μ = overall mean; τ_i = effect of treatment; and e_{ij} = random error associated with each observation.

Results

For DMI, no differences ($P>0.05$) were found between treatments. No interaction ($P>0.05$) between treatment and day was found. At the end of the trial, ADG was greater ($P<0.05$) for the control group, although no effect ($P>0.05$) for G:F was found. For the apparent digestibility of DM, CP, and NDF, no differences ($P>0.05$) were found between treatments. Due to the equal DMI and similar digestibility, digestible dry matter intake (DDMI), digestible crude protein intake (DCPI), and digestible neutral detergent fiber intake (DNDFI) were similar ($P>0.05$) between treatments.

Discussion

The diets used in the present experiment are common for feedlot cattle in the central and northern parts of Mexico. The results of the present experiment are consistent with other authors. Different studies did not find variations in DMI when steers received probiotics in the diet (Vasconcelos et al. 2008; Stephens et al., 2010; Narvaez et al. 2014; Cull, et al., 2015; Kenney et al., 2015; Wilson et al., 2016). In these studies, additives based only on probiotics were provided (lactate acid-producing bacteria, lactate acid-utilizing bacteria, or both in combination). In those experiments, bacteria such as *Enterococcus faecium*, *Lactobacillus acidophilus*, and *Propionibacterium freudenreichii* were the primary cultures used. Similar results for DMI were reported by Swyers et al. (2014) when *Saccharomyces cerevisiae* was used in feedlot cattle. These authors attributed the similarities to animal discomfort during the experiment compared with studies in which animals were not subjected to stressful factors. During this experiment, steers were exposed to constant rain, which produced stress and had an impact on animal performance. These stress factors should be an effect in which the DFM could improve animal performance, due to an increase in fiber digestibility. Yeast feeding is a common practice in feedlot reception for both steers and heifers due to the resulting improvement in DMI (Lesmeister et al., 2004) and the positive impact on ruminal microbiota, increasing dry matter digestibility (Brown and Nagaraja, 2009), and reducing the effect of stress. In the present experiment, steers were adapted to the feedlot management at the beginning of the experiment. It is assumed that DFM did not have an impact on ruminal microbiota. Conversely, Ponce et al. (2011) reported an improvement in DMI and ADG when heifers fed in feedlot received diets with a mixture of lactate acid-producing bacteria and digestive enzymes, which differ from the results of the present experiment. There are similarities between the results of this study and the work of Stephens et al. (2010), in which a combination of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* was given to feedlot cattle and led to no differences reported between a group using DFM and a control group for DMI, ADG, and G:F. When enzymes such as amylase were added to the additive used in this study, an improvement in nutrient digestibility was expected. Due to this improvement, an improvement in animal performance was also expected. Nonetheless, DFM did not lead to better performance. The data presented here agree with those reported by Krehbiel et al. (2003), who concluded that the response of animals that received DFM is inconsistent for DMI.

As mentioned above, similar ADG between treatments for each period was obtained. However, an unexpectedly higher ADG was obtained in the control group at the end of the trial and associated with the tendency towards higher DMI for this group (Table 3). The reason for a reduced ADG in animals of the group fed DFM is not clear. Similar results were shown by Swyers et al. (2014), who found a reduction in ADG in steers receiving *Saccharomyces cerevisiae* during 125 days of a finishing feedlot diet; they reported that stress factors were not present during the experiment. The conditions differed from those of this study, in which the presence of mud represented a stress factor for a long period of time during the experiment. Ponce et al. (2011) reported a greater ADG in steers with diets supplemented by a mixture of lactate acid-producing bacteria and digestive enzymes for 140 days in the feedlot compared with a control group. To our knowledge, information about the effect of using DFM-enzyme mixtures is limited. It was assumed that their use would increase nutrient digestibility, which could in turn improve animal performance, but a different effect was found. The use of DFM usually increases nutrient digestibility in animals fed diets with high content of fiber, which differ

Table 3 - Performance of steers fed diet supplemented or not with direct-fed microbials (DFM)

Item	Control	DFM[1]	SEM	P-value
DMI (kg/day)				
1-28	9.79a	9.20a	0.39	0.281
29-56	11.76a	11.29a	0.39	0.386
57-84	12.36a	11.82a	0.40	0.338
85-112	12.10a	11.42a	0.40	0.227
113-140	11.77a	11.64a	0.40	0.810
Average	11.56a	11.07a	0.31	0.284
ADG (kg)				
1-28	2.30a	1.83b	0.12	0.005
29-56	2.00a	1.88a	0.12	0.485
57-84	1.79a	1.82a	0.12	0.874
85-112	1.77a	1.45a	0.12	0.065
113-140	0.94a	0.82a	0.12	0.486
Average	1.76a	1.56b	0.06	0.026
G:F				
1-28	0.233a	0.200b	0.01	0.018
29-56	0.172a	0.168a	0.01	0.806
57-84	0.143a	0.152a	0.01	0.535
85-112	0.146a	0.127a	0.01	0.182
113-140	0.078a	0.065a	0.01	0.355
Average	0.154a	0.143a	0.01	0.114

DMI - dry matter intake; ADG - average daily gain; G:F - feed efficiency; SEM - standard error of the mean.
[1]Effect of the addition of a mixture of DFM, yeast, and digestive enzymes.
Means in the same row with different superscripts are significantly different ($P<0.05$).

from the conditions of the present experiment. Similar results have been reported by several other authors, who did not find differences in ADG between treatments as a result of DFM use in animal feeding (Neuhold et al., 2012; Narvaez et al., 2014; Cull et al., 2015; Kenney et al., 2015).

Reports for G:F are not consistent. Ponce et al. (2011) found similar G:F when feedlot cattle received a mixture of DFM and digestive enzymes (amylase, proteases, hemi-cellulases, phytase, cellulose, lipase, pectinase, and glucanase), and results were compared with a control group; these results are in agreement with our experiment. Additionally, recent studies have not found improvements in G:F associated with use of DFM (Neuhold et al., 2012; Narvaez et al., 2014; Kenney et al., 2015; Wilson et al., 2016). Similarly, when *Saccharomyces cerevisiae* (Swyers et al., 2014; Carrasco et al., 2016), or its combination with *Lactobacillus acidophilus* (Stephens et al., 2010) were given as supplements to feedlot cattle, these supplementations did not have an effect on G:F. However, Aydin et al. (2009) reported an increase in G:F when Holstein steers received a combination of DFM and digestive enzymes. Vasconcelos et al. (2008) and Cull et al. (2015) added *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* to a feedlot cattle diet and reported an associated improvement in G:F. Krehbiel et al. (2003) reported that the use of DFM increased ADG and G:F at levels of 5 and 2.5%, respectively, compared with a control group. Due to the inconsistency in the literature, it is recommended that for the use of DFM in ruminant feeding, one should consider factors such as health status, stress factors, diets, and dosage, among others.

In contrast, Tricarico et al. (2007) supplemented amylase in a diet for feedlot steers and found no increase in G:F, which is in agreement with the results found in this experiment. In this study, due to the characteristics of the supplement, it was expected that the use of DFM, yeast, and digestive enzymes might increase digestibility of DM, CP, and NDF, which could have an impact on animal performance. Similar results have been reported when *Lactobacillus acidophilus* and *Enterococcus faecium* (Kenney et al., 2015), *Enterococcus faecium* or its combination with *Saccharomyces cerevisiae* (Beauchemin et al., 2003), *Saccharomyces cerevisiae* (Monnert et al., 2013), *Enterococcus faecium* and *Propionibacterium* (Ghorbani et al., 2002), and a mixture of lactate acid-producing bacteria and digestive enzymes (Ponce et al., 2011) were supplemented to diets of feedlot cattle; however, this research reported no differences in nutrient digestibility compared with a control group (Table 4). To our knowledge, information related to digestible DM intake, digestible CP intake, and digestible NDF intake is not yet available. Due to the relationship between DMI and nutrient digestibility, the supplementation of this study was expected to increase the availability of nutrients for the ruminant and, consequently, to markedly improve animal performance; these results were not found.

Table 4 - Nutrient digestibility and their intake by steers supplemented with and without direct-fed microbials (DFM)

Item	Control	DFM[1]	SEM	P-value
Aparent DMD (g kg^{-1} of DM)	830.47	834.11	1.109	0.819
Aparent CPD (g kg^{-1} of DM)	769.31	774.81	1.354	0.777
Aparent NDFD (g kg^{-1} of DM)	543.59	558.85	3.223	0.741
Digestible DMI (g kg^{-1} of DM)	87.48	91.69	0.520	0.574
Digestible CPI (g kg^{-1} of DM)	8.13	8.542	0.050	0.571
Digestible NDFI (g kg^{-1} of DM)	9.90	10.64	0.066	0.445

DM - dry matter; DMD - dry matter digestibility; CPD - crude protein digestibility; NDFD - neutral detergent fiber digestibility; DMI - dry matter intake; CPI - crude protein intake; NDFI - neutral detergent fiber intake; SEM - standard error of the mean.
[1]Effect of the addition of a mixture of DFM and digestive enzymes.

Conclusions

Supplementing feedlot cattle diets with a mixture of direct-fed microbials and digestive enzymes does not improve animal performance and nutrient digestibility. Its use for finishing feedlot cattle is not recommended.

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