

ORIGINAL RESEARCH



EFFECT OF FLAX SEED SUPPLEMENTATION ON MILK PRODUCTION, CHEMICAL COMPOSITION, PHYSICAL ATTRIBUTES AND FATTY ACID PROFILE OF MILK IN LACTATING GOATS OF HALAYEB-SHALATEEN-ABU RAMAD TRIANGLE OF EGYPT

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ABSTRACT

Production and composition of goat milk, especially fatty acids profile are important factors that influence the economic upshot, nutritivity and health prospective of the product. There is an increasing thrust on research for evolving dairy products with reduced saturated fatty acids (SFAs) responsible for undesirable hyper-cholesterolemic effects culminating in hypertension, coronary heart disease, diabetes and cancer in humans. Earlier research works have revealed that milk fat composition can be altered through dietary interventions. This work focuses on the effect of dietary supplementation of flax seed (whole) to lactating desert goats of Egypt on milk production, chemical composition, physical properties and fatty acid profiles of milk, since there is dearth of desired information on this subject. Twenty lactating local desert goats were randomly distributed into two groups, viz., control group and experimental group (n=10) for the experiment. The lactating goats were let out for grazing on the natural pastures during day, and received supplemental feeding of concentrate mixtures @ 500 gm/ head (NRC, 2007) in equal amounts twice daily at 06.00 h and at 16.00 h. The concentrate mixture in the diet of the experimental goats was replaced with 14% whole flax seed (*Linum usitatissimum* L). Goats were milked by hand twice daily for 12 weeks for estimation of average daily milk yield, chemical composition (Fat%, Protein%, Lactose%, Ash%, SNF%, TS%), physical attributes (Sp. Gr., Freezing point, pH, Conductivity) in different weeks (1, 4, 8, and 12), along with fatty acid profile as percentage of the total fatty acids in milk. Results indicated that the average daily milk yield (g/animal/day⁻¹) of FS supplemented goats in week 1 (569.0±48.8) was higher ($P \leq 0.05$) than the control by a margin of 48.5%. There were no differences ($P \geq 0.05$) in milk yields as well as energy corrected milk yields between the groups in other weeks and over the experimental period. The FS supplemented group showed higher ($P \leq 0.05$) proportions over the control in respect of protein (3.77±0.18 vs. 3.43±0.22), lactose (4.56±0.25 vs. 4.32±0.15), SNF (9.16±0.28 vs. 8.56±0.28) and TS (12.59±0.55 vs. 12.01±0.33), but lower freezing point (-0.57±0.06 vs. -0.53±0.06, Celsius). FS supplementation diminished ($P \leq 0.05$) Σ saturated fatty acids (64.12±0.23) and enhanced ($P \leq 0.05$) Σ unsaturated fatty acids (3.38±0.036) in milk over the control. In addition, there was increase ($P \leq 0.05$) in Omega-6/Omega-3 FAs (2.39±0.001) and reduction ($P \leq 0.05$) of total conjugated linoleic acid (1.79±0.013) in the milk of goats fed on FS supplemented diet. It may be concluded from this study that dietary supplementation of flax seeds to lactating goats would enhance milk production and its nutritivity, beneficial for boosting growth and immunity of neonatal kids and help in promotion of stink free and health bolstering dairy products through moderation of harmful hyper-cholesterolemic effects of high level of LDL cholesterol in milk.

KEYWORDS

Chemical composition, Desert goat, Egypt, Fatty acids, Flaxseed, Milk yield, Physical properties

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INTRODUCTION

Goat is of significant importance for survival and sustenance of nomadic Bedouins living in middle-eastern deserts because of the superior ability of goats to adapt to difficult environmental conditions. Milk products from goats are important for proper human nutrition due to their unique composition and high digestibility, especially for people with food allergies, and it is more suitable for those living in desert areas where cow's milk is not easy to produce (Ceballos et al., 2009). The goat population in Egypt between the period of 2000 and 2009 has been estimated as 3.43-4.55 million (Abbas et al., 2017).

In spite of production of substantial amount of goat milk in Egypt (125 Tons/ year- 2014) as per the report of Hanan (2016), and its numerous virtues, consumers do not like it due to its offensive goaty (musky) odour, which is in fact imparted by the bucks during breeding season, in case the bucks are allowed to stay with the does. Otherwise, it is indistinguishable in taste and odour from cow milk (Okeyo, 1997). The consumer acceptability score of goat milk is 3.75 like that of cow milk (3.75), which is marginally below than the very much liking score of 4 (Wanjekeche et al., 2016).

Fat content and fatty acids (FAs) profile of goat milk reflect its nutritivity and plays an important role in goat cheese making technology. A characteristic goatish (musky) flavour of goat milk and milk products differentiating it from cow milk and milk products is also attributed to its fatty acids (FAs) profile, as goat milk contains about twice the amount of volatile branched-chain fatty acids (C_{4:0} through C_{10:0}) compared to cow milk fat (Ibrahim and Soryal, 2014).

Goat milk production and composition, especially (FAs profile and flavour) is a genetic characteristic, but it can be altered by the type of dietary fat and feed offered as well as the genetic diversity of the animals, besides geo-climatic conditions of the breeding tract (Nudda et al., 2006).

There is an increasing research interest towards manufacture of dairy products with relegated saturated fatty acids (SFAs), especially myristic acid (C_{14:0}) and palmitic acid (C_{16:0}) have demonstrated undesirable hyper-cholesterolemic effects, responsible for hypertension and cardiovascular & coronary heart diseases (Wright et al., 1998).

Polyunsaturated fatty acids (PUFAs) such as conjugated linoleic acid (CLA), i.e., Rumenic acid, C_{18:2} cis-9 trans-11 and omega-3 FAs have antithrombotic and anti-inflammatory effects. CLA and isomers of omega-3 FAs are not synthesized by humans although considered as the most important intermediary product of ruminal bio-hydrogenation of dietary lipids. Furthermore, similar to CLA, isomers of omega-3 FAs are now well known as anti-atherosclerotic, antioxidant, antibacteriogenic and immunomodulator (Waghmare, 2013).

Consumers are now increasingly aware of the potential health benefits of CLA and omega-3 isomers in reducing the risk factor of coronary heart disease. Therefore, potential exists to reduce the concentrations of C_{12:0} to C_{16:0} and increase beneficial Polyunsaturated fatty acids (PUFAs) in milk for higher consumer acceptability due to the presence of healthy milk fats (Joyce et al., 2009).

It is widely recognized that milk fat composition can be substantially altered through adoption of proper feeding strategies. The most effective strategies include supplementation of animal feed with different oilseeds or lipid supplementation, rich in bio-hydrogenation of unsaturated fatty acids (UFA), e.g., soybean and flaxseed oils, which can be used to modify the undesirable fatty acid profile for human health benefits (Chilliard et al., 2003; Morsy et al., 2015).

The interest in using flaxseed in dairy goat diets has increased over the past few years, and among all oilseeds, flaxseed is preferred as it has the highest proportion of n-3 α -linolenic acid constituting 54.4 ± 4.7 g/100 g of total fatty acids (Kholif et al., 2016) so that the addition of flaxseed to the diet of dairy goat could improve PUFA and conjugated FAs contents which is synthesized in the rumen through the bio-hydrogenation of linoleic acid by effects on the transcription of the major lipogenic mRNA abundances of genes involved in the *de novo* synthesis genes and also $\Delta 9$ -desaturation in the mammary gland tissue (Bernard et al., 2008; Shingfield et al., 2010).

Milk fat composition can be changed dramatically through feeding strategies and the most effective strategies involved is supplementing animal feed with different oilseeds or lipid supplementation rich in bio-hydrogenation unsaturated fatty acids (UFA), e.g., soybean and flaxseed oils; which can be used to modify the undesirable FA profile for human consumption (Morsy et al., 2015; Kholif, et al., 2016).

Several studies have been published on the effect of supplementing flaxseed to dairy goat's diet in different forms, e.g., flaxseed oils, flaxseed cakes, and crushed as well as whole flaxseeds (Chilliard and Ferlay, 2004; Nudda et al., 2006; Marín et al., 2012; Kholif et al., 2016; Kholif et al., 2018), in which the authors have stated that feeding flaxseed to the dairy goat enhances milk yield and makes a favorable and significant increase in milk fat proportions of UFA, especially Omega-3 FAs, C_{18:2}, cis-9 and trans-11CLA, along with significant drop (about 2-fold) in short-chain FAs (SCFA) and medium-chain fatty acids (MCFA) especially C_{12:0} to C_{16:0} as well as n-6/n-3 ratio in milk fat which can add extra values to dairy goat products.

However, to the best of our knowledge, there are no studies reporting information about supplementing the diet of local desert goats with whole flaxseed under extensive and range grazing conditions.

Therefore, the current study was conducted to determine the effect of supplementation of whole flaxseed to the diet of local desert goats in Ras-Hederba valley of Halayeb-Shalateen-Abu ramad triangle of Egypt on milk production, milk composition, physical properties, and fatty acids profile of milk. Furthermore, this research can lead to the development of natural, consumer-friendly strategies and milk processing systems to produce Omega-3 Polyunsaturated fatty acid (PUFA) and octadecadienoic acid (CLA) enriched goat milk products with desirable nutritional properties.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments: The experiment was carried out in the farm of Shalateen research station-desert research center, a district in Ras-Hederba valley located in Halaieb-Shalateen-AbouRamad triangle, Red Sea Governorate, Egypt.

Twenty lactating healthy local desert goats in their first and second lactation, with 27.9 ± 2.1 kg of average body weight, and aged between 2-3 years were randomly divided by single-factor randomized design into two equal groups ($n=10$). Both the treatment groups were allowed grazing in natural pasture, mostly consisting of *Panicumturgidum* (one of the most drought resistant plants in this desert region) and received supplementary feeding of concentrate mixture 500 g/head/day on return from pasture at the recommended level of the milk goats feeding standards (NRC, 2007).

The experimental diets were formulated to cover their nutritional requirements. The concentrate supplement mixture consisted of 31% wheat bran, 26% cottonseed meal, 25% barley grain, 4% molasses, 2.5% limestone, 10% rice bran, 1% common salt, and 0.5% minerals and vitamins premix. The average of chemical composition and nutritive values of the concentrate mixture were, 94.5% Dry matter (DM g/kg wet material), 91.0% Organic matter (OM), 17.38% Crude protein (CP), 2.26% Ether extract (EE), 47.5% Neutral detergent fiber (NDF), 14.5% Acid detergent fiber (ADF), 5.62% Acid detergent lignin (ADL) and 1.08% Acid insoluble ash (AIA).

The first experimental group served as the control, while the feed of second experimental group was enriched by partial replacement of concentrate supplement mixture by adding 14% whole flaxseed (*Linum usitatissimum L*). The flaxseed enriched concentrate mixture contained 92.5% DM, 91.6% OM, 16.9% CP, 6.1% EE, 42.2% NDF, 13.2% ADF, 5.44% ADL and 1.5% AIA.

Milking operation and Sample collection: The data were recorded for two years (2017 & 2018). Diets (Control and Flaxseed enriched) were offered in equal amounts twice daily at 06.00 and 16.00 h. Goats were milked twice daily at 07.00 h and 17.00 h.. The experimental period was composed of 12 weeks. All the kids were separated one day before milking. After milking, the kids were allowed to suckle their mothers for one hour and separated again before each milking. The goats were milked by hand and the milk yield was calculated and recorded for each goat on monthly basis. Milk samples from both morning and afternoon milking were collected once every 4 weeks, and were mixed in equal proportion (1:1 v/v) for chemical analysis.

Milk samples were divided into two groups for preservation. The first batch was preserved with a solution of (3.3 μ l/ml) of 2-Bromo-2-Nitro-1,3-propanediol Tablet (D&F Control System, San Ramon, CA, USA) as an antimicrobial (Sigma-Aldrich) at -20 °C for chemical composition determination and the second batch was kept without preservative for analysis of milk fat and fatty acid (FA) profile.

Physico-Chemical Properties: Physico-Chemical composition of goat milk in terms of protein, fat, lactose, ash and total solids contents (%), and physical properties such as specific gravity, freezing point (°C) and conductivity (mS/cm) at 18°C were analyzed in triplicate for each sample by using Lactoscan milk analyzer (Model Lactoscan SL, Milkotronic Ltd, Bulgaria) calibrated for goat milk. The pHs of the samples was determined using a digital pH meter (model pH 211, Hanna Instruments).

Chemical composition of Dietary components: The chemical composition of feed ingredients and flaxseed were analyzed for Dry Matter ([AOAC International, 2006](#); Method 930.15), Ash ([AOAC International, 2000](#); Method 942.05), Crude protein (CP) by Kjeldahl method for nitrogen ([AOAC International, 2000](#); Method 990.03), Ether extract (EE) content by using petroleum ether in a Soxtec device ([AOAC International, 1990](#); Method 7.060), and Neutral Detergent Fibre (NDF) & Acid Detergent Fibre ([Van Soest et al.,1991](#); [AOAC International, 2000](#); Method 973.18). Organic matter (OM) was calculated with the following formula: $OM = 100 - \text{Crude ash} (\%)$.

Fatty acids profile: One hundred (100) ml from each of four bulk milk samples of the experimental and the control groups (n=10) were collected every 30 days, and were centrifuged 2,500×g for 30 min at 8°C for removal of the fat cake. Milk fat was extracted according to the procedure of International Dairy Federation ([ISO-IDF, 2001](#)) using a volume of 18 ml of hexane and isopropanol (3:2, v/v) /g of fat cake. After vortexing, 12 ml of sodium sulfate solution (NaSO₄ 6.7% in distilled H₂O) was added to 300 mg of fat cake and allowed to settle for 10 min. The hexane layer was transferred to a tube containing 1 g of NaSO₄, and after 30 min, the hexane layer was removed and dried under nitrogen condition and stored at –20°C until methylation.

Fatty acid methyl esters (FAMES) were prepared by direct methylation by base-catalysed methanolysis of the glycerides (KOH in methanol) as per the procedure laid down by International Dairy Federation ([ISO-IDF, 2002](#)). Approximately 20 mg of lipid extract was added to 0.2 ml of 2N methanolic sodium hydroxide into a 10 ml reaction tube, and then heated at 100°C for 5 min with occasional shaking, followed by cooling to room temperature. After methylation was completed, the solution was centrifuged at 2,500 ×g for 10 min at 5°C and the top hexane layer was removed to use for fatty acid methyl esters (FAME) quantification by gas chromatograph (GC). Gas chromatography (Ultra trace GC DSQ Thermo scientific, USA) with TR-FAME capillary column (30 m length × 0.22 mm ID × 0.25-micron thickness, Thermo scientific USA) was used for analyzing FAMES under the following operating conditions:

The column inlet pressure was set at 53.5 kpa that caused a helium (carrier) gas flow-rate of 1.0 ml/min (He 99.9%) and hydrogen 40 m/min., flame ionization detector (FID) at 240°C, the injection volume of 1µl and the split ratio was (100:1). The initial column temperature was set at 70°C, increased to 200°C at 25°C/min, and finally increased to 250°C for 10 min and kept for 15 min. Individual FAME peaks were identified using pure methyl ester standards (Sigma Chemical Co., St. Louis, MO). Scan mode was full scanning mass 50-650 and the FAs amount was expressed as percent of total FAs of relative area.

Calculations and statistical analyses: Energy corrected milk (ECM) was determined following the equation of [Flores et al. \(2009\)](#) as follows:

$$ECM = (0.327 \times \text{milk yield}) + (12.86 \times \text{fat yield}) + (7.65 \times \text{protein yield}).$$

Milk atherogenicity index (AI) was calculated according to [Ulbricht and Southgate \(1991\)](#) as: $(C_{12:0} + 4 \times C_{14:0} + C_{16:0}) / \sum (\text{MUFA} + \text{PUFA})$

Where, MUFA = Monounsaturated fatty acids and PUFA = polyunsaturated fatty acids.

The data were subjected to statistical analysis (ANOVA) using SPSS statistical software version 20.0 for Windows. The means which showed significant differences at the probability level of ($P \leq 0.05$) were compared with each other by using LSD.

RESULTS AND DISCUSSION

Milk yield: The effect of flaxseed supplementation to dairy goat's diets on milk production is shown in Table-1. Results indicated that the average of daily milk yield of the goats supplemented with flax seed (14%) in the first week (569.0 ± 48.8 g) was significantly ($P \leq 0.05$) higher than the control goats (383.3 ± 31.6 g). However, the milk yield of the goats on FS supplemented diet did not show significant improvement ($P \geq 0.05$) over the control in subsequent weeks (4 wk & 8 wk). Moreover, there was significant ($P \leq 0.05$) decline in 12 wk milk yield of FS supplemented goats (330.0 ± 64.9 g) over the control (448.8 ± 38.9 g). The difference between the overall mean milk yield of FS supplemented goats (444.3 ± 46.0 g) and the control goats (430.5 ± 84.8 g) was non-significant ($P \geq 0.05$). Besides, the difference between the overall mean of energy corrected milk yield (Kcal/kg) of FS supplemented goats (72.9 ± 0.43) and the control goats (71.4 ± 0.42) was also non-significant ($P \geq 0.05$).

Higher milk production of milk in FS supplemented goats by 48.5% in our study in the first week of lactation over the control indicates positive action of FS in stimulating kid's growth, as kids need more nutrition and maternal immunity from milk during the early age for proper growth and resistance against diseases. [Petit et al. \(2004\)](#) have stated that supplementation of diet of dairy cows after calving with whole flax seed @ 9.7% of dietary dry matter increased milk production by 29%. [Akraim et al. \(2007\)](#) have observed that interaction between stage of lactation and the amount of dietary lipid supplementation had increased milk production of dairy cows in the early stage of lactation.

[Kholif et al. \(2014\)](#) have observed that increased milk yield with flax seed treatments may be due to increase in acetic acid along with decrease in butyric acid in the rumen of goats. [Abuelfatah et al. \(2016\)](#) have stated that inclusion of whole linseed in the diet of goats increase the concentration of polyunsaturated fatty acids (PUFA) in the rumen, and decrease the population of cellulolytic bacteria (*F. succinogenes* & *R. flavefaciens*), methanogens and protozoa in rumen liquid of goats. [Kholif et al. \(2016\)](#) have reported increase in milk yield and decrease in milk-fat contents ($P \leq 0.05$) with supplementation of 20 ml/day of flaxseed oil or Soyabean oil.

Our results are in agreement with [Kholif et al. \(2018\)](#), who reported that goats fed with flaxseed supplementation at different levels (0 to 15% of DM) showed higher milk production. They surmised that increased milk production in goats fed with flaxseed supplementation could be due to improved ruminal fermentation kinetics which might have positively altered ruminal digestibility. [Petit et al. \(2004\)](#) had observed that feeding on oil plant sources increases the energy density of the diet and energy intake, causing greater fat mobilization leading to increase in milk yield.

On the other hand, energy corrected milk (ECM) is milk that is corrected for fat content (fat-protein correction) of milk. Therefore, these changes in yield of protein and lactose, as our results showed were in effect indicated the milk energy output.

Table 1: Daily milk yield (g/animal/day¹) and Energy corrected milk (Kcal/kg) of goats fed on flaxseed supplemented diet (Mean ± SD)

Dietary Treatment LAC Period (Week)	Daily milk yield	Minimum daily milk yield	Maximum daily milk yield	Energy corrected milk
Control Diet				
Week 1	383.3 ^b ±31.6	232	650	70.51 ^a ±0.21
Week 4	399.3 ^b ±20.8	240	700	71.85 ^a ±0.33
Week 8	471.4 ^{ab} ±82.0	210	705	69.42 ^a ±0.21
Week 12	448.8 ^{ab} ±38.9	330	595	70.34 ^a ±0.25
Diet supplemented with 14% Flaxseed (FS)				
Week 1	569.0 ^a ±48.8	390	1080	72.82 ^a ±0.34
Week 4	485.6 ^{ab} ±66.3	280	850	72.83 ^a ±0.34
Week 8	384.4 ^b ±80.3	230	620	72.63 ^a ±0.61
Week 12	330.0 ^c ±64.9	200	470	73.89 ^a ±0.27
Main Effect				
Control Diet	430.5 ^a ±84.8	210	705	71.4 ^a ±0.42
FS suppl. Diet	444.3 ^a ±46.0	200	1080	72.9 ^a ±0.43

^{a,b} Means in the same column with different superscripts differ significantly ($P \leq 0.05$)

Our results indicated that ECM did not differ among treatments during lactation periods and showed that flaxseed supplementation increased the ECM (72.9±0.43 Kcal/kg), but it did not differ ($P \geq 0.05$) from the ECM of control group (71.4±0.42 Kcal/kg). Our results are in agreement with the results of Flores et al. (2009) and Marín et al. (2012). They too found that supplementing flaxseed to the dairy goat's diet increased the energy corrected milk compared to a control diet.

From the breeder point of view, these results are encouraging for enhancing the milk yield of local goats reared under hot climate (Ranging 37-47 °C) in Halayeb-Shalateen-Abu Ramad triangle of Egypt. Using diets supplemented with flaxseed is considered a way to overcome the problem of the milk shortage in this area.

Although, the data on milk yield of local breed goats were lower than that reported for Saanen and Nubian goats reared under intensive system (Serradilla, 2001; Haenlein, 2007), the milk production of local goats of Egypt under hot climate and conventional management system is steady. Therefore they are greatly appreciated by local breeders.

Chemical Composition of Milk: The protein (3.77 ± 0.18 %), lactose (4.56 ± 0.25 %), SNF (9.16 ± 0.28 %) and TS (12.59 ± 0.55 %) contents in the milk of goats fed on flaxseed supplemented diet were significantly ($P \leq 0.05$) higher than the protein (3.43 ± 0.22 %), lactose (4.32 ± 0.15 %), SNF ($8.56 \pm 0.28\%$) and TS (12.01 ± 0.33 %) contents in the milk of control goats (Table 2). The difference between the two groups in respect of fat content and ash content were non-significant ($P \geq 0.05$). .

Our findings are in agreement with the findings of Petit and Benchaar (2007) and Kholif et al. (2014) who have found higher amount of protein in goat and cow's milk fed with linseed oil or whole flaxseed, which is attributed to improvement in ruminal microbial protein synthesis.

Table 2: Chemical composition (%) of milk of goats fed on flaxseed supplemented diet (Mean \pm SD)

Diet Lact.Period	Fat	Protein	Lactose	Ash	SNF	Total Solid
Control Diet						
Week 1	3.43 ^a \pm 0.26	3.45 ^b \pm 0.32	4.31 ^{ab} \pm 0.28	0.82 ^a \pm 0.05	8.58 ^b \pm 0.57	12.02 ^b \pm 0.63
Week 4	3.54 ^a \pm 0.18	3.44 ^b \pm 0.23	4.31 ^{ab} \pm 0.12	0.80 ^a \pm 0.07	8.55 ^b \pm 0.17	12.08 ^{ab} \pm 0.23
Week 8	3.38 ^a \pm 0.32	3.39 ^b \pm 0.20	4.38 ^{ab} \pm 0.17	0.81 ^a \pm 0.07	8.57 ^b \pm 0.29	11.96 ^b \pm 0.31
Week 12	3.41 ^a \pm 0.28	3.46 ^b \pm 0.18	4.27 ^b \pm 0.05	0.82 ^a \pm 0.01	8.56 ^b \pm 0.17	11.97 ^b \pm 0.26
Diet supplemented with 14% Flaxseed (FS)						
Week 1	3.43 ^a \pm 0.37	3.75 ^a \pm 0.17	4.51 ^{ab} \pm 0.17	0.85 ^a \pm 0.05	9.11 ^a \pm 0.23	12.54 ^{ab} \pm 0.41
Week 4	3.39 ^a \pm 0.15	3.82 ^a \pm 0.16	4.59 ^a \pm 0.25	0.83 ^a \pm 0.03	9.24 ^a \pm 0.24	12.63 ^a \pm 0.32
Week 8	3.44 ^a \pm 0.62	3.71 ^a \pm 0.21	4.56 ^{ab} \pm 0.31	0.81 ^a \pm 0.05	9.09 ^a \pm 0.34	12.53 ^{ab} \pm 0.78
Week 12	3.49 ^a \pm 0.63	3.79 ^a \pm 0.15	4.55 ^{ab} \pm 0.26	0.83 ^a \pm 0.05	9.18 ^a \pm 0.34	12.67 ^a \pm 0.60
Main Effect						
Control	3.45 ^a \pm 0.26	3.43 ^b \pm 0.22	4.32 ^b \pm 0.15	0.81 ^a \pm 0.06	8.56 ^b \pm 0.28	12.01 ^b \pm 0.33
Suppl. Diet	3.43 ^a \pm 0.45	3.77 ^a \pm 0.18	4.56 ^a \pm 0.25	0.83 ^a \pm 0.05	9.16 ^a \pm 0.28	12.59 ^a \pm 0.55

^{ab}Means in the same column with different superscripts differ significantly ($P \leq 0.05$)

Our result is comparable with that of [Caroprese et al. \(2010\)](#) who have suggested that cows fed with whole flaxseed have a positive impact on milk protein synthesis and the protein yield, which was increased by 7% in cow's milk fed with flaxseed compared to milk from control cows. They attributed it to decrease in the ruminal protein degradability of the whole flaxseed supplemented diet, due to increased flow of nitrogen (N) to the duodenum because of its greater bypass protein content. As a consequence, the availability of N for protein synthesis in the mammary gland was increased.

Our results also indicated that flaxseed maintained fat percentage of milk un-changed throughout the trial. It was 3.43 ± 0.45 % vs. 3.45 ± 0.26 % for flaxseed supplemented group and the control group, respectively. These results are in agreement with [Secchiari et al. \(2003\)](#) and [Petit and Gagnon, \(2009\)](#) who have reported consistence in fat percentages of dairy cow milk throughout the experiment, fed on flaxseed supplemented diet. They attributed it to increased fat mobilization. In addition, they observed that dairy cows fed on flaxseed supplemented diet caused improvement in energy utilization for milk synthesis, which induced depression of milk fat during high ambient temperature in summer months.

Our results further indicated that milk lactose, SNF and total solids (TS) concentrations significantly ($P \leq 0.05$) increased in goats fed on whole flaxseed supplemented diet. Similar results were also reported by [Rigout et al. \(2003\)](#) and [Kholif et al. \(2018\)](#) who noticed significant ($P \leq 0.001$) increase in the concentrations of total solids and lactose contents in the milk of Nubian goats fed with flaxseed and flaxseed oil in the diet compared to the control goats. They also explained that higher lactose content in milk may be related to the increased propionate production which is the main precursor for gluconeogenesis and lactose synthesis and has a favorable effect on the milk lactose content.

Physical properties of milk: Few studies were done for estimating the effect of feeding flaxseed supplemented diet on the physical properties of goat's milk. So, some physical properties were focused in this study (Table 3).

Results of our study showed lower freezing point (-0.57 ± 0.06 °C) and lower conductivity (4.97 ± 0.31 mS/cm at 18°C) of milk in the goats fed on FS supplemented diet compared to the control ($P \leq 0.05$). These values are within the range reported by [Abbas et al. \(2014\)](#) and [Park et al. \(2007\)](#). The variation in the FP values of goat milk between the two dietary groups is caused by a higher content of non-fat solids in the flaxseed group ([Janštová et al., 2007](#)). Our results also indicated non-significant differences ($P \geq 0.05$) in average specific gravity between the two dietary groups. Similar results were reported for goat milk by [Al-kanhal \(1993\)](#).

On the contrary, the average of conductivity (mS/cm at 18 °C) of milk in flaxseed supplemented goats (4.97 ± 0.31) was less ($P \geq 0.05$) than in the control (5.13 ± 0.30). These results are in line with earlier studies ([Leitner et al., 2004](#); [Diaz et al., 2011](#)). They attributed increase in conductivity (>60%) to a decrease in lactose concentration and increase in inorganic salts, such as chlorides, sodium, and potassium in milk, since higher transfer of these minerals from blood to milk increases the conductivity.

[Hamann and Zecconi \(1998\)](#) noticed that variation is also caused due to variations in feeding and management systems in the farm, which are considered as the main factors responsible for variation in milk composition and thus, in milk conductivity too.

Table (3): Physical properties of milk of goats fed on flaxseed supplemented diet (Mean \pm SD)

Diet Lact.Period	Specific Gravity	Freezing point ($^{\circ}$ C)	pH	Conductivity (mS/cm) at 18 $^{\circ}$ C
Control Diet				
Week 1	1.033 ^a \pm 0.002	-0.54 ^{ab} \pm 0.05	6.61 ^b \pm 0.13	5.19 ^a \pm 0.35
Week 4	1.031 ^a \pm 0.003	-0.51 ^b \pm 0.06	6.64 ^{ab} \pm 0.15	5.20 ^a \pm 0.36
Week 8	1.031 ^a \pm 0.003	-0.53 ^{ab} \pm 0.06	6.64 ^{ab} \pm 0.08	5.10 ^a \pm 0.27
Week 12	1.032 ^a \pm 0.004	-0.54 ^a \pm 0.05	6.65 ^a \pm 0.15	5.04 ^a \pm 0.28
Diet supplemented with 14% Flaxseed (FS)				
Week 1	1.030 ^a \pm 0.003	-0.57 ^a \pm 0.08	6.71 ^a \pm 0.08	4.86 ^b \pm 0.33
Week 4	1.032 ^a \pm 0.003	-0.56 ^a \pm 0.06	6.67 ^a \pm 0.12	4.99 ^{ab} \pm 0.37
Week 8	1.032 ^a \pm 0.002	-0.58 ^a \pm 0.05	6.68 ^a \pm 0.09	4.90 ^{ab} \pm 0.22
Week 12	1.032 ^a \pm 0.002	-0.57 ^a \pm 0.04	6.68 ^a \pm 0.07	5.20 ^a \pm 0.26
Main Effect				
Control	1.032 ^a \pm 0.003	-0.53 ^b \pm 0.06	6.65 ^a \pm 0.12	5.13 ^a \pm 0.30
Suppl. Diet	1.032 ^a \pm 0.002	-0.57 ^a \pm 0.06	6.68 ^a \pm 0.10	4.97 ^a \pm 0.31

^{ab}, Means in the same column with different superscripts letters differ significantly (P \leq 0.05)

Milk Fatty acids composition: The results of milk fatty acid composition of goat's milk fed on whole flaxseed diet and on control diet are summarized in Table 4.

Milk fat contains approximately 400 different fatty acids, which make it the most complex of all natural fats. The milk fatty acids are derived almost equally from two sources, the feed and the microbial activity in the rumen of the cow and the lipids in bovine milk are mainly present in globules as an oil-in-water emulsion.

It is reported (Mansson, 2008) that almost 70% of the fat in bovine milk in Sweden contains saturated fatty acids, out of which around 11% comprises short-chain fatty acids, and almost half of which is butyric acid. Approximately 25% of the fatty acids in milk are mono-unsaturated fatty acids and 2.3% are poly-unsaturated fatty acids with omega-6/omega-3 ratio around 2.3. Approximately 2.7% are trans-fatty acids. Our results showed that main fatty acids (C_{8:0}, C_{10:0}, C_{12:0}, C_{14:0}, C_{15:0}, C_{16:0}, C_{17:0} and C_{18:0}) were significantly (P \leq 0.05) reduced in the milk of goats on FS supplemented diet compared to the control.

Table 4: Main fatty acids as % of total fatty acids in milk of goats fed on flaxseed supplemented diet (Mean \pm SD)

Fatty acid	Name	Control	FS Suppl.	P
C _{4:0}	Butyric acid	1.65 ^a \pm 0.01	1.63 ^a \pm 0.08	NS
C _{6:0}	Caproic acid	1.17 ^a \pm 0.01	1.16 ^a \pm 0.01	NS
C _{8:0}	Caprylic acid	2.53 ^a \pm 0.02	2.44 ^b \pm 0.03	***
C _{10:0}	Capric acid	6.95 ^a \pm 0.07	5.11 ^b \pm 0.13	***
C _{11:0}	Undecanoic acid	0.16 ^a \pm 0.01	0.17 ^b \pm 0.01	***
C _{12:0}	Lauric acid	5.79 ^a \pm 0.01	4.49 ^b \pm 0.01	*
C _{14:0}	Myristic acid	9.65 ^a \pm 0.01	8.73 ^b \pm 0.08	*
C _{15:0}	Pentadecanoic acid	1.52 ^a \pm 0.01	1.34 ^b \pm 0.03	*
C _{16:0}	Palmitic acid	26.60 ^a \pm 0.14	24.76 ^b \pm 0.27	*
C _{17:0}	Margaric acid	1.39 ^a \pm 0.01	1.25 ^b \pm 0.01	**
C _{18:0}	Stearic acid	13.41 ^a \pm 0.01	11.92 ^b \pm 0.07	*
C _{20:0}	Arachidic acid	0.68 ^a \pm 0.01	0.65 ^b \pm 0.01	***
C _{22:0}	Behenic acid	0.49 ^a \pm 0.01	0.48 ^b \pm 0.01	***
C _{14:1 n5}	Myristoleic acid	0.121 ^b \pm 0.001	0.163 ^a \pm 0.004	*
C _{16:1}	Palmitoleic acid	0.765 ^b \pm 0.021	0.950 ^a \pm 0.042	*
C _{18:1 trans-9}	Elaidic acid	1.265 ^b \pm 0.021	1.470 ^a \pm 0.028	*
C _{18:1 cis-9}	Oleic acid	17.75 ^b \pm 0.071	20.63 ^a \pm 0.262	*
C _{18:1 trans-11}	Vaccenic acid	0.740 ^b \pm 0.057	1.290 ^a \pm 0.028	*
C _{18:1 trans-7}	IsoVaccenic acid	0.785 ^b \pm 0.007	1.180 ^a \pm 0.085	*
C _{20:1 n5 cis-11}	Eicosenoic	0.004 ^a \pm 0.0001	0.006 ^b \pm 0.0001	***
C _{20:1 n7 cis 9}	Eicosaenoic acid	0.003 ^a \pm 0.0001	0.003 ^a \pm 0.0001	NS
C _{22:1 n9}	Erucic acid	0.004 ^a \pm 0.0001	0.005 ^b \pm 0.0001	***
C _{22:1 n11}	Docosenoic acid	0.002 ^a \pm 0.0001	0.003 ^b \pm 0.0001	***

C_{16:3}	Hexagonic acid	0.022^a±0.002	0.023^a±0.003	NS
C_{18:2 trans-6}	Linolelaidic	2.150^b±0.028	2.620^a±0.042	*
C_{18:2 cis-6}	Linoleic	1.160^b±0.028	1.470^a±0.028	*
C_{18:2 cis-9, trans-11}	Rumenic acid CLA	1.055^b±0.064	2.055^a±0.021	*
C_{18:2 trans-10, cis-12}	Octadecadienoic acidCLA	0.154^b±0.005	0.335^a±0.021	*
C_{18:3 n-3}	α- Linolenic acid	0.335^b±0.007	0.455^a±0.006	*
C_{18:3 n-6}	γ- Linolenic acid	0.143^b±0.004	0.256^a±0.008	*
C_{18:4 n3}	α-Octadecatetraenoic acid	0.315^b±0.007	0.355^a±0.006	*
C_{20:2 cis-11,14}	Eicosadienoic	0.005^b±0.0001	0.006^a±0.001	*
C_{20:3 n6}	Dihomogammalinolenic acid	0.013^a±0.0001	0.014^b±0.0001	**
C_{20:3 n3 cis-11,14,17}	Eicosatrienoic	0.004^b±0.001	0.016^a±0.001	*
C_{20:4 n6}	Arachidonic	0.013^b±0.001	0.017^a±0.001	**
∑ Total fatty acids		98.76±0.14	98.43±0.21	---
∑ Unidentified		1.24±0.02	1.57±0.02	---
∑ Short chain fatty acids (SCSFA)		2.81^a±0.01	2.79^a±0.01	NS
∑ Medium chain fatty acids (MCSFA)		25.07^a±0.14	20.93^b±0.12	*
∑ Long chain fatty acids (LCSFA)		44.07^a±0.13	40.39^b±0.11	*
∑ <i>de novo</i> FAs		29.40^a±0.13	25.07^b±0.16	*
∑ Saturated fatty acids (SFA)		71.95^a±0.21	64.12^b±0.23	*
∑ Mono- unsaturated fatty acids (MUSFA)		21.44^b±0.10	25.69^a±0.12	*
∑ Poly-unsaturated fatty acids (PUSFA)		5.37^b±0.13	8.62^a±0.17	*
Unsaturated fatty acids USFA		3.48^a±0.031	3.38^b±0.036	*
Total Omega-6		0.65^b±0.011	0.83^a±0.012	*
Total Omega-3		5.32^a±0.011	4.52^b±0.013	*
Omega-6/Omega-3		1.21^b±0.001	2.39^a±0.001	*
Total conjugated linoleic acid (CLA)		3.18^a±0.015	1.79^b±0.013	*
Atherogenicity index (IA)		5.37^b±0.13	8.62^a±0.17	*

a,b,c Means with the same letter are significantly different at *P≤ 0.05, **P≤ 0.01, *P≤ 0.001. NS= (Non-Significant).**

SFA=saturated fatty acids, **MUFA**=monounsaturated fatty acids, **PUFA**= polyunsaturated fatty acids, **USFA= MUSFA+ PUSFASCFA**= short-chain fatty acids, **MCFA**= medium-chain fatty acids, **LCFA**= long-chain fatty acids, **CLA**=conjugated linolenic acids. **Omega-6**= (C_{18:2} trans-6, C_{18:2} cis-6, C_{18:3} n-6, C_{20:3} n6 and C_{20:4} n6), **Omega-3**= (C_{18:3} n-3, C_{18:4} n3 and C_{20:3} n3), **SCFA** = Butyric (4:0) + Caproic (6:0), **MCFA**=(8:0 to14:0), **LCSFA** =(15:0 to22:0), **de novo FAs**= (C_{4:0}-C_{16:0})

Chilliard et al. (2003 & 2006) had also found lower values of saturated fatty acids (SFAs) in goat's milk, when they used whole or oil-free flaxseed supplements. After absorption, they noticed reduced synthesis of C_{4:0} and C_{16:0} fatty acids (mostly synthesized *de novo* FAs) in the mammary gland, and also by converting C_{18:0} (Stearic acid) to C_{18:1} cis-9 through the action of Δ⁹-desaturase. Kholif et al. (2016) have explained another reason for the lowered SFAs concentration, which was due to the inhibitory effect of trans-18 isomers produced during bio-hydrogenation on the *de novo* synthesis of SFAs.

Decrease in SFAs especially C_{12:0}, C_{14:0} and C_{16:0} (responsible for increasing LDL), the low-density lipoprotein plasma levels can provide nutritional benefit from human health perspective, because they raise the bad cholesterol levels, associated with hypertension and coronary heart disease, certain cancers, obesity and type-2 diabetes (Haug et al., 2007; Mills et al., 2011).

Many reports carried out in dairy ruminants showed that animals fed on diets rich in unsaturated fatty acids (UFA) diminishes the content of SFAs in milk fat. This could be due to decrease in production of acetate and butyrate VFAs in the rumen due to dietary UFA, can cause a decrease in the amount of substrate for *de novo* FAs synthesis in the mammary cells, as well as the long-chain FAs taken up by the mammary gland inhibits enzymatic activities in the pathways of FAs synthesis in the mammary cell (Bernard et al., 2009a; Chilliard and Ferlay, 2004).

Moreover, our study demonstrated that flaxseed feeding produced non-significant changes in milk fat proportions of short-chain FAs (C_{4:0} and C_{6:0}). A similar result was reported by Nudda et al. (2006); Bouattour et al. (2008); Marín et al. (2012). They showed non-changes (negative or positive) in short chain FAs (C_{4:0} and C_{6:0}) response to plant lipid supplementation in goat experiments.

Furthermore, our results indicated that adding flaxseed to the goat's diets resulted in increasing UFAs in milk. The majority of 18-carbon FAs in milk fat were affected by the dietary supplementation with flaxseed and caused an increase C_{18:1} trans-9, C_{18:1} cis-9, C_{18:1} trans-11, C_{18:1} trans-7, C_{18:2} cis-6, C_{18:2} cis-9, trans-11, C_{18:2} trans- 10, cis-12, C_{18:3} n-3, C_{18:3} n-6, C_{18:4} n3 isomers in milk fat compared to the control diet.

Zhang et al. (2006) have reported that ewes fed on flaxseed supplemented diets rich in C_{18:3}, produced milk with markedly higher proportions of C_{18:1}, C_{18:2} and C_{18:3}, and a lower proportion of C₁₆. According to Petit et al. (2002) and Chilliard et al. (2009) about 58% of 18-carbon linoleic and linolenic acids are bio-hydrogenated in the rumen, since flaxseed contains a high oil level (40% of total seed weight), and α-linolenic acid (n-3 FAs) constituting 55% of total fatty acids.

Feeding on flaxseed diet increased the proportion of 18-carbon FAs isomers in milk fat and also the concentrations of n-3 FA which reached 14% of total FAs in milk fat, resulting from a rapid release of oil from seeds and higher infusion in the small intestine bypassing the rumen.

Concerning of fatty acid profile, the sum of the *de novo* FAs (C_{4:0}, C_{6:0}, C_{8:0}, C_{10:0}, C_{12:0}, C_{14:0} and C_{16:0}) in milk fat has been affected by flaxseed supplementation. Lower concentrations of $\sum de novo$ FAs were observed in goat milk fat fed on flaxseed (25.07 ± 0.16%) vis-à-vis control diet (29.40±0.13%).

[Akraim et al. \(2007\)](#), [Weisbjerg et al. \(2013\)](#) and [Cívico et al. \(2017\)](#) have explained from their studies that supplementing the diets of animals with PUFA rich oil sources (e.g., flaxseed) are typically associated with inhibition of $\sum de novo$ FAs (C_{4:0}-C_{16:0}) synthesis of milk fatty acids in the mammary gland by the inhibition or reduction of enzyme in *de novo* lipogenesis pathways including palmitic acid in the mammary gland.

Moreover, the milk contents of SCSFA (C_{4:0} and C_{6:0}) FAs did not differ significantly ($P \leq 0.05$) between the flaxseed and control diets, which were 2.79 ± 0.01 and 2.81 ± 0.01 percents, respectively. Indeed, significantly lower contents of MCSFA (C_{8:0} up to C_{14:0}) FAs were observed in the current study in milk from goats fed on flaxseed (20.93 ± 0.12 %) than in the control diet (25.07 ± 0.14 %).

Similar results were reported by [Petit and Côrtes \(2010\)](#) and [Lerch et al. \(2012\)](#). They noticed that the addition of flaxseed results in a decrease in MCFA concentration. These results are in agreement with [Nudda et al. \(2006\)](#) and [Jóźwik et al. \(2010\)](#). They reported that the addition of plant oil rich in the PUFA in the diet had no effect on the SCFA content of goat milk.

Comparison between flaxseed and control treatments revealed that flaxseed was characterized by significant ($P \leq 0.05$) decrease in milk SFA (64.12±0.23 %) and increase in fat milk UFA (34.31 ± 0.21 %) compared to the control group diet (71.95 ± 0.21% and 26.80 ± 0.18%, respectively). In addition, compared to MUFA, PUFA accounted for a smaller portion of the total UFA. Our results showed higher and significant ($P \leq 0.05$) total MUFA and PUFA ratio in flaxseed groups compared to the control group (25.69 ± 0.12 % vs. 21.44±0.10 %) and (8.62 ± 0.17% vs. 5.37±0.13 %), respectively. Similar result was found in the study of [Marín et al. \(2012\)](#) and [Kholif et al. \(2016\)](#) who reported an increase in the MUFA, PUFA, and total UFA concentrations of milk fat and a decrease in SFA concentration when flaxseed supplements were used in goat feed.

On the other hand, goats fed on whole flaxseed had significantly ($P \leq 0.05$) higher proportion of CLA content (2.39 ± 0.001 % vs. 1.21 ± 0.001 %) compared to the control treatment. Those results are in agreement with [Bernard et al. \(2009\)](#) and [Caroprese et al. \(2010\)](#) who have confirmed significant increase in C_{18:2 cis-9, trans-11} CLA content with addition of flaxseed to goat's diet.

This observation can be explained by the higher concentration of bio-hydrogenation in flaxseed of C_{18:2 cis-9cis-12}, which are intermediates of total CLA, and approximately 80 % of CLA appearing in milk fat is synthesized in the mammary gland via decreasing Δ^9 -desaturase mRNA abundance of the mammary gland ([Chilliard et al., 2001](#); [Caroprese et al., 2010](#)).

Therefore, increasing dietary contents of oils rich in linoleic and linolenic acids is the main reason for increased milk content of CLA. From the point of view of nutrition, increasing CLA concentration in milk is an important issue because of its anticarcinogenic properties. Therefore, feeding flaxseed under hot climate could be considered a more efficient method to increase CLA concentrations in goat milk.

Our results indicated that the content of omega-3 PUFA was significantly ($P \leq 0.05$) higher in milk (0.83 ± 0.012 %) in flaxseed supplemented goats than in milk fat from control goats (0.65 ± 0.011 %). Moreover, feeding flaxseed supplemented diet significantly ($P \leq 0.05$) decreased omega-6 fatty acid concentrations in milk fat (3.38 ± 0.036 % vs. 3.48 ± 0.031 %) in control diets, respectively. A decrease of omega-6 FAs along side with an increase of omega-3 FA led to a decrease of omega-6 FAs to omega-3 FAs ratio in milk of goats fed on flaxseed (4.52 ± 0.013 % vs. 5.32 ± 0.011 %) compared to the control. This finding is in agreement with [Abedi and Sahari \(2014\)](#) and [Kholif et al. \(2016\)](#), who reported lowering in the ratio of omega-6/omega-3 and increase in omega-3 concentration to prevent or modulate certain diseases in humans because of its anticarcinogenic properties.

On the other hand, the atherogenicity index (AI) was significantly ($P \leq 0.05$) lower in flaxseed group (1.79 ± 0.013) than in the control (3.18 ± 0.015), which indicated that healthy milk is produced by feeding flaxseed. According to [Kholif et al. \(2016\)](#), feeding on flaxseed supplemented diet decreased the atherogenicity index (about 19 to 24 %) than the control diet. Furthermore, [Chilliard and Ferlay \(2004\)](#) and [Marín et al. \(2012\)](#) attributed that decrease in AI was in higher proportions of PUFA or trans FAs in animals fed with oil-supplemented diets.

From a dairy product maker's view point, this modification in milk FAs profiles by supplementation of diet with flaxseed caused a reduction in SCF FAs, which led to improvement not only the nutritional quality of milk, but also the organoleptic properties (flavors) in some dairy products such as cream cheese or butter.

Moreover, this modification in milk FAs profiles can affect the rheological properties (spreadability, firmness and texture) of butter or cream cheese, which is especially true where its texture attributes to fat contents. Increased concentration of palmitic acid C16:0 combined with a decrease in SCF FAs lead to lower butter spreadability and firmness ([Williams, 2000](#); [Chilliard and Ferlay, 2004](#)). Hence, flaxseed can lead to more spreadable butter with a reduction in SCF FAs.

These data also indicate that technology is available to improve the profile of milk fatty acid via supplementation of goat diet with flaxseed, resulting in favorable changes in the FAs profile like reduction of SFA and increase in PUFA, especially CLA and Omega-3 concentrations which are beneficial for health and have positive effects in the prevention of cancers and atherosclerosis in milk consumers.

CONCLUSIONS

The present study indicated that, supplementation of flaxseed to lactating goats has a significant effect on milk yield especially at early lactation. The flaxseed supplemented group showed significant increase in milk protein, lactose, pH, freeze point values and total solids, while the effect was non-significant on fat content.

Analyses of total fatty acids profile indicated reduction in total SFAs content, especially C_{12:0}, C_{14:0} and C_{16:0}, *de novo* FAs (C_{4:0}-C_{16:0}) and enhancement of nutritionally desirable USFA content. In addition, milk of goats fed on flaxseed led to increased proportions of Conjugated linoleic acid (CLA) and significant reductions of the ratio of Omega-6/Omega-3 FAs indicating improvement in the nutritional quality of milk with positive impact on human health. Furthermore, this study confirms the feasibility of using flaxseed as a nutritional strategy to reduce SFAs and increase USFA in milk and also enhance production of CLA and Omega-3 enriched goats milk without negative effects on milk yield and solids (TS) content.

Therefore, it is recommended that supplementation of diet of lactating local goats of Halayeb-Shalateen-Abu Ramad Triangle of Egypt with 14% whole flaxseed is a likely panacea to overcome the problem of milk deficiency in this area. Moreover, the modification of FAs profiles of goat milk fat may help to correct the dieticians' criticism against dairy products for their ability to raise LDL cholesterol and to improve the health prospective and organoleptic properties with conventional repulsive image of goat milk due to off flavour as perceived by the consumers and nutritionists.

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UNDERTAKING



It is certified that the research paper ‘**EFFECT OF FLAX SEED SUPPLEMENTATION ON MILK PRODUCTION, CHEMICAL COMPOSITION, PHYSICAL ATTRIBUTES AND FATTY ACID PROFILE OF MILK IN LACTATING GOATS OF HALAYEB-SHALATEEN-ABU RAMAD TRIANGLE OF EGYPT**’ is an original research work carried out by the authors in the Department of Animal and Poultry Breeding, Desert Research Center, Cairo, Egypt. It has neither been published nor contemplated for publication elsewhere.

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