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Effect of calcium propionate and monensin on *in vitro* digestibility and gas production

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ABSTRACT - An evaluation of the effects of monensin and calcium propionate on the *in vitro* kinetics of gas production, digestibility, carbon dioxide, and minor gas production on different days was performed using the ruminal fluid from eight Suffolk lamb donors, after receiving additives for 1, 10, and 20 days. Treatments consisted of a control ration of 40% grain; 30 mg/kg of monensin in a diet with 40% grain; 10 g/kg calcium propionate in a diet with 30% grain; and the combination of both additives in a diet with 30% grain. The gas production was measured up to 72 h of incubation and all incubation procedures were repeated three times on days 1, 10, and 20. On incubation day 20, the volume and production of methane and minor gases were measured. There was an interaction between calcium propionate and monensin for maximum gas production, *in vitro* dry matter digestibility (IVDMD), carbon dioxide, and minor gases. Monensin reduced gas production on days 1 and 20, whereas calcium propionate increased gas production (Vm) on day 1. The rate of gas production (s) was reduced by calcium propionate on day 1 and by the combination of additives on day 10. Lag time was reduced by monensin on day 10; however, it declined linearly with the feeding time of the additives. Monensin had no effect on IVDMD (62.29 vs. 62.24%), while calcium propionate increased the IVDMD (60.00 vs. 64.53%). The inclusion of monensin increased CO_2; however, the combination of monensin and calcium propionate had no effect on CO_2 production. Monensin reduced methane (25.37 vs. 20.29%) and increased CO_2. None of the additives showed consistent effects on the kinetic parameters of *in vitro* gas production over time. The treatments with monensin and calcium propionate showed a significant reduction in methane production, with a higher fermentation efficiency since the IVDMD was increased. Both additives are a strategy to consider to reduce methane emissions without affecting the ruminal fermentation.

Key Words: additive feeding, carbon dioxide, ionophore, methane, rumen fermentation

Introduction

To maximize the production efficiency and reduce metabolic problems, it is a common practice to include feed additives in rations for high-producing animals. Certain products could have more advantages, such as reducing the greenhouse gas production without affecting animal performance. Ionophores are an alternative to reduce ruminal methane production (Ellis et al., 2012); monensin is a predominant antibiotic given to ruminants (Kim et al., 2014a), although its use is restricted in many countries. Alternatively, the use of calcium propionate may change the acetate to propionate ratio (Ferraro et al., 2009), allowing for a reduction in the grain level of the ration (Lee et al., 2012). The combination of both additives could reduce methane emissions (Moss et al., 2000; Wang et al., 2009) without affecting ruminant productivity and could be an interesting feeding option.

It is well known that monensin increases the production of propionate (Ellis et al., 2012); therefore, its combination with a calcium propionate could be a better alternative for reducing methane emissions. It is important to have a previous evaluation of the ruminal additives before their use in production that can be performed with an *in vitro* gas production technique, which is a precise and fast procedure (Getachew et al., 2004). Therefore, the objective of this study was to evaluate monensin, with or without calcium propionate, in complete diets in which grain was reduced to 100 g/kg using *in vitro* gas techniques to estimate CO_2 and minor gas production (CH_4, N_2, H_2 and others), and

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incubating at different days of the additive adaptation of the inoculum donors.

Material and Methods

The present investigation was performed in Texcoco, Estado de Mexico, Mexico (19°29'23"N and 98°53'37"W), following the guidelines of the local Academic Committee, under the Law of Animal Production of the State of Mexico.

Eight male Suffolk sheep (24 kg body weight, six months old) were ruminally cannulated and divided into four treatments; then, they were fed the experimental diets with or without additives (Table 1) and used as donors of rumen fluid. The animals were housed and fed in individual pens. Before the beginning of the experiment, the animals were fed a 50:50 (silage:concentrate) ration. Then, they were adapted to the experimental rations gradually during a 10-day period. The experiment lasted twenty days after the adaptation period. Sampling was performed three times during the experimental period on days 1, 10, and 20, in which the animals were fed diets with additives.

The sheep were fed 1.2 kg of an experimental diet twice daily (at 08.00 and 15.00 h) and water was provided *ad libitum*. Experimental diets were offered over 20 days and ruminal fluid was collected on days 1, 10, and 20.

The dietary treatments consisted of a control ration with 40% grain; 30 mg/kg of monensin sodium (Rumensin 200 g Elanco Animal Health, Mexico D.F.) in a diet with 40% grain; 10 g/kg calcium propionate (65%, Paniplus Mexico) in a diet with 30% grain; and 30 mg/kg of monensin sodium plus 10 g/kg calcium propionate in a diet with 30% grain. Diets were analyzed for dry matter, organic matter, crude protein, and ether extract according to AOAC (1990) and neutral detergent fiber procedures (Van Soest et al., 1991) (Table 1).

Table 1 - Experimental diets and chemical compositions

	Control	Ca-Pr	Monensin	Ca-Pr + Monensin
Ingredient (g/kg)				
Corn grain	400	309.5	400	309.2
Corn gluten	95	100	95	100
Sugarcane molasses	100	110	110	110
Corn stover	380	445	369.7	445
Urea	10	10.5	10	10.5
Buffer[1]	10	10	10	10
Minerals[2]	5	5	5	5
Calcium propionate[3]	0	10	0	10
Monensin	0	0	0.3	0.3
Chemical composition (g/kg)				
Dry matter	880.4	868.8	888.4	878
Organic matter	838.0	845.0	846.4	843.5
Crude protein	148.6	146.1	148.4	147.1
Ether extract	25.5	25.0	24.5	28
Neutral detergent fiber	307.9	321.5	301.2	332.5

Ca-Pr - calcium propionate.
[1] Acid buff: 750 g $CaCO_3$ and 190 g $MgCO_3$.
[2] Mycotoxin absorbent, 2,000 g; antirust, 200 g; Buff, 6,000 g; NaCl, 3,000 g; Co, 75 mg; Cu, 5,000 mg; Cr, 200 ppb; P, 40 g; Fe, 30,000 mg; Yeast, 3,000 g; Mn, 2,000 mg; monensin, sodium 200 g; flavoring, 200 g; Se, 100 mg; vitamin A, 6,800,000 IU; vitamin D, 630,000 IU; vitamin E, 16,500 IU; I, 125 mg; and Zn, 10,500 mg.
[3] Propionic acid, 650 g and Ca, 350 g.

Rumen fluid was collected (100 mL) using a Tygon tube connected to a vacuum pump and a 1000-mL Erlenmeyer flask. Rumen fluid was transported to the laboratory in sterile plastic containers (150 mL) at 39 °C and the rumen fluid obtained from both donors for each treatment was obtained individually and then mixed for later use.

Ninety millilitres of reduced mineral solution and rumen fluid (9:1) were added to amber flasks (120 mL) containing 0.5 g of each diet and incubated under anaerobic conditions in a water bath at 39 °C. Each incubation was performed in triplicate, using each experimental diets as a substrate. Gas production was measured up to 72 h of incubation using the *in vitro* gas production procedure described by Menke and Steingas (1988).

The reduced mineral solution per liter of solution contained Ca_2CO_3 (4 g); K_2HPO_4 (0.45 g); KH_2PO_4 (0.45 g); $(NH_4)_2SO_4$ (0.45 g); NaCl (0.90 g); $MgSO_4$ (0.18 g); $CaCl_2$ (0.07 g); Na_2SO_4 (0.5 g); L-cysteine (0.5 g); distilled water (50 mL); NaOH 1N (2 mL); and one drop of rezarsurin (Ferraro et al., 2009).

After incubation (72 h), the residual was filtered to determine *in vitro* dry matter digestibility. All incubation procedures were repeated three times on days 1, 10, and 20. Gas volume data and incubation time were used to obtain the parameters of the kinetics of gas production: maximum volume of gas produced (Vm), lag phase (L), and rate of gas production (S), using the logistic model $Vo = Vm / (1 + \exp^{(2-4 * s * (t L))})$ described by Pitt et al. (1999).

On incubation day 20, the volume and production of CO_2 after 24 h of fermentation were measured volumetrically, estimating the methane and minor gases by the difference in these values (Singh and Mohini, 1999). Thirty millilitres of reduced mineral solution and rumen fluid (2:1) were added into amber flasks (60 mL) containing 0.25 g of the each diet. Gas production was measured at 0, 6, 12, and 24 h with a 150-mL glass syringe to determine the production of CO_2 and minor gases. Flasks (60 mL) were prepared with 40 mL 1 N potassium hydroxide to capture the gas and then titrated with 0.55 N hydrochloric acid using 1 mL potassium hydroxide, one drop of barium chloride, and one drop of rezarsurine to determine CO_2 mmol/g = (B – S) × N × 1000/2 (weight sample), in which B = mL HCl used in the blank, S = mL HCl used in the

sample (mL), and N = normality of HCl. The percentage of CO_2 was estimated as a function of the total maximum volume.

Results were analyzed as a completely randomized design with a 2 × 2 factorial arrangement (factors were the additive source at two levels) with the repeated measure procedure (days 0, 10, and 20). A Tukey's test was used to compare treatment means using the JMP software (Sall et al., 2012).

The model included the fixed effects of treatments and its interaction. Data were analyzed with the repeated measures over time (Littell et al., 1998) according to the model:

$$Yijklm = \mu + \alpha_i + \tau_k + (\alpha\tau)_{ik} + d_k(ij) + \gamma m + (\alpha\gamma)im + (\tau\gamma)km + (\alpha\tau\gamma)ikm + \varepsilon ijklm,$$

in which *Yijklm* is the dependent variable; μ is the overall mean; α_i is the fixed effect of calcium propionate (i =1, 2); τ_k is the fixed effect of monensin level (k = 1, 2); $(\alpha\tau)_{ik}$ is the calcium propionate × monensin; $d_k(ij)$ is the random effect of the k-th subject (animal) nested within treatment (i.e., the calcium propionate × monensin level interaction); γm is the fixed effect of the day of evaluation (m = 1, 2, 3); $(\alpha\gamma)im$ is the Ca-Pr × day of evaluation interaction; $(\tau\gamma)km$ is the monensin level × day of evaluation interaction; $(\alpha\tau\gamma)ikm$ is the Ca-Pr × monensin × day of evaluation interaction; and $\varepsilon ijklm$ is the random error.

Results

There was an interaction (P<0.05) between calcium propionate and monensin for maximum gas production (Vm), *in vitro* dry matter digestibility (IVDMD), CO_2, and methane and minor gases (Table 2). Monensin reduced Vm on days 1 and 20 (P<0.05), whereas calcium propionate increased Vm on day 1 (P<0.05). The rate of gas production was reduced by calcium propionate on day 1 by 1 and by the combination of additives on day 10 (P<0.05). Lag time was reduced by monensin on day 10 (P<0.01) and declined linearly with adaptation time (Figure 1).

Monensin had no effect on *in vitro* digestibility (62.29 vs. 62.24%), whereas calcium propionate increased this value (60.00 vs. 64.53%) (P<0.01). There was a linear increase in digestibility according to the adaptation time (P<0.0001) (Figure 2).

The inclusion of monensin increased CO_2 (74.62 vs. 79.70%), whereas the combination of monensin and calcium propionate avoided this increment (interaction P<0.008). Methane production was reduced with monensin (25.37 vs. 20.29%), whereas its combination with calcium propionate had no effect (Table 2). Likewise, calcium propionate did not affect methane production. A longer duration of additive feeding improved the digestibility,

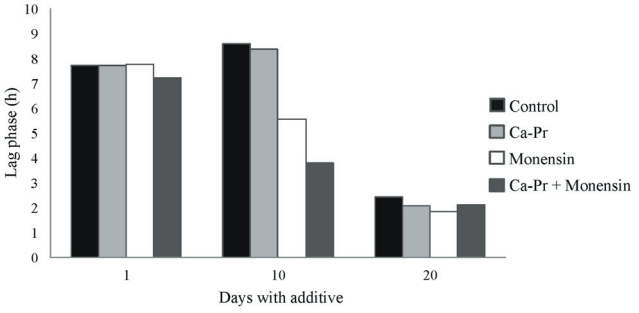


Figure 1 - Effect of day with feed additives on the lag time during *in vitro* gas production.

Table 2 - Effect of monensin and calcium propionate on *in vitro* gas production kinetics, digestibility, methane, and carbon dioxide production

Days with additive		Control	Ca-Pr	Monensin	Ca-Pr + Monensin	SEM	P-value
1	Vm (mL/g)	299.58b	330.20a	235.98c	284.63b	7.48	0.0001
	s (mL/g)	0.035a	0.029b	0.031ab	0.032ab	0.0009	0.002
	L (h)	7.72a	7.73a	7.75a	7.22a	0.51	0.86
	IVDMD (%)	48.61b	52.19ab	48.55b	57.69a	2.13	0.0002
10	Vm (mL/g)	312.22a	324.20a	315.90a	337.52a	9.15	0.24
	s (mL/g)	0.034ab	0.036a	0.034a	0.031b	0.0007	0.0009
	L (h)	8.59a	8.36a	5.56b	3.78c	0.37	0.0001
	IVDMD (%)	61.39a	61.47a	56.55b	61.05a	0.73	0.020
20	Vm (mL/g)	300.75a	282.67ab	264.90b	302.02a	7.14	0.004
	s (mL/g)	0.040a	0.038a	0.041a	0.041a	0.001	0.09
	L (h)	2.43a	2.07a	1.87a	2.11a	0.19	0.26
	IVDMD (%)	72.83b	77.25a	72.11b	77.52a	0.65	0.0001
	CO_2 (%)	71.65b	77.60ab	82.09a	77.31ab	2.38	0.045
	CH_4 (%)	28.35a	22.39ab	17.90b	22.68ab	2.38	0.045

Ca-Pr - calcium propionate; SEM - standard error of the mean; Vm - maximum volume of gas produced; s - rate of gas production; L - lag phase; IVDMD - *in vitro* dry matter digestibility.
Means with different letters are statistically different (P<0.05).

rate of gas production, and lag time parameters of the lambs (Figure 3 and Table 3).

Discussion

Because of the antibiotic nature of monensin, we expected it to have an inhibitory effect on all kinetic parameters of gas production and digestibility. The effect of monensin is a function of the dosage. Two *in vitro* experiments reported that monensin decreased gas production and that the doses were higher than those used in animal feeds (Smith, 2010; Kim et al., 2014a). In contrast, Singh and Mohani (1999) did not find any effect on *in vitro* gas production using 50 or 100 mg/d of monensin in rumen fluid donors. The lack of effects of monensin in this study agrees with molecular studies that did not reveal large shifts in the relative abundance of Gram-positive bacteria in response to monensin from *in vivo* samples (Kim et al., 2014b).

The absence of effects on *in vitro* dry matter agrees with findings of Singh and Mohani (1999), without changes in *in vivo* organic and dry matter digestibility. Smith (2010) reported that the *in vitro* digestibility was not affected by monensin, even when microbial growth was depressed. Similar results were reported by Garcia et al. (2000), Gonzales et al. (2009), and Meyer et al. (2009) with different levels of monensin (30 mg/kg or 90 mg/kg DM). Schelling (1984) concluded that monensin have no effect on cellulose digestibility when animals were adapted for 21 days, which agrees with the current study, because sheep had more time receiving the ionophore. Consequently, the linear response on time confirms the adaptation for all additives on day 20.

It is not clear why the addition of calcium propionate increased *in vitro* gas production; its inclusion probably produced a change in pH and osmotic pressure. Greater amylolytic activity has been observed in similar conditions (Mendoza et al., 1999), because the dissociation of calcium propionate contributes to the production of volatile fatty acids. Lee et al. (2012) reported that the addition of calcium propionate in diets for lambs resulted in a higher proportion of propionic acid in lambs. Miranda et al. (2016) did not find the stimulatory effect of calcium propionate on the volume of gas production with similar grain levels. Other glycogenic substrates, such as glycerol, reduced *in vitro* gas production (Young et al., 2011; Ferraro et al., 2009); however, the kinetics of degradation was affected by the adaptation period. Young et al. (2011) observed that after

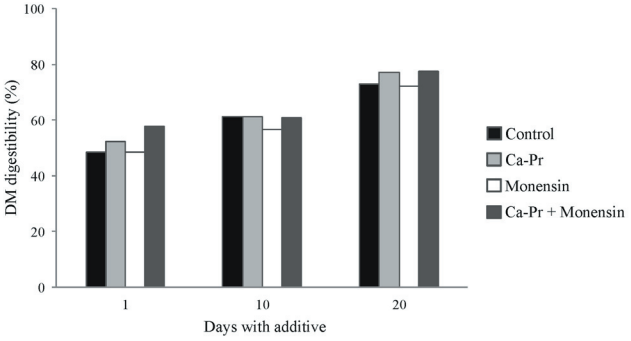
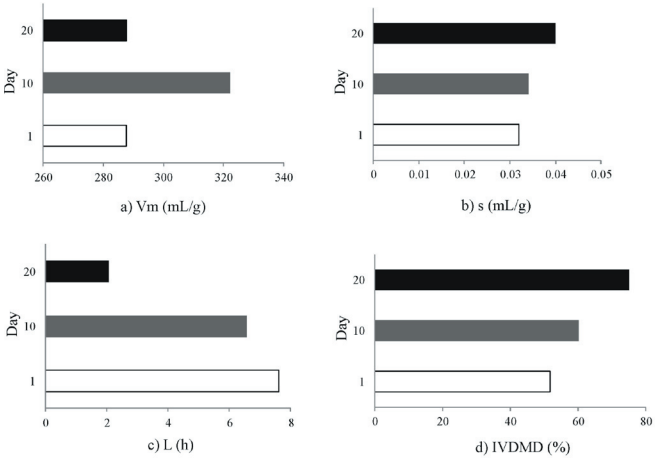


Figure 2 - Effect of adaptation time on digestibility.



Data are presented repeated at time by treatment: a) maximum volume of gas (Vm); b) rate of gas production (s); c) lag phase (L); and d) *in vitro* dry matter digestibility (IVDMD).

Figure 3 - Effect of time (days 1, 10, and 20) on fermentation kinetics variables and IVDMD.

Table 3 - Effect of treatments and day on fermentation kinetics variables

Item	Treatment (T)				Day (D)			P-value	SEM	Effect		
	Control	Monensin	Ca-Pr	Ca-Pr + Monensin	1	10	20			T	D	T × D
Vm (mL/g)	304.38a	272.20b	312.30a	308.06a	287.61b	322.47a	287.62b	0.003	17.28	0.006	0.002	0.03
s (mL/g)	0.03a	0.03a	0.035a	0.035a	0.032b	0.034b	0.040a	0.005	0.002	0.65	<0.0001	0.19
L (h)	6.18a	5.06a	6.05a	4.37a	7.60a	6.57a	2.07b	<0.001	1.14	0.05	<0.0001	0.08
IVDMD (%)	60.94bc	59.05c	63.63ba	65.42a	51.76c	60.10b	74.93a	<0.001	2.38	0.002	<0.0001	0.25

Ca-Pr - calcium propionate; SEM - standard error of the mean; Vm - maximum volume of gas produced; s - rate of gas production; L - lag phase; IVDMD - *in vitro* dry matter digestibility.
Means with different letters are statistically different (P<0.05).

an adaptation, ruminal bacteria can easily ferment glycerol, whereas ruminal fluid from unadapted donors shows a considerably longer lag phase.

The estimation indicates that monensin reduced methane production by 10% in comparison with the control, which is similar to that reported in other studies (Guan et al., 2006; Johnson et al., 2009; Ellis et al*.,* 2012), and can be explained by the effect on bacteria susceptible to ionophores, which are the main producers of acetate, butyrate, and H_2, and CO_2 substrates for methanogenesis (Moss et al., 2000). Singh and Mohani (1999) reported reductions of methane between 23 and 28% with slightly higher doses than those reported in this experiment. In contrast, Kim et al. (2014a) observed a dramatic reduction in methane (53%) with doses not biologically useful for animals.

The addition of calcium propionate could reduce methane production because it is metabolized into propionic acid. The formation of propionate requires hydrogen and does not produce CO_2 (D'Mello, 2000); however, this effect was not observed by Miranda et al. (2016), who studied grain level and calcium propionate.

Callaway and Martin (1996) incubated corn with organic acid plus monensin (5 mg/kg), which increased CO_2 production because of lactate fermentation to propionate via the succinate propionate pathway, with carbon dioxide as the end-product. It has been demonstrated that ionophores select for major propionate producers such as *Selenomonas rumiantium,* which ferment lactate using that pathway (Mackie et al*.,* 1984).

There is evidence that monensin reduces Gram-positive bacteria groups, rumen protozoa, and fungi, whereas some bacteria strains are more sensitive than others and some are resistant, mainly due to the structure of the cell wall of organisms (Ellis et al., 2012, Mendoza et al., 1993). Research indicates that monensin may decrease the number of protozoa by 4 to 63% (Shelling, 1984).

Conclusions

The use of monensin and calcium propionate during a given period of time shows an improved *in vitro* dry matter digestibility, reducing methane production, resulting in a better fermentative efficiency. Both additives are a viable nutritional strategy for the reduction of Greenhouse gases such as methane.

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References

AOAC - Association of Official Analytical Chemists. 1990. Official methods of analysis 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Callaway, T. R. and Martin, S. A. 1996. Effects of organic acid and monensin treatment on *in vitro* mixed ruminal microorganism fermentation of cracked corn. Journal of Animal Science 74:1982-1989.

D´Mello, F. P. J. 2000. Farm animal metabolism and nutrition. 1st ed. CABI Publishing, New York. USA.

Ellis, J. L.; Dijkstra, J.; Bannink, A.; Kebreab, E.; Hook, S. E.; Archibeque, S. and Franc, J. 2012. Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high-grain-fed beef cattle. Journal of Animal Science 90:2717-2726.

Ferraro, M. S.; Mendoza, G. D.; Miranda, L. A. and Gutiérrez, C. G. 2009. In vitro gas production and ruminal fermentation of glycerol, propylene glycol and molasses. Animal Feed Science and Technology 154:112-118.

García, C. C. G.; Mendoza, G. D.; González, S. M.; Cobos, M.; Ortega, M. E. and Ramírez, R. L. 2000. Effect of a yeast culture *Saccharomyces cerevisiae* and monensina on ruminal fermentation and digestion in sheep. Animal Feed Science and Technology 83:165-170.

Getachew, G.; DePeters, E. J. and Robinson, P. H. 2004. In vitro gas production provides effective method for assessing ruminant feeds. California Agriculture 58:54-58.

Gonzáles, M. M. L.; Kawas, R.; García, R.; González, C.; Aguirre, J.; Hernández, G.; Fimbres, H.; Picón, F. J. and Lud, C. D. 2009. Nutrient intake, digestibility, mastication and ruminal fermentation of Pelibuey lambs fed finishing diets with ionophore (monensin or lasalocid) and sodium malate. Small Ruminant Research 83:1-6.

Guan, H.; Wittenberg, K. M.; Ominski, K. H. and Krause, D. O. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. Journal of Animal Science 84:1896-1906.

Herrera, J. G. and García, C. 2010. Bioestadística en Ciencias Veterinarias, Procedimientos de análisis de datos con SAS. 1.ed. Editorial Universidad Complutense de Madrid, Madrid.

Johnson, M. C.; Devine, A. A.; Ellis, J. C.; Grunden, A. M. and Fellner, V. 2009. Effects of antibiotics and oil on microbial profiles and fermentation in mixed cultures of ruminal microorganisms. Journal of Dairy Science 92:4467-4480.

Kim, D. H.; Mizing, K. M.; Kube, J. C.; Friesen, K. G.; McLeod, K. R. and Harmon, D. L. 2014a. Influence of monensin and lauric acid distillate or palm oil on *in vitro* fermentation kinetics and metabolites produced using forage and high concentrate substrates. Animal Feed Science and Technology 189:19-29.

Kim, M.; Eastridge, M. L. and Yu, Z. 2014b. Investigation of ruminal bacterial diversity in dairy cattle fed supplementary monensin alone and in combination with fat, using pyrosequencing analysis. Canadian Journal of Microbiology 60:65-71.

Lee, R. H. A.; Mendoza, G. D. and Gonzalez, S. S. 2012. Effect of calcium propionate and sorgum level on lamb performance. Animal Feed Science and Technology 177:237-241.

Mackie, R. I.; Gilchrist, F. M. C. and Heath, S. 1984. An *in vivo* study of ruminal microorganisms influencing lactate turnover and its contribution to volatile fatty acid production. Journal of Agricultural Science 103:37-51.

Mendoza, G. D.; Britton, R. A. and Stock, R. A. 1993. Influence of ruminal protozoa on site and extent of starch digestion and ruminal fermentation. Journal of Animal Science 71:1572-1578.

Mendoza, G. D.; Britton, R. A. and Stock, R. A. 1999. Effect of feeding mixtures of high moisture corn and dry-rolled grain sorghum on ruminal fermentation and starch digestion. Small Ruminant Research 32:113-118.

Menke, K. H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research Development Journal 27:7-55.

Meyer, N. F.; Erickson, G. E.; Klopfenstein, T. J.; Greenquist, M. A.; Luebbe, M. K.; Williams, P. and Engstrom, M. A. 2009. Effect of essential oils, tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. Journal of Animal Science 87:2346-2354.

Miranda, L. A.; Lee, R. H. A.; Mendoza, M. G. D.; Crosby, G. M. M; Relling, A. E.; Pinos, R. J. M.; Rojo, R. R. and González, M. 2016. Influence of calcium propionate on *in vitro* fermentation of sorghum-based diets. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Available at: <http://revista.fca.uncu.edu.ar/>. Accessed on: Apr. 26, 2017.

Moss, R. A.; Jouany, J. P. and Newbold, J. 2000. Methane production by ruminants: its contribution to global warming. Annales de Zootechnie 49:231-253.

Pinos, R. J. M.; González, S.; Mendoza, G. D.; Bárcena, G. R. and Martínez, G. A. 2002. Análisis estadísticos de experimentos de digestibilidad *in vitro* con forrajes. Interciencia 27:143-146.

Pitt, R. E.; Cross, T. L.; Pell A. N.; Shofield P. and Doane P. H. 1999. Use of *in vitro* gas production models in ruminal kinetics. Mathematical Biosciences 159:145-163.

Russell, B. J. and Strobel, J. H. 1989. Effect of ionophores on ruminal fermentation. Applied Environmental Microbiology 55:1-6.

Sall, J.; Lehman A.; Stephens M. and Creighton, L. 2012. JMP® Start Statistics: A guide to statistics and data analysis. 5th ed. SAS Institute Inc, Cary, NC, USA.

Shelling, T. G. 1984. Monensin mode of action in the rumen. Journal of Animal Science 58:1518-1527.

Singh, F. P. and Mohini, M. 1999. Effect of different levels of rumensin in diet on rumen fermentation, nutrient digestibility and methane production in cattle. Asian-Australasian Journal Animal Science 12:1215-1221.

Smith, D. R.; DiLorenzo, N.; Leibovich, J.; May, M. L.; Quinn, M. J.; Homm, J. W. and Galyean, M. L. 2010. Effects of sulphur and monensin concentrations on *in vitro* dry matter disappearance, hydrogen sulphide production, and volatile fatty acid concentrations in batch culture ruminal fermentations. Journal of Animal Science 88:1503-1512.

Van Soest, P. J.; Robertson, J. B. and Lewis, A. B. 1991. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. Methods for dietary fibre neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597.

Wang, Y.; Zhang, Y.; Wang, J. and Meng, L. 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass and Bioenergy 33:848-853.

Young, L. S.; Moon, L. S.; Bae, Y. C.; Keun, K. D.; Cheol, L. S.; Hyun, K. C. and Seo, S. 2011. Glycerol as a feed supplement for ruminants: *In vitro* fermentation characteristics and methane production. Animal Feed Science and Technology 166-167:269-274.