



Decreasing Nitrogen Leaching and Increasing Canola Forage Yield in a Sandy Soil by Application of Natural Zeolite

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ABSTRACT

Selection of an appropriate forage species is an important first step in successful forage production. Among forage species, canola (*Brassica napus* L.) can be cut for hay or silage to cover certain costs of growing the crop. Because forage production is largely controlled by the environment and management, this experiment was conducted in a semiarid region of Iran during the 2006–2007 and 2008–2009 growing seasons to investigate whether canola forage yield and its nutritive value affected by different rates of N and natural zeolite. The experimental treatments were arranged in randomized complete blocks with three replications and comprised a factorial combination of three N levels (90, 180, and 270 kg N ha⁻¹) and four zeolite rates (0, 3, 6, and 9 t zeolite ha⁻¹). The results showed that the enhanced N application from 90 to 270 kg N ha⁻¹ resulted in a 49% rise in forage yield for the first year and a 39% increase for the second year. The converse effects from N and zeolite yielded a significant interaction on the forage nitrogen concentration (FNC) so that the N and zeolite application enhancement led to an increase and decrease, respectively, in the FNC. The integration of the minimum N level with the maximum zeolite application generated the highest forage calcium concentration (FCC) (1.13% in dry matter). In contrast, N₂₇₀Z₀ treatment yielded the lowest FCC (0.54% in dry matter). There was a linear response between N application rate and its leaching loss whereas in the zeolite treatments, minimum N leaching was observed after the Z₉ treatment. A combined application of zeolite and chemical N for canola production in a poor sandy soil is recommended to ensure an acceptable forage yield and for soil protection from excess N leaching loss.

THE IMPORTANCE OF forage crops in livestock feeding and human food production is undeniable. Generally, several crop species such as alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], and varieties of clover are raised for green-chop forage production in different parts of the world. However, certain climatic limitations such as drought and the special requirements of these crops have generated doubts and questions regarding the relative efficiency of these crops compared to other sources. Traditionally, canola is grown as an oilseed crop with a low level of saturated fat, but with a new approach, it could produce considerable levels of green forage especially at that time when additional forage sources cannot be exploited. It will not compete in yield with alfalfa and corn, but grazed and ensiled winter canola offers high nutritional value and digestibility. In addition, there are many reasons for growing canola as forage, including the considerable levels of fresh and dried forage (Gholamhoseini et al., 2008); optimum use of rainfall in autumn and winter (McRae et al., 2006); high levels of crude protein, nutritional value, and good palatability especially for the ruminants (Amin et al., 2002); and finally high potential for beekeeping and honey production (Zarinabadi et al., 2010).

Thus, for energy and protein, canola could be considered an excellent forage source.

Among the agronomic factors that may affect the yield and quality of forage in forage crop production, the application of N is considered to be the most important. One of the main criteria of forage quality is the crude protein (CP) because CP increases the digestibility of forage (Peyraud and Astigarraga, 1998). Many researchers reported that N fertilization increases CP and dry matter yield in forage crops (Polat et al., 2007; Delaby et al., 1996). Kobayashi et al. (2002) found that N fertilizer significantly increased fiber concentration in the forage of Italian ryegrass (*Lolium multiflorum* Lam.). Also Delagarde et al. (1997) observed that neutral detergent fiber (NDF) concentration was inconsistently affected by the application of N fertilizer in crop forage. Because canola is a strong N consumer, N availability plays an important and critical role in seed and dry matter yield (Rathke et al., 2006). Hence, determining the optimal utilization of N in an agroecosystem is important. Given the low N efficiency for this crop (Dreccer et al., 2000) and the high rainfall in autumn and winter, significant N leaching is expected, and this problem encourages irregular and unreasonable use of N fertilizers by farmers. Excessive N fertilizer application regardless of glucosinolate accumulation (Evans and Islam, 1990) increases nitrate loss from leaching and finally contaminates the ground water. This event is a prevalent and widespread problem for sandy soils. Gholamhoseini et al. (2010) showed that in light texture farm approximately 50% of applied N could be leached. Thus, providing a way to

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Published in Agron. J. 104:1467–1475 (2012)

Posted online 3 Aug. 2012

doi:10.2134/agronj2012.0145

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Abbreviations: ADF, acid detergent fiber; CEC, cation exchange capacity; CP, crude protein; FCC, forage calcium concentration; FMC, forage magnesium concentration; FNC, forage N concentration; FSC, forage sodium concentration; GTRI, grass tetany ratio index; LAI, leaf area index; NDF, neutral detergent fiber; TDR, time domain reflectometry.

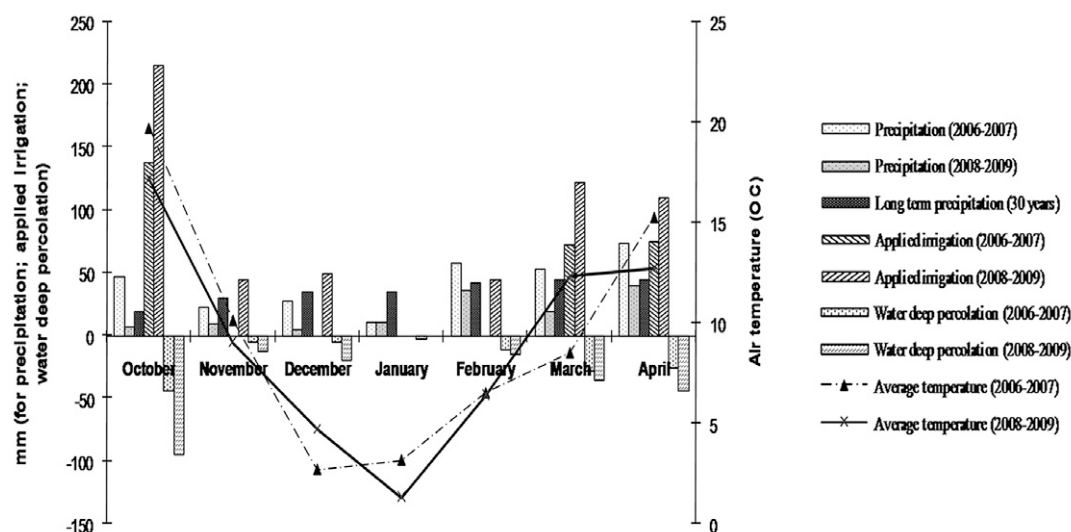


Fig. 1. Monthly precipitation, applied irrigation, water deep percolation during the first and second years of experiment, long-term monthly mean precipitation and average monthly temperature of the growth seasons (2006–2007 and 2008–2009).

control the consumption of N fertilizers and increase their efficiency in parallel with producing a desirable yield is necessary, especially in sandy soils.

Among the effective solutions for increasing N fertilizer efficiency and preventing chemical fertilizer waste, the application of natural minerals such as zeolite has been suggested (Baerlocher et al., 2001). Zeolites are hydrated aluminosilicates, characterized by a three-dimensional network of SiO_4 and AlO_4 , which are linked by shared oxygen atoms. Partial substitution of Si^{+4} and Al^{+3} results in an excessive negative charge, which is compensated by cations. These cations are located with water molecules in the cavities and channels inside the aluminosilicate framework. Water and cations can be removed or replaced by other cations (Rehakova et al., 2004; Murphy et al., 2005). Selective absorption and controlled release of cations by zeolite enhances nutrient availability and improves plant growth and development. Zeolite has certain unique features such as high cation exchange capacity ($200\text{--}300\text{ cmol}_c\text{ kg}^{-1}$) (Leggo et al., 2006), selective absorption, slow release of ammonium (He et al., 2002), structure stability over the long term (Hung and petrovic, 1994). Regarding zeolite availability, Mumpton (1999) reported that extensive deposits of zeolite have been found in western United States, Bulgaria, Hungary, Japan, Australia, China, and Iran. In Iran zeolite cost is approximately 2.5 cents per kg so application of zeolite would be economical. Recently, other researchers have conducted experiments about integrated use of organic fertilizer with zeolite and their results have shown that the efficiency of organic fertilizer in combination with zeolite is increased significantly (Daryaei et al., 2011; Khodaei Joghani et al., 2012). Therefore, it is expected that the application of zeolite with chemical fertilizers especially N fertilizers can increase N use efficiency and optimize chemical fertilizer use.

Considering these advantages, a field study was conducted using an integrated nutrient management practice including zeolite and N fertilizer. The primary objective of this research was to study the effect of N fertilization and zeolite application on green-chop forage productivity as well as the quality of winter canola, grown in a semiarid environment and sandy soil. Specifically, this study compared canola forage yield and

nutritive value as well as N leaching based on this hypothesis that zeolite application decreases NO_3^- leaching and N fertilization along with zeolite would improve growth and canola forage yield in sandy soils.

MATERIALS AND METHODS

Experiment Location and General Methodology

The experiment was conducted in the 2006–2007 and 2008–2009 growing seasons at the research farm in the Tarbiat Modares University, Tehran, Iran ($35^\circ41' \text{ N}$, $51^\circ19' \text{ E}$ and 1215 m asl). This region is characterized as semiarid with a 298 mm mean annual precipitation, which primarily falls during the autumn and winter months. The daily meteorological data on precipitation and air temperature were collected at the nearest weather station (500 m from the experimental site) (Fig. 1). Before planting, several soil samples were taken at 0- to 30- and 30- to 60-cm depths to prepare composite samples for each depth. These samples were air dried, crushed, and tested for physical and chemical properties (Table 1). The soil texture was sandy loam based on the textural triangle classification.

Land Preparation and Treatments

For both years of the experiment, canola was planted in different parts of the field. The preceding crop was sweet corn in the first year and field corn in the second year. The field was prepared by shallow plowing followed by disking in autumn. To control weed growth in the area, Trifluralin (3.5 L ha^{-1}) was sprayed and then incorporated into the soil using a disk. Each experimental unit was 6 m long with 10 rows 0.3 m apart. There were 2-m gaps between the blocks, and between each plot, a 1.5-m alley was established to prevent lateral water movement and other interferences. A polyethylene pipeline and counter were installed to control irrigation. The experiment was conducted using a randomized complete-block design with a factorial arrangement of treatments through three replications. The first factor included three N rates (90, 180, and 270 kg N ha^{-1}) for the urea, and the second factor included four amounts of natural zeolite (0, 3, 6, and $9\text{ t zeolite ha}^{-1}$).

Table 1. Soil chemical properties at experimental site.

Properties	Depth			
	0–30 cm		30–60 cm	
	2006–2007	2008–2009	2006–2007	2008–2009
Organic matter, %	0.8	0.4	0.7	0.3
pH/water 1:2 ratio	7.7	7.2	7.2	7.00
Sand, %	65	76	58	61
Silt, %	23	17	25	21
Clay, %	12	7	17	17
EC, dS m ⁻¹	1.5	1.6	1.7	1.6
N, %	0.09	0.08	0.11	0.09
P, mg kg ⁻¹	25	20	nd†	nd
K, mg kg ⁻¹	480	375	nd	nd
Fe, mg kg ⁻¹	7.6	7.8	nd	nd
Zn, mg kg ⁻¹	1	1.2	nd	nd
CaCO ₃ , %	4.9	5.5	nd	nd
CEC‡, cmol _c per kg	6.4	5.9	nd	nd

† nd, not determined.

‡ CEC, cation exchange capacity.

Treatments and Planting

A zeolitized volcanic tuff was used, which contained K–Ca clinoptilolite with an exchange capacity of approximately 200 cmol_c kg⁻¹. Zeolite was sourced from a quarry in the Mianeh city in Northwest Iran and comprised approximately 90% clinoptilolite; the remainder comprised volcanic glass, clay minerals, quartz, and feldspar. The results from X-ray spectrophotometer analysis (dispersive spectra) showed that the clinoptilolite from Mianeh was a K–Ca type with low Na ion content. The full chemical analysis of applied zeolite is shown in Table 2. In the levels determined by the treatments, zeolite was distributed on the soil surface of each plot and incorporated into the top 0 to 30 cm of the soil. For both years, K and P fertilizers were not applied because the nutrient levels in the soil were adequate. Furthermore, the N fertilizer was divided into three equal quantities and distributed in the three applications: one-third before sowing, one-third at the beginning of the stem-elongation stage, and the remainder at the flowering stage.

The canola seeds (*Brassica napus* L. cultivar Okapi) were sown by hand at a 3-cm depth on 4 and 7 October in the first and second years, respectively. To ensure good emergence, the experimental plots were overseeded and then thinned (4 cm apart) to the recommended plant density 830,000 plants ha⁻¹ at the three-leaves stage. Immediately after sowing, the soil was irrigated. The irrigation cycle for each plot was closed to avoid run-off. The irrigation was scheduled according to the daily changes in soil water content (ΔSW) at the root development depth. A deficit approach was used to estimate irrigation requirements (soil water content at the field capacity (FC) is representative of a condition with no water deficit). When the daily deficit value was 50% of the available water at the root development depth (80 cm for these experiments), the plots were irrigated. The 80-cm depth was chosen according to Kjellstrom (1991), wherein the highest levels of dry matter and the root number for winter canola were established at a 0- to 80-cm soil depth.

Tube access probes (TRIME-FM, Haslemere, England) based on time domain reflectometry (TDR) were used to measure soil-water content (Θ_v) at a 0- to 80-cm soil depth in the experimental plots (at 0.2-m intervals). Soil volumetric water

Table 2. Chemical analysis of the applied zeolite (%).

SiO ₂	Al ₂ O ₃	K ₂ O	Na ₂ O	MgO	CaO
65	12.02	3	1.08	0.1	2.3
Fe ₂ O ₃	MnO	TiO ₂	P ₂ O ₅	CEC†	
1.5	0.04	0.03	0.01	200 cmol _c kg ⁻¹	

† CEC, cation exchange capacity

content data were collected daily during the growing season, but during winter, the measurements were registered weekly.

Before sowing the seeds and during the TDR tube access probe installations, vertical holes 5 to 6 cm in diameter and 80 cm in depth were created using a hand auger in the middle of each plot, and then soil water sampler tubes (Model 1900, Soil Moisture Equipment Co., Santa, Barbara, CA) were inserted into the holes. To avoid possible contamination, the ceramic caps for the soil water sampler tubes were washed before insertion, and to facilitate a good contact between the ceramic cap and soil, the gaps were filled with fine soil.

Soil Water Sampling and Analysis

Determination of solute leaching loss requires two sets of information: (i) the quantity of drainage flux and (ii) the solute concentration in the drainage solution. A portable vacuum pump (Model 2005 G2, Soil Moisture Equipment Co.) was used to apply a –30 KPa tension to collect water samples every 4 to 6 d or when drainage was suspected, such as after rain events or irrigation (when soil-water content exceeds field capacity). Water samples were taken from the soil-water sampler tubes using a thin collection vessel, vacuum erlen, and vacuum hand pump. The samples were acidified with sulfuric acid (1 mL L⁻¹) and stored in a refrigerator. Water samples were analyzed for NH₄⁺ and NO₃⁻ concentrations using the salicylate and Cd methods, respectively, and a spectrophotometer (Model dr/2500, Hach Co.). In this paper, we ignored the NH₄⁺–N concentration in the percolate solution because NH₄⁺ was very low, so the result was reported as a NO₃⁻–N leaching loss. For daily measurements of deep percolation, the water-balance equation (Errebhi et al., 1998) was used (Eq. [1]).

$$\text{Daily deep percolation} = P + I - \Delta SW - ET_C - R \quad [1]$$

P is the precipitation (mm), I is the irrigation water applied (mm), ΔSW is the daily change in soil water content (mm) at the root development depth (measured by TDR), ET_C is the evapotranspiration (mm), and R is the runoff (mm). There was no runoff because each plot had a closed irrigation cycle. Percolation occurs whenever the sum ($P + I$) is higher than $\Delta SW + ET_C$ (Vazquez et al., 2005). Water input from irrigation and rainfall were measured at the experiment site. The crop evapotranspiration was calculated daily using Eq. [2].

$$ET_C = ET_0 \times K_C \quad [2]$$

ET_0 refers to evapotranspiration calculated by the FAO–Penman–Monteith method (Allen et al., 1998) and depended on the daily weather conditions at the site; however, the crop coefficient K_C depended on the crop growth stage. The initial water

storage was equal to the soil water holding capacity to an 80-cm depth (before sowing when the soil profile was fully charged), and the subsequent ΔSW was determined on a daily basis.

The NO_3^- mass in the leachate was determined as follows (Gheysari et al., 2009) (Eq.[3]).

$$NO_3^- \text{ mass (kg ha}^{-1}\text{)} = NC \times WDP \times 0.01 \quad [3]$$

NC is the concentration of nitrate in the leachate ($mg L^{-1}$), WDP is the level of water-deep percolation (mm) and 0.01 is the conversion factor for $mg L^{-1}$ to $kg ha^{-1}$.

Plant Measurements

To determine the actual whole forage yield, $4 m^2$ from each plot was harvested on 10 Apr. 2007 and 15 Apr. 2009, which is approximately 190 d after planting. In both years, the crop was harvested at pod setting stage. Canola forage was collected in paper bags and weighed to estimate wet yield per hectare. To estimate the leaf and stem dry matter, subsamples (eight plants) were taken from each plot, the leaf area was measured using a leaf area meter (ΔT , England) and then the samples were oven dried at $72^\circ C$ for 48 h until the weight was constant. Dry samples (whole plant) were ground to 2 mm using an electrical mill and stored at room temperature for further nutritive value analysis.

Total N was determined through a titration method using a Kjeltac instrument (Auto 1030 Analyzer, Tecator, Westovers, AL). Further, K and Na were measured using a flame-photometer (JenWay PFP7, Burlington, NJ, respectively). The total Ca content was measured using an atomic absorption method (Model GBC 932). In addition, the ash and organic matter were determined using an AOAC (1990) method. The CP levels were calculated by multiplying the percentage of N by 6.25 as suggested by AOAC (1990), and then protein yield was estimated by multiplying CP by dry matter yield. In the second year, the Mg content was measured using an atomic absorption method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated in accordance with Van Soest et al. (1991) and the grass tetany ratio index (GTRI) for the forage was calculated using the ratio of K to (Ca + Mg) on an equivalence basis.

Statistical Analysis of the Data

All of the data were subjected to ANOVA using SAS software (SAS Institute 2002). The Bartlett test showed homogeneity in the variance of all traits in both years. The F test indicated statistical significance at $P < 0.01$ or $P < 0.05$, and the orthogonal polynomial contrasts was used to separate main and interaction effects. The discussion of the results in the following sections is based on the order of statistical significance, which ranges from the highest-level interactions (year \times N \times zeolite) to the main effects of treatments (year, N, and zeolite). For statistically significant interactions, the main effects of treatments involved in the interaction are not presented. By contrast, if the studied trait showed no significant interactions but did show significant main effects, the main effects were discussed.

RESULTS AND DISCUSSION

Weather Conditions

According to the meteorological data, the total precipitation was 291 mm in the first year and 127 mm in the second year;

compared with the long-term average (250 mm), there was a 16% increase and 49% decrease in the first and second years, respectively (Fig. 1). More than 63% of the rainfall in the first year and 75% of the rainfall in the second year was in February, March, and April. For the long-term data, these months (February, March, and April) received approximately 53% of the total precipitation (Fig. 1). The water drainage measurements from each year showed that the lowest level of drainage water was generated from November to February. It is worth mentioning that these months (November–February) had the least irrigation.

In both years, the maximum and minimum temperatures were registered in June and January, respectively (Fig. 1). According to the long-term data, these months were the hottest and the coldest months, respectively.

Forage Yield

According to the data analysis, the effects from the year, N, and zeolite on the canola forage yield were significant (Table 3); furthermore, the year \times N interaction was significant for forage yield (Tables 3 and 4), which arises from the stronger influence from various N rates on the dry weight for the first year compared with the second year. The enhanced N application from 90 to 270 $kg N ha^{-1}$ resulted in a 49% rise in forage yield for the first year and a 39% increase for the second year (Table 4). Enhanced N fertilizer application enhanced the forage yield such that in both years, the highest dry weight (11,319 $kg ha^{-1}$ in the first year and 6974 $kg ha^{-1}$ in the second year) was from the 270 $kg N ha^{-1}$ treatment, and the lowest dry weight (7697 $kg ha^{-1}$ in first year and 5000 $kg ha^{-1}$ in second year) was from the 90 $kg N ha^{-1}$ treatment (Table 4). Several researchers have reported that N increases crop biomass through increase of green area resulting in higher N assimilation (Colnenne et al., 2002; Svecnjak and Rengel, 2006). Nitrogen assimilation enhancement is closely linked to an increase in net photosynthesis that finally results in increased plant dry weight. The results demonstrate that enhanced zeolite application increased the forage yield linearly for both years (Table 5). Because the zeolite application increases the fertilizer efficiency (Ok et al., 2003), enhanced forage yield from higher zeolite treatments (Z_6 and Z_9) is reasonable. Further, the ability of zeolite to supply more nutritional elements during the plant growth period resulted in enhanced forage canola yield. Also research has shown that soil cation exchange capacity (CEC) has increased on account of zeolite application (Ok et al., 2003; Bigelow et al., 2003), in that case long-term availability of N, will have a positive effect on crop growth. In both years of the experiment, a significant and direct correlation between the total dry weight and leaf dry weight was observed ($r^2_{\text{first year}} = 0.65^{**}$, $r^2_{\text{second year}} = 0.62^{**}$). Ideally, those treatments that enhanced leaf dry weight ($N_{270}+Z_9$ and $N_{270}+Z_6$ in both years and $N_{180}+Z_9$ in the second year (data are not shown)) because of their good palatability and digestibility can be categorized as optimum treatments. The changes of forage yield in response to different N levels were linear and quadratic in the first and second year, respectively (Table 4). The linear relationship between dry matter and N reflects the tendency of canola to exhibit an indeterminate growth habit when nutrients are essentially unlimited (Jackson, 2000).

Table 3. Analysis of variance of N and zeolite (Z) effects on canola forage yield, forage quality, and N leaching loss.

Source of variance	df	FY†	LAI	FNC	PY	FCC	FSC	NLL
Year (Yr)	1	220,073,752.2*	24.00**	5.21**	15,955,917.00**	1.136**	0.438**	19,245.38**
Rep×Yr	4	18,401,705.5	0.408	0.158	557,029.59	0.010	0.004	208.08
N	2	49,263,019.0**	15.36**	1.729**	3,971,560.05**	0.456**	0.087**	7,853.46**
Z	3	31,475,346.9**	9.59**	2.676**	259,191.05ns‡	0.316**	0.062**	1,418.95**
N × Yr	2	4,600,893.7*	0.162ns	0.007ns	380,692.91*	0.050ns	0.002ns	1,065.06**
Z × Yr	3	1,157,708.8ns	0.016ns	0.165ns	33,024.35ns	0.013ns	0.014**	195.73ns
N × Z	6	849,883.3ns	0.352ns	0.439**	176,894.88ns	0.079**	0.001ns	153.46ns
Year × N × Z	6	316,308.0ns	0.070ns	0.013ns	17,563.51ns	0.027 ns	0.0009ns	16.16ns
Error	44	1,442,456.1	0.374	0.122	97,556.49	0.019	0.002	95.40
CV, %		15.36	22.95	10.86	19.65	19.04	8.59	21.68
Contrast								
N_{XZ_0} vs. N_{XZ_X} §		**	**	**	**	ns	ns	**
N_{XZ_9} vs. N_{XZ_X}		**	**	**	**	ns	ns	**
N_{270Z_9} vs. N_{XZ_X}		**	**	**	**	ns	*	**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† FY: forage yield, LAI: leaf area index, FNC: forage nitrogen concentration, PY: protein yield, FCC: forage calcium concentration, FSC: forage sodium concentration, NLL: nitrogen leaching loss.

‡ ns: not significant.

§ × index shows different amount of N or zeolite.

Table 4. Mean comparison of interaction effects of Year × N and Year × zeolite (Z).

Year × N effect sliced by year for FY†			Year × N effects by year for PY			Year × N effect sliced by year for NLL			Year × Z effect sliced by year for FSC		
Year	df	MS‡	Year	df	MS	Year	df	MS	Year	df	MS
1	2	41,961,027**	1	2	3,403,531**	1	2	1628**	1	3	0.068**
2	2	11,902,882**	2	2	948713**	2	2	7290**	2	3	0.008*
Year	N (kg ha ⁻¹)	FY (kg ha ⁻¹)	Year	N (kg ha ⁻¹)	PY (kg ha ⁻¹)	Year	N (kg ha ⁻¹)	NLL (kg ha ⁻¹)	Year	Z (t ha ⁻¹)	FSC (% dry matter)
1	90	7697	1	90	1,487	1	90	18	1	0	0.53
	180	9772		180	2,153		180	27		3	0.61
	270	11,319		270	2,539		270	41		6	0.68
Linear		**	Linear		**	Linear		**		9	0.73
Quadratic		ns§	Quadratic		ns	Quadratic		ns	Linear		*
									Quadratic		ns
									Cubic		ns
2	90	5,000	2	90	811	2	90	35	2	0	0.45
	180	6,222		180	1,181		180	63		3	0.47
	270	6,974		270	1,363		270	85		6	0.49
Linear		*	Linear		ns	Linear		**		9	0.52
Quadratic		**	Quadratic		**	Quadratic		ns	Linear		ns
									Quadratic		*
									Cubic		ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† FY: forage yield, PY: protein yield, NLL: nitrogen leaching loss, FSC: forage sodium concentration.

‡ MS: mean square.

§ ns: not significant.

Leaf Area Index

Canola leaf area index (LAI) was significantly affected by year, N, and the zeolite rates ($p \leq 0.01$). An increase in the N fertilizer rates from 90 to 180 and 180 to 270 kg N ha⁻¹ enhanced the canola leaf area index by 41 and 24%, respectively, in the first year and 56 and 32%, respectively, in the second year (Table 6). In fact, N efficiency was reduced by higher levels of this fertilizer. In both years, application of 90 kg N ha⁻¹ compared with other N treatments produced less LAI compared with the additional N treatments (Table 6).

According to Radin and Boyer (1982), the effect of N on leaf size is mediated by cell size. They attributed the slower leaf expansion under the N-limiting conditions to decreased hydraulic conductance, which limits water delivery to growing leaves. In contrast, an enhancement in leaf area where N is more available depends on leaf number and leaf size. Ozer (2003) reported that N prolongs the life of leaves, improves LAI and increases overall crop assimilation, thus contributing to increased dry matter yield. In both years, the Z₆ and Z₉ treatments produced higher LAI levels than the other zeolite

Table 5. Mean comparison of zeolite (Z) main effect.

Traits treatment	Forage yield		Leaf area index		Protein yield		Nitrogen leaching loss	
	kg ha ⁻¹				kg ha ⁻¹			
Year	2006–2007	2008–2009	2006–2007	2008–2009	2006–2007	2008–2009	2006–2007	2008–2009
Main effect								
Z rate, t ha ⁻¹								
0	8025	5068	2.36	1.22	2091	1080	33.10	72.78
3	8427	5256	2.93	1.73	1850	993	33.05	69.01
6	10586	6670	3.81	2.73	2201	1181	27.30	57.95
9	11210	7268	3.86	2.65	2097	1218	21.28	45.76
Linear	**	**	**	**	ns†	ns	**	*
Quadratic	ns	ns	ns	ns	ns	ns	ns	ns
Cubic	ns	ns	ns	ns	ns	ns	ns	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† ns: not significant.

Table 6. Mean comparison of N main effect.

Traits treatment	Leaf area index		Forage sodium concentration, percentage in dry matter	
	2006–2007	2008–2009	2006–2007	2008–2009
Year				
Main effect				
N Rate, kg ha ⁻¹				
90	2.33	1.35	0.58	0.42
180	3.29	2.11	0.63	0.49
270	4.09	2.79	0.71	0.54
Linear	**	**	**	**
Quadratic	ns†	ns	ns	ns

** Significant at the 0.01 probability level.

† ns: not significant.

application rates (Z₀ and Z₃) (Table 5). The application of zeolite in soil decreased N leaching and increased LAI by supplying more N during the plant growth period. Our results indicate that LAI significantly and positively correlates with total dry weight ($r^2_{\text{first year}} = 0.70^{**}$, $r^2_{\text{second year}} = 0.63^{**}$). It is significant that sufficient N availability in canola enhances N assimilation, which increases the LAI. Further, in accordance with the direct relationship between LAI and plant photosynthetic capacity, it is expected that increased N availability for canola enhances LAI and total dry matter.

The Effect of Treatment on Qualitative Canola Forage Traits

Forage Nitrogen Concentration

The effects of year and the N × zeolite interaction on the FNC were significant (Table 3). The converse effects from N and zeolite especially in 90 and 180 kg N ha⁻¹ levels yielded a significant interaction on the FNC (Table 7). Nitrogen and zeolite application enhancement led to an increase and decrease, respectively, in the FNC. The maximum FNC (4.29% in dry matter) was observed for those plots that received 270 kg N ha⁻¹ with no zeolite, and the minimum FNC (2.47% in dry matter) was obtained from application of the lowest levels of N fertilizer with highest levels of zeolite (Table 7). The dominant reasons for this result are (i) N absorption by zeolite when N is more available (by applying N fertilizer) and (ii) the slow release of N by zeolite during the plant growth period. Rehakova et al. (2004) stated that the combination of zeolite and chemical N fertilizer reduced the N concentration in barley.

Table 7. Mean comparison of interaction effects of N × zeolite (Z).

N × Z effect sliced by N for FNC†			N × Z effect sliced by N for FCC†		
N level	df	MS‡	N level	df	MS‡
90	3	0.995 **	90	3	0.345 **
180	3	0.266 ns§	180	3	0.058 *
270	3	2.292 **	270	3	0.071 *
FNC, % dry matter			FCC, % dry matter		
N, kg ha ⁻¹	Z, t ha ⁻¹		N, kg ha ⁻¹	Z, t ha ⁻¹	
90	0	3.27	90	0	0.58
	3	3.24		3	0.81
	6	2.65		6	0.99
	9	2.47		9	1.13
Linear		ns	Linear		ns
Quadratic		*	Quadratic		*
Cubic		ns	Cubic		ns
180	0	3.32	180	0	0.54
	3	3.27		3	0.72
	6	3.15		6	0.75
	9	3.20		9	0.76
Linear		ns	Linear		ns
Quadratic		ns	Quadratic		*
Cubic		ns	Cubic		ns
270	0	4.29	270	0	0.47
	3	3.68		3	0.74
	6	3.26		6	0.59
	9	2.83		9	0.62
Linear		*	Linear		ns
Quadratic		ns	Quadratic		*
Cubic		ns	Cubic		ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† FNC: forage nitrogen concentration. FCC: forage calcium concentration.

‡ Mean square.

§ ns: not significant.

Protein Yield

Crude protein is one of the major nutritious compounds in livestock feeding, and its deficiency in forage could reduce livestock production yield (Peyraud and Astigarraga, 1998). Moreover, protein yield reflects not only forage quality but also production quantity; thus, in this paper, the protein yield was considered instead of the forage CP.

The effects of year, N, and their interaction on protein yield were significant (Table 3). The application of 270 kg N ha⁻¹ in the first year increased protein yield to the maximum level

(2540 kg ha⁻¹), which was 86% higher compared with the same treatment in the second year (Table 4). The optimum distribution and high precipitation level (291 mm) during first year lowered the irrigation applied in this year; whereas the low precipitation level in the second year (127 mm) forced us to use more water through irrigation (286 mm irrigation ha⁻¹ in the second year vs. 158 mm irrigation ha⁻¹ in the first year). The enhanced irrigation increased water deep percolation and N leaching loss in the second year (28.68 kg leached N ha⁻¹ in the first year vs. 61.38 kg leached N ha⁻¹ in the second year). Enhanced N leakage not only decreased plant dry weight production but also reduced the plant mass N concentration and forage crude protein. By contrast, the increase in protein yield with N fertilization is in line with finding of Almodares et al. (2009) and Delagarde et al. (1997). The protein yield enhancement with increasing in fertilizer levels may be due to enhancement in amino acid formation affected by fertilization and N availability.

In both years, there was no significant difference in protein yield from the zeolite treatments (Tables 3 and 5). However, the percentage of forage CP (data are not shown) significantly decreased with an increase in the zeolite rates due to N absorption by zeolite when N was more available in soil after N distribution. However, the reduction in forage CP percentage from zeolite treatments is negligible because (i) N leaching decreased by 35% (first year) and 37% (second year) after the Z₉ treatment compared with the Z₀ treatment and (ii) the protein yield differences between zeolite treatments were not significant (Table 5). In both years, higher dry matter yield from the Z₉ treatment compensated for the reduced forage CP content with this treatment; hence, no significant difference between the Z₉ and Z₀ (containing the maximum N concentration and forage crude protein) treatments was observed.

Forage Calcium Concentration

There was a significant difference in FCC between the 2 yr. Further, the effect of the N × zeolite interaction on this trait was significant (Table 3). The initial difference in soil Ca concentration for these 2 yr likely caused a significant difference in the FCC (4.5% soil Ca in the first year vs. 5.5% soil Ca in the second year). The integration of the minimum N level (90 kg N ha⁻¹) with the maximum zeolite application (9 t zeolite ha⁻¹) generated the highest FCC (1.13% in dry matter). In contrast, N₂₇₀Z₀ treatment yielded the lowest FCC (0.54% in dry matter) (Table 7). An antagonistic relationship between Ca and N has been reported by Barker and Pilbeam (2009). They have stated that the N availability in the root zone reduced Ca absorption in the plant. The negative correlation between N and Ca concentration in plant mass ($r^2_{\text{first year}} = -0.66^{**}$, $r^2_{\text{second year}} = -0.52^{*}$) from our results agrees with their report.

Forage Sodium Concentration

The effects from year, N, zeolite, and the year × zeolite interaction were significant for the forage sodium concentration (FSC) (Table 3). In both years, an enhanced N application enhanced the Na concentration for the forage (Table 6). On the other hand, application of more N increased FSC linearly (Table 6). It is possible that N fertilization could have increased root proliferation, Na uptake per unit root weight and/or the

translocation of Na to the aboveground portion of the plant. According to Table 4, the maximum FSC (0.73% dry matter) was observed after the application of 9 t zeolite ha⁻¹ in the first year, and the minimum FSC (0.45% dry matter) was observed after the nonzeolite application in the second year. Because Na is a major constituent of the zeolite structure (Baerlocher et al., 2001), a higher Na accumulation in the forage after the zeolite application is expected.

Forage Magnesium Concentration and the Grass Tetany Ratio Index

The effects of N and zeolite on the forage magnesium concentration (FMC) and grass tetany ratio index (GTRI) were significant (Table 8). In N treatments the highest and lowest FMC values (2.78 and 1.84% dry matter, respectively) were generated using the N₂₇₀ and N₉₀ treatments, respectively (Table 9). Cohen et al. (2004) reported that high levels of N fertilization can reduce FMC through exchangeable base leaching from the plant root zone. Further, Grunes et al. (1970) indicated that the application of more N fertilizer to produce higher forage levels could increase the N and K concentrations in plants such that the livestock digestive system is disrupted

Table 8. Analysis of variance (mean square) of N and zeolite (Z) effects on forage quality traits (measured only in second year of experiment).

Source of variance	df	FMC†	GTRI	NDF	ADF
Replication	2	0.0002ns‡	0.047ns	34.49ns	188.25**
N	2	0.0064**	3.323 **	2.597ns	7.982ns
Z	3	0.0048**	0.656 *	2.170ns	1.682ns
N × Z	6	0.0001ns	0.183ns	1.017ns	7.702ns
Error	22	0.0005	0.155	58.64	21.13
CV, %		13.09	17.01	17.09	12.29

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† FMC: forage magnesium concentration, GTRI: grass tetany ratio index, NDF: neutral detergent fiber, ADF: acid detergent fiber.

‡ ns: not significant.

Table 9. Mean comparison of canola forage quality traits (measured only in second year of experiment).

Trait treatments	FMC†	GTRI	NDF	ADF
% in dry matter				
Main effect				
N rate, kg ha ⁻¹				
90	0.21	1.84	45.23	38.24
180	0.17	2.42	44.85	36.91
270	0.16	2.78	44.30	36.94
Linear	**	**	ns‡	ns
Quadratic	ns	ns	ns	ns
Main effect				
Zeolite rate, t ha ⁻¹				
0	0.16	2.73	45.27	37.74
3	0.16	2.00	45.12	36.97
6	0.20	2.40	44.58	37.80
9	0.20	2.26	44.21	37.07
Linear	ns	ns	ns	ns
Quadratic	**	**	ns	ns

** Significant at the 0.01 probability level.

† FMC: forage magnesium concentration, GTRI: grass tetany ratio index, NDF: neutral detergent fiber, ADF: acid detergent fiber.

‡ ns: not significant.

for Mg absorbance in forage. In contrast, application of zeolite abrogated the decreasing FMC such that the highest FMC was observed from the highest application amount of zeolite (9 t ha^{-1}) compared with other zeolite levels (Table 9). The changes of FMC in response to different N and zeolite levels were linear and quadratic, respectively (Table 9).

Mayland et al. (1975) suggested that a GTRI, which is the ratio of K to (Ca+Mg) in the forage, >2.2 (standard upper GTRI limit) could be a sign of grass tetany induction. During this research period, the GTRI fluctuated between 1.45 (for the N_{90}Z_9 treatment) and 3.03 (for the N_{270}Z_0 treatment). Furthermore, each increasing step of the N rate enhanced the GTRI such that this index exceeded the threshold limit (2.2) after the 180 kg N ha^{-1} treatment (Table 9). In contrast, with the exception of Z_3 among zeolite treatments the lowest GTRI (2.26) was observed after the Z_9 treatment (Table 9). In this plant, greater N availability (because of the enhanced N fertilizer application) resulted in a higher K absorption (Jackson, 2000) but lower Ca (Vuckovic et al., 2005) and Mg uptake (Madhava Rao et al., 2006). Hence, an enhanced GTRI could be expected in N-rich treatments. However, zeolite inhibits irregular N absorption in plants (Gholamhoseini et al., 2010; AghaAlikhani et al., 2011) and evidently promotes an equal uptake of nutrients.

Neutral Detergent Fiber and Acid Detergent Fiber Percentage

The amount of neutral detergent fiber (NDF) in the feed is an indication of cell wall quantity, and the forage digestion coefficient can be predicted from the cell wall percentage. Further, the forage acid detergent fiber (ADF) is an appropriate index to determine forage digestibility because it contains a high lignin ratio; thus, greater forage ADF decreased the digestibility of feed dry matter (NRC, 2001). Experimental treatments did not affect the NDF and ADF percentages in forage (Table 8). Because the experimental factors (N and zeolite) primarily influenced the available N in the plants (N fertilizer treatments directly and zeolite treatments indirectly) and carbohydrates are the most crucial factor for cell wall percentage (NDF and ADF), not significant effects of experimental treatments are reasonable. Also Valk et al. (2000) stated that NDF and ADF content of forage are more influenced by stage of maturity than by N fertilization. Although, the effects of N and zeolite on NDF percentage were not statistically significant, enhancement of N and zeolite application slightly decreased NDF (Table 9). Suyama et al. (2007) reported that generally NDF value is closely and negatively correlated with dry matter intake of ruminants, as NDF includes the structural cell wall components of plants (except pectins) and consists of the slowest digesting fractions (cellulose, hemicelluloses, lignin, and cutin). Therefore, it could be stated that high NDF restricts average daily body weight gains of cattle (*Bos taurus*). No one has reported the influence of zeolite on the forage cell wall percentage.

Organic Matter Percentage and Forage Crude Ash

The experimental treatments did not affect these two traits (the data are not shown). The organic matter (87.3 and 88.22% in the first and second years, respectively) and crude ash results (10.6 and 8.52% in the first and second years, respectively) yielded the same range in a standard table of forage properties. The canola forage organic matter and crude ash have been recorded as 88.6 and 11.4%, respectively (MAFF, 1990).

Nitrogen Leaching Loss from the Canola Root Zone

The effects of year, N, zeolite, and the year \times N interaction on the N leaching loss from the root zone were significant ($p \leq 0.01$) (Table 3). The high level of irrigation was applied to compensate for the low precipitation in the second year and caused more N leaching in this year compared with the first year (Table 4). In the first year, 15% of the applied N had leached from the plants treated with high N (270 kg N ha^{-1}), and was more than double (32%) in the second year. In each year, the enhanced N that was applied was contemporaneous with high N leaching. There was a linear response between N application rate and its leaching loss, so that 270 kg N ha^{-1} treatment had 2.5 and 1.4-fold more N losses compared with 90 and 180 kg N ha^{-1} , respectively (Table 4). Li et al. (2007) have also shown that N leaching was enhanced with a high N application. In the zeolite treatments, minimum N leaching was observed after the Z_9 treatment. Compared with the nonzeolite application (Z_0), this treatment caused a 36 and 37% decrease in N leaching loss in the first and second years, respectively (Table 5). Decreased N leaching from the zeolite application could be attributed to the unique physiochemical properties of this natural mineral substance. The clinoptilolite zeolite canals are so large that cations such as ammonium are located therein, but bacteria, particularly nitrifican bacteria, could not be located in zeolite canals (Baerlocher et al., 2001). Therefore, after urea application in soil and its conversion to ammonium, clinoptilolite zeolite, which has a selective absorption characteristic (Mumpton, 1999), it absorbs ammonium and renders it unavailable to nitrobacteria, which are active in well-aerated sandy soils. Thus, the transformation of ammonium to nitrate (which is prone to leaching) will decrease with zeolite activity, and finally, N leaching will control.

CONCLUSION

The results herein indicate that the application of 6 to $9 \text{ t zeolite ha}^{-1}$ were considerably more effective than the zeolite-free treatment for the most quantitative canola forage traits. Although the N concentration in forage decreased with a concurrent zeolite application, the forage yield quality and quantity did not decline. Furthermore, the application of high N levels (180 and 270 kg N ha^{-1}) significantly enhanced the qualitative and quantitative canola forage traits. A combined application of zeolite and chemical N for canola production in a poor sandy soil is recommended to ensure an acceptable forage yield (agronomic importance) and for soil protection from excess N leaching loss (economical and environmental importance). However, to precisely determine the way that zeolite performs, more research (evaluating different crop and cultivars as well as integrated application of zeolite with other minerals and organic N sources) is necessary.

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