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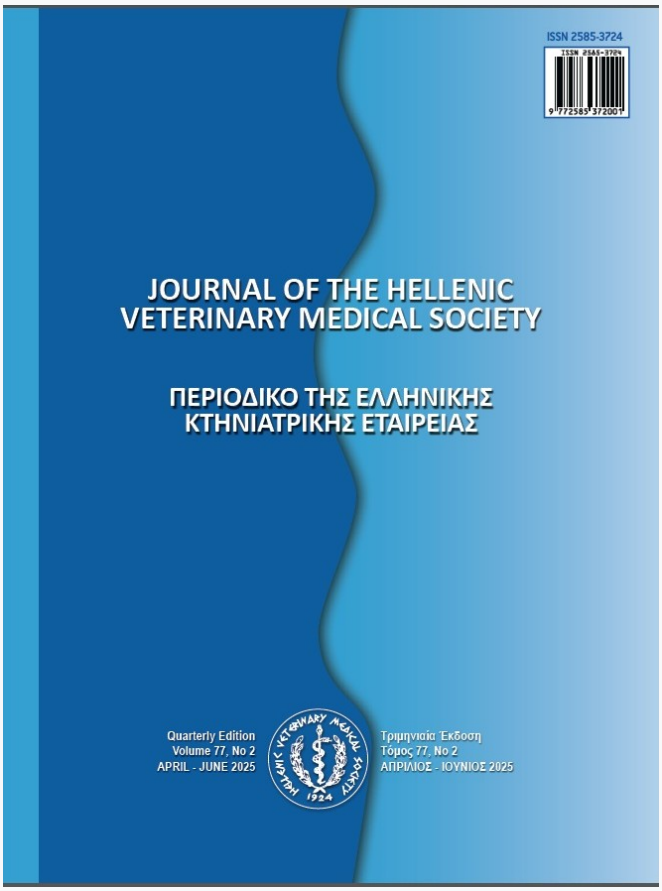

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AMM Mani

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Changes in Rumen Parameters and Microbial Communities Over Inclusion Crud Glycerin in Goat Diet

Ali MM Mani[1*]

Department of Public Health, College of Veterinary, Medicine, Al-Qasim Green University,51001, Babylon, Iraq.

ABSTRACT: Performance and ruminal parameters (pH, NH3, and volatile fatty acid) and microbial community of twenty-four male goats (initial weight 19.5 ± 2.2 kg BW and 120.0 ±10 days of age) fed diets containing crude glycerin (CG) in replacement of corn were investigated. Goats were distributed in a completely randomized design with three levels CG: 0, 100, 150 g/kg of dry matter, six replications, and kept individually in separate yards, experimental diets were formulated to be isonitrogen (CP content 14.5%), isoenergy (ME content 2.19 Mcal/kg) and the basal diet consisted of alfalfa hay (300 g/kg) and concentrate (700 g/kg containing corn and soybean) for 60-days period. The inclusion of CG in the diet did not affect performance trials (final BW and total gain), but at high levels increased final weight (29.8 ±1.8 and 29.6±1.7) mathematically in treated groups than control. No significant differences in DM intake were observed, while improved FCR by (7.46 ±0.32, 6.93 ±1.7, and 6.79 ±1.7) in treated groups. Significantly ($P \leq 0.05$) decreased ruminal pH and ammonia for goats fed the CG diet. There is a significant ($P \leq 0.05$) increase in propionic and butyric acid content in line with the higher level of CG provision, unlike acetic acids, which showed a difference significantly ($P \leq 0.05$) decrease compared with control group. Iisobutyrate, valerate, and isovalerate concentrations and acetic acid to propionic acid ratio (A/P) were not affected by the different levels of CG. Our results showed that incorporation of CG in the diet minor changes in microbial community profile .The treated groups exhibited a decrease in total bacterial genera content with the higher level of CG provision(8.32 ± 0.26,8.01 ± 0.16 and 7.46 ± 0.18)respectively. The relative abundance of Fibrobacter succinogenes ($p = 0.021$) was lower in the treated groups(4.01 ± 0.20, 3.63 ± 0.23 and 3.22 ± 0.18). In contrast, the relative abundance of methanogenic archea ($p = 0.018$) and protozoa ($p = 0.028$) was higher in the control group (4.11 ± 0.18, 4.03 ± 0.18 2.66 ± 0.13) and (3.31 ± 0.19, 3.02 ± 0.15 and 2.96 ± 0.11) respectively. In conclusion, the inclusion of CG in goat diet causes shifts in the rumen microbiota and fermentation by increasing the formation of propionic and butyric acid content in line with the higher level of CG provision, unlike acetic acids.

Keyword: Crud Glycerin; biodiesel by product; microbial communities; Real-time PCR.

Correspondence author:
Ali MM Mani,
Department of Public Health, College of Veterinary,
Medicine, Al-Qasim Green University,51001, Babylon, Iraq.
E-mail address: alimani197971@gmail.com

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INTRODUCTION

Global warming is affected by the emission of greenhouse gases (GHG) such as methane, carbon dioxide,and nitrous oxide due to their ability to absorb infrared radiation. The main source of GHG emissions is the agriculture (livestock)sector, ruminants are responsible for the global productionof CH4, which represents nearly 33% of anthropogenic CH4 emissions (Nugrahaeningtyas et al., 2024). The end product of food fermentation in rumen is enteric methane, it has a 100-year global warming potential that is twenty-five times greater than carbon dioxide (equivalent amount) (Morgavi et al., 2023). Methane formation by methanogenic bacteria represents an energy loss estimated at 6–18% of total energy intake (Homem et al., 2017).

Studies indicate that diet modification will reduce the emissions of CH4 by affecting rumen microbes and fermentation (Greening et al., 2019). Initially, the use of glycerin in the diet of dairy cows emerged as an alternative to preventing and controlling ketosis. In 2004, it was approved to use glycerin in animal diets; in 2016, EU legislation approved glycerol and propylene glycol (secondary biodiesel by-products) as animal feed additives without restrictions on animals, with a recommendation on pollutants (methanol and NaCL) (Younes et al., 2018). In ruminants, inclusion of glycerin in diets leads to increased energy efficiency without any adverse effects (Lee et al., 2011; Wilbert et al., 2013). In ruminants, glycerol is completely fermented by ruminal fermentation to volatile fatty acids, especially propionate and butyrate, by bacteria(de Souza Silva et al., 2022). Considering the inverse relationship between methane (CH4) and propionate production (Mitsumori and Sun 2008), the inclusion of glycerin in ruminant diets may reduce CH4 emissions. Almeida etal., (2021) reported that supplement or pasture DM intakes, slaughter BW, and carcass traits were not influenced ($P > 0.05$) by increasing levels of CG.But dos Santos et al., (2024) show linear decrease ($P < 0.05$) was observed in DM, crude protein (CP), neutral detergent fiber (NDF), and non-fibrous carbohydrates intake with increasing CG levels in the diet, with a significant reduction ($P < 0.05$) noted at 100 and 150 g/kg of CG.

On the other hand, glycerol may affect the efficacy and activity of ciliary protozoa and thus reduce CH4 emission due to reduced production of acetic acid (Rémond et al., 1993), which is the main precursor of rumen methane, or reduction of the amount of H2 to availability for methanogenic microorganisms in the rumen (Villar et al., 2020). Since methanogens are related ecto–and endosymbiotically with ciliate protozoa, they play a specific role in interspecies hydrogen transfer. Finlay et al. (1994) reported that the percentage of methane related to ciliate protozoans' ranges between 10-20% of the total methanogens, but Newbold et al. (1995) report that the percentage is 9–25%. Microbes are crucial when considering CH4 mitigation strategies for ruminant without disrupting normal digestive function and reducing productivity. Based on the literature, ruminants can use crud glycerin (CG) at levels of 10–15% (basic dry matter) without adversely affecting their performance. In light of this information, we hypothesize that feeding inclusion of CG will improve understanding of the rumen microbial ecosystem and reduction of GHG).

MATERIALS AND METHODS

Ethical Considerations and Location.

All animal handling practices and care were performed in compliance with the local faculty of veterinary medicine (protocol No. 1511/P.G dated 2 January 2023). The trial was carried out in goat breeding farms in the private sector, located in the Wasit province, Iraq from January to March 2023, an adaptation period of 14 days was allowed preceding the experimental period of 60 days.

Animals, Experimental Design, and Diets

Twenty-four healthy male goats (19.5 ± 2.2 kg BW and 120.0 ±10 days of age) were used in three dietary treatments with 6 replications (complete randomized design). Goats were kept individually in separate yards (200×100 cm) supplied with water and feed bunks.The experimental diets were isonitrogen (CP content 14.5%), isoenergy (ME content 2.19 Mcal/kg),and forage-to-concentrate ratio 30:60.

The control diet (CON) was formulated with corn grain, whereas the other diets replaced a percentage of corn grain with crude glycerin (CG; 10% and 15%). The levels of CG were derived from the previous studies that used CG levels between 10–15 % without adverse effects on animals (Bora and İsmail 2022). Diets were presented in pellet form appropriate amounts of crude glycerin (semi-aquase form) for each diet were mixed with corn and added to the other dietary ingredients to obtain a homogeneous diet. The feeding experiment period lasted for 60 days. All goats were fed an experimental diet at 3% of live body weight, in two periods at 8:30 AM and 3:30 PM.

The body weight was measured before the start of the trial (initial), thereafter weekly for adjustment of the offered diet during the feeding trial in the morning and to calculate the weights of animal feeds that were offered. The residues from the previous day were weighed to calculate the amount of feed consumed (offered - refused) and feed conversion ratio(FCR).

Sample collection and Analytical methods.
Glycerin was acquired from a vegetable oil-based biodiesel supplied and analyzed by Biofuels Division, in Al-Dura, Baghdad (Table 1).

The experimental diets were analyzed according to the A.O.A.C. (1997) in the nutrition research center. Neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined according to the procedures of Goering and Van Soest (1970). Table (3) summarizes the ingredientsand analysis of the dietary formulation. Rumen fluid samples were collected on day 57 morning (after 2 hours of feed) using a pump system (esophageal vacuum pump) Tagliapietra *et al.,* (2012). One sample (approximately 200 mL) of rumen fluid was collected and ruminal pH was measured immediately using a pH electrode (Model PB-20, Sartorius, Germany),then filtered and strained through four layers of compressed gauze.

Aliquots of 10 mL of filtered ruminal fluid were immediately sealed and stored at -80°C in liquid nitrogen for analysis (eukaryotic diversity). Ammonia nitrogen concentration (NH3-N) and the total volatile fatty acids (TVFA's) (gas chromatography) were evaluated A.O.A.C (1997) and (Jiao et al., 2015) respectively.

DNA extraction and microorganism communities
The real-time PCR was done to determine rumen microbial communities, the DNA extracted from ruminal liquor samples by specialized kit (stool DNA) (OMEGA Bio-Tekno, Norcross, GA, USA) to determine the population of total bacteria, *Fibrobacter succinogenes*, *methanogens archaea*, and protozoa. Species-specific PCR primers and CFX 96 system were used for amplification 16S rDNA regions and detection by real-time PCR (Bio-Rad, GA, USA) (Table 2). The amplification mixture consisted of 25 µl (12.5 µl Maxima SYBR Green qPCR Master Mix, 1 µl of each species-microbial PCR forward primer and reverse primer, 2 µl of DNA elution, and 8.5 µl RNAse-free distilled water). Amplification performed at 95 °C was an initial denaturation for 5 seconds, followed by 39 cycles of denaturing for the same time, 30 s at annealing temperature, and 20 s at 72 °C for an extension. The agarose gels were purified by QIAquick Gel extraction kit (Qiagen, Venlo, The Netherlands) to extract PCR products. A Nanodrop ND-1000 spectrophotometer was used to measure the concentration of the result product. The number of copies of a template DNA per ml of elution buffer was calculate by an online formula (Navidshad, *et al.,* 2012). Finally, standard curves were constructed of each microbial group by using a serial dilution of plasmid DNA.

Table 1. chemical composition of crude glycerin

Items	Content	Analytical method
Glycerol %	74.2	AOAC method
Methanol%	4.26	Gas chromatography
Moisture	11.6	AOAC method
CP	0.3	AOAC method
EE	26.6	AOAC method
GE	2,215	Bomb calorimeter
Sodium	1.23	AOAC method
Phosphorus	< 0.005	AOAC method
Calcium	0.084	AOAC method

Table 2. The PCR primers used to determine of microbial communities in goat

Microbes	Primers	
	Forward	Reverse
General bacteria	5'-CGGCAACGAGCGCAACCC-3′	5'-CCATGTAGCACGTGTGTAGCC-3′
Fibrobacter succinogenes	5'-GTTCGGAATTACTGGGCGTAAA-3′	5'-CGCCTGCCCCTGAACTATC-3′
Methanogenic archaea	5'-TTCGGTGGATCDCARAGRGC-3′	5'-GBARGTCGWAWCCGTAGAATCC-3′
Protozoa	5'-GCTTTCGWTGGTAGTGTATT-3′	5'-CTTGCCCTCYAATCGTWCT-3′

Statistical analysis

Statistically analyze data was used general linear model (GLM) procedure. The Duncan multiple range test was used to further compare means at P≤ 0.05 and Mean differences determined.

RESULTS

Chemical analysis of diets

The analysis of the experimental diets was similar (sort of thing) since the CG was replaced by an unequal amount of corn (Table 3). The differences in EE and NDF concentrations can be related to differences in the ingredients among experimental diets, especially EE in CG (26.6%).

Growth performance:

Table 4 illustrated effect of crud glycerin on performance trials in goats. The initial, final BW, and total gain were not significantly affected (p≥0.05) by CG content in experimental diets (10% to 15% of CG) compared with the control. The final body weight of goats fed included CG in diets was higher than control group but not significant (p≥0.05). No significant differences attributed to CG were observed in total DMI; although the experimental diet(15%) was showed numerically lower.The average dry matter intake (kg/d) of three experimental diets was 1.23 ±0.6,1.14 ±0.08,and1.11 ±0.3 respectively, and the DMI of treated diet with CG was similar (P = 0.061; Table 4). Because similarity in the dry matter intake, goats had a similar average daily gain (P = 0.053), and final BW (P = 0.07). Results clearly indicated that the high energy content of CG was effect on DMI.Ruminants fed diets containing high energy, tend to regulate the intake depending on the amount of energy consumed (Table 3). Likewise, FCR was not affected (p≥0.05) by inclusion of CG in the diets.

Ruminal parameters:

The values of the main fermentation parameters for experimental diets are shown in Table 5. It can be seen from the data presented that treated groups show ruminal pH was decreased in all treatments (P=0.048). The inclusion of CG in diet significantly (P≤0.05) decreased the concentration of ruminal pH with increase of CG. The lowest pH levels (6.12 and 6.27) were recorded for %15 and %10 respectively. Regarding NH3-N concentration, there was significant decrease (P ≤ 0.05) among the groups. where, control recorded the highest NH3-N concentration

Table 3. Ingredients and chemical composition of experimental diets.

Composition	Crud glycerin (g Kg^{-1} DM)		
	0	10	15
Ingredient proportion (g Kg^{-1} DM)			
Alfalfa hay	30	30	30
Corn	42	30	20
Barn (wheat)	10	10	10
Soya meal	15	17	22
Crude glycerin	---	10	15
Salt	1.5	1.5	1.5
Limestone	0.5	0.5	0.5
Mineral–vitamin premix	1	1	1
Chemical composition (g Kg^{-1} DM)			
Dry matter	86.4	86.0	86.1
Crude protein	14.4	14.6	14.7
Crud fiber	12	11.8	1.4
Ether extract	2.4	3.6	4.1
NDF	28.4	27.1	27.6
ADF	14.2	13.7	13.4
Gross energy	2129.5	2210.5	2220

Table 4. Effect of dietary crude glycerin on the performance of goats

Item	Crud glycerin			SEM	*p-Value*
	0%	*10%*	*15%*		
Initial body weight (kg)	19.8±1.9	19.6±2.0	19.3±2.2	1.68	0.73
Final body weight (kg)	29.1 ±1.2	29.8 ±1.8	29.6±1.7	1.25	0.07
Total gain (kg)	9.3±0.43	10.2±0.84	10.3±1.55	0.92	0.058
Average daily gain (g)*	155 ±4.60	170 ±5.32	171 ±5.90	4.41	0.053
Dry matter intake kg/day	1.23 ±0.6	1.14 ±0.05	1.11 ±0.3	0.12	0.061
FCR	7.46 ±0.32	6.93 ±1.7	6.79 ±1.7	0.9	0.073

(22.33 mg/d), but CG15% showed the lowest NH3-N concentration (21.89mg/dl). Results show that the inclusion of CG in diet affects VFA. The total VFA concentration (mmol) was significantly ($P \leq 0.05$) increased in treated groups compared to the control group (Table 5). There is a significant ($P \leq 0.05$) increasein propionic and butyric acid content in line with the higher level of CG provision, unlike acetic acids, which showed a difference significantly ($P \leq 0.05$) decrease compared with control groups. isobutyrate, valerate, and isovalerate concentrations and acetic acid to propionic acid ratio (A/P) were not affected by the different levels of CG.

Rumen microbial profile:

The effects of GC on rumen microbial population are presented in Table (6). Reduction numbers of total bacteria could be observed in the treated groups compared to control although not statistically significant ($P \geq 0.05$). No differences in the proportion of F. succinogenes were observed between the CG fed goats and the control group ($P \geq 0.05$), although the lowest level 3.22 ± 0.18 was recorded in 15%. groups.Dietary crude glycerin also had no effect ($P \geq 0.05$) on archaea population and protozoa, despite of the numbers was reduction by inclusion CG.

DISCUSSION

The chemical composition of the experimental diets appears similar (Table 3). In contrast, several researchers have reported that use of CG as an alternative for energetic components in animal feed has shown promising results and is an acceptable feed ingredient for ruminants and can used up to

Table 5. Effects of crude glycerin on ruminal fermentation in goats

Item	*Crud glycerin*			*p-Value*
	0%	**10%**	**15%**	
Fermentation parameter	Means ± SE	Means ± SE	Means ± SE	
pH	6.43 ±0.6 a	6.17 ±0.48 b	6.12±0.42 b	0.048
NH3-N (mg/dl)	22.33 ±1.7 a	22.17±2.1 b	21.89 ±2.6 c	0.041
Total volatile fatty acids (mmol/d)	66±3.2 c	69±2.1 b	72±1.8 a	0.021
Molar proportions (mol/100mol)				
Acetate	62.87 ±2.1 a	58.26 ±1.9 b	55.02 ±1.8 c	0.023
Propionate	19.11±1.3 c	22.65±1.6 b	26.01±1.7 a	0.0166
Butyrate	12.31±0.9 b	13.27±1.1 a	13.44 ±1.4 a	0.048
Isobutyrate	1.38 ±0.2	1.5 ± 0.21	1.6±0.13	0.094
Isovalerate	1.02±0.13	1.06 ± 0.12	1.03±0.11	0.063
Valerate	0.9±0.14	1.1 ± 0.20	1.06±0.19	0.074
Caproate	0.12±0.03	0.13 ±0.06	0.13±0.05	0.088
Acetate/propionate (mol/mol)	3.27±0.08	2.53±0.12	2.1±0.11	0.07

Table 6. Effects of crude glycerin on ruminal microbial community in goats

DNA Copies/µL (log10)	*Crud glycerin*			*SEM*	*p-Value*
	0%	*10%*	*15%*		
General bacteria	8.32 ± 0.26	8.01 ± 0.16	7.46 ± 0.18	0.18	0.32
Fibrobacter succinogenes	4.01 ± 0.20	3.63 ± 0.23	3.22 ± 0.18	0.21	0.21
Methanogenic archaea	4.11 ± 0.18	4.03 ± 0.18	2.66 ± 0.13	0.14	0.18
Protozoa	3.31 ± 0.19	3.02 ± 0.15	2.96 ± 0.11	0.13	0.28

levels of 10%–15% without adverse effects.These results similar Chanjula et al. (2014) who studied the suitability of glycerin as an energy source and evaluated the effects of replacing corn with CG (0, 5, 10, and 20%) in goat diets.In addition, glycerol content energy close to that of corn (1.98–2.29 Mcal/kg dry matter (DM) for ruminant diets, this feed can partially replace starchy feeds, especially corn (Krehbiel, 2008).

Although there was no statistical difference in animal performance results, we observed improving performance with the increased level of glycerin in the diet. The improvement of final weight comes back to improving the efficient use of the feed, due to providing a gluconeogenic substrate and increased propionic acid in the rumen. Similar responses were reported by Whitney et al. (2000) in beef heifers. Glycerin in diets may also improve nutrition digestibility and increase the microbial protein synthesis in the rumen. These results concur with Wang et al., (2009) in cattle. Crude glycerin may have fermentative characteristics lead to stimulated rumen growth, by increasing the total VFA production, which enhances the growth and proliferation of papillae of rumen, with increased absorption area, thus improving the animal performance.

The improved final weight of treated groups will probably be high fat deposition in subcutaneous and visceral parts due to glycerin converted into propionate in the rumen which accelerated the metabolism of pyruvate in the adipose tissue. Similar responses were reported by Costa etal (2008).In two separate studies, Romanzini et al. (2018) and Almeida et al. (2017) reported average daily gains of 250g/day and 332g/day when including 12.5% and 10% crude glycerin,respectively.

The inclusion glycerin in the goat diets did not significantly reduce dry matter intake. The possible causes, more supply of propionate to the liver increases more EE content in diets, Thus, contributing to physiology satiety and, consequently, a lower DM intake. Similar responses were reported by Lage et al. (2014), Saleem and Singer, (2018). Andrade etal., (2018) also reported a reduction in DMI when supplementing crude glycerin in diet for lambs and goats. On the other hand, Mach et al. (2009) observe no differences in DMI when inclusion CG up to 12% and 30% in diets. Gunn etal., (2010) reported increased DMI and performance in the first 14 days when inclusion of crude glycerin up to 15% in lamb's diets. Moreover, (Pyatt, etal., 2007 and Parsons, etal.,2009) observed improve the feed conversion ratio in ruminants when used of glycerin in10-15%. Furthermore, in sheep and cattle many researchers reported improved feed efficiency when crude glycerin was included up to 12%.

The decrease in ruminal pH with the higher level of CG provision results from increases in the production of fatty acids (propionic and butyric) that result from increased levels of fermentation in rumen. Similar responses were reported by Lage et al. (2017) in goats, and Wang et al. (2009) in calves. Van Cleef etal. (2018) observe increase inclusion CG in lamb diets lead to ruminal pH increased, but Abo El-Nor et al. (2010) did not find an effect on ruminal pH in Holstein cows fed glycerin (108 g kg-1), the difference in the values of pH in previous studies is due to a difference diet ingredient rather than CG level.

In ruminants, the inclusion of glycerin in feed causes modifies fermentation such as ruminal NH3-N concentration (reduction). The reduction in NH3-N concentration could be explained by reduced proteolytic activity in rumen, some protozoa which responsible the recycling of nitrogen and excretion of NH3-N and amino acids into the rumen. This result agree with Syahniar et al. (2016) observed decreased ammonia level in rumen with increased nutritional glycerol level.Glycerol reduced the proteolytic activity by about 20% in rumen fluid. A Similar response was reported by Shin et al. (2012), in lactating cows fed CG to replace corn silage- or

cottonseed. Chanjula et al. (2014) in goat fed 20% CG. However, El-Nor et al. (2010) did not find a significant change in the NH3–N level in vitro fermenters.

There is an increase in propionic and butyric acid content in line with the higher level of CG provision, unlike acetic acids, the inclusion of CG 10-15 % has shown a difference significant compared with control groups. Glycerol is completely fermented in the rumen to volatile fatty acids (VFA), especially propionate and butyrate. This result agrees with (Rémond etal 1993; Wang etal 2009 and Silva et al.,2014).

The increase in VFA concentration in response to glycerol addition suggests that glycerol was used as anenergy source, and the decrease in the concentration of acetate because CG is converted to propionate in the rumen commonly. Benedeti et al. (2015) observed increased propionate and TVFA linearly in parallel with the increase in the CG during vitro study that used different levels of CG (0,10, 30%).Chanjula et al (2014) reportedno difference between the ruminal TVFA concentration of goats fed different levels of CG (0, 5, 10, and 20%) and also, molar ratios of butyrate and other volatile fatty acids. In the present study, no effect was observed for iso-butyrate, iso-valerate, valerate, and caproate. Similar responses were reported by (Chanjula et al., 2014. and Van Cleef et al., 2015).

In ruminants, the adaptation to a wide range of dietary management strategies (Dietary changes) requires an adaptation period to facilitate the stability of the rumen ecosystem, the variety of microbiomes in the rumen plays an important role in improving and ability to adapt. The inclusion of CG in the diet did not significantly affect ($p > 0.05$) the studied ruminal total bacteria (Table 6), the total bacteria were less changed for all of the treatments, and the 15% CG addition decreased the bacteria slightly.That indicates that these microorganisms are sensitive to CG or shift in bacterial population. These results can be explained by the inclusion of high levels of CG in the diet reduces the population densities of ruminal bacteria especially, in particular, cellulolytic bacteria (gram-positive), which may be related to three main factors: the development of an environment not favorable to the multiplication of these bacteria, such as osmolarity and pH; encapsulation of the fibrous particles, preventing adhesion of the bacteria; and competition or predilection for another substrate (D'Aurea et al., 2017), but F. succinogenes is less sensitive that is a gram-negative bacterium and its cell membranes differ from those positive bacteria. The *Fibrobacter succinogenes* plays acritical role in the rumen by degrading cellulose into metabolic products that are available to non-cellulolytic species such as *Streptococcus bovis, Selenomonas ruminantium, and Treponema bryantii,* which have been shown to grow on cellulose in the presence of F. succinogenes (Nouaille et al.,2005).

These results agree with Castagnino et al. (2018) whom they reported the inclusion of glycerin to the diets of beef cattle (10%) did not changes in *Fibrobacter succinogenes* bacteria.The vitro fermentation of glycerol has shown that growth of *Fibrobacter succinogenes* can be inhibited when glycerol concentrations are 2 to 5% in a medium with cellobiose as the main substrate (Roger et al., 1992).

The protozoa are present in higher numbers in animals on forage-based diets, and its reduction can be linked to the negative effect of CG inclusion on microbial adhesion to the fiber or reduction of the amount of starch in the diet. A similar observation was also reported by (Newbold et al., 2015). San vito, etal., 2016 reported that crude glycerin (CG) inclusion in feed supplements (0, 70, 140, 210, and 280 g/kg DM basis of supplement) did not change the number of total protozoa.

The reduction of protozoa may be related to a low rumen pH which leads to inhibit growth and or activity. Brossard et al., (2004) showed ciliate protozoa inhibition via pH reduction. According to Clarke (1977) rumen ciliated protozoa decreaseif pH below 5.0 and cannot survive an increase above 7.8 because sensitive to pH changes.

The population of protozoa in rumen often correlates to the population of methanogens. reported that reduction in protozoa led to reduced methanogens population since methanogens live in association with protozoa, linked by hydrogen transfer within the interspecies (Moa, etal.,2010).

with CG inclusion might be due to the observed shift in bacterial population, possibly resulting in decreased production of hydrogen or format of the methanogenic substrates.

The addition of CG did not affect the methanogenic archaea population, which is consistent with previous studies (Danielsson et al., 2014; Castagnino et al., 2015). This may be because protozoa live in the rumen in a symbiotic relationship with Archaea. Thus, the maintenance of numbers of total ciliated

protozoa may have contributed to the lack of effects on the methanogenic proportions.

Depression of methanogenesis may be related to a long lag time for glycerol fermentation (Lee et al.,2011) so rumen microbes especially methanogens need more time to adapt. San vito, et al., 2016 reported the crude glycerin (CG) inclusion in feed supplements (0, 70, 140, 210, and 280 g/kg DM basis of supplement) did not affect F. succinogenes (P=0.420) or the methanogens (P=0.150). The reduction observed in rumen microbes bacteria, methanogens, and protozoa populations in the present study suggests that the ruminal microbial populations shifted over time due to the addition of CG or may also be sensitive to CG.

CONCLUSIONS

The inclusion of crude glycerin in diet formulations provides a precursor to gluconeogenesis, thus emerging as an alternative feed in goat diets. Any attempt to improve goat nutrition must consider the fact that any alterations in feed composition will lead to a change in the rumen ecosystem. The inclusion of CG in diet did not alter the bacteria and total microbial populations in the present study, indicating that these microorganisms are not sensitive to CG significantly.

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