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In vitro Utilizable Crude Protein at the Duodenum of Dairy Cows of Various Ecotypes of *Kochia scoparia* Fertilized with Nitrogen

Research Article

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ABSTRACT

The aim of this study was to determine possible differences between first cut and second cut forage of Birjand ecotype of *Kochia scoparia* for chemical composition and fermentability, and to evaluate the changes may be induced by N fertilization in chemical composition, fermentability, and utilizable crude protein (uCP) supply at the duodenum of dairy cows of Sabzevar ecotype of Kochia. Birjand ecotype was sampled in mid bloom and Sabzevar ecotype was sampled in first bloom stage of maturity. Different *in vitro* gas production methods were used to determine gas production kinetics and uCP and effective uCP (EuCP) supply of the forage samples. Results of the first experiment showed that there is no significant difference between first cut and second cut forage of *Kochia scoparia* for gas production kinetics (P>0.05), but significantly lower content (g/kg DM basis) of neutral detergent fiber (NDF) (451.1) and hemicellulose (233.7) and higher content of CP (100.1) and ash (133.4) were observed in the first cut than those of the second cut plants (P<0.01). The results of second experiment indicated that the application of moderate levels of the N fertilizer (115 kg N/hectare) on Sabzevar stands of *Kochia scoparia* induced considerable changes in chemical composition, fermentability, and protein degradability of forge, so that, dry matter (DM), NDF, and hemicellulose declined and CP and ash content of forage increased (P<0.05). Moreover, the gas production kinetics decreased and supplied amounts of uCP and EuCP increased in association with increase in CP and true protein content (P<0.05).

KEY WORDS fermentability, forage cut, *Kochia scoparia*, metabolizable protein.

INTRODUCTION

Halophytes show good potential to adapt to drought conditions and the high salinity of both soil and water. In the northeast and east of Iran, 70% of halophyte species are used as forage (Rezvani Moghaddam and Koocheki, 2003). *Kochia scoparia* (*Bassia scoparia*, Kadereit and Freitag, 2011) is an annual halophytic plant native to Eurasia, grows rapidly, up to 2 meters in height, and turns into a bushy, pyramid shape. The stems are erect, green, and much branched, and the alternate leaves are hairy. *Kochia scoparia*, a C4 species, only reproduces by seed and can survive in very harsh conditions, including sandy and alkaline soils, drought, and salinity. It has been used as livestock feed in arid to semiarid regions (Friesen *et al.* 2009). The palatability of immature kochia is better than that of many other types of forage, but slightly worse than that of alfalfa hay (Undersander *et al.* 1990; Vavra *et al.* 1977). Kochia produces hay yields of 1 to 4 tons dry matter (DM)/acre/cut. Three or four cuttings are possible in a growing season

(Undersander *et al.* 1990). High-quality kochia hay contains up to 60% leaves and 11-22% crude protein (CP) (Undersander *et al.* 1990).

Toxic substances identified in kochia are saponins, alkaloids, oxalates, nitrates, and sulfates (Friesen *et al.* 2009). Scientific reports on the toxic effects of kochia are discrepant, however it is indicated that kochia can not cause poisoning when comprise less than 50% of the ruminant diets or when harvest at early stages of the maturity (Mir *et al.* 1991; Souto and Milano, 1966). The chemical composition of kochia changes depending on the maturity stage, ecotype, environmental conditions, and fertilization (Sherrod, 1971; Mir *et al.* 1991; Mengistu and Messersmith, 2002; Barnes *et al.* 1990; Lugg *et al.* 1983; Steppuhn *et al.* 1994).

With respect to the semiarid climatic condition, it is possible to cultivate forage kochia after harvesting the grain cereals and produce at least two cut of forage in a growing season. However, we could not found any scientific publication on chemical composition and nutritional value of kochia forage harvested in different cuts.

Kochia forage containing high amounts of nitrogen (N), therefore N fertilization of the cultivated plants is necessary to achieve maximum forage growth rate and produce high quality forage (Lugg *et al.* 1983). So far, the effects of N fertilization on agronomic traits of kochia were explored in some studies, but all of these publications have been neglected the nutritional properties of this forage plant. In addition, these articles provided poor description of the effects of N fertilization on chemical composition of this forage (Lugg *et al.* 1983; Steppuhn *et al.* 1994).

The application of non-invasive *in vitro* techniques are now becoming widespread for estimating nutritional value of animal feedstuffs due to low costs, controlled conditions, speed and ease of doing. *In vitro* gas production technique is one of the known methods for evaluation of rumen fermentability of feedstuffs. Several papers have been reported a high correlation between *in vivo* digestibility and predicted from *in vitro* rumen gas production technique in combination with chemical composition (Getachew *et al.* 2005). The gas production technique provides kinetic data that describes extent and rate of ruminal digestion (Menke and Steingass, 1988; Getachew *et al.* 2005). Edmunds *et al.* (2012) described a new gas production technique that facilitates to estimate sum of microbial protein and ruminally undegraded protein (that is approximately equivalent with the utilizable CP at the duodenum (uCP)) in the incubation medium. This technique eliminate the confounding effects of various parameters such as de novo synthesis of microbial protein, microbial markers, loss of the feed particles through bag pores, soluble proteins and endogenous N on evaluation of feed protein (Hedqvist and Udén, 2006; Karlsson *et al.* 2009).

Moreover, this new gas production technique estimates the nutritional value of feed proteins using CP content of feed sample and concentration of ammonia N in the incubation medium which are easily measurable.

Optimum application of kochia forage in ruminant diet requires further research on the nutritional characteristics of this plant when fertilized and harvested at different cuts. Results of such studies can be utilized to develop management strategies for producing high quality and quantities of kochia forage in each growing season.

Therefore, two independent experiments were conducted to determine differences between first cut and second cut of kochia and N fertilized and non-fertilized kochia using new *in vitro* gas production techniques.

MATERIALS AND METHODS

Growth conditions and sampling

These experiments were performed in the spring and summer of 2015 at the educational site of the agricultural department of Ferdowsi University of Mashhad located in the east of Mashhad in Khorasan Razavi province, northeastern Iran (36˚ 15´N, 59˚ 28´E).

First experiment

In the first experiment Birjand ecotype of *Kochia scoparia* was planted in a plot of 500 square meters. The field was irrigated on a weekly basis and rainfall was negligible. Two weeks after germination, the weeds were removed manually. No fertilizing or spraying was done in the field. Forage samples of first and second cuts were randomly picked in the mid bloom stage of maturity (56 and 95 days after the sowing date, respectively). The plants were cut 10 cm above the soil surface. The forage samples were immediately transferred to the laboratory and dried in a forced-air oven (60 ˚C, 48 h). Then the chemical composition and *in vitro* gas production kinetics of the first cut forages were compared to those of the second cut samples.

Second experiment

In the second experiment, the Sabzevar ecotype of *Kochia scoparia* was planted in two plots of 1000 square meters. One of the plots was fertilized by 125 kg/hectare of urea two and four weeks after first irrigation (total urea=250 kg/hectare). The irrigation program and the removal of the weeds were performed as the first experiment. Forage samples were randomly peaked at the first bloom stage of maturity (49 days after the sowing date). The plants were cut 5 cm above the soil surface. The forage samples dried as mentioned above. Then, the chemical composition of non fertilized samples were compared to the fertilized samples. Moreover, *in vitro* gas production kinetics and uCP of the non-fertilized and fertilized samples, alfalfa hay (50% bloom, harvested at summer of 2014), kochia sample of Birjand ecotype (first bloom, harvested at summer of 2014), and kochia sample of Esfahan ecotype (pre-bloom, harvested at summer of 2014) were compared.

In vitro gas production technique

The mineral solutions were prepared as described by Menke and Steingass (1988). The gas production test was performed using 120 mL serum bottles. Rumen fluid was obtained manually before the morning feeding from two rumen fistulated lactating Holstein dairy cows (657±31 kg, body weight, 290 days in milk (DIM) and 21 kg milk production). They were fed a totally mixed ration (TMR) composed of (DM basis) 268.9 g alfalfa hay, 198.6 g corn silage, 133.4 g barley grain, 154.9 g corn grain, 40.6 g cottonseed meal, 122.3 g soybean meal, 73.9 g wheat bran, and 7.5 g mineral and vitamin premix/kg. Animals were offered the TMR twice a day at 8 am and 8 pm. They had free access to fresh water. The rumen fluid was filtered through four layers of cheesecloth and mixed with buffered mineral solution as 1:2 of rumen fluid to mineral buffer ratio (V/V). All handling of ruminal inoculums happened under a constant stream of CO_2. Then, 200 mg of feed samples and 30 mL buffered rumen fluid (BRF) were added to the bottles, and the sealed bottles were incubated in a water bath at 39 ˚C. The blanks contained only BRF. The blanks and standard hay were treated in the same manner as the samples. The feed samples, standard hay, and the blanks were incubated in four repeats and three runs. At 2, 4, 6, 8, 12, 16, 24, 30, 36, 48, 72, and 96 hours post-incubation, the gas production volume was recorded by converting gas pressure built inside the bottles, measured by an electronic pressure transducer (Pressure Sensor, PSA-01, Autonics) to the volume using an experimentally calibrated curve (Jahani-Azizabadi *et al.* 2011). The kinetic parameters of gas production was determined for each feed sample by fitting gas production data to the nonlinear equation $Y = a + b\ (1–e^{[-ct]})$ (Ørskov and Mc Donald, 1979), in which y was the volume of gas produced at time t, a (mL) was the initial gas production, b (mL) was the gas production during incubation, a + b (mL) was the potential gas production, and *c* was the fractional rate of gas production per hours.

Utilizable crude protein at the duodenum of ruminant

Utilizable crude protein at the duodenum was determined using a new gas production technique described by Edmunds *et al.* (2012) as follows; the gas production procedure was performed as explained above. The incubations were performed in a water bath at 39 ˚C for 8 and 48 h. Blanks were contained only BRF and treated in the same manner as the samples.

Half of the replicates of each treatment (and blanks) at 8 hours and remained bottles at 48 hours post incubation were transferred to a water-ice mixture to stop fermentation. Afterwards, the bottles were opened and 5 mL of liquid phase was pipetted into 50 mL serum bottles that were contained 5 mL of 0.2 N HCl. Then, these bottles were sealed with rubber stopper and aluminum cap and stored in refrigerator at 4 ˚C for measurement of ammonia concentration. For each of feed samples the uCP value was calculated according to this equation (Edmunds *et al.* 2012):

$$\text{uCP (g/kg DM)} = (NH_3N_{blank} + N_{sample} - NH_3N_{sample}/\text{weight (mg DM)}) \times 6.25 \times 1000$$

Where:
NH_3N_{blank}: average amount (mg) of ammonia N in the blanks at the time of interest.
N_{sample}: amount (mg) of N that inserted into the incubation bottles from feed sample.
$NH3N_{sample}$: amount (mg) of ammonia N in the sample containing bottles.
Weight: amount of feed sample weighted into bottles.

To calculate effective uCP (EuCP) value of each feed sample, at the first, uCP values of 8 and 48 hours incubation were plotted against a log time (ln(t)) scale, where 't' is the time of incubation. Thereafter, the slope (a) and intercept (y) of the resulted regression equation placed in the following equation (Edmunds *et al.* 2012):

$$\text{EuCP (g/kg DM)} = y + a \times \ln(1/K_p)$$

Where:
K_P: assumed passage rate.

Chemical composition

The dried samples were ground using a hammer mill (Toos Shekan Khorasan, Mashhad, Iran) to pass a 1.5 mm screen and stored at room temperature. Kochia samples were analyzed for CP (Kjeldahl method, Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hogans, Sweden), neutral detergent fiber (NDF), (without sodium sulfite and alpha amylase, expressed inclusive of the residual ash), acid detergent fiber (ADF), (Van Soest *et al.* 1991), ether extracts (EE), (AOAC, 2000; ID 920.39), and ash (Thiex *et al.* 2012). Sequential method was used to measure the NDF and ADF. Hemicellulose was estimated as the difference between NDF and ADF content. Tungstic acid soluble protein (non protein N) was calculated as the difference between the total N and true protein N precipitated with tungstic acid (Licitra *et al.* 1996).

The liquid samples were centrifuged (3000×g at 5 ˚C for 10 minute) and supernatant was collected. Ammonia N concentration was measured in supernatant using a phenol-hypochlorite reaction (Weatherburn, 1967).

Statistical analysis

The data of the first experiment including plants height, chemical composition, and gas production were analyzed by independent t-test using t-test procedure of SAS (2002). In the second experiment paired t-test was used to analyze the data. Gas production kinetics, uCP, and EuCP data of the second experiment were analyzed as a completely randomized design using the general linear model (GLM) procedure of SAS (2002). The differences were considered to be significant at P < 0.05. In second experiment, Tukey's test was used to compare the differences between the means.

RESULTS AND DISCUSSION

First experiment

Height and chemical composition of the first cut and second cut forage from the Birjand ecotype of *Kochia scoparia* are given in Table 1.

The height of the first cut plants was significantly higher than that of the second cut plants (P<0.001). First cut plants had higher content of CP and ash and lower content of NDF and hemicellulose compared to the second cut plants (P<0.05). On an average, the ADF content of first cut plants was 3.75% less than that of the second cut plants, but the difference was not significant (P>0.05).

Gas production by the first and second cut samples of Birjand ecotype of kochia at 24, 36 and 96 hours post-incubation and related kinetics of gas production are shown in Table 2.

Mean gas production volume at 24 and 48 hours post-incubation and total gas production after 96 hours post-incubation were not significantly different between samples (P>0.05). The kinetic parameters of the fermentability were not significantly different, but the first cut plants had numerically greater initial gas production and fractional rate of gas production than those of the second cut plants.

Second experiment

Height and chemical composition of the non-fertilized and fertilized forge of the Sabzevar ecotype of *Kochia scoparia* are given in Table 3. On average, the fertilized plants grown 19.14 cm more than non-fertilized plants (P< 0.001).

On the other hand, the DM matter content of fertilized plants was approximately half that of the non-fertilized plants (P<0.001). The CP content of the fertilized plants was 202.8 g/kg DM which was almost twice as much as the non fertilized plants (P<0.001). The effect of the N fertilization on the NDF, hemicellulose, and ash content was significant and fertilized plants had higher ash and lower NDF and hemicellulose than non-fertilized plants (P<0.05). The effect of the urea fertilizer on the ADF content of the kochia samples showed a marginal trend toward significance (P=0.079).

The cumulative gas production of the non fertilized and fertilized kochia samples and alfalfa hay at different incubation times and their related kinetic parameters appear in Table 4.

The N fertilized plants had significantly lower gas production at 24 hours post-incubation than that of the non fertilized samples and alfalfa hay (P<0.05).

On an average, final volume of cumulative gas production of the non-fertilized kochia was 26.22% greater than that of the fertilized kochia, but difference was not significant (P>0.05).

The kinetic parameters of gas production significantly were affected by N fertilization (P<0.05). However, the fertilized kochia had higher initial gas production than non-fertilized kochia, but the gas production during incubation (b), the potential gas production (a+b), and the fractional rate of gas production (c) were higher for non-fertilized samples.

The results of the tungstic acid soluble protein, true protein, uCP, and EuCP of the non fertilized and fertilized samples in comparison with alfalfa hay and two selected samples of the *Kochia scoparia* are presented in Table 5.

The effect of N fertilization on protein fractions and uCP supply of the kochia was significant, so that the fertilized kochia had the highest amounts of CP, tungstic acid soluble protein, true protein, uCP, and EuCP (at different rumen passage rates) among different forage samples (P<0.05).

The differences between non-fertilized and fertilized kochia samples were significant, except for uCP measurement at 48 hours post incubation.

First experiment

The results of the first experiment on the fermentability of mid bloom kochia of Birjand ecotype revealed that the gas production characteristics of the first and second cut plants, especially potential gas production (a+b), which had the highest correlation with voluntary feed intake (Blummel and Becker, 1997), were not significantly different. However, the first cut plants had numerically higher initial gas production and fractional rate of gas production, which may be due to higher accumulation of the soluble, readily degradable components (Campos *et al*. 2004).

The soluble carbohydrates are more rapidly degradable and thus may affect fermentation processes (Downing *et al*. 2008).

Table 1 Dry matter (g/kg of fresh forage), height (cm), and chemical composition (g/kg of DM) of the first cut and second cut of the Birjand ecotype of *Kochia scoparia*

Items	Different forage cuts				Equality of variances	Pr > |t|
	First cut		Second cut			
	Mean	SD	Mean	SD		
Height	71.8	11.5	57.7	7.2	0.39	< 0.000
DM	269.9	24.9	279.5	28.8	0.91	0.666
CP	100.1	11.0	81.3	8.0	0.50	0.007
Ash	133.4	11.3	107.8	1.7	0.00	0.002
NDF	451.1	15.0	499.1	10.3	0.43	< 0.000
ADF	217.4	10.6	227.6	16.1	0.38	0.224
Hemicellulose	233.7	17.1	271.4	11.6	0.41	0.001
EE	13.9	7.7	14.6	5.2	0.42	0.922

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber and EE: ether extracts.
SD: standard deviation.

Table 2 *In vitro* fermentability characteristics of the first cut and second cut of the Birjand ecotype of *Kochia scoparia*

Items	Different forage cuts				Equality of variances	Pr > |t|
	First cut		Second cut			
	Mean	SD	Mean	SD		
Gas production (24 hours)	45.55	2.77	44.11	2.05	0.71	0.51
Gas production (36 hours)	50.43	2.59	50.25	3.97	0.60	0.95
Gas production (96 hours)	54.74	2.27	56.07	4.27	0.44	0.66
a	2.62	1.05	1.75	0.27	0.12	0.23
b	50.93	2.27	52.99	2.20	0.96	0.32
a + b	53.56	1.28	54.75	2.40	0.44	0.49
c	0.0872	0.0008	0.0769	0.0068	0.03	0.11

a: initial gas production (mL); b: gas production during incubation (mL); a + b: potential gas production (mL/200 mg DM) and c: fractional rate of gas production per hour.
SD: standard deviation.

Table 3 Dry matter (g/kg of fresh forage), height (cm), and chemical composition (g/kg of DM) of N fertilized and non-fertilized samples of Sabzevar ecotype of *Kochia scoparia* in first bloom stage of maturity

Items	Kochia samples				t	Pr > |t|
	Non fertilized		Fertilized			
	Mean	SD	Mean	SD		
Height	54.1	16.1	73.2	14.4	-5.43	< 0.000
DM	304.8	16.3	169.4	15.3	16.60	0.000
CP	107.4	3.8	202.8	2.3	-27.10	0.001
Ash	145.2	2.5	187.5	2.3	-30.57	0.001
NDF	410.1	11.3	326.3	24.8	6.73	0.021
ADF	187.2	2.1	169.3	7.9	3.34	0.079
Hemicellulose	222.8	10.6	157.00	17.00	8.87	0.012
EE	13.2	7.7	21.8	11.0	-1.21	0.350

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber and EE: ether extracts.
SD: standard deviation.

Table 4 Cumulative gas production at 24, 36, and 48 hours post-incubation and gas production kinetics of the N fertilized and non-fertilized kochia forage of Sabzevar ecotype and alfalfa hay

Items	Forage samples			SEM	P-value
	Kochia hay		Alfalfa hay		
	Non-SKS	F-SKS			
Gas production at 24 hours	47.54[a]	36.70[b]	46.96[a]	2.19	0.021
Gas production at 36 hours	53.09	42.06	52.87	3.24	0.102
Gas production at 96 hours	58.42	48.93	56.68	3.63	0.224
a	0.68[c]	2.74[b]	8.74[a]	0.24	< 0.000
b	56.44[a]	45.02[b]	47.03[b]	1.82	0.009
a + b	57.12[a]	47.77[b]	55.77[ab]	1.86	0.024
c	0.081[a]	0.067[b]	0.084[a]	0.00	0.008

non-SKS: non fertilized forage of Sabzevar ecotype of *Kochia scoparia* and F-SKS: N fertilized forage of Sabzevar ecotype of *Kochia scoparia*.
a: initial gas production (mL); b: gas production during incubation (mL); a + b: potential gas production (mL/200 mg DM) and c: fractional rate of gas production per hour.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

On the other hand, it is indicated that the ruminants prefer forages with higher amounts of soluble carbohydrates and are able to distinguish between two forage samples that differ by as little as 0.5% soluble sugar (Mayland *et al.* 2005).

Unlike the gas production kinetics, the chemical composition results indicated that considerable differences were exist between first cut and second cut kochia. First cut kochia had higher amounts of CP and ash and lower amounts of NDF and hemicellulose compared with those of the second cut forage.

A high concentration of ash may increase rumen osmolality and stimulate water consumption, which may cause a decrease in DM intake and ruminal digestibility of the plant cell wall (Carter and Grovum, 1990; De Waal *et al.* 1989). While, a decrease in ash content may causes an increase in the voluntary intake of DM and organic matter (OM) by ruminants (Carter and Grovum, 1990).

Also, it is indicated that an increase in ADF and NDF content of forage may depress the OM digestibility and non-ammonia N flow into the duodenum (Mambrini and Peyraud, 1994).

It is indicated that the CP content of forages had a high correlation with rumen degradable DM and rumen degradable CP (r=+0.86, Hoffman *et al.* 1993; Du *et al.* 2016), while the NDF contents of forage had negative correlation with rumen degradable DM and rumen degradable CP (r=-0.91 and r=-0.81, respectively, Hoffman *et al.* 1993; Du *et al.* 2016).

Consequently, the higher amounts of NDF and lower amounts of CP in second cut kochia than those of the first cut forage may to reduce its nutritional value for ruminants. The observed increase in NDF, hemicellulose, and ADF content of the second cut plants may be somewhat explained by the growth strategy of the halophytes. To counteract salinity stress, these species employ a rapid growth strategy in the advanced growth stages to dilute salt concentrations, which minimizes salt stress (Salehi and Kafi, 2011; López-Aguilar *et al.* 2013).

Therefore, the growth and maturity development in the lower parts of the stems, which were cut 10 cm above the soil surface in first cut to facilitate branching and regrowth of the plants, may cause to increase in fiber and decrease in protein content of the second cut plants. Moreover, a reasonable explanation for non statistically different kinetic parameters of the gas production may be refer to the higher amounts of OM in the second cut plants, which can offset the negative effects of higher NDF contents on forage degradability.

In the second experiment**,** the application of 115 kg of N/hectare in the form of urea to the field induced considerable changes in the chemical composition of the first bloom Sabzevar ecotype of kochia (Table 3). So that, CP and ash content strongly increased and DM, NDF, and hemicellulose content decreased dramatically as a result of the N application. In the current study, application of the N fertilizer resulted in more than 88% increase in CP content of the first bloom kochia.

Our results confirmed Steppuhn *et al.* (1994) who evaluated the effects of applying 0-168 kg N/hectare on forage production of mature kochia and reported a linear increase (up to 64%) in N content of kochia with increasing N fertilization. Lovett *et al.* (2004) with perennial ryegrass, indicated that N fertilization decreased DM content, and increased NDF, ADF, CP, and ash concentrations.

Other researchers with different forage species reported an increase (Namihira *et al.* 2010) or a decrease (Li *et al.* 2016; Islam *et al.* 2012; Kaplan *et al.* 2016; Wilman and Wright, 1978; Wilman, 1975; Lovett *et al.* 2004) in DM content when N fertilizer was applied.

Nevertheless, the DM content of N fertilized kochia (169.4 g/kg) was less than the critical level of 247 g DM/kg of fresh forage for good ensiling conditions (Castle and Watson, 1973), which may limit ensiling process and DM intake/head/day.

However, as described above, higher contents of CP and lower amounts of NDF and ADF might increase rumen digestibility of N fertilized kochia than non-fertilized kochia (Hoffman *et al.* 1993; Du *et al.* 2016).

In the gas production test, the fermentability of the N fertilized kochia was compared with the non-fertilized kochia and alfalfa hay as a common forage source for dairy cows. These results revealed that unexpectedly N fertilized kochia with lower content of NDF and ADF had lower potential gas production than non-fertilized kochia.

The most likely explanation for these observations may be related to the higher content of hemicellulose and fermentable OM in non-fertilized kochia than fertilized Kochai. It is accepted that, ADF content is an important limiting factor for forage DM digestibility (Hoffman *et al.* 1993; Du *et al.* 2016).

There was no considerable increase in the ADF content of the non-fertilized kochia than that of fertilized plants, while, the increases in hemicellulose, potentially digestible fraction of plant cell wall, greatly was significant. Cui *et al.* (2016), investigated the effects of different levels of urea fertilizer on forage oat straw and reported a linear reduction in 72 hours cumulative gas production and potential gas production (a+b) with increasing urea fertilizer application.

The results indicated a linear increases in NDFom, ADFom, cellulose, and lignin content and no change in hemicellulose content of oat straw in response to graduated increase in urea application, which may demonstrate the cause of decreasing in fermentability.

Table 5 Protein fractions, duodenal utilizable crude protein supply (uCP), and effective uCP (EuCP) supply of the N fertilized and non fertilized kochia forage of Sabzevar ecotype, kochia forage of Birjand ecotype, kochia forage of Esfahan ecotype, and alfalfa hay (g/kg of DM)

Items	Forage samples					SEM	P-value
	Kochia hay				Alfalfa hay		
	EKS	BKS	Non-SKS	F-SKS			
CP	162.5	114.3	107.4	202.8	173.0		
TP	138.4^{b}	88.3^{d}	104.2^{c}	162.8^{a}	139.0^{b}	1.75	< 0.000
Tungstic acid soluble protein	24.0^{b}	26.0^{b}	3.2^{c}	40.0^{a}	34.0^{a}	1.75	< 0.000
uCP in 8 hours post incubation	174.1^{ab}	137.3^{c}	163.2^{bc}	201.1^{a}	173.9^{ab}	8.10	0.001
uCP in 48 hours post incubation	101.5^{ab}	57.3^{b}	74.0^{ab}	117.1^{a}	86.1^{ab}	12.76	0.038
EuCP$_{0.02}$	99.9^{ab}	55.5^{d}	72.0^{cd}	115.0^{a}	84.1^{bc}	4.32	< 0.000
EuCP$_{0.04}$	127.9^{b}	86.4^{d}	106.5^{c}	147.6^{a}	118.1^{bc}	3.11	< 0.000
EuCP$_{0.06}$	144.4^{b}	104.5^{d}	126.7^{c}	166.6^{a}	138.0^{bc}	2.65	< 0.000

BKS: Birjand ecotype of *Kochia scoparia*; EKS: Esfahan ecotype of *Kochia scoparia*; Non-SKS: non-fertilized forage of Sabzevar ecotype of *Kochia scoparia* and F-SKS: N fertilized forage of Sabzevar ecotype of *Kochia scoparia*.
TP: true protein; EuCP $_{0.02}$: effective uCP at assumed rumen passage rate of 0.02/hour; EuCP $_{0.04}$: effective uCP at assumed rumen passage rate of 0.04/hour and EuCP $_{0.06}$: effective uCP at assumed rumen passage rate of 0.06/hour.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Moreover, Lovett *et al.* (2004) reported a decline in total gas production of perennial ryegrass when N fertilizer level increased from 0.0 to 160 kg/hectare.

In addition, high concentration of ash in N fertilized plants may affect fiber digestibility. Bilbro *et al.* (1991) reported the silica concentration in kochia at 1.5%.

Research with rice straw (low in lignin and high in silica) indicated that the silica content of hay reduced bacterial colonization and fiber digestibility (Bae *et al.* 1997; Van Soest, 2006) and increased NDF lag time (Agbagla-Dohnani *et al.* 2003). Kaplan *et al.* (2016) whit ensiled corn and Abbasi *et al.* (2012) with halophytic plant of *Amaranthus hypochondriacus* reported an increase in gas production of N fertilized than non-fertilized plants. While Islam *et al.* (2012) with forage rape reported a negative correlation between CP and gas production. Nevertheless, N fertilized kochia had significantly higher initial gas production than non-fertilized kochia which may be due to increases in soluble sugars and readily fermentable carbohydrates (Islam *et al.* 2012; Campos *et al.* 2004). Current results indicated that *Kochia scoparia*, depending on growth condition, may be comparable to alfalfa hay in terms of fermentability and nutritional value.

We determined true protein, uCP value, and EuCP of the Esfahan ecotype of kochia in pre-bloom stage, Birjand ecotype of *Kochia* in first bloom stage, alfalfa hay in 50% blooming, and N fertilized and non-fertilized Sabzevar ecotype of kochia in first bloom stage of maturity. The Esfahan and Birjand samples were selected from samples of each ecotype at different maturity stages, according to the potential gas production. Results revealed that true protein content of different non fertilized sample of different ecotypes of *Kochia scoparia* was in the range of 77.21 to 97.05 percent of the CP content. While, the true protein content of the N fertilized kochia was 80.28% of CP, which was not lower than that of non-fertilized kochia and was comparable to that of alfalfa. The average true protein content of the N fertilized Sabzevar ecotype of kochia was 162.8 g/kg DM, which was 56.22% greater than the average true protein content of the non-fertilized stands. On the other hand, the tungstic acid soluble protein content of the fertilized plants (40.0 g/kg DM) was 12.65 folds greater than that of the non fertilized kochia, which may to increase the risk of the nitrate poisoning in ruminants (Boyer *et al.* 2014).

However, the tungstic acid soluble protein (non protein N) mainly consisted of different nitrogenous compounds including; amino acids, and amides, such as glutamine and asparagine, nucleic acids, nitrates, and ammonia (Pacheco and Waghorn, 2008).

Steppuhn *et al.* (1994) reported an exponential increases in nitrate content of kochia, when the application of N fertilizer increased from 0.0 to 168 kg/hectare, but the maximum concentration of the accumulated nitrates was 0.5 g/kg DM, which was less than the safe limit of 1.1 g/kg DM to avoid livestock poisoning (Guide to farm practice in Saskatchewan Agricultural Services Co-ordinating Committee, 1987). The current results confirmed Chase *et al.* (1976) and Reid and Strachan, (1974) who reported an increase in true protein, tungstic acid soluble protein, and nitrate in alfalfa and grass forages in response to different levels of N fertilizer application.

However, tungstic acid soluble protein of the N fertilized kochia was 19.7% of total N that was in the normal range of 20-30% (Mangan, 1982). Tungstic acid soluble protein accumulation increase in plant tissues when application rate of N fertilizer exceeded from maximum N requirements for maximum DM yield/hectare, which was calculated 159.7 kg N/hectare by Steppuhn *et al*. (1994).

The highest estimates of uCP at both 8 and 48 hours post-incubation was related to the N fertilized kochia, followed by Esfahan ecotype, alfalfa, non fertilized kochia, and Birjand ecotype, but with increasing incubation time, the differences decreased. The uCP value reflects the undegraded feed proteins plus microbial protein synthesis during fermentation. Therefore, uCP results of 8 hours incubation are consistent with higher initial gas production and higher content of true protein of N fertilized kochia than those of non fertilized kochia. But, with elongation of the incubation time, the depletion of fermentable substances and microbial lysis caused to increase in protein degradation, increase in ammonia N, and consequently decrease in uCP amounts.

The Pearson correlation coefficient between uCP measurements at 8 and 48 hours post-incubation and CP, true protein, and tungstic acid soluble protein were; 0.87 and 0.89, 0.96 and 0.96, and 0.48 and 0.52, respectively. These results indicate that the CP and true protein content of a forage sample are good measurements to evaluate forage quality in terms of the supplying amounts of uCP to allocate forages of different qualities to various groups of dairy cows.

According to Table 5, the EuCP increased with increasing rumen assumed passage rates from 0.02 to 0.06/hours. Increasing in rumen passage rate means lower rumen retention time and lower degradation of feed, which cause to higher EuCP, as described about uCP amounts at 8 and 48 hours post incubation. Our estimates of alfalfa uCP was close to the values reported by Edmunds *et al*. (2012), but overall average uCP values of 23 forage samples that determined by them at the assumed passage rates of 0.02, 0.04, and 0.06/hours were 112.7, 148.0, and 168.6 g/kg DM, respectively, which was higher than those values obtained in the current study. The higher average CP (180.6 g/kg DM) in the forage samples were used by Edmunds *et al*. (2012) than the average CP content of the forage samples used in the present study (152 g/kg DM) may explain the main reason of the lower values observed here.

The N fertilization cause to increase CP, true protein, and tungstic acid soluble protein in the different forage plants (Peyraud and Astigarraga, 1998; Abbasi *et al*. 2012). As reviewed by Peyraud and Astigarraga (1998), low N fertilization reduced solubility of CP and increased the portion of cell wall associated N of the total grasses N, while high levels of N fertilization increase the CP solubility and slightly increase non ammonia N flow to the small intestine, without increase in percentage of the NDF associated CP. Moreover, with alfalfa it is indicated that N fertilization cause to increase in CP and rumen solubility of proteins without any increase in *in situ* rumen degradability of CP (Cherney *et al*. 1995). Abbasi *et al*. (2012) with amaranths, a halophytic plant which similar to kochia is belong to the *Amaranthaceae* family, reported higher CP, true protein, and protein solubility, without considerable increases in the content of NDF associated protein in consequence of N fertilization. Therefore, the higher uCP supplied by N fertilized kochia than non-fertilized kochia may be due to higher amounts of true proteins and higher resistance of soluble proteins to rumen degradability. Higher solubility of true protein in N fertilized plants increase the possibility of protein bypass through rumen without degradation and cause to increase in non-ammonia N flow to the duodenum, especially when DM intake is high.

CONCLUSION

In general, the results of the first experiment have revealed that there is no significant difference between first cut and second cut forage of Birjand ecotype of *Kochi scoparia* for gas production kinetics, but the lower content of NDF and hemicellulose and higher content of CP in the first cut than those of the second cut plants are more desirable for ruminant nutrition. The results of second experiment indicate that the application of moderate levels of the N fertilizer on Sabzevar stands of the *Kochia scoparia* induce considerable changes in chemical composition, fermentability, and protein degradability of forge, so that, DM , NDF, and hemicellulose declined and CP and ash content of forage increased. Moreover, the gas production kinetics decreased and supplied amounts of uCP and effective uCP increased in association with CP and true protein content. Chemical composition, gas production, and uCP production of N fertilized kochia was comparable to those of alfalfa hay.

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