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Effects of nitrogen and phosphorus fertilizers on nutritive value of safflower forage and silage

F. Asgharzadeh[1*], M. H. Fathi Nasri[1], M. A. Behdani[2]

[1]*Department of Animal Science, University of Birjand, Iran*
[2]*Department of Agronomy, University of Birjand, Iran*

Abstract

This experiment was conducted to determine the effects of nitrogen and phosphorus fertilizers on nutritive value of safflower forage and silage by in situ and in vitro methods. The safflower forage was harvested at flowering stage, chopped to 3-5 cm particles and then ensiled in the laboratory silos for 60 days. The experimental treatments included: 1. Unfertilized forage (control), 2. Phosphorus fertilized forage (100 kg per ha), 3. Nitrogen fertilized forage (300 kg per ha), and 4. Nitrogen and phosphorus fertilized forage (300 kg N and 100 kg P per ha). The effect of Nitrogen and phosphorus fertilizers on reduction of Dry mater, Neutral detergent fiber and Acid detergent fiber and increasing of ash, Calcium and Phosphorus of forage and silage was significant. The Nitrogen fertilizer significantly increased Crude protein, Non-protein nitrogen and Buffer soluble protein and decreased the Acid detergent insoluble nitrogen content of silage. The Nitrogen and phosphorus fertilizers caused the significant increase of the degradability of both safflower forage and silage. The highest production of gas after 96 h of incubation belonged to treatment 4 and the lowest was for treatment 1 (for both forage and silage). The effect of Nitrogen and phosphorus fertilizers on gas production of forage and silage was significant. Applying the Nitrogen and phosphorus fertilizers caused the significant increase of the gas production parameters including b and c fractions and also Metabolism energy content of forage and silage. Nitrogen and phosphorus fertilizers application caused the production of the higher quality forage for making silage and also improving the Dry mater digestibility of silage.

Keywords: Nitrogen fractionations, Ruminal degradability, Gas production, Safflower forage

*Correspondence author: Tel: +989151639479; Fax: +985614310922
E-mail address: asgharzadeh.samira@gmail.com

Introduction

Oily plants have a significant role in providing energy requirements for both human and livestock. The safflower which is an oily general purpose plant from *Asteraceae* family is used as forage for livestock feeding in addition to supplying oil for human. This plant has very deep and strong roots reaching to 3.7 m of the ground depth and generating several lateral roots. This intimacy helps the plant have higher absorbing power and utilize the moisture reservation in the depth of the ground (Henderson, 1981). The safflower also has a relatively high stability in salty soils according to salinity tolerance ranks after barley, sugar beet and cotton; hence, it is possible to cultivate it in salty farmlands (Zeynali, 1999). Safflower planting has a long antiquity in Iran and as a tolerant plant against the unfavorable environmental conditions, it likes water shortage, cold, saline and alkaline grounds and has good potentials in providing the forage for ruminants in such conditions.

An approach for increasing the nutritive value of forage crops is to meet their nutritional requirements to grow better and to prevent their destruction (Khoshgoftar-manesh, 2003). The nitrogen element is the first preference of many plants including safflower for desirable growth. Nitrogen has a structural impression to make oil and protein; therefore, providing enough N especially in oily plants is of myriad importance. Phosphorus also plays many important roles in plants and especially has deserving impression on energy transmission processes. The influence of safflower supplementing with N and P fertilizers on many of its agronomical aspects has been deeply studied, but, as the authors are aware, the effects of supplementing safflower with these two important elements on nutritional value of its forage for ruminant is not well evaluated. So, the objective of this study was to illustrate the effects of applying N and P fertilizers to safflower plants on the nutritional value of its forage and silage applying *in situ* and *in vitro* methods.

Materials and methods

Testing environment

Safflower (cv. Esfahan) was grown at agricultural research station of Birjand University (latitude 32° 52′ 20″ N, longitude 59° 9′ 52″ E); it was sown in autumn 2009. The field was randomly divided into 4 × 3 m^2 plots which received N fertilizer at 0 or 300 kg ha^{-1} or P fertilizer at 0 or 100 kg ha^{-1}. There were four replicate plots for each fertilization level in a completely randomized design and with factorial arrangement. In late June 2010 the safflower forage of different treatment plots were harvested at the flowering stage. The plants from the replicate fertilization treatments were pooled. The forages were chopped to 3-cm particles with a chopper (Fimaks Turkish) and some were ensiled in 3 L anaerobic plastic buckets (with density of 600 kg m^{-3}) and stored at 25°C for 60 days. There were six buckets per fertilization treatments. Some of the fresh forage was also oven dried for the next chemical analysis. The forage samples were subjected to the chemical analyses including DM (dry matter), CP (crude protein), NDF, ADF, ash, Ca, P, water soluble carbohydrate (WSC), buffering capacity, tannins and N fractionations (i.e. NPN = non-protein nitrogen),

buffer soluble protein (BSP), NDIN (neutral detergent insoluble nitrogen), and ADIN (acid detergent insoluble nitrogen). The silage samples were also analyzed for chemical compositions including DM, CP, NDF, ADF, ash, Ca, P, lactic acid, WSC, NH_3-N, CO_2 production, pH, tannins and N fractionations.

Analytical procedures

The forage and silage samples were dried using a forced-air oven for 48 h at 60 ˚C and then grounded to pass through a 2 mm screen. Crude protein was determined by the Kjeldahl method. Neutral detergent fiber (inclusive of ash, assayed using sodium sulfate but without amylase) and ADF (inclusive of ash) were determined according to van Soest et al. (1991). Ash, Ca and P content of feed samples were analyzed according to AOAC (1990) methods. The water soluble carbohydrate content was determined by the phenol-sulfuric acid method according to Dubois et al. (1956). The buffering capacity of the fresh safflower plants was determined according to Playne and McDonald (1966). Lactic acid was determined by the spectrophotometric method of Barker and Summerson (1941). Filya (2003) method was used for determining the NH_3-N. Tannin content of samples was measured according to Makkar et al. (1995). The CO_2 production of the silage samples which served as a spoilage indicator was determined by the "bottle"system, as described by Ashbell et al. (1990). The NPN of the samples was estimated by incubating 2 g DM equivalent of feed samples in 3 M sodium tungstate solution and determining N in residual sediments (NPN was then calculated as the total N minus residual sediments N). Buffer soluble protein was determined similarly, but borate-phosphate solution was used. Neutral detergent insoluble nitrogen and ADIN were measured according to Licitra et al. (1996).

In situ DM degradability

Ruminal DM disappearance of feed samples was evaluated using the *in situ* technique. Two ruminally fistulated Holstein heifers (400 ± 10 kg BW) were used in the study. Heifers were fed on a totally mixed ration (TMR) twice daily (0800 and 1700 h). The TMR included (on DM basis) 1.8 kg alfalfa hay, 0.5 kg maize silage, 1.8 kg wheat straw and 1.8 kg concentrate (barley 635.0, cottonseed meal 58.0, beet pulp 173.0, wheat bran 115.0, limestone 10.0, salt 4.0 and vitamin-mineral supplement 5.0 g kg DM^{-1}) per heifer per day. In order to determine DM ruminal degradability coefficients, 5 g DM equivalent of each feed sample (ground with a 2 mm screen mill) was placed in the individual nylon bags (made of artificial silk cloth with a 50 µm pore size and averaged 12 cm × 19 cm). The incubation times were 2, 4, 8, 16, 24, 48, 72 and 96 h. The bags were placed in the dorsal sac of the reticulorumen of heifers (2 bags per each feed sample in each heifer) after morning feeding. Immediately after incubation, the bags were hand washed thoroughly in cold running water. Two bags of each feed sample were washed without incubation in the rumen (0 h samples). The bags were dried in a forced-air oven (60 ˚C, 48 h) and weighed to determine DM disappearance. The exponential equation of Ørskov and McDonald (1979) was fitted to values for ruminal degradation of DM versus time. Curves were fitted using the NLIN procedure of SAS (SAS, 1989) to estimate the soluble (a) and insoluble potentially degradable (b) fractions, and rate of degradation (c).

Effective degradation (ED) was calculated as a function of degradation and passage, assuming a constant fractional passage rate (kp) of 0.08 h^{-1}: ED = a + [(b × c)/(c + kp)].

Gas production test

Rumen liquor which was obtained from two ruminally fistulated Holstein heifers, 1 h before the morning feeding, was strained through 4 layers of cheese cloth and the filtrate was maintained at 39 °C under CO_2 reflux before use. The diet of heifers and feeding times was as described at *in situ* study. The inoculum was prepared as described by Menke and Stingass (1988). It consisted of the rumen liquor mixed with anaerobic artificial saliva (1:2 V/V). The latter included, for a final volume of 1 liter, 237 ml of buffer solution, 237 ml of a main element solution, 0.12 ml of a trace element solution, 1.22 ml of resazurin solution (100 mg resazurin made up to 100 ml distilled water), 49.5 ml of a reduction solution (prepared fresh and separately and consisting of 2 ml of NaOH 1 N, 285 mg of Na_2S-$7H_2O$ and 47.5 ml distilled water for 1 L saliva), completed by 475 ml of distilled water. The buffer solution consisted of $NaHCO_3$ 35 g L^{-1} and NH_4HCO_3 4 g L^{-1}. The main element solution consisted of Na_2HPO_4 5.70 g L^{-1}, KH_2PO_4 6.20 g L^{-1} and $MgSO_4$-$7H_2O$ 0.60 g L^{-1}. The trace element solution consisted of $CaCl_2$-$2H_2O$ 13.20 g, $MnCl_2$-$4H_2O$ 10.00 g, $CoCl_2$-$6H_2O$ 1.00 g and $FeCl_2$-$6H_2O$ 0.80 g made up to 100 ml with distilled water. The rumen liquor was incorporated in the medium once the reduction process was achieved (resazurin decoloration after adding the reduction solution). To measure the gas production of samples, about 200 ± 10 mg DM of substrate was weighted into 100 ml calibrated glass syringes. The syringes, pre-warmed at 39 °C and their pistons lubricated by vaseline to prevent gas from escaping, were then inoculated by 30 ml inoculum under continuous CO_2 reflux. They were incubated in a water bath at 39 °C for 96 h. The gas production (GP) of each syring was recorded after 2, 4, 8, 16, 24, 48, 72 and 96 h of incubation. Each substrate was incubated in duplicate in three different runs in order to generate 6 measurements per substrate sample. Each run included in triplicate and a blank (syringes incubated with the inoculum alone). The corrected GP values were fitted with the model $y= b (1-e^{-ct})$, where "b" is the gas produced from the slowly fermented DM and "c" is the rate constant of DM fermentation.

Statistical analyses

The experiment was designed as completely randomized by a factorial arrangement of treatments and the date was analyzed using the General Linear Models procedure of SAS (SAS, 1989), with the factors in the model consisting of N fertilization (2 level), P fertilization (2 level) and their interactions. The main effects of factors and their interactions were compared using Tukey-Kramer test.

Results and Discussion

Chemical compositions of forage

The results demonstrated that a significant statistical difference ($P < 0.05$) existed for DM of different treatments (Table 1). The effect of fertilizing by N and P was significant ($P < 0.05$) for decreasing the DM of treatments. The main reason for this reduction of forage DM came from N and P fertilizer effect in the plant growing motivation, specially the aerial members.

Using the N fertilizer leads to increase the leaves (Kamal, 1973; Nasr et al., 1978) and thus cause the DM forage increment. Applying the P fertilizer redounds to increase root growth and aerial members and also create great leaves (Dahnke et al., 1990). Crude protein content of the safflower forage increased significantly ($P < 0.05$) by N fertilizing. The crude protein is one of the main factors affecting the forage quality of (Lawlor et al., 2001). Phosphorus fertilizers also commonly caused the increase in the forage protein content (Dahnke et al., 1990) and usually conduce to improve the CP content. The phosphorus fertilizer also caused the increase CP of the forage, but not significantly. The effect of N and P fertilizing in reduction of NDF and ADF of the forage was significant ($P < 0.05$).

Applying the N and P fertilizers motivated the vegetative stage and hindered the plant maturation that lead to lowering the NDF and ADF.

The safflower forage contained high amounts of ash and fertilizing caused the increase of ash content too. There was a significant ($P < 0.05$) difference between the control group and fertilized forages and the effect of N and P fertilizing on increment of Ca and P content of forages was significant ($P < 0.05$). The nitrogen fertilizer increases the uptake of K, Na, Ca and Mg by plant and P fertilizer enhances P content of the plant (Najarnejad-Mashhadi, 2007), so the enhancement of the ash, Ca, and P of forage by fertilizing was expected.

Table 1. The chemical comparison of safflower forage through difference fertilizing treatment

	Treatment					Effect of N fertilizer			Effect of P fertilizer			Significant level of factors effects		
	1	2	3	4	SEM	0	300	SEM	0	100	SEM	N	P	N*
DM	38.0^{a}	37.0^{ab}	36.0^{ab}	34.3^{c}	0.39	37.5^{a}	35.2^{b}	0.27	37.0^{a}	35.7^{b}	0.27	*	*	ns
CP	9.5^{c}	10.8^{bc}	13.0^{ab}	13.8^{a}	0.53	10.1^{b}	13.4^{a}	0.37	11.2	12.3	0.37	*	ns	ns
NDF[1]	42.1^{a}	41.5^{a}	37.7^{b}	37.2^{b}	0.16	41.8^{a}	37.4^{b}	0.11	39.9^{a}	39.3^{b}	0.11	*	*	ns
ADF[2]	35.7^{a}	34.9^{b}	32.8^{c}	32.6^{c}	0.05	35.3^{a}	32.7^{b}	0.04	34.2^{a}	33.7^{b}	0.04	*	*	*
Ash	6.0^{c}	7.0^{b}	11.0^{a}	11.7^{a}	0.33	8.0^{b}	11.3^{a}	0.23	90.0^{b}	10.3^{a}	0.23	*	*	ns
Ca	1.0^{c}	1.1^{b}	1.2^{a}	1.2^{a}	0.18	1.0^{b}	1.2^{a}	0.13	1.1^{b}	1.2^{a}	0.13	*	*	ns
P	0.29^{d}	0.37^{c}	0.38^{b}	0.39^{a}	0.0015	0.33^{b}	0.38^{a}	0.0012	0.33^{b}	0.37^{a}	0.001	*	*	*
WSC[3]	5.4	5.2	5.3	5.2	0.02	5.3	5.3	0.02	5.4	5.3	0.02	ns	ns	ns
BC[4]	34.0^{d}	40.7^{c}	45.3^{b}	50.0^{a}	0.84	37.3^{b}	47.7^{a}	0.6	39.7^{b}	45.3^{a}	0.6	*	*	ns
Tannin[5]	0.73	0.65	0.50	0.40	0.11	0.69	0.45	0.77	0.61	0.52	0.77	ns	ns	ns
NPN[6]	0.22^{b}	0.30^{ab}	0.44^{ab}	0.65^{a}	0.09	0.26^{b}	0.54^{a}	0.06	0.33	0.47	0.06	*	ns	ns
BSP[7]	0.25^{b}	0.29^{b}	0.43^{ab}	0.69^{a}	0.06	0.27^{b}	0.56^{a}	0.047	0.34	0.49	0.047	*	ns	ns
NDIN[8]	0.28	0.29	0.29	0.30	0.04	0.27	0.29	0.02	0.29	0.30	0.02	ns	ns	ns
ADIN[9]	0.0049^{a}	0.045^{ab}	0.039^{bc}	0.036^{c}	0.002	0.047^{a}	0.037^{b}	0.001	0.044	0.040	0.001	*	ns	ns

Treatment: 1. Unfertilized forage (control), 2. Fertilized forage with P (100 kg per ha), 3. Fertilized forage with N (300kg per ha), 4. Fertilized forage with N and P (300kg N and 100kg P per ha).

[1]insulable fiber in neutral detergent (DM); [2]insulable fiber in acid detergent (DM); [3]sulable carbohydrates in water (DM); [4]buffer capacity (mequiv); [5]tannin (DM); [6]non protein nitrogen (gr N per total gr N); [7]sulable protein in buffer phosphate boret (gr N per total gr N); [8]insulable nitrogen in neutral detergent (gr N per total gr N); [9]insulable nitrogen in acid detergent (gr N per total gr N).

NS: not significant and *: $P < 0.05$; unlike letters in each row show the significant statistical difference.

The WSC content of safflower forage was unaffected significantly by fertilizing. The WSC of plant usually decreases following the N fertilizer applying (van Soest et al., 1991) which the result of fructans is lowering. The crucial level of WSC in forages for production of the desirable silage is about 2.5-3 percentage of the fresh forage (Wilkinson, 1990). The WSC density of all experimental treatments in the present study was higher than the suggested criteria. The buffering capacity was increased significantly ($P < 0.05$) by applying fertilizers. Most of the buffering characteristics of the forages are attributed to their CP and anions (salts of organic acids, orthophosphates, sulfates, nitrates and chlorides) content. The buffering capacity of the forages usually rises with the CP level enhancement. Furthermore, increasing the soil mineral contents leads to increase the buffering capacity of the forages (McDonald et al., 1995). Thus, the effect of N and P fertilizers on buffering capacity in increasing the safflower forage was the result of raising its CP and ash content. The total tannin content of the forages was reduced by implementing the fertilizers but not significantly. The NPN and BSP of the forages was significantly ($P < 0.05$) increased in treatment 4 compared to the control group. The NPN content of the forages is variable according to their physiological stage. So, if the growth conditions of the plant are desirable, the NPN content will be greater (McDonald et al., 1995). As implementing the N fertilizer improves the plant growth, the higher NPN content of N fertilized forage is expected. Fertilizing with P also increased this fraction of the forage N, but it was not considerable. The NDIN content of the forage was increased by applying fertilizers. However, it was not significant, but the ADIN content decreased significantly ($P < 0.05$) by the N fertilizer application. Lowering the cell wall content of the plant by using the N fertilizer may have been the main reason of this reduction.

Chemical composition of silage

The results showed that there was a significant difference ($P < 0.05$) between DM of treatments (Table 2). As fertilizing with the N and P fertilizers decreased DM of the forage, this reduction was also observed for ensiled forages. Applying the N fertilizer caused to increase the CP content of safflower silage ($P < 0.05$) but the effect of the P fertilizer on increasing the CP content was not significant. The effect of the N fertilizer on decreasing the NDF of silage was significant, but this was not the case for the P fertilizer. The reason was probably the better hydrolysis of hemicellulose of the forage during the fermentation process in the silo by adding the N fertilizer. The hemicellulose degradation in the silo occurs by the action of the hemicellulase enzymes of the forage, bacterial hemicellulases and hydrolysis by acids of the silo (McDonald et al., 1991). Nitrogen contributes to the catalyst of the chemical reaction that hemicellulase enzymes involve in (Robb and Pierpon, 1983). The ADF content of both the N fertilized and P fertilized silages was significantly ($P < 0.05$) reduced, which was due to the better hydrolysis of the cell wall.

The effect of the N and P fertilizers on increasing the ash content of the safflower silage was significant ($P < 0.05$) and the main reason was the effect of the fertilizers on the ash content escalation of the forages. The effect of the N fertilizer on increment of the P and Ca content of the safflower silage was significant ($P < 0.05$), but the P fertilizer only increased the P content. Applying the N and P fertilizers did not impress the

pH of the safflower silage. Lactic acid content of the silage was significantly ($P < 0.05$) increased by the N and P fertilizers.

Table 2. The chemical comparison of safflower silage through difference fertilizing treatment

	Treatment					Effect of N fertilizer			Effect of P fertilizer			Significant level of factors effects		
	1	2	3	4	SEM	0	300	SEM	0	100	SEM	N	P	N*P
DM	43.3^{a}	36.3^{ab}	34.3^{ab}	29.0^{b}	2.61	39.8^{a}	31.6^{b}	1.84	38.8^{a}	32.6^{b}	1.84	*	*	ns
CP	12.3^{b}	12.4^{b}	14.4^{ab}	14.8^{a}	4.8	12.3^{b}	14.6^{a}	3.41	13.4	13.6	3.41	*	ns	ns
NDF[1]	49.0^{a}	48.4^{a}	45.3^{b}	46.0^{b}	1.62	48.7^{a}	45.6^{b}	0.11	47.1	47.2	0.11	*	*	ns
ADF[2]	42.1^{a}	41.5^{a}	37.6^{b}	37.2^{b}	1.64	41.8^{a}	37.4^{b}	0.11	39.8^{a}	39.3^{b}	0.11	*	*	*
Ash	9.0^{c}	10.3^{b}	11.0^{b}	12.3^{a}	0.23	9.6^{b}	11.6^{a}	0.16	10.0^{b}	10.3^{a}	0.16	*	*	ns
Ca	1.0^{c}	1.2^{b}	1.2^{ab}	1.3^{a}	0.28	11.1^{b}	12.8^{a}	0.2	11.3^{b}	12.6^{a}	0.2	*	*	ns
P	0.35^{b}	0.3^{b}	0.40^{a}	0.42^{a}	0.08	0.35^{b}	0.41^{a}	0.057	0.38	0.39	0.057	*	*	*
pH	4.8	4.7	4.9	4.9	0.22	4.8	4.9	0.15	4.9	4.8	0.15	ns	ns	ns
LA[3]	9.0^{c}	10.2^{b}	13.0^{a}	13.0^{a}	0.22	9.1^{b}	13.3^{a}	0.16	11.0^{b}	11.9^{a}	0.16	*	*	ns
WSC[4]	2.8	2.2	2.0	1.9	0.18	2.4^{a}	1.9^{b}	0.13	2.3	2.0	0.13	ns	ns	ns
N-NH_3[5]	18.3^{b}	20.0^{b}	24.3^{ab}	27.7^{a}	1.65	19.1^{b}	26.0^{a}	1.17	21.3	23.8	1.17	*	ns	ns
CO_2[6]	5.3	5.0	3.8	3.2	0.74	5.1	3.5	0.52	4.6	4.1	0.52	*	ns	ns
Tannin[7]	0.08	0.08	0.06	0.04	0.005	0.08	0.05	0.003	0.07	0.06	0.003	ns	ns	ns
NPN[8]	0.4^{b}	0.5^{ab}	0.7^{ab}	0.8^{a}	0.08	0.4^{b}	0.8^{a}	0.05	0.5	0.7	0.05	*	ns	ns
BSP[9]	0.63^{b}	0.81^{ab}	0.85^{ab}	0.89^{a}	0.058	0.72^{b}	0.87^{a}	0.04	0.74	0.85	0.04	*	ns	ns
NDIN[10]	0.23	0.24	0.25	0.25	0.004	0.24	0.25	0.005	0.24	0.25	0.005	*	ns	ns
ADIN[11]	0.029^{b}	0.028^{ab}	0.028^{ab}	0.027^{a}	0.0002	0.029^{a}	0.028^{b}	0.0003	0.028	0.028	0.0003	*	ns	ns

Treatment: 1. Unfertilized forage (control), 2. Fertilized forage with P (100 kg per ha), 3. Fertilized forage with N (300 kg per ha), 4. Fertilized forage with N and P (300 kg N and 100kg P per ha).

[1]insulable fiber in neutral detergent (DM); [2]insulable fiber in acid detergent (DM); [3]lactic acid (DM); [4]sulable carbohydrates in water (DM); [5]N-NH3 (DM); [6]CO2 (gr/kg DM); [7]tannin (DM); [8]non protein nitrogen (gr N per total gr N); [9]sulable protein in buffer phosphate boret (gr N per total gr N); [10]insulable nitrogen in neutral detergent (gr N per total gr N); [11]insulable nitrogen in acid detergent (gr N per total gr N).

NS: not significant and *: $P < 0.05$

Unlike letters in each row show the significant statistical difference.

The fertilizers may have improved the number of initial lactic acid bacteria. Particularly they have the high capacity for acid production (McDonald et al., 1995). Nitrogen fertilizer decreased the WSC content of the silage. The increment of LA in the ensiled N fertilized forage was 46% (compared to control), while this increment in the ensiled P fertilized forage was only 8.2%, so this may explain why N fertilizing reduced the silage WSC significantly, but P fertilizing reduced it just numerically. Water soluble carbohydrates of the forage are used by the silo s microorganisms, especially lactic acid bacteria. Thus, their contents were less in the silage compared to the fresh forages. The N fertilizer increased the NH_3 content of the safflower silage significantly ($P < 0.05$), but this effect was not observed for the P fertilizer. Applying the N fertilizer presumably has increased the decomposition of amino acid to ammonia. The CO_2 gas production, after placing silage in exposure to air, is an index of silage aerobic stability and was reduced numerically but not significantly by the N and P fertilizers. Applying the N fertilizer led to lower the tannin content of the silage which maybe was due to increasing the ratio of the leaf to stem in the forage. The N fertilizer significantly ($P < 0.05$) increased the NPN and BSP content of the silage, but the P fertilizer increased them just numerically which was probably because of the physiological conditions of the plant in the harvesting stage. The N fertilizer caused significantly ($P < 0.05$) the decrease of the ADIN content of the silage, but the P fertilizer did not affect it. The higher degradation of ADF and higher breakage of the bonds between the protein and cell wall could be the reasons for reduction of ADIN by N fertilizing.

The DM ruminal degradability of safflower forage and silage

Dry matter ruminal degradability parameters of safflower forage and silage are shown in Tables 3 and 4. The reason of this raise could be related to the reduction of fiber contents and increments of CP content of the forage by the N fertilizer. There is a negative relation between the DM soluble fraction and the

Table 3. The comparison of degradability coefficients and effective DM ruminal degradability of safflower forage through different fertilizing treatment

		Degradability[1]			Effective degradability		
					Transmission rate (per hour)		
Level N	Level P	a	b	c (per hour)	0.02	0.05	0.08
0	0	0.22^{b}	0.35	0.08^{c}	0.51^{c}	0.44^{c}	0.40^{c}
	100	0.23^{ab}	0.35	0.10^{b}	0.53^{b}	0.47^{b}	0.43^{b}
300	0	0.24^{a}	0.35	0.10^{b}	0.54^{b}	0.48^{b}	0.44^{b}
	100	0.25^{a}	0.36	0.13^{a}	0.58^{a}	0.51^{a}	0.47^{a}
SEM		0.003	0.005	0.003	0.01	0.003	0.002
N fertilizer	0	0.23^{b}	0.35	0.09^{b}	0.52^{b}	0.46^{b}	0.42^{b}
	300	0.24^{a}	0.35	0.11^{a}	0.56^{a}	0.50^{a}	0.46^{a}
SEM		0.002	0.004	0.002	0.007	0.002	0.002
P fertilizer	0	0.23	0.35	0.09^{b}	0.52^{b}	0.46^{b}	0.42^{b}
	100	0.24	0.35	0.11^{a}	0.55^{a}	0.49^{a}	0.45^{a}
SEM		0.002	0.004	0.002	0.007	0.002	0.002
Effect of N fertilizer (A)		*	ns	*	*	*	*
Effect of P fertilizer (B)		ns	ns	*	*	*	*
A*B		ns	ns	ns	ns	ns	ns

[1]-a: Quickly degradable fraction, b: slowly degradable fraction, c: degradability rate constant
NS: not significant and *: $P < 0.05$
Unlike letters in each column show the significant statistical difference.

Table 4. The comparison of DM ruminal degradability coefficients and effective DM ruminal degradability of safflower forage through different fertilizing treatment

		Degradability[1]			Effective degradability		
					Outflow rate (per hour)		
Level N	Level P	a	b	C (per hour)	0.02	0.05	0.08
0	0	0.23^{c}	0.35	0.11^{c}	0.53^{d}	0.48^{c}	0.44^{c}
	100	0.24^{b}	0.35	0.14^{b}	0.55^{c}	0.50^{b}	0.46^{b}
300	0	0.25^{a}	0.35	0.17^{a}	0.56^{b}	0.52^{a}	0.49^{a}
	100	0.25^{a}	0.36	0.17^{a}	0.59^{a}	0.54^{a}	0.50^{a}
SEM		0.002	0.004	0.003	0.007	0.003	0.002
N fertilizer	0	0.23^{b}	0.35	0.13^{b}	0.54^{b}	0.49^{b}	0.45^{b}
	300	0.25^{a}	0.36	0.17^{a}	0.58^{a}	0.53^{a}	0.50^{a}
SEM		0.001	0.002	0.002	0.005	0.002	0.002
P fertilizer	0	0.24^{b}	0.35	0.14^{b}	0.55^{b}	0.50^{b}	0.46^{b}
	100	0.25^{a}	0.36	0.16^{a}	0.57^{a}	0.52^{a}	0.48^{a}
SEM		0.001	0.002	0.002	0.005	0.002	0.002
Effect of N fertilizer (A)		*	ns	*	*	*	*
Effect of P fertilizer (B)		*	ns	*	*	*	*
A*B		ns	ns	ns	ns	Ns	ns

[1]-a: Quickly degradable fraction, b: slowly degradable fraction, c: degradability rate constant
NS: not significant and *: $P < 0.05$
Unlike letters in each column show the significant statistical difference

cell wall content of forages (Ayed et al., 2001). Also, the forages that contain higher mineral content have more DM soluble fractions (Mayne, 1993).

The same trend was noted for the DM soluble fraction of the silage, so the highest value belonged to fertilized ensiled forages with N plus P (treatment 4). The effect of both the N and P fertilizers on this fraction was significant ($P < 0.05$). Application of the chemical fertilizers to forage enhances the growth of cell wall degrading microorganisms in the ensiled forage and thereby the DM soluble fraction increases. The insoluble but potentially degradable fraction (*b*) of the DM was not effected by fertilizing in both the forage

and silage, but the DM degradation rate (*c*) significantly ($P < 0.05$) increased by the N and P fertilizers in both the forage and silage which possibly was related to the reduction of the cell wall content.

The DM effective degradability (ED) of the silage and forage significantly increased in all ruminal outflow rates (0.02, 0.05 and 0.08 per h) by using the N and P fertilizers which could be associated with the

Table 5. The comparison of gas production, OMD and ME of safflower forage and silage through different fertilizing treatment

		Forage					Silage				
Level N	Level P	Gas production[1]	b[2]	c[3]	OMD[4]	ME[5]	Gas production	b	c	OMD	ME
0	0	49.7^{b}	0.45^{d}	0.052^{c}	57.09^{d}	8.46^{d}	53.2^{d}	0.51^{b}	0.05^{c}	56.2^{c}	8.2^{d}
	100	52.7^{ab}	0.53^{c}	0.061^{b}	61.47^{c}	0.12^{c}	58.0^{c}	0.51^{b}	0.06^{b}	59.6^{b}	8.7^{c}
300	0	54.8^{a}	0.57^{b}	0.062^{b}	65.56^{b}	9.71^{b}	61.9^{b}	0.54^{a}	0.06^{b}	63.6^{a}	9.3^{b}
	100	57.3^{a}	0.65^{a}	0.064^{a}	68.20^{a}	10.04^{a}	63.5^{a}	0.56^{a}	0.062^{a}	65.4^{a}	9.6^{a}
SEM		1.03	0.015	0.0003	0.24	0.032	0.057	0.039	0.0007	0.39	0.05
N fertilizer	0	51.2^{b}	0.53^{b}	0.054^{b}	59.28^{b}	8.79^{b}	55.6^{b}	0.51^{b}	0.05^{b}	57.9^{b}	8.5^{b}
	300	56.0^{a}	0.57^{a}	0.060^{a}	66.88^{a}	9.88^{a}	62.7^{a}	0.55^{a}	0.06^{a}	64.5^{a}	9.5^{a}
SEM		0.73	0.010	0.0002	0.17	0.022	0.040	0.028	0.0005	0.27	0.03
P fertilizer	0	52.2^{b}	0.54^{b}	0.056^{b}	61.3^{b}	9.09^{b}	57.5^{b}	0.52^{b}	0.05^{b}	59.9^{b}	8.8^{b}
	100	55.0^{a}	0.56^{a}	0.058^{a}	64.8^{a}	9.58^{a}	60.7^{a}	0.53^{a}	0.06^{a}	62.5^{a}	9.2^{a}
SEM		0.73	0.010	0.0002	0.17	0.022	0.040	0.028	0.0005	0.27	0.03
Effect of N fertilizer		*	*	*	*	*	*	*	*	*	*
Effect of P fertilizer		*	*	*	*	*	*	*	*	*	*
A*B		ns	*	*	*	*	*	ns	*	ns	*

[1]Gas production rate during 96 hours incubation (ml per 200 mg DM); [2]Gas production of insoluble fraction; [3]Gas production rate constant (per hour); [4]Organic matter digestibility (DM percentage); [5]Metabolisable energy (MJ per kg DM).
NS: not significant and *: $P < 0.05$
Unlike letters in each column show the significant statistical difference.

higher DM soluble fraction of the silage and forage by the application of fertilizers. Also, this increment in ED could be a result of the reduction of the fiber content of the forage. As studies show there is a negative relationship between the fiber content and ED of DM (Yan and Agnew, 2001).

The gas production of safflower forage and silage

Gas production parameters of the safflower forage and silage are demonstrated in Table 5. The greatest amount of gas production during 96 h incubation belonged to N plus P fertilized forages and the lowest amount was for the unfertilized forage.

The effect of the N and P fertilizers on gas production was significant ($P < 0.05$). Fertilizing with N and P brought significant ($P < 0.05$) increase of the gas produced from the slowly fermented DM fraction (*b*), the rate of the fermentation of DM (*c*), ME and OMD of the forage. Higher ADF and NDF and lower CP content apparently have been the main reasons for lower ME and OMD of unfertilized forage.

There is a significant negative relation between the cell wall content and ME and OMD (Kamalak et al., 2005). The results of the current experiment was in accordance with the results of Mansuri et al. (2003) who reported that among the gas production of alfalfa forage, bamboo hay and wheat straw, the highest amount was for alfalfa with CP around 14.9 percent rather than the bamboo hay and wheat straw with 7.3 and 3.6 percent CP, respectively.

The ensiling process caused the significant ($P < 0.05$) decrease of the gas production during all incubation periods. The reduction of WSC content of the forage and converting a part of nitrogenous compounds to ammonia in the silo could be the reason of decrease of gas production of the safflower silage compared with the safflower forage. The highest amount of gas production was for N plus P ensiled

fertilized forage and the lowest was for ensiled unfertilized silage. The effect of both N and P fertilizers on silage gas production was significant ($P < 0.05$). Results of Filya (2004) illustrated that gas production of the corn silage was influenced by its CP, NDF and ADF contents. Fertilizing forage by N and P also caused significant ($P < 0.05$) increase in b, c, ME and OMD of its silage. The main reason for the higher gas production of the ensiled fertilized forage was possibly its higher CP content and lower ADF and NDF contents.

Conclusion

The results of this study beyond the demonstration of supreme features of the nutritive value and DM ruminal degradability characteristics of the safflower forage and silage which certainly are useful for the future researches showed that the forage and silage of this plant is of great value in ruminant nutrition. The application of the N and P fertilizers considerably improved the quality of ruminal fermentation of the safflower forage and silage. The safflower silage characteristics are comparable with the corn silage and its forage has high potentials for ensiling.

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