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Effects of Feeding Different Levels of Whole and Ground Flaxseed in Late Pregnancy on the Performance of Karakul Ewes

Research Article

S.H. Hosseini[1], M.R. Dehghani[1*], A. Karimi[2], M. Yousef Elahi[1] and M.J. Abarghuei[2]

[1] Department of Animal Science, Faculty of Agriculture, University of Zabol, Zabol, Iran
[2] Department of Animal Science, Fars Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran

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*Correspondence E-mail: mr.dehghani@uoz.ac.ir

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ABSTRACT

This study were conducted to determine the effect of different levels of flaxseed, whole and ground (8 and 15%), in late pregnancy on the performance of ewes. 30 Karakul ewes (ages between 2 and 2.5 years old, mean weight of 55±2.5 kg) were used in a completely randomised design with five treatments and six replicates. Treatments include: 1) control diet, a diet without flaxseed; 2) a diet containing 8% whole flaxseed (WFS-L); 3) a diet containing 15% whole flaxseed (WFS-H); 4) a diet containing 8% ground flaxseed (GFS-L); and 5) a diet containing 15% ground flaxseed (GFS-H). The addition of flaxseed (whole and ground) had no significant effect on dry matter intakes or body weight changes in ewes and lambs (P>0.05), but in comparison, crude protein and ether extract digestibility was significantly (P<0.05) higher in ewes fed whole and ground flaxseed compared to those fed the control diet. The yield and composition of colostrum were not affected by experimental treatments (P>0.05), except for colostrum protein, which increased by using treatments with whole flaxseed (P<0.05). Glucose and cholesterol in blood plasma were significantly increased in ewes fed whole and ground flaxseed compared to the control treatment. (P<0.05), and the highest blood glucose and cholesterol levels were observed in the treatment of 15% whole flaxseed. The research showed that whole flaxseed (15%) can be added to sheep's diets, and it can be concluded that processing flaxseed for ewes in late pregnancy might not be necessary to improve performance.

KEY WORDS apparent digestibility, flaxseed, Karakul ewe, performance, pregnancy.

INTRODUCTION

Given that 80% of fetus growth takes place in the last two months of pregnancy, the nutrient requirements of ewes increase significantly during this period (Dawson *et al.* 1999). The nutrition management of ewes during late pregnancy plays a crucial role in determining various factors, such as lambs' birth weight and good-quality colostrum, and early detection of colostrum quality in post-lambing is also extremely important for lamb health and performance (Karakus and Atmac, 2016). Petit and Benchaar (2007) observed that feeding cows with flaxseed as a source of omega-3 is an effective way to improve feed intake and energy balance. Flaxseed (*Linum usitatissimum*), of Mesopotamic origin, has been cultivated since 5000 BC, being used until the 1990s principally for the fabrication of cloths and papers. Today it is cultivated in over 2.6 million ha and the important linseed growing countries are India, China, United States, Ethiopia. Canada with 614000 metric tonnes of flaxseed produced in the year 2013-2014, is the world's largest producer of flax and accounts for nearly 80% of the global trade in flaxseed (Oomah, 2001; Bhatty, 1995). Flaxseed contains approximately 20% crude protein (CP) and 40% oil on a dry matter (DM) basis (Petit, 2010), and it is also a rich source of essential fatty acids, of which 50–70% is alpha-linolenic acid (ALA), a rich source of n-3 polyunsaturated fatty acids (PUFA) (Xu *et al*. 2013). Greco *et al.* (2015) stated that reducing the ratio of n-6 to n-3 FA in the diet of dairy cows increased dry matter intake (DMI). Rosa Velazquez *et al*. (2020) also observed that supplementation of the diet with omega-3 (10.1 g/kg dry matter) resources such as flaxseed has beneficial effects on the development and growth of the lambs. Moreover, some studies showed that when flaxseed was used in the ruminant diet, disease resistance increased due to the effect of alpha-lipoic acid (ALA) on the immune system. In other words, the level of immunity against diseases in livestock increased (Nudda *et al*. 2015). Farmer *et al*. (2010) reported that feeding flaxseed meals in late gestation supplementation improved the post weaning growth of piglets, possibly due to increased carcass glycogen on day 1. Thus, we hypothesized that supplementing pregnant ewes' diets with flaxseeds in late gestation may enhance the performance of them. There is also little research on direct comparisons of the use of whole and ground flaxseed in late pregnancy on the performance of Karakul ewes. Therefore, the main objective of the present study was to investigate the effects of feeding diets with flaxseed (whole or ground) during late pregnancy on performance, nutrient digestibility, colostrum yield, colostrum composition, and some plasma metabolites of Karakul ewes.

MATERIALS AND METHODS

Animals, experimental treatments, and nutrition management

This experiment was carried out in the Fars Agricultural and Natural Resources Research and Training Center in the fall of 2018 for 8 weeks before calving (including 2 weeks of habituation and 6 weeks of testing period) and 48 hours after calving. In this experiment, 30 Karakul ewes (ages between 2 and 3 years old and mean of weight of 55±2.5 kg) were used in a completely randomized design with five treatments and six replicates. Treatments include 1) control diet, a diet without flaxseed; 2) a diet containing 8% whole flaxseed (WFS-L); 3) a diet containing 15% whole flaxseed (WFS-H); 4) a diet containing 8% ground flaxseed (GFS-L); and 5) a diet containing 15% ground flaxseed (GFS-H). Experimental diets were balanced based on NRC (2001) to meet the recommended nutrient requirements and adjusted based on DM using small ruminant feeding system software (SRNS, 2010). The body weight of ewes was recorded and they were randomly assigned to one of five experimental treatments inside an individual cage. The experiment was conducted during the last two months of pregnancy. During the experiment, the ewes were nourished twice a day (8:00 and 16:00 h) and had free access to fresh drinking water. Ingredients and chemical composition of diets and flaxseed during late pregnancy are shown in Tables 1 and 2, respectively.

Performance ewes

The amount of feed intake and daily residue were recorded to calculate the DMI. The ewes of each treatment were weighed at intervals two months before lambing and one hour after lambing. The newborn lambs were allowed to be licked and dried by the ewes and within one hour were weighed, their sex was determined, and ear-tagged (Hashemi *et al*. 2008).

Apparent digestibility by the acid-insoluble ash method

A digestibility trial was performed during late pregnancy (7 days before the expected birth date). On three consecutive days, the faeces were collected using the faecal grab samples method in all ewes three times a day (7:00, 14:00, and 18:00) (Meteab *et al*. 2020). The faeces were mixed thoroughly by hand and 10% sub-sample was retained and stored at -15 ^{o}C. At the end of the collection period, the sample of feed and refusal were dried at 65 ^{o}C for 48h and faeces were dried at 65 ^{o}C until constant weight. Acid-insoluble ash was used as an internal marker to estimate faecal output and nutrient digestibility. The digestibility coefficient of a given nutrient was calculated according to the following formula (Van Keulen and Young, 1977).

Digestibility= 100 – (% indicator in the feed/% indicator in the feces) × (% nutrient in the feces/% nutrient in the feed)

Colostrum production

The measure of colostrum was determined by measuring the difference between the pre- and post-sucking weights of lambs. The rest of the colostrum was milked and weighed. The colostrum yield can be calculated by adding the colostrum consumed by the lambs to the milked colostrum. The colostrum yield of each ewe was recorded at 12, 24, and 48 h after birth (Ocak *et al*. 2005).

Blood metabolites

Blood samples were taken from the jugular vein using heparinized plastic syringes 3 hours after the morning feeding (Ababakri *et al*. 2021), 30 days after the beginning of the experiment (Hashemi *et al*. 2008). Blood samples were withdrawn from the jugular vein into vacutainer tubes containing EDTA. The plasma was isolated by centrifuging at 3000 rpm for 20 minutes and frozen at −20 °C until the following analysis. Blood serum samples were analyzed using Pars Azmon kits and spectrophotometer (Jenway 6300, UK).

Table 1 Ingredients and composition of the experimental diets

Ingredients (%)	Treatments				
	Control	WFS-L	WFS-H	GFS-L	GFS-H
Alfalfa hay	33.34	33.34	33.34	33.34	33.34
Wheat straw	27.01	27.01	27.01	27.01	27.01
Flaxseed	-	8	15	8	15
Wheat bran	16	16	16	16	16
Barley grains	9.33	8.53	8.13	8.53	8.13
Soybean meal	7.33	4.93	2.53	4.93	2.53
Corn grain	5.33	5.33	5.33	5.33	5.33
CaCO3	0.67	0.67	0.67	0.67	0.67
Salt	0.33	0.33	0.33	0.33	0.33
Sodium bicarbonate	0.33	0.33	0.33	0.33	0.33
Mineral and vitamin supplement[1]	0.33	0.33	0.33	0.33	0.33
Chemical composition					
Metabolizable energy (Mcal/kg DM)	2.14	2.17	2.19	2.17	2.19
Dry matter (%)	91.28	91.32	91.74	91.32	91.74
Crude protein (%)	16.86	16.47	16.04	16.47	16.04
Ether extract (%)	3.09	4.02	4.83	4.02	4.83
Neutral detergent fiber (%)	39.11	39.46	39.77	39.46	39.77
Acid detergent fiber (%)	24.32	24.63	24.89	24.63	24.89
Ca (%)	0.88	0.80	0.82	0.80	0.82
P (%)	0.31	0.30	0.30	0.30	0.30

[1] Each kilogram of vitamin–mineral premix contained: vitamin A: 50,000 IU; vitamin D_3: 10,000 IU; vitamin E: 1000 IU; Ca: 196 g; P: 96 g; Na: 71 g; Mg: 19 g; Fe: 3 g; Cu: 0.3 g; Mn: 2 g; Zn: 3 g; Co: 0.1 g; I: 0.1 g and Se: 0.001 g.
Control: a diet without flaxseed; WFS-L: a diet containing 8% whole flaxseed; WFS-H: a diet containing 15% whole flaxseed; GFS-L: a diet containing 8% ground flaxseed and GFS-H: a diet containing 15% ground flaxseed.

Table 2 Compounds of flaxseed

Ingredients	(% Dry matter, DM)
DM (% of fresh weight)	93
Crude protein (% of DM)	19.3
Ether extract (% of DM)	35
Crude fiber (% of DM)	26
Ash (% of DM)	2.92

Determination of chemical composition of feed and feces

Sample of feed and feces were oven-dried at 60 °C for 48 h and milled to pass through a 1-mm mesh (Wiley mill, Swedesboro, NJ). Samples of diet were analyzed for dry matter (DM), Crude protein (CP), Ether extract (EE), organic matter (OM) Ash and acid detergent fiber (ADF) according to AOAC (2000) procedures. Also, the determination of neutral detergent fiber (NDF) was measured by Van Soest *et al*. (1991) method.

Statistical analysis

The data underwent analysis of variance (ANOVA) using a completely randomized design. Mean comparisons were performed using Duncan's 1995 test, with a statistical significance level of up to 5%, utilizing the PROC MIXED module in SAS 9.4 software (SAS, 2013). The analysis followed the model outlined below:

$$\hat{Y}ij = \mu + Ti + \varepsilon ij$$

Where:
$\hat{Y}ij$: observed value of the dependent variable.
μ: overall mean.
Ti: signifies the effect of the treatment.
εij: accounts for the experimental random error associated with each observation.

RESULTS AND DISCUSSION

The results of the effect of different levels of whole and ground flaxseed on feed intake, the ewes' weight, and the birth weight of their lambs are given in Table 3. During the experimental period, different levels of whole and ground flaxseed supplementation in late pregnancy had no significant effect on feed intake, change the ewes' weight or birth weight of their lambs (P>0.05).

Results from Table 4 revealed that whole and ground flaxseed supplementation in late pregnancy had no significant effect on dry matter (DM), organic matter (OM), acid detergent fiber (ADF), and neutral detergent fiber (NDF) digestibility (P>0.05), but the crude protein (CP) digestibility was significantly (P<0.05) higher in diets containing whole and ground flaxseed compared to the control diet.

Table 3 The effect of feeding whole flaxseed (WFS) or ground flaxseed (GFS) on feed intake, ewe's body weight change of ewes in late pregnancy, and lambs birth weight

Parameter	Treatments					SEM	P-value
	Control	WFS-L	WFS-H	GFS-L	GFS-H		
Feed intake ewes	1.66	1.77	1.80	1.73	1.76	0.05	0.56
Ewe live weight (kg)							
56 days before lambing	56.50	54.15	55.25	57	55.70	2.65	0.94
1 h after lambing	54.75	52.10	53.67	55.37	54.15	2.58	0.90
Ewe weight loss (kg)	1.75	2.05	1.57	1.62	1.55	0.69	098
Lamb birth weight (kg)	4.80	5.42	5.55	5	5.13	0.20	0.21

SEM: standard error of the means.

Table 4 Effects of feeding whole flaxseed (WFS) or ground flaxseed (GFS) on apparent digestibility of ewes in late pregnancy

Parameter	Treatments					SEM	P-value
	Control	WFS-L	WFS-H	GFS-L	GFS-H		
Digestibility (% DM)							
DM	69.72	68.68	75.41	72.65	70.40	2.33	0.32
OM	69.62	71.68	73.72	71.50	70.92	2.09	0.73
CP	69.68[c]	83.48[a]	85.10[a]	74.92[b]	75.35[b]	1.52	0.001
EE	66.29[b]	77.06[a]	80.61[a]	74.57[a]	76.56[a]	2.27	0.01
NDF	44.46	53.44	46.47	43.50	40.18	3.86	0.24
ADF	34.46	40.11	36.47	33.50	30.84	3.32	0.41

SEM: standard error of the means.

The CP digestibility in treatments with whole flaxseed (especially the 15%) was significantly better than in treatments with ground flaxseed. According to the results in Table 4, the EE digestibility in ewes fed whole and ground flaxseed diets improved compared to those fed the control diet (P<0.05). In the treatment with 15% whole flaxseed, the highest amount of EE digestibility in ewes was observed.

The effects of the levels and physical form of flaxseed on the blood metabolites of pregnant ewes are inserted in Table 5. According to Table 5, the blood glucose and cholesterol levels of ewes were affected by experimental treatments (P<0.05), so that the highest blood glucose and cholesterol levels were observed in the treatment of 15% whole flaxseed. The blood triglycerides and albumin levels of ewes were not affected by experimental treatments (P>0.05). The application of whole or ground flaxseed had no significant effect on ewes' yield, fat, protein, or solids non-fat (SNF) of colostrum in ewes supplemented with whole flaxseed or ground flaxseed (P>0.05; Table 6), Table 6), but colostrum protein was significantly increased in the ewes fed with whole flaxseed compared to other animals (P<0.05). The highest colostrum protein was observed in the treatment of 15% whole flaxseed.

Consistent with our results, Danesh Mesgaran and Stern (2005) reported that fat in the type of oil seeds had no effect on DMI. Also, similar to our results, in a study conducted by Bell *et al.* (2006), fish oil supplementation as another rich source of polyunsaturated fatty acids (PUFA), administered 21 days before lambing up to 10 days after lambing, had no effect on DMI in ewes.

Gandra *et al.* (2016) reported that the DMI of cows fed whole flaxseed was greater than that of those fed no flaxseed during the prepartum period. Also, Meteab *et al.* (2020) observed that the application of flaxseed in diet increased DMI during the lactation period and suggested that this may be attributed to the increment in nutrient digestibility, which promotes rumen discharge and consequently forces the animal to eat a lot. Grummer *et al.* (2004) stated that fat supplementation increases dietary energy density, and if the DMI is not altered, the net energy for lactation (NE_L) intake would be greater and, consequently, could diminish the negative energy balance of early lactating cows (Gandra *et al.* 2016).

In the present study, flaxseed supplementation (especially the whole form) improved CP digestibility. The protein in flaxseed is a matrix that surrounds the fat droplets, leading to protection of the protein from deterioration in the rumen and, therefore, greater availability of protein in the intestinal tract, thus increasing its digestibility (Khorasani *et al.* 1992). Also, oilseeds have been shown to increase the secretion and activity of pancreatic enzymes, enhancing digestion and absorption of proteins in the small intestine and the efficiency of protein utilisation (Mir *et al.* 2000).

The higher digestibility of fat increased in the current study with the inclusion of dietary flaxseeds. These results are similar to the results of Micek *et al.* (2004), who reported higher ether extract intake in groups containing 10% crushed linseed supplemental as compared to the control groups. Also, this result was similar to the finding of Alcalde *et al.* (2011) who reported that nutrient intake increased in goat kids fed flaxseed, sunflower, or canola.

Table 5 Effects of fed diets containing whole flaxseed (WFS) or ground (GFS) flaxseed on blood plasma biochemical metabolites of ewes in late pregnancy

Parameter (mg/dL)	Treatments					SEM	P-value
	Control	WFS-L	WFS-H	GFS-L	GFS-H		
Glucose	60.75[b]	70.62[a]	72.09[a]	66.87[a]	68.51[a]	1.67	0.006
Triglycerides	34.53	35.86	37.84	36.98	37.32	1.24	0.39
Cholesterol	69.59[b]	75.13[a]	78.84[a]	75.07[a]	76.00[a]	1.17	0.003
Albumin	3.77	3.54	3.69	3.64	3.53	0.09	0.38

SEM: standard error of the means.

Table 6 Effects of fed diets containing whole flaxseed (WFS) or ground flaxseed (GFS) on yield and composition of colostrum collected 1 h after lambing of ewes

Parameter	Treatments					SEM	P-value
	Control	WFS-L	WFS-H	GFS-L	GFS-H		
Colostrum yield (g/d)	290	295	298.25	292.25	290.50	34.34	0.99
Fat (%)	11.87	13.42	14.12	12.12	12.25	1.26	0.67
Protein (%)	13.25[b]	16.75[a]	17.75[a]	14.50[b]	14.75[b]	0.54	0.001
Solids non-fat (SNF, %)	25.72	27.48	28.36	27.87	27.68	1.25	0.63
Total fat (g/d)	34.52	40.25	42.84	32.89	35.08	5.09	0.61
Total protein (g/d)	38.82	49.56	53.32	42.72	42.49	6.09	0.45
SNF (g/d)	57.44	63.01	67.75	61.89	67.73	4.45	0.50

SEM: standard error of the means.

Although in the current study flaxseed supplementation had no effect on the digestibility of NDF and ADF, there was a tendency to increase the digestibility of these nutrients. This may be attributed to the influence of flaxseed inclusion, which is characterised by a small, flat, and oval-shaped (2×5 mm); consequently, it has a higher possibility of escaping from mastication as well as an increased passage rate from the rumen (Meteab *et al.* 2019). Moreover, the flaxseed fat contents are protected by the seed coats, which gives partial protection against a microbial attack or limits the negative effects of oil on ruminal microbes or both, leading to a negligible effect on the digestion of fibres (Kim *et al.* 2004). Similarly, another's studies showed that the reduction in fibre digestibility in the animals supplemented with 10 and 5% full-fat soybean compared to the animals supplemented with 10% flaxseed may have been that the fats in full-fat soy are not protected and have a negative effect on rumen function and cellulolytic bacteria, which led to decreased fibre digestion, whereas on the contrary, for flaxseed, the fat is protected (Khorasani *et al.* 1992; Kim *et al.* 2004). In agreement with our findings, Wachira *et al.* (2000) concluded that diets with 10.5% flaxseed had no significant effect on NDF digestion in ewes. In contrast with the results, Da Silva *et al.* (2007) reported that cows fed whole flaxseed showed higher digestibility of ADF but lower CP and EE digestibility than treatments with ground flaxseed. These differences may be due to the animal's species used, flaxseed level, and diet composition.

Increases in blood glucose concentrations in ewes fed flaxseed are in agreement with experiments.

Ababakri *et al.* (2021) observed that the ewes fed flaxseed had a higher glucose concentration than other groups during pre-lambing periods, and Gandra *et al.* (2016) reported that feeding whole flaxseed to cows increased blood glucose in transition dairy cows. Glycerol from fat hydrolysis in oilseeds was converted to propionate, which increased serum glucose through the process of gluconeogenesis (Hess *et al.* 2008). Also, Qin *et al.* (2018) reported that the increase in glucose concentrations in fat-supplemented groups might be a consequence of higher somatotropin concentrations, as somatotropin was found to stimulate hepatic gluconeogenesis to supply the energy demand of the lactating mammary gland (Knapp *et al.* 1992). The findings of the present study showed that feeding whole and ground flaxseed increased cholesterol levels in ewes. In agreement with the results of this study, Ye *et al.* (2009) reported that the inclusion of 2% flaxseed in Holstein dairy cows resulted in an increase in cholesterol and triglyceride concentrations. The increased cholesterol concentrations could be due to a decrease in the circulation of low-density lipoprotein cholesterol in the blood via flaxseed supplementation (Pan *et al.* 2009). In contrast with the results of this study, Meteab *et al.* (2019) reported that flaxseed supplementation in goat diets during the gestation period reduced cholesterol and triglyceride levels.

In the present study, the application of whole flaxseed increased colostrum protein concentration in experimental ewes. Similar to our results, Nudda *et al.* (2013) reported that flaxseed supplementation in goats led to increased milk protein concentrations as a result of higher protein avail ability in the intestine. Also, Meteab *et al*. (2020) suggested that milk protein concentration was increased in goats fed 10% flaxseed and could be due to the improved digestibility. Mir *et al*. (2000) stated that oil seeds increase the secretion and activity of pancreatic enzymes, enhancing digestion and absorption of proteins in the small intestine and the efficiency of protein utilization. Petit (2003) reported no change in milk yield with the feeding of whole flaxseed. In addition, Ababakri *et al*. (2021) observed that during pre-lambing periods, the ewes fed whole and extruded flaxseed had no effect on the yield, fat, or protein content of the colostrum.

CONCLUSION

In general, the results of this study showed that ewes fed 15% whole flaxseed had the best CP and EE digestibility and the highest glucose and cholesterol in the blood plasma. Also, 15% whole flaxseed increases protein concentration in colostrum ewes, which may be beneficial to immunity in their offspring. Therefore, 15% whole flaxseed can be added to sheep's diets in late pregnancy.

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