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Effect of different levels of nitrogen fertilizer on silage quality of Artichoke (*Cynara scolymus* L.)

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ABSTRACT

To study the effect of different levels of nitrogen fertilizer on silage quality of artichoke (*Cynara scolymus* L.) an experiment was conducted using a randomized complete block design with three replications in Isfahan, Iran in 2014. The N application rates were 0, 100, 150 and 200 kg ha^{-1}. Plants were harvested at vegetative rosette stage and submitted to ensiling in experimental silos for 75 days. The results showed that different levels of N fertilizer significantly affected silage quality. Crude protein (CP), ash, organic matter digestibility (OMD), metabolizable energy (ME) and net energy for lactation (NE_L) increased by the increment of N fertilizer application while neutral detergent fiber (NDF) and crude fat (CF) reduced. The highest CP (19.25 %), ash (18.66 %), OMD (49.65 %), ME (7.25 MJ kg^{-1} DM) and NE_L (4.16 MJ kg^{-1} DM) were obtained by the treatment of 200 kg N ha^{-1}. The control treatment had the maximum values of NDF (35.24 %) and CF (2.25 %). Water-soluble carbohydrates (WSC) increased by use of N fertilizer from 0 to 100 kg N ha^{-1}, but more fertilizer consumption decreased its value. In general, the nitrogen fertilizer at the rate of 150 kg N ha^{-1} can be recommended as the best treatment to obtain high-quality silage.

INTRODUCTION

Soil fertility is the capacity to supply plant nutrients in adequate amount and in suitable proportion. The fertility develops by application of organic manure and chemical fertilizers (Sedaghat et al. 2013). The use of chemical fertilizers is an integral part of practices for increasing the agricultural production (Poudel et al. 2001). Higher yields and quality of products have often been related to the appropriate contribution of nutrients and mineral nutrient application becomes essential to satisfy nutrient uptake (Negro et al. 2016). Buxton et al. (1996) mentioned that soil fertility, especially in terms of nitrogen and phosphorus has a significant influence on the nutritive value of forage crops. Among the major nutrients, nitrogen is the most important element in plant nutrition and the addition of nitrogen fertilizer usually improves plant growth and yield (Barker and Mills 2011). The amount of nitrogen fertilizer has a key role in obtaining the maximum yield and nutritive value of forage (Yousefzadeh et al. 2009) and determination of the optimized level of nitrogen is very important to achieve livestock feed with no nitrate toxicity (Sedaghat et al. 2013).

Silage is defined as a moist forage in the absence of air and preserved by fermentation (McDonald et al. 1991). Fermentation is carried out by bacteria which feed on the carbohydrates in the chopped forage and rapidly produce volatile acids and lactic acid. When the production of this acid reaches a certain level, the preserved feed is called silage **(**Meneses et al. 2005). The success of silage depends on a number of key factors such as species and variety selection, soil type, soil pH, fertilizer regime, pest control, optimal harvest time and storage losses (O'Donvan et al. 2000; Ross et al. 2004 a and b; King, 2007). The high silage quality increases digestibility and voluntary intake by animals and is achieved by the adequate amount of N fertilization. Also, the management of N fertilization changes the chemical composition of forage and consequently affects the fermentation quality of silage in the process of ensiling (McDonald et al. 1991).

Artichoke (*Cynara scolymus* L.) is a perennial plant with edible flower buds belongs to the family *Compositae,* widely cultivated in the Mediterranean

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region and central Europe (Jiménez-Escrig et al. 2003). Different studies have pointed to the good quality of artichoke wastes. Fresh artichoke by-products are suitable for ensiling with a pleasant smell, good silage characteristics, crude protein content 88 g kg^{-1} dry matter and fiber content 509 g kg^{-1} dry matter (Meneses et al. 2007) and can be used in ruminant feeding (Meneses et al. 2005). Also, this silage can be replaced conventional roughage sources such as hay (Gasa et al. 1989; Sallam et al. 2008). Nutritional value and energy content of artichoke silage are comparable to other silage (Gul et al. 2001). Fateh et al. (2009) noted that silage quality of this forage was less than alfalfa silage but it is as much as corn silage with lower seed contents and was more than sorghum silage. Sadeghian et al. (2015) reported that the palatability of this plant increased after ensiling, and also the use of urea and molasses can increase the quality of silage.

Few studies evaluated the silage quality of artichoke and for the first time, we measured some quality indices of artichoke silage, including metabolizable energy and net energy for lactation. Also, the optimal application of N chemical fertilizers was so important especially with consider to increase expense and environmental pollution, resulting from their incorrect application. So, this research was conducted to study the effects of nitrogen application rates on artichoke silage quality.

MATERIALS AND METHODS

Experimental site

The current study was carried out to investigate the effect of nitrogen fertilization on quality of artichoke silage in Isfahan (32° 37′ N, 51° 28′E and an altitude of 1612 m), Iran in 2014. The results of soil analysis are shown in Table 1.

Table 1. Some physical and chemical characteristics of the experimental soil

Texture	TN(%)	P (mg kg^{-1})	K (mg kg^{-1})	OC (%)	pH	EC ($dS.m^{-1}$)
Clay loam	0.04	15	250	0.065	7.7	2.8

Design type and Treatments

The experiment was laid out in randomized complete block design in three replications. Treatments were different doses of nitrogen fertilizer (0, 100, 150, and 200 kg of N ha^{-1}).

Farm preparation

N fertilizer was applied (½ before the time of planting+ ½ at 7-8 leaf stage) from urea (46% pure nitrogen) sources. There was no need to use phosphorus and potassium with considering to results of soil experiment. Artichoke seed was sown on 26 April. Plot size was 5 m ×3.5 m. Rows were spaced 60 cm apart and the intra-row of artichoke plant was 30 cm. All plots were irrigated after planting. Weeds were controlled by hand.

Evaluated traits

Some silage characteristics such as crude protein (CP), crude fat (CF), water-soluble carbohydrates (WSC), neutral detergent fiber (NDF) organic matter digestibility (OMD), metabolizable energy (ME), net energy for lactation (NE_L) and ash content were measured.

Silage making

Plants were harvested at vegetative rosette stage (23 August 2014), chopped at a particle length of 2.5 cm by a forage chopper and ensiled in micro-silos. Silo samples were placed at room temperature (25°C) and in a dark environment for 75 days.

Methods

WSC was measured by the phenol-sulfuric acid method **(**Kochert 1978). NDF was evaluated using the methods of Van Soest et al. (1991). The ash contents, crude protein (CP) and crude fat (CF) were determined according to AOAC (2007). Water displacement technique was used for gas production measurement (Fedorak and Hrudey 1983). The amount of gas produced from the fermentation of feed was recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hours after incubation, respectively. The following equations were used for estimation of OMD, ME and NE_L according to Menke and Steingas (1988).

OMD (% DM) = 9.00 + 0.9991 GP + 0.0595 CP +0.0181 CA (n=200, r^2 = 0.92) (1)

ME (MJ Kg^{-1} DM) = 1.06+0.1570 GP + 0.0084 CP+ 0.0220 CF – 0.0081 CA (n = 200, r^2 = 0.94) (2)

NE_L (MJ Kg^{-1} DM) = -0.36 + 0.1149 GP + 0.0054 CP + 0.0139 CF – 0.0054 CA (n=200, r^2 = 0.93) (3)

GP: gas production at 24 h after incubation (mL), CP: crude protein, CA: crude ash and CF: crude fat

Statistical analysis

The obtained data were analyzed by SAS software and the mean of treatments was compared using Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Crude Protein (CP)

Soil fertilization treatments significantly ($p \leq 0.01$) affected crude protein of artichoke silage

(Table 2). The CP content increased by increasing N application (Table 3). CP varied from 7.68 % in control treatment to 19.25 % in the 200 kg N ha^{-1}. The lower CP percent in control treatment compared with other treatments was probably due to lower nitrogen content in the soil. The amount of protein in the forage depends on different factors such as the kind of plant, the age of plant and the amount of N fertilizer (Ordas and Stucker 1977). Nitrogen is a major element that is essential for the synthesis of amino acids, proteins, enzymes, nucleic acids, cell walls, chlorophyll, phytohormones, and secondary metabolites (Hawksford et al. 2012). Almodares et al. (2009) stated that the increase in protein contents with increasing in N fertilizer levels may be the result of enhancement in the amino acid formation due to fertilization. Mengel (2001) noted that increasing nitrogen fertilizer generate increases in CP content and, consequently, increases in silage, as this nutrient accounts for approximately 16% of the constitution of these molecules. Nitrogen is important for growth, yield and protein levels in artichoke. But excess N applications will increase nitrate and reduce sugar levels in silage and may have a negative impact on silage fermentation and animal health. The positive effects of nitrogen fertilization on crude protein percent of artichoke have been discussed in previous studies. Fateh et al. (2009) reported that chemical and integrated systems increased crude protein content in globe artichoke. Similar results reported by researchers in other silage. As nutrient uptake increases, the protein synthesis in the plants tends to increase (Amin 2011). Nitrogen fertilizers can increase the contents of crude protein in grass species silage (King et al. 2013). Neumann et al. (2017) found that increasing nitrogen dose in cover fertilization provided an increase in protein value of corn silage. Nitrogen doses increased the crude protein concentration in maize and sorghum crops (Damian et al. 2017).

Crude Fat (CF)

According to (Table 2), N chemical fertilizer rates significantly ($p \leq 0.01$) influenced the silage crude fat content. CF decreased by increasing of N fertilizer, and the highest amount of it obtained in the control treatment (2.25%). Reports on the effect of N fertilizer levels on the crude fat content of herbages have been inconsistent. Alexander et al. (1963) noted that an increase in fertilizer (N-P-K) rate showed little effect on the crude fat of corn silage. As the N fertilizer application rate increased, the contents of crude fat increased in both the pre-ensiled herbage and silage of Italian ryegrass (Lv et al. 2017).

Ash

Ash percentage was significantly ($p \leq 0.01$) influenced by N rates (Table 2). Ash content increased by increasing the applied N rate (Table 3). The ash content was increased due to increase in mineral uptake. The most amount of ash percent (18.66 %) obtained in consumption of 200 kg N ha^{-1}, whereas the lowest amount of it (10.63 %) observed in control treatment. Ash percent will give a measure of the mineral content of silage. The researchers presented similar results on the effects of nitrogen fertilizer on other plants. Fateh et al. (2009) stated that the use of chemical treatments increased the ash of artichoke silage compared to control. Asgharzadeh et al. (2014) reported that application of nitrogen and phosphorus increased ash in safflower silage. In contrast, Kaplan et al. (2016) noted that crude ash content of corn silage decreased with increasing nitrogen doses.

Neutral Detergent Fiber (NDF)

Different levels of nitrogen fertilizer significantly ($p \leq 0.01$) affected neutral detergent fiber percent (Table 2). The highest and lowest NDF values were obtained in control and 200 kg N ha^{-1} treatments with 35.24 and 26.11 %, respectively (Table 3). Increasing NDF ratios make the digestion difficult and ultimately result in decreased crude protein, gas production, metabolic energy and digestible organic matter content (Kamalak et al. 2011). Also, McDonald et al. (1991) indicated that this trait is negatively correlated with digestibility and thus affects the amount of available energy for ruminants. The

Table 2. Analysis of variance for some silage chemical properties of artichoke as affected by different levels of nitrogen fertilizer

Source of variable	df	Mean squares (MS)							
		CP	CF	Ash	NDF	WSC	OMD	ME	NE_L
Replication	2	1.018*	0.11*	2.22**	0.16 ns	0.416 *	0.943*	0.029*	0.016*
Nitrogen fertilizer	3	78.10**	0.361**	35.64**	55.42**	4.58 **	94.99**	2.09**	1.11**
Error	6	0.161	0.011	0.106	0.037	0.041	0.12	0.003	0.002

**significant at 1%,* significant at 5%, ns: not significant

Table 3. Mean comparison of some chemical characteristics of silage artichoke at different levels of nitrogen fertilizer

N (kg ha^{-1})	CP (%)	CF (%)	Ash (%)	NDF (%)	WSC (%)	OMD (%)	ME (MJ Kg^{-1} DM)	NE_L (MJ Kg^{-1} DM)
0	7.68 d	2.25 a	10.63 d	35.24 d	7.23 c	37.01 d	5.38 d	2.80 d
100	15.67 c	1.80 b	15.08 c	27.28 c	10 a	46.15 c	6.75 c	3.80 c
150	17.46 b	1.62 c	16.87 b	26.70 b	9.63 ab	47.90 b	7 b	3.98 b
200	19.25 a	1.45 d	18.66 a	26.11 a	9.26 b	49.65 a	7.25 a	4.16 a

Means in the same column followed by different letter(s) are significantly different using Duncan's multiple range test ($p \leq 0.05$)

higher level of nitrogen fertilizer leads to a reduction in NDF value in artichoke because this element has a critical role in photosynthetic activity and plant growth. On the basis of Velho et al. (2007) the silage NDF contents below 50% encouraged greater consumption by animals, because of the lower this value in the feed, the higher the fermentation rate, and the faster the animal rumen gets emptied. In general, its high levels have a negative effect on the silage quality. Asgharzadeh et al. (2014) showed that nitrogen fertilizer decreased NDF of safflower silage. Li et al. (2016) noted that the contents of crude fiber (NDF and ADF) in wheat silage tended to decrease with increasing N application rate. In the contrary, Islam et al. (2012) mentioned that there was a non-significant trend towards a decrease in NDF contents of maize silage with increased application of both pre and post-sown N fertilizer.

Water-Soluble Carbohydrates (WSC)

Nitrogen fertilizer significantly ($p \leq 0.01$) affected the water-soluble carbohydrates of silage. Increase use of N fertilizer from 0 to 100 kg N ha^{-1} caused to increase the WSC % in the artichoke silage, but more fertilizer consumption decreased its value. So that the least amount of WSC (7.23 %) produced in control treatment and the most amount of that (10 %) belonged to 100 kg N ha^{-1}. The treatment of N = 100 and N = 150 kg ha^{-1} were at the same statistical groups by 10 and 9.63 WSC%, respectively (Table 3). The water-soluble carbohydrates in forage are important for microbial activities in the rumen. If the WSC is increased before ensiling, the silage pH will increase and silage quality will decrease (Van Soest 1991). Previous studies showed that N fertilization had a different effect on WSC content of various plant species. N fertilizer could increase the WSC contents of corn silage (Islam et al. 2012). Li et al. (2016) mentioned that WSC contents increased significantly as the N application rose from 0 to 225 kg ha^{-1}, but more fertilizer use reduced it. Heeren et al. (2014) found that increasing N fertilizer levels decreased the water-soluble carbohydrate content in ryegrass silage. Lv et al. (2017) investigated the effects of nitrogen fertilizer application on silage quality of Italian ryegrass. They said that increasing N fertilizer levels decreased the water-soluble carbohydrate content.

Organic Matter Digestibility (OMD)

N fertilization significantly ($p \leq 0.01$) affected organic matter digestibility in artichoke silage (Table 2). Three levels of nitrogen fertilizer increased the OMD% compared to control treatment. OMD values varied from 37.01 % in control to 49.65 % in 200 kg N ha^{-1} (Table 3). Increasing in OMD with the application of N fertilizer is related to the effect of nitrogen on increasing of CP and ash content (there is a positive significant relation among CP and ash with OMD) (Table 4). Application of nitrogen fertilizers improved the organic matter digestibility of artichoke silage by changing the amount of gas production, crude protein and ash content. Different

Table 4. Pearson correlation coefficients among different traits at different levels of nitrogen fertilizer in artichoke

Traits	CP	CF	Ash	NDF	WSC	OMD	ME	NE_L
CP	1							
CF	-0.92**	1						
Ash	0.95**	-0.87**	1					
NDF	-0.98**	0.86**	-0.92**	1				
WSC	0.81**	-0.61*	0.69*	-0.90**	1			
OMD	0.98**	-0.86**	0.94**	-0.98**	0.85**	1		
ME (MJ Kg^{-1} DM)	0.98**	-0.85**	0.93**	-0.98**	0.86**	1**	1	
NE_L (MJ Kg^{-1} DM)	0.98**	-0.85**	0.93**	-0.98**	0.86**	1**	1**	1

**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level.

results have been reported regarding the effect of nitrogen application on digestibility. Bernardi et al. (2011) found that there were no significant differences between levels of N fertilization and in vitro digestibility in corn silage. Increasing nitrogen doses increased organic matter digestibility of corn silage (Kaplan et al. 2016).

Metabolizable Energy (ME)

As shown in Table 2, the influence of N consumption on metabolizable energy was significant ($p \leq 0.01$). The amount of this trait was increased with application of nitrogen which increased the quality of the silage (Table 3). The least amount of ME observed in control treatment and the most amount of it related to 200 kg N ha^{-1} with means of 5.38 and 7.25 MJ Kg^{-1} DM, respectively (Table 3). ME is an estimate of the feed's available energy for maintenance and growth (Robinson et al. 1998). This trait increased due to a reduction in NDF percent and increasing crude protein content by applying fertilizer. A review of the published research showed that there was little research on the effect of fertilizers on the amount metabolizable energy and net energy for lactation. Islam et al. (2012) indicated that application of both pre-sown and post-sown N fertilizer increased ME of maize silage, due mainly to an increase in CP content. Martins et al. (2014) stated that nitrogen fertilization increased the ME of maize silage. Increasing nitrogen doses increased the metabolic energy of corn silage (Kaplan et al. 2016).

Net energy For Lactation (NE_L)

Net energy for lactation significantly ($p \leq 0.01$) affected by nitrogen rates (Table 2). Increasing of nitrogen fertilizer caused an increase in NE_L (Table 3). The highest and the lowest amount of NE_L obtained from consumption of 0 and 200 kg N ha^{-1} with values of 2.8 and 4.16 MJ Kg^{-1} DM, respectively (Table 3). NE_L is an estimate of the energy value of the forage when used for milk production during lactation (Van Alfen 2014). The amount of net energy for lactation, as well as metabolizable energy, is related to the amount of crude protein, crude fat, ash content, and gas production. Higher values of NE_L indicate a better quality of silage. Previous Studies in other plant showed that NE_L were improved as nitrogen fertilizers levels increased. For example, Martins et al. (2014) showed that nitrogen fertilizer enhanced the NE_L in maize silage.

Correlation coefficients

The correlation among traits is given in Table 4. CP had the highest correlation with ash, OMD, ME and NE_L, but it had a strong negative correlation with NDF and CF. CF showed a weak negative correlation with all the traits except NDF. There was a negative correlation between ash and NDF, but there was a positive correlation between ash and other traits. WSC, OMD, ME and NE_L had a significant and negative correlation with NDF. Correlation between WSC and OMD was found positive, also, ME and NE_L showed a positive correlation with WSC. OMD had a strong positive correlation with ME and NE_L. The correlation between ME and NE_L was strongly positive. Ash percentage had a positive and significant relation with crude protein content.

CONCLUSIONS

This study indicated that artichoke silage significantly affected by different levels of N fertilizer. Crude protein, ash, organic matter digestibility, metabolizable energy and net energy for lactation increased with increasing N fertilizer rate while neutral detergent fiber and crude fat decreased. WSC increased by use of N fertilizer from 0 to 100 kg N ha^{-1}, but more fertilizer consumption decreased its value. It could be concluded that nitrogen fertilizer at the rate of 150 kg N ha^{-1} showed the best results concerning silage quality and composition. Further investigations are required about acid composition (lactic, acetic, propionic and butyric acid), minerals (e.g., calcium and phosphorus) and the other silage characteristics of artichoke. In addition, animal feeding test should be carried out to assess palatability and feed intake of artichoke silage.

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