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Effect of adding linseed oil on in vitro gas and methane production and some fermentation characteristics

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Abstract:

This experiment was carried out in animal feeding laboratories in the Animal Production Department of the Faculty of Agriculture, University of Baghdad / Jadriya for the period from 1/12/2016 to 1/5/2017, where food supplements were used with different percentages of Linseed oil. 0,70,140,280 µl / kg dry matter to a mixture of concentrated feed and coarse feed to study its effect on total gas production and methane gas and fermentation properties ((pH, volatile fatty acid ratios (Acetic, propionic and butyric), ammonia nitrogen ratio)). Results:

1-. Using the gas production technology in the laboratory, a significant reduction in the total production of gas and methane in T2, T3, containing 70,140 µl / kg dry material from LO oil at incubation time after 12 hours in vitro compared to T1and T4 (supplemented with 280 µl / kg dry matter of LO oil). After 24 hours of in vitro incubation, T2 treatment was significantly reduced ($p<0.01$) in total gas production compared to T3 and T4, For the production of methane, it was significantly lower ($p<0.01$) for T2 compared to T3 and T4 respectively. The total volume of gas after 48 hours of in vitro incubation was significantly decreased ($p<0.01$) for T2, T3 and T4 compared to the control treatment , the same T6 and T7 were significantly lower ($p<0.01$) in methane compared to T2 and after 72 hours of laboratory incubation, T2 and T4 showed a significant decrease ($p<0.01$) in total gas production while T6 was central between them and there were no differences In the size of methane in the models withdrawn after 72 hours of in vitro incubation.

2- For fermentation characteristics, the results showed the following:

2-1 There were significant differences (P<0.01) in the pH values of the withdrawn samples after 12 hours of in vitro incubation compared to the control treatment, the T4 was significantly reduced (P<0.01) in the pH values in the withdrawn models after 24 and after 48 and 72 hours of laboratory incubation. The T4 was significantly lower (P<0.01) in the pH value compared to T2 and T3 respectively.

2-2 As for the concentration of ammonia nitrogen, The results showed that there were significant differences (P<0.01) between the LO oil added to the hay in the concentration of ammonia nitrogen where T2, T3 and T4 containing LO were significantly reduced (P<0.01) in nitrogen concentration Ammonia compared to T1 treatment of the withdrawn samples after 12 and 24 hours of laboratory incubation. The results also showed a significant decrease in (p<0.01) in the ammonia nitrogen concentration of T3 and T4 for the withdrawn samples after 48 and 72 hours of laboratory incubation compared to T2 treatment.

2-3 The results showed that there were significant differences (p<0.01) between the LO oil of the hay in the concentration of volatile fatty acids (acetic acid, butyric acid, propionic), where T2 and T3 were significantly lower (p<0.01) 12 days of incubation in vitro compared to T4 treatment, while no significant differences in acid concentration were observed 24 hours of laboratory incubation. T2 was significantly lower (p<0.01) in acetic acid concentration compared to T3 and T4 respectively, There were significant differences(p<0.01) in acid concentration In the samples withdrawn after 72 hours of in vitro incubation. As for propionic acid, LO (T5) showed a significant reduction(p<0.01) in the concentration of propionic acid after 12 in vitro incubation compared with T1 while T3 and T4 were significant(p<0.01) The concentration of propionic acid after 24 and 48 hours of in vitro incubation, while the concentration of acid and high morale (p<0.01) in the treatment T2 compared with the treatment T4, and the results showed no significant differences of LO oil treatments in the concentration of butyric acid has 12 and 24 hours incubation in the laboratory, Results showed a high morale (p<0.01) concentration Butyric acid for treatment T2 compared to control treatment after 48 and 72 hours of incubation laboratory.

Key words: linseed oil, in vitro gas and methane production, fermentation characteristics

INTRODUCTION

Animal production has increased the use of synthetic chemicals as feed additives for animal feed (Calsamiglia et al., 2006), which improves growth and microbial metabolic processes (Fellner, 2002) and improves milk management for ruminants (cattle, sheep, Goats), but recently there have been concerns about the use of these synthetic chemicals as food supplements in animal feed because of potential toxic hazards resulting from the use of these Artificial chemicals, Which increased the

prediction of the risks of these chemicals to the environment and health associated with the use of these synthetic chemicals. All these obstacles made the governmental institutions and regulatory bodies, including the European Union in 2006, to consider limiting the use of some synthetic and chemical materials in animal feed. Chemicals are antibiotics (Barton, 2000), prompting researchers and animal production professionals to find food alternatives of plant origin that are derived mainly from vegetable sources, such as **vegetable oils** (Cowan, 1999), They are usually extracted from plant materials by boiling water and distillation of steam, but other methods include the use of solvents and the use of supercritical carbon dioxide. The expression of vegetable oils (Benchaar and Greathead, 2011) has been used to be a safe alternative to antibiotics in Ruminants, where there are a number of types of vegetable oils and these oils Flaxseed oil (Usitatissimum) or flaxseed oil is the oil extracted from the flaxseed through the times followed by extraction with organic solvents, is a colorless liquid to yellowish and rich in unsaturated fatty acids Arabic for body and knowledge Popularly called the hot oil or Also with drying oil due to the possibility of conversion into solid form by polymerization. Flaxseed oil can be used alone or mixed with a mixture of other oils, resins or solvents as a suppressed seed (Thompson, 2003). linseed oil is used as a dietary supplement in the diet because it is a major source of α-phenylenolic acid, (Vereshagin and Novitskaya, 1965) (between 42-48% ALA "C18: 3") (Diller and Diller, 2004), This acid is reduced in the body to a limited amount of Eicosapentaenoic acid (EPA) or known omega-3 is the same acid found in fish oil, which in turn turns into three series of prostaglandins and is a semi-hormones that are manufactured in many parts of the body. **The purpose of this study** was to add linseed oil with different percentages to the ruminants' diet consisting of 80% alfalfa hay with 20% of concentrated feed and to study its effect on the total production of gas and methane in the laboratory and measure some

fermentation properties such as volatile fatty acids, ammonia nitrogen, pH After different incubation laboratory periods.

MATERIAL AND METHODS

1- **Experiment Plan**: The experiment was carried out in the nutrition laboratories of the Faculty of Agriculture / University of Baghdad-Jadriya, to study the effect of adding different levels of flaxseed oil by 140.70 and 280 µl / kg to a mixture of concentrated feed and coarse feed. The effect of feed type and addition of castor oil on the pattern of rumen fermentation, total gas production and methane gas in the laboratory.

2- **Experiment Diet**: The components of the experimental bush used in the study were provided from the animal field of the University of Baghdad / Faculty of Agriculture / Animal Resources Department consisting of 80% of alfalfa hay + concentrated feed 20% with the addition of linseed oil (140,70 and 280 micro liters / kg DM). In this study, the use of alfalfa hay should be crushed with a laboratory jar and a diameter of 1 mm in order to facilitate a mixture with concentrated feed and oil with completely before starting the experiment

Table 1: Percentage and components of raw materials included in the composition of concentrated nutritional diets used in the experiment.

Raw Materials	**%**
Barley	23
Wheat bran	22
Soybean Meal	13
yellow corn	40
Minerals and vitamins	1
Salt	1
Crud protein*	14.62
ME(MJ/Kg DM)**	11.23

***Calculated from the chemical analysis table for Iraqi feed materials (Al-Khawaja, 1978) (on DM% basis)**

**** ME(MJ/Kg DM)=0.012 × CP + 0.031 × EE + 0.005 × CF + 0.014 × NFE**

(MAFF, 1975)

3-Chemical composition of primary feed materials and experimental diets Table (2 and 3) shows the chemical composition of the concentrates of the concentrates and the experimental grits with the added LO oil. The dry matter(DM), organic matter(OM), crud protein(CP), crud fiber(CF), Ether Extract(EE) and nitrogen-free extract(NFE) were estimated by AOAC (1984) In the nutrition laboratory of the Faculty of Agriculture - University of Baghdad / Department of Animal Production. As for the metabolizable energy, has been calculated under the following equation:

ME(MJ/Kg DM)=0.012XCP+0.031XEE+0.005XCF+0.014XNFE

According to in vitro digestibility of DM* and OM** by method (Tilley and Terry,1963).

Table 2: shows the chemical composition of concentrated and roughage feed in the composition of the nutritional diets used in the experiment % .

factor / Ingredient	DM % of fresh	OM%	Ash%	CP%	CF%	EE%	NFE** %	ME*(MJ/Kg DM)
Barley	**91.431**	**86.896**	**4.535**	**11.678**	**7.410**	**2.059**	**65.749**	**11.6150**
yellow corn	**90.196**	**87.245**	**2.951**	**10.092**	**2.879**	**4.890**	**69.384**	**12.5846**
Soybean Meal	**90.122**	**85.125**	**4.997**	**48.455**	**6.575**	**2.237**	**27.858**	**10.7369**
Wheat bran	**90.151**	**84.479**	**5.672**	**17.114**	**11.998**	**4.563**	**50.804**	**11.1806**
alfalfa hay	**96.415**	**85.841**	**10.574**	**14.312**	**18.094**	**1.95**	**51.485**	**10.4345**

***ME(MJ/Kg DM)=0.012XCP+0.031XEE+0.005XCF+0.014XNFE**

****NFE(%OFDM)=%OM-(%CP+%CF+%EE)**

Table 3: : shows Chemical composition and metabolizable energy (MJ / kg DM) for 80 % alfalfa hay with 20% concentrated feed and adding Linseed oil at 0.70,140 and 280 µl* / kg DM

factor / Treatments	DM % of fresh	OM%	Ash%	CP%	CF%	EE%	NFE %	ME**(MJ/Kg DM)
T1(control)	**92.948**	**82.418**	**10.530**	**12.046**	**20.850**	**1.221**	**48.299**	**9.6286**
T2 add 70 µl LO	**92.958**	**83.218**	**09.740**	**11.068**	**19.360**	**1.122**	**51.666**	**9.8775**
T3 add 140 µl LO	**92.912**	**83.404**	**09.508**	**11.010**	**20.116**	**1.163**	**51.112**	**9.8436**
T4 add 280 µl LO	**93.804**	**83.568**	**10.235**	**11.582**	**20.095**	**1.187**	**50.702**	**8.6139**

T1=control (without any addition)

T2= treated adding Linseed oil at 70 µl/ kg DM

T3= treated adding Linseed oil at 140 µl/ kg DM
T4= treated adding Linseed oil at 140 µl/ kg DM
*µl=micro liters
**** ME(MJ/Kg DM)=0.012X CP+0.031XEE+0.005XCF+0.014XNFE (MAFF, 1975)**

RESULTS AND DISCUSSION

1-Production of total gas and methane gas in the laboratory

The results of Table 4 showed a significant decrease in ($p<0.01$) in total gas production after 12 hours of laboratory incubation when LO oil was added, That lowest quantity was 31.75 ml / 200 mg dry matter in T2 followed by T4 (33.25 ml / 200 mg DM) compared with T4 35.45 ml / 200 mg dry matter. The results showed a significant decrease in the production of methane gas for T2 and T3 (3.43 and 3.40 mg / 200 mg dry matter respectively) compared with T4 (4. 50 ml / 200 mg dry matter), In the same as that significant decrease ($P <0.01$) in total gas production when LO oil was added after 24 hours, As T3 and T4 were recording the highest amount ($P <0.01$) 39.75 and 39.65 ml / 200 mg dry matter respectively) compared with T2 treatment (36.25 ml / 200 mg dry matter). As for the amount of methane after 24 hours of laboratory incubation, Table 4 revealed the existence of significant differences between the transactions in the volume of methane added by the LO oil. The transaction T2 recorded a high morale ($p<0.01$) with an average of 4.50 ml / 200 Mg dry matter) in methane production compared to T3 and T4 (5.00 and 5.20 ml / 200 mg dry matter, respectively) The results of Table 4 also showed that the highest significant volume ($p <0.01$) of total gas when adding LO oil after 48 hours of laboratory incubation was in T1 (45.25 ml / 200 mg dry matter) compared with treatment in T2 (36.25 ml / 200 mg) followed(T3 and T4 39.75 and 41.35 ml / 200 mg dry matter respectively). For the LO oil treatments, the results of Table 4 showed a significant decrease ($p <0.01$) for T3 and T4 (5.00 and 5.00 ml / 200 mg dry matter) compared to T2 (5.71

mg / 200 mg dry matter). These results are consistent with those of Marino et al. (2013), **At the same time** These results do not agree with Moreira et al. (2014), which indicated that adding different levels of vegetable oils by 0, 30, 50 and 70 resulted in a significant reduction in methane production, The reason for the decrease in the production of gas when adding oil to the crops may be due to the modification of the fermentation process and the decrease in its effectiveness due to the presence of a cover of oil on the feed material, which is considered as a basic food for rumen bacteria (Manso et al., 2009) For the feed material or perhaps the added oil contains some toxic substances that are harmful to the Microorganisms, especially cellulose and cellulose fibers in the rumen.

Table 4. Effect of linseed oil supplementation by 0, 70, 140 and 280 µl / kg DM on total gas production and methane (200 mg / DM) for the 80% alfalfa hay with 20% concentrated feed at different laboratory incubation periods.

	Std ± mean							
Studied Trait / Treatments	Total gas Volume	Methane Volume	Total gas Volume	Methane Volume	Total gas Volume	Methane Volume	Total gas Volume	Methane Volume
	Incubation periods(hour)							
	12	12	24	24	48	48	72	72
T1(control)	36.70±0.01 a	3.50±0.28 b	38.00±0.40 b	4.50±0.17 b	45.25±0.47 a	5.05±0.05 b	51.00±1.47 a	5.15±0.33
T2 add 70 µl LO	31.75±1.25 c	3.43±0.82 b	36.25±0. 25 b	4.50±0.28 b	36.25±0.62 d	5.71±0.44 a	41.50±0.64 c	5.15±0.49
T3 add 140 µl LO	33.25±0.47 b	3.40±0.35 b	39.75±0.47 a	5.00±0.01 a	39.75±0.47 c	5.00±0.57 b	43.00±1.08 bc	5.50±0.64
T4 add 280 µl LO	35.45±0.49 a	4.50±0.28 a	39.65±0.47 a	5.20±0.46 a	41.75±0.62 b	5.00±1.01 b	45.50±0.64 b	4.90±0.75
Significant	**	**	**	**	**	**	**	N.S

T1: control(without addition), **T2**: Diet adding linseed oil by 70 micro liters / kg DM, **T3**: Diet Adding linseed oil by 140 micro liters / kg DM, **T4**: Diet Adding linseed oil by 280 micro liters / kg DM, **N.S** indicates not significant, ****** indicates significant differences at the probability level($P < 0.01$).

DM=dry matter

LO=linseed oil

The results also show that when LO oil was added to the diet, a significant reduction ($P<0.01$)was achieved in the amount of total gas produced after 72 hours of in vitro incubation for T2

treatment (41.50 ml / 200 mg dry matter) compared to T4 (45.50 ml / 200 mg dry matter),These results do not agree with Morgavi et al.(2012),at the same time The results also showed no significant differences in methane production after 72 hours of in vitro incubation in the addition of LO oil, which is consistent with Murply et al. (1990) and inconsistent with Mizubuti et al. (2015).) This may be due to the reflection of the effectiveness and extent of the degradation of food and feed digestibility which containing a high percentage of lignin that affect the effectiveness of the microbiology of analyzed fiber and thus affect the amount of gas produced in the rumen.

2- PH measurement

The results of the statistical analysis at the pH measurement shown in Table (5) show the effect of the addition of LO oil by 0, 70, 140 and 280 µl / kg dry matter of the 80% alfalfa hay with 20% of concentrated feed in incubation periods 12,24,48 and 72 hours in vitro, there T3 and T4 were significantly higher in ($P < 0.01$) after 12 hours of laboratory incubation (6.65,6.60) than T1 at (6.35) while T2 was intermediate between them. Also The results indicated that T4 recorded the lowest pH value(6.60) in the models ($P<0.01$) compared with T2 and T3 (7.05 and 7.10) compared T1(6.55), after 24 hours in vitro incubation. while the pH of T4 and T1 were significantly reduced ($P<0.01$) (6.75,6.70) compared with T2 and T3 (7.10 and 7.20),As after 48 hours in vitro incubation, As for the samples with taken after 72 hours of laboratory incubation, the T4 was significantly lower ($P<0.01$) at (6.75) compared to T2 and T3 containing LO oil (7.00 and 6.95 respectively). These results were similar to those of Shingfield (2003) and similar to (Musibau et al., 2015) because their research was based on the addition of different percentages of lipid supplementation with one of the antibiotics(monensein) to the roughages feed, The results were similar with those of Jalc et al. (2007) when two types of **vegetable oils** (LO oil and

soybean oil) were added, and they were not similar (Sterk et al., 2011), which indicated that the concentration of pH decreased with the addition of vegetable oil This may be due to the survival of microorganisms in Crash with increased estrogen degradation and increased concentration of volt fatty acids at a time added by the oil to the diets .

Table 5. The effect of adding linseed oil by 0, 70, 140 and 280 µl / kg dry matter, on the pH of the alfalfa hay (80%) with the concentrated feed (20%) in different incubation periods.

Std ± mean				
Studied Trait / Treatments	PH			
	Incubation periods(hour)			
	12	24	48	72
T1(control)	0.05b ±6.35	6.55±0.05b	6.70±0.005b	6.85±0.05ab
T2 add 70 µl LO	6.45±0.05ab	7.05±0.05a	7.10±0.10a	7.00±0.005a
T3 add 140 µl LO	6.65±0.05a	7.10±0.10a	7.20±0.10a	6.95±0.15a
T4 add 280 µl LO	6.60±0.005a	6.60±0.10b	6.75±0.05b	6.75±0.005b
Significant	**	**	**	**

T1: control(without addition), **T2**: Diet adding linseed oil by 70 micro liters / kg DM, **T3**: Diet Adding linseed oil by 140 .micro liters / kg DM, **T4**: Diet Adding linseed oil by 280 micro liters / kg DM, ****** indicates .significant differences at the probability level($P < 0.01$).

3- Measuring the concentration of Nitrogen ammonia

The results of Table 6 show the concentration of ammonia nitrogen concentration in the rumen liquid for 80% alfalfa hay with 20% of concentrated feed at the effect of adding LO oil of 0, 70, 140 and 280 µl / kg dry matter in incubation periods after 12,24,48 and 72 h in vitro, The results showed significant differences ($P<0.01$) between the concentrations of nitrogen ammonia in the samples with taken after 12 hours in laboratory incubation, that T3 and T4 showed a significant decrease in ammonia nitrogen concentration ($P<0.01$) at (31.05 and 30.05 mg / 100), compared with T1 and T2(32.85 and 32.45 mg / 100 ml) .Also The results showed a significant reduction ($P<0.01$) for T2, T3 and T4, which contained LO oil of (31.65,31.85 and 30.00 respectively), compared with the treatment of T1 (32.85 mg / 100 ml), After 24 hours of

laboratory incubation, These results were **consistent** with the findings of Szumacher et al. (2004) when adding different levels of **vegetable oils** to ruminants' ruminants, also similar to those of Gunal et al. (2014) when adding vegetable oils at different levels 125, 250 and 500 µl / kg dry matter to calves Which resulted in reduced nitrogen concentration of ammonia in the diet, while T2 was highest of ammonia nitrogen concentration($P<0.01$) at (31.35 mg / 100 ml) compared with the treatments of T1,T3 and T4(30.00, 30.60 and 30.70 respectively) In sample taken after 48 hours of in vitro incubation. after 72 hours of in vitro incubation, The results showed that treatment T3 and T4 were significantly lower ($P<0.01$) at (30.55 and 29.75 mg / 100 ml) than T2 (31.25 mg / 100 ml), which was supported by Mertens (2002). However, these results were not consistent with Vargas et al. (2011) when adding LO oil by 50 g / Kg dry matter which were reduced to the concentration of ammonia nitrogen to the rumen fluid, and in the same context Dorea et al. (1994), In addition vegetable oils to feeds that reduces the nitrogen ammonia of concentration to the rumen, a number of researchers, such as Wallace (2002), have suggested that the presence of **vegetable oils** as supplements or dietary supplements that contain different levels of fatty acids, which acts as a inhibitory agent for the reduce active of ammonia-producing bacteria and harmful bacteria In addition to it reduces the effectiveness of the process by removing the amino group of proteins and this cycle reduces the rate of production of nitrogen ammonia, thereby increasing the use of protein microbial .

Table 6. Effect of the addition of linseed oil by 0, 70, 140 and 280 µl / kg dry matter on nitrogen ammonia for 80% alfalfa hay with 20% concentrated feed in different laboratory incubation periods.

Std ± mean				
Studied Trait / Treatments	Nitrogen ammonia			
	Incubation periods(hour)			
	12	24	48	72
T1(control)	32.85±0.05a	32.25±0.55a	30.00±1.10b	30.75±1.05b
T2 add 70 µl LO	32.45±0.05a	31.65±0.05b	31.35±0.15a	31.25±0.05a
T3 add 140 µl LO	31.05±0.05b	31.85±0.05b	30.60±0.005b	30.55±0.05b
T4 add 280 µl LO	30.05±0.05c	30.00±0.005c	30.70±0.20b	29.25±0.05c
Significant	**	**	**	**

4. **Measuring the concentration of volatile fatty acids (VFA)**

The results of Table 7 show the concentration of volatile fatty acids (acetic, propionic, butyric acids) in the rumen liquid of the 80% alfalfa hay with 20% concentrated feed when added LO oil by 0, 70, 140 and 280 micro liters / kg dry material in vitro periods after 12,24 , 48 and 72 h, The treatment T4 (62.90 mmol / L) was significantly higher (P<0.01) in concentration of **acetic acid** on T2 and T3 (62.13 and 62.20 mmol / L) In the samples taken from the rumen fluid after 12 hours of laboratory incubation, While there was no significant difference in the concentration of acetic acid between the treatment which were containing LO oil After 24 hours of laboratory incubation. After 48 hours of laboratory incubation, the concentration acetic acid was significantly decreased(P<0.01) in the T2 which was containing the (70 µl) LO oil at (65.26 mmol / L) compared with T3 and T4 (66.30 and 66.36) mmol / L respectively). The results showed significant differences between LO oil by 0.70, 140 and 280 µl / kg dry matter after 72 hours of laboratory incubation, that concentration acetic acid was significantly decreased(P<0.01) in the T1,T2 and T3 at (65.30,65.00 and 65.40 mmol / L) compared with T4(66.46 mmol/L), These results were consistent with Toral et al. (2009),As These results are not consistent with Atkinson et al. (2006), which added that the **vegetable oil** to the diet had a

positive relationship to increase the concentration of acid and increase the incubation period up to 96 hours in the laboratory. The Results (Table 7) showed significant differences (P<0.01) between the treatment in the concentration of **propionic acid** in the samples taken after 12 hours. A significant decrease was observed (P<0.01) in T2,T3 and T4 which were 26.36,2640 and 26.46 mmol / L Containing 70,140 and 280 µl / kg dry matter of LO oil, compared with T1(27.82 mmol / L). These results were similar to Oliveira et al. (2013), **At the same time** These results are not consistent with Martin et al. (2010), which was shown when adding glycerol to the diets and three types, including glycerol digested and pure glycerol and the mixture between the two types and 15% of each type of glycerol with addition **vegetable oil** to the relationship has increased the concentration of propionic acid after 24 hours of laboratory incubation , While the results also showed no significant differences in the concentration of propionic acid between the LO oil treatment after 24 and 48 hours of laboratory incubation, These results are not consistent with Getachew et al.(2004),that were using in our experiment with addition vegetable oil the mixture with a diet containing 50% alfalfa hay, 18% of all straw, corn stalks, and 6.9% of soybean flour by increasing propionic acid in alfalfa hay. The results showed a high significantly (P<0.01) in T4 (30.93 mmol / L) when LO oil was added after 72 hours of laboratory incubation compared to T2 andT3 (29.66 and 29.73 mmol / L) while T1 was significant decrease (P<0.01). These results are consistent Benchaar et al. (2006). At the same time the results are not consistent Dohme et al. (2011), Perhaps it was because which added that lipids, have significantly affected the ratios of volatile fatty acids as they reduced the concentration of propionic acid in rumen fluid. The results (Table 7) showed significant effect from the addition of LO oil to the alfalfa hay on the concentration of **butyric acid** after 12, whereas a significant decrease was observed (P<0.01) in T2,T3 and T4 which were (9.26,9.33 and 9.36 mmol /

L) Containing 70,140 and 280 µl / kg dry matter of LO oil, compared with T1(10.08 mmol / L). while The also results showed no significant after 24 hours of laboratory incubation, While the results showed that were significant differences (P<0.01) in the concentration of butyric acid in the samples taken after 48 and 72 hours. A significant decrease was observed (P<0.01) in the concentration of butyric acid, decreased in treatment T2,T3 and T4 significantly in the concentration of butyric acid for both periods at (11.33,11.40,1156 and 11.46,11.80 mmol / L) except T4 from period 72 h that was Recorded higher concentration of butyric acid(12.00 mmol / L) compared with control treatment T1 (11.86 and 12.13 mmol / L), These results were Similar to those obtained by Doreau and Chilliard (1997), but are not consistent with Aghajanzadeh-Golshani et al. (2015b), which they mentioned that the addition of **vegetable oils** was significant in rumen fermentation with microorganism survival and high volatile fatty acid content and precipitation, As well These results are conformity to those obtained by Kristensen and Harmon (2004), whereas The addition of vegetable oils has improved the concentration of butyric acid in the mixture by keeping the activity of microorganisms in rumen with increasing the decomposition of the Astria bonds in rumen.

Table 7. Effect of linseed oil supplementation by 0, 70, 140 and 280 µl / kg dry matter on the concentration of volatile fatty acids (acetic, butyric and propionic acid mmol / L) for the 80% alfalfa hay with 20% concentrated feed in different laboratory incubation periods.

Studied Trait / Treatments	Std ± mean concentration of volatile fatty acids											
	Acetic acid(mmol/L)				propionic acid(mmol/L)				Butyric acid(mmol/L)			
	Incubation periods(hour)											
	12	24	48	72	12	24	48	72	12	24	48	72
T1(control)	62.73±0.08 a	64.26±0.03	66.30±0.05 a	65.30±0.05 b	27.82±0.06 a	28.26±0.08	29.20±0.20	30.90±0.05 a	10.08±0.03 a	10.33±0.03	12.06±0.03 a	12.13±0.03 a
T2 add 70 µl LO	62.13±0.16 b	64.13±0.31	65.26±0.03 b	65.00±0.35 b	26.36±0.03 b	28.40±0.05	29.33±0.03	29.66±0.08 b	9.26±0.03 b	10.26±0.03	11.33±0.06 b	11.76±0.03 b
T3 add 140 µl LO	62.20±0.05 b	64.23±0.06	66.30±0.05 a	65.40±0.17 b	26.40±0.01 b	28.36±0.03	29.20±0.20	29.73±0.03 b	9.33±0.03 b	10.33±0.03	11.40±0.05 b	11.80±0.05 b
T4 add 280 µl LO	62.90±0.15 a	64.46±0.03	66.36±0.03 a	66.46±0.96 a	26.46±0.03 b	28.33±0.03	29.50±0.05	30.93±0.08 a	9.33±0.03 b	10.36±0.03	11.56±0.8 b	12.00±0.10 a
Significant	**	N.S	**	**	**	N.S	N.S	**	**	N.S	**	**

T1: control(without addition), **T2**: Diet adding linseed oil by 70 micro liters / kg DM, **T3**: Diet Adding linseed oil by 140 micro liters / kg DM, **T4**: Diet Adding linseed oil by 280 micro liters / kg DM, **N.S** indicates not significant, ****** indicates significant differences at the probability level(P <0.01).

REFERENCES

1. **Castillejos, L., Calsamiglia, S. and Ferret, A.2006.** Effect of essential oils active compound on rumen microbial fermentation and nutrient flow in-Vitro System .J. Dairy.Sci.,89:2649-2658.
2. **Fellner, V. 2002**. Rumen microbes and nutrient management. North Carolina state university. ARPAS Conference.
3. **Barton, M.D. 2000.** Antibiotic use in animal feed and Its impact on human health**.**NUTR,Res,Rev.,13:279-299.
4. **Cowan, M.M.1999.** Plant products as antimicrobial agent. Clin., Microbial Rev.,12:564-582.
5. **Chaouki, Benchaar and Henry Greathead. 2011.** Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Volumes 166–167, 23 June 2011, Pages 338-355.
6. **Thompson, Lilian U and Cunnane, Stephen C. eds (2003).** *Flaxseed in human nutrition. 2nd ed*. AOCS Press. pp. 8–11. ISBN 1-893997- 38-3**.**
7. **Vereshagin, A. G. and Novitskaya, G. V .1965.** The triglyceride composition of linseed oil. Journal of the American Oil Chemists Society 42, 970-974**.**
8. **Diller, S. and J. Diller. 2004**. Craftsman's**.** Construction Installation Encyclopedia, Craftsman Book Company, p. 503.
9. **Khawaja, Ali Kazem, Elham Abdullah and Samir Abdel Ahad (1978).** Chemical composition and nutritional value of Iraqi feed materials Bulletin issued by the Nutrition Section of the General Livestock Directorate of the Ministry of Agriculture and Agrarian Reform, Republic of Iraq.
10. **MAFF.1975.**Ministry of Agric.,Fisheries and Food Dept. of Agric. and Fisheries for Scotland energy allowances and feed system for ruminants, Technical Bulletin,33.
11. **Marino, C. T., M. J. Ruiz-Moreno, T. M. Schulmeister, F. M. Ciriaco, D. D. Henry, V. R. G. Mercadante, G. C. Lamb, N. DiLorenzo,2013** .Effects of extracts of cashew nut shell and castor oil on in vitro ruminal fermentation, gas production kinetics, and methane production. J. Dairy Sci. 96(E-Suppl. 1):[page number]. or J. Anim. Sci. 91(E-Suppl. 2)
12. **Moreira, M. N., Silva, A. M. A., Carneiro, H., Bezerra, L. R., Morais, R. K. O. & Medeiros, F. F. (2014).** In vitro degradability and total gas production of biodiesel chain byproducts used as a replacement for cane sugar feed., Acta Scientiarum. Animal Sciences. 36(4), 399-403.

13. **Manso,T.,R.Bodas,T.Castro,V.Jimeno,A.R.Mantecon.2009)**Animal performance and fatty acid composition of lamb fed with Different vegetable oils.Meat.Sci.83:511-516.
14. **Morgavi, D.P., C. Martin, J.P. Jouany, and M.J. Ranilla. 2012.** Rumen protozoa and methanogenesis: not a simple cause-effect relationship. British Journal of Nutrition 107:388-397.
15. **Murphy, D.J.1990.** Strong lipid Bodies in the plant and other organisms. Prog lipid Res.29(4):299-324.
16. **Musibau A. B., I. M. Ogunade, F. Amaro, Y. Jiang, T. F. Bernardes, D. D. Henry, V. R. Vasconcelos, F. O. Ugiagbe, U. J. Ikhatua, N. DiLorenzo, and A. T. Adesogan.2015.** Methanogenesis reduction ability of monensin and essential oils from two Nigerian citrus species. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2**.**
17. **Jalc, D0, Certik M., Kundrikova K. and Namestkova P.,2007.** Effect of unsaturated C-18 fatty acids(oleic linoleic and alph-linlenic acid)on ruminal fermentation and production of fatty acid isomers in arti- ficial rumen. Veterinarni Medicina,52.p.87-94.
18. **Sterk A, Johansson BEO, Taweel HZH, Murphy M, Van Vuuren AM, Hendriks WH and Dijkstra J 2011.**Effects of forage type, forage to concentrate ratio, and crushed linseed supplementation on milk fatty acid profile in lactating dairy cows. Journal of Dairy Science 94, 6078–6091.
19. **Szumacher, S. M., S.A. Martin, A. Pothanski, A. Cielak and J. Kowalczyk.2004.** Changes in fermentation processes as the effect of vegetable oil supplementation in vitro studies. J. Anim. Feed..Sci., 13: 215-218.
20. **Gunal, M., A. Ishlak, A.A. AbuGhazaleh , and W. Khattab.2014.** Essential oils effect on rumen fermentation and biohydrogenation under in vitro. conditions**.** Czech J. Anim. Sci., 59, 2014 (10): 450–459**.**
21. **Mertens, D. R. 2002.** Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. of AOAC. Inter, 85(6), 1217-1240.
22. **Vargas, J.E.,S. Andres, D. R. Yanez R and S.Lopez.2011.**The effect of olive, sunflower or linseed oils on the fermentation pattern and methane production in the rumen simulating technique, ptionsMediterraneennes,A,99:163-168.
23. **Doreau, M. and A. Ferlay. 1994.** Digestion and utilization of fatty-acid by ruminants. Anim .feed Sci. Technol.,45:379 -396.
24. **Wallace, R.J.N.R.F. McEwan, M. Mclntoosh, B. Teferedegne and C. J.Newbold, 2002.** Natural products as mainpulators of rumen fermentation .Asian-Aust. J. Anim .Sci 15:1458-1468.

25. **Toral, P.G., A. Belenguer and P. Frutos. 2009.**Effect of the supplementation of a high –concentrate diet with sunflower and.Fish oils on ruminal fermentation in sheep. Small Ruminant Research,81:1119- 125.
26. **Atkinson, R.L., E.J. Scholljegerd, and S.L. Lak. 2006.** Esterifed fatty acid.in sheep fed a high-concentrate diet supplemented with site and extent.of digestion, duodenal flow, and intestinal disappearance of total and. high-linoleate sunflower oil. J. of Anim. Scie.,84:387-396.
27. **Oliveira, J. S., Antoniassi, R., Freitas, S. C. & Müller, M. D. (2013).** Chemical composition of glycerin produced by biodiesel plants in Brazil and potential utilization in animal feeding. Ciencia Rural, 43(3), 509-512.
28. **Martin, A., Varona, S., Navarrete, A., Cocero,M.J., 2010**. Encapsulation and co-precipitation processes with supercritical fluids: application with essentials oils. open Chem.Eng.J.,4,31-41.
29. **Getachew, G. P.H. Robinson, E. J.Depeters and S. J.Taylor. 2004.** Relation between chemical composition, Dry matter dehydration and in vitro gas production of several ruminant feeds. Animal and Technology, Amsterdam,111(1-4):57-71.
30. **Benchaar, C., Duynisveld, J. L. and E .Charmley., 2006.** Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef.cattle. Canadian Journal of Animal Science, 86, 91-96.
31. **Dohme, F., A. Machmüller, A. Wasserfallen. and M. Kreuzer. 2011.** Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. *Letters in Applied Microbiology*.: 32(1):47–51.
32. **Doreau, M and Y.Chilliard. 1997.** Digestion and metabolism of dietary fat in farm animals.Br.J.Nutr.78:S15-S35.
33. **Aghajanzadeh-Golshani A., Naser Maheri-Sis., Ramin S. Doust- Nobar.,Y. Ebrahimnezhad. and A. Ghorbani**. **2015b** Comparing fermentation kinetics and nutritional value of alfalfa hay using rumen and faeces liquor as inocula for in vitro gas production technique. Journal of Biodiversity and Environmental Sciences. Vol. 5, No.(3) : 308-315.
34. **Kristensen, N.B.; Harmon, D.L. 2004.** Effect of increasing ruminal butyrate absorption on splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. Journal of Animal Science, Champaign, v.82, n.12, p.3549-59.