



CRUDE GLYCEROL (1, 2,3-PROPANETRIOL) FROM *JATROPHA CURCAS* L. ITS EFFECT ON FERMENTATION DYNAMICS AND RUMEN ECOLOGY

GLICEROL CRUDO (1,2, 3 PROPANOTRIOL) DE *JATROPHA CURCAS* L. SU EFECTO EN LA DINÁMICA FERMENTATIVA Y ECOLOGÍA DEL RUMEN

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To evaluate the effect of crude glycerol from *Jatropha curcas* L. on the fermentation dynamics and rumen ecology, an *in vitro* experiment was conducted. The treatments were: 1) star grass + concentrate without glycerol (control), 2) star grass + concentrate with 3 % glycerol and 3) star grass + concentrate with 6 % glycerol. Sampling was carried out at 0 hours (before incubation) and at 3 and 6 hours after the start of incubation. The populations of total viable bacteria, cellulolytic, proteolytic and cellulolytic fungi were determined according to a completely random design in factorial arrangement. Nonparametric analysis of variance was performed. The populations of total viable bacteria, cellulolytic bacteria, and cellulolytic fungi were higher with 3 % glycerol, and there was no effect on the population of proteolytic bacteria. There were changes in the concentration of total short-chain fatty acids, their molar proportions and the acetic/propionic acid ratio. The estimated glucose release was 10788.67, 11200.33 and 12110.00 g. The methane produced was 751.56, 708.04 and 726.67 g and the microbial biomass was 4126.2, 4347.7 and 5621.5 g for the control (without glycerol), 3 and 6 %, respectively. It is concluded that glycerol modifies microbial populations and fermentative processes in the rumen. These studies are the first to use glycerol from *J. curcas* in the rumen.

Para evaluar el efecto del glicerol crudo de *Jatropha curcas* L. en la dinámica fermentativa y ecología del rumen se condujo un experimento *in vitro*. Los tratamientos fueron: 1) pasto estrella + concentrado sin glicerol (control), 2) pasto estrella + concentrado con 3 % de glicerol y 3) pasto estrella + concentrado con 6 % de glicerol. Los muestreos se realizaron a las 0 horas (antes de incubación) y a las 3 y 6 horas posteriores al inicio de la incubación. Se determinaron las poblaciones de bacterias viables totales, celulolíticas, proteolíticas y hongos celulolíticos, según diseño completamente aleatorizado en arreglo factorial. Se realizó análisis de varianza no paramétrico. Las poblaciones de bacterias viables totales, celulolíticas y hongos celulolíticos fueron superiores con 3 % del glicerol y no hubo efectos en la población de bacterias proteolíticas. Se encontraron modificaciones en la concentración de ácidos grasos de cadena corta totales, sus proporciones molares y la relación acético/propiónico. La liberación de glucosa estimada fue de 10788.67, 11200.33 y 12110.00 g. El metano que se produjo fue 751.56, 708.04 y 726.67 g y la biomasa microbiana fue 4126.2, 4347.7 y 5621.5 g para el control (sin glicerol), 3 y 6 %, respectivamente. Se concluye que el glicerol modifica las poblaciones microbianas y procesos fermentativos del rumen. Estos estudios constituyen los primeros con la utilización del glicerol de *J. curcas* en el rumen.

Key words: bacteria, glucose, rumen, SCFA

Palabras clave: AGCC, bacterias, glucosa, rumen

Introduction

Glycerol, also known as glycerin or 1, 2,3 propanetriol ($C_3H_8O_3$), is an alcoholic compound with three -OH groups (hydroxyls), a byproduct generated in biodiesel manufacturing processes (Awogbemi and Desai 2025) and

which negatively impacts the environment (Ningaraju *et al.* 2022). Therefore, its use in animal feeding solves the environmental problem and provides energy to the diet (Zacaroni *et al.* 2022, Zhang *et al.* 2022 and Fraga *et al.* 2024).

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In Guantánamo, Cuba, projects were carried out in which glycerol was obtained from the *J. curcas* seeds (Sotolongo et al. 2021) and its use in nutritional physiology and milk production was evaluated (Gaillard et al. 2018 and Bonis et al. 2024).

The use of glycerol in the feeding of ruminant animals has important advantages. The beneficial effect of glycerol is reported as a preventive strategy for metabolic disorders, characterized by nutritional deficiencies or imbalances: ketosis, fatty liver, puerperal hypocalcemia and hypomagnesemic tetany, called production diseases (Delgado et al. 2018). This is because glycerol is characterized as an exogenous glucogenic precursor, which supplies energy needs and controls fat mobilization by stimulating the release of insulin, which has an inhibitory effect on lipolysis. Its addition allows the reduction of the negative energy balance of animals as well as the catabolism of body fat and serum levels of non-esterified fatty acids (Gómez and Campos 2016). The inclusion of 10 % glycerol in the dry matter of the diet of high-producing Holstein cows was also reported, allowing the replacement of corn with glycerol (Delgado et al. 2016).

Glycerol is mainly used as an alternative for animal supplementation. It is considered a partial substitute for corn in high-production bovine diets, given its similarity in net energy value (Filho et al. 2024). Other studies conducted by Clariget et al. (2016) showed that there are no significant differences between animals fed with corn, compared to glycerol.

There are no studies on the application of *J. curcas* glycerol to rumen physiology, specifically to its microbial populations, so the objective of this study was to evaluate the effect of crude glycerol from *Jatropha curcas* L. on the fermentation dynamics and rumen ecology with star grass under *in vitro* conditions.

Materials and Methods

Origin of vegetable oil: The vegetable oil from *J. curcas*, used in the process of obtaining glycerol, was obtained during the biodiesel production process at Paraguay Biodiesel Plant, belonging to LABIOFAM, in the coastal region of Guantánamo province.

Procedure for cleaning, extraction and transesterification: The ripe fruits were harvested, sun-dried and shelled to obtain the seed. To extract the oil, the seeds were pressed using an expeller machine with a power of 7.5 kW, a speed of 1,400 rpm, and a capacity of 200 kg of seeds per hour. The crude oil obtained was filtered through a filter press, which guaranteed a 25 micron product. Subsequently, the oil was subjected to a heating process at 105 °C to extract all soluble and volatile impurities, including water (Piloto et al. 2021 and Sotolongo et al. 2021).

Indicators, test methods and international quality standards: The tests were carried out at the Energy and Refrigeration Studies Center of the Faculty of Mechanical and Industrial Energy from Universidad de Oriente. They were described by Bonis et al. (2024), as well as the physicochemical characterization of crude glycerol, obtained in the transesterification process of *J. curcas* L. oil.

The research was conducted in the rumen microbiology laboratories of the Unidad Central de Laboratorios (UCELAB) from Instituto de Ciencia Animal, located at km 47½, in San José de las Lajas, Mayabeque, Cuba. The *in vitro* gas production technique described by Theodorou et al. (1994) was applied. The experimental units consisted of 100 mL bottles, into which 1 g of the food to be evaluated and 80 mL of the rumen fluid/buffer solution of Menke and Steingass (1988) were introduced in a 1:3 ratio. Four repetitions were set up in the experiment.

Treatments: Treatments were compared according to the level of corn by glycerol substitution in the feed: a) control (no glycerol), b) 3 % glycerol, and c) 6 % glycerol. The composition of the experimental diets is shown in table 1.

Star grass (*C. nlemfuensis*) was used as a base food. It was collected in a similar way to animal bite and the leaves were taken with their petioles. The material was oven-dried at 60 °C until constant weight was reached. Then, it was ground in a hammer mill to a particle size of 1.0 mm and stored in hermetically sealed in glass bottles. The chemical composition of star grass was 11.90, 41.06, 39.95, 0.54 and 0.08 % of the DM of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca) and phosphorus (P), respectively (AOAC 2016).

Table 1. Description of the concentrate, according to the level of glycerol incorporation in the diet in dry matter, kg (%)*

Source	Control	3 %	6 %
Corn	1.5 (60.0)	1.27 (50.8)	0.98 (39.2)
Glycerol	-	0.3 (12.0)	0.6 (24.0)
Soy bean	0.415 (16.6)	0.414 (16.7)	0.455 (18.2)
Wheat bran	0.357 (14.3)	0.276 (11.0)	0.219 (8.9)
Urea	0.015 (0.6)	0.027 (1.1)	0.033 (1.3)
Minerals	0.125 (5.0)	0.125 (5.0)	0.125 (5.0)
Common salt	0.088 (3.5)	0.088 (3.5)	0.088 (3.5)

*Percentage in relation to the total concentrate

Sampling: Sampling was carried out dynamically at 0 h (before incubation) and at 3 and 6 h after the start of incubation. The microbiological indicators: population of total viable bacteria, cellulolytic, proteolytic and cellulolytic fungi were determined. For the culture of viable bacteria, the [Hungate \(1950\)](#) microbial culture technique was used in rolled tubes and under strict anaerobic conditions. The culture of totally viable, cellulolytic and proteolytic bacteria was carried out in the culture media of [Caldwell and Bryant \(1966\)](#), modified by [Elias \(1971\)](#). In the case of proteolytic bacteria, 10 % sterile skimmed milk was added, according to [Galindo \(1988\)](#). [Joblin \(1981\)](#) culture medium was used to determine the fungal population.

Colony counts of total viable bacteria, cellulolytic, proteolytic and fungal bacteria were performed by placing the rolled tubes under a magnifying glass and counting all colonies for total viable bacteria and for proteolytic, cellulolytic and cellulolytic fungi, only those that showed a digestion halo. The results were expressed as colony forming units (CFU) for bacteria and thallus forming units (TFU) for fungi.

Animals donating rumen fluid: To obtain the ruminal fluid inoculum, two Holstein-Zebu crossbred cows were used, cannulated in the dorsal rumen sac. They were kept in stable conditions and intake low-quality forage *ad libitum* and 2 kg of commercial concentrate for dairy cows, with free access to water and mineral salts. Ruminal fluid was extracted from fasting animals through a cannula using a vacuum pump. It was kept in vacuum flask with a hermetic seal to guarantee the temperature conditions (39 °C) and anaerobiosis during the transfer to the laboratory, where it was filtered through muslin before use. The production of short chain fatty acids (SCFA) was determined by gas chromatography, according to [Cottyn and Boucque \(1968\)](#).

Stoichiometric balance determinations:

- Based on the results of SCFA and using the BALANCE and RUMETANO programs ([Stuart 2016](#)), the stoichiometric balance of ruminal fermentation was determined.
- Determination of bacterial biomass (BB) and fermented organic matter (fOM), according to [Smith \(1975\)](#).

Experimental design and statistical processing: For the analysis of the variables total bacteria, proteolytic, cellulolytic and fungi, the methodology proposed by [Herrera et al. \(2015\)](#) was applied. First, the theoretical assumptions of the classic ANOVA, normality of the residues by the [Shapiro-Wilk test \(1965\)](#) and homogeneity of variance by [Levene \(1960\)](#) were verified, both of which were not fulfilled. Subsequently, they were transformed by $\ln$ and these assumptions did not improve, so a non-parametric analysis of variance was performed according to a completely random design ([Kruskal Wallis 1952](#)). For the comparison of mean ranges, [Conover \(1999\)](#) test was used for $p < 0.05$. The statistical package InfoStat, version 2012 ([Balzarini et al. 2012](#)) was used.

Results and Discussion

There was no interaction between the effect of glycerol treatment and sampling time in this experiment. These reasons justify presenting the effects of glycerol and sampling time separately.

Table 2 shows the results of the effect of glycerol on the populations of total viable bacteria, proteolytic bacteria, as well as cellulolytic bacteria and fungi in the rumen. In the treatment where 3 % glycerol was used in the concentrate, the population of total viable bacteria was higher than the control, and did not differ from the treatment with 6 % glycerol. Therefore, populations of cellulolytic bacteria and fungi were more numerous when the 3 % glycerol level was used in the feed, while the control treatment without glycerol and the one in which 6 % was used did not showed differences. However, there were no effects of glycerol level on the population of proteolytic bacteria.

Ruminococcus albus, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* are known to be the most important cellulolytic bacteria in the rumen. Studies conducted by [Dengke et al. \(2022\)](#) showed that certain rumen bacteria are capable of degrading the cellulose intake by ruminants into glucose and cellobiose, as well as using other carbon compounds, including maltose, lactose, xylose and starch, and also producing acetate and succinate, while requiring biotin and p-aminobenzoic acid. Additionally,

Table 2. Effect of glycerol level of *J. curcas* on the *in vitro* microbial population of star grass (*C. nlemfuensis*)

Microbial groups	Glycerol, %			Signif.
	0	3	6	
Total viable bacteria, 10^{11} CFU.mL ⁻¹	31.02 ^b (7.00), SD=4.41	50.16 ^a (10.00), SD=8.81	43.00 ^{ab} (12.00), SD=22.28	p=0.0107
Proteolytic bacteria, 10^6 CFU.mL ⁻¹	47.39 (11.00), SD=6.07	40.38 (7.50), SD=6.64	36.78 (7.00), SD=3.86	p=0.2476
Cellulolytic bacteria, 10^6 CFU.mL ⁻¹	28.50 ^b (7.00), SD=4.17	53.36 ^a (13.00), SD=5.81	40.22 ^b (10.00), SD=5.09	p=0.0005
Cellulolytic fungi, 10^5 TFU.mL ⁻¹	31.73 ^b (4.00), SD=2.68	55.68 ^a (10.00), SD=6.10	34.73 ^b (5.00), SD=2.87	p=0.0005

() Data between parenthesis in the median, SD: Standard deviation

cellulolytic activity has also been reported in studies conducted by metagenomic technologies from the fungi *Neocallimastix frontalis*, *Neocallimastix patriciarum* and *Neocallimastix joyonii*, among others, and also from the protozoa *Eudiplodinium maggie*, *Ostracodinium album* and *Epidinium caudatum*.

In studies performed by Bonis et al. (2024), where the effect of glycerol from *J. curcas* was evaluated on Siboney de Cuba dairy cows from, grazing pitilla (*Sporobolus indicus* L.) of low nutritional level, increases of 1.23 L milk/cow/d were recorded. These are the first reports of glycerol use in Cuba for milk production, although Nivia-Osuna et al. (2020) previously reported that the effect of glycerol on *J. curcas* has not been conclusive enough. Further studies are needed to evaluate different basic diets.

The effect of glycerol on the population of lipolytic bacteria was not evaluated in this research. However, this group is in the population of total viable bacteria, which were more numerous when glycerol was used.

Lipolytic bacteria, including *Anaerovibrio lipolyticus*, are capable of using and hydrolyzing glycerol in the fat molecule. This group also includes organisms that hydrogenate unsaturated fatty acids and those that metabolize long-chain fatty acids to ketone bodies (Kansagara et al. 2022).

In this regard, Hidalgo-Hernández et al. (2018) showed that lipolytic bacteria, such as *Selenomonas ruminantium* and *Selenomonas dextrinosolvens*, are the groups with the greatest participation in the fermentation of glycerol to propionic acid, mainly. *Selenomonas ruminantium* is also one of the main rumen bacteria that ferment starches that reach the organ from food, and its population increases when the animals' diets are based on starches, but it is also capable of fermenting glycerol.

In the study carried out on the chemical composition by GC-DM of glycerol from *J. curcas* L., it was found that 5.5 % of the long-chain unsaturated fatty acids are C18:1c9 (oleic) and C18:2 c9 c12 (linoleic), which is of great importance for the health of the animals that intake it (Galindo et al. 2025).

Vesga et al. (2024) alluded to the fact that unsaturated fatty acids could exert toxic effects on some rumen microorganisms, including cellulolytic microorganisms and some protozoan species, while other researchers (Granja-Salcedo et al. 2017) report that the addition of vegetable oils with conjugated linoleic acid increases the disposition of UFA in the duodenum.

When analyzing the effect of fermentation time on the populations of rumen microorganisms evaluated, there were only differences between fermentation times for proteolytic bacteria, with higher concentrations at time zero (before incubation) and three hours after incubation. The study showed that the maximum population of these microbial groups was found three hours after the fermentation began (table 3).

The results of the study with total short-chain fatty acids (SCFAs) seem to show that glycerol produced modifications in its total concentration in the rumen, as well as in the acetic/propionic acid ratio (table 4). However, the information is not entirely accurate, because technical difficulties arose in the laboratory that prevented the completion of the information necessary to carry out the statistical studies. However, it was decided to use the information obtained due to its relative importance in relation to other studies.

It is observed that glycerol is able of increasing the total production of short-chain fatty acids *in vitro*, fundamentally the production of propionic acid, as reported by Li et al. (2022).

Table 3. Effect of fermentation time on some physiological groups of bacteria and cellulolytic fungi of the rumen with glycerol

Microbial groups	Fermentation time, h			
	0 (before start)	3 h	6 h	Signif.
Total viable bacteria, 10 ¹¹ CFU.mL ⁻¹	35.37 (7.00), SD=8.46	47.67 (11.00), SD=22.44	41.43 (10.00), SD=8.65	p=0.1643
Proteolytic bacteria, 10 ⁶ CFU.mL ⁻¹	42.00 ^a (7.00), SD=6.19	54.37 ^a (11.00), SD=5.92	28.61 ^b (6.00), SD=3.29	p=0.0003
Cellulolytic bacteria, 10 ⁶ CFU.mL ⁻¹	43.69 (10.00), SD=5.97	45.38 (10.50), SD=5.46	34.34 (8.00), SD=5.14	p=0.1722
Cellulolytic fungi, 10 ⁵ TFU.mL ⁻¹	43.19 (6.00), SD=5.15	35.62 (4.00), SD=6.20	43.89 (5.00), SD=3.38	p=0.1722

() Data between parenthesis in the median, SD: Standard deviation

Table 4. Total short-chain fatty acids using two levels of glycerol compared to the control without glycerol

Indicator	Glycerol, %		
	Control (0)	3	6
TSCFA, meq.L ⁻¹	149.0	157.9	203.7
Acetic: propionic	3.10	2.59	2.77

It is well established in the scientific literature that glycerol is fermented to short-chain fatty acids (SCFAs) in the rumen. The first studies of glycerol fermentation show that virtually all glycerol is fermented to propionate (Valencia-Echavarría *et al.* 2024).

It is known from previous studies that glycerol can enter the glycolytic way, converting it into pyruvate, which generates propionate by two different routes: succinate or acrylate. This justifies the increase in propionate, by adding glycerol to the ruminants diet (Vera *et al.* 2025). Likewise, it is known that propionate is of vital importance for glucose synthesis in ruminant animals (Arita-Portillo y Elizondo-Salazar 2023).

Previous studies indicates increases in acetic acid and propionic acid, increases in propionic acid and butyric acid or both (van-Cleef *et al.* 2016) with decreases in acetic acid, which contributes to the depreciation in the acetic:propionic ratio, as reported by Marchelli *et al.* (2015). These authors reported that most of the glycerol is fermented to SCFAs through glycolytic way with a small production of lactic acid. Thus, fermentation of glycerol in the rumen increases the concentration of propionic and butyric acid, while acetic acid decreases.

Considering that propionic acid, like glycerol itself, are potent neoglucogenic agents (Ladeira *et al.* 2016), it is reasonable to use glycerol as an energy supplement for milk production in the transition period. It could even be more recommendable than other energy sources, because it has a metabolic advantage over its traditional counterparts, especially propionate and propylene glycol, due it enters in the gluconeogenesis at the level of phosphate isomerase, metabolically closer to glucose (Delgado *et al.* 2016 and Delgado *et al.* 2018).

According to Valencia-Echavarría *et al.* (2024), glycerol entering the rumen produces propionic acid, as the main short-chain volatile fatty acid. Additionally, glycerol itself can be absorbed through the rumen mucosa and transformed into glucose in the liver. This is of great importance because it contributes to energy production in cellular metabolism.

Hejna *et al.* (2016) report that propionic acid produced by ruminal fermentation is the main substrate for gluconeogenesis in high-producing dairy cows. This way provides between 50 and 60 % of the total glucose required for metabolism. Studies performed by Chanjula *et al.* (2016) reduced rumen ammonia nitrogen levels by incorporating 6 % glycerol into the diet. However, in the studies conducted by Correa and Moreno (2019), blood urea nitrogen content was not modified.

Table 5 shows the analysis of the estimated stoichiometric balance, according to Stuart (2016). The fermentation pattern shows a marked tendency toward propionic acid production when glycerol is included compared to the control. The reduction in methane with the inclusion of glycerol could have a positive impact on intermediary metabolism.

Conclusions

It can be concluded that glycerol produces modifications in the microbial populations of the rumen and fermentative processes in the organ. Microbial biomass increases and some physiological groups of bacteria are selectively affected. These results presented are the first studies obtained with the use of glycerol from *J. curcas* at the level of rumen microbial populations. Further researches will be required to show its participation in the intermediary metabolism of animals.

Table 5. Effect of glycerol on the stoichiometric balance of ruminal fermentation, fermented organic matter and microbial biomass under *in vitro* conditions

Indicator	Glycerol, %		
	0 (control)	3	6
Acetic acid, % molar	68.01	64.14	64.96
Propionic acid, % molar	21.93	24.80	23.47
Butyric acid, % molar	10.06	11.06	11.58
Glucose released, g	10788.67	11200.33	12110.00
CO ₂ produced, moles	54.58	54.86	55.71
CH ₄ produced, g	751.56	708.04	726.67
H ₂ O produced, moles	21.02	23.46	23.31
Microbial biomass, g	4126.2	4347.7	5621.5
Fermented organic matter, g	11461.5	12076.9	15669.2

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References

- Aita-Portillo, C.R. & Elizondo-Salazar, J.A. 2023. Efecto del uso de una mezcla de compuestos gluconeogénicos en vacas lecheras en transición. *Agronomía Costarricense*, 47(2): 11-120, ISSN: 2215-2202. <https://dx.doi.org/10.15517/rac.v47i2.56136>.
- AOAC. 2016. Official methods of analysis of AOAC International. 20. ed. ed., Rockville MD: AOAC International., Latimer, George W. Jr., ISBN: 9780935584875, Available at: <http://www.worldcat.org/title/official-methods-of-analysis-of-aoac-international/oclc/981578728?referer=null&ht=edition>, [Consulted: April 05, 2018].
- Awogbemi, O. & Desai, D.A. 2025. Progress in the conversion of biodiesel-derived crude glycerol into biofuels and other bioproducts. *Bioresource Technology Reports*, 30(1): 102106, ISSN: 2589-014X. <https://doi.org/10.1016/j.bi-teb.2025.102106>.
- Bonis, R., Valenciaga, D., García López, R., Sotolongo, J.A. & Galindo, J.L. 2024. Effect of crude glycerol from *Jatropha curcas* L. oil on the production and quality of cattle milk. *Cuban Journal of Agricultural Science*, 58: e21, ISSN: 2079-3480. <https://cu-id.com/1996/v58e21>, <https://www.ejascience.com/index.php/CJAS/article/view/1156>.
- Caldwell, D.R. & Bryant, M.P. 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Applied Microbiology*, 14(5): 794-801, ISSN: 2717-5936. <https://doi.org/10.1128/am.14.5.794-801>.
- Chanjula, P., Pongprayoon, S., Kongpan, S. & Cherdthong, A. 2016. Effects of crude glycerin from waste vegetable oil supplementation on feed intake, ruminal fermentation characteristics, and nitrogen utilization of goats. *Tropical Animal Health and Production*, 48(5): 995-1004 ISSN: 1573-7438. <https://doi.org/10.1007/s11250-016-1047-0>.
- Clariget, J.M., Pérez-Clariget R., Álvarez-Oxiley, A., Bentancur, O. & Bruni, M.A. 2016. Suplementación con glicerina cruda y afrechillo de arroz entero a vacas de carne pastoreando campo natural. *Agrociencia Uruguay*, 20(2): 121-131, ISSN: 2301-1548. <https://agrocienciauruguay uy/index.php/agrociencia/article/view/212>.
- Conover, W.J. 1999. Practical Nonparametric Statistics. Third Edition, John Wiley & Sons, New York. 608 pp. ISBN 9780471160687/0471160687.
- Correa, C. & Moreno, L. 2019. Evaluación de la producción de leche, nitrógeno ureico en sangre y algunos componentes de la leche en vacas Holstein suplementadas con glicerol y palmiste en la dieta. *Revista Colombiana de Zootecnia*, 5(10): 38-47, ISSN: 2462-8050. <http://www.anzoo.org/publicaciones/index.php/anzoo/article/view/95/91>.
- Cottyn, B.G. & Boucque, C.V. 1968. Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry*, 16(1): 105-107, ISSN: 1520-5118. <https://doi.org/10.1021/jf60155a002>.
- Delgado, A., Bruni, M.A., Galindo, J.L., Marchelli, J.P., Rodríguez, D. & Chilibroste, P. 2016. Efectos del glicerol al inicio de la lactancia en la producción y calidad de la leche de vacas Holando en pastoreo. *Avances en Investigación Agropecuaria*, 20(2): 5-18, ISSN: 2683-1716. <https://www.redalyc.org/journal/837/83754343002/83754343002.pdf>.
- Delgado, A., Bruni, M.A., Galindo, J.L., Marchelli, J.P., Rodríguez, D. & Chilibroste, P. 2018. Efecto de la sustitución de maíz por glicerol crudo sobre el consumo de materia seca, en vacas Holando en pastoreo. *Pastos y Forrajes*, 41(2): 131-137, ISSN: 2078-8452. http://scielo.sld.cu/scielo.php?script=sci_art-text&pid=S0864-03942018000200007&Ing=es&nrm=iso.
- Elias, A. 1971. The rumen bacteria of animals fed on a high-molasses urea diet. Thesis PhD. Aberdeen.
- Filho, R.S.F., Rebelo, L.R., Zanchetin, M., Silva, A.S., de Paula, N.F., Zervoudakis, J.T., da Silva Cabral, L. & Galati, R.L. 2024. Partial replacement of corn grain with levels of crude glycerin on feed intake, digestibility, ruminal fermentation, nitrogen utilization, and performance of feedlot lambs. *Tropical Animal Health and Production*, 56(9): 401, ISSN: 1573-7438. <https://doi.org/10.1007/s11250-024-04245-y>.
- Fraga, D. da Rosa, Ulsenheimer, B.C., Pereira, E.A., da Silva, J.A.G., Baroni, J.I., Pereira, S.N., de Oliveira, L., Huttra, A.P. & Viégas, J. 2024. Milk composition and productivity of Holstein cows in Ryegrass grazing and crude glycerin in the diet. *Revista de Gestão Social e Ambiental*, 18(2): e03635, ISSN: 1981-982X. <https://doi.org/10.24857/rgsa.v18n2-077>.
- Gaillard, C., Sorensen, M., Vestergaard, M.R., Weisbjerg, M.K., Larsen, H., Martinussen, U. & Sehested, J. 2018. Effect of substituting barley with glycerol as energy feed on feed intake, milk production and milk quality in dairy cows in mid or late lactation. *Livestock Science*, 209: 25-31, ISSN: 1878-0490. <https://doi.org/10.1016/j.livsci.2018.01.006>.
- Galindo, J. 1988. Efecto de la zeolita en la población de bacterias celulolíticas y su actividad en vacas que consumen ensilaje. Tesis en opción al grado científico de Dr.C. Veterinarias. Instituto de Ciencia Animal, Cuba.

- Galindo, J., Bonis, R., Valenciaga, D., Sotolongo, J.A., Soca, M., Delgado, A., García, R., Herrera, M. & Suárez, J. 2025. Evaluación del glicerol (1,2, 3 propanotriol), procedente del aceite de *Jatropha curcas*: su efecto en la ecología ruminal y producción de leche vacuna. Informe Final de Proyecto de investigación PN131LH001.48. Evaluación del glicerol (1,2,3 propanotriol), procedente de *Jatropha curcas* en la fisiología ruminal y comportamiento productivo en vacunos de leche y carne. Instituto de Ciencia Animal. Mayabeque, Cuba.
- Gómez, L. & Campos, R. 2016. Control del balance energético negativo y comportamiento productivo y metabólico en vacas doble propósito bajo suplementación energética. *Revista de Investigación Agraria y Ambiental*, 7(1): 147-156, ISSN: 2145-6453. <https://doi.org/10.1016/10.22490/21456453.1545>.
- Granja-Salcedo Y.T., de Souza V.C., Dias A.V., Gomez-Insuasti A.S., Messana J.D. & Berchielli T.T. 2017. Diet containing glycerine and soybean oil can reduce ruminal biohydrogenation in Nellore steers. *Animal Feed Science and Technology*, 225: 195-204, ISSN: 1873-2216. <https://doi.org/10.1016/j.anifeedsci.2017.01.021>.
- Hejna, A., Kosmela, P., Formela, K., Piszczyk, Ł. & Haponiuk, J.T. 2016. Potential applications of crude glycerol in polymer technology-Current state and perspectives. *Renewable and Sustainable Energy Reviews*, 66(C): 449-475, ISSN: 1879-0690. <http://dx.doi.org/10.1016/j.rser.2016.08.020>.
- Herrera, M. Bustillo, C.W. & Torres, V. 2015. Metodología para el análisis estadístico de diferentes de tipos de variables que se miden en las investigaciones que utilizan diseños experimentales relacionados con los modelos de análisis de varianza paramétrico y no paramétrico. ISBN 978-959-7171-57-7. Editorial EDICA, Instituto de Ciencia Animal, Cuba.
- Hidalgo-Hernández, U., Ortega-Cerrilla, M.E., Herrera-Haro, J.G., Ramírez-Mella, M. & Zetina-Córdoba, P. 2018. Glicerol una alternativa para la alimentación de rumiantes. *Agro Productividad*, 11(5): 124-129, ISSN: 2594-0252. <https://doi.org/10.22004/ag.econ.352916>.
- Hungate, R.G. 1950. The anaerobic, mesophilic cellulolytic bacteria. *Bacteriological Reviews*, 14(1): 1-49, ISSN: 2691-9443. <https://doi.org/10.1128/br.14.1.1-49.1950>.
- Balzarini, M., Di Rienzo, A., Cazanove, F., González, L., Tablada, M., Guzmán, W. & Robeldo, W. 2012. InfoStat paquete estadístico InfoStat versión 2012.
- Joblin, K.N. 1981. Isolation, enumeration and maintenance of rumen anaerobic fungi in roll tubes. *Applied and Environmental Microbiology*, 42(6): 1119-1122, ISSN: 1098-5336. <https://doi.org/10.1128/aem.42.6.1119-1122.1981>.
- Kansagara, Y.G., Savsani, H.H., Chavda, M.R., Chavda, J.A., Belim, S.Y., Makwana, K. & Kansagara, B.K. 2022. Rumen microbiota and nutrient metabolism: A Review. *Bhartiya Krishi Anusandhan Patrika*, 37(4): 320- 327, ISSN: 0976-4631. <https://doi.org/10.18805/BKAP486>.
- Kruskal, W. & Wallis, W. 1952. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, 47(260): 583-621, ISSN: 1537-274X. <https://doi.org/10.2307/2280779>.
- Ladeira, M.M., Carvalho, J.R.R., Chizzotti, M.L., Teixeira, P.D., Dias, J.C.O., Gionbelli, T.R.S., Rodrigues, A.C. & Oliveira, D.M. 2016. Effect of increasing levels of glycerin on growth rate, carcass traits and liver gluconeogenesis in young bulls. *Animal Feed Science and Technology*, 219: 241-248, ISSN: 1873-2216. <http://dx.doi.org/10.1016/j.anifeedsci.2016.06.010>.
- Levene, H. 1960. Robust tests for the equality of variance. Contributions to Probability and Statistics. Stanford University Press.
- Li, Y., Wang, H., Zhang, Y., Li, X., Jiang, X. & Ding, H. 2022. Effects of dietary supplementation with glycerol monolaurate (GML) or the combination of GML and tributyrin on growth performance and rumen microbiome of weaned lambs. *Animals*, 12(10): 1309, ISSN: 2076-2615. <http://doi.org/10.3390/ani12101309>.
- Marchelli, J.P., Bruni, M.A. & Chilbroste, P. 2015. Efecto de la sustitución de grano de maíz por glicerol crudo sobre el consumo y patrón de fermentación. *Archivos Latinoamericanos de Producción Animal*, 23(5): 79-80, ISSN: 2075-8359. http://www.alpa.org.ve/ojs/index.php/ojs_files/article/viewFile/2516/903.
- Menke, K.H. & Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Development*, 28: 7-55, ISSN: 0340-3165.
- Ningaraju, C., Yatish, K. & Sakar, M. 2022. Simultaneous refining of biodiesel-derived crude glycerol and synthesis of value-added powdered catalysts for biodiesel production: a green chemistry approach for sustainable biodiesel industries. *Journal of Cleaner Production*, 363: 132448, ISSN: 0959-6526. <https://doi.org/10.1016/j.jclepro.2022.132448>.
- Nivia-Osuna, A., Ramírez-Peña, A., Porras-Sánchez, C.J. & Marentes-Barrantes, D.L. 2020. Glicerol: Suplemento alimenticio y su respuesta en bovinos de leche. *Agronomía Mesoamericana*, 31(3): 821-833, ISSN: 2215-3608. <http://doi.org/10.15517/am.v31i3.39259>.
- Piloto, R., Sotolongo, J.A., Díaz, Y. & Suárez, J. 2021. Extracción de aceite de origen vegetal. En: Biodiésel: producción y uso. Capítulo 2. Editor: Dr.C. Ramón Piloto Rodríguez, Dr.C. Jesús Suárez Hernández y M.Sc. José Angel Sotolongo Pérez. ISBN: 978-959-7138-48-8.
- Shapiro, S. & Wilk, B. 1965. An analysis of variance test for normality (complete simples). *Biometrika*, 52(3-4): 591-611, ISSN: 1464-3510. <https://doi.org/10.2307/2333709>.

- Smith, R.H. 1975. Nitrogen metabolism in the rumen and the composition and nutritive value of nitrogen compounds entering the duodenum. En: Digestion and metabolism in the Ruminant. W. McDonald & A.C.I. Warner (eds.) New England University. Publishing Unit. Armidale. Australia. p. 399
- Sotolongo J.Á., Piloto R., Díaz A. & Hernández J. 2021. Producción de biodiésel. En Libro Biodiésel: producción y uso. Capítulo 4 Editor: Dr.C. Ramón Piloto Rodríguez, Dr.C. Jesús Suárez Hernández y M.Sc. José Ángel Sotolongo Pérez. ISBN 978-959-7138-48-8.
- Stuart, R. 2016. BALANCE-RUMETANO: programa estadístico para el cálculo del balance estequiométrico de la fermentación ruminal. Mayabeque, Cuba: Instituto de Ciencia Animal.
- Theodorou, M.K., Williams, B. A., Dhanoa, M.S., McAllan, A.B. & France, J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, 48(3): 185-197, ISSN: 2321-1628. [https://doi.org/10.1016/0377-8401\(94\)90171-6](https://doi.org/10.1016/0377-8401(94)90171-6).
- Valencia-Echavarría, D.M., Granja-Salcedo, Y.T., Noriega-Marquez, J.G., Valderrama, L.A.G., Vargas, J.A.C. & Berchielli, T.T. 2024. Crude glycerol increase neutral detergent fiber degradability and modulates rumen fermentative dynamics of kikuyu grass in non-lactating Holstein cows raised in tropical conditions. *Dairy*, 5(3): 480-490, ISSN: 2624-862X. <https://doi.org/10.3390/dairy5030037>.
- Van-Cleef, E.H.C.B., Sencanari, J.B.D., Silva, Z.F., D'Aurea, A.P., Favaro, V.R., van Cleef, F.O.S., Homem Júnior, A.C. & Ezequiel. J.M.B. 2016. High concentrations of crude glycerin on ruminal parameters, microbial yield, and in vitro greenhouse gases production in dairy cows. *Canadian Journal of Animal Science*, 96(4): 461-465, ISSN: 1918-1825. <https://doi.org/10.1139/cjas-2015-0170>.
- Vera, N. Suescun-Ospina, T., Gutierrez-Gomez, C., Olms-Salvo, V. & Aviala-Stagno, J. 2025. Effects of linseed and glycerol inclusion in concentrate ruminant diets on methane production using a Rusitec semicontinuous system. *Chilean Journal of Agricultural Research*, 85(3): 434-444, ISSN: 0718-5839. <http://dx.doi.org/10.4067/s0718-58392025000300434>.
- Vesga, D.A., Granja-Salcedo, Y.T., Costa, R.V., Gomes, K.L., Carvalho Alves, Narvaez, H.J. & Berchielli, T.T. 2024. Changes in ruminal fermentation and rumen bacteria population in feedlot cattle during a high lipid diet adaptation. *Animal Science Papers and Reports*, 42(3): 255-270, ISSN: 230-8342. <https://doi.org/10.2478/aspr-2023-0035>.
- Zacaroni, O.F., Lopes, L.M., Júnior, G.S.D., DeVries, T.J., Pereira, R.A., Donkin, S.S. & Pereira, M.N. 2022. Complete replacement of corn grain with crude glycerin for dairy cows. *Livestock Science*, 258: 104893, ISSN: 1878-0490. <https://doi.org/10.1016/j.livsci.2022.104893>.