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# Effect of lipid source; Linseed or soybean in diets, on rumen and blood fatty acids profiles in Damascus goats

**Abeer M. El-Essawy**

Animal and Poultry Nutrition Department, Desert Research Center, El-Matarya, Cairo, Egypt

**Correspondence Author:** Abeer M. El-Essawy Animal and Poultry Nutrition Department, Desert Research Center, El-Matarya, Cairo, Egypt.
E-mail: - abeerateek@hotmail.com

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**Abstract**

**This research aims to evaluate the effect of feeding different levels of whole linseed (L); as a lipid source rich in linolenic fatty acid, and full fat soybean (S); as a lipid source rich in linoleic fatty acid; on ruminal and plasma fatty acids profiles, rumen fermentation, and microorganism population. Twenty-four Damascus goats were assigned to 3 dietary treatments; S, S+L and L groups; contained 90% basal diet which consisted of 56.67% Concentrate feed mixture (CFM) and 33.33% Alfalfa hay with either 10% full fat soybean, 5% full fat soybean + 5% whole linseed or 10% whole linseed, respectively. Inclusion of linseed to diets increased (P<0.01) the total volatile fatty acids (TVFA's) and ammonia-nitrogen ($NH_3$-N) (P<0.05) compared to animals fed on soybean diet whereas ruminal pH was not affected by dietary treatments. Significant increases (P<0.01) were detected in the populations of *Cellulomonas cellulasea, Bacillus sp., Thermonospora fusca, Acetobacter xylinum, Ruminococcus albus* and *Clostridium cellulovorans* in goats fed on linseed compared to those fed on soybean diets. The populations of *R. albus* and *C. cellulovorans* were only affected with the high inclusion of linseed.**
**The number of ciliated sp. (*Holotrichs)* decreased (P< 0.01) while *Entodinomorphs sp*. increased (P<0.05) with linseed . There was a significant increase (P<0.05) in the total number of protozoa compared to that of goats which received soybean only. The ruminal and blood plasma fatty acids (FA) profiles of the experimental animals showed considerable modifications. Ruminal stearic acid showed the highest (P<0.01) percentage with linseed feeding while palmitic (P<0.01) and oleic acids (P<0.05) were predominant with soybean feeding. Saturated fatty acids (SFA) detected higher percentage than unsaturated ones on both levels of linseeds. Soybean inclusion increased (P<0.01) percentage of polyunsaturated fatty acids (PUFA) compared to other two groups. In blood plasma; the predominant FA were palmitic, oleic then stearic acids with soybean, either supplied alone or with linseed (S and S+L groups), whereas oleic, palmitic then linoleic acids were predominant with linseed (P<0.01). The percentage of the absorbed UFA with linseed supply was more than SFA in the same group. Plasma total protein, albumin (P<0.01) and urea (P<0.05) were increased by linseed inclusion while createnine (P<0.05) increased with soybean. Linseed inclusion resulted in decreased blood cholesterol (P<0.01), triglycerides (P<0.05), low density lipoprotein (P<0.01) and increased high density lipoprotein (P<0.01) and total antioxidant capacity (P<0.01).**
**Results indicated that linseed inclusion in diets resulted in considerable variations in rumen and plasma FA which indicates health promoting effects. Therefore, fatty acids composition of animal's products resulted in more satisfying and healthful properties for consumer**

**Keywords:** Linseed - full-fat soybean - Damascus goats- fatty acids

## INTRODUCTION

Recently, most studies have focused on increasing the n-3 poly unsaturated fatty acids (PUFA) and the conjugated linoleic acid (CLA) in animal's products (Atti et al., 2013), because of it's potential health benefits and positive effects on human health (Yang et al., 2009). The rumen is a complex ecosystem in which all nutrients consumed anaerobically by microorganisms such as bacteria, protozoa, and fungi (Castillo-Gonzalez et al., 2014). The study of the impact of PUFA supplementation on ruminal bacteria should be made by examining specific bacterial species rather than the total number of bacteria (Liu et al., 2012).

Dietary oils reduce cellulolytic bacteria, protozoa count, and total ruminal volatile fatty acids (VFA) (Yang et al., 2009). Lipids supplementation influences the fatty acids concentrations as well as the types of ruminant products. The rumen bacteria convert linoleic acid (C18:2) and linolenic acid (C18:3) into stearic acid (C18:0) through biohydrogenation (Harfoot & Hazlewood, 1988). The conjugated linoleic acid (CLA) isomer cis 9, trans 11- C18:2 (C9,t11-CLA) is formed as an intermediate

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product during rumen biohydrogenation of linoleic acid (C18:2) to trans vaccenic acid, trans 11 – C18:1 ( TVA) and stearic acid (C18:0) by rumen microorganisms (Harfoot and Hazlewood, 1988). Biohydrogenation is either defined as the reduction of double bonds in the fat molecule or as the addition of hydrogen atoms by the action of isomerases and reductases (Jenkins, 1993). However, high levels of unsaturated fat as supplements in the diet can cause an incomplete biohydrogenation of linoleic acid, which leads to the production of isomers like trans-vaccenic acid (C18:l *trans*-11), consequently leading to a reduction in stearic acid (C18:0) content (Harfoot et al. 1973). Rumen biohydrogenation of dietary PUFA is affected by several factors, such as the amount and type of the lipid supplement (Harfoot and Hazlewood, 1997).

In ruminant feeding, large amounts of vegetable oils inhibit ruminal fermentation (Jenkins, 1993) and decrease the counts of ruminal bacteria and protozoa (Dohme et al., 2001). Some oilseeds and their oils, either protected or unprotected from rumen digestion, seem to be suitable fat sources that are considered beneficial, such as polyunsaturated fatty acids (PUFA) of the n-3 series (Ashes *et al.*, 1997; Chilliard *et al.*, 2001).

Linseed, as a source of linolenic acid, contains 40% oil and about 50 -60 % of this oil is Linolenic acid. Therefore, linseed is one of the richest plant sources of n-3 fatty acids (Nassu et al., 2011). Full fat soybean, as a source of linoleic acid, contains 17 – 20 % oil on dry matter basis. The negative effect of PUFA on ruminal fermentation parameters is explained by reductions in cellulolytic bacteria concentration. However, Maia et al. (2007) had evaluated the effect of different fatty acids on the growth of different strains of ruminal bacteria and found that linolenic acid was more toxic than linoleic acid. Reports documenting the effects of feeding whole linseed and soybean on ruminal microorganisms are rare.

Thus, this experiment was conducted to evaluate the influence of feeding different levels of the whole linseed as a source of α-linolenic acid, and soybean as a sole or combined source of linoleic acid on ruminal microbial population, fermentation parameters, ruminal and plasma fatty acid profiles of Damascus goats. Also, the effects of dietary treatments on some blood parameters were determined.

## MATERIALS AND METHODS

### Animals, diets and experimental design

This experiment was conducted at Mary out Research Station (30 km to Alexandria), Egypt. Twenty-four, non-lactating, non-pregnant Damascus goats with initial body weight (means and SE) of 31.40 ±0.50 kg and age between (2-3 years), were used. Goats were randomly divided into three equal groups (eight animals each). All animals were housed in individual pens with separate facilities for feeding and watering. The experimental groups were fed on 90% basal diet (consisted of 56.67% concentrate feed mixture (CFM) and 33.33% alfalfa hay with either 10% full-fat soya (S), or 5% whole linseed + 5% full-fat soya (S+L) or 10% whole linseed (L). The experimental rations were formulated to cover goats requirements, according to NRC (1981).

The experimental diets werebeing mixed once a day and offered daily in two equal portions at 7 am and 4 pm. Oilseeds (fat source) were stored in a shaded place and were manually mixed with diets as well. Fresh water was available *ad libitum*. The feeding trial lasted for 120 days.

### Measurements, sample collection and analysis

#### a) Feed analysis

Proximate chemical analysis of feeding ingredients and experimental diets were determined according to the standard methods of the Association of Official Analytical Chemists (AOAC,2007). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by Goering and Van Soest (1970). Each analysis was performed in triplicate.

#### b) Oilseeds analysis

Fatty acids contents of soybean and linseedwere analyzed according to AOAC (2000) using Ultra Gas Chromatographs.

### Rumen liquor sample

At the end of the feeding trial; ruminal fluid was collected from animals, 3 hours after the morning feeding using a stomach tube via the esophagus. The samples were monitored visually to ensure they were not polluted with saliva. About 100 ml of liquor was obtained from each animal.

### Preparation of bacterial cultures

Six strains of cellulolytic bacteria were isolated from rumen fluid and were grown in a purely cultural. The separated strains were *Cellulomonas cellulase, Acetobacter xylinum, Thermonospora fusca, Ruminococcus albums, Bacillus sp., and Clostridium cellulovorans.* The isolation of species was carried out using the pour-plate technique for pure preparation of cultures, according to ATCC (1992). The rumen samples were immediately gassed with CO2. Viable counts of rumen cellulolytic bacteria were determined according to the method described by Gall et al. (1949), Moir (1951) and their classification were done according to Pounden and Hibs (1948).

### Ruminal fermentation characteristics

On day 120, the ruminal pH of rumen liquid was measured immediately after sampling; using a portable pH meter (Mettler-Toledo Ltd., England). Rumen liquid was taken and squeezed through four layers of cheesecloth to remove the feed particles. The filtered rumen was divided into subsamples for the determination of total volatile fatty acids TVFA's (Warner, 1964), ammonia – N ($NH_3$-N) (AOAC, 1997) and for the counting of protozoa that had been classified into *Entodiniomorphs* and *Holotrichs.* For the

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protozoal count: 1ml of the filtered rumen fluid was added to 4 ml of methyl green – formalin – saline solution. The number of protozoa was counted microscopically (100x) in a drop of rumen fluid with the defined volume, with the division on the *Holotricha* and *Entodiomorpha* groups (Szumacher-Strabel et al., 2002). Duplicate measurements were made from each sample and the average of these measurements was used to determine the number of protozoa present in the initial sample.

Fatty acids in filtered rumen liquor and plasma were extracted by using 100% ether where the sample was mixed with 100% ether in the ratio of 1:10 (v/v). The mixture was agitated manually for the 20s and centrifuged at high speed for 10 min. This step was repeated three times with the aqueous phase (Ferraz et al. 2004). Saturated, unsaturated, and total fatty acids were determined by using methyl esters boron tri fluoride method (AOAC, 2000). Fatty acids were methylated with boron tri fluoride in methanol, extracted with heptane and determined on a Gas Chromatograph with FID detector (PE Auto System XL) with autosampler and Ezchrom integration system. The carrier gas (He); ca. 25 Psi – air 450 ml/min – Hydrogen 45 ml – split 100 ml/min. Oven temperature 200 ˚C injectors and detector 250˚C.

**Biochemical blood analysis**

Blood samples were collected from the jugular vein at the end of the trial, 3 hours post feeding. All samples were centrifuged at 4.000 rpm for 15 minutes, and the collected plasma were immediately frozen at -20˚C for subsequent analysis of total protein, albumin, globulin (subtracting the total proteins values from the albumin values) , urea –N, creatinine, ammonia, cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) , total lipids (TL), lipase enzyme, Alkaline phosphatase, Alanine amino transferase (ALT) and aspartate amino transferase (AST) and total antioxidant capacity (TAC) using Biodiagnostic laboratory kits.

**Statistical analysis**

Analysis of variance (ANOVA) used to test the obtained data using the general linear modeling procedure (SAS, 2000). One-way analysis for data used for ruminal parameters and blood metabolites. Duncan multiple range tests (1955) was used for comparison of means.

## RESULTS

**Diets**

Chemical composition of feed ingredients and experimental diets are presented in Table (1). Diets of S, L, and S+L are comparable in their proximate analysis. On the other hand, full-fat soybean contained higher crude protein (CP) than linseed (37.64 vs 20.06%) while higher contents of crude fat and crude fiber were recorded in linseed (40.24 and 28.55% vs 16.28 and 10.23%, respectively); consequently elevated neutral detergent fiber (NDF) and acid detergent fiber (ADF) are present in linseed.

**Table (1):** Chemical composition and Ingredients of diets (% on DM basis).

| Item | Linseed | Concentrate | Full fat soya | Alfalfa hay | S | S+L | L |
|---|---|---|---|---|---|---|---|
| Dry matter | 95.93 | 90.76 | 93.58 | 92.66 | 91.68 | 91.79 | 91.91 |
| Organic matter | 96.00 | 92.50 | 92.80 | 87.00 | 89.88 | 90.07 | 90.26 |
| Crude protein | 20.06 | 16.73 | 37.64 | 16.28 | 20.37 | 19.38 | 18.4 |
| Crude fat | 40.24 | 3.30 | 16.28 | 2.19 | 4.61 | 5.91 | 7.21 |
| Crude fiber | 28.55 | 13.16 | 10.23 | 30.83 | 20.46 | 21.43 | 22.40 |
| Nitrogen Free Extract | 7.20 | 59.30 | 28.60 | 37.80 | 44.44 | 43.35 | 42.25 |
| Ash | 4.00 | 7.50 | 7.20 | 13.00 | 10.12 | 9.93 | 9.74 |
| NDF | 48.43 | 30.76 | 31.89 | 46.31 | 39.33 | 40.18 | 41.03 |
| ADF | 32.32 | 14.71 | 13.65 | 30.78 | 21.77 | 22.76 | 23.75 |
| ADL | 0.73 | 1.64 | 0.48 | 5.20 | 2.96 | 2.97 | 2.98 |

L: linseed supplemented diet S: soybean supplemented diet S+L: soybean + linseed supplemented diets. NDF: Neutral detergent fiber, ADF: acid detergent fiber, ADL: Acid detergent lignin

**Fatty acid composition of oil seeds**

Fatty acid (FA) profiles of the two oilseeds are completely different, as shown in Table (2). In the whole linseed, linolenic acid (C18:3n-3) was the most abundant fatty acid (53.4% of the total fatty acids) followed by oleic (C18:1n-9), linoleic (C18:2n-6), palmitic (C16:0), then stearic acid (C18:0) as (19.4, 14.73, 5.52 and 4.90%, respectively). On the other hand, linoleic acid is the most abundant fatty acid in soybean being 50.36% and the rest of FA which are oleic, palmitic, stearic and linolenic acids accounting for formed 23.6, 13.9, 5.72 and 4.53% of the total FA, respectively.

**Ruminal fermentation parameters**

The ruminal pH, TVFA's, and $NH_3$ –N of goats fed on diets containing different lipid sources are presented in Table (3). The pH was not affected ($P>0.05$) by different fat sources. However, whole linseed inclusion as 10 or 5% in (L or S+L) diets had increased ($P< 0.01$) the TVFA's and $NH_3$ –N ($P< 0.05$) in goat's rumen compared to those were being fed on soybean (S group).

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**Table (2):** Fatty acids content (% of total FA) of the experimental oilseeds.

| | Oilseeds | |
|---|---|---|
| Fatty acid | Linseed | soybean |
| C16:0, Palmitic acid | 5.52 | 13.90 |
| C18:0, Stearic acid | 4.90 | 5.72 |
| C18:1n-9, Oleic acid | 19.4 | 23.6 |
| C18:1n-7, Vaccinic acid | 0.74 | 1.30 |
| C18:2n- 6, Linoleic acid | 14.73 | 50.36 |
| C18:3n-4, | 0.20 | ND |
| C18:3n-3, Linolenic acid | 53.4 | 4.53 |
| C20:0, Arachidic acid | 0.18 | 0.40 |
| C20:1n-9, Gadolic acid | 0.13 | ND |
| C22:0, Behenic acid | 0.15 | 0.24 |
| Non identified fatty acids | 0.65% | ND |

ND: not detected FA: fatty acids

**Table (3):** Effect of feeding different lipid sources on rumen fermentation and microbial population of goats.

| | Experimental diets | | | | |
|---|---|---|---|---|---|
| Item | **S** | **S+L** | **L** | **SEM** | **P<** |
| **Fermentation** | | | | | |
| PH | 6.57 | 6.55 | 6.24 | 0.12 | NS |
| Total volatile fatty acids (TVFA's) | $5.38^{b}$ | $7.90^{a}$ | $8.63^{a}$ | 0.21 | 0.01 |
| Ammonia-nitrogen ($NH_3$-N) | $4.20^{b}$ | $5.95^{b}$ | $6.38^{a}$ | 0.22 | 0.05 |
| **Cellulolytic Bactria ( $\times 10^5$)** | | | | | |
| *Cellulomonase cellulasea* | $1.51^{c}$ | $1.79^{b}$ | $2.05^{a}$ | 0.04 | 0.01 |
| *Bacillus sp.* | $0.23^{b}$ | $0.27^{a}$ | $0.27^{a}$ | 0.01 | 0.01 |
| *Thermonospora fusca* | $0.59^{c}$ | $0.73^{b}$ | $0.86^{a}$ | 0.01 | 0.01 |
| *Acetobact xylinum* | $1.54^{c}$ | $1.76^{b}$ | $2.11^{a}$ | 0.04 | 0.01 |
| *Ruminococcus albus* | $2.26^{b}$ | $2.40^{b}$ | $2.81^{a}$ | 0.06 | 0.01 |
| *Clostridium cellulovorans* | $0.92^{b}$ | $0.96^{b}$ | $1.42^{a}$ | 0.03 | 0.01 |
| **Protozoa** | | | | | |
| *Holotrichs* ($\times 10^{2}$) | $9.50^{a}$ | $1.44^{b}$ | $3.63^{b}$ | 138.24 | 0.01 |
| *Entodiniomorphs* ($\times 10^{4}$) | $6.13^{b}$ | $8.36^{b}$ | $8.70^{a}$ | 6633.64 | 0.05 |
| Total count ( $\times 10^{4}$) | $6.22^{b}$ | $8.38^{a}$ | $8.74^{a}$ | 7666.71 | 0.05 |

SEM: standard error of means

a, b and c : values with different letters in the same row means statistically significant at (P<0.05), NS: non-significant

L: linseed supplemented diet S: soybean supplemented diet S+L: soybean + linseed supplemented diets,

**Rumen microbial populations**

The effect of feeding on different oilseeds as a source of linolenic acid (C18:3n-3) and linoleic acid (C18:2n-6) on rumen microbial populations of goats is presented in Table (3). In the present study, linseed supplementation (5 or 10%) increased (P< 0.01) the cellulolytic bacterial species compared to goats fed only on soybean. Concerning protozoa, the concentration of ciliates from the genus *Holotrichs* was higher (P<0.01) in goats fed on soybean compared to S+L or L groups whereas, the concentration of non – ciliated sp. *Entodiniomorphs, andthe total count of protozoa*, were decreased (P< 0.05) in both inclusion levels of soybean (S (10%) and S+L (5%) compared to L group.

**Ruminal and blood plasma fatty acid profiles**

The effect of using diets with different lipid sources on ruminal FA profiles of goats is presented in Table (4). In rumen, it is clear that the most abundant (P<0.01) FA for all experimental groups was stearic acid (C18:0), followed by palmitic acid (C16:0) (P<0.01) then oleic acid (C18:1n-9) (P<0.05). The main changes in rumen liquor FA content were induced by soybean supply, which was able to increase the liquor contents of FA as myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), oleic (C18:1n-9), linoleic acid (C18:2n-6), alpha octadecatetraenoic (C18:4n-3), UFA, MUFA and PUFA. Besides, traces of gamma linolenic acid (C18:3n-6) (0.33% and 0.22%), linolenic acid (C18:3n-3) (0.64% and 0.28%) (P<0.01) and eicosaenoic (C20:1n-11) (0.19 and 0.06) were detected in ruminal liquor of goats fed on S and S+L diets respectively, while these last three FA were not detected in rumen of goats fed on L diet. Vaccenic acid (C18:1n- 7) was detected only with a combination of both oilseeds.

Generally, in rumen liquor, the experimental diets increased (P<0.01) levels of saturated fatty acids (SFA) and decreased (P> 0.05) unsaturated ones.

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Furthermore, in blood plasma (Table 5) the main effect of soybean at both inclusion levels increased the supply of palmitic acid (C16:0), stearic acid (C18:0), linoleic acid and total SFA (P<0.01) compared to the other of treatments. On the other hand, the main linseed effect was to increase the supply of oleic acid (C18:1n-9) and UFA (P<0.01) compared to other treatments.

**Table (4):** Effect of feeding different lipid sources on rumen fatty acids profile of goats

| Fatty acid | S | S+L | L | SEM | P< |
|---|---|---|---|---|---|
| C10:0, Capric | 1.15[a] | 0.44[b] | ND | 0.07 | 0.01 |
| C12:0, Lauric | 0.86[a] | 0.22[b] | ND | 0.05 | 0.01 |
| C14:0, Myristic | 7.44[a] | 2.22[b] | 1.93[b] | 0.33 | 0.01 |
| C15:0, Pentadecanoic | 14.30[a] | 2.33[b] | 2.09[b] | 0.44 | 0.01 |
| C16:0, Palmitic | 25.36[a] | 16.13[b] | 17.11[b] | 0.59 | 0.01 |
| C18:0, Stearic | 34.40[b] | 67.82[a] | 67.70[a] | 1 | 0.01 |
| C18:1n-9, Oleic | 10.59[a] | 8.45[b] | 7.42[b] | 0.66 | 0.05 |
| C18:1n-7, Vaccinic | ND | 1.60[a] | ND | 0.22 | 0.01 |
| C18:2n-6, Linoleic | 3.25[a] | 0.22[c] | 1.64[b] | 0.31 | 0.01 |
| C18:2n-4, | 0.22[b] | ND | 1.26[a] | 0.13 | 0.01 |
| C18:3n-4, | 0.25[a] | ND | 0.05[b] | 0.04 | 0.01 |
| C18:3n-6, Gamma linolenic | 0.33[a] | 0.22[a] | ND | 0.04 | 0.01 |
| C18:3n-3, Linolenic | 0.64[a] | 0.28[b] | ND | 0.03 | 0.01 |
| C18:4n-3, Alpha octa decatetraenoic | 1.01[a] | ND | 0.08[b] | 0.04 | 0.01 |
| C20:0, Arachidic acid | ND | ND | 0.72[a] | 0.14 | 0.05 |
| C20:1n-11, Eicosaenoic | 0.19[a] | 0.06[b] | ND | 0.04 | 0.05 |
| UFA, Unsaturated fatty acids | 16.49 | 14.24 | 10.42 | 2.09 | NS |
| S F A, Saturated fatty acids | 83.51[b] | 89.16[a] | 89.54[a] | 0.58 | 0.01 |
| M U F A, Monounsaturated | 10.78[a] | 10.12[a] | 7.42[b] | 0.75 | 0.05 |
| P U F A, Polyunsaturated | 5.71[a] | 0.96[c] | 3.00[b] | 0.39 | 0.01 |

ND: not determined, L: linseed supplemented diet S: soybean supplemented diet S+L: soybean + linseed supplemented diets, SEM: standard error of means, a, b and c: values with different letters in the same row means statistically significant at(P<0.05). NS: nonsignificant

**Table (5):** Effect of feeding different lipid sources on blood plasma fatty acids profile of goats

| Fatty acids | S | S+L | L | SEM | P< |
|---|---|---|---|---|---|
| C10:0, Capric | ND | ND | 0.65[a] | 0 | 0.01 |
| C12:0, Lauric | ND | ND | 2.90[a] | 0.04 | 0.01 |
| C14:0, Myristic | 2.43[b] | 3.42[b] | 6.69[a] | 0.29 | 0.01 |
| C15:0, Pentadecanoic | 5.87[b] | 7.71[b] | 10.69[a] | 0.19 | 0.01 |
| C16:0, Palmitic | 32.63b | 36.31[a] | 20.90[c] | 0.89 | 0.01 |
| C16:1n-7, Palmitoleic | ND | 0 | 0.14 | 0.08 | NS |
| C17:0, Heptadecanoic | ND | 0 | 0.69 | 0.01 | 0.01 |
| C18:0, Stearic | 19.08[a] | 12.65[b] | 5.33[c] | 0.36 | 0.01 |
| C18:1n-9, Oleic | 20.44[c] | 28.59[b] | 36.09[a] | 0.25 | 0.01 |
| C18:2n-6, Linoleic | 15.35[a] | 7.97[c] | 13.58[b] | 0.39 | 0.01 |
| C18:3n-4, | 2.02[a] | 2.01[a] | 0.34[b] | 0.04 | 0.01 |
| C18:3n-3, Linolenic | 2.14a | ND | ND | 0.05 | 0.01 |
| C20:0, Arachidic | 0 | 1.3 | 0.7 | 0.75 | NS |
| C20:1n-11, Eicosaenoic | ND | ND | 1.21 | 0.04 | 0.01 |
| UFA, Unsaturated fatty acids | 40.37[b] | 38.58[b] | 51.37[a] | 0.56 | 0.01 |
| SFA, Saturated fatty acids | 60.03[a] | 61.41[a] | 48.56[b] | 0.56 | 0.01 |
| MUFA, Monounsaturated fatty acids | 20.44[c] | 28.59[b] | 37.44[a] | 0.3 | 0.01 |
| PUFA, Polyunsaturated fatty acids | 19.52[a] | 9.99[c] | 13.92[b] | 0.42 | 0.01 |

ND: not determined, L: linseed supplemented diet S: soybean supplemented diet S+L: soybean + linseed supplemented diets, SEM: standard error of means, a, b and c : values with different letters in the same row means statistically significant at( P<0.05) NS: nonsignificant

**Blood plasma metabolites**

Some blood metabolites of goats that fed on diets containing different lipid sources are presented in Table (6). Plasma total protein (TP) and albumin were affected (P<0.01) by fat sources. Therefore, goats fed on L diet had numerically higher values than goats fed on other diets.

Urea values were increased (P<0.05), however creatinine values were decreased (P<0.05) in goats fed on L diet. There was a measurable effect of the whole linseed on the blood lipids. Goats fed on L diets had lower values of total cholesterol (TC) (199.73 mg/dl; P<0.01), triglycerides (TG) (114.01 md/dl; P<0.05) , low density lipoproteins (LDL) (106.84 mg/dl; P<0.01) and higher

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values of high density lipoproteins (HDL) (59.02 mg/dl; P< 0.01). Moreover, goats fed on S+L or L diets had higher lipase activity (78.74 or 67.6 u/l, respectively; P<0.01). Supplementary fat in diets, either linseed or soybean + linseed increased total antioxidants capacity (TAC) (0.35 or 0.27 mM/l respectively, P<0.01). However, no significant (P>0.05) effect has been observed of fat supplements on serum globulin, albumin/globulin ratio, ammonia, total lipids (TL), alkaline phosphatase, aspartate amino transferase (AST) and alanine amino transferase (ALT).

**Table** (6). Effect of feeding different lipid sources on blood plasma metabolites of goats

| **Item** | **S** | **S+L** | **L** | **SEM** | **P<** |
|---|---|---|---|---|---|
| Total protein, g/dl | $8.36^{b}$ | $8.27^{b}$ | $9.08^{a}$ | 0.07 | 0.01 |
| Albumin, g/dl | $4.64^{b}$ | $4.55^{b}$ | $5.04^{a}$ | 0.08 | 0.01 |
| Globulin, g/dl | 3.72 | 3.71 | 4.04 | 0.12 | NS |
| Albumin/globulin | 1.25 | 1.23 | 1.24 | 0.06 | NS |
| Urea, mg/dl | $50.60^{b}$ | $51.91^{b}$ | $61.09^{a}$ | 2.21 | 0.05 |
| Creatinine, mg/dl | $1.06^{a}$ | $0.83^{b}$ | $0.74^{b}$ | 0.06 | 0.05 |
| Ammonia µmol/l | 89.27 | 82.82 | 97.02 | 13.75 | NS |
| Cholesterol, mg/dl | $226.08^{a}$ | $220.10^{a}$ | $199.73^{b}$ | 4.12 | 0.01 |
| Triglycerides, mg/dl | $135.90^{a}$ | $131.44^{a}$ | $114.01^{b}$ | 4.55 | 0.05 |
| High-density lipoprotein, mg/dl | $41.01^{b}$ | $39.84^{b}$ | $59.02^{a}$ | 2.31 | 0.01 |
| Low-density lipoprotein, mg/dl | $149.52^{a}$ | $128.06^{b}$ | $106.84^{c}$ | 3.59 | 0.01 |
| Lipase U/L | $40.11^{c}$ | $78.74^{a}$ | $67.60^{b}$ | 1.96 | 0.01 |
| Total lipids, mg/dl | 584.64 | 566.41 | 472.66 | 60.50 | NS |
| Alkaline phosphatase, U / L | 70.88 | 66.29 | 57.58 | 3.91 | NS |
| Aspartate aminotransferase, U /L | 38.52 | 38.85 | 41.81 | 2.89 | NS |
| Alanine aminotransferase, U /L | 15.86 | 15.19 | 15.98 | 0.37 | NS |
| Total antioxidant capacity, mM/L | $0.19^{c}$ | $0.27^{b}$ | $0.35^{a}$ | 0.02 | 0.01 |

L: linseed supplemented diet S: soybean supplemented diet S+L: soybean + linseed supplemented diets, SEM: standard error of means NS: nonsignificant

a, b and c: values with different letters in the same row means statistically significant at(P<0.05)

## DISCUSSION

### Ruminal fermentation

Linseed inclusion at both levels (L and S+L) as a source of PUFA in diets of goats resulted in increasing the concentration of total volatile fatty acids and ammonia – nitrogen, while pH is not influenced. Improving ruminal fermentation in this study might be due to the enhancement of cellulolytic bacteria and protozoa activity. Fiorentini et al. (2013) demonstrated that the reduction in protozoa concentration leads to a decrease in $NH_3$-N concentration in the rumen. In contrast, Kim et al. (2007) in sheep and Abuelfatah et al. (2016) in goats did not detect any influence of feeding linseed on ruminal pH or concentration of VFA. However, Czerkawski et al. (1975) found a decrease in the level of TVFA with 90 g linseed oil /d. On the other hand, elevated ruminal ammonia –nitrogen ($NH_3$-N) was in line with the previous studies of Yang et al. (2009) in dairy cows ; Brokaw et al. (2001) and Ueda et al. (2003) in steers used diets supplemented with soybean or linseed oils, respectively, while Broudiscou et al. (1994) observed a decrease in ruminal $NH_3$-N in sheep that received linseed oil as a supplement.

### Ruminal microbial population

Studies on the effect of lipids, especially PUFA on rumen microbes have a pronounced interest due to human health aspects. The increment of the population of six strains of cellulolytic bacteria, total protozoa, and *Entodiomorphussp*. With a decrease in *Holotrichs sp.* were recorded when linseed supplemented in diets at both levels (5 and 10%) than with soybean. Meteab et al. (2018) using similar animals and supplements have noticed a significant increase in digestibility of CF, NDF and ADF. This result may explain the increase of cellulolytic bacterial count in this study. Indeed, Loor et al. (2007) agreed with our study because they had found a decrease in the number of cellulolytic bacteria in the rumen with low fiber digestibility of the diet.

Furthermore, R. *albus*, in the current study, which is the most important cellulolytic bacteria, had increased with linseed inclusion. This finding is in agreement with Ivan et al. (2012) and Ebrahimi (2012) when cattle and goats respectively were fed PUFA diet. The influence of oilseeds inclusion on microbial population is controversial. Zhang et al. (2008); Liu et al. (2012) and Abuelfatah et al. (2016) found that population of *R. albus* was not affected negatively by linseed. Other authors (Ferlay et al., 1993; Yang et al., 2009) reported a negative effect of PUFA on cellulolytic bacteria. It is clear that the effect of PUFA on bacterial population is not the same. Besides this, ruminal pH has a great influence on the activity of cellulolytic bacteria which is sensitive to acidic conditions. In line with this concept, Martin and Jenkins (2002) suggested that ruminal pH has to be maintained above 6.0 and that agrees with the pH results in this study. Regarding the decrease of protozoa count with soybean inclusion, it is attributed to high content of linoleic acid (50.36%) which is toxic to ruminal protozoa. Previous researchers (Sutton et al., 1983; Hirstov et al., 2004; Yang et al., 2009) are consistent with us. Additionally, Hirstov et al. (2004) proved that different levels of oleic acid in vitro decreased protozoal count. Moreover, oleic acid is greater in soybean than in linseed ( 23.6% vs 19.4%), respectively. In this connection, Fiorentini et al. (2013) concluded that animals fed on protected fat diet had a larger protozoal number in rumen than those supplemented with soybean grain or soybean oil.

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**Rumen fatty acids**

The present results of ruminal FA (Table 4) show that the extent of biohydrogenation of both C18:2 and C18:3 was not affected either by linseed nor soybean. This agrees with Gonthier et al. (2004) and disagrees with Hussein et al. (1996), who detected a decrease in ruminal biohydrogenation of the same fatty acids with whole canola seed supplementation. This result is explained by the presence ofa seed coat, which reduced the accessibility of ruminal bacteria to PUFA of the seed. Increasing stearic acid (C18:0) and decreasing 18-carbon unsaturated fatty acids (UFA) indicated that a considerable amount of 18 – carbon UFA was subjected to biohydrogenation. This agreed with Harfoot and Hazlewood, 1997; Varadyova et al. 2007 and McKain et al. 2010) but not with others (Wu et al., 1991 and Pantoja et al., 1996). Increasing stearic acid percentage leads to a significant ($P<0.01$) increment of total SFA and a reduction of total USFA, especially with linseed supply. Nassu et al. (2011) who reported that biohydrogenation leads to extensive loss of UFA. This explanation is supported by the presence of a higher percentage of total PUFA (5.71%) in the rumen liquor of goats fed on soybean compared to others.

In the present study, absence of linolenic (C18:3n-3) in the rumen and blood plasma (Table 5) may be due to the high level of ruminal biohydrogenation for this acid, or to its high rate of incorporation into other tissue lipids. Also, Doreau and Ferlay (1994) reported ruminal biohydrogenation of linolenic acid with an average of 92% (range, 85% to 100%) and biohydrogenation of linoleic acid with 80% (range, 70% to 95%). Moreover, Chilliard et al. (2003) observed that goats presented higher ruminal passage rates.

**Blood plasma fatty acids and some metabolites**

In the current study, oilseeds inclusion affected plasma fatty acids profile where soybean supply (S and S+L) increased palmitic (C16:0) and oleic acids (C18:1n- 9) significantly ($P<0.01$) in plasma compared to linseed supply (L) due to their high contents in soybean. As reviewed by Almeida et al. (2019), levels of linoleic acid in blood plasma were enhanced by soybean, probably due to its high content from this FA. Concerning the higher concentration of UFA in blood plasma with linseed supply, it could be attributed to the high absorption of UFA in the intestine. Almeida et al. (2019) reported increases in absorption of UFA from the diets to milk thus reducing the available SFA for absorption and incorporation into other tissues.

Interestingly, Doreau and Ferlay, (1994) and Romo et al. (2000) supported our findings; they found that fatty acid digestibility increased with the degree of unsaturation. In addition to that, lipids absorbed in the small intestine transported to different tissues and organs of the animal where fatty acids are subjected to further metabolic modifications (Brzozowska and Oprzadek, 2016). Regarding other plasma parameters, Table (6) indicated that including linseed in feeding (L) increased the total protein (TP), albumin and urea, and these findings matched with improving crude protein digestibility when linseed is included in the diet. Meteab et al. (2018) suggested that oilseeds increased the secretion and activity of pancreatic enzymes, enhancing digestion and absorption of proteins in the small intestine hence improved protein utilization (Mir et al., 2000). Interestingly, there were measurable effects of linseed on blood lipid profile as reducing triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL), while increased high-density lipoprotein (HDL) as a result of enrichment of linseed with omega-3 fatty acids. Results are in harmony with Singh et al. (2011) who reported that Omega-3 fatty acids present in linseed help in reducing blood triglycerides, blood pressure and increasing blood HDL cholesterol (Cunnane et al., 1993; Li et al., 1999) and reducing lipoprotein (Bloedon et al., 2008). Additionally, Weill et al. (2002) observed a repeated decrease in the fat content of animal products as a result of the inhibition of lipogenesis by alpha-linolenic acid (Price et al., 2000). Moreover, linseed inclusion allowed higher lipase activity in both groups (L and S+L) and this finding consistent with our results of increased cellulolytic bacterial population because both bacteria and fungi are predominant microbial sources of lipase (Sangeetha et al. 2011).

Lipolytic bacteria is particularly specialized in the production of lipase, Moharrery, and Das (2001) showed that oil enhanced microbial production, and this could change the pattern of enzymes in the rumen of sheep.

Moreover, Ertugrul et al. (2007) observed secretion of intra- and extracellular lipase by *Bacillus sp.* to the external medium through different secretory systems, thus supporting the present results. On the other hand, liver enzymes, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase recorded normal levels. Results indicated that diets, including linseed, induced significant modifications of goat's plasma total antioxidant capacity (TAC), which helps to improve their immunity. These findings are consistent with prior results reported by Singh et al. (2011) and (Waghmare, 2013) that linseed favorably affects immunity besides, α- linolenic acids and lignans in linseed modulate the immune response and may play a beneficial role in the clinical management of autoimmune diseases (Javed, 1999).

## CONCLUSION

Therefore, it was concluded that for improving the nutritional quality of ruminant-derived products, linseed inclusion resulted in considerable variations in responses to the supplemented FA, indicating health-promoting effects. Therefore, fatty acids composition of animal's products resulted in more satisfying healthful properties for the consumer.

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