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*Original Article*

# Effects of Flaxseed-rich Diet on Reproductive Performance in Estrous-synchronized Baluchi Ewes

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**ABSTRACT**

The purpose of this study was to compare the effects of using different levels of flaxseed in diets on the reproductive performance of estrous-synchronized Baluchi ewes (para 3). Diets contained either basal diet (control) or different levels of extruded flaxseed (2%, 5%, 7%, 10%, and 12%) and were fed from lambing to 60 days after lambing. The ewe estrus cycles were synchronized using controlled internal drug release (CIDR) for 14 days starting from day 16 of fat supplementation. The rams were introduced 24 h after CIDR removal. The ewes fed control diets had the highest mean dry matter intake (1,800±35 g) which was declined with the increase of flaxseed levels. The experimental diets exerted no effects of urea concentration in blood plasma. However, plasma glucose concentration was lower ($P<0.05$) in the ewes fed the control diet and 2% flaxseed, compared to those in other groups. Nonetheless, there was no difference among the ewes fed 5%, 7%, 10%, and 12% flaxseed in terms of plasma glucose concentrations ($P<0.05$). The ewes fed 2% flaxseed had the highest level of plasma triglyceride concentration among other groups. In addition, the control group had the lowest level of plasma total cholesterol and low-density lipoproteins concentration in comparison to other groups ($P<0.05$). However, plasma nonesterified fatty acids and β-hydroxybutyrate concentrations were similar among the groups ($P>0.05$). The mean interval between CIDR removal and the exhibition of estrus ranged from 30 to 40 h with the shortest interval being recorded in the ewes fed 12% flaxseed ($P<0.05$). The control group had the lowest number of follicles on estrus day among other groups ($P<0.05$). Furthermore, the ewes fed 10% and 12% flaxseed had the highest ovulation, pregnancy, and lambing rates, compared to other groups ($P<0.05$). In conclusion, the findings revealed that feeding the ewes with 10% and 12% flaxseed resulted in the improvement of reproductive performance.

**Keywords:** Flaxseed, Ovulation, Reproduction, Sheep, Ultrasonography

**Effets d'un Régime Riche en Graines de Lin sur les Performances Reproductives des Brebis de Race Baloutchi avec œstrus Synchronisé**

**Résumé:** Le but de cette étude était de comparer les effets de l'utilisation de différentes quantités de graines de lin dans les régimes alimentaires sur les performances de reproduction des brebis de race Baloutchi avec œstrus synchronisé (paragraphe 3). Les régimes alimentaires contenaient soit un régime de base (témoin), soit différentes quantités de graines de lin extrudées (2 %, 5%, 7%, 10% et 12%) et ont été nourris de l'agnelage jusqu'à 60 jours après l'agnelage. Les cycles d'œstrus de la brebis ont été synchronisés en utilisant la libération interne contrôlée de médicament (CIDR) pendant 14 jours à partir du jour 16 de la supplémentation en graisses. Les béliers ont été introduits 24 h après l'élimination du CIDR. Les brebis nourries avec des régimes témoins montraient la plus forte consommation moyenne de matière sèche (1,800±35 g), qui a été diminuée avec

l'augmentation de l'apport en graines de lin. Les régimes expérimentaux n'ont exercé aucun effet sur la concentration d'urée dans le sang. Cependant, la concentration plasmque de glucose était plus faible (P <0,05) chez les brebis nourries avec le régime témoin et 2% de graines de lin, par rapport à celles des autres groupes. Néanmoins, il n'y avait aucune différence entre les brebis nourries avec 5%, 7%, 10% et 12% de graines de lin en termes de concentrations plasmiques de glucose (P <0,05). Les brebis nourries avec 2% de graines de lin montraient les concentrations plasmiques de triglycérides les plus élevées. En outre, le groupe témoin avait le plus faible taux de cholestérol total et de lipoprotéines de basse densité par rapport aux autres groupes (P<0,05). Cependant, les concentrations plasmiques d'acides gras non estérifiés et de β-hydroxybutyrate étaient similaires dans les différents groupes (P>0,05). L'intervalle moyen entre l'élimination du CIDR et l'exposition d'œstrus variait de 30 à 40 h, l'intervalle le plus court étant enregistré chez les brebis nourries avec 12% de graines de lin (P<0,05). Le groupe témoin avait le plus petit nombre de follicules au jour de l'œstrus parmi les autres groupes (P<0,05). En conclusion, les résultats ont révélé qu'un régime alimentaire comprenant 10% et 12% de graines de lin entraînait une amélioration des performances de reproduction chez les brebis.
**Mots clés:** Graines de lin, Ovulation, Reproduction, Mouton, Échographie

## INTRODUCTION

Several methods have been researched for enhancing the reproductive performance of ewes, such as nutritional manipulation and estrous synchronization (Evans and Maxwell., 1990). Studies are indicative of the beneficial effect of nutritional flushing on the ovulation rate in ewes. The addition of protected fat to a ruminant diet may decrease negative energy balance or shorten the duration of this period. The use of energy-rich additives, such as fat supplements, may modulate the recrudescence of hypothalamic and pituitary function and therefore ovarian activity through affecting the overall energy status of the female. Furthermore, greater dietary fat ingestion has direct effects on the ovarian structures (Dirandeh et al., 2013a; Dirandeh et al., 2013b; Dirandeh et al., 2015). Based on the evidence, feeding cows (Esposito et al., 2014) and sheep (Scaramuzzi et al., 2006) with fatty acids results in an improvement in their reproductive performance (Fouladi-Nashta et al., 2009). One of the reasons explaining this I the alteration in the follicular fluid fatty acid composition, granulosa, and vitelline membrane. Consequently, it may lead to a betterment in the interaction between ligand and the corresponding membrane receptors, as well as the resulting gene expression. The other two reasons raised for this effect include the improvement of folliculogenesis, oocyte cytoplasmic maturation, steroidogenesis, and ovulation rate and the enhancement of embryonic survival and pregnancy maintenance. The purpose of this study was to investigate the reproductive parameters of estrous-synchronized Baluchi ewes fed different levels of flaxseed.

## MATERIAL AND METHODS

This study was conducted on 90 Baluchi ewes, which were in their second or third pregnancy, with the mean body weight of 45±3 kg about 105±10 days post-lambing, from May to December 2017. The animals were allotted into six groups (n=20), housed in individual pens (2×2.5 m), and fed one of the six experimental diets. Water was freely available, and 2 weeks were allowed for adaptation to the experimental conditions. The experimental diets (71% dry matter, 2.42 Mcal metabolizable energy per kg/dry matter) contained various proportions of starch and fat, with or without flaxseeds (Table 1). The control group was fed

a basic ration without flaxseed, while the other groups were subjected to 2%, 5%, 7%, 10%, or 12% flaxseed-rich diet. In order to ensure ad libitum intake, feeding was performed in two equal portions at 7 a.m. and 4 p.m. Feed intake was recorded daily until the end of the synchronization period. Estrous was synchronized using controlled internal drug release (CIDR, Newzeland), containing 0.3 g progesterone, inserted on day 30 of the experiment, which was removed after 14 days. In order to induce ovulation, an injection of 300 IU eCG was performed on the day of CIDR removal. The standing estrous was detected during a 4-day period using castrated rams by observing the footage recorded with surveillance cameras in each barn and video recorder. The ewes were subjected to intracervical insemination using a mixture of semen samples from four fertile Baluchi rams. The semen was collected by the artificial vagina, and estrous ewes were used to sexually stimulate the rams. Ultrasonography was performed (ECM model, France; 8 MHz probe) to determine the number of follicles (>4 mm) at the time of CIDR removal and diagnose pregnant ewes 34 days after artificial insemination (Table 4). The number of ewes having corpus luteum was divided by the total number of ewes to calculate the ovulation rate. In addition, the calculation of the rate of pregnancy was accompanied by dividing the number of pregnant ewes by the number of inseminated ewes. To calculate the estrous rate, the number of estrous ewes was divided by the total number of ewes in different experimental groups. The number of ewes showing estrus and the interval between the removal of CIDR and the onset of estrus were calculated in each treatment on a daily basis. Lambing rate was also based on the number of lambs born per ewe lambing, and the twinning rate was mentioned as the number of twin-bearing ewes divided by the number of ewes lambing. In the final week of the experiment, jugular blood was collected in EDTA-containing vacuum tubes at 9:00 a.m. (2 h after the morning feeding). Blood samples were centrifuged at 1500× g for 15 min, and plasma was stored at -20 °C. The samples were then used for the determination of cholesterol, glucose, urea, triglycerides, low-density lipoproteins (LDL), and high-density lipoproteins (HDL), using the commercially available kits (Pars Azmoon, Tehran, Iran). Furthermore, β-hydroxybutyrate (BHBA, Abbott Diabetes Care Ltd., Witney, UK) and nonesterified fatty acids (NEFA, FA 115, Randox Laboratories Ltd., Antrim, UK) were determined enzymatically using a spectrophotometer (Shimadzu 2100, Kyoto, Japan). The PROC GLM was used to analyze the data in SAS software (Windows Version Release 8.02, SAS Inst., Inc., Cary, NC). In addition, the K-score test was employed to compare the reproductive parameters calculated as percentages. The comparison of other mean values was also performed using the Tukey's test. The significance level was set at $< 0.05$.

## RESULTS

**Dry matter intake.** The ewes fed the control diet (1,800±35 g) had the highest level of dry matter intake, which was declined with an increase in flaxseed levels. The mean scores of dry matter intake were obtained as 1,750±25, 1,740±33, 1,750±28, 1710±16, and 1720±11 g in the groups fed 2%, 5%, 7%, 10%, and 12% flaxseed, respectively.

**Blood metabolites.** The concentrations of blood metabolites are shown in Table 2. Urea concentration in blood plasma was not affected by the experimental diets. Plasma glucose concentration was lower ($P<0.05$) in the ewes fed the control diet and 2% flaxseed, compared with those in other groups. There was no difference among the ewes fed 5%, 7%, 10%, and 12% flaxseed in terms of plasma glucose concentrations ($P<0.05$, Table 2). The ewes fed 2% flaxseed had the highest level of plasma triglyceride concentration. Furthermore, the control group had the lowest levels of plasma total cholesterol and LDL concentrations as compared with other groups ($P<0.05$, Table 2). Plasma NEFA and BHBA concentrations were found to be similar among the research groups ($P>0.05$, Table 2).

**Reproductive responses.** The effect of experimental diets on the reproductive characteristics of the ewes is shown in Table 3. The proportion of the ewes showing estrus was similar among groups (P>0.05; Table 3). The mean interval between CIDR removal and the exhibition of estrus ranged from 30 to 40 h with the shortest interval being recorded for the ewes fed 12% flaxseed (P<0.05; Table 3). The ewes fed 10% and 12% flaxseed had the lowest number of follicles on the CIDR operation day, compared to other groups (P<0.05; Table 3). However, on the estrus day, the control group had the lowest number of follicles in comparison to other groups (P<0.05; Table 3). Furthermore, the ewes fed 10% and 12% flaxseed had the highest rates of ovulation, pregnancy, and lambing in comparison to other groups (P<0.05, Table 5).

## DISCUSSION

The dry matter intake of the estrus-synchronized ewes was evaluated in the present study. The results confirmed the findings obtained by Bell et al. (2006), who reported that the reduction of dry matter intake was caused by the negative effects of fat, especially fats with high levels of unsaturated fatty acids, on ruminal microorganisms and rumen fermentation. However, there are also reports indicating that fat in the type of oil seeds had no effect on dry matter intake (Danesh Mesgaran and Stern, 2005). In a study conducted by Bell et al. (2006), fish oil supplementation, administered 21 days before lambing up to 10 days after lambing, had no effect on dry matter intake in ewes. The present study involved the description of major differences in the blood metabolites of the ewes subjected to estrus induction and high-starch or -fat diets. Our results regarding the increased glucose concentration are in line with those reported by Ghattas and Nasra (2010) and Daghigh Kia et al. (2012). Glucose is one of the most important ingredients for a suitable reproductive performance and affects the hypothalamus-pituitary axis (Downing et al., 1995). The increased plasma cholesterol concentration in the ewes fed flaxseed-rich diets may be attributed to increased cholesterol absorption and production of LDLs in the epithelial cells lining the small intestine and liver cells. Increased cholesterol concentration in the animals fed flaxseed, compared with that in the

**Table 1.** Ingredient of experimental diets with or without flaxseed (%DM)

| | Experimental diets | | | | | |
|---|---|---|---|---|---|---|
| | | Flaxseed levels (%) | | | | |
| Ingredient | Control | 2% | 5% | 7% | 10% | 12% |
| Alfalfa hay | 19.5 | 19.6 | 19.6 | 19.5 | 19.5 | 19.5 |
| Corn silage | 17.9 | 17.9 | 17.9 | 17.9 | 17.9 | 17.9 |
| Whole barley silage | 12.8 | 12.8 | 12.8 | 12.8 | 12.8 | 12.8 |
| Barley grain | 20.4 | 12.8 | 12.8 | 12.6 | 12.8 | 12.8 |
| Corn grain | 10.7 | 0.0 | 0.0 | 0.0 | 5.4 | 3.1 |
| Sugar beet pulp | 0.0 | 0.0 | 13.7 | 13.1 | 4.3 | 6.3 |
| Soybean meal | 10.0 | 7.49 | 0.0 | 0.0 | 5.0 | 3.1 |
| Cottonseed meal | 0.0 | 10.0 | 8.3 | 8.2 | 2.6 | 2.1 |
| Flaxseed | 0 | 2 | 5 | 7 | 10 | 12 |
| protected fat | 00.0 | 2.1 | 1.8 | 0.3 | 1.6 | 0.2 |
| Wheat bran | 6.2 | 12.6 | 5.4 | 5.9 | 5.4 | 7.4 |
| Calcium carbonate | 0.6 | 0.8 | 0.8 | 0.8 | 0.8 | 0.9 |
| Sodium bicarbonate | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Min supp. A and vitl | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| **Chemical composition** | | | | | | |
| Energy (MJ/kg) | 11.89 | 11.92 | 11.94 | 11.92 | 12.15 | 12.20 |
| Crud protein, % DM | 14.0 | 14.0 | 14.0 | 14.0 | 14.0 | 14.0 |
| Ether extract, % DM | 2.9 | 3.7 | 5.5 | 6.7 | 4.2 | 5.7 |
| Neutral detergent fiber, % DM | 36.1 | 40.7 | 42.6 | 42.8 | 40.3 | 41.1 |
| Non-fiber carbohydrates, % DM | 37.4 | 31.2 | 31 | 30.4 | 34.2 | 31.7 |
| Ash, % DM | 10 | 10 | 10 | 10.2 | 10 | 10.2 |
| Calcium, % DM | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Phosphate, % DM | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |

control group, can be due to the enhanced concentration of fatty acids in the small intestine. This is because micelle formation and absorption of fatty acids in the small intestine, as well as their

**Table 2:** Least square means of blood concentrations of various metabolites in ewes fed experimental diets

| | Experimental diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Flaxseed levels (%) | | | | | |
| Variable | Control | 2% | 5% | 7% | 10% | 12% | SEM |
| Urea | 33.43 | 33.45 | 32.87 | 32.45 | 31.45 | 30.20 | 2.57 |
| Glucose | 63.67 [b] | 63.85 [b] | 68.43 [a] | 67.45[a] | 67.21 [a] | 71.21 [a] | 3.44 |
| Triglycerides | 42.12[b] | 47.21 [a] | 42.06 [b] | 44.84[b] | 41.96 [b] | 43.11 [b] | 0.92 |
| Total cholesterol | 70.61 [b] | 76.15 [a] | 75.45[a] | 77.35 [a] | 74.29 [a] | 76.39 [b] | 1.25 |
| HDL-cholesterol | 40.21 [c] | 46.12 [a] | 40.35 [c] | 42.17 [c] | 40.11 [c] | 35.23 [b] | 3.52 |
| LDL-cholesterol | 30.40 [b] | 40.03 [a] | 35.10[a] | 35.18[a] | 44.18[a] | 41.16 [a] | 3.11 |
| NEFA | 0.298 | 0.340 | 0.346 | 0.324 | 0.323 | 0.293 | 0.054 |
| BHBA | 0.183 | 0.242 | 0.234 | 0.233 | 0.194 | 0.196 | 0.510 |

HDL: high-density lipoprotein, LDL: low-density lipoprotein, NEFA: non-esterified fatty acids
† The concentrations of urea, glucose, triglycerides, total cholesterol, high-density lipoprotein, and low-density lipoprotein are expressed as mg/dl of plasma.
The concentrations of non-esterified fatty acids and beta-hydroxybutyrate are expressed as mmol/L of plasma.
a,b,c: Dissimilar letters in each row indicate a significant difference (P<0.05).

**Table 3.** Estrus rate and controlled internal drug release removal up to estrus (time) in ewes fed experimental diets

| | Experimental diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Flaxseed levels (%) | | | | | |
| Variable | Control | 2 | 5 | 7 | 10 | 12 | SEM |
| Estrus rate (%) | 100 | 93.3 | 100 | 93.3 | 100 | 100 | - |
| CIDR removal Up to estrus (day) | 37.45 [a] | 40.21 [a] | 38.41 [a] | 34.45 [a] | 35.42 [a] | 30.12 [b] | 1.21 |

CIDR: controlled internal drug release removal
The concentrations of urea, glucose, triglycerides, total cholesterol, high-density lipoprotein, and low-density lipoprotein are expressed as mg/dl of plasma.
The concentrations of non-esterified fatty acids and beta-hydroxybutyrate are expressed as mmol/L of plasma.
a,b,c: Dissimilar letters in each row indicate a significant difference (P<0.05).

**Table 4.** Number of follicles on estrus and controlled internal drug release removal day in ewes fed experimental diets

| | Experimental diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Flaxseed levels (%) | | | | | |
| Variable | Control | 2 | 5 | 7 | 10 | 12 | SEM |
| Number of follicles on day CIDR operation | 1.43[b] | 1.48[b] | 1.45[b] | 1.56[b] | 2.32[a] | 2.1 [a] | 0.09 |
| Number of follicles on estrus | 2.45[b] | 1.98[b] | 3.41[a] | 3.20[a] | 4.3 [a] | 4.42[a] | 0.21 |

a,b,c: Dissimilar letters in each row indicate a significant difference (P<0.05).

**Table 5.** Ovulation, pregnancy, lambing, and twin delivery rates in ewes fed experimental diets

| | Experimental diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Flaxseed levels (%) | | | | | |
| Variable† | Control | 2 | 5 | 7 | 10 | 12 | SEM |
| Ovulation rate | 75[b] | 80 [b] | 75[b] | 70[b] | 100[a] | 95[a] | - |
| Pregnancy rates | 75[b] | 80 [b] | 75[b] | 70[b] | 100[a] | 95[a] | 6.32 |
| Lambing rate | 75 [b] | 80 [b] | 75[b] | 75[b] | 120[a] | 115[a] | 0.43 |

a,b,c: Dissimilar letters in each row indicate a significant difference (P<0.05).

transformation into lipoprotein, require cholesterol. Therefore, by increasing the amount of fatty acid for absorption in the small intestine, the needs for the synthesis and uptake of cholesterol may also increase. A higher level of total cholesterol as a result of increased fat levels in the diet could be also attributed to a reduction in the excretion of cholesterol and its metabolic products (i.e., bile acids) or to the increased level of lipids in the diet (Petit, 2002). It has been demonstrated that the beef cows fed diets supplemented with fish oil, soybean oil, or fat have higher levels of total cholesterol, HDL cholesterol, and triglycerides. Furthermore, the concentrations of total cholesterol and HDL cholesterol in the plasma of cows fed soybean oil-rich diets were reported to be higher than those in the cows fed diets containing fish oil (Petit, 2002). Dietary treatments had no effect on the plasma concentrations of NEFA and BHBA. The blood concentration of NEFA is an index of body fat mobilization (Roberts et al., 1981) and is related to the energy balance of dairy cows. Bertics et al. (1992) reported that dry matter intake was inversely related to the concentrations of NEFA and BHBA in the plasma and liver. In a study performed by Zeleke et al. (2005) on sheep outside the breeding season, it was reported that the mean numbers of large follicles before and after progesterone treatment were not different between the groups subjected to glucogenic and lipogenic diets. However, Bertics et al. (1992) observed an increase in the number of large follicles before and after CIDER removal and feeding glucogenic diet in the mating season. Sakaguchi et al. (2004) reported that the use of omega-3 fatty acids may increase the ovarian follicles and oocytes in sheep. They also reported that these fatty acids may increase the number of oocytes and improve the physical properties of oocytes and follicles by increasing the unsaturated fatty acids. In the mentioned experiment, the size of the ovulatory follicle was affected by experimental diets and was significantly greater in omega-3 and omega-6 groups as compared with that in the control group. This was ascribed to the higher concentration of estradiol in the two groups at the time of estrus. However, there was no significant difference between omega-3 and omega-6 groups. Dietary fat has been shown to directly affect dairy cow fertility by modifying the energy status. In the present study, the use of 10% and 12% flaxseed diets resulted in better reproductive performance. The use of the sources of supplemented fat as oil or oilseeds resulted in an improved lambing performance in sheep (Daghigh Kia

et al., 2012). The addition of supplemented fat to flushing diets in ewes may have a significant impact on their reproductive performance. This could be due to the elevation of the plasma concentrations of progesterone (Badiei et al., 2014; Jahani-Moghadam et al., 2015) and also an increase in the supply of the lipoproteins involved in the regulation of steroidogenesis system in the ovaries. Polyunsaturated fatty acids, n-3 and n-6 FA, may decrease the embryonic loss (Dirandeh et al., 2015) and increase the number of small follicles, size of pre-ovulatory follicle (Ambrose et al., 2006), and conception rate (Ambrose et al., 2006; Dirandeh et al., 2013a). Oocyte quality may also be improved by n-3 FA. However, fish oil and flaxseed diets were also reported to decrease the size of the corpus luteum and its progesterone synthesis, thereby resulting in a less favorable uterine environment and reduced fertility.

The results of the present study showed that feeding a starch-rich diet as a part of estrus synchronization protocol may improve the ovine reproductive performance. However, there was no improvement in reproductive performance by adding a source of unsaturated fatty acids to the glucogenic diet.

## Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Authors' Contribution

Study concept and design: Didarkhah, M.
Acquisition of data: Dadashpour Davachi, N.
Analysis and interpretation of data: Vatandoost, M.
Drafting of the manuscript: Didarkhah, M.
Critical revision of the manuscript for important intellectual content: Dadashpour Davachi, N.
Statistical analysis: Dirandeh, E.
Administrative, technical, and material support: Didarkhah, M.

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