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The replacement value of corn silage with wild pistachio (*Pistacia khinjuk*) leaf in the diet of sheep and its effect on digestibility of fiber and protein

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Abstract The aim of present experiment was to investigate the feeding value of wild pistachio leaf (khinjuk leaf, KL) for Arabi sheep. The digestibility and fermentation of diets containing different amounts of khinjuk (0, 5, 10, 15, 20, 25 and 30%) were measured. Then, the effect of presence of KL in the diet and its tannin content on the digestibility of alfalfa hay (Alfa), wheat straw (WS) and soybean meal (SBM) was studied by gas production (GP) technique. There was significant difference between the digestibility of dry matter (DM) and neutral detergent fiber (NDF) in diets containing KL compared with control ($P<0.05$). By increasing the amount of KL in the diet, digestibility of DM and NDF was decreased; the most and least digestibility belonged to the control and diet containing 30% KL, respectively. The digestibility of DM and NDF, up to 10% replacement of corn silage (CS) with khinjuk, did not have any difference with the control diet. There were no significant differences in the potential (b) and rate (c) of GP of WS, and potential of GP of Alfa between diets, but the rate of the GP of Alfa significantly declined ($P<0.05$). The GP potential and microbial biomass of SBM in control was more than diets containing KL. The amounts of PF, microbial biomass production and biomass production efficiency of WS ($P>0.05$) and Alfa ($P<0.05$) in the diets containing KL were higher than those of the control. Conversely, all of these indexes were greater for SBM in control diet, but only for biomass production efficiency the difference was significant ($P<0.05$). Overall, the results indicated that using KL in the diets of sheep, had no significant negative effect on digestion and fermentation of fiber and protein resources but microbial biomass production and its efficiency were improved.

Keywords: Arabi sheep, digestion and fermentation, microbial protein, soybean meal, tannin

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Introduction

Providing fodder for animal husbandry industry is one of the fundamental requirements, so usage of inexpensive feed is concerned. Some of these feed sources are the edible leaf of deciduous trees; they relatively make massive amounts of hay-like and are an important source for feeding sheep and goats. In terms of nutritional value, the leaves are similar to average quality forage, due to resources constraints of forage in Iran, optimal using of them in feeding of sheep and goat is important (Fazaeli, 2012).

It seems that native tropical plants due to the high growth rate, high protein content, their ability to fix nitrogen, on the one hand reduce the need for expensive nitrogen fertilizers, on the other hand some of them are the useful supplement for other roughage (Tieman et al., 2008). However, most of the plants in these areas contain high concentrations of tannin. Tannins are plant secondary metabolites, and polyphenolic compounds (Kumar & Singh, 1984) that can be bound to protein and carbohydrate and reduced their digestion in the gastrointestinal tract of ruminants (Min et al., 2003). In addition, the effect of tannins on ruminants depends on the type of tannin, its chemical structure and molecular weight, amount of consumption and animal species; therefore it may be have beneficial or harmful effects (Frutos et al., 2004). It has been shown that tannins through inhibition of the production of methane, degradation of protein and reduction of bio-hydrogenation process in the rumen, can improve the rumen metabolism and cause to increase the flow of amino acids and unsaturated fatty acids to duodenum. The tannins can reduce fiber digestion by junction with lignocellulosic materials, therefore prevent from microbial digestion, or directly affect the activity of cellulolytic microorganisms and fibrolytic enzymes or both of them (Teman, et al., 2008).

The wild pistachio or khinjuk *(Pistacia khinjuk*) belongs to the family of *Anacardiaceae* and is native to the Middle East. The wild pistachio mainly is dispersed in the Zagros Mountains (Ghaemmaghami et al., 2009), and contains some phenolic compounds (such as tannin) (Safarzadeh, 2000). Currently, the khinjuk provides parts of sheep and goats feed by grazing on pastures and forests during cold seasons, and the influence of its phenolic compounds (tannins) on animal nutrition is unknown. There was no information about the effects of the KL tannins and its compounds in animal nutrition. Therefore, the aim of present experiment was to study the digestibility and replacement values of diets containing different amounts of khinjuk leaves replaced with corn silage and its effect on fiber and protein digestion in Arabi sheep.

Materials and Methods

Preparation of diets

The khinjuk leaf (KL) was collected from the forests of north east of Khuzestan province and dried in shade. The different amounts of khinjuk leaf at 0, 5, 10, 15, 20, 25 and 30% in the diet of Arabi sheep was replaced by corn silage (Table 1). The diets were formulated based on live weight using standard requirements tables (NRC, 2007).

Digestibility (determine the optimum level of replacement)

In order to determine the *in vitro* digestibility of diets containing different levels of KL, 0.5 g sample was weighed into glass test tubes. The rumen fluid was collected using a stomach tube from four sheep before morning feeding, and mixed them with each other. The sheep were feeding by forage base diet at maintenance level. The rumen fluid mixed with McDougall's artificial saliva (at a ratio of 1:4, vol/vol). Then 50 ml from the rumen fluid and artificial saliva was added to each tube under carbon dioxide gas, to create anaerobic conditions, and the tube stoppered immediately. After 48 hours incubation, 6 ml from 20% HCl was added to each tube. Then 5 ml pepsin enzyme (Merck, 1:3300) solution (0.5g per 100 ml HCl 0.1 N) was added, and tubes were placed on the water bath again. After 48 hours incubation, the digestibility of DM and NDF was calculated (Tilley and Terry, 1963).

Investigation the effect of KL in sheep diet on fiber and protein digestion with in vitro gas production (GP) technique

The rumen fluid was obtained through stomach tube from the sheep (5 sheep per treatment) fed with control diet (contain corn silage) and diet contain 30% KL for 6 weeks, and rumen fluid was mixed for all sheep per tre-

Table 1. The feed ingredients and chemical composition of experimental diets

	Diets (khinjuk leaf, per %DM of diet)						
	0	5	10	15	20	25	30
Ingredient, % of DM							
Barley	19.5	19.5	19.5	19.5	19.5	19.5	19.5
Soybean meal	2.0	1.9	1.6	1.3	1.0	0.7	0.4
Wheat bran	8.0	8.1	8.4	8.7	9.0	9.3	9.6
Corn silage	30.0	25.0	20.0	15.0	10.0	5.0	0.0
Khinjuk leaf	0.0	5.0	10.0	15.0	20.0	25.0	30.0
Alfalfa hay	28.0	28.0	28.0	28.0	28.0	28.0	28.0
Straw	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Mineral & Vitamin*	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition							
Dry matter	51.85	57.50	65.14	71.87	78.52	86.25	91.86
Organic matter	91.56	91.55	91.54	91.52	91.50	91.44	91.40
ME	2.40	02.31	02.13	01.87	01.92	01.83	01.92
CP % of DM	11.00	11.03	11.32	11.61	11.70	11.82	11.85
NDF % of DM	57.72	56.60	56.45	55.85	53.10	52.77	52.60
ADF % of DM	28.54	28.99	29.46	29.92	30.39	30.85	31.32
Ash % of DM	08.44	08.45	08.46	08.48	08.50	08.56	08.60

* Minerals: Ca, P, Mg, Co, Cu, I and Se; Vitamins: A, E and D3.

atment. The alfalfa hay (Alfa) and wheat straw (WS) and soybean meal (SBM) were used as fiber and protein sources, respectively. The GP method was performed according to Menke and Steingass (1988). Briefly, 300 mg dried WS, Alfa and SBM was weighed, combined with rumen fluid and artificial saliva (1:2 ratio), and incubated in 100 ml vials. The volume of gas produced at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours were recorded by using a digital pressure gauge. The data were fitted using modified model of Orskov and McDonald (1979).

$P=b(1–e^{-ct})$. Where: P=gas produced in time t, b=GP from the fermentable fraction (ml), c; the GP rate constant (ml/h), t=the incubation time (h)

Results and Discussion

In vitro digestibility of the experimental diet (determine optimum level)

There were significant differences in digestibility of DM and NDF between the control and diets containing 5, 10, 15, 20, 25 and 30% KL (P<0.05) (Table 2). By increasing the KL in diets, the digestibility of DM and NDF were decreased and control diet had the highest DM and NDF digestibility. In compared with control diet, replacement up to 10% KL with corn silage, did not affect the digestibility of DM and NDF of diets. There was no significant difference between the diets contain 15, 20, 25 and 30% KL for dry matter digestibility. The studies of Van hoven & Furstenburg (1992) showed that, using *in vitro* tannin led to decreased cell wall digestibility; the tannins react with bacterial cell wall and microbial extracellular enzymes (Scalbert, 1991), this process prevents the transfer of nutrients into bacteria cells, thereby reducing their growth rate, and consequently reduction of the digestibility of DM and NDF. Probably part of this low digestibility was referred to higher percentage of ADF and ADL of KL compared to corn silage (Aminifard et al., 2013). Therefore, it seems that tannin, fiber and lignin content of KL were effective in decreasing of the DM and NDF digestibility of diets by increasing the levels of KL.

Table 2. The *in vitro* digestibility of sheep diets containing different levels of khinjuk leaf

Diets (percent of khinjuk leaf)	Digestibility of DM (%)	Digestibility of NDF (%)
0 (without khinjuk)	71.37[a]	45.05[a]
5	68.51[ab]	44.40[a]
10	68.03[ab]	43.33[ab]
15	64.94[c]	42.14[c]
20	62.60[c]	40.60[d]
25	62.51[c]	40.26[d]
30	61.14[c]	38.16[e]
SEM	01.600	0.279
P value	0.0060	0.0001

SEM: standard error of mean; the means with different letters within each row are significantly differ (P<0.05).

After analyzing experimental data from the first stage, the control (without KL) and the diet containing 30% replaced leaf of khinjuk with corn silage, were selected and used to feeding the sheep; and their rumen fluid was used for study the digestion and fermentation of Alfa, WS and SBM by *in vitro* GP method.

Table 3. The gas production parameters of incubated wheat straw with rumen fluid of sheep fed diets contain khinjuk leaf

Parameters	Corn silage (control)	30% Khinjuk	SEM	P value
Wheat straw				
b (ml/300mg)	74.24	70.51	3.86	0.518
c (ml/h)	0.018	0.014	0.001	0.267
PF	5.875	7.088	0.536	0.256
Microbial biomass production	103.58	119.31	9.347	0.366
Efficiency of microbial production	62.1	68.86	0.031	0.271
Alfalfa hay				
b (ml/300mg)	113.66	111.96	08.109	0.887
c (ml/h)	0.028[a]	0.015[b]	0.003	0.025
PF	03.37[b]	04.46[a]	0.098	0.016
Microbial biomass production	55.89[b]	94.47[a]	04.49	0.026
Efficiency of microbial production	34.70[b]	50.60[a]	0.018	0.026

b: GP from the fermentable fraction (ml), c: the GP rate constant (ml/h), PF: partitioning factor, SEM: standard error of means, The means with different letters within each row are significantly differ (P<0.05).

Table 4. The gas production parameters of incubated soybean meal with rumen fluid of sheep fed diets contain khinjuk leaf

parameters	Corn silage (control)	30% khinjuk	SEM	P value
b (ml/300mg)	132.36[a]	116.2[b]	4.66	0.049
c (ml/h)	0.026	0.019	0.003	0.21
PF	3.69	3.41	0.076	0.23
Microbial biomass production	78.46[a]	57.22[b]	2.54	0.027
Efficiency of microbial production	38.8	36.6	0.014	0.243

b: GP from the fermentable fraction (ml), c: the GP rate constant (ml/h), PF: partitioning factor, SEM: standard error of means, The means with different letters within each row are significantly differ (P<0.05).

GP parameters of WS and Alfa with the rumen fluid of sheep fed diets containing KL

The results showed there was no significant difference in the potential and rate of GP of WS between experimental diets (P>0.05) (Table 3). The potential of GP was not affected by leaf of khinjuk in diet. This may be related to the negative effect of tannin on the protozoa population because there is a beneficial symbiosis between rumen protozoa and methanogens microorganism, which inhibition of protozoa reduces methanogens activity, methane production followed by reduced GP. The condensed tannins can reduce ruminal degradation of organic matter content and waste energy as methane (Waghorn et al., 1994). Hassan Sallam et al (2010) indicated that tannins reduce GP in the rumen, which is related to connection of microorganisms to feed particles (Mc Alaster et al., 1994) and inhibit the growth of microorganisms and the activity of the microbial enzy mes (Mcsweeny et al., 2001). Principally, the main part of GP is through direct production of carbon dioxide and methane. Therefore, any factor that reduces methane and carbon dioxide may affect GP (Johnson, 1995).

In the present experiment, the significant reduction in GP rate of Alfa was significant (P<0.05) that may be related to interaction of the tannin with the protein of alfalfa, which is 4 times more than WS. Agreed with our results, Hervas et al (2003) reported that 8.2% tannin extract in the diet had no effect on potential of GP of WS, but the potential and rate of GP of Alfa was reduced; therefore, the effect of tannins on GP parameters depends on the type of incubated feed. In general, according to the literature reports, in most of the plant species containing tannins, GP had negative correlation with tannins (Khazaal et al., 1994), which the present study confirms their results.

The amounts of PF, microbial biomass production and microbial biomass production efficiency of WS (Table 3) were not affected by treatments (P>0.05), but for Alfa (Table 3) they were significantly higher in diets containing KL (P<0.05).

Typically, the PF value is in the range of 2.7 to 4.4. In samples containing high tannin, usually PF is more than 4.41, because of the solution of tannin during fermentation and thereby reducing dry matter without participation in GP and limiting GP and loss of the soluble content of cell. (Blummel et al., 1997). In the present experiment, the PF of incubated WS and Alfa with rumen fluid of sheep fed the 30% KL was higher than control diet. Similar to the GP rate, in the present experiment, PF, production of microbial biomass and microbial biomass production efficiency of Alfa was more than WS, because the interaction of the tannin with protein of Alfa, which is 4 times more than that of WS. Angagi et al (2011) reported that the presence of tannin in the feed increases the PF; that is a useful positive factor in protein nutrition of animals; because more part from the digested materials companies in microbial protein synthesis than GP and produces short chain fatty acids, confirming the present findings.

GP parameters of incubated SBM with the rumen fluid of sheep fed diets containing KL are presented in Table 4. The potential of GP of SBM in control diet was more than the diet containing KL (P<0.05). The GP rate of the diet containing KL was lower than control diet (P>0.05). The tannins bind the proteins and making them unavailable for microorganisms, consequently the growth of microorganisms and the GP will be reduced. Hervas et al (2000) studied the effect of tannic acid (0, 1, 5, 10, 15 and 25%) on ruminal and after ruminal degradation of SBM; they reported that ruminal nitrogen degradation reduced by increasing the level of tannic acid. They also reported intestinal digestion of proteins was significantly decreased at 15 and 20% of tannic acid. It has been reported that fermentation of proteins may not lead to a lot of GP in comparison to carbohydrates (Khazaal et al., 1994). So the reduction in the potential and rate of GP of soybean may be due to the tannin of khinjuk.

The PF and microbial biomass production efficiency did not affect by diets (P>0.05); but the control diet had greater microbial biomass production (P<0.05).The PF is an index from microbial biomass production efficien-

Table 5. The trend of GP of fiber and protein resources incubated with the rumen fluid of sheep fed diets contain khinjuk leaf

Time(h)	Wheat straw				Alfalfa				Soybean meal			
	Corn silage		Khinjuk leaf		Corn silage		khinjuk leaf		Corn silage		khinjuk leaf	
	GP (ml)	ratio*	GP (ml)	ratio	GP (ml)	ratio	GP (ml)	ratio	GP (ml)	ratio	GP (ml)	ratio
0	0	0	0	0	0	0	0	0	0	0	0	0
2	0.74	0.01	0.44	0.55	5.86	5.74	2.06	2.34	4.57	3.82	0.55	0.41
4	0.90	0.29	0.98	1.06	10.26	4.44	5.36	4.07	10.04	4.74	5.58	5.32
6	2.50	2.81	2.66	3.33	14.45	4.22	9.88	5.58	16.56	5.64	12.05	6.86
8	4.70	3.85	4.78	4.20	16.90	2.47	12.41	3.12	23.43	5.96	16.50	4.69
10	9.44	8.30	7.38	5.15	24.11	07.27	15.34	3.62	30.01	5.7	22.41	6.25
24	24.5	26.41	18.01	21.10	52.40	28.55	26.86	14.23	54.46	21.21	36.00	14.36
36	38.01	23.7	31.20	36.13	73.87	21.67	45.38	22.88	75.86	18.56	58.39	23.68
48	46.36	14.64	40.31	18.10	86.76	13.00	61.21	19.56	95.68	17.18	75.07	17.64
72	53.31	12.18	47.12	13.50	95.99	9.32	74.50	16.42	107.53	10.27	88.51	14.21
96	57.01	6.50	50.42	6.54	99.07	3.1	80.93	7.95	115.28	6.73	94.53	6.37

*GP ratio: the difference between GP per each hour from prior hour divided by the total produced gas, GP: gas production.

cy or efficiency of microbial protein synthesis, which is defined as mg true organic matter digestion per ml GP (Blümmel et al., 1997). The PF represents the proportion of the substrate that degraded to short chain fatty acid, gases, or converted to microbial biomass.

The potential of GP of SBM in KL diet was less than control diet; therefore, it can be concluded that in present study the tannin of KL, made SBM protected against rumen microbial digestion, partially, which it was beneficial effects of tannin in the diet. The SBM has high protein content, optimum balance of amino acids, bio-peptides, so is affective in milk production of high producing dairy cattle and feed lot cattle with high growth rate. This meal frequently is used as a protein supplement, so that, protecting SBM against rumen microbes and convert it to resources of amino acid and undegradable protein is important.

The GP trend is shown in Table 5. According to the results for all feedstuffs, the most percentage from the gas in the control diet was produced up to time 24 of incubation. Therefore, probably the tannin of pistachio leaf caused to slowing the rate of fermentation and degradation rate in the rumen. Agreeing with the present experiments, Frutos et al (2002) reported that the tannins caused to reduce the rate of digestion and fermentation of feed. Therefore, theory about the role of tannins on delay of degradation and fermentation of nutrients is confirmed.

Conclusions

In vitro two steps digestibility was shown that up to10% replacement of corn silage with the KL had no adverse effect on DM and NDF digestibility. The replacement up to 30% KL in diet of sheep had no significant effect on the potential and rate of GP of WS and Alfa. The PF, microbial biomass production and efficiency of WS and alfalfa in diets containing leaf khinjuk was higher than that of the control. Therefore, using up to 30% KL as replacement by corn silage in the diet of sheep had no undesirable effect on digestion of the fiber and protein materials. Therefore, KL can be used as feed ingredient in diets of sheep, providing an inexpensive resource of fodder.

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چکیده آزمایش حاضر با هدف بررسی قابلیت هضم جیره‌های حاوی سطوح مختلف برگ پسته وحشی (برگ خنجوک) جایگزین شده با سیلاژ ذرت و تأثیر آن بر هضم و تخمیر منابع الیافی و پروتئینی در گوسفندان عربی انجام شد. در مرحله اول تاثیر وجود مقادیر مختلف خنجوک (صفر، ۵، ۱۰، ۱۵، ۲۰، ۲۵ و ۳۰ درصد) در جیره گوسفندان عربی بر خصوصیات هضم و تخمیر جیره‌ها مورد بررسی قرار گرفت. در مرحله بعد تأثیر حضور برگ خنجوک و تانن آن در جیره بر هضم‌پذیری یونجه، کاه گندم و کنجاله سویا با تکنیک تولید گاز مطالعه گردید. تفاوت معنی‌داری بین قابلیت هضم ماده خشک و الیاف نامحلول در شوینده خنثی جیره شاهد با جیره‌های حاوی برگ خنجوک جایگزین شده با سیلاژ ذرت وجود داشت ($P<0/05$). با افزایش مقدار برگ خنجوک در جیره، هضم‌پذیری ماده خشک و الیاف نامحلول در شوینده خنثی روند کاهشی داشت؛ بیشترین مقدار هضم آنها مربوط به تیمار شاهد و کمترین مقدار مربوط به جیره دارای ۳۰ درصد برگ خنجوک بود. برای هضم‌پذیری ماده خشک و الیاف نامحلول در شوینده خنثی، تا ۱۰ درصد جایگزینی خنجوک با سیلاژ ذرت، اختلافی با جیره شاهد مشاهده نشد. تفاوت معنی‌داری در پتانسیل و نرخ تولید گاز کاه گندم و پتانسیل تولید گاز یونجه خشک در جیره‌های آزمایشی مشاهده نشد، اما کاهش نرخ تولید گاز در یونجه محسوس بود ($P<0/05$). پتانسیل تولید گاز و تولید توده میکروبی کنجاله‌سویا در جیره شاهد بیشتر از جیره حاوی برگ خنجوک بود. مقادیر PF، تولید توده میکروبی و بازده تولید توده میکروبی در کاه گندم ($P>0/05$) و یونجه خشک ($P<0/05$) در جیره حاوی برگ خنجوک بیشتر بود. بر عکس، این موارد برای کنجاله سویا در جیره شاهد بیشتر بود؛ اما فقط برای بازده تولید توده میکروبی معنی‌داری شد. به طور کلی نتایج مشخص کرد که استفاده از برگ خنجوک در جیره گوسفندان اثر منفی قابل ملاحظه‌ای بر هضم و تخمیر مواد فیبری و پروتئینی مورد آزمایش نداشت و حتی بازده تولید توده میکروبی را نیز بهبود بخشید.