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Impact of different additives on ruminal acidosis and fermentation of lams

Impacto de diferentes aditivos sobre la acidosis y fermentación ruminal en corderos

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ABSTRACT

The objective was to evaluate the ruminal fermentation and acidosis on finishing lambs supplemented with different additives. Four ruminal cannulated lambs were used. Treatments were 1) Control (Basal diet); 2) LEV (Basal diet + *Saccharomyces cerevisiae*); 3) ION (Basal diet + Sodic monensin); 4) TE (Basal diet + Experimental Treatment). Diet contained at least 14 % de CP and 2.962 Mcal/kg de ME. Dry matter intake (DMI) was evaluated. In ruminal fluid samples, pH, ammonia concentration (NH_3), and volatile fatty acids concentration (VFA) were evaluated. Data was analyzed in a 4 x 4 latin square design. Ruminal pH, VFA concentration and acetic:propionic ratio was not different (P>0.05) among treatments. Dry matter intake was similar (P>0.05) among treatments. Ruminal pH was different (P < 0.01) among hours. Interaction treatment x hour was different (P<0.05) for NH_3 concentration, acetic, propionic, butyric acids, and total VFA´s concentrations. The use of a mixture of probiotics, prebiotics and symbiotic, did not improve ruminal fermentation of finishing hair lambs.
Keywords: ovine, feed lot, probiotics, prebiotics, symbiotics.

RESUMEN

El objetivo fue evaluar la acidosis y fermentación ruminal en ovinos en fase de finalización, suplementados con diferentes aditivos. Se usaron 4 borregos fistulados en rumen. Los tratamientos fueron: 1) Control (Dieta basal); 2) LEV (Dieta basal + *Saccharomyces cerevisiae*); 3) ION (Dieta basal + Monensina sódica); 4) TE (Dieta basal + Mezcla de probióticos, prebióticos, sinbióticos y enzimas). La dieta contenía 14 % de PC y 2.962 Mcal/kg de EM. Se evaluó el consumo de materia seca (CMS. En muestras de líquido ruminal se evaluó el pH, la concentración de nitrógeno amoniacal (NH_3), y la concentración de ácidos grasos volátiles (AGV´s). La información fue analizada mediante un diseño en cuadrado latino 4 x 4. El pH, la concentración de AGV´s, y la relación acético:propiónico no fueron diferentes (P>0.05) entre tratamientos. El CMS fue igual entre tratamientos (P>0.05). El pH fue diferente (P < 0.01) entre hora. La interacción tratamiento x hora (P<0.05) fue diferente para NH_3. Para las concentraciones de ácido acético, propiónico butírico y total de ácidos grasos volátiles se encontró diferencia (P< 0.05) para la interacción tratamiento × hora. El uso de la mezcla de probióticos, prebióticos y simbióticos no mejora la fermentación ruminal de corderos en la fase de finalización.
Palabras clave: ovinos, corral de engorda, probióticos, prebióticos, simbióticos.

INTRODUCTION

Currently, sheep production systems use feed additives to improve ruminal fermentation and feed digestion. These improvements increase productive performance (Kiran & Deswal, 2020), in addition to mitigating ruminal acidosis and tympanism (Chiquette, 2009). Among the additives commonly used in production systems are ionophores, which are a group of anthobiotics that selectively modify the ruminal microbiota, improving feed efficiency (Guan *et al*., 2006); probiotics (Chiquette, 2009), which promote microbial balance through three mechanisms: 1) competitive exclusion; 2) bacterial antagonism; 3) immuno-modulation (Molina, 2019); and prebiotics (Zhang *et al*., 2014) which are non-digestible substances that provide a benefit to the host by selectively stimulating the growth of a group of indigenous bacteria (Guzmán *et al*., 2012). Given the ability to modulate the rumen environment (Jiao *et al*., 2018), the use of these products has become widespread, especially when animals are fed high-grain diets.

The high intake of rapidly fermenting carbohydrates results in an accumulation of organic acids in the rumen, which brings about a decrease in ruminal pH (Nagaraja & Lechtenberg, 2007). The decrease in ruminal pH generates a subclinical acidosis or in a more severe case acute acidosis, which can result in the animal death (Granja *et al*., 2012). The inclusion of certain additives such as ionophores in ruminant feed has a positive effect in mitigating ruminal acidosis (Chiquette, 2009). Different additives such as probiotics and prebiotics have been used to improve animal performance, covering important aspects in production systems such as animal health and increased production (Vyas *et al*., 2014). Nowadays, the use of symbiotics has been explored in monogastrics, however, the impact of these products on ruminal fermentation is unknown. Derived from this, it was hypothesized that the inclusion of a mixture of additives (probiotics, prebiotics and symbiotics) alters ruminal fermentation mitigating ruminal acidosis in high concentrate diets. The objective was to evaluate ruminal acidosis and ruminal fermentation in sheep in the finishing phase supplemented with different additives.

MATERIAL AND METHODS

All procedures used in the present experiment were performed according to the Mexican Official Standards NOM-051-ZOO-1995, Standard for humane treatment in the mobilization of animals; NOM-024-ZOO-1995, Animal health specifications and characteristics for the transport of animals, their products and by-products, chemical, pharmaceutical, biological and food products for use in animals or consumption by them; NOM-062-ZOO-1999. Technical specifications for the production, care and use of laboratory animals, and to the institutional code for the regulation of bioethics and animal welfare (CFTZYE-ACTA-101/2015: AGREEMENT 4.2). The study was conducted at the School of Animal Husbandry and Ecology, Autonomous University of Chihuahua (latitude 28° 35' 10.9'' north; longitude 106° 6' 26.6'' west; altitude 1440 m a.s.l).

Animals, treatments and feeding

Before starting the experiment, animals were dewormed against external and internal parasites (Iverfull®; Aranda Salud Animal, Querétaro, Querétaro, Mexico), vaccinated (Bacterina triple bovina®; Bio-Zoo) and vitaminized (Vigatol ADE Fuerte®; Bayer); the drugs were applied intramuscularly. Four 10-month-old rumen-fistulated Pelibuey bighorn sheep with an average initial weight of 45 kg ± 2 kg were used. The sheep were randomly assigned to a metabolic cage. Treatments were: 1) Control (basal diet; Table 1); 2) LEV (basal diet + .20 g of *Saccharomyces cerevisiae*/animal/day; 3) ION (basal diet + 0.10 g of Monensin sodium/animal/day); 4) HP (basal diet + 10 g of Mixture of probiotics, prebiotics, symbiotics and enzyme (Hp Ruminal Health)/animal/day; Table 2). The additives were fed daily in two feedings through the rumen fistula prior to the offering of each feed.

Table 1. Ingredients and chemical composition of the basal diet

Ingredient	Total diet %
Rolled corn	63.80
Canola	11.26
Molasses	2.38
Miceral premix	0.48
Salt	0.48
Calcium Carbonate	0.23
Sodium Bicarbonate	0.843
Alfalfa	20.84
Calculated chemical composition	
CP, %	14.00
ME, mcal/kg	2.962

Lambs were subjected to an individual feeding scheme. The experiment was carried out under a Latin square experimental design with four periods (12 days per period). The animals received a nine-day adaptation to the diets at the beginning of each period. Treatments were randomly rotated for each of the sheep during the four periods, with each treatment represented by one animal per period.

Table 2. Chemical composition of the mixture

Ingredients	Quantity[1]
Amylase (units)	1.000
Protease (units)	133.33
Cellulases (units)	53.33
Lipase (units)	40
Peptinase (units)	26.66
Lactase (units)	0.60
Lactobacillus acidophilus (ufc)	1.2×10^7
Bifidobacterium thermophilum (ufc)	1.2×10^7
Bifidobacterium longum (ufc)	1.2×10^7
Enterococcus faecium (ufc)	1.2×10^7
Sacharomyces cerevisiae (ufc)	2.6×10^6

cfu: colony forming units
[1]Amount of microorganisms provided directly in the feed, yeasts, and digestive enzymes per 10 grams of product

Feed was offered twice a day (0800 and 1800 h), adjusting to a 5-10 % rejection. The lambs were provided with clean water throughout the day. The forage-concentrate ratio was 20-80 % respectively. The diet was formulated to contain at least 14 % CP and ME of 2.962 Mcal/kg (Table 1). Concentrates were made and mixed at one time for the total test. Diets were prepared with locally available ingredients, having rolled corn as the concentrate base and alfalfa as the forage source.

Variables evaluated and sampling

Dry matter intake (DMI; g) was evaluated daily from the tenth day of each period and during the following days, which corresponded to rumen fluid sampling days. On the first day of sampling (tenth day of each period), a 200 mL sample of rumen liquid was obtained under the following schedule: 0, 1, 2, 4, 8, 12, 18, 24 hours post feeding; considering zero hours before the morning offering of feed (0800 h).

The liquid sample was evaluated for pH, concentration (m/M/lt) of ammoniacal nitrogen (NH_3), and concentration (mM/lt) of volatile fatty acids (acetic, propionic and butyric) in which the fermentative balance allowed estimating the production of methane and carbon dioxide by the conversion of dietary carbohydrates to VFA's (Wolin, 1960).

Laboratory analysis

The pH of the rumen liquid was measured with a potentiometer (UltraBASIC pH/mV Meter; Denver Instrument), immediately after the sample was extracted. Subsequently, four subsamples of 15 ml of ruminal liquid were obtained in sterilized tubes containing 1 ml of 50 % sulfuric acid for subsequent analysis in the chromatograph.

A new sample of rumen liquid was also extracted, which was filtered on a screen and then filtered again with 601 grade filter paper. These samples were immediately frozen at -20 °C after collection until further analysis. The NH_3 concentration was then determined (Broderick & Kang, 1980).

The concentration of volatile fatty acids was measured: acetic, propionic and butyric acids, for which a previous thawing and centrifugation was carried out for 20 minutes at 13800 ×g of the samples; with a temperature of 4 ºC, to again carry out a filtering process (Ahlstrom filter paper grade 601). From the surplus, the sample was prepared with 25% metaphosphoric acid, in a sample: acid ratio of 5:1.

The determination of VFA concentrations was carried out by gas chromatography (GC) with flame ionization detection (Galyean, 2010). For this purpose, a Claurus 400® gas chromatograph (Perkin Elmer) using a Varian capillary CP-wax58(FFAP)CB(15 m × 0.53 mm, 0.5 um) column was used.

Statistical analysis

The data collected for the variables pH, NH_3, molar percentage of VFA's, CH_4 and CO_2 were analyzed by a 4 × 4 Latin square design using the MIXED procedure of SAS (Statistical Analysis System version 9.1.3) fitting a model that included the effects of treatment, period, time and the treatment by time interaction. Sheep, period, time and treatment were considered as classificatory variables. On the other hand, the effect of sheep within treatment was considered random.

The model used was:

$yijk = \mu + \tau i + \rho j + \sigma k + \Theta ik + eijk.$

Where: yijk = observed value of the response variable; μ = overall mean; τi = is the effect of treatment); ρj = effect of period; σk = effect of time; Θik = interaction between treatment × time; eijk = Random error associated with each observation.

Least squares means and standard error were reported for these variables. Differences were denoted when ($P < 0.05$).

For DMI the information was analyzed by a 4 × 4 Latin square design using the MIXED procedure of SAS (Statistical Analysis System version 9.1.3) fitting a model that included the effects of treatment, period, day and the treatment × day interaction. Sheep, period, day and treatment were considered as classificatory variables. On the other hand, the sheep within-treatment effect was considered random.

The model used was:

$y_{ijk} = \mu + \tau_i + \rho_j + \sigma_k + \Theta_{ik} + e_{ijk}$.

Where: y_{ijk} = observed value of the response variable; μ = overall mean; τ_i = is the treatment effect; ρ_j = period effect; dk = day effect; Θ_{ik} = treatment × day interaction effect; e_{ikj} = random error associated with each observation.

Least-squares means and standard error were reported for this variable. Differences were denoted when ($P < 0.05$).

RESULTS AND DISCUSSION

For pH, no difference was found between treatments, period and treatment × hour interaction ($P > 0.05$), however, a difference ($P < 0.01$) was found in the hour effect. The values found (Figure 1) show that the pH behavior after two hours of feeding decreased from 6.0; in addition, at hour 12 the CON and TE treatments decreased from 5.5, putting the animals at risk of subacute acidosis. Several studies have concluded that a pH below 5.5 directly impacts the rumen health of lambs (Jaramillo-López *et al*., 2017; Harlow *et al*., 2017). A factor to consider in ruminants when fed high grain diets is the time in which pH is below two levels (5 and 5.5; Hibbard *et al*., 1995) as this is when animals enter a stage of acute or sub-acute acidosis, respectively (Harlow *et al*., 2017). In the case of the present experiment, the animals of the different treatments remained above 5 (Figure 1), resulting in no symptomatic manifestations of acute acidosis.

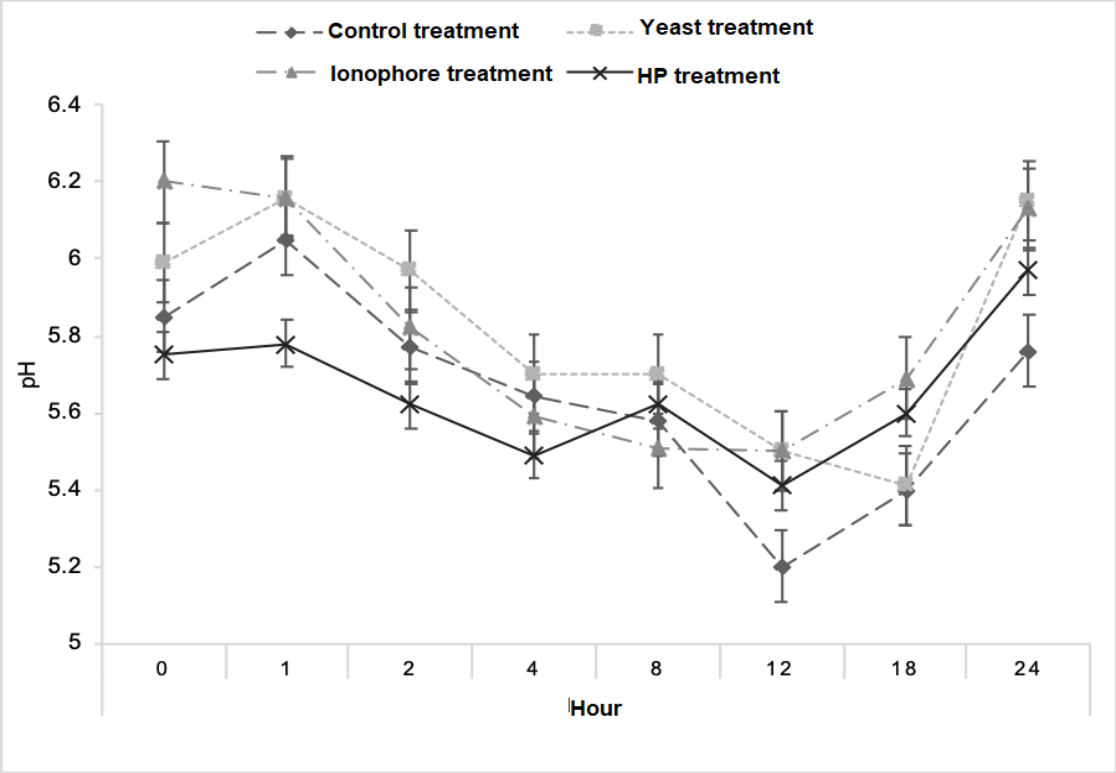

Figure 1. Average pH of each treatment per hour

According to the values found, we can see that lambs of all treatments reached the threshold of subacute acidosis (Figure 1). It was observed that the decreases occurred from 8 h after the first offering of feed (Figure 1). A period of 111 to 180 min during 24 h, where pH remains below 5.5 and above 5.0 is sufficient to declare subacute acidosis (Jaramillo-López *et al*., 2017). Given the established sampling schedules, such behavior is shown between hours 12 and 18 after the first feed offering, however, the precise time of the feeding was not recorded. The presence of subacute acidosis may have clinical symptomatology involving the presence of diarrhea and loss of appetite, which generates a decrease in GDP (Jaramillo-López *et al.*, 2017; Vyas *et al*., 2014). This apparent symptomatology was not recorded in the present experiment. One of the risks associated with this type of acidosis is the increase of lactic acid bacteria in the rumen (Devant, 2015). These stop the activity of different bacterial populations (Kleen *et al*., 2003). Such changes in the populations result in an affection in the final fermentation products, which directly impacts the productive behavior of the animals (Commun *et al*., 2009; Kleen *et al*., 2003). As reported (Jaramillo-López *et al*., 2017; Jimeno *et al*., 2004; Kleen *et al*., 2003), the main factor associated with the presence of acidosis is the amount of starch and NDF present in the diet, which agrees with the results found in this study.

For DMI, no difference was found between treatments (P>0.05) or in the rest of the factors considered in the model. Diets used in the present experiment are common in intensive sheep fattening schemes in northern Mexico. In a study by Castillo Rangel *et al*. (2017) reported DMI similar to that found in this work. In another study by Álvarez *et al*., (2018) found no differences between treatments when they provided an additive based on probiotics and digestive enzymes to steers in the finishing phase. Such product was similar to the one used in this experiment. Data on the use of these types of additives is inconsistent (Swyers *et al*., 2014; Cull *et al*.,2015). One of the conditions that directly impact this variable is the type of diet provided.

Several studies in ruminants have found no difference in DMI when probiotics were provided in the diet (Cull *et al*., 2015; Wilson *et al* 2016). In those experiments the additives were based solely on probiotics (lactic acid producing bacteria, lactic acid utilizing bacteria or the combination between them). In addition, similar results have been reported for DMI when *Saccharomyces cerevisiae* strains were used in feedlot cattle diets (Swyers *et al*., 2014). In studies conducted in sheep, Mir & Mir (1994) found a reduction in DMI in lambs fed a high grain diet when compared to high forage diets. In this study, it can be observed how the increase in the level of soluble carbohydrates in the rumen has a direct impact on this variable. The accumulation of organic fatty acids in the rumen occurs due to the consumption of fast fermenting carbohydrates in large amounts (Commun *et al*., 2009). This type of diets can lead animals to fall into ruminal acidosis (Nagaraja & Letchenberg, 2007). It has been evidenced that this is a disorder that directly affects dry matter intake (Devant, 2015; Kleen *et al*., 2003; Granja *et al.*, 2012). In its subclinical form, ruminal acidosis directly affects productive behavior (Devant, 2015, Granja *et al.*, 2012). Such decrease is linked to alterations in fermentation patterns and the impact of decreased DMI (Commun *et al.*, 2009). As observed in the results, the additives employed were not able to prevent the decrease in ruminal pH for prolonged periods of time. It is assumed that this directly affected the behavior of the sheep, since the animals were in ruminal pH ranges considered as borderline or at risk of falling into subclinical acidosis (Chiquette, 2009).

The data for ammonia nitrogen concentration show that there was an effect for hour and treatment × hour interaction ($P < 0.05$). The behavior in rumen NH_3 concentration shows that the addition of *Saccharomyces cerevisiae* yeast provides greater stability in the rate of protein degradation and in the solubility of non-protein nitrogen. Similarly, it can be observed that the HP treatment gives a greater variation throughout the day in NH_3 concentration (Figure 2). The NH_3concentration in the rumen is a function of both the rate of degradation and the concentration of rumen degradable protein (RDP), in addition to the needs of microbial populations and the amount of energy available to rumen microorganisms (Hirstov, 2004). Increasing the amount of CP in the diet or the percentage of RDP results in an increase in NH_3 concentration (Davidson *et al*., 2003).

In this study, using the same basal diet in each treatment, the lambs were fed isonitrogenous feed, so we can infer that the variation in NH_3 concentration over time was a direct effect of the impact of the additives.

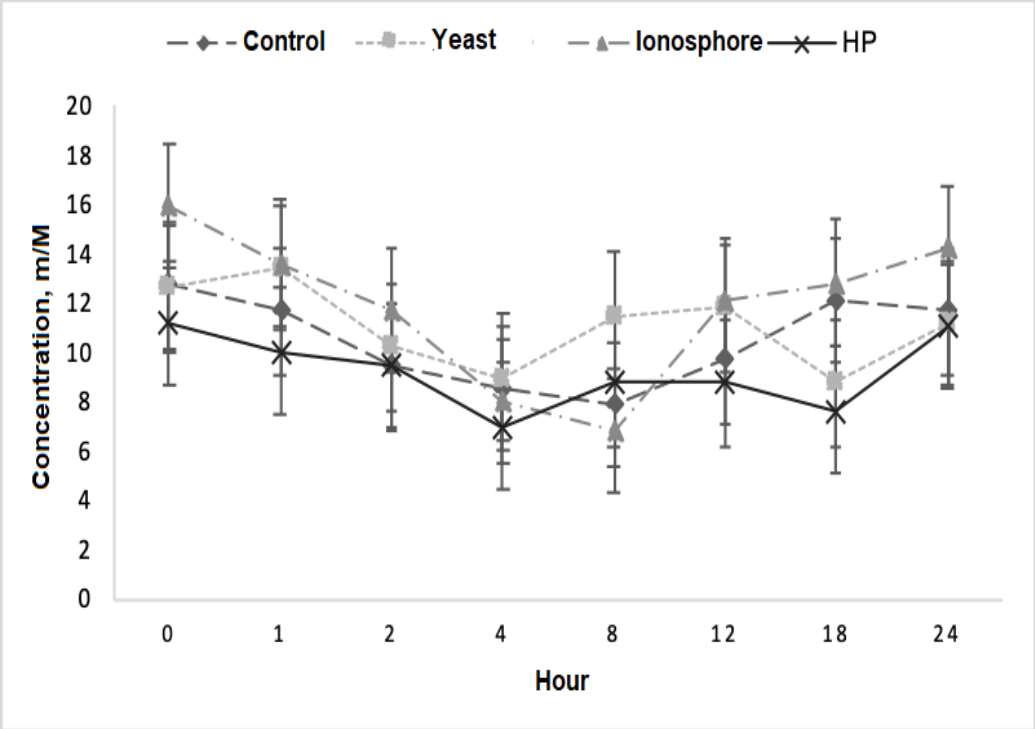

Figure 2. Hourly ammonia nitrogen concentration

One of the main effects of the treatments used is the one it exerts on microbial populations (Farghaly & Hamdon, 2018, Molina, 2019). Within the most important intrinsic factors that impact the development of microbial populations is pH. It has been observed that proteolytic bacteria are sensitive to pH below 6.0. Among the main benefits of the use of microbial additives added to the diet is the increase in DM (Jiao *et al*., 2017) and NDF (Gang *et al*., 2020) digestibility, resulting from their positive effect on the growth of cellulolytic microbial populations (Ruiz *et al*., 2016); however, their impact on CP digestibility is not clear (Baloyi *et al*., 2018). It has been reported, that for the best expression of the positive results of this type of additives (pre- and probiotics), is the use of diets high in forage (Mir & Mir, 1994, *Vyas et al*., 2014), however when used with diets high in concentrate and with optimal levels of CP and RDP the results have been inconsistent (Anele *et al*., 2017, Ellerman *et al*., 2017), which has been reflected in studies of ruminant productive behavior, where homogeneous results are not reported when high starch diets are used (Alvarez *et al*., 2018).

The information corresponding to acetic, propionic and butyric acid concentrations, total volatile fatty acids and acetic: propionic ratio, shows that there were no differences between treatments ($P > 0.05$; Table 3). However, for acetic, propionic, and butyric acid concentration, and total volatile fatty acids, a difference was found ($P < 0.05$) for the effects of period, time, and the treatment × time interaction. In the case of the acetic:propionic ratio, the effects of treatment and period were not different ($P > 0.05$). CH_4 production showed an effect for time and treatment × time interaction ($P < 0.05$) while treatment and period effects were equal ($P > 0.05$). In the case of CO_2 the effects that made a difference ($P < 0.05$) were period, hour and treatment × hour interaction, while there was no effect ($P > 0.05$) of treatment. One of the factors that is directly related to fermentation patterns is the digestibility of dry matter and neutral detergent fiber (Scholljegerdes, 2020). The inclusion of additives that include microorganisms or enzymes in their composition have a direct impact on the digestibility of the fibrous portion of the feed (Vyas *et al.*, 2014). In this experiment the diet provided was finisher, so the amount of grain had a direct impact on fermentation patterns. Such fermentation patterns directly impact the productive behavior of the animals (Shimada, 2015).

The use of yeast-based additives, probiotics and prebiotics is a common practice during the reception phase, as it allows the increase of DMI (Lesmeister *et al*., 2004) derived from the improvement in the digestibility of the fibrous portion of the feed that occurs due to changes in the ruminal microbiota (Brown & Nagaraja, 2009). In this study, animals were adapted to the addition of the additives for a period of nine days, and they also received a period of adaptation to the diet prior to the experiment beginning. Given the results obtained in the fermentation patterns, it is assumed that the additives did not have an impact on the rumen microbiota of animals. It has been observed that when high grain diets are provided to ruminants, fed with additives similar to those in the present work, productive behavior is not altered (Cull *et al*., 2015; Kenney *et al*., 2015; Álvarez *et al*., 2018).

Table 3. Concentrations of volatile fatty acids, CH_4 and CO_2

Treatment	Control	YEAST	ION	HP	SE	Value P Treatment	Value P Interaction	Value P Hour
AAC	49.8	54.4	45.9	53.9	5.1	0.6243	<0.0001	<0.0001
PAC	25.8	26.7	26.3	29.9	2.4	0.6221	<0.0001	<0.0001
BAC	18.3	20.1	19.5	20.8	2.9	0.9944	<.00001	<0.0001
APR	2.08	2.13	1.89	1.85	0.23	0.8771	<0.0001	<0.0001
TVFA	99.9	101.1	91.5	104.8	8.05	0.7887	<0.0001	<0.0001
CO_2	60.8	62.8	58.3	61.4	5	0.6273	0.0004	<0.0001
CH_4	28.9	30.3	27.7	27.8	1.6	0.9326	<0.0001	<0.0001

AAC: Acetic acid concentration (mM/lt), PAC: Propionic acid concentration (mM/lt), BAC: Butyric acid concentration (mM/lt), APR: Acetic:propionic ratio, TVFA: Total volatile fatty acids (mM/lt), CO_2:Carbon dioxide, CH_4: Methane

There is consistent information to the findings of this study (Anele *et al*., 2017), where the addition of this type of additives did not have an impact on fermentation patterns when animals were fed high concentrate diets. It has been reported that one of the factors directly influencing VFA production is pH (Christophersen *et al*., 2008; Commun *et al*., 2009). A pH below 6.0 affects cellulolytic bacteria, promoting the growth of lactic acid producing bacteria strains (Zanine *et al*. 2016). This results in the use of carbonaceous structures in their production not favoring the production of desirable VFA's (acetic, propionic and butyric). The concentration of propionic acid is one of the aspects to be noted in the present work, since it was similar to that produced by the treatment that contemplated the use of monensin sodium. It has been reported that the use of ionophores increases the concentration of propionic acid (Burnett *et al*., 2016), which was not reflected in this experiment. Such similarities in individual concentrations resulted in no difference in the acetic: propionic ratio, given the levels of starch present in the diet which favors acetic acid production (Ran *et al.*, 2021).

No differences ($P > 0.05$) were found between treatments for CH_4 and CO_2 concentration. Currently, limited information is available on methane production. Since it is a topic of current interest, there is little related information. Pelchen & Peters (1998) reported that there is no difference in daily methane production in ruminants when fed diets with a dry matter digestibility percentage between 60-80 %. In the present study, the diet provided had a forage: concentrate ratio of 20-80 %, which can have apparent digestibility levels

above 65 % (Ferrell *et al*., 2001). In addition, Pelchen & Peters (1998) found that when digestibility is less than 60 %, differences in this variable are found.

Manipulation of the ruminant diet is considered a viable alternative to mitigate methane production, since it can reduce energy losses. The use of additives and good management in animal feed can improve fermentative characteristics at the rumen level, reflecting a decrease in methane emissions (Carmona *et al*., 2005). However, in the present study, no difference in fermentative parameters was observed, reflecting the same concentrations of greenhouse gases between treatments.

CONCLUSIONS

The addition of the probiotic, prebiotic and symbiotic mixture did not improve ruminal pH conditions. Levels of pH recorded were indicative of subclinical acidosis. On the other hand, there was no difference in ruminal fermentation parameters between treatments. These results suggest that the use of the additives does not have an impact on VFA production and therefore will not be reflected in improved feed efficiency. The use of the proposed mixture in the feeding of lambs with high grain diets is not recommended. It is suggested to analyze the inclusion concentrations of each of the components of the mixture within the additive, in order to improve its impact on the feeding of ruminants with high concentrate diets.

ACKNOWLEDGMENTS

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Errata Erratum

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