Plant Archives Volume 20 No. 2, 2020 pp. 5570-5574 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF LINSEED AND SUNFLOWER OIL SUPPLEMENTATION TO RATION ON THE ESTROGEN AND PROGESTERONE HORMONES LEVELS IN AWASSI EWES DURING THE LAST STAGE OF PREGNANCY AND LACTATION PERIOD

Noor M. Hayawy and Abdulnassir Th. M. Alkhashab*

Department of Animal Production, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.

Abstract

This study was conducted in the field of animal production, department of animal production/College of Agriculture and Forestry/University of Mosul. 16 Awassi ewes were used, at the age of 2-3 years with an average live weight of 60 ± 1.25 Kg. The ewes were randomly divided in to 3 groups (6 ewes each group) and treated as follow. The first group was regard as control group supplied with 1 Kg concentrate ration + 500 g roughage ration (wheat straw), second group supplied with 1 Kg concentrate ration + 500 g roughage ration (wheat straw) + 4% linseed oil , third group supplied with 1 Kg concentrate ration + 500 g roughage ration (wheat straw) + 4% sunflower oil. Blood samples were taken from animals during the different stages of study (Fifth pregnancy month, postpartum, lactating months) for measuring estrogen and progesterone hormone levels in blood serum of the ewes. The results showed an a significant effect of oils supplementation to ration on the estrogen hormone level and no effect on progesterone hormone level during the different stages of study.

***Key words*:** linseed oil, sunflower oil, estrogen H., Progesterone H., Awassi ewes.

Introduction

Several studies of researchers showed that fatty acids especially omega-3 fatty acids in ruminant diet improve the animal's reproductive performance and this improvement may be due to a state of energy balance in the diet and an increase in production of steroid hormones like (progesterone hormone) (Meteab, 2019) and that increase in the level of progesterone hormone is necessary in maintaining the pregnancy in the animal and reducing prostaglandin $PGF_2\alpha$ level which defused from uterus , and leads to a reduction in miscarriage or newborn deaths (Ambrose *et al.,* 2006). The animals reproductive performance is greatly affected by the amount of fatty acids processed in the diet more than the level of energy equipped (Staples *et al.,* 2005) and these fats added to the diet increase the concentration of cholesterol in blood plasma and thus increase the steroid hormones, as cholesterol is the main compound in the process of synthesis these hormones (estrogen and progesterone) and the high level of cholesterol in diet may lead to an increase the level of these hormones and reduce the process of lower hormones levels in blood (Hawkins *et al.,* 1995). As well known, estrogen and progesterone had a role in growth and development of the mammary gland during puberty and pregnancy and the level of the hormone in blood reflects the reproductive state of animal and the period after lamping in animals is characterized by the restoration of uterine recession of its natural state and the revitalization of ovarian activity that must occur and prepare for pregnancy and new birth (Qureshi *et al.,* 2000). It is known that linseed oil is considered as one of the sources rich in unsaturated fatty acids of omega-3 as contains a high percentage of alpha linolenic acid (Xu *et al.,* 2016), so it was used in ruminant diets to improve reproductive and productive performance (Dirandeh *et al.,* 2013) and also sunflower oil was used in ruminant diets which is consider one of oils rich in unsaturated fatty acids as omega – 6 especially linoleic acid (Elsetiha *et al.,* 2015). So this study aimed to find out the effect of linseed oil and sunflower oil supplementation to the ration as energy sources and

***Author for correspondence :** E-mail : drnasiralkhashab@gmail.com

unsaturated fatty acids on estrogen and progesterone levels in the last pregnancy, postpartum period and lactation period.

Materials and Methods

This experiment was conducted in the animal field of animal production department, college of Agriculture and forestry, University of Mosul during the period from 12/9/2019 to 20/1/2020 Eighteen Awassi ewes aged 2-3 years and 60 ± 1.5 kg average live body weight, the ewes taken from the sheep flock of animal production department/college of Agriculture and Forestry. The ewes was in the last stage of pregnancy (110 days) after conducting the process of Estrus synchronization by using vaginal sponges containing progesterone hormone . The ewes were subjected to health care to ensure their safety from disease according to preventive program in animal field.

Experimental design

Eighteen pregnant Awassi ewes (110days) were randomly allocated in to three equal groups (6 head each group) and the ewes kept in three similar (3×4 m) pens assigned to experiment and were placed under the same environmental conditions . All the ewes were fed for week as initial period on stander diet composed of barley seeds, Wheat bran, soybean meal, urea, salts, vitamins and limestone. Then the ewes were distributed to the following treatments : first group fed 1 kg concentrate ration + 500 g / head wheat straw as roughage without additive , second group fed 1 kg concentrate ration + 500 g/head wheat straw with 4% linseed oil , third group fed 1 kg concentrate ration + 500 g/h. Wheat straw with 4% sunflower oil. The experimental animals had free access to water and mineral block. Feed was offered to animals twice daily at (8:00 h am and 4:00 h pm) totally at 2.5% BW. Table 1 shows ingredient and nutrient composition of experimental groups.

Blood samples were taken in tenth day of prepartum, 24 hours postpartum and every month of lactation period. 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. Serum estrogen (E_2), progesterone (P_4) levels were assayed using (cobas E 411) German device originating from Roche company . This device works with chemiluminescence technique to measure hormone concentrations.

Statistical Analysis

The results were analyzed by the electronic calculator by applying statistical analysis system (Anonymous , 2000) using the complete random design (CRD) according to the following mathematical model:

$yij = \mu + ti + eij$

As:

Yij = The transaction value of j for transaction i

μ = The general mean of the studied character.

ti = Effect of the studied treatments (the study included three treatments).

Eijk = Random error which is distributed naturally at an average of zero and a variance of $2e\sigma$.

The averages were compared using Duncan (1955) Test.

Results and Discussion

The result of statistical analysis table 2 showed a significant ($p \leq 0.05$) effects of treatments on estrogen (E_2) level in the last month of pregnancy between experimental treatments, as the level of hormone concentration reach (69.52 pg/ml) in linseed oil treatment group which was significantly ($p \leq 0.05$) differ than (53.07 pg/ml) in sunflower oil treatment and (45.62 pg/ml) in control group. Then estrogen level decreased to lowest level after 24 h. of postpartum in experimental groups, but there was no significant variance in its level between treatments . Estrogen levels remained low during the first

Table 1: Ingredient and chemical composition of experimental rations.

Ingredient	Control g.	Linseed oil g.	Sunflower oil g.
Barley seeds	70	50	50
Wheat brand %	23	39	39
Soya meal %	5	5	5
Urea %	1	1	1
Limestone %	0.5	0.5	0.5
Salt %	0.5	0.5	0.5
Linseed oil %	0	4	
Sunflower oil %			4
Chemical composition of rations %			
DM*	92.6	91.2	91.2
OM*	93.23	92.4	92.4
CP**	15.57	15.6	15.6
E.E*	4.20	2.31	2.31
CF**	9.92	8.92	8.92
ME(Mcal./kgDM)***	2.762	2.893	2.893

*Estimated laboratory on dry mater basis according to A.O.A.C.(2007).

**Calculated according to (Al-Khawaja et al ,1978).

***The energy of oils supplementation calculated according to (NRC.2001).

month of lactation, however, hormone level during first month of lactation was significantly ($p \leq 0.05$) higher in control group compared to its level in sunflower oil treatment groups, while estrogen hormone level be increased significantly ($p \leq 0.05$) during the second and third month of lactation in linseed oil treatment compared with its level in sunflower oil treatment or control group . The estrogen level which obtained in this study was below the (98.65 pg/ml) level obtained by Alwan *et al.,* (2010) in prepartum and decreased to (4.1pg/ml) in postpartum in Iraqi sheep. The high level of estrogen in groups treated with oils, especially linseed oil during the last month of pregnancy agree with Wanhong *et al.,* (2017) who found that adding 4% linseed oil which it rich in unsaturated fatty acids led to a significant increase in estrogen concentration level. Also the results in agreement with Elsetiha (2015) which got an increase in estrogen level in rabbit fed diets supplemented with 1%, 2%, 3% sunflower oil than control group. Fats supplementation to the diet of ruminants may lead to an improvement in reproductive and productive performance of the animals and that the reproductive performance is greatly affected by the amount of fatty acids added to diet more than energy level (Staples *et al.,* 2005) and the oils adding to diets increase the concentration of cholesterol , which is considered the basis or the synthesis of steroid hormones and the high level of cholesterol in blood may lead to an increase in steroid hormones levels (Estrogen and progesterone) (Hawkins *et al.,* 1995).

The result about progesterone hormone (P4) in table 3 showed that there were no significant differences between treatments in all experimental stages (fifth month of pregnancy, postpartum, three months of lactation) in ewes, despite the absence of significant differences between treatments, however progesterone level was arithmetically high in groups treated with oils compared with control group and linseed oil group exceeded mathematically in P4 level than its level in sunflower oil group. The results in agreement with Mahmoud *et al.,* (2019) who noticed that adding linseed oil to ration had no significant effect on P4 level in ewes during the last pregnancy compared to control group, also results in agreement with Elsetiha (2015) who did not found a significant variance in P4 level in rabbits fed on 1%, 2%, 3% sunflower oil, also Wannacott *et al.,* (2010) indicated that P4 concentration level was higher in sheep fed ration rich in omega-3 fatty acids than sheep fed ration rich in omega-6 fatty acids and the P4 level depend on the fat percentage in corpus luteum cells and total steroids percentage depends on the amount of fat supplementation in ration and the concentration of cholesterol in blood serum (Hawkins *et al.,* 1995). In contrast of our results Amir *et al.,* (2014) found that the group fed on 5% linseed oil recorded a significant increase in P4 level during pregnancy compared with that fed 5% sun flower oil and control group. Also Asgari *et al.,* (2013) notice a significant increase in P4 level in ewes fed line seed oil and significantly differ than sunflower oil and control groups in the first stage of pregnancy and then insignificantly decreased after lamping, however, the level of hormone was higher in treatment groups than in control group. This is consistent with EL- Shahat *et al.,* (2009) who indicated that adding unsaturated fatty acids in the diet leads to an improvement in P4 level in blood plasma.

Table 2: Effect of oils treatments on Estrogen hormone level in blood plasma (means ± stander error).

No.	Period Groups	Fifth month of pregnancy Pg/ml	Post partum Pg/ml	First month of lactation Pg/ml	Second month of lactation Pg/ml	Third month of lactation Pg/ml
1	Conttrol	45.62 ± 5.66 b	6.84 ± 1.14 a	6.66 ± 2.21 a	7.72± 1.13 b	29.63± 3.72 b
2	Linseed oil	69.52± 31.51 a	5.10± 0.44 a	5.95± 1.13 ab	9.84± 1.22 a	44.03 ± 3.26 a
3	Sunflower oil	53.07± 4.32 b	7.32± 1.13 a	5.45± 0.44 b	8.1± 1.03 b	33.5± 2.66 b

a.b. means in the same column have the different superscript are significantly different at ($p \leq 0.05$) .

Table 3: Effect of oils treatments on Progesterone hormone level in blood plasma (mean ± stander error).

No.	Period Groups	Fifth month of pregnancy ng/ml	Post partum ng/ml	First month of lactation ng/ml	Second month of lactation ng/ml	Third month of lactation ng/ml
1	Control	5.34± 1.21 a	0.65± 0.22 a	0.58± 0.06 a	0.42± 0.15 a	0.40±0.12 a
2	Linseed oil4 %4	6.54± 1.22 a	1.38± 0.17 a	0.52± 0.29 a	0.41± 0.08 a	0.45± 0.08 a
3	Sunflower oil %4	6.44± 1.07 a	1.1 5± 0.24 a	0.47± 0.11 a	0.37± 0.17 a	0.39± 0.06 a

a.b. means in the same column have the different superscript are significantly different at ($p \leq 0.05$).

It was also noted from the results that P4 level in control group close to the value (0.6 ng/ml) obtained by Alwan *et al.,* (2010) after lamping in Iraqi sheep, but Medan and Deck (2015) indicate that P4 concentrate level keep it lower than (1ng/ml) during postpartum to three months of lactation, While our results were slightly higher than (3.47 ng/ml) P4 level in fifth month of pregnancy and decreased to 1.36 ng/ml after lamping, which founded by Kamil (2018) in Turkish Awassi ewes, but our results of P4 level in lactation months was slightly lower than the researcher obtained. Finally the level of P4 indicates the activity of the ovary and the occurrence of estrus cycle after lamping and the P4 concentration in blood plasma after lamping is an indication of activity of corpus luteum in ovaries because this hormone is created from the cells of corpus luteum (Hileman *et al.,* 2011), but the level of P4 remains low along period after lamping indicates that ewes are affected by season and the rise of prolactin hormone as well as the effect of feeding factor and lactation factor of newborns (Medan and Dack, 2015) (Schirar *et al.,* 1989).

Conclusion

The results of this study showed that 4% supplementation of linseed or sunflower oils had a significant effect on Estrogen hormone level during the last month of pregnancy, postpartum and the last two months of lactation, but no effect on P4 level during all experimental stages.

Acknowledgments

The authors thanks the presidency of the University of Mosul and the dean of the college of Agriculture and forestry and thank the head of the animal production department and those working in the field of animal production department.

References

Al-Khawaja, A.K., I.A. Al-Bayati and S. Abdul-Ahad (1978). Chemical composition and nutritional value of Iraqi feed materials. Third Edition. Department of Nutrition - Ministry of Agriculture and Agrarian Reform. The Republic of Iraq. (In Arabic).

Alwan, A.F., F.A.M. Amin and N.S. Ibrahim (2010). Blood Progesterone and Estrogen Hormones Level During Pregnancy and after birth in IRAQI Sheep and Goat. *Bas. J. Vet. Res.,* **10(2):** 153–157.

Anonymous (2002). Users Guide : tatistics. Version 8 Edition. SAS Institute Inc., Cary, NC., USA.

Ambrose, D.J., J.P. Kastelic, R. Corbett, P.A. Pitney, H.V. Petit, J.A. Small *et al.,* (2006). Lower pregnancy losses in lacting dairy cows fed a diet enriched in α-linolenic acid. *J. Dairy Sci.,* **89:** 3066–74.

Amir, H.A.S., N. Dayyani and N.M. Kor (2014). Effect of fatty acids on reproduction and blood hormones in Ashwari ewes. *Online J. Vet. Research,* **18(5):** 432-437.

AOAC (2007). Official method of analysis 17th ed. (Association of official analytic chemists), Washington, DC.,USA.

Asgari safdar, A., H. Daghigh Kia, G. Moghaddam, A. Khani and S. Alijani (2013). Reproductive Performance and Blood Metabolites Concentration in Iranian Afshari Ewes Fed Calcium Salts of Fatty Acids (CSFA) in Flushing Period, *International Journal of Advanced Biological and Biomedical Research,* **1:** 669–676.

Duncan, D.B (1955). Multiple range and multiple F-test. *Biometrics,* **11:** 1-42.

Dirandeh, E., A. Towhidi, S. Zeinoaldini, M. Ganjkhanlou, Z. Ansari Pirsaraei and A. Fouladi-Nashta (2013a). Effects of different polyunsaturated fatty acid supplementations during the postpartum periods of early lactating dairy cows on milk yield, metabolic re-sponses and reproductive performances. *J. Anim. Sci.,* **91:** 713–721.

Elsetiha, W.E.H. (2015). The productive performance of Rabbits fed on diets supplemented with sunflower oil. Thesis of master Sci. Fac. Agri. Tanta Univ.

El-Shahat, K.H. and M. Abo-El maatyAmal (2009). He effect of dietary supplementation with calcium salts of long chain fatty acids and/or l-carnitine on ovarian activity of Rahmani ewes. *Animal. Reproduction Science,* **117:** 78–82.

Fuentes, M.C., S. Calsamiglia, C. Sanchez, A. Gonzalez, J.R. Newbold, J.E.P. Santos, L.M. Rodriguez-Alcala, J. Fontecha (2008). Effect of extruded linseed on productive and reproductive performance of lactating dairy cows. *Livestock Science,* **113:** p.144-154,. DOI: 10.1016/ j.livsci.2007.03.005.

Hawkins, D.E., K.D. Niswender, G.M. Oss, C.L. Moeller, K.G. Odde, H.R. Sawyer and G.D. Niswender (1995). An increase in serum lipids increases luteal lipid content and alters the disappearance rate of progesterone in cows. *J. Anim. Sci.,* **73:** 541–545.

Hileman, McManus C.J., R.L. Goodman and H.T. Jansen (2011). Neurons of the lateral preoptic area/rostral anterior hypothalamic area are required for photoperiodic inhibition of estrous cyclicity in Sheep. *Biol. Reprod.,* **85:** 1057–1065.

Kamil, M.A. (2018). Progestrone level of lactating ewes influenced by parity order, type of lambing and lamb sex. *Advance in animal and veterinary sci.,* **7(4):** 295–300.

NRC (2001). Nutrient Requirements of Small Ruminants. Natl. Acad. Of Sci., Washington, DC. USA.

Mahmoud, S., El-Tarabany Mostafa, A. Atta, Sana S. Emara and Mohamed M. Mostafa (2019). Folic acid and flaxseed oil supplements in Ossimi ewes: effect on body weight changes, progesterone profile, blood chemistry and litter traits. *Tropical Animal Health and Production,* **52(1):**

301–308.

Medan, El-Daek T. (2015). Uterine involution and progesterone level during the postpartum period in Barbary ewes in north Libya. *Open Vet. J.,* **5(1):** 18-22.

Meteab, M.I., H.M. El-Sayed, M. Abeer, EL-Essawy, M.S. Nassar and N.E. El-Bordeny (2019). Effect of dietary incremental levels of flaxseed supplementation on productive performance of lactating Damascus goats. *International Journal of Environment, Agriculture and Biotechnology,* **5(1):**.

Qureshi, Habib G., G. Nawab, M.M. Siddiqui, N. Ahmad and H.A. Samad (2000). Milk progesterone profiles in various reproductive states in dairy buffaloes under field conditions. *Proc. Natl. Sci. Counc. ROC (B).,* **24(2):** 70-75.

Schirar, A., Y. Cognie, F. Louault, N. Poulin, M.C. Levasseur and J. Martinet (1989). Resumption of estrus behavior and cyclic ovarian activity in suckling ewes and non-suckling ewes. *J. Reprod. Fertil.,* **87:** 789-794.

Staples, C.R. and W.W. Thatcher (2005). Effects of fatty acids on reproduction of dairy cows. Pages 229-256 in Recent Advances in Animal Nutrition, ed. P.C. Garnsworthy and J. Wiseman, Nottingham University Press, England.

Wanhong L., D. Tang, F. Li, H. Tian, X. Tue, Fe. Li, X. Weng, W. Sun, W. Wang and F. Mo (2017). Supplementation with dietary linseed oil during peri-puberty stimulates steroidogenesis and testis development in rams. *Thriogenology,* **102:** 10–15.

Wonnacott, K.E., W.Y. Kwong, J. Hughes, A.M. Salter, R.G. Lea, P.C. Garnsworthy and K.D. Sinclair (2010). Dietary omega-3 and -6 polyunsaturated fatty acids affect the composition and development of sheep granulosa cells, oocytes and embryos. *Reproduction,* **139(1):** (January 2010), pp. 57-69. ISSN 1470-1626.

Xu, J., H. Gao, L. Song, W. Yang, C. Chen, Q. Deng, Q. Huang, J. Yang and F. Huang (2013). Flaxseed oil and α-lipoic acid combination ameliorates hepatic oxidative stress and lipid accumulation in comparison to lard. *Lipids Health Dis.,* **1(12):** 58.