

Effect of different levels of ground flaxseed on *In vitro* and *In vivo* digestibility in Karadi lambs

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Abstract

This study was conducted to determine the effect of different levels of flaxseed (0, 3, 6 or 9%) on *in vitro* and *in vivo* apparent digestibility of dry and organic matter and voluntary feed intake on growth of Karadi lambs. Sixteen Karadi male lambs of eight months and weighing 45.6 ± 0.481 kg were randomly divided into four treatments and individually penned for a period of 84 days. Results revealed that there were no significant differences among treatments in the daily intake of DM, OM, CP, CF, and NFE, ME. Ether extract (EE) was higher ($P < 0.05$) in lambs fed 9% flaxseed both *in vitro* and *in vivo* digestibility trials. The *in vivo* apparent and *in vitro* digestibility of dry matter (DM), organic matter (OM), total digestible nutrient (TDN), digestible energy (DE) and metabolizable energy was not influenced by the addition of flaxseed to the diet. In conclusion, the addition of 6 or 9% flaxseed to the diets does not improve intake and digestibility of nutrients. However, significantly higher digestibility of EE was noticed in lambs fed 9% flaxseed.

Keywords: Flaxseed; digestibility; Karadi lambs

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Introduction

Inclusion of flaxseed into the ruminant diets increases the fat content, which may decrease fibre digestibility in the rumen (Micek et al., 2004). On the other hand, the addition of fat increases the energy concentration of the diet and helps to limit energy losses during fermentation by restricting the number of methane-producing bacteria, which may have a positive effect on the magnitude of microbiological protein synthesis (Machmüller et al., 2000). Supplemental flaxseed, containing fat rich in polyunsaturated fatty acid, may therefore alter the digestibility of dietary nutrients and influence the energy and protein metabolism in the body (Jenkins, 1993). Feeding flaxseed, which is high in linolenic acid, is a viable option to enrich the n-3 fatty acid concentration in livestock diets and subsequent in ruminant products (Soita et al., 2003; Kronberg et al., 2006; Maddock et

al., 2006). However, there are limitations on the level of fat that can be added to ruminant diets due to reductions in intake and digestibility (Schauff and Clark, 1992). This is especially true for fats high in polyunsaturated fatty acids (Palmquist and Jenkins, 1980; Jenkins, 1993). Furthermore, flaxseed seems to be unique in this regard as indicated by Zhang et al. (2007) who showed an increase in dry matter digestibility for lactating ewes fed a silage-based diet containing 8% flaxseed compared with 7.3% canola seeds or no oilseeds. In feedlot diets, flaxseed did not influence (Maddock et al., 2006) or increases dietary intake, and an overall improvement in average daily gain was observed with inclusions rate of 5 to 8% flaxseed in the diet (Drouillard et al., 2004).

The aim of this study was to determine the effect of supplementation of flaxseed to the diets on daily feed intake, *in vitro* and *in vivo* digestibility of Karadi male lambs.

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Materials and Methods

This study was carried out at the Animal Farm, Department of Animal Production, Faculty of Agricultural Sciences, University of Sulaimani, Bakrajo, Sulaimani, Kurdistan, Iraq.

Animals

Sixteen Karadi male lambs weighing 45.6 ± 0.083 kg and of eight months old were individually housed and treated against ecto- and endo-parasites. After an adaptation periods of 14 days, lambs were randomly divided into four treatments for a period of 84 days.

Diets

Four rations were used in this experiment each contained 0, 3, 6 or 9% of flaxseed. Formulation and ingredients' of the diets are presented in Table 1.

Lambs of the control group received a basal diet with 0% flaxseed, whereas lambs in T1, T2 and T3 received flaxseed at a rate of 3, 6 or 9% (Table 1). All lambs were fed concentrate at a rate of 3% of their body weight once daily, and the refusal of the diet was collected and weighed before offering the feed in the next morning. Straw was given *ad libitum*. Clean water was available constantly. Lambs were weighed at weekly intervals.

Faeces collection and apparent digestibility

The digestibility trial was determined from the total collection of faeces for 7 days at 11 weeks of feeding trial. Faeces from the individual lambs were collected and weighed every morning by fitting lambs collection bags (Saeed, 2011). The faeces were mixed thoroughly by hand and 10% sub-sample was retained and stored at -15°C . At the end of the collection period, the sample of feed and refusal were dried at 65°C for 48 h and faeces were dried at 65°C until constant weight. The dried samples were ground through 1 mm mash. Aliquots of the samples from each day were pooled and analyzed chemically. The apparent digestibility coefficient of feed nutrients was determined according to McDonald et al. (2002).

Chemical analysis

Proximate chemical analysis of concentrate samples in triplicate per each determination was carried out for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash content according to the A.O.A.C. (1995). The nitrogen free extract (NFE) was calculated by subtracting the summation percentages of CP, EE, CF and ash from one hundred. The *In vitro* digestibility of DM and OM for treatments was determined using the method of Telley and Terry (1963).

Table 1: Formulation and chemical composition of experimental diets

Ingredients (%)	Control	T1	T2	T3
Barley	40	40	40	40
Wheat	27	27	27	27
Yellow Corn	15	15	15	15
Soybean meal	15	12	9	6
Flaxseed	0	3	6	9
Salt	1	1	1	1
Minerals and vitamins	2	2	2	2
Chemical composition%				
Dry matter (DM)	94.5	94.0	94.0	94.0
Organic matter (OM)	93.7	93.6	93.4	93.3
Crude protein (CP)	16.2	16.1	16.1	16.1
Ether extract (EE)	2.3	3.1	4.1	4.7
Total Ash	6.3	6.4	6.6	6.7
Crude fiber (CF)	5.4	4.8	4.2	4.4
Nitrogen free extract (NFE)	69.8	69.6	69.0	68.1
Metabolizable energy ME (MJ/Kg)*	12.7	12.8	13.1	13.1

*ME (MJ/ kg DM) = $0.012 \text{ CP} + 0.031 \text{ EE} + 0.005 \text{ CF} + 0.014 \text{ NFE}$ (MAFF, 1977). T1-T3 was fed flaxseed at the rate of 3, 6 and 9%.

Statistical analysis

The obtained data were analyzed according to Addinsoft XLSTAT (2007) for one way analysis of variance. Differences among means were carried out by using Duncan multiple range tests (Duncan, 1955). Data from experiment were analyzed according to the following model:

$$Y_{ij} = \mu + t_i + e_{ij}$$

Where: Y_{ij} = the dependent variable, μ = overall mean, T_i = effect of the treatment (i = control, 3% flaxseed, 6% flaxseed or 9% flaxseed), e_{ij} = random residual error.

Results

In vitro dry matter and organic matter digestibility

Data related to the *in vitro* digestibility of dry matter and organic matter is given in Table 2. There was no effects ($P > 0.05$) of different levels of flaxseed on *in vitro* digestibility of DM and OM among treatments.

Total daily nutrient intake during the digestibility trial

Data of daily nutrient intake is given in Table 3. It can be seen from this table that DMD, OMD, CP, CF, Ash, NFE and ME intake are the same for all treatments. The EE intake was significantly ($P < 0.05$) higher in T3 as compared to control group. However, marginally higher DM and OM were observed in T1 followed by T2 and T3.

Numerically, higher ME intake was observed in T2 treatment followed by T1 and T3. However, all treatments were not significant.

Table 2: Effect of different levels of flaxseed on *in vitro* dry matter and organic matter digestibility and daily nutrient intake of lambs during the digestibility trial

Item	Control	T1	T2	T3
Live body weight (kg)	45.3±1.89 ^a	45.97±1.61 ^a	46.0±1.67 ^a	45.1±1.91 ^a
Metabolic weight (W ^{0.75})	17.46±0.41 ^a	17.65±0.24 ^a	17.66±0.37 ^a	17.4±0.49 ^a
IVDMD	66.3±0.61 ^a	66.8±0.96 ^a	66.2±0.51 ^a	65.7±0.45 ^a
IVOMD	68.2±0.93 ^a	68.4±0.27 ^a	68.9±0.60 ^a	67.5±0.38 ^a
DMI (g/day)	1382±1.90 ^a	1392±1.83 ^a	1388±1.48 ^a	1384±2.21 ^a
DMI (g/kg W ^{0.75})	79.15±0.48 ^a	78.87±0.74 ^a	78.56±0.89 ^a	79.68±0.97 ^a
OMI (g/day)	1295±2.39 ^a	1303±2.40 ^a	1297±2.73 ^a	1291±2.28 ^a
OMI (g/kg W ^{0.75})	74.16±0.76 ^a	73.82±0.70 ^a	73.36±1.10 ^a	74.33±1.71 ^a
CP (g/day)	224.0±1.51 ^a	224.0±1.93 ^a	224.0±1.88 ^a	222.6±1.99 ^a
CP (g/kg W ^{0.75})	12.82±0.17 ^a	12.69±0.29 ^a	12.65±0.52 ^a	12.8±0.59 ^a
EE (g/day)	31.8±1.23 ^c	43.2±1.32 ^{bc}	57.0±1.42 ^{ab}	64.7±1.29 ^a
EE (g/kg W ^{0.75})	1.82±0.19 ^c	2.43±0.30 ^{bc}	3.22±0.28 ^{ab}	3.73±0.33 ^a
Ash (g/day)	87.0±1.64 ^a	89.0±1.01 ^a	91.0±1.56 ^a	93.0±1.91 ^a
Ash (g/kg W ^{0.75})	4.98±0.26 ^a	5.04±0.04 ^a	5.17±0.15 ^a	5.33±0.39 ^a
CF (g/day)	74.6±2.39 ^a	67.0±2.67 ^a	58.0±2.00 ^a	61.0±2.17 ^a
CF (g/kg W ^{0.75})	4.27±0.46 ^a	3.78±0.49 ^a	3.29±0.31 ^a	3.52±0.33 ^a
NFE (g/day)	964.6±3.36 ^a	968.8±4.11 ^a	958.0±4.80 ^a	942.7±3.67 ^a
NFE (g/kg W ^{0.75})	55.25±0.87 ^a	54.90±0.91 ^a	54.21±0.97 ^a	54.27±0.88 ^a
ME MJ/kg DM	17.6±0.21 ^a	17.82±0.10 ^a	18.2±0.15 ^a	18.1±0.23 ^a
ME (g/kg W ^{0.75})	0.727±0.01 ^a	0.725±0.01 ^a	0.742±0.01 ^a	0.755±0.04 ^a

Means followed by different superscripts within a row are significant (P<0.05); T1-T3 were fed flaxseed at the rate of 3, 6 and 9%. IVDMD= In vitro dry matter digestibility, IVOMD= In vitro organic matter digestibility, DMI= Dry matter intake, OMI= Organic matter intake, CP= Crude Protein, EE= Ether extract, CF=Crude fiber, NFE= Nitrogen free extract, Me= Metabolizable energy

Table 3: Effect of different levels of ground flaxseed on the *in vivo* digestibility of the nutrient (%)

Item	Control	T1	T2	T3
DMD	67.4±0.25 ^a	67.9±0.28 ^a	67.3±0.21 ^a	66.9±0.23 ^a
OMD	69.7±0.37 ^a	70.1±0.39 ^a	69.3±0.30 ^a	68.6±0.22 ^a
CPD	69.1±0.29 ^a	69.5±0.36 ^a	70.1±0.34 ^a	68.6±0.20 ^a
EED	68.4±0.67 ^c	73.2±0.77 ^b	79.5±0.87 ^a	81.3±0.92 ^a
CFD	44.2±0.19 ^a	43.6±0.22 ^a	45.1±0.26 ^a	43.6±0.20 ^a
NFED	74.8±0.29 ^a	75.1±0.17 ^a	73.6±0.21 ^a	74.0±0.18 ^a

Means followed by different superscripts within a row are significant (P<0.05); DMD = Dry matter digestibility, OMD = Organic matter digestibility, CPD = crude protein digestibility, EED = Ether extract digestibility, CFD = Crude fibre digestibility, NFED = Nitrogen free extract digestibility; T1-T3 were fed flaxseed at the rate of 3, 6 and 9%.

In vivo digestibility of the nutrients

The apparent *in vivo* digestibility of all nutrients are presented in Table 3. In the digestibility experiment, the *in vivo* apparent dry matter digestibility (DMD) ranged between 66.9 to 76.9%, and the differences among treatment were not significant (P>0.05). The organic matter digestibility (OMD) was marginally higher in T1 followed by T2 and T3. Moreover, the digestibility of CP was marginally higher in T2 followed by T1 and T3.

Result indicated that the ether extract digestibility (EED) was significantly (P<0.05) higher in T2 and T3 as compared to T1.

Furthermore, the mean value of crude fibre digestibility (CFD) was not significantly (P>0.05) different among treatment. On the other hand, the *in vivo* apparent nitrogen free extract digestibility (NFED)

was not significant (P>0.05) among treatments. However, digestibility of NFED was numerically higher in T1 (75.1%) followed by T3 and T2.

The energetic values of diets

The energetic values of the diets such as total digestible nutrient (TDN), digestible energy (DE) and metabolizable energy (ME) are presented in Table 4. In the current work, TDN, DE and ME are almost similar in all treatments (P>0.05).

Discussion

In the present work, it was clear that the use of flaxseed cannot be used as an energy and protein source in animal. Similar result was observed by Macdonald (1994) and Mustafa et al. (2003). Similar results were also reported by Astellini et al. (2004), Petit et al. (2003) and Gonthier et al. (2004). The high percentage of EE in T3 diet is reasonable since linseed contains about 40% oil (Legrand et al., 2010). These results are similar to the results of Micek et al. (2004) who reported higher ether extract intake in contained 10% crushed linseed supplemental, as compared to the control groups.

The higher digestibility of ether extract increased in the current study by the inclusion of dietary flaxseeds. This result was similar to the finding of Alcalde et al. (2011) who reported that nutrient intake increased in trialon Boer and Saanen goat kids fed on flaxseed, sunflower or canola.

Table 4: Effect of different levels of ground flaxseed on the energetic value of experimental diets

Item	Control	T1	T2	T3
Total digestible nutrient (%DM) TDN ¹	67.39±0.41 a	67.84±0.44 a	67.23±0.40 a	67.19±0.38 a
Digestible energy (Mcal /kg DM) DE ²	2.971±0.05 a	2.991±0.03 a	2.964±0.06 a	2.962±0.04 a
Metabolizable energy (Mcal / kg DM) ME ³	2.437±0.04 a	2.453±0.02 a	2.431±0.06 a	2.429±0.03a

Means followed by the same superscripts within a row are not significant (P<0.05); ¹TDN (DM) = DCP+DEE × 2.25+TDC (Sniffen *et al.*, 1992); ²DE = %TDN × 0.04409 (NRC,1996); ³ME = DE × 0. 82 (NRC, 1996); T1-T3 were fed flaxseed at the rate of 3, 6 and 9%.

No significant difference was found in dry matter in the current study. These findings are in confirmation with the findings of the Micek *et al.* (2004) and Walkunde and Adangale (2011) who reported non-significant difference in dry matter digestibility among treatments when 50, 100 and 150 g linseed supplementation was done in Osman abadi goat kids and who observed non-significant difference in dry matter digestibility when 3% linseed oil supplementation was done in dairy cows (Ueda *et al.*, 2003). However, Machmüller *et al.* (2000) evaluated the supplementation with squeezed canola, sunflower or flaxseed in growing lambs fed on corn silage, grass hay and concentrate (5.6% EE vs. 3.1% control) and observed that dietary sunflower seed reduced the digestibility of neutral detergent fibre, acid detergent fibre and organic matter. Diets were formulated to provide similar total digestible nutrients and crude protein contents in order to avoid composition variations and changes in rumen environment. The major variation was observed in the ether extract content, which showed significant change (Table 3). These results were similar to the results of Mustafa *et al.* (2002), Gonthier *et al.* (2004) and Alemu *et al.* (2010) who reported higher CP digestibility in linseed cake supplemented ration than a control ration of Sidama Goat. It may be due to higher solubility of heat treated of linseed protein which might favour higher digestibility. The perusal of the data indicated that linseed protein can be efficiently used by ruminants without affecting overall digestibility of experimental ration. Moreover, EE content of diets with oilseeds (3.1 and 4.7% on average) is not considered at level high enough to damage nutrient digestibility, mainly crude fibre digestibility. In general, these fatty acids show better digestibility than saturated fatty acids and are more harmful to rumen fermentation (Palmquist and Mattos, 2006). The findings of present study envisage that digestibility of various nutrients under different treatments supplemented with different levels of flaxseed were similar and comparable with control indicating no adverse effect on nutrient digestibility. The perusal of data indicates that there was no specific trend in NFE digestibility.

There was no comparable data for energy contents of the flaxseed in the diets. These results are similar to the findings of Alcalde *et al.* (2011) who reported that an increase of 2.32% in ether extract compared with the

control. Chilliard (1993) reported that diets with 3.9% of lipids show higher (9%) energy values than control diets. Among the nutrients, lipids have the highest energetic value (NRC, 2007). Therefore, the highest intake and digestibility for ether extract provided by flaxseeds could alter the energy value of diets, but this was not observed in this experiment. This may be related to levels of inclusion of flaxseed and also to the balancing of the diets which presented similar values of metabolizable energy in their final composition. The increased content of polyunsaturated fatty acid in linseed-based diets may cause a slight decrease in cellulolytic activity in the rumen, which was observed in the present study by the decrease of the VFA content (Micek *et al.*, 2004). They reported that 10% addition of linseed to concentrate caused a decrease in VFA in the rumen fluid. Furthermore, the control diet had higher nitrogen free extract, which probably offset the additional energy contained in flaxseed diets.

Conclusions

Feeding diets with different levels of flaxseed (3, 6 and 9%), have no beneficial effect in Karadi male lambs except improvement in digestibility of ether extract.

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