EFFECT OF LINSEED OIL BEADS ADDITION WITH VITAMIN E ON PERFORMANCE, BLOOD METABOLITES AND MILK YIELD OF LACTATING GOATS

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SUMMARY

This study aimed to determine the effects of addition of capsulated linseed oil (beads) alone or in combination with vitamin E (α-tocophyrol acetate) as feed additives on animal performance, blood plasma and milk fatty acid (FA) profiles during lactation period. Twenty four Damascus pregnant doses of about 44.5 ± 1.2 kg live body weight were randomly allotted to 3 groups of 8 doses each and assigned to receive one of three experimental diets: control group: where goats were received berseem hay and concentrate feed mixture (50:50) without any additives, (CON), LO group: goats were offered control diet plus 2.5 gm beads/head/day and LO+VE group: goats were offered control diet plus 2.5 gm beads plus 600 IU VE/head/day. The experiment lasted for about 135 days (from mid pregnancy and lasted 60 days post-partum). Results showed that LO beads inclusion strongly increased milk yield, fat ($P<0.01$), protein, lactose, total solids yield ($P<0.05$) and milk fat % ($P<0.05$) compared with control. Blood plasma γ-linolenic acid was positively affected by beads while C20:2ω6 was increased ($P<0.05$) by beads plus VE. Moreover, beads inclusion reduced lauric (C12:0), myristic (C14:0) and palmitic (C16:0) ($P<0.05$) acids while increased contents of monounsaturated fatty acids (MUFA) as oleic (C18:1ω9) and polyunsaturated fatty acids (PUFA) as γ-linoleic (C18:3ω3) ($P<0.01$) and C18:4ω3 ($PP<0.05$) compared with control milk. As a consequence reduction of the proportions of total SFA ($P<0.01$), total omega-6 FAs ($P<0.05$) and omega-6: omega-3 ratio and enhanced the proportions of unsaturated FAs (UFA) and omega-3 FAs ($P<0.01$) resulted in health quality of goat's milk was improved. In addition, some of blood plasma metabolites were improved with beads where plasma createnine, cholesterol ($PP<0.05$) and lipase activity ($P<0.01$) were decreased compared with control animals. Plasma urea ($P<0.05$), triglycerides (TG) ($P<0.01$), low density lipoprotein (LDL) ($P<0.05$), high density lipoprotein (HDL) ($P<0.01$) and total antioxidant capacity (TAC) ($P<0.05$) were increased while lipase activity was decreased with VE addition. The inclusion of VE combined with LO beads didn't result in benefits in goat's products. Further in vivo studies should be undertaken to explore suitable level of vitamin E in association with protected form of useful and healthy fatty acids.

***Keywords**: Linseed, fatty acid profile, milk, vitamin E and goats.*

INTRODUCTION

Animal products provide a considerable percentage of saturated fats that affecting human health. Nutrition constitutes a natural way to modulate animal's products fatty acids (FA) composition. Addition of linseeds to ruminant diets is useful to increase the concentration of polyunsaturated FA in dairy products and meat. Ruminant products contain a variety of FA, particularly polyunsaturated fatty acids (PUFA), which are considered beneficial to human health (Doreau and Ferlay, 2015). The inclusion of linseed as a source of PUFA in the diet has been used to enhance these beneficial FA in animal products (Abuelfatah *et al.*, 2016). Milk fatty acid profile is influenced by dietary fatty acids and ruminal fatty acid

metabolism including lipolysis and biohydrogenation of UFA (Bai *et al.*, 2018). Thus, the use of fat sources protected from rumen biohydrogenation could be a more practical approach (Scott *et al.*, 1971).

Many attempts aimed to protect PUFA against rumen biohydrogenation to improve dairy products and meat fatty acid content. So, different protection methods have been applied such as encapsulation of lipids in a coat of proteins treated with formaldehyde (Doreau *et al.*, 2011; and Fievez *et al.*, 2007). However, this technique is not used, because of it's high cost, and possible adverse effects of excessive amounts of polyunsaturated fatty acids on animal health and product quality (Doreau and Ferlay, 2015). Commercially supplementation with FA calcium salts is used although saturated and monounsaturated (MUFA) react with calcium forming an insoluble product that resist rumen biohydrogenation but PUFA do not react to form calcium salts then no protection against biohydrogenation of PUFA (Lundy *et al.*, 2004; and Castañeda-Gutiérrez *et al.*, 2007). Therefore, Gawad *et al.* (2015) found a new encapsulation method using biopolymers to protect FAs in linseed oil from rumen biohydrogenation. An increase in milk PUFA content increased it's susceptibility to oxidation, so it may require dietary supply of antioxidants to prevent oxidative damage of the fatty acids. Vitamin E being one of the most powerful antioxidants, it plays an important role in the prevention of lipid oxidation in cell membranes (Deaville *et al.*, 2004). Vitamin E is used in animal feed mainly due to its lipid-soluble antioxidant function and because it assists in reducing the effects of oxidative stress. Therefore, the uses of dietary antioxidants with dairy animals increase the oxidative stability of milk (Santos *et al.*, 2014).

The overall objective of the current study is to determine the effects of protected lipid supply alone or in combination with vitamin E on Damascus does performance during lactation period and on blood plasma and milk fatty acid profiles.

MATERIALS AND METHODS

Preparing linseed oil beads:

Encapsulated linseed oil was prepared according to the method described by Gawad *et al.* (2015). Briefly, 2.5% (w/v) alginate/k-carrageenan solution was prepared by dissolving sodium alginate/k-carrageenan gel (1:1 w: w) in distilled water using an overhead mechanical stirrer. Then, linseed oil emulsion gel was prepared by mixing linseed oil with alginate/carrageenan solution (20%; v/v) using Tween 80 as an emulsifier (0.5 ml /100 ml gel). Uniform linseed oil beads were prepared using Encapsulator instrument (model IE-50 R was purchased from Encap. Biosys., Switzerland). Linseed emulsion gel solution was injected in encapsulater and let solution for dripping under specific conditions as follow: Nozzle: 1ml, frequency 1700 HZ, flow rate: 4 ml min^{-1} and air pressure of 1 bar. The formed beads were received in 2.5% $CaCl_2$ (w/v) and left in hardening solution for up to 30 min for more hardening. Then, linseed oil beads were filtered and dried using oven dryer (45 ^{o}c).

Fatty acids profile of linseed oil and linseed oil beads:

Fatty acids profile of linseed oil before and after encapsulation was analyzed according to AOAC (2000) using Ultra Gas Chromatographs.

Animals, diets and experimental design:

Twenty-four Damascus goats in mid pregnant stage with an initial body weight (BW) of 44.5 ± 1.2 kg were divided into three groups. Goats within groups (8 goats each) were assigned randomly to one of three treatments. The three dietary treatments were; control treatment (CON) where the animals were fed basal diet without any additive. The second; linseed oil beads treatment (LO) where the experimental animals were fed basal diet plus a daily dose of 2.5 g /goat/d of linseed oil beads. The third, LO+VE, the animals were fed basal diet plus a daily dose of 2.5 g /goat/d of linseed oil beads + 600 IU /goat/d of vitamin E (α-tocopherol acetate). The daily dose of vitamin E was orally administered once daily directly after feeding with the aid of a syringe. The experimental animals had free access to water and mineral block.Goats with their kids were housed in individual concrete pens, fed individually, and milked. The experiment was extended from mid pregnancy and lasted 60 days post-partum. The experimental diets were formulated to meet the animals' nutrient requirements according to NRC (2007) recommendations. Diets were offered once daily at 08.00 h. The beads were top dressed and mixed with a fork into the basal diet. Before starting the experiment, goats were vaccinated against internal and external parasites and intro-toxemia. Live body weights of does and their kids were measured biweekly and body weight changes were calculated.

Feed intake and chemical analysis:

Feed intake was measured throughout the experimental period by weighing the offered diets and refusals from the previous day. The feed samples were dried in a forced-air oven at 65 °C for 72 h, and ground in a Willey mill with a 1.0-mm screen. Samples were analyzed for DM (method 930.15), ash (method 942.05), Nitrogen (method 954.01) and ether extract (EE; method 920.39), according to AOAC, (1997) official methods. Neutral detergent fiber was determined by the procedure of Van Soest *et al.* (1991) without use of an alpha amylase but with sodium sulfite and expressed exclusive of residual ash. Acid detergent fiber was analyzed according to AOAC, (1997) and expressed exclusive of residual ash. The chemical composition of the basal diet is shown in Table (1).

Table (1): Nutrient composition of the basal diet (% on DM basis).

Item	Concentrate feed mixture	Berseem hay
Dry matter	90.45	88.32
Organic matter	97.16	87.65
Crude protein	17.32	13.22
Ether extract	4.25	2.96
Ash	2.82	12.35
Neutral detergent fiber	17.82	55.38
Acid detergent fiber	10.80	38.01

Rumen liquor sampling and analysis:

At the end of the experiment, ruminal contents were sampled randomly from four goats in each group at 4 h after the morning feeding by stomach tube. Approximately 100 mL of rumen fluid were collected and strained through 4 layers of cheesecloth. The pH of ruminal fluid was measured immediately with a pH meter (Accumet Model 15, Fisher Scientific, USA). A 50-mL aliquot of ruminal fluid was acidified with 2.5 mL of 6 N HCl and frozen (–20°C) for subsequent determination of ammonia-N concentration calorimetrically using commercial kit (Konitzer and Voigt, 1963) and total volatile fatty acids (TVFA) (Warner, 1964).

Milk sampling, milk composition and fatty acid analysis:

Milk production was determined biweekly as follow: kids were separated from their dams, then does were milked completely by hand at 8.00 and 18.00 h and kids were return to their dams from the second week of lactation till the end of the experiment. The collected milk was recorded, and a subsample was taken from individual does at each milking and mixed as a constant percentage of the morning and eve ning to obtain the sample of each does, then stored at –20 °C until further analysis. Milk samples were analyzed for total solids, fat, protein, and lactose using infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark). Yield of fat-corrected milk was calculated according to the equation reported by Gravert (1987): FCM (3.5%) = 0.433MY + 16.218 FY, where: FCM: fat-corrected milk; MY: milk yield (kg/day); FY: fat yield (kg/day).

Blood plasma sampling and analysis:

At the end of the experiment, 10-mL blood samples were taken before morning feeding from the jugular vein of each animal into a clean tube containing EDTA . The plasma were separated by centrifuging at 3000 rpm for 20 minutes and frozen at −20 °C up to subsequent analysis. Blood plasma samples were analyzed using commercial kits. All plasma samples were analyzed for total protein, albumin, globulin (by subtraction the total proteins values from the albumin values) , urea –N, createnine, total lipids (TL), cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) , lipase enzyme, total antioxidant capacity (TAC), alanine amino transferase (ALT) and aspartate amino transferase (AST) using Biodiagnostic laboratory kits.

Milk and plasma fatty acids analysis:

The milk samples were pooled per does, resulting in one milk sample per does during the experiment. Milk lipids were determined according to the method of AOAC (2000), where FAs are methylated with boron trifluoride in methanol, extracted with heptane and determined on a gas chromatograph with FID detector (PE Auto System XL) with auto sampler and Ezchrom integration system. Oven temperature 200 ˚C, injector and detector 250 ˚C. On the other hand, plasma lipids were extracted by using 100% ether /

sample in the ratio of 1:10 (v/v) as detailed by Ferraz *et al.* (2004) then methylated and determined by the same procedure referred to milk FAs.

Statistical analysis:

The DMI, milk yield, fat-corrected milk and milk composition data were analyzed as repeated measurements over time (weeks) using mixed procedure of SAS (2006) (Statistical Analysis System, version 9.2 for Windows; SAS Institute, Cary, NC, USA). The model used was the following: Yij= μ + Di + bj + Hk + Di × Hk + eij; where, Yij=dependent variable; μ =overall mean; Di=effect of diet i, i=1 to 3; bj=effect of block j, j=1 to 8; Hk=effect to the week k, k=2 to 8; Di×Hk=interaction of diet i×week k; eij=random effect. The initial BW, final BW, BW change of the does and kids throughout the experiment, rumen parameters, blood plasma parameters and the FA profile of the milk fat and of plasma were analyzed using MIXED procedure of SAS (Statistical Analysis System, version 9.2 for Windows; SAS Institute, Cary, NC, USA). The model used was the following: Yij = μ + Di + bj + eij, where μ = overall mean, Di = effect of diet, bi = effect of block and eij = random effect. Orthogonal contrasts were used to compare the effects of: linseed oil beads (CON vs. LO) and the association between linseed oil beads and vitamin E (LO vs. LO+VE). The effects were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Fatty acids composition of beads:

Results of Table (2) indicated that encapsulation process did not affect FAs content of oil especially

Table (2): Fatty acids composition of linseed oil and linseed oil beads.

Fatty acids %	Linseed oil	Linseed oil beads
SFA		
C6:0, Caproic	0.63	0.67
C8:0, Caprlyic	0.33	0.37
C14:0, Myristic	0.25	0.27
C15:0, Pentaenoic	0.18	0.2
C16:0, Palmitic	7.46	7.98
C17:0, Heptadecanoic	0.19	0.14
C18:0, Stearic	5	3.9
C20:0, Arachidic	0.33	0.24
C22:0, Behenic	0.3	0.36
∑Saturated fatty acids	14.67	14.13
MUFA		
C15:1ω6, 10-Pentadecanoic	0.48	0.48
C16:1ω7, Palmitoleic	0.1	0.21
C18:1ω9, Oleic	18.43	18.41
C18:1ω7, Vaccinic	0.94	0.97
C20:1ω9, Gadolic	0.3	0.27
C20:1ω7, 9-eicosaenoic	ND	0.15
C20:1ω5, 11-eicosaenoic	ND	0.25
C22:1ω9, Erucic	0.21	0.45
∑Monounsaturated	20.46	21.19
PUFA		
C18:2ω6, Linoleic	13.47	14.1
C18:2ω4,	ND	0.18
C18:3ω3, Linolenic	51.4	50.4
∑Poly unsaturated fatty acids	64.87	64.68
Unsaturated fatty acids	85.33	85.87
Omega-3 FA	51.4	50.4
Omega-6 FA	13.95	14.58

ND: non detectable

PUFA fractions. Therefore, linolenic (C18:3ω3) and linoleic (C18:2ω6) acids which are the most important PUFA were not affected by encapsulation process. Their values were 51.4 and 50.4% for linolenic acid and 13.47 and 14.1% for linoleic acid in linseed oil and it's beads, respectively. The present results are in agreement with Gawad *et al.* (2015), who used the same technology of encapsulation.

Table (3): Effect of supplementation of linseed oil beads with or without vitamin E on does performance.

Item	Treatment[1]			SEM	*P*- value[2]	
	CON	LO	LO + VE		CON *vs.* LO	LO *vs.* VE
No. of does	8	8	8			
Body weight of does, kg						
Initial	44.06	44.56	44.81	1.20	0.901	0.949
Final	41.71	42.38	42.75	1.23	0.872	0.927
Body weight changes	-2.46	-2.19	-2.06	0.09	0.147	0.630
Dry matter Intake, kg	1.73	1.71	1.69	0.07	0.864	0.833
Production, g/d						
Milk	1574	1807	1824	35.68	0.001	0.794
FCM[3]	1484	1802	1798	43.77	0.003	0.960
Fat	49.49	62.85	62.15	1.78	0.005	0.810
Protein	49.27	59.47	56.87	1.46	0.016	0.440
Lactose	71.92	84.27	85.87	2.10	0.013	0.729
Total solids	183.5	222.4	219.9	5.89	0.010	0.846
Milk composition, %						
Fat	3.14	3.48	3.41	0.05	0.033	0.340
Protein	3.15	3.29	3.12	0.04	0.194	0.153
Lactose	4.56	4.67	4.71	0.07	0.645	0.742
Total solids	11.64	12.30	12.06	0.15	0.213	0.420

1Treatments: Control group fed on basal diet without additives; LO group: basal diet with linseed oil beads; LO+VE group: fed on basal diet with linseed oil beads and vitamin E. 2 CON vs. LO: control diet vs basal diet and linseed oil beads; LO vs. VE: basal diet + linseed oil beads vs basal diet + linseed oil beads + vitamin E. SEM, standard error of means.

Table (4): Effect of supplementation of linseed oil beads with or without vitamin E on sulking kids.

Item	Treatment[1]			SEM	*P*- value[2]	
	CON	LO	LO + VE		CON *vs.* LO	LO *vs.* VE
No. of kids	11	11	11			
Birth weight, kg	3.80	3.84	3.77	0.15	0.925	0.760
Body weight, kg						
After 30 days	8.54	8.71	8.79	0.17	0.443	0.766
After 60 days	13.51	14.13	14.41	0.26	0.374	0.648
Average daily gain, g/d						
After 30 days	158.1	162.6	167.3	6.13	0.697	0.659
After 60 days	165.6	180.3	187.3	6.79	0.422	0.685

[1]Treatments: Control group fed on basal diet without additives; LO group: basal diet with linseed oil beads; LO+VE group: fed on basal diet with linseed oil beads and vitamin E. [2] CON vs. LO : control diet vs basal diet and linseed oil beads; LO vs. VE : basal diet + linseed oil beads vs basal diet + linseed oil beads + vitamin E. SEM, standard error of means.

Milk production and composition:

Milk yield was increased with LO supplied goats compared with CON goats (Table 3) and this increment agree with findings of Gomez – Cortes *et al.* (2009), Benchaar *et al.* (2012) and Kholif *et al.* (2015), who used different forms of linseed or it's oil. This increase in milk production may be attributed to that LO resulted in higher volatile fatty acids concentration in the rumen of the supplemented goats as matched with the following present results in Table (4). Moreover, LO supply may affect mammary gland metabolism. Petit (2003) illustrated that greater milk production could be a result of greater dietary amino

acids available for absorption by the animal. Crawford and Hooverm (1984) and Petit (2003) agreed with the present data. On the other hand, Zened *et al.* (2012), Suksombat *et al.* (2014) and Almeida *et al.* (2019) found that milk yield did not affect by oil diet with or without VE, Petit *et al.* (2005) recorded a reduction in milk production. All of the macro components yield were affected by LO supply compared with milk of CON goats. Milk fat corrected milk (FCM) was increased by LO treatment comparing with milk of CON goats (225.1 vs 185.6), respectively. Milk fat yield and it's percentage were increased with LO supply and this may be attributed to effective oils protection against ruminal bio hydrogenation increased fat yield (Ashes *et al.*, 1992) while ineffective protection (Petit *et al.*, 2002) or low level of added fat (Tymchuk *et al.*, 1998) had no effect on milk fat yield. Similarly, Knapp *et al.* (1991); Kim *et al.* (1993) and Petit and Gagnon, (2009) observed higher yield and percentage of milk fat when cows were fed whole oilseeds or protected fats. This may be due to increased dietary FA being taken up by mammary gland for milk fat synthesis (Knapp *et al.*, 1991). Generally, supplemental fat may elevate milk yield and milk fat but sources and types of fat affecting differently on such parameters (Chouinard *et al.*, 1997). Elevated concentrations of milk protein with LO supply may be due to increased oil supply of amino acids for synthesis of milk protein. Petit (2002) and Petit (2003) were in agreement with the present findings. Kholif *et al.* (2014) attributed the increase of milk protein to improvement of ruminal microbial protein synthesis. Some of the previous studies such as Chilliard *et al.* (2009) and Radivojević *et al.* (2011) reported no change in milk protein whereas, Pires *et al.* (1996) and Miller *et al.* (2009) found that milk protein percentage was decreased particularly for cows fed on extruded oilseeds. In the same line, the lactose production was increased with LO treatment. Similar result was reported by Petit (2003) with formaldehyde treated oilseeds while, Almeida *et al.* (2019) suggested that lactose production is rarely changed by diet. All of the significant effects noted previously with LO beads supply were suppressed when vitamin E supplied with beads. Focant *et al.* (1998) reported that vitamin E supply increased the concentration of this vitamin in milk by 45% resulting in higher resistance of such milk fat to oxidation.

Kids growth rates:

No differences were identified between lactating doses fed on either LO or LO+VE ($p>0.05$) and control ones regarding their kids birth weight or change in body weight till weaning (Table 4). These observations in terms of average daily gain are consistent with the results of the previous studies on growing lambs supplemented with different vegetable oils (Miltko *et al.* 2019), fattening lambs fed on soybean meal or extruded linseed (Facciolongo *et al.* 2018) and weaned male lambs of Tan sheep supplemented with vitamin E (Zhao *et al.* 2013).

Ruminal parameters:

Results of Table (5) indicated comparable levels of ruminal ammonia ($p>0.05$) on addition of LO or LO + VE while feeding of linseed oil beads increased the concentration of volatile fatty acids (VFA's) ($P<0.05$) compared to control goats while their concentrations were decreased ($P<0.01$) when VE incorporated compared to LO fed goats. These results may be due to the anaerobic fermentation of linseed oil or crushed linseed that increased yielding of VFA's (Kholif *et al.*, 2015) and / or to improvement of ruminal fermentation due to enhancement of cellulolytic bacteria and protozoa activities with linseed (El–Essawy, 2019). In contrast, Kim *et al.* (2007) in sheep, Benchaar *et al.* (2012) in dairy cows and Abuelfatah *et al.* (2016) in goats demonstrated no effect of linseed or linseed oil supplementation on pH and VFA's. These differences in concentrations of TVFA were probably attributed to differences in rumen volume (Chikunya *et al.*, 2004) or to changes in the rumen species composition of microbes in response to inclusion of unsaturated fatty acids in linseed that being toxic to cellulolytic and methanogenic bacteria (Broudiscou *et al.*, 1994).

Table (5): Effect of supplementation of linseed oil beads with or without vitamin E on rumen fermentation.

Item	Treatment[1]			SEM	*P*- value[2]	
	CON	LO	LO + VE		CON *vs.* LO	LO *vs.* VE
pH	6.11	6.22	6.21	0.02	0.050	0.891
Ammonia-N, mg /dl	18.13	19.74	16.85	0.241	0.499	0.224
Volatile fatty acid, mg /dl	7.1	8.14	6.49	1.42	0.018	0.0003

[1]Control, basal diet without oil supplement; LO, basal diet with linseed oil beads; LO+VE basal diet with linseed oil beads and vitamin E. [2] CON vs. LO = control diet vs diet supplemented with linseed oil; LO vs. VE = diet supplemented with linseed oil vs diet supplemented with linseed oil and vitamin E. SEM, standard error of the means.

An increased tendency of rumen pH is recorded (p=0.05) with LO addition compared to CON values whereas VE inclusion did not change pH values compared to those fed on LO. Numerically higher ruminal pH on feeding LO was in agreement with previous studies using different lipid sources including fish oil in diets of sheep (Wachira *et al.,* 2000), in diet of cows (Shingfield *et al.,* 2003) and both of these studies attributed this increase to associated decreases in DMI and also in diets of cattle fed cottonseed alone or combined with vitamin E (Nogueira *et al.,* 2019) and they explained higher pH to higher NDF content of cottonseed. On the other hand, Gawad *et al.* (2015) recorded lower ruminal pH values in vitro with different levels of linseed oil beads but they suggested that the mean of pH values remained within normal range of pH which is between (6 and 6.7). Other studies reported a lack of change in pH values with whole linseed inclusion compared to a protected palm oil (Scollan *et al.,* 2001) or compared to soybean (El–Essawy, 2019).

Blood plasma metabolites and fatty acids:

Regarding LO supply in feeding resulted in decreasing blood plasma createnine, cholesterol (TC) (P<0.05) and lipase activity (P<0.01) (Table 6). The reduced cholesterolemia probably attributed to enrichment of linseed with omega-3 fatty acids. These results are in harmony with El-Essawy (2019) who found a reduction in createnine and TC with linseed supply due to enrichment of linseed with omega-3 fatty acids. In the same line, Weill *et al.* (2002) observed a repeated decrease in fat content of animal products as a result of inhibition of lipogenesis by alpha linolenic acid (Price *et al.,* 2000) and also linoleic acid that known to reduce TC (Weill *et al.,* 2002). Another study showed that oilseed supplementation increased the concentration of TC in bovine blood (Gonthier *et al.,* 2005) and these variations may be attributed to different sources and quantity of oilseed (Liu *et al.,* 2008). However, LO supply allowed lower lipase activity may be attributed to increase cellulolytic bacterial population hence both bacteria and fungi are predominant microbial sources of lipase (Sangeetha *et al.* 2011). Vitamin E supply, resulted in elevated levels of urea, triglycerides (TG), low – density lipoprotein (LDL), high – density lipoprotein (HDL) and total antioxidant capacity while lipase activity was considerably decreased.

Table (6): Effect of supplementation of linseed oil beads with or without vitamin E on blood plasma measurements.

Item	Treatment[1]			SEM	*P*- value[2]	
	CON	LO	LO + VE		CON *vs.* LO	LO *vs.* VE
Total protein, g/dl	7.89	9.02	10.50	0.62	0.487	0.331
Albumin, g/dl	3.83	3.90	3.62	0.13	0.651	0.545
Globulin, g/dl	4.05	5.12	6.89	0.68	0.508	0.293
Urea, mg/dl	67.63	58.75	70.6	2.45	0.235	0.026
Creatinine, mg/dl	1.44	0.98	0.86	0.09	0.019	0.211
Total lipid, mg/dl	75.17	74.74	74.79	0.75	0.863	0.804
Triglycerides, mg/dl	10.50	11.36	20.43	0.25	0.549	0.003
Cholesterol, mg/dl	88.33	74.41	83.18	2.07	0.010	0.137
LDL-cholesterol, mg/l	40.69	39.60	43.73	0.55	0.224	0.019
HDL-cholesterol, mg/dl	63.42	63.73	72.90	1.39	0.878	0.009
Lipase, U/l	150.0	116.7	91.33	8.66	0.005	0.013
Total antioxidant capacity, mM/l	0.334	0.350	0.497	0.02	0.656	0.014
Aspartate amino transferase, U/l	16.2	15.2	15.6	0.44	0.446	0.740
Alanine amino transferase, U/l	16	17.4	17.6	0.53	0.206	0.923

[1]*Control, basal diet without oil supplement; LO, basal diet with linseed oil beads; LO+VE basal diet with linseed oil beads and vitamin E.* [2] *CON vs. LO = control diet vs diet supplemented with linseed oil; LO vs. VE = diet supplemented with linseed oil vs diet supplemented with linseed oil and vitamin E. SEM, standard error of means.*

El–Essawy (2019) supported our findings where she reported increased blood urea due to improved CP digestibility with linseed inclusion whereas Sharma *et al. (*1972) found reduced values of blood urea were related to decreased N degradability. Petit (2003) found that blood urea was not affected by different oilseeds treated with formaldehyde.

Fatty acid composition in blood plasma and milk:

Saturated fatty acids (SFA):

The SFA in blood plasma were not affected significantly (p>0.05) by any of feed additives, (Table 7) although most of them showed insignificant decrease on feeding of LO compared to CON goats as capric (C10:0), undecanoic (C11:0), tridecanoic (C13:0), myristic (C14:0), palmitic (C16:0) and heptadecanoic (C17:0) acids. As a consequence of this reduction, the total SFA was decreased numerically in LO fed goats compared to CON ones (33.79 vs. 37.82) instead vitamin E supply, increased total SFA compared to LO fed group (36.39 vs. 33.79, respectively) indicating that BH of PUFA and its transformation to SFA was decreased with LO addition. So, many studies by Chikunya *et al.* (2004) and Fiorentini *et al.* (2015) concluded that the process of bio hydrogenation is the main cause of reduction of PUFA leaving the rumen. Consequently, SFA's especially lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids were also decreased (P<0.05) therefore, total SFA's were decreased (P<0.01) significantly in goat's milk fed LO treatment (Table 8). Chichlowski *et al.* (2005) and Liu *et al.* (2008) found similar trend with different oilseeds sources. Indeed, reduction of SFA in animal products especially C14:0 and C16:0 with LO supply prevent many physiological problems for consumer such as elevated blood cholesterol (Suksombat *et al.*, 2014) because C16:0 (palmitic acid) has hypercholesterolemic properties (Kennelly, 1996).

Table (7): Effect of supplementation of linseed oil beads with or without vitamin E on fatty acids profile (g of fatty acid/ 100 g) in blood plasma.

	Treatment[1]				*P*- value[2]	
Fatty acid %	CON	LO	LO + VE	SEM	CON *vs.* LO	LO *vs.* VE
SFA						
C10:0, capric	0.51	0.22	0.22	0.18	0.423	0.994
C11:0, undecanoic	2.8	2.3	2.08	0.23	0.478	0.837
C12:0, lauric	0.94	1.22	0.8	0.22	0.776	0.253
C13:0, tridecanoic	1.91	1.84	1.96	0.58	0.965	0.957
C14:0, myristic	2.43	1.71	1.51	0.27	0.379	0.256
C15:0, pentadecanoic	1.56	1.71	2	0.14	0.758	0.224
C16:0, palmitic	8.01	7.41	10.91	0.84	0.658	0.289
C17:0, heptadecanoic	12.09	9.7	9.09	0.76	0.275	0.855
C18:0, stearic	7.57	7.68	7.83	0.24	0.897	0.778
∑Saturated	37.82	33.79	36.39	1.04	0.414	0.375
MUFA						
C18:1ω7, vaccinic	0.8	1.17	2.09	0.38	0.368	0.431
C18:1ω9, oleic	7.91	7.7	8.88	0.82	0.881	0.757
∑Monounsaturated	8.71	8.87	10.97	1.16	0.928	0.666
PUFA						
C16:4ω3	13.96	15.36	12.97	0.8	0.442	0.394
C18:2ω6, linoleic	15.56	15.39	15.56	0.54	0.918	0.882
C18:3ω3, ɣlinolenic	1.64	5.29	5.28	0.64	0.036	0.995
C18:4ω3, alpha Octadecatetraenoic	10.03	9.94	10.14	0.51	0.942	0.903
C20:2ω6	6.5	7	5.94	0.35	0.698	0.031
C20:4ω3, ecosatartrienoic	4.18	3.87	1.85	0.8	0.891	0.182
∑Polyunsaturated	52.66	56.85	51.74	1.66	0.48	0.089
∑Unsaturated	61.37	65.72	62.71	1.27	0.484	0.5
Other fatty acids	0.81	0.49	0.9	1.29	0.607	0.377
∑Omega-6	22.06	22.39	21.5	0.31	0.652	0.399
∑Omega-3	30.6	34.45	30.87	1.81	0.521	0.237
Omega-6: omega-3	0.79	0.65	0.7	0.06	0.52	0.55

[1]Control, basal diet without oil supplement; LO, basal diet with linseed oil beads; LO+VE basal diet with linseed oil beads and vitamin E. [2] CON vs. LO = control diet vs diet supplemented with linseed oil; LO vs. VE = diet supplemented with linseed oil vs diet supplemented with linseed oil and vitamin E.SEM, standard error of the means. ∑ Saturated fatty acids: total saturated fatty acids, ∑ Unsaturated fatty acids: total unsaturated fatty acids, ∑ Mono unsaturated fatty acids: total mono unsaturated fatty acids, ∑ Poly unsaturated fatty acids: total poly unsaturated fatty acids

Monounsaturated fatty acids (MUFA):

Vaccinic acid (C18:1ω7) was increased numerically (p=0.36) in blood plasma (Table 7) and in milk (p= 0.25) (Table 8) with beads supply compared to control goats (1.17 *vs.* 0.8) in blood and (0.30 *vs.* 0.22) in milk, respectively indicating an incomplete biohydrogenation by microbial activity (Corl *et al.*, 2001). Also, vaccinic acid is known as the main precursor of conjugate linoleic acid (CLA), which is an isomers group of linoleic acid that has the capacity to perform several metabolic benefits in human as inhibition of lipogenesis, blood cholesterol, cancer incidence reduction and changes in muscle tissues growth (Rice *et al.,* 2012). Almeida *et al.* (2019) found the same trend of the present results with fish oil supplemented to goats and also, oleic acid was increased (P<0.01) in milk in the same group. Oleic acid (C18:1ω9) concentration as a main MUFA was not significantly affected by dietary additives in blood whereas, it increased (P<0.01) in milk of goats fed LO compared to CON goats. This could be due to higher uptake from blood and/ or higher synthesis from stearic acid as observed by Bas *et al.* (2007). As a result, the concentration of total MUFA was not affected in blood (p>0.05) however, it increased (P<0.01) in milk of goats fed on LO compared to CON goats. These results are in agreement with Gawad *et al.* (2015) who used batch culture system and demonstrated that protected forms of linseed oil (beads) didn't affect MUFA levels after 24h of incubation.

Table (8): Effect of supplementation of linseed oil beads with or without vitamin E on fatty acids profile (g of fatty acid/ 100 g of milk fat) in milk.

Fatty acid %	Treatment[1]			SEM	*P*- value[2]	
	CON	LO	LO + VE		CON *vs.* LO	LO *vs.* VE
SFA						
C8:0, caprlyric	2.05	1.89	1.6	0.09	0.392	0.348
C10:0, capric	7.73	6.25	6.21	0.29	0.083	0.757
C12:0, lauric	3.74	3.1	2.91	0.13	0.049	0.421
C14:0, myristic	8.87	8.18	8.34	0.11	0.015	0.495
C15:0, pentadecanoic	1.58	1.44	1.38	0.05	0.213	0.661
C16:0, palmitic	27.88	26.99	27.28	0.15	0.016	0.52
C17:0, heptadecanoic	1.95	2.19	2.1	0.08	0.291	0.584
C18:0, stearic	15.87	15.21	15.16	0.16	0.186	0.807
C20:0, arachidic	0.23	0.24	0.28	0.01	0.391	0.108
∑ Saturated fatty acid	69.9	65.49	65.26	0.68	0.008	0.558
MUFA						
C16:1ω7, palmitoleic	1.67	1.59	1.46	0.05	0.663	0.404
C18:1ω7, vaccinic	0.22	0.3	0.34	0.02	0.257	0.416
C18:1ω9, oleic	24.49	28.98	28.89	0.66	0.001	0.859
∑Monounsaturated	26.38	30.87	30.69	0.66	0.001	0.738
PUFA						
C16:3ω4, hexagonic	0.26	0.33	0.28	0.01	0.116	0.317
C18:2ω6, linoleic	2.39	2.28	2.37	0.05	0.521	0.682
C18:3ω3, ɣ-linolenic	0.19	0.37	0.33	0.02	0.001	0.147
C18:4ω3, alpha Octadecatetraenoic	0.23	0.37	0.38	0.02	0.028	0.892
C20:4ω6, arachidonic	0.29	0.2	0.17	0.02	0.118	0.238
∑Polyunsaturated	3.36	3.55	3.53	0.05	0.229	0.908
∑Unsaturated fatty acid	29.74	34.42	34.22	0.68	0.001	0.689
Other fatty acids	0.36	0.09	0.52	0.46	0.079	0.735
∑ Omega-6	2.68	2.48	2.53	0.04	0.048	0.569
∑ Omega-3	0.42	0.74	0.71	0.05	0.004	0.559
Omega-6: omega-3 ratio	6.45	3.37	3.59	0.44	0.005	0.582

1Control, basal diet without oil supplement; LO, basal diet with linseed oil beads; LO+VE basal diet with linseed oil beads and vitamin E. 2 CON vs. LO = control diet vs diet supplemented with linseed oil; LO vs. VE = diet supplemented with linseed oil vs diet supplemented with linseed oil and vitamin E. SEM, standard error of the means. ∑ Saturated fatty acids: total saturated fatty acids, ∑ Unsaturated fatty acids: total unsaturated fatty acids, ∑ Monounsaturated fatty acids: total monounsaturated fatty acids, ∑ Poly unsaturated fatty acids: total poly unsaturated fatty acids.

Polyunsaturated fatty acids (PUFA):

Linseed oil beads treatment resulted in greater concentration of linolenic acid (C18:3ω3) in blood plasma ($P<0.05$) (Table 7) and in milk ($P<0.01$) (Table 8) compared to CON group. This finding was expected as a consequence of its high dietary level in oil beads composition (Table 2) where part of this FA passed through the rumen without suffering biohydrogenation, then incorporated into the milk. Results of Almeida *et al.* (2019) agree with the present finding. The linoleic acid (C18:2ω6) supply for blood plasma was comparable among treatments ($p>0.05$), consequently, the concentration of this acid was not changed on goat's milk among treatments (Table 8). Although, alpha octadecatetraenoic (C18:4ω3) acid was not affected in blood plasma but it's concentration was increased ($P<0.05$) in milk on LO feeding compared to control group (0.37 vs. 0.23), respectively. Kholif *et al.* (2015) concluded that feeding dairy goats with fat source affecting indirectly lipogenesis in the mammary glands. The total PUFA proportion was numerically influenced by LO beads in blood plasma and in milk ($p>0.05$). There were no significant differences between dietary groups in blood plasma ($p>0.05$) regarding the total of omega-6, omega-3 or omega-6/omega-3 ratio while in milk, the total omega-6 and omega-6/omega-3 ratio were decreased significantly ($P<0.05$ and $P<0.01$, respectively) with a desirable increase in concentration of total omega-3 ($P<0.01$) due to beneficial effects of this FA for human health (Ruxton, 2007) compared to CON milk. Gawad *et al.* (2015) proved that the content of PUFA, omega-3 and omega-6 were increased with beads supply and decreased ratio of omega-6/ omega-3 after incubation with batch culture system. The higher concentrations of unsaturated fatty acids (UFA) supplied for the blood plasma ($P>0.05$) and milk ($P>0.01$) of goats supplied with LO may be attributed to greater fat content in feed additives resulting in increased absorption of UFA then transferred from diets to milk (Almeida *et al.*, 2019). Thus, it is a considerable gain in nutritional value of milk in terms of UFA especially omega-3 fatty acids contents. A similar trend was recorded in the recent study of Almeida *et al.* (2019) with different oilseeds sources. Lastly, an effect of vitamin E on FA profiles is limited. Chikunya *et al.* (2004) consistent with the current results where they proved that vitamin E supplementation to the sheep diet didn't affect the efficiency of rumen BH nor the trans C18:1 proportion among FAs.

CONCLUSION

Encapsulated beads supply as a source of polyunsaturated fatty acids did not affect the DMI, dose weight, birth weight and weight after 60 days of kids but increased milk production and the yield of all macro compounds of it as fat corrected milk, fat, protein, lactose and total solids and fat% only. Also, dietary supply of PUFA increased TVFAs, changed the FAs profile in blood plasma and in milk. Linseed oil beads promoted the increment of unsaturated FA particularly omega-3 FA and reduced SFA, omega-6 FA and omega6: omega-3 ratio in milk. Consequently, milk FA profile was improved and became healthier for consumers. However, the inclusion of vitamin E combined with linseed oil beads did not result in benefits to goat's milk. Further in vivo studies should be undertaken to explore suitable level of vitamin E in association with protected form of useful and healthy fatty acids to improve milk FA profile.

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تأثير إضافة كبسولات زيت بذرة الكتان مع فيتامين هـ علي أداء و مشتقات الدم و كمية اللبن في الماعز الحلابة

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تهدف هذه الدراسة ألي تقييم تأثير إضافة كبسولات زيت بذرة الكتان فقط أو مع فيتامين هـ كإضافات غذائية علي أداء الحيوان و تركيب الأحماض الدهنية في بلازما الدم و في اللبن أثناء فترة الرضاعة. تم تقسيم 24 من المعز الدمشقي العشار في منتصف الحمل (متوسط وزن 44.5 ± 1.2 كجم) الي ثلاث مجموعات (8 ماعز للمجموعة). المجموعة الأولي (المقارنة) تغذت علي دريس البرسيم و مخلوط العلف المركز بنسبة 50:50 بدون اي إضافات ، المجموعة الثانية تغذت علي نفس غذاء مجموعة المقارنة + 2.5 جم كبسولات زيت بذرة الكتان لكل حيوان و المجموعة الثالثة تغذت مثل مجموعة المقارنة + 2.5 جم كبسولات زيت الكتان + 600 وحدة دولية من فيتامين هـ لكل حيوان. استمرت التجربة 135 يوم من منتصف الحمل حتي 60 يوم بعد الولادة . أظهرت النتائج أن إضافة كبسولات زيت الكتان نتج عنها زيادة معنوية كبيرة في كمية اللبن و كمية الدهون و البروتين و سكر اللاكتوز و المكونات الصلبة باللبن و كذلك النسبة المئوية للدهن مقارنة بمجموعة المقارنة. ارتفع مستوي حمض الأوميجا -3 بيلازما الدم مع الكبسولات و كذلك أحد احماض الأوميجا -6 مع إضافة الفيتامين. و في اللبن :إنخفض مستوي الأحماض الدهنية المشبعة بينما ارتفع مستوي الأحماض غير المشبعة الأحادية و المتعددة التشبع مع إضافة الكبسولات مما نتج عنه انخفاض النسبة الكلية للأحماض الدهنية المشبعة و أحماض الأوميجا-6 الكلية و النسبة بين أوميجا-6 : أوميجا -3 مع ارتفاع نسبة الأحماض الدهنية الغير مشبعة و أحماض الأوميجا -3 . مما ترتب عليه إنتاج لبن صحي و أكثر فائدة بدرجة كبيرة. أيضا تحسنت بعض مشتقات الدم حيث انخفض مستوي الكوليستيرول و الكرياتينين و مستوي انزيم الليبيز مقارنة بمجموعة المقارنة. و علي الجانب الأخر فقد ارتفعت في الدم نسبة البولينا و الدهون الثلاثية والبروتينات الدهنية قليلة و عالية الكثافة و مضادات الأكسدة الكلية بينما انخفض نشاط انزيم الليبيز مع إضافة فيتامين هـ. نستخلص مما سبق ان إضافة كبسولات زيت الكتان أسهمت في زيادة انتاج اللبن ذو مواصفات صحية مرتفعة للمستهلك لأن زيت الكتان يرفع من القيمة الغذائية للبن بزيادة الأحماض الدهنية المفيدة به. تبين ان إضافة فيتامين هـ مع الكبسولات أدي الي تحسن في منتجات الماعز محل الدراسة. لهذا فمن المهم زيادة الأبحاث و الدراسات علي الحيوان للوصول للمستوي المناسب لمنتج أفضل من كبسولات زيت الكتان و الفيتامين.