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Substitution of garlic leaves to alfalfa hay and its effect on *in vitro* ruminal fermentation

Sustitución de heno de alfalfa por hojas de ajo y su efecto en la fermentación ruminal *in vitro*

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ABSTRACT

This research aimed to evaluate the substitution of alfalfa hay to garlic leaves during the *in vitro* gas and methane production, as well as in ruminal fermentation patterns. There were four treatments: (T1) alfalfa hay (50%); (T2) alfalfa hay (33%) + raw garlic leaves (17%); (T3) alfalfa hay (17%) + raw garlic leaves (33%) and (T4) raw garlic leaves (50%). The highest values of "a" (gas production from the soluble fraction); "b" (gas production from the insoluble fraction) and "c" (gas production rate) were found in T4 (mL). While lower in T1 ($P<0.05$); but no differences were observed between T2 and T3 ($P>0.05$).The highest ammonia-nitrogen (NH_3-N) concentration was recorded in T4 and the lowest in T1 ($P<0.05$). The propionate concentrations increased with T4 and decreased with T1, whereas the acetate decreased with T4 and increased with T1 ($P<0.05$). The highest methane production was recorded in T1 and the lowest in T4 ($P<0.05$). It is concluded that substitution of alfalfa hay to garlic leaves in beef cattle diets, improved the ruminal fermentation and decreased methane production under *in vitro* conditions.
Keywords: garlic leaves, gas production, ruminal fermentation, methane.

RESUMEN

Esta investigación tuvo como objetivo evaluar la sustitución de heno de alfalfa por hojas de ajo en la producción de gas *in vitro* y metano, así como en los patrones de fermentación ruminal. Se evaluaron cuatro tratamientos: (T1) heno de alfalfa (50%); (T2) heno de alfalfa (33%) + hojas de ajo crudo (17%); (T3) heno de alfalfa (17%) + hojas de ajo crudo (33%) y (T4) hojas de ajo crudo (50%). Los valores más altos de "a" (producción de gas a partir de la fracción soluble); "b" (producción de gas a partir de la fracción insoluble) y "c" (tasa de producción de gas) se encontraron en T4 (mL); mientras que fueron menores en T1 ($P <0.05$); pero no se observaron diferencias entre T2 y T3 ($P> 0.05$). La concentración más alta de nitrógeno amoniacal (N-NH_3)

se registró en T4 y la más baja en T1 (P <0.05). Las concentraciones de propionato aumentaron con T4 y disminuyeron con T1, mientras que las de acetato disminuyeron con T4 y aumentaron con T1 (P <0.05). La producción de metano más alta se registró en T1 y la más baja en T4 (P <0.05). Se concluye, que la sustitución de heno de alfalfa por hojas de ajo en dietas de bovinos carne, mejoró la fermentación ruminal y disminuyó la producción de metano en condiciones *in vitro*.
Palabras clave: hojas de ajo, producción de gas, fermentación ruminal y metano.

INTRODUCTION

There is a trend in global demand for garlic consumption, which has generated employment in food, particularly ruminants, of its main by-products such as husks, leaves and straws (Kallel and Ellouz, 2017). In various countries, the garlic leaves obtained during harvest are incinerated, and consequently there are problems associated with disposal costs and environmental contamination (Han *et al*., 2013). In this sense, it can be said that the methane emissions by ruminants only cause serious environmental problems, but also represent an important source of energy loss for animals (Gallegos *et al*., 2019; Lan and Yang, 2019). The production of greenhouse gases (GHG) in different livestock systems and their impact on climate changes are a major concern throughout the world. Enteric methane has been reported as the most GHG emitted (50-60%), in the production systems in ruminants (Tapio *et al*., 2017; Haque, 2018).

Because of this problem, many attempts have been to modify ruminal fermentation and decrease methane production made; among them is the incorporation into ruminant diets of good quality forages, such as alfalfa hay. Garlic and some of its components have also been shown to decrease methane production under *in vivo* and *in vitro* conditions (Kamra *et al*., 2012). In fact, so far few studies have investigated the effects of garlic and its harvest by-products on ruminal fermentation and methane production.

Based on the above, it assumes that the substitution of garlic leaves for alfalfa hay in beef cattle diets produces similar changes in the rumen fermentation patterns and methane production during *in vitro* fermentation. Therefore, the objective of the present work was to evaluate the substitution of alfalfa hay for garlic leaves in the production of *in vitro* gas and methane, as well as in the ruminal fermentation patterns.

MATERIAL AND METHODS

The experiment was in the metabolic unit and in the Animal Nutrition laboratory of the Faculty of Veterinary Medicine and Zootechnics of the Juárez University of Durango state (Mexico) carried out.

Raw garlic leaf sampling. The samples (25 cm long leaves) of raw garlic (*Allium sativum*) used in this study were collected in the northern region of Mexico. To ensure representative sampling, the samples were collected five times, between January and May 2019. Before chemical analysis and *in vitro* tests, the samples were dried and then ground through a 1 mm mesh.

Experimental treatments. In each experimental treatment, alfalfa hay and raw garlic leaf hay were used as forage sources. The chemical composition of raw alfalfa hay and garlic leaf hay is shown in Table 1.

Table 1. Chemical composition of alfalfa hay and garlic leaves (DM%)

	Alfalfa hay	Garlic leaves
DM	90.2	89.5
OM	88.9	90.1
CP	18.0	18.1
EE	1.6	2.1
NDF	33.2	35.1
ADF	23.7	26.7
TCH	68.8	66.3
NFC	35.6	31.2
L	5.7	6.8

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; TCH = total carbohydrates, NFC = non-fibrous carbohydrates; L = lignin

Four treatments were evaluated: (T1) alfalfa hay (50% DM) + raw garlic leaves (0% DM); (T2) alfalfa hay (33% DM) + raw garlic leaves (17% DM); T3 alfalfa hay (17% DM) + raw garlic leaves (33% DM); T4 alfalfa hay (0% DM) + raw garlic leaves (50% DM). Similar proportions of ground corn, cottonseed and minerals were used in all treatments. The nutritional composition of the experimental treatments is shown in Table 2.

Nutritional composition. Each sample of the experimental treatments was analyzed in triplicate for dry matter (DM), crude protein (CP), ether extract (EE) and organic matter (OM) (AOAC, 2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) analyzes were determined using the filter bag technique with a fiber analyzer (ANKOM Technology, Fairport, NY, USA). The non-fibrous carbohydrate content (NFC) was calculated using the difference between the total carbohydrates (TCH) and the % of NDF. The *in vitro* digestibility of dry matter (DIVDM) and organic matter (DIVOM) was determined using the Daisy incubator (ANKOM Technology, Fairport, NY, USA).

Table 2. Nutritional composition of the experimental treatments

	Treatments			
	T1	T2	T3	T4
Alfalfa hay	50	33	17	0
Garlic leaves	0	17	33	50
Ground corn	39	39	39	39
Flour	10	10	10	10
Minerals	1	1	1	1
	Chemical composition (DM%)			
DM	96.1	95.6	95.6	93.9
OM	92.5	91.8	92.0	93.0
CP	15.0	14.6	14.7	15.6
EE	2.0	2.0	2.5	2.1
DIVDM	58.2	60.3	62.2	64.2
DIVOM	56.1	58.5	59.1	62.2
NDF	53.3	49.3	50.9	47.1
ADF	28.3	23.3	21.7	19.2
NFC	21.5	27.2	23.9	28.2

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; DIVDM = *in vitro* digestibility of dry matter, DIVOM = *in vitro* digestibility of organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fibrous carbohydrates

***In vitro* gas, ruminal fermentation patterns and methane production.** *In vitro* gas production was measured using the ANKOM gas production system. Ruminal fluid was collected approximately 3 h after the morning feeding of two fistulated steers in the rumen, fed open access with alfalfa hay. The ruminal fluid was immediately through four layers of gauze filtered and it was transported to the laboratory in a thermos sealed. The resulting ruminal fluid was purged with CO_2 before use as an inoculum. Approximately 1 g of dried and ground samples from each treatment were in triplicate weighed and placed in glass modules. Ruminal liquid was into each module pipetted and mixed with McDougall's saliva (120 mL).

Gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation. The gas production kinetics (PD) was adjusted to the non-linear model proposed by Ørskov and McDonald (1979). It is the following: PD (t) = a+b × (1-exp^{-ct}); where "**a**" is the gas production from the soluble fraction (mL), "**b**" is the gas production from the insoluble fraction, (mL), "**c**" is the constant rate of gas production (mL/h) and "**t**" is the incubation time.

For the determination of methane, after 24 h of incubation, 10 mL of gas were collected from the upper space of the modules of the gas production system, from which 3 mL subsamples were taken and the methane production was then determined in a gas chromatograph (Kara, 2015).

Likewise, after the end of the 24 h incubation and before filtration, two samples (5 mL) of the liquid from each module were collected, and the pH was immediately measured and stored at -20 °C until subsequent analysis of ammoniacal nitrogen (N-NH3) and volatile fatty acids (VFA) (Ammar *et al*., 2005).

Statistical analysis. An analysis of variance was carried out for a completely randomized design to compare the parameters of *in vitro* gas production, methane and ruminal fermentation patterns with the MIXED procedure of SAS (2002). Individual means were separated by Tukey's multiple range test. The mean differences were considered significant at P <0.05.

RESULTS AND DISCUSSION

***In vitro* gas production parameters.** The highest values of "a", "b" and "c" (table 3), were found in T4; while the lowest values in T1 (P <0.05); but no differences were observed between T2 and T3 (P> 0.05). Differences in the value "a + b" were observed between treatments (P <0.05). The results are consistent with those reported by Tag El-Dini *et al*. (2012), who found an "a" value of 0.60% when garlic pulp was fermented under *in vitro* conditions at a level of 3% of the concentrate.

In this study, the differences observed between treatments in the value "a" can be attributed to the different concentrations of the treatments in non-fibrous carbohydrates (La O *et al*., 2008). The average values of "b" and a+b found in the present study were 116.0 mL 200 mg^{-1} DM and 116.5 mL 200 mg^{-1} DM, respectively. These values coincide with those reported by Sahli *et al*. (2018) when garlic powder, in doses of 32 mg, were incubated *in vitro* in a diet composed of 50% ryegrass hay and 50% commercial concentrate.

The "c" value registered with T4 (9.2 mL h^{-1}) was higher than that found by Anassori *et al*. (2012) (3.5 mL h^{-1}), who evaluated the *in vitro* gas production kinetics of raw garlic bulb.

In the current study, the high constant rate of gas production "c" registered in T4 indicates a high availability of nutrients for ruminal microorganisms; while the lower "c" values observed in T1 could be the result of a high content of NDF, whose chemical components could decrease the fermentation rate of the substrate (Fievez *et al*., 2005).

Table 3. Gas production parameters of the experimental treatments

	Treatments					
	T1	T2	T3	T4	mean	SEM
a (mL 0.2 g^{-1} DM)	0.56^{c}	0.70^{b}	0.72^{b}	0.76^{a}	0.68	0.12
b (mL 0.2 g^{-1} DM)	110.7^{c}	117.7^{b}	115.1^{b}	120.2^{a}	116	0.81
c (mL h^{-1})	6.0^{c}	8.0^{b}	8.0^{b}	9.2^{a}	5.8	0.025
a+b (mL 0.2 g^{-1} DM)	111.2^{d}	118.4^{b}	115.8^{c}	120.9^{a}	116.5	1.10

^{abc} Values with different letters in the same row are statistically different (P <0.05).

a = Production of gas from the soluble fraction, b = Production of gas from the insoluble fraction; c = constant rate of gas production; a + b = Potential gas production; SEM: Standard error of the mean

Ruminal fermentation patterns and methane production. Ruminal fermentation patterns and methane production are in Table 4 presented. The concentration of N-NH3 ranged from 15.1 to 18.1 mg/dL. The highest concentration of N-NH3 was recorded in T4 and the lowest in T1 (P <0.05). There were no differences between T2, T3 and T4 in total VFA concentrations (P> 0.05); but both treatments were different at T1 (P <0.05).

The highest acetate concentration was recorded in T1 and the lowest in T4 (P <0.05), while the highest concentration of propionate was registered in T4 and the lowest in T1 (P <0.05). There was no difference between T1, T2 and T3 in the acetate: propionate ratio (P> 0.05); but both treatments were different at T4 (P <0.05). The highest methane production was recorded in T1 and the lowest in T4 (P <0.05).

The ruminal concentrations of N-NH3 registered in all the evaluated treatments were kept within the suggested range for optimal microbial growth. According to Wanapat and Pimpa (1999), a range of 15 to 30 mg/100 mL is the minimum concentrations required for optimal microbial protein synthesis.

In contrast, with the results obtained in this study, several *in vitro* and *in vivo* studies report that garlic oil and garlic powder reduce or have no effect on the concentration of N-NH3 in ruminal fluid (Cardozo *et al*., 2004). However, Yang *et al*. (2007) found an increase in the concentration of ruminal N-NH3, when raw garlic was incorporated into the diets of lactating cows. In this study, the higher concentration of N-NH3 obtained with T4 could be explained by the high content of crude protein supplied by garlic leaves (Panthee *et al*., 2017).

Table 4. Ruminal fermentation patterns and methane production of the experimental treatments

	Treatments					
	T1	T2	T3	T4	Mean	SEM
pH	6.7[a]	6.7[a]	6.6[a]	6.5[a]	6.6	0.07
$N\text{-}NH_3$, mg dL^{-1}	15.1[c]	17.1[b]	17.5[b]	18.1[a]	16.7	0.03
Total VFA, mM	86.6[b]	104.4[a]	104.6[a]	104.8[a]	100	0.36
Acetate VFA, mol 100 mol^{-1}	52.8[a]	50.5[b]	50.8[b]	48.1[c]	50.5	0.10
Propionate mol 100 mol^{-1}	24.8[c]	28.0[b]	27.8[b]	33.0[a]	28.4	0.08
Butyrate mol 100 mol^{-1}	12.2[a]	12.6[a]	13.5[a]	13.2[a]	12.8	0.02
A:P ratio	2.1[a]	1.8[a]	1.8[a]	1.4[b]	1.7	0.03
Methane (mL g^{-1} DM)	19.1[a]	17.0[b]	16.8[b]	9.5[c]	15.6	0.53

[abc] Values with different letters in the same row are statistically different (P <0.05).
SEM: Standard error of the mean

The total VFA concentrations obtained with T2, T3 and T4 are consistent with other *in vitro* studies, where they did not differ with the addition of garlic oils (Klevenhusen *et al*., 2011). The propionate concentration observed in T4 was higher than in the other treatments; this is evidenced by the decrease in the acetate: propionate ratio.

Furthermore, the increase in propionate concentration could be attributed to the amount of non-fibrous carbohydrates supplied by T4 (Van Soest, 1994). Similarly, the reduction in the ratio of acetate to propionate registered in T4 indicates an improvement in the net energy contribution (Zhong *et al*., 2019). Similar results are reported by Mirzaei-Aghsaghali and Maheri-Sis (2011) who found that garlic oil increased the proportions of propionate and butyrate, but reduced the proportion of acetate. In this study, the low concentration of methane was obtained with T4 (high in garlic leaves); and coincides with the results found by Kongmun *et al*., (2010), who report a decrease in methane production when garlic powder was evaluated in ruminant diets. In our study, the methane values recorded in the four treatments were lower than those reported by Zafarian and Manafi (2013), who evaluated *in vitro* conditions, the addition of different doses of garlic powder (2, 4 and 6% DM) in diets with 50% fiber and 50% concentrate.

In this study, the reduction in methane production registered in T4 in comparison with the other treatments could be attributed to the increase in the concentration of propionate, because the formation of propionate consumes reducing equivalents; while the formation of acetate generates H2 for methanogenesis (Moss *et al*., 2000). Any component or variable in the diet that causes a change in favor of propionate production will be by a reduction in methane production per unit of fermented diet accompanied while the opposite is observed for acetate and butyrate (Pinares-Patiño, 2003). However, the differences observed in this study in methane production, compared to those reported in the literature, may be related to different genetic varieties and possibly to the phenological state of the garlic plant; which results in different contents of the cell wall and as a consequence in the ruminal fermentation patterns (Lee *et al*., 2017).

CONCLUSIONS

The substitution of alfalfa hay for garlic leaves in beef cattle diets improved some indicators of nutritional importance, such as the production of gas from the soluble fraction and the constant rate of gas production. Similarly, it increased the concentrations of N-NH3, total VFA, and reduced the production of methane. Further research is needed to evaluate the effects of garlic leaves in ruminant diets on ruminal fermentation patterns and methane production under *in vivo* conditions; as well as to evaluate the sustainability of garlic leaf supplementation to mitigate rumen methanogenesis without harmful effects on animal performance.

LITERATURE CITED

AMMAR H, López S, González JS. 2005. Assessment of the digestibility of some Mediterranean shrubs by *in vitro* techniques. *Animal Feed Science and Technology.* 119: 323-331. https://doi.org/10.1016/j.anifeedsci.2004.12.013

ANASSORI E, Dalir–Naghadeh B, Pirmohammadi R, Taghizadeh A, Asri-Rezaei S, Farahmand-Azar S, Besharati M, Tahmoozi M. 2012. *In vitro* assessment of the digestibility of forage based sheep diet, supplemented with raw garlic, garlic oil and monensin. *Veterinary Research Forum*. 3: 5–11. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4312812/

AOAC. 2000. Official Methods of Analysis, Association of AOAC International. 17th Ed. Association of Official Analytical Chemists, Arlington, USA. Disponible: https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/

CARDOZO P W, Calsamiglia S, Ferret A, Kamel C. 2004. Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. *Journal of Animal Science*. 82:(11) 3230-3236. https://doi.org/10.2527/2004.82113230x

FIEVEZ V, Babayemi OJ, Demeyer D. 2005. Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production that requires minimal laboratory facilities. *Animal Feed Science and Technology*. 123-124: 197-210. https://doi.org/10.1016/j.anifeedsci.2005.05.001

GALLEGOS FP, Bañuelos VR, Delgadillo RL, Echavarría CF, Meza LC, Rodríguez TD. 2019. Differential evaluation of oregano extracts in the production of volatile fatty acids and methane during ruminal fermentation *in vitro*. *Abanico Veterinario*. *9*(1):1-18. http://dx.doi.org/10.21929/abavet2019.91

HAN X, Cheng Z, Meng H, Yang X, Ahmad I. 2013. Allelopathic effect of decomposed garlic (*Allium sativum L*.) stalk on lettuce (*L. Sativa Var. Crispa L*.). *Pakistan Journal of Botany*. 45:225-233. https://www.pakbs.org/pjbot/PDFs/45(1)/32.pdf

HAQUE MN. 2018. Dietary manipulation: a sustainable way to mitigate methane emissions from Ruminants. *Journal Animal Science and Technology*. 60: 15. https://doi.org/10.1186/s40781-018-0175-7

KALLEL F, Ellouz CS. 2017. Perspective of garlic processing wastes as low-cost substrates for production of high-added value products: a review. *Environmental Progress and Sustainable Energy*. 36: 1765-1777. https://doi.org/10.1002/ep.12649

KAMRA DN, Agarwal N, Sakthivel PC, Chaudhary LC. 2012. Garlic as a rumen modifier for eco-friendly and economic livestock production. *Journal of Applied Animal Research*. 40: 90 96. https://doi.org/10.1080/09712119.2011.607764

KARA K. 2015*.* *In vitro* methane production and quality of corn silage treated with maleic acid. *Italian Journal of Animal Science*. 14: 718-722. https://doi.org/10.4081/ijas.2015.3994

KLEVENHUSEN F, Zeitz JO, Duval S, Kreuzer M, Soliva CR. 2011. Garlic oil and its principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. *Animal Feed Science and Technology*. 166–167: 356–363. https://doi.org/10.1016/j.anifeedsci.2011.04.071

KONGMUN P, Wanapat M, Pakdee P, Navanukraw C. 2010. Effect of coconut oil and garlic powder on *in vitro* fermentation using gas production technique. *Livestock Science*. 127: 38-44. https://doi.org/10.1016/j.livsci.2009.08.008

LA O, García R, Ruiz O, Castillo Y, Muro A, Rodríguez C, Arzola C, González H, Ortiz B. 2008. Potencial fermentativo ruminal *in vitro* de dos árboles (Pithecellobium dulce y Tamarindos indica) de importancia para la ganadería en ecosistemas frágiles, salinos y de alta sequía, situados en el Oriente de Cuba. *Revista Cubana de Ciencia Agrícola*. 42: 57-61. https://www.redalyc.org/articulo.oa?id=193015413009

LAN W, Yang C. 2019. Ruminal methane production: Associated microorganisms and the potential of applying hydrogen-utilizing bacteria for mitigation. *Science of the Total Environment*. 654: 1270-1283. https://doi.org/10.1016/j.scitotenv.2018.11.180

LEE YH, Kim YI, Oh YK, Ahmadi F, Kwak WS. 2017.Yield survey and nutritional evaluation of garlic stalk for ruminant feed. *Journal of Animal Science and Technology*. 59: 22. https://doi.org/10.1186/s40781-017-0147-3

MIRZAEI-AGHSAGHALI A, Maheri-Sis N. 2011. Factors affecting mitigation of methane emission from ruminants I: Feeding strategies. *Asian Journal of Animal and Veterinary Advances*. 6:888-908. http://dx.doi.org/10.14269/2318-1265/jabb.v4n1p22-31

MOSS AR, Jouany JP, Newbold J. 2000. Methane production by ruminants: its contribution to global warming. *Annals of Zootechnie*. 49: 231-253. https://doi.org/10.1051/animres:2000119

ØRSKOV ER, McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science.* 92: 499-503. https://www.cambridge.org/core/services/aop-cambridge-core/content/view/E2DB4F2290E374E10E9800E512D127A7/S0021859600063048a.pdf/estimation_of_protein_degradability_in_the_rumen_from_incubation_measurements_weighted_according_to_rate_of_passage.pdf

PANTHEE A, Matsuno A, Al-Mamun M, Sano H. 2017. Effect of feeding garlic leaves on rumen fermentation, methane emission, plasma glucose kinetics, and nitrogen utilization in sheep. *Journal of Animal Science and Technology*. 59: 14. https://doi.org/10.1186/s40781-017-0139-3

PINARES-PATIÑO CS, Ulyatt MJ, Waghorn GC, Lassey KR, Barry TN, Holmes CW, Johnson DE. 2003. Methane emissions by alpaca and sheep fed on lucerne hay or grazed on pastures of perennial ryegrass/white clover or birdsfoot trefoil. *Journal of Agricultural Science*. 140: 215-226. https://doi.org/10.1017/S002185960300306X

SAHLI F, Darej C, Moujahed N. 2018. Potential of white garlic powder (*Allium sativum L*.) to modify *in vitro* ruminal fermentation. *South African Journal of Animal Science*. 48: 253-260. http://dx.doi.org/10.4314/sajas.v48i2.6

SAS. System Analytics Static. 2002. User's Guide: Statistics Ver 9.0. SAS Inst. Inc., Cary, NC, USA.

TAG EL-Dini AE, Mohara MS, Nour AA, Nasser ME. 2012. Effect of some herbs on the rumen fermentation: 1- Effect of ginger (zingiber officinale) and garlic (allium sativum) on gas production, energy values, organic matter digestibility and methane emission, *in vitro*. *Journal Agriculture and Enviromental Science, Damanhoure University Egypt*. 11: 33-52. http://www.damanhour.edu.eg/pdf/agrfac/Root1/Vol.11-2-2.pdf

TAPIO I, Snelling TJ, Strozzi F, Wallace RJ. 2017. The ruminal microbiome associated with methane emissions from ruminant livestock. *Journal of Animal Science and Biotechnology*. 8: 7. https://doi.org/10.1186/s40104-017-0141-0

VAN Soest PJ. 1994. Nutritional Ecology of the Ruminant. Ithaca, Cornell University Press, USA. https://books.google.es/books?hl=es&lr=&id=TlluDwAAQBAJ&oi=fnd&pg=PP1&dq=Van+Soest+P+J.+(1994).+Nutritional+Ecology+of+the+Ruminant.+Ithaca,+Cornell+University+Press,+USA.&ots=lnCgEloYfy&sig=KEkNXOzwc9BFqFueYB25lK5Hvk8#v=onepage&q&f=false

WANAPAT M, Pimpa O. 1999. Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. *Asian-Australasian Journal of Animal Sciences*. 12: 904–907. https://doi.org/10.5713/ajas.1999.904

YANG WZ, Benchaar C, Ametaj BN, Chaves AV, He ML, McAllister TA. 2007. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. *Journal of Dairy Science*. 90: 5671–5681. https://doi.org/10.3168/jds.2007-0369

ZAFARIAN R, Manafi M. 2013. Effect of Garlic Powder on Methane Production, Rumen Fermentation and Milk Production of Buffaloes. *Annual Review and Research in Biology*. 3:1013-1019. http://www.journalarrb.com/index.php/ARRB/article/view/24989

ZHONG RZ, Xiang H, Cheng L, Zhao C, Wang F, Zhao X, Fang Y. 2019. Effects of feeding garlic powder on growth performance, rumen fermentation, and the health status of lambs infected by gastrointestinal nematodes. *Animals*. 9: 2-10. https://doi.org/10.3390/ani9030102