AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of sunflower oil (*Helianthus annuus*) on *in vitro* ruminal fermentation and emission of gases

Crosby-Galván, María M.[1]; Torres-Salado, Nicolás[2]; Sánchez-Santillán, Paulino[2]; Salinas-Ríos, Teodulo[3]; Ayala Monter, Marco A.[2]; Herrera-Pérez, Jerónimo[2*]

1 Colegio de Postgraduados, Campus Montecillo, Posgrado en Recursos Genéticos y Productividad, Ganadería, Texcoco, Estado de México, C.P. 56230.
2 Universidad Autónoma de Guerrero, Facultad de Medicina Veterinaria y Zootecnia No. 2. Km 197 Carretera Acapulco-Pinotepa Nacional, Cuajinicuilapa, Guerrero, México, C. P. 41940.
3 Universidad Autónoma Benito Juárez de Oaxaca, Facultad de Medicina Veterinaria y Zootecnia, Oaxaca, México, CP. 68120.
* Correspondence: mvzjero@hotmail.com

ABSTRACT

Objective: To evaluate the production of total gas, methane (CH_4), and *in vitro* fermentative variables in diets for sheep with 1 and 2% sunflower oil.

Design/Methodology/Approach: Serological pipettes with 0.5 g of treatment and 50 ml of culture medium were incubated at 39 °C for 24, 48, and 72 h. The production of total gas, CH_4, and degradation of dry matter (DEGDM), neutral detergent fiber (DEGNDF), acid detergent fiber (DEGADF), as well as the production of volatile fatty acids (VFA) and ammoniacal nitrogen ($N\text{-}NH_3$) were estimated. The experimental design was completely randomized.

Results: Total gas production increased ($p<0.05$) at 48 and 72 h and decreased ($p<0.05$) at 24 h as the oil increased. CH_4 production at 24 and 48 h did not present differences ($p>0.05$); a linear decrease ($p<0.05$) was quantified at 72 h. DEGDM increased ($p<0.05$) at 24 and 48 h and decreased ($p<0.05$) at 72 h. DEGNDF and DEGADF increased ($p<0.05$) at 48 h. Butyric acid content and $N\text{-}NH_3$ decreased ($p<0.05$) at 48 h.

Study Limitation/Implications: A >2% inclusion of sunflower oil in the diet can reduce the degradability of the food and the microbial protein.

Findings/Conclusions: Including up to 2% of sunflower oil in diets for lambs does not affect the fiber degradation and is an alternative to reduce the amount of methane emissions released into the environment.

Keywords: degradation, bacteria, methane, *in vitro*, lambs.

Citation: Crosby-Galván, M. M., Torres-Salado, N., Sánchez-Santillán, P., Salinas-Ríos, T., Ayala Monter, M. A., & Herrera-Pérez, J. (2022). Effect of sunflower oil (*Helianthus annuus*) on *in vitro* ruminal fermentation and emission of gases. *Agro Productividad*. https://doi.org/10.32854/ agrop.v15i7.2327

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: February 14, 2022.
Accepted: June 19, 2022.
Published on-line: August 02, 2022.

Agro Productividad, 15(7). July. 2022. pp: 119-126.

INTRODUCTION

An increase in greenhouse gasses (GG) is a significant contributor to climate change (Patra, 2014; Hill *et al.*, 2016). Livestock farming contributes 18% of global greenhouse gas emissions, of which the enteric methane (CH_4) of ruminants makes up 37% of the total anthropogenic production (Steinfeld *et al.*, 2006; Key & Tallard, 2012; Kumar *et al.*, 2014). CH_4 is produced during rumen fermentation and represents a loss of 2 to 15% of

Image by Susanne Jutzeler, Schweiz, at Pixabay

consumed energy (Kumar *et al.*, 2009; Eckard *et al.*, 2010). Climate change and energy loss during food fermentation in ruminants awakens an interest in the study of different biotechnological alternatives that minimize the production of enteric CH_4 (Eckard *et al.*, 2010; Patra, 2014), without affecting rumen metabolism.

A strategy to reduce CH_4 production in ruminants is the use of fats and oils in diet (Grainger and Beauchemin, 2011; Bodas *et al.*, 2012; Hristov *et al.*, 2013). Peanut, rapeseed, corn, and soy oils affect fatty metabolism in rumen and act as CH_4 suppressors (Wang *et al.*, 2016). Other researchers have used coconut oil to reduce CH_4 production in sheep (Machmuller *et al.*, 2000) and heifers (Jordan *et al.*, 2006). Therefore, this study aims to evaluate the *in vitro* production of total gas, methane, and fermentative variables in the diets of fattening lambs with two levels of sunflower oil.

MATERIALS AND METHODS

Location of study area

The study was carried out in the laboratory of the Nutrición Animal y Microbiología Ruminal y Genética Microbiana del Posgrado en Recursos Genéticos y Productividad – Ganadería, Campus Montecillo, Colegio de Postgraduados, Montecillo, Texcoco, State of México.

Treatments

Treatments consisted of three iso protein diets (Table 1), developed based on NRC (2007), in order to meet the nutritional requirements of finishing lambs. The ingredients of these diets (Table 1) were ground on a mill (Thomas Willey, USA) with a 1-mm mesh.

Table 1. Experimental diets for finishing lambs.

Ingredients	T1	T2	T3
Composition (g kg^{-1} MS)			
Alfalfa	120	120	120
Corn grain	550	550	550
Corn stubble	150	140	130
Soybean meal	100	100	100
Sunflower oil	0	10	20
Wheat bran	60	60	60
Mineral premix	20	20	20
Chemical composition			
Dry matter, %	90.85	91.41	92.66
Crude protein, %	15.47	15.23	15.15
Ether extract, %	3.74	5.28	7.74
Neutral detergent fiber, %	33.14	30.75	31.29
Acid detergent fiber, %	20.07	18.13	18.56
Ashes, %	6.38	6.71	6.81

T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil.

Chemical analysis

In treatments, the following elements were determined: dry matter (DM), crude protein (CP), ashes (A), ether extract (EE), according to AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified using an ANKOM 200/220 analyzer (USA), based on the method of Van Soest *et al.* (1991).

Culture medium, traps for the capture of biogas and biodigesters

They were prepared following the methods described by Sánchez-Santillán *et al.* (2015), Hernadez-Morales *et al.* (2018), and Torres-Salado *et al.* (2018).

Total gas and methane production

Total gas production was quantified by displacing saturated saline solution at 24, 48, and 72 h of incubation, according to the description of Torres-Salado *et al.* (2018). CH_4 production was measured at 24, 48, and 72 h in accordance with the technique described by Prada-Matiz and Cortés-Castillo (2011).

Variables of rumen fermentation

In vitro degradation of dry matter (DEGDM) was calculated according to Hernández-Morales *et al.* (2018). ANKOM® bags were heat sealed to determine NDF, using the ANKOM® Technology Method in accordance with Van Soest *et al.* (1991).

Volatile fatty acids (VFA) were analyzed in accordance with the description of Sánchez-Santillán and Cobos-Peralta (2016).

For the concentration of total bacteria, a direct counting technique was employed in a Petroff-Hausser® chamber (Hausser #39000, Electron Microscopy Sciences, USA) and an Olympus® EX51 microscope (Olympus, USA), at 1000X magnification, in accordance with the description of Sánchez-Santillán and Cobos-Peralta (2016).

Statistical analysis

The experimental design was completely randomized (eight independent repetitions per treatment). Data was analyzed using the GLM procedure of SAS® (SAS, 2011). Comparison of means was done with Tukey's test ($\alpha=0.05$). Orthogonal polynomials were used in order to test the linear and quadratic effect resulting from the gradual increase of sunflower oil.

RESULTS AND DISCUSSION

The production of partial gas and total gas increased ($p<0.05$) lineally at 48 and 72 h of incubation, as sunflower oil content increased in treatments (Table 2). This increase is assumed to be the consequence of a greater digestibility of diets, since Menke *et al.* (1979) mention a high correlation between *in vitro* gas production and apparent degradability of dry matter. At 24 h of incubation, partial gas production decreased ($p<0.05$) lineally, perhaps as a result of cellulolitic bacteria, protozoa, and methanogenic archaea toxicity (Dohme *et al.*, 1999).

Partial production at 24 and 48 h and accumulated production displayed no difference among treatments ($p>0.05$). However, at 72 h of incubation, CH_4 production decreased ($p<0.05$) lineally. Long chain fatty acids in oils are toxic to methanogenic archaea in rumen, which brings about a decrease in CH_4 production (Patra & Yu, 2013).

In vitro degradation of dry matter (DEGDM) increased ($p<0.05$) lineally at 24 and 48 h of incubation as sunflower oil content in the diet increased (Table 3). However, DEGDM decreased ($p<0.05$) lineally at 72 h of incubation. The increase in DEGDM up to 48 h of fermentation is attributed to the fermentation of non-structural carbohydrates, while at 72 h, the linear decrease could be caused by the slow fermentation of carbohydrates in diet fiber (Gatachew *et al.*, 1998; Toral *et al.*, 2011).

In vitro degradation of neutral detergent fiber (DEGNDF) and acid detergent fiber (DEGADF) show no difference between treatments ($p>0.05$) at 24 and 72 h of incubation. However, DEGNDF and DEGADF increased ($p<0.05$) lineally at 48 h of incubation. This effect could be attributed to a low percentage of sunflower oil in diet, as it has been reported that >6% DM contents in diet have decreased fiber digestibility in rumen — through the direct action of fatty acids on the cellular membrane of bacteria or through indirect effects in the availability of Ca^{2+} and Mg^{2+} (Palmquist, 1991).

Table 3 shows that, on the one hand, pH increased ($p<0.05$) lineally as the level of sunflower oil rose at 24 and 48 h. This could be the result of grain content in diets, given that they have fest fermenting carbohydrates. On the other hand, pH decreased ($p<0.05$) at 72 h of incubation, which may be related to the accumulation of organic acids in the *in vitro* system, where there is no flow of organic acids towards other systems (Dehority *et al.*, 2003). Total bacteria count decreased ($p<0.05$) lineally at 24 and 72 h of incubation between treatments. Ley de Coss *et al.* (2013) reported a total bacteria concentration of 109 ml^{-1}, while Dehority (2003) published values of 1010 to 1012 bacteria mL^{-1} in rumen. These values are higher than those obtained in this study, since high fat content

Table 2. Production of partial gas and total gas and methane totals in diets prepared with sunflower oil.

Incubation (h)	Treatment			SEM	Value of p	
	T1	T2	T3		Linear	Quadratic
Total gas production (ml g^{-1} MS)						
24	102.85[a]	83.94[b]	91.70[ab]	2.5640	0.0429	0.0063
48	37.93[b]	47.66[a]	49.14[a]	1.6980	0.0044	0.1841
72	9.43[b]	12.36[ab]	15.11[a]	0.6640	0.0001	0.9337
Total	143.19[b]	151.60[ab]	162.16[a]	3.0350	0.0086	0.8560
Methane gas production (ml g^{-1} MS)						
24	38.09	36.54	40.06	1.5470	0.6230	0.4570
48	13.25	13.48	13.18	0.3590	0.9442	0.7359
72	6.96[a]	4.72[b]	4.55[b]	0.3120	0.0002	0.0332
Total	61.79	56.80	58.88	1.554	0.4512	0.3011

[a,b] Mean values with different letters in a row indicate differences ($p\leq0.05$).
T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil; SEM: Standard error of the mean.

Table 3. Fermentative characteristics of diets prepared with sunflower oil.

Incubation (h)	Treatment			SEM	Value of p	
	T1	T2	T3		Linear	Quadratic
In vitro degradability of dry matter (%)						
24	41.80^{b}	41.49^{b}	47.85^{a}	1.0030	0.0050	0.0410
48	48.08^{b}	61.38^{a}	68.48^{a}	2.7750	0.0002	0.3414
72	72.75^{a}	67.48^{b}	68.98^{ab}	0.8650	0.0430	0.0370
In vitro degradability of neutral detergent fiber (%)						
24	43.51	47.90	45.06	1.087	0.5564	0.1456
48	45.85^{b}	52.29^{a}	54.85^{a}	1.252	0.0009	0.2719
72	57.87	55.66	56.98	0.499	0.4553	0.1057
In vitro degradability of acid detergent fiber (%)						
24	20.30	14.44	22.06	1.367	0.5314	0.0224
48	25.14^{b}	37.19^{a}	44.81^{a}	2.578	0.0001	0.3976
72	49.79^{a}	46.28^{b}	50.39^{a}	0.674	0.6396	0.004
pH						
24	5.48^{b}	5.52^{ab}	5.58^{a}	0.015	0.0041	0.6903
48	5.54	5.52	5.58	0.013	0.2149	0.1564
72	5.64	5.60	5.60	0.009	0.0687	0.2707
Total bacteria (10^{-9} cells mL^{-1})						
24	6.16^{a}	5.68^{a}	4.56^{b}	0.233	0.0016	0.3676
48	5.68	6.20	6.88	0.492	0.3435	0.9451
72	6.00	5.30	5.12	0.170	0.0243	0.4274

^{a,b} Mean values with different letters in a row indicate differences ($p \leq 0.05$).
T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil; SEM: Standard error of the mean.

in diet reduces the number of protozoa as well as bacterial concentration in rumen (Yang *et al.*, 2009), especially fibrolytic bacteria, which are more sensitive to fat content in diet (Patra and Yu, 2013). Table 4 shows that the acetic and propionic acid ratio did not display any difference between treatments at 24 and 72 h of incubation ($p>0.05$). Butyric acid content decreased ($p<0.05$) lineally at 48 h of incubation. The inclusion of oil in diets did not result in the decrease of the total concentration of fatty acids during the measured incubation times ($p>0.05$). On the contrary, Toral *et al.* (2011) reported that the total fatty acid content diminished with diets including 20 g kg^{-1} of sunflower oil and 10 g kg^{-1} of fish oil in adult sheep. The H_2 generated, along with the presence of methanogenic archaea, increase methane production by using H_2 and CO_2 as a source of energy (Kim *et al.*, 2012). Meanwhile, Jordan *et al.* (2006) have observed that using coconut oil decreases the total VFA concentration, because it reduces the digestibility of dry matter and NDF and ADF components.

Table 4. Volatile fatty acid (mM L^{-1}) and ammoniacal nitrogen (mg dL^{-1}) concentration in the *in vitro* fermentation of sunflower oil-based diets for sheep.

Incubation (h)	Treatment			SEM	Value of p	
	T1	T2	T3		Linear	Quadratic
Acetic (mM L^{-1})						
24	36.76	35.38	37.23	0.451	0.663	0.102
48	41.78	44.44	40.81	0.687	0.515	0.024
72	42.68	42.12	41.64	0.404	0.331	0.965
Propionic (mM L^{-1})						
24	21.70	20.84	21.61	0.280	0.891	0.194
48	24.02	24.90	23.31	0.420	0.494	0.186
72	28.64	29.22	28.45	0.196	0.685	0.118
Butyric (mM L^{-1})						
24	15.38	15.33	14.63	0.182	0.098	0.379
48	16.54[a]	17.08[a]	15.51[b]	0.211	0.008	0.002
72	15.92	16.12	16.07	0.122	0.631	0.648
A:P Ratio						
24	1.69	1.70	1.73	0.025	0.608	0.930
48	1.79	1.79	1.75	0.020	0.543	0.659
72	1.49	1.44	1.46	0.015	0.495	0.295
Ammoniacal nitrogen (mg dL^{-1})						
24	23.62	24.73	24.49	0.485	0.496	0.541
48	31.44[a]	27.41[b]	27.22[b]	0.666	0.002	0.066
72	29.49[b]	35.27[a]	32.40[ab]	1.030	0.196	0.031

[a,b] Mean values with different letters in a row indicate differences ($p \leq 0.05$).
T1: Complete diet without sunflower oil; T2: Complete diet with 1% sunflower oil; T3: Complete diet with 2% sunflower oil; A:P acetic-propionic; SEM: Standard error of the mean.

CONCLUSIONS

The inclusion of sunflower oil in sheep diet has had a positive influence on the *in vitro* degradability of dry matter, without affecting fiber degradability. Therefore, it could be used as an alternative to reduce methane emissions in ruminants.

ACKNOWLEDGMENTS

The authors would like to thank the Colegio de Postgraduados - Campus Montecillo for their technical support, and the UAGRO-CA-183 academic group (sustainable production of ruminants in the tropics) for their support in the development of this research.

REFERENCES

AOAC. (2005). Official Methods of Analysis of AOAC International. (18td Ed). AOAC International, Gaithersburg, MD, USA, Official Method.

Bodas, R., Prieto, N., Garcia-Gonzalez, R., Andres, S., Giráldez, F.J., López, S. (2012). Manipulation of rumen fermentation and methane production with plant secondary metabolites. *Animal Feed Science and Technology. 176*(1-4), 78–93. Doi: 10.1016/j.anifeedsci.2012.07.010

Dohme, F., Machmüller, A., Estermann, B.L., Pfister, P., Wasserfallen, A., Kreuzer, M. (1999). The role of the rumen ciliate protozoa for methane suppression caused by coconut oil. *Letters in Applied Microbiology. 29*(3), 187-192. Doi: 10.1046/j.1365-2672.1999.00614.x

Eckard, R.J., Grainger, C., de Klein M.C.A. (2010). Options for the abatement of methane and nitrous oxide from ruminant production: *A review, Livestock Science. 130*(1-3), 47-56. Doi: 10.1016/j.livsci.2010.02.010

Gatachew, G., Blümmel, B., Makkar, H.P.S., Becker, K. (1988). *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. *Animal Feed Science and Technol., 72*(3-4). 261-281. Doi: 10.1016/S0377-8401(97)00189-2

Grainger, C., Beauchemin, K.A. (2011). Can enteric methane emissions from ruminants be lowered without lowering their production? *Animal Feed Science and Technology*. 166-167. 308-320. Doi: 10.1016/j.anifeedsci.2011.04.021

Hernández-Morales, J., Sánchez-Santillán, P., Torres-Salado, N., Herrera-Pérez, J., Rojas-García, A.R., Reyes-Vázquez, I., Mendoza-Nuñez, M.A. (2018). Composición química y degradaciones *in vitro* de vainas y hojas de leguminosas arbóreas del trópico seco mexicano. *Revista Méxicana de Ciencias Pecuarias. 9*(1), 105-115. Doi: 10.22319/rmcp.v9i1.4332

Hill, J.C., McSweeney, G.A., Wright, G., Bishop-Hurley., Kalantar-Zedeh, K. (2016). Measuring methane production from ruminants. *Trends in Biotechnology. 34*(1), 26-35. Doi: 10.1016/j.tibtech.2015.10.004

Hristov, A.N., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A., Yang, W., Tricarico, J., Kebreab, E., Waghorn, G., Dijkstra, J., Oosting, S. (2013). Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO_2 emissions. Edited by Pierre J. Gerber, Benjamin Henderson and Harinder P.S. Makkar.

FAO Animal Production and Health Paper. 177-206. FAO, Rome, Italy

Jalc, D., Certik, M., Kundrikova, K., Namestkova, P. (2007). Effect of unsaturated C-18 fatty acids (oleic, linoleic and alpha-linolenic acid) on ruminal fermentation and production of fatty acid isomers in an artificial rumen. *Veterinarni Medicina. 52*(3), 87–94. Doi: 10.17221/2012-VETMED

Jordan, E., Lovett, D.K., Monahan, F.J., Callan, J., Flynn, B., O'Mara, F.P. (2006). Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. *Journal of Animal Science. 84*(1), 162–170. Doi: 10.2527/2006.841162x

Key, N. and Tallard, G. (2012). Mitigating methane emissions from livestocks: a global analysis of sector policies. *Climatic Change. 112*(2), 387–414. Doi: 10.1007/s10584-011-0206-6

Kim, M.J., Lee, J.S., Kumar, S., Rahman, M.M., Shin, J.S., Ra, C.S. (2012). Indirect estimation of CH_4 from livestock feeds through TOCs evaluation. *Australasian Journal of Animal Science. 25*(4). 496-501. Doi: 10.5713/ajas.2011.11352

Kumar, S., Choudhury, K.P., Carro, D.M., Griffith, W.G., Dagar, S.S., Puniya, M., Calabro, S., Ravella, S.R., Dhewa, T., Upadhyay, C.R., Sirohi, S.K., Kundu, S.S., Wanapar, M., Puniya, A.K. (2014). New aspects and strategies for methane mitigation from ruminants, *Applied Microbiology and Biotechnology. 98*(1), 31-44. Doi: 10.1007/s00253-013-5365-0

Kumar, S., Puniya, A.K., Puniya, M., Dagar, S.S., Sirohi, S.K., Singh, K., Griffith, G.W. (2009). Factors affecting rumen methanogens and methane mitigation strategies. *World Journal of Microbiology and Biotechnology. 25*(9), 1557-1566. Doi: 10.1007/s11274-009-0041-3

Ley de Coss, A., Arce-Espino, C., Cobos-Peralta, M., Hernández-Sánchez, D., Pinto-Ruiz, R. (2013). Estudio comparativo entre la cepa de *Pediococcus acidilactici* aislada del rumen de borregos y un consorcio de bacterias ruminales. *Agrociencia. 47*(6), 567-578.

Machmuller, A., Ossowski, D.A., Kreuzer, M. (2000). Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. *Animal Feed Science and Technology. 85*(1-2), 41–60. Doi: 10.1016/S0377-8401(00)00126-7

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *The Journal of Agricultural. Science. 93*(1), 217-222. Doi: 10.1017/S0021859600086305

NRC, (2007). National Research Council. Nutriment Requirements of Small Ruminants, Sheep, Goats, Cervids and New World Camelids. Washington, D.C. USA. The National Academics Press. 362 p.

Palmquist, D.L. (1991). Influence of source and amount of dietary fat on digestibility in lactating cows. *Journal of Dairy Science, 74*(4), 1354-1360. Doi: 10.3168/jds.S0022-0302(91)78290-8

Patra, A.K. (2014). A meta-analysis of the effect of dietary fat on enteric methane production, digestibility and rumen fermentation in sheep, and a comparison of these responses between cattle and sheep. *Livestock Science*. 162, 97-103. Doi: 10.1016/j.livsci.2014.01.007

Patra, A.K., Yu, Z. (2013). Effects of coconut and fish oils on ruminal methanogenesis, fermentation, and abundance and diversity of microbial populations *in vitro*. *Journal of Dairy Science. 96*(3), 1782-1792. Doi: 10.3168/jds.2012-6159

Prada-Matiz, A., Cortés-Castillo, C.E. (2011). Experiencias en la captura de los gases de combustión de la cascarilla de arroz con soluciones alcalinas. *Orinoquia. 15*(1), 16-30.

Sánchez-Santillán, P., Cobos-Peralta, M.A. (2016). Producción *in vitro* de ácidos grasos volátiles de bacterias celulolíticas reactivadas y bacterias ruminales totales en sustratos celulósicos. *Agrociencia. 50*(5). 565-574.

Sánchez-Santillán, P., Meneses-Mayo, M., Miranda-Romero, L., Santellano-Estrada, E., Alarcón-Zuñiga, B. (2015). Actividad fibrolítica y producción de gas de *Pleurotusostreatus*-IES y *Fomes fomentarius*-EUM1 en bagazo de caña. *Revista MVZ Cordoba. 20*(1). 4907-4916.

SAS. (2011). SAS/STAT Software, Version 9.3. Cary NC SAS USA: Institute INC.

Steinfeld, H., Gerber, P., Wassenaar, T. D., Castel, V., Rosales, M., Rosales, M., de Haan, C. (2006). Livestock's long shadow: environmental issues and options. Food & Agriculture Org.

Toral, P.G., Hervás, G., Bichi, E., Belenguer, A., Frutos, P. (2011). Tannins as feed additives to modulate ruminal biohydrogenation: Effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil. *Animal Feed Science and Technology. 164*(3-4), 199-206. Doi: 10.1016/j.anifeedsci.2011.01.011

Torres-Salado, N., Sánchez-Santillán, P., Rojas-Garcia, R.A., Herrera-Pérez, J., Hernandez-Morales, J. (2018). Producción de gases de efecto invernadero *in vitro* de leguminosas arbóreas del trópico seco mexicano. *Archivos de Zootecnia. 67*(257), 55-59. DOI: Doi: 10.21071/az.v67i257.3491

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal Dairy Science. 74*(10), 3583-3597. Doi: 10.3168/jds.S0022-0302(91)78551-2

Wang, M.Z., Jing, Y.J., Liu, S.M., Gao, J., Shi, L.F., Vercoe, P. (2016). Soybeam oil suppresses ruminal methane production and reduces content of coenzyme F-420 *in vitro* fermentation. *Animal Production Science. 56*(3), 627-633. Doi: 10.1071/AN15553

Yang, S.L., Bu, D.P., Wang, J.Q., Hu, Z.Y., Li, D., Wei, H.Y., Zhou, L.Y., Loor, J.J. (2009). Soybean oil and linseed oil supplementation effect profiles of ruminal microorganisms in dairy cows. *Animal. 3*(11), 1562-1569. Doi: 10.1017 / S1751731109990462.