ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

**ISSN (print ):**2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

# EFFECT OF CRUDE GLYCEROL SUPPLEMENTATION VIA DRINKING WATER ON LAMBS PERFORMANCE, SOME BLOOD HAEMATOLOGY, AND BIOCHEMICAL METABOLITES

Abdulhadi Omar. Qoja[1] , Abdulqader Ahmed. Hussein[*2]
1Department of Veterinary, Shaqlawa Technical College, Erbil Polytechnic University-Erbil, Iraq
2Department of Animal Resources, Agricultural engineering Science College, Salahaddin University-Erbil, Iraq

**A B S T R A C T:**

This study was carried out to investigate the effect of crude glycerol (CG) supplementation via drinking on lambs performance, haematological and biochemical metabolites. Twenty-four Arabi lambs were randomly (35.38 ± 0.79 kg) assigned to one of four treatments (Six lambs per treatment). Lambs were fed (total mixed ration) as group feeding for 63 days. Diet was composed of concentrate and roughage (straw) at ratio of 70:30 (concentrate:forage) for the categories, being these similar among the treatments, though the CG differed for the supplements in (0, 50, 100, 150 ml/5L/lambs) being It added to the animals drinking water. There was no effect (P>0.05) of CG on lambs performance (weekly live weigh and weight gain). Although, lambs fed G1 and G2 diets had numerically higher ADWG 4 and 6%, with lower DMI 17 and 18% compared to control group. There was no difference (P>0.05) among treatments in haematological variables. Whereas, supplementing CG elevated (P<0.05) cholesterol, triglyceride, and glucose, while serum insulin was not affected (P>0.05). The CG supplementation via drinking water did not affect negatively live weight gain, which was ≥ 0.260 kg/d, but reduced DMI. However, cholesterol, triglyceride, and glucose serum levels were significantly increased by the end of the study.

KEY WORDS: crude glycerol, Arabi lamb, performance, haematology, serum metabolites.
DOI: http://dx.doi.org/10.21271/ZJPAS.34.1.11
ZJPAS (2022) , 34(1);110-119 .

## 1. INTRODUCTION:

Recent growth in the biofuel industry has led to an unstable futures market, which has increased the cost of grains traditionally used in feedlot feed. Livestock producers of all sectors are looking for ways to reduce production costs while maintaining profitability and thriving in their respective industries. Alternative feed sources like as glycerin (or glycerol) have become a main focus for the animal husbandry 2001). industry due to rising costs of energy-rich feedstuffs. Since feed is the highest cost in any production system, the use of inexpensive alternative feeds can be one way to maintain or increase profitability.

* **Corresponding Author:**
Abdulqader Ahmed. Hussein
E-mail: abdulqader.hussein@su.edu.krd
**Article History:**
Received: 13/10/2021
Accepted: 20/12/2021
Published: 24/02/2022

As a result, livestock feeding industries have been widely accepted alternative feed ingredients in order to maintain or reduce gain costs (Almeida et al., 2017; Almeida and Paschoaloto 2019).

The crude glycerol (CG) is the main by-product of biodiesel synthesis that is obtained via the transesterification of triacylglycerols from plant oils or animal fats, generally using methanol and a catalyst (sodium methylate). CG is a “generally recognized as safe” for use in animal diets (Sellers, 2008). The CG contains approximately 80% glycerol, but this level can vary depending on its source. Glycerol, the 3-carbon backbone of a triglyceride, is a gluconeogenesis substrate (Thompson and He, 2006). In rumen, CG converts via microorganisms to mainly propionate and butyrate rather than acetate, or it can be directly absorbed via rumen wall, acting as a precursor for

gluconeogenesis in the liver (Krehbiel, 2008). Reports have showed its energetic potential in ruminant diets (Chung et al., 2007; Osborne et al., 2009). The use of CG has been investigated for lambs (Barros et al., 2015; Borghi et al., 2016) There are many varying results regarding the CG supplementation in ruminant diets. Promising results concerning an improvement in lambs performance have been shown for supplementation CG for lambs (Volpi-Lagreca and Duckett, 2017). However, the addition of CG could decrease lambs performance (Almeida et al., 2017). The different response in lambs performance to CG inclusion has been attributed to the degree of purity of the CG used (Almeida and Paschoaloto, 2019).

Blood serum metabolites such as triglycrides, glucose, and insulin has been used as indicators for CG supplementation to animals, and its effect to produce energy via conversion of CG to volatile fatty acids in the liver and consequently to these parameters (Radostits et al., 2000; Gardner et al., 2001; Volpi-Lagreca and Duckett, 2017). Moreover, haematological parameters has been used to assess their overall health of animals (Jones and Allison, 2007; Hussein et al., 2017), and to investigate the effect of adding CG on RBC and animal immunity system represented by WBC and Hct (Lopez et al., 2018; Porcu et al., 2020). Former authors observed that dietary CG supplementation didn't change WBC and Hct values. However, later authors demonstrated that high level of dietary CG inclusion could negatively affect animal health by altering the RBC indices and functions.

Though, glycerol is supplied to ruminants and reaches the rumen, digested into volatile fatty acids, and there is little rumen escape of these substrates into the tissues (Garza and Owens, 1989). According to these authors, 60 to 80 % of drinking water escapes the rumen. There was no response to oral drench technique to evaluate CG influence on calves performance, and other techniques such as CG supplementation via drinking water was recommended to evaluate CG effectiveness (Lopez et al., 2018). However, to the best of our knowledge, there is little, if any, literature concerning CG supplementing finishing local lambs CG via drinking water in local lambs. Therefore, the objective of this study was to evaluate the effects of different levels of CG supplementation via drinking water on performance and blood parameters of Arabi lambs.

## 2. MATERIALS AND METHODS

### 2.1. Animal Ethic

All protocols for this study were approved by the Erbil Polytechnique University through Animal Research Centre Committee. The experiment was conducted from April through July 2021 at the Erbil Polytechnique University Scientific Research Centre.

### 2.2. Location, Animals, and experimental design

A study was carried out at Scientific Research Centre - Erbil Polytechnic University during the period from 20$^{th}$ of April to 4$^{th}$ of July 2021 using 24 Arabi ram lambs with an initial body weight of 35.38± 0.79 kg over a period of 9 weeks with 14 d of adaptation to the installation, location, and diet. Lambs were randomly allocated (according to live weight (LW) to one of four treatments, with 6 lambs per treatment. The lambs were housed in a well-ventilated shed in groups and bedded on wood shavings. They had free accessed to water and minerals.

### 2.3. Diets

Lambs were offered diet with a concentrate to wheat straw ratio of 70:30 (DM basis). The diet was formulated to support weight gain 0.250 kg/d (AFRC, 1993). The forage was wheat straw and the concentrate was dried pelleted feed from commercial feed manufacture (Top Feed, livestock feed manufacture, Erbil, Iraq), including soyabean meal (12.3%), corn grain (19.0%), wheat flour (24.0%), wheat bran (28.5%), wheat (9.5%), urea (0.5%), limeston (2.9%), sodium bicarbonate (0.44%), Salt (0.28%), toxin binder (0.1%), premix (2.5%). Feed samples (straw and concentrate) were analysed at commercial feed laboratory (Afnan feed laboratory for chemical analysis for livestock feed, Erbil, Iraq) for DM (91.5,90.3 g/kg), Ash (59,63), CP (31,123), EE (14,30), and crude fiber (45,36) contents (g/kg DM) respectively. The diets were formulated to be similar across all dietary treatments. The

difference was only CG supplementation. The predicted metabolisable energy (ME) as a total mixed ration (TMR) for experimental diets was 11.3 MJ/kg DM (AFRC, 1993).

### 2.4. Treatments

Lambs were allocated by LW to one of four dietary treatments (Table 1.1). The supplemented CG (5L/lamb per day) was dissolved in drinking water daily. Therefore, CG for G1(300ml), G2 (600ml), and G3 (900ml) were dissolved in 30L of water for each group. The drinking water were changed daily.

**Table 1.1. Dietary treatments**

| Code | Treatments |
|---|---|
| **Control (C)** | **no addition CG[1]** |
| **G1** | **CG 50ml/5L/lamb** |
| **G2** | **CG 100ml/5L/lamb** |
| **G3** | **CG 150ml/5L/lamb** |

[1] Glycerol 80%; Moisture 15%; Ash 10%; Methanol 8%; Matter Organic Non-Glycerol 5%. ASB Biodiesel (Hong Kong) limited.

### 2.5. Experimental Routine

All lambs in each group were offered feed twice a day at 08:00 and 18:30 h. Concentrate and wheat straw at 70:30 ratio was manually mixed and put into fodder. Feed refusals were collected twice a week (every Sunday and Wednesday throughout the period of experiment) to estimate group feed intake. The diet was offered *ad libitum* as group-feeding.

### 2.6. Live Weight Determination

Lambs were weighed fortnightly on Sundays at 11:00 using a digital weigh. The scale was calibrated prior to use and every 6 weighing using standard weights for precision and accuracy. Daily live weight gain (DLWG) was calculated using regression analysis.

### 2.7. Blood Sample Collection and Analysis

Blood samples were collected via jugular venipuncture using a 20 gauge 1.5″ needle (Becton Dickinson Vacutainer Systems, Plymouth, UK) on week 0, 5, and 9 at 11:00h for haematological and blood biochemical parameters. Tubes containing $K_2$EDTA (7.2 mg/tube) were used for the determination of whole blood haematology. Tubes containing silica, which had been sprayed onto the inner walls of the tube to accelerate the clotting process, were used to collect blood serum for the determination of glucose and insulin. Blood samples that were collected in the second set of tubes were left overnight in a refrigerator to coagulate and all tubes were then centrifuged at 1000 g for 15 min at a 4 ºC using a (Beckman Avanti 30). The serum was then pipetted off with plastic pipettes into 2 ml bijou tubes and stored at – 20 ºC for subsequent cholesterol, triglyceride, glucose and insulin analysis.

White blood cells (WBC) and red blood cells (RBC), haemoglobin (Hb), and haematocrit (Hct) were analyzed using a Vet Animal Blood Counter (MCL 3800, China). The blood samples were mixed thoroughly for 15 min and then for haematological parameters in Agricultural Engineering Sciences College, Salahaddin University-Erbil. Blood serum metabolite parameters were determined in commercial laboratory (Med-Line laboratory for disease diagnosis, Erbil, Iraq), using Cobas analyzer (Roche Cobas®6000 analyzer series -Roche-Diagnostics, Japan). Serum cholesterol, triglyceride, glucose, and insulin were analysed using kits such as (cholesterol Gen.2; 0303977 190, TRIGL; 20767107 322, Glucose HK Gen.3; 04404483 190, and Elecsys Insulin; 12017547 122) respectively.

### 2.8. Statistical Analysis

All statistical analysis was conducted using GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Daily Live Weight Gain (DLWG) was calculated by regression analysis. Blood haematological and biochemical variables data were analysed as repeated-measures using the same procedure of SAS, and time (week) of observation and interaction diets ·time (week) were included in the model. Treatment means were computed with the LSMEANS option and significance was defined as $P < 0.05$ and trends as $0.05 \leq P \leq 0.10$.

## 3. RESULTS AND DISCUSSION

### 3.1. Animal Performance

The currents demonstrated that the mean ADWG was 0.255±0.01 kg/d across all glycerol supplemented lambs which was close to 0.250 kg/d as predicted by AFRC (1993). Despite statistical analysis was not applied for DMI due to

group feeding. Daily dry matter intake (DDMI) for all dietary treatment groups were 1.766 (C), 1.459 (G1), 1.456 (G2), and 1.418 (G3) kg/day. Implying, lambs DDMI (kg/d) in CG supplemented groups (G1 17% (0.307), G2 18% (0.310), and G3 20% (0.348)) were lower compared to control groups. Similar results for DMI were reported when CG (90% purity) was added to the market lambs (Musselman et al., 2008). Moreover, supplementation of CG (85% purity) via drinking water to sheep at the end of gestation has been used as a strategy to reduce DM consumption and occupy less physical space. (Sá et al., 2017). Pillmore et al. (2017) also demonstrated that supplementation of CG (85% purity) up to 50 g/L to lambs did not significantly change DMI, while 75 g/L negatively affected DMI of corn silage-based diet. However, Lopez et al. (2018) reported CG administration via drinking water to calves didn't affect DMI. The fact that glycerol might improve animal metabolic condition is another plausible explanation for DMI decrease (Lopez et al., 2018). The higher energy intake from glucose, along with increased propionate and a lowered acetate/propionate ratio in the rumen, satiates the animal in terms of energy (Krehbiel, 2008; Almeida et al., 2017). Similarly, in current study, the decrease in DMI in G3 was greater compared to G1and G2. Furthermore, the low DMI in ewes supplemented with CG has been attributed to physiological mechanisms, in which intake is a reciprocal function of feed characteristics, such as fill effect and energy content to meet the energy requirements of the animal (Sá., 2017).

The CG recognised as precursor for dietary energy sources (Donkin, 2008). Results of this study suggested that performance and physiological state requirements were met by the chemostatic regulation factors. Therefore, the reduction on DMI observed can be explained by the high CG content in supplemented water provided to the lambs. Ruminants are capable to control their energy intake (similar to non-ruminant animals) as long as the dietary energy density is high enough to prevent interference by physical limitations (Forbes, 1993). The all four diets of current study were similar and differed only by the amount of CG supplemented via drinking water provided to the lambs, therefore lambs were capable to alter their intake based on their energy requirements. Sá et al. (2017) observed a reduction in DMI when similar diets supplemented with different levels of CG. Moreover, the deleterious selection of fibrolytic bacteria, which are known to be sensitive to glycerin, may explain the DMI reduction. Glycerol, in previous studies, has been shown to provide a selection of rumen bacteria, mainly fibrolytic ones, that affect NDF digestibility. Moreover, according to Roger et al. (1992) glycerin inhibits microorganism activity, cell membrane permeability, and bacterial adhesion in feed. As a result of these facts, the feed flow rate through the rumen may be reduced. This causes ruminal filling, also known as repletion state (Allen, 2000), which decreases DMI.

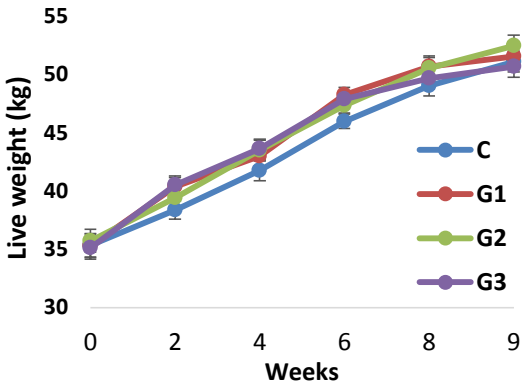


**Figure 1. Weekly live weight (kg) of lambs fed diets supplemented with crude glycerol 0, 50, 100, 150 ml/5L/lamb. Error bars indicates SEM.**

There was no effect (P>0.05) of CG supplementation on both weekly LW and average daily weight gain (ADWG) (Figure 1 and 2). Though, numerically the addition of CG in G1 and G2 4 and 6% increased ADWG and 3% decreased in G3 in comparison with control groups.

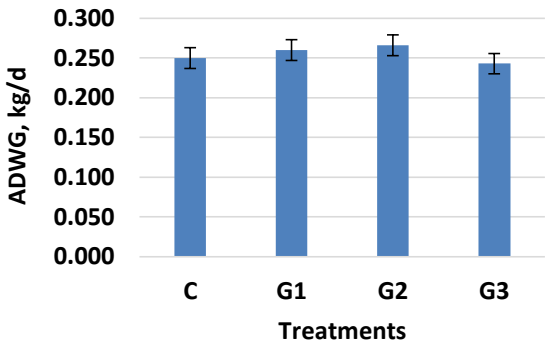


**Figure 2. Average Daily Weight Gain (g/d) of lambs fed diets supplemented with crude glycerol 0, 50, 100, 150 ml/5L/lambs. Error bars indicates SEM.**

There are many varying results regarding the CG supplementation in ruminant diets. Chanjula et al. (2016) and Romanzini et al. (2018) reported non-significant differences in ADWG in lambs and steers supplemented with CG (83 and 88% purity respectively). Promising results concerning an improvement in lambs performance have been shown for CG supplementation (87% purity) for lambs (Volpi-Lagreca and Duckett, 2017). However, the addition of CG (90 and 83% purity) decreased lambs ADWG (Musselman et al., 2008; Almeida et al., 2017). The different response in lamb's performance to CG supplementation has been attributed to the degree of purity of the used CG (Gomes et al., 2011; Almeida and Paschoaloto, 2019). Moreover, Romanzini et al. (2018) stated a no significant difference in ADWG between lambs supplemented with and without CG could be due to the similar composition and nutrient value, including CP and ME, of CG supplemented and unsupplemented diets, were also important factors in the ADG results. As a result, the similarity between diets with and without CG inclusion may determine the possibility of their use for maintaining or improving animal performance. Results from the current study suggested that feeding CG 50 and 100 ml/5l/lambs/day via drinking water might have a positive impact (no significant) on lambs performance. Hence, it can be suggested that despite of reduction in DMI of CG supplementation via drinking water of lambs (G1, G2, and G3). Those animals were able to avoid decrease in LW. This result is reflecting improved energetic efficiency with CG feeding (Almeida et al., 2020). It is observed that CG supplementation via drinking water resulted in a decrease in DMI of ewes. Though, those animals were able to avoid changes in LW and body condition score (Almeida et al., 2020).

Finally, the performance results from the current study demonstrated that despite of lower dietary ME (11.3 MJ/Kg DM) than required ME to support 0.250 kg/d. Lambs from CG supplemented groups gave similar ADWG with similar DMI as presented above. This may be due to the fact that CG according to literature giving minimum 3.0 MJ/kg (NRC, 2007), which altogether were giving 14.0MJ/Kg DM. According to AFRC (1993), lambs at 35-45 kg LW required 14 MJ/Kg DM, with DDMI 1.350-1500 kg/d to support ADWG 250 g/d. In other word, lambs in control group, that had a deficit in ME and not supplemented with CG, gave ADWG as 0.250 kg/d but consumed 1.766 kg/d which is higher than stated in AFRC (1993). Therefore, better performance (lower DMI and greater weight gain) especially in lambs in G1 and G2 groups compared to control lambs that consumed more DM to get predicted ADWG. This effect may have attributed to energetic potential from CG. Similarly, Duff and Galyean (2007) suggested that better animal performance may be possible through increased caloric intake.

### 3.2. Haematological Parameters

Blood haematological parameters have been estimated to evaluate the effect of CG on lambs health status (Cruz et al., 2014; Porcu et al., 2020). In current study, repeated measures analysis showed no effect ($P>0.05$) of time, and time x treatment on RBC and Hb. and WBC. However, there was an effect of time on Hct%, while increased by the end of the study in treatment groups but not time x treatment interaction. Blood haematology parameters such as Hct (%), Hb (g/dL), RBC ($10^6$/mm$^3$), and WBC ($10^3$/mm3) were not affected ($P>0.05$) by dietary CG supplementation (Table 2). All blood haematological parameters in this study were in line with the normal ranges described by Jackson and Cockcroft (2002) and Abdulkareem et al. (2020). These results are in agreement with Almeida et al. (2020) who showed that a 10% CG supplementation via drinking water to Santa Inês ewes did not significantly change the hematological and biochemical variables, which were within the normal ranges. There was also no effect of CG supplementation at 200 and 400 g/L on heifers Hct % and WBC (Lopez et al., 2018). Additionally, dietary CG supplementation for bulls exhibited non-significant difference among treatments in blood haematological parameters such as Hct%, Hb, RBC, and WBC (Cruz et al., 2014). Although, high dose of glycerol (greater than 22.55 of DM of offer) could result in alteration in RBC indices (Porcu et al., 2020).

### 3.3. Serum Metabolites

Triglyceride level tended ($P=0.080$) to increase by the end of study (Table 3). There was a time x treatment interaction where in G1 and G3

triglyceride increase but in C and G2 decreased with the time. At week 9, CG supplementation in G1, G2, and G3 were higher (P<0.001) compared to C group. In addition, serum cholesterol was rose by the time (P<0.001), and there was a trend time x treatment (P=0.082) for higher cholesterol level in G3 lambs compared to other dietary groups. At week 5, serum cholesterol was greater (P<0.05) in G3 in comparison with other groups. The energy status of the animal has been assessed by both triglycerides and cholesterol measurements. Glycerol metabolism in the liver can promote triglyceride synthesis, resulting in changes in blood triglyceride levels (Carvalho et al., 2012). This may explain a significant (P<0.05) increase in triglyceride of lambs on G1, G2, G3 groups in comparison with lambs on control group.

In the current study, the cholesterol level for G3 lambs on week 5 were higher (P<0.05) compared to control lambs. Correspondently, Ribeiro et al. (2018) observed the change in the lamb's cholesterol levels supplemented with CG. This outcome has been can be explained by the higher lipid content in the diets with added CG (Krebs and Lund, 1966). Thus, it was considered that higher dietary lipid concentrations enhanced the availability of absorbed fatty acids, which were then converted to acetate and then to cholesterol production (Costa et al., 2016).

The CG has been applied as a source of additional dietary energy in cattle diets (Parsons et al., 2009). Glycerin alters rumen fermentation to promote propionate production (Lopez et al., 2017), elevates glucose concentrations (Linke, 2005). Glucose is an energy source that is required for animal production and reproduction (Radostits et al., 2000). Blood glucose levels has been suggested to use as an indicator for energy state (Chanjula et al., 2014). There was time, and time x treatment effect on serum glucose concentration. Which was elevated by the time (P<0.001), and the rate of elevation at week 5 was higher in lambs at G3 compared to other treatment groups (P<0.05). In addition, the main effect of treatment resulted in an increasing serum glucose concentrations (at week 5) relative to increasing in CG concentrations administrated via drinking water to lambs (P<0.05; Table 2). Gardner et al. (2001) also demonstrated that lambs fed low-energy diets offering water containing 3.5% glycerol resulted in an increase plasma glucose concentration.

**Table 2. Haematological parameters of the Arabi lambs fed diet supplemented with different levels of crude glycerol via drinking water (0. 50, 100, and 150 ml/5L/lambs)[1]**

| Parameters[2] | C | G1 | G2 | G3 | SEM | Significance |
|---|---|---|---|---|---|---|
| Hct, % | | | | | | |
| week0 | 24.81 | 24.57 | 25.20 | 24.89 | 1.885 | 0.996 |
| week5 | 25.21 | 25.78 | 25.88 | 25.75 | 1.331 | 0.982 |
| week9 | 26.93 | 27.56 | 27.25 | 27.45 | 1.053 | 0.976 |
| | | Repeated measures analysis | | | | P-value |
| | | Time | | | | 0.045 |
| | | Time. Treatment | | | | 0.991 |
| Hb, g/dL | | | | | | |
| week0 | 9.58 | 9.73 | 9.60 | 9.82 | 1.395 | 0.999 |
| week5 | 10.49 | 10.70 | 10.20 | 10.13 | 1.246 | 0.987 |
| week9 | 12.46 | 12.18 | 12.23 | 12.23 | 2.085 | 0.999 |
| | | Repeated measures analysis | | | | P-value |
| | | Time | | | | 0.156 |
| | | Time. Treatment | | | | 0.996 |
| RBC, $10^6$/mm$^3$ | | | | | | |
| week0 | 10.63 | 10.63 | 10.39 | 10.88 | 0.740 | 0.976 |

| | | | | | | |
|---|---|---|---|---|---|---|
| week5 | 11.24 | 11.95 | 11.02 | 11.30 | 1.572 | 0.978 |
| week9 | 10.91 | 10.94 | 10.78 | 10.67 | 0.627 | 0.990 |
| | | Repeated measures analysis | | | | P-value |
| | | Time | | | | 0.624 |
| | | Time. Treatment | | | | 0.976 |
| | | WBC, $10^3/mm^3$ | | | | |
| week0 | 10.16 | 10.29 | 10.66 | 10.20 | 1.743 | 0.948 |
| week5 | 10.67 | 10.04 | 10.13 | 10.06 | 1.645 | 0.992 |
| week9 | 9.27 | 9.23 | 9.22 | 10.22 | 1.845 | 0.837 |
| | | Repeated measures analysis | | | | P-value |
| | | Time | | | | 0.893 |
| | | Time. Treatment | | | | 0.756 |

**[1]C= control (no CG supplementation), G1= (50ml/5L/lamb CG supplementation), G2= (100 ml/5L/lamb CG supplementation), and G3=(150 ml/5L/lamb CG supplementation)**
**[2]Hct=haematocrit; Hb=Haemoglobin; RBC=Red Blood Cell; WBC=White Blood Cell**

**Table 3. Blood biochemical parameters of Arabi lambs supplemented with different levels of crude glycerol via drinking water (0. 50, 100, and 150 ml/5L/lambs)[1]**

| Items | C | G1 | G2 | G3 | SEM | Significance[2] |
|---|---|---|---|---|---|---|
| | | | Cholesterol, mg/dL | | | |
| week0 | 38.17 | 42.33 | 42.00 | 51.20 | 4.144 | 0.185 |
| week5 | 39.36[b] | 41.06[b] | 46.23[ab] | 53.18[a] | 2.684 | 0.007 |
| week9 | 51.20 | 56.78 | 56.78 | 60.24 | 3.439 | 0.341 |
| | | Repeated measures analysis | | | | P-value |
| | | Time | | | | <.001 |
| | | Time. Treatment | | | | 0.082 |
| | | | Triglyceride, g/dL | | | |
| week0 | 14.99 | 14.00 | 16.50 | 16.16 | 1.182 | 0.241 |
| week5 | 15.98 | 15.066 | 13.75 | 17.66 | 1.668 | 0.179 |
| week9 | 11.96b | 16.12a | 16.11a | 21.05a | 1.687 | 0.007 |
| | | Repeated measures analysis | | | | P-value |
| | | Time | | | | 0.080 |
| | | Time. Treatment | | | | <.001 |
| | | | Glucose, µU/ml | | | |
| week0 | 35.67 | 46.5 | 38.33 | 37 | 3.821 | 0.2149 |
| week5 | 55.86[b] | 73.32[a] | 74.88[a] | 82.78[a] | 2.525 | <.0001 |
| week9 | 65.68 | 65.88 | 73.17 | 71.4 | 2.422 | 0.0896 |
| | | Repeated measures analysis | | | | P-value |
| | | Time | | | | <.001 |
| | | Time. Treatment | | | | 0.003 |
| | | | Insulin, mg/dL | | | |
| week0 | 4.46 | 4.63 | 4.74 | 4.87 | 0.832 | 0.987 |
| week5 | 9.82 | 13.78 | 10.13 | 10.09 | 1.701 | 0.325 |
| week9 | 11.27 | 10.01 | 14.17 | 11.27 | 1.352 | 0.199 |

| Repeated measures analysis | P-value |
|---|---|
| Time | <.001 |
| Time. Treatment | 0.089 |

**[1]C= control (no CG supplementation), G1= (50ml/5L/lamb CG supplementation), G2= (100 ml/5L/lamb CG supplementation), and G3=(150 ml/5L/lamb CG supplementation)**
**[2]Means within a row with different superscripts are significantly different (P<0.05).**

Moreover, Volpi-Lagreca and Duckett (2017) also reported that supplementation of glycerol through drinking water to grazing lambs increased blood glucose compared to control lambs. In general, glycerin has been reported rapidly fermented and converted to propionic acid, and it is easily absorbed across the rumen wall (Kijora, 1998). As, glycerin is directly gluconeogenic, any glycerin that didn't convert to propionic acid immediately expected to be transported to the liver for gluconeogenesis. Subsequently, the glycerol component can be converted to glucose via the liver and kidneys (Krebs and Lund, 1966) to provide energy for cellular metabolism. As a result, an increase in dietary CG would result in a rise in circulating serum glucose levels.

Repeated measures analysis showed an effect (P<0.001) of time on insulin concentration that was increased by the time. There was a trend (P=0.089) for greater increase in insulin of G2 lambs compared to other treatments. There was no effect (P>0.05) of CG supplementation on serum insulin at any time points of the week. Circulating insulin levels usually correspond to changes in circulating glucose levels (Evans et al., 1975). Insulin secretion, on the other hand, is influenced by a variety of factors and has been shown that insulin levels were unrelated to glucose levels (Gunn et al., 2010).

## 3. CONCLUSION

This scenario, control lambs in comparison with CG 50, 100, 150 ml/5L/lambs supplementation via drinking water (statistically) didn't significantly change the lambs performance, blood haematology. Though, numerically resulted in decrease DMI by 17 and 18 % DMI of G1 and G2, and increase DLWG by 3 and 6% of G1 and G2 lambs respectively. This would result in better economic results. Furthermore, some blood serum parameters such as cholesterol, triglyceride, and glucose were increased by supplementing CG, while blood serum insulin wasn't affected.

## 4. ACKNOWLEDGEMENT

Authors are grateful to Research Centre Technical Institute Polytechnic University –Erbil –Iraq for providing a place to conduct this study. Many thanks to Agricultural Engineering Sciences College–Salahaddin University/Erbil-Iraq for providing the facilities to do the present work in their laboratory.

## 5. REFERENCES

Abdulkareem, T.A., Eidan, S.M., Shubber, A.M., Ibrahim, F.F., Ali, M.D. and Mohammed, O.A. 2020. Reference Physiological Values in Different Animal Species. Ministry of Agriculture, Iraq, 96-97.

Agricultural and Food Research Council (AFRC). 1993. Energy and Protein Requirements of Ruminants. Advisory Manual, Agricultural and Food Research Council Technical Committee on Responses to Nutrients. CAB International, Wallingford, Oxon, UK.

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J Dairy Sci. 83(7), 1598-1624.

Almeida, M.M.S., de Araújo, M.J., da Costa Torreão, J.N., Marques, C.A.T., Nogueira, A.F.S., Pereira, E.A., Cavalcante, T.V., Dias-Silva, T.P. and V. Sejian. 2020. Crude glycerin inclusion in Santa Inês ewes' diet before and during the breeding season: its effects on physiological parameters, hematological variables, and reproductive performance. Semin Cienc Agrar. 41(5), 1735-1772.

Almeida, M.T.C. and J.R. Paschoaloto. 2019. Inclusion of crude glycerin Yin Diets for Sheep. In *Glycerine Production and Transformation-An Innovative Platform for Sustainable Biorefinery and Energy*.1-14. IntechOpen.

Almeida, M.T.C., Ezequiel, J.M.B., Paschoaloto, J.R., Perez, H.L., de Carvalho, V.B., Castro Filho, E.S. and E.H.C.B van Cleef. 2017. Effects of high concentrations of crude glycerin in diets for feedlot lambs: feeding behaviour, growth performance, carcass and non-carcass traits. Anim. Prod. Sci. 58(7), 1271-1278.

Barros, M. C. C., J. de A. Marques, F. F. da Silva, R. R. Silva, G. S. Guimaraes, L. L. da Silva, and F. L. de Araujo. 2015. Crude glycerin in diets for wethers in

feedlot: intake, digestibility, performance, carcass and meat traits. Semina Ci. Agr. 36:453–466.

Borghi, T. H., A. G. da Silva Sobrinho, N. M. B. L. Zeola, F. A. de Almeida, L. G. A. Cirne, and A. R. C. Lima. 2016. Dietary glycerin does not affect meat quality of Ile de France lambs. R. Bras. Zootec. 45,554–562.

Carvalho, P.L.D.O., Moreira, I., Martins, E.N., Piano, L.M., Toledo, J.B. and Costa C.D.L. Filho. 2012. Crude glycerine in diets for piglets. R. Bras. Zootec. 41: 1654-1661.

Chanjula, P., Pakdeechanuan, P. and S. Wattanasit. 2014. Effects of dietary crude glycerin supplementation on nutrient digestibility, ruminal fermentation, blood metabolites, and nitrogen balance of goats. Asian-Australasian J. Anim. Scie. 27(3), 365.

Chanjula, P., Raungprim, T., Yimmongkol, S., Poonko, S., Majarune, S. and W. Maitreejet. 2016. Effects of elevated crude glycerin concentrations on feedlot performance and carcass characteristics in finishing steers. Asian-australas. J. Anim. Sci.29(1), 80.

Chung, Y.H., Rico, D.E., Martinez, C.M., Cassidy, T.W., Noirot, V., Ames, A. and G.A. Varga. 2007. Effects of feeding dry glycerin to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. J. Dairy Sci. 90(12), 5682-5691.

Costa, J.B., Oliveira, R.L., Silva, T.M., Ribeiro, R.D.X., Silva, A.M., Leão, A.G., Bezerra, L.R. and T.C. Rocha. 2016. Intake, digestibility, nitrogen balance, performance, and carcass yield of lambs fed licuri cake. J. of Anim. Sci. 94(7), 2973-2980.

Cruz, O.T.B., Valero, M.V., Zawadzki, F., Rivaroli, D.C., do Prado, R.M., Lima, B.S. and I.N. do Prado. 2014. Effect of glycerine and essential oils (Anacardiumoccidentale and Ricinuscommunis) on animal performance, feed efficiency and carcass characteristics of crossbred bulls finished in a feedlot system. Italian, J. Anim. Sci.13(4), 3492.

Donkin, S.S. 2008. Glycerol from biodiesel production: the new corn for dairy cattle. Rev. Bras. Zootec. 37, 280-286.

Duff, G.C. and M.L. Galyean. 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85(3), 823-840.

Evans, E., Buchanan-Smith, J.G. and G.K. Macleod. 1975. Postprandial patterns of plasma glucose, insulin and volatile fatty acids in ruminants fed low-and high-roughage diets.J. Anim. Sci. 41(5), 1474-1479.

Forbes, J. M. 1993. Voluntary feed intake. In: Forbes, J. M.; France, J. (Ed.). Quantitative aspects of ruminant digestion and metabolism. Cambridge: University Press, pp. 479-494.

Gardner, G.E., McIntyre, B.L., Tudor, G.D. and D.W. Pethick. 2001. Nutritional influences on muscle glycogen recovery following exercise in sheep and cattle. Rec. Adv. Anim. Nutr. 13, 145-151.

Garza, F. and F.N. Owens. 1989. Quantitative origin of ruminal liquid with various diets and feed intakes. Miscellaneous publication-Agricultural Experiment Station. Univ. Okla. (USA).

Gomes, M.A.B., Moraes, G.V.D., Mataveli, M., Macedo, F.D.A.F.D., Carneiro, T.C. and R.M. Rossi. 2011. Performance and carcass characteristics of lambs fed on diets supplemented with glycerin from biodiesel production. Rev. Bras. Zootec. 40, 2211-2219.

Gunn, P.J., Schultz, A.F., Van Emon, M.L., Neary, M.K., Lemenager, R.P., Rusk, C.P. and S.L. Lake. 2010. Effects of elevated crude glycerin concentrations on feedlot performance, carcass characteristics, and serum metabolite and hormone concentrations in finishing ewe and wether lambs. Prof. Anim. Sci. 26(3), 298-306.

Hussein A A, 2017. The Effects of Forage Type and Antagonists on Copper Metabolism in Sheep. Ph.D. Thesis, Harper Adams University, UK.

Jackson, P. and P. Cockcroft. 2002. Clinical examination of farm animals. Oxford, UK, Blackwell Science. pp 303-305.

Jones, M.L. and P. Allison. 2007. Evaluation of the ruminant complete blood cell count. Veterinary Clinics of North America: Food Anim. Pract. 23(3):377-402.

Kijora C, Bergner H, Götz KP, Bartelt J, Szakács J, Sommer A. 1998. Research note: investigation on the metabolism of glycerol in the rumen of bulls. Arch Tierernahr. 51(4), 341-8

Krebs, H.A. and P. Lund. 1966. Formation of glucose from hexoses, pentoses, polyols and related substances in kidney cortex. Biochem. J. 98(1), 210-214.

Krehbiel, C.R., 2008. Ruminal and physiological metabolism of glycerin. J. Anim. Sci. 86(2), 392.

Linke, P. 2005. Ruminal and plasma responses in dairy cows to drenching or feeding glycerol. J Undergrad Res. 3(1), 8.

Lopez, F.A., Oosthuysen, E.R., Duff, G.C., Richeson, J.T., Samuelson, K.L., Hubbert, M.E. and C.A. Löest. 2018. Health, performance, and complete blood counts of newly received feedlot heifers in response to an oral drench of water and crude glycerin. Translat. Anim. Sci. 2(1), S74-S78.

Lopez, F.A., Samuelson, K.L., Carey, R.E., Pillmore, S.L., Brooks, J.M., Klump, L.T., Oosthuysen, E.R. and C.A. Löest. 2017. Supplemental glycerin alters rumen fermentation and in situ degradation in steers exposed to an endotoxin. Proc. Am. Soc. Anim., West Sect. 68, 310-315.

Musselman, A.F., Van Emon, M.L., Gunn, P.J., Rusk, C.P., Neary, M.K., Lemenager, R.P. and S.L. Lake. 2008. Effects of crude glycerin on feedlot performance and carcass characteristics of market lambs. Proc. Amer. Soc. Anim. Sci. W. Sec. 59, 353-355.

National Research Council (NRC). 2007. Nutrient Requirements of Small Ruminants, 7th ed. National Academies Press, Washington, DC, USA.

Osborne, V.R., Odongo, N.E., Cant, J.P., Swanson, K.C. and B.W. McBride. 2009. Effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, energy balance, and production performance of periparturient dairy cows. J. Dairy Sci. 92(2):698 -707.

Parsons, G.L., Shelor, M.K. and J.S. Drouillard. 2009. Performance and carcass traits of finishing heifers fed crude glycerin. J. Anim. Sci. 87(2):653-657.

Pillmore, S.L., Carey, R.E., Samuelson, K.L., Klump, L.T., Lopez, F.A. and Löest, C.A. 2017. Supplementation of crude glycerin via drinking

water alters feed intake of sheep. Amer. Soc. Anim. Sci. W. Sec. 324.

Porcu, C., Sotgiu, F.D., Pasciu, V., Cappai, M.G., Barbero-Fernández, A., Gonzalez-Bulnes, A., Dattena, M., Gallus, M., Molle, G. and F. Berlinguer. 2020. Administration of glycerol-based formulations in sheep results in similar ovulation rate to crude glycerin but red blood cell indices may be affected. BMC Vet. Res.16(1), 1-15.

Radostits, O. M., C. C. Gay, D. C. Blood, and K. W. Hinchcliff. 2000. Veterinary medicine, 9th ed. London, Harcourt Publishers. 1417-1420.

Ribeiro, R.D., Carvalho, G.G., Silva, T.M., Costa, J.B., Bezerra, L.R., Cambuí, G.B., Barbosa, A.M. and R.L. Oliveira. 2018. Effects of crude glycerin from biodiesel on the diets of lambs: intake, digestibility, performance, feeding behavior, and serum metabolites. J. Anim Sci.96(5), 1952-1961.

Roger, V., Fonty, G., Andre, C. and P. Gouet. 1992. Effects of glycerol on the growth, adhesion, and cellulolytic activity of rumen cellulolytic bacteria and anaerobic fungi.Curr. Microbiol. 25(4), 197-201.

Romanzini, E.P., da Silva Sobrinho, A.G., de Lima Valença, R., Borghi, T.H., de Andrade, N. and P.A. Bernardes. 2018. Feedlot of lambs fed biodiesel co-products: performance, commercial cuts and economic evaluation. Trop. Anim. Health. Prod. 50(1), 155-160.

Sá, H.C.M., Borges, I., Junior, G.D.L.M., Santos, F.S., Cavalcanti, L.F.L., Alvarenga, F.A.P., Martins, T.L.T. and Campolina, J.P. 2017. Evaluation of dairy sheep lambs supplemented with crude glycerin conveyed in water during pregnancy and lactation. Semina: Ciências Agrárias, 38(4), 2681-2690.

Sellers, R.S. 2008. Glycerin as a feed ingredient, official definition (s) and approvals. J. Anim. Sci. 86(2), 392.

Thompson, J. C. and He. B. B. 2006. Characterization of CGfrom biodiesel production from multiple feed stocks. Appl. Eng. Agric. 22, 261-265.

Volpi-Lagreca, G. and S.K. Duckett. 2017. Supplementation of glycerol or fructose via drinking water to grazing lambs on tissue glycogen level and lipogenesis. J. Anim. Sci. 95(6), 2558-2575.