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Application of cattle manure, zeolite and leonardite improves hay yield and quality of annual ryegrass (*Lolium multiflorum* Lam.) under semiarid conditions

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Abstract

A field experiment was conducted under semiarid conditions to investigate the effect of cattle manure (20 and 40 t ha^{-1}), zeolite (250, 500 and 750 kg ha^{-1}), and leonardite (250, 500 and 750 kg ha^{-1}) applications on hay yield and nutrient quality of annual ryegrass. Application of manure, leonardite, and zeolite increased ryegrass hay yield by 4, 24 and 47%, respectively compared with the control. In all fertilizer levels, the crude protein (CP) content was greater than the control and increased as the level of each fertilizer was increased. Most fertilizer treatments (except from the first level of cattle manure) gave higher CP yield than the control. All fertilizers increased the content of K, S, Ca, Mg, Fe, Mn and B of ryegrass hay as compared with the control, whereas they had no significant effect on Cu and Zn content. The content of most minerals increased as the amount of manure and leonardite applications increased. In conclusion, all fertilizers (especially zeolite) showed great potential for use in organic agriculture as they can improve plant growth and soil conditions in the long term.

Keywords: Crude protein, hay yield, *Lolium multiflorum*, minerals.
Abbreviations: ADF - Acid detergent fiber; CP - Crude protein; DM - Dry matter; L_1 - 250 kg ha^{-1} leonardite; L_2 - 500 kg ha^{-1} leonardite; L_3 - 750 kg ha^{-1} leonardite; M_1 - 20 t ha^{-1} manure; M_2 - 40 t ha^{-1} manure; NDF - Neutral detergent fiber; Z_1 - 250 kg ha^{-1} zeolite; Z_2 - 500 kg ha^{-1} zeolite; Z_3 - 750 kg ha^{-1} zeolite.

Introduction

Annual ryegrass (*Lolium multiflorum* Lam.) is utilized for hay, pasture, soil protection, pellet feed and green area, and its hay has high nourishment quality, early drying property (Serin and Tan, 2004), plentiful leaf and high yield (Serin et al., 1996). Dry matter digestibility may be high as 80% early in the season of growth, particularly in more temperate areas, and as the season progresses, digestibility is decreased but still remains higher than 65% for much of the grazing season (Balosko et al., 1995). In semiarid conditions of Turkey, most soils are very poor in organic matter and nutritional minerals for crops. Also, very strong water and wind erosions have made the soils extremely poor in organic matter and nutrients in those areas which are generally rough and undulating, and mostly bared. Therefore, those regions of Turkey are suitable and profitable for keeping livestock in agricultural systems. In addition, concerns about the overuse of manufacturing fertilizers leading to environmental pollution, especially to fresh springs and other natural sources, are often expressed in the country. After excessive use of easily soluble chemicals in conventional agricultural production, health problems in humans, animals and plants have been dramatically increased. Therefore, organic forage crop production is receiving attention the last few years. Fertilizations in these areas are different from those in conventional areas. For all the above reasons, crop rotation systems, intercropping mixtures, green manures, solid and liquid farmyard manures, poultry manures, compost, leonardite, zeolite and biological fertilizers have taken over in the organic agriculture instead of chemical fertilizers (Yolcu, 2010a; Lithourgidis et al., 2011). Studies on how to use efficient practices in those regions without nutrient losses and with improvement of nutrient and water use efficiency to maintain or increase crop yields of high quality have been popular in the last few years (Oosterhuis and Howard, 2008). Many studies have been conducted on materials which could be beneficial for increasing nutrient use efficiency and water holding capacity of soils for various crop plants (Akinremi et al., 2000; Erdal et al., 2000; Gevrek at al., 2009; Mahmoodabadi et al, 2009; Ahmed et al., 2010). In this regard, leornadite, zeolite and manure may have potential in fertilizer management programs in those regions. Leonardite is naturally oxidized lignite characterized by high content of humic and fulvic acids which are used in the agricultural production (Broughton, 1972, Lee and Barlett, 1976). A zeolite is a crystalline, hydrated aluminosilicate of alkali and alkaline earth cation having an infinite, open, three-dimensional structure and this mineral has high cation exchange capacity, high water holding capacity and high adsorption capacity (Mumpton, 1999). Also, it can decrease the toxic effects of some elements on crops (Mahmoodabadi et al., 2009). Although the effects of various organic fertilizations have been studied in several crops (Tan and Nopamornbodi, 1979; Lithourgidis et al., 2007; Dordas et al., 2008; Yolcu, 2010a; Bilalis et al., 2010; Rokhzadi and Toashih, 2011), there is not

Table 1. Chemical properties of the experimental field before showing.

Soil Properties	Units	0-30cm depth	30-60 depth
pH	(1:2 s/w)	7.63	7.72
Organic matter	%	2.78	1.72
Cation exchangeable capacity	$cmol_c\ kg^{-1}$	27.74	27.83
$CaCO_3$	%	23.98	23.92
N	$mg\ kg^{-1}$	20.42	12.63
Available P	$mg\ kg^{-1}$	15.31	10.74
Exchangeable K	$cmol_c\ kg^{-1}$	8.79	8.77
Exchangeable Ca	$cmol_c\ kg^{-1}$	21.14	21.09
Exchangeable Mg	$cmol_c\ kg^{-1}$	5.45	5.44
Exchangeable Na	$cmol_c\ kg^{-1}$	1.12	1.32
Available Fe	$mg\ kg^{-1}$	2.14	1.50
Available Mn	$mg\ kg^{-1}$	1.45	1.02
Available Zn	$mg\ kg^{-1}$	3.22	2.25
Available Cu	$mg\ kg^{-1}$	1.23	0.86

Table 2. Climatic data of the location in 2008, 2009 and long-term average (1986-2006) at Kelkit, Turkey.

	J	F	M	A	M	J	J	A	S	O	N	D	
Years	Total Precipitation (mm) (Monthly)												Total
2008	40.8	23.3	38.4	51.4	28.4	35.8	2.6	20.0	30.3	35.2	21.1	34.4	361.7
2009	21.3	45.6	57.9	96.3	63.6	25.3	37.4	0.0	71.0	35.1	127.2	33.0	613.7
1986-2006	33.1	35.5	38.3	57.7	68.3	45.1	14.8	13.8	26.3	50.6	45.1	37.6	466.2
	Mean air temperature (°C) (Monthly)												Mean
2008	-6.1	-4.4	8.1	11.6	11.7	16.6	20.1	21.5	17.1	11.9	6.6	-0.7	9.5
2009	-0.2	3.2	3.8	7.8	12.9	18.1	19.6	18.0	14.8	13.0	4.7	4.2	10.0
1986-2006	-1.8	-1.0	3.1	9.4	13.3	16.8	20.2	20.1	16.3	11.3	4.4	0.5	9.4
	Mean relative humidity (%) (Monthly)												Mean
2008	70.7	71.4	63.0	65.0	68.3	69.6	68.5	69.4	68.3	73.0	72.9	73.2	69.4
2009	71.2	68.7	67.9	63.5	65.5	66.4	67.6	65.8	72.8	67.7	77.2	73.7	69.4

enough information regarding the effect of leonardite, zeolite and manure on ryegrass forage production under semiarid conditions. Thus, this study evaluated the effects of different application rates of leonardite, zeolite and manure on the hay yield and other nutritive properties of annual ryegrass under semiarid conditions.

Results

Hay yield and quality

The greatest hay yield was obtained after fertilization of ryegrass with the Z_1 application level followed by L_1 and Z_3 applications, whereas all the other fertilizer levels gave similar hay yield to the control (Table 4). In particular, Z_1, L_1 and Z_3 fertilizer applications produced 31 to 97% higher ryegrass yield than the control. In all fertilizer levels, the CP content was greater relative to control and increased as the level of each fertilizer was increased (Table 4). CP content of ryegrass was the highest in the L_3 application (14.4%) followed by L_2, M_2 and Z_3 (13.5 to 13.7%) treatments. Furthermore, most fertilizer treatments, with the exception of M_1, gave higher CP yield (192 to 309 kg ha^{-1}) as compared to the control (140 kg ha^{-1}) (Table 4). In terms of ADF and NDF concentrations of ryegrass no significant differences were found among treatments (Table 4). The single degree of freedom contrasts indicated 4, 24 and 47% greater ryegrass hay yield for manure, leonardite and zeolite application, respectively, relative to control (Table 4). In addition, all fertilizers significantly increased the CP content of ryegrass (12.8 to 13.6) as compared to unfertilizer plots (10.6). Moreover, zeolite application resulted the highest CP yield of ryegrass (245 kg ha^{-1}) followed by leonardite application (221 kg ha^{-1}), whereas no significant differences were observed between the manure application (140 kg ha^{-1}) and the control (178 kg ha^{-1}) (Table 4).

Mineral contents

Macro- and micro-nutrients concentrations of the annual ryegrass hay showed significant variation after manure, zeolite and leonardite applications at different levels (Table 5). In most cases, the treatments significantly increased the mineral contents of ryegrass. In particular, in all levels of each fertilizer the content of K, S, Ca, Fe and Mn in the hay were higher relative to control. Similarly, all manure and leonardite applications gave higher Mg, Na, Zn and B contents as compared with control. However, in some levels of treatments (especially in zeolite) the content of P, Mg, Na, Cu, Zn and B were similar to control, and only in the cases of Z_3 level, and Z_3 and L_3 levels the Na and Cu contents, respectively, was lower than that of control. The content of most minerals (i.e. S, Mg, Na, Cu, Fe, Mn, Zn and B) increased as the amount of manure application increased (Table 5). Therefore, the maximum content of these minerals were obtained with M_2 level, however at this manure level the content of K and Ca decreased. Similar trends were observed with leonardite applications. In particular, the content of P, K, S, Ca, Mg, Fe, Mn, Zn and B increased as the levels of leonardite application increased. On the other hand, only the Mn and Zn contents increased with increasing the levels of zeolite application. The single degree of freedom contrasts showed that all fertilizers significantly increased the content of K, S, Ca, Mg, Fe, Mn and B of the ryegrass hay as compared with the control, whereas they had no significant effect on Cu and Zn content (Table 5). In addition, there were no significant differences between zeolite and the control for the P and Na content. Also, no differences were observed for

Table 3. Some properties of fertilizers used in the experiment.

Properties	Units	Manure	Zeolite	Leonardite
pH	(1:5 s/w)	7.32	4.24	6.70
Organic matter	%	25.6	14.2	50.6
Humic acid + Fulvic acid	%	5	40	41
N	%	0.32	0.16	1.12
P	%	0.16	0.04	0.09
K	%	0.12	0.32	0.51
S	%	0.21	1.20	0.11
Ca	mg kg^{-1}	3200	6500	14000
Mg	mg kg^{-1}	990	1800	2400
Na	mg kg^{-1}	680	1420	960
Fe	mg kg^{-1}	452	192	6800
Mn	mg kg^{-1}	180	65	255
Zn	mg kg^{-1}	554	224	685
Cu	mg kg^{-1}	268	45	120

Table 4. Hay yield, crude protein content and yield, acid detergent fiber (ADF) and neutral detergent fiber (NDF) of annual ryegrass fertilized with manure (M), zeolite (Z) and leonardite (L).

Fertilizers	Amount (t ha^{-1})	Hay yield (kg ha^{-1})	Crude protein content (%)	Crude protein yield (kg ha^{-1})	ADF (%)	NDF (%)
Control	0.00	1318 DE	10.6 F	140 E	37.2	54.4
M_1	20.00	1232 E	12.1 E	149 E	35.7	55.1
M_2	40.00	1507 CD	13.7 B	206 CD	36.9	55.6
Z_1	0.25	2599 A	11.9 E	309 A	38.2	52.0
Z_2	0.50	1482 DE	13.0 CD	192 D	35.5	54.1
Z_3	0.75	1731 C	13.5 BC	234 BC	35.2	54.9
L_1	0.25	2012 B	12.7 D	256 B	36.3	54.7
L_2	0.50	1429 DE	13.7 B	196 CD	36.3	53.3
L_3	0.75	1473 DE	14.4 A	212 CD	37.3	57.9
Single degree of freedom contrasts						
Control		1318 c	10.6 B	140 C	37.2	54.4
Manure (M)		1370 b	12.9 A	178 BC	36.3	55.3
Zeolite (Z)		1938 a	12.8 A	245 A	36.3	53.7
Leonardite (L)		1638 ab	13.6 A	221 AB	36.6	55.3

Values followed by small and capital letters in a column shows significantly differences at $P < 0.05$ and $P < 0.01$ levels, respectively.

Fe and Mg ryegrass concentrations among three fertilizers. In most cases, manure application gave higher mineral contents than that of leonardite and zeolite.

Discussion

Hay yield and quality

Zeolite applications were more effective on the hay and crude protein yield of annual ryegrass than the other two fertilizers. Similarly, Gul et al. (2009) reported higher shoot yield of common vetch after zeolite application as compared with leonardite or manure applications. Also, Bernardi et al. (2008) found that the DM production of *Citrus limonia* was significantly increased with NPK-enriched zeolite application. This could be attributed to the positive effects of zeolite on the physical and chemical properties of soil (Akbar et al., 1999; Aleksiev et al., 2000), high available water holding and high adsorption capacities (Unver et al., 1984; Mumpton, 1999), improvement of nutrient use efficiency by increasing the availability and uptake of macro and micro plant minerals (Weber et al., 1983; Gworek, 1992; Kavoosia, 2007). When choosing the appropriate forage to be grown, farmers should consider the need for protein for animal feed stuff. In this study, most fertilizer treatments contributed higher CP content and yield of ryegrass than the control. Also, the CP content increased as the application level of each fertilizer increased. Similarly, Yolcu (2010c) reported that CP content of common vetch-barley mixtures increased as the amount of solid cattle manure application increased. Also, Akinremi et al. (2000) found higher N content of canola with increasing the amounts of leonardite applications. However, the highest hay and CP yields were obtained with the lowest level of zeolite (Z_1). These findings are in agreement with the results of Yolcu (2010b). On the contrary, Mahmoodabadi et al. (2009) reported that the highest shoot DM of soybean was obtained with the highest level of zeolite. In addition, Ahmed et al. (2010) reported no differences between treatment levels of zeolite for DM yield in maize. These differences were probably due to the plant species or/and to the various soil and climatic conditions of the experimentation sites.

Mineral contents

All fertilizers (manure, zeolite and leonardite), in most application levels, provided higher mineral contents of ryegrass hay relative to control, whereas only in some treatments the contents of few minerals were similar or lower to control. Also, in most cases, manure application gave higher mineral contents than that of leonardite and zeolite. Similarly, several studies reported that humic acid, manure, leonardite or zeolite applications increased most macro- and micro-nutrient concentrations in many crops (Akinremi et al., 2000; Cimrin et al, 2001; Matsi et al., 2003; Butler and Muir,2006; Gevrek et al, 2009; Mahmoodabadi et al., 2009).

Table 5. Mineral contents (mg kg^{-1}) of annual ryegrass fertilized with manure (M), zeolite (Z) and leonardite (L).

Fertilizers	Amount (t ha^{-1})	P	K	S	Ca	Mg	Na	Cu	Fe	Mn	Zn	B
Control	0.00	1000 CD	11522 G	585 G	4845 G	1551 D	441 E	61.4 D	615 F	55.4 G	27.4 F	41.1 F
M_1	20.00	1246 B	16812 A	898 E	7512 B	1733 C	661 B	64.9 CD	749 D	78.5 C	32.3 E	68.1 C
M_2	40.00	1197 B	15400 C	1064 C	7294 C	2187 A	767 A	76.5 B	940 A	89.2 A	45.1 B	83.3A
Z_1	0.25	1046 C	12830 F	1198 AB	8228 A	2259 A	485 D	75.1 B	892 BC	70.8 DE	25.5 F	57.5 E
Z_2	0.50	1050 C	15946 B	1239 A	8096 A	2195 A	437 E	64.3 CD	869 C	77.9 C	36.4 D	60.8 D
Z_3	0.75	945 D	13956 E	1157 B	6025 E	1592 D	381 F	54.9 E	675 E	85.1 B	44.1 BC	42.1 F
L_1	0.25	1081 C	12796 F	798 F	5806 F	1878 B	655 B	82.9 A	764 D	64.6 F	31.9 E	65.7 C
L_2	0.50	1238 B	15142 CD	972 D	6342 D	2216 A	623 C	69.2 C	867 C	68.5 E	41.4 C	60.3 DE
L_3	0.75	1343 A	14912 D	1084 C	6438 D	2194 A	505 D	49.2 E	932 AB	72.7 D	59.0 A	77.7 B
Single degree of freedom contrasts												
Control (0)		1000 B	11522 C	585 C	4845 C	1551 b	441 C	61.4	615 B	55.4 C	27.4	41.1 C
Manure (M)		1222 A	16106 A	981 B	7403 A	1960 a	714 A	70.7	845 A	83.9 A	38.7	75.7 A
Zeolite (Z)		1014 B	14244 B	1198 A	7450 A	2015 a	434 C	64.7	812 A	77.9 A	35.3	53.5 B
Leonardite (L)		1221 A	14283 B	951 B	6195 B	2096 a	594 B	67.1	854 A	68.6 B	44.1	67.9 A

Values followed by small and capital letters in a column shows significantly differences at $P < 0.05$ and $P < 0.01$ levels, respectively.

On the contrary, Walker and Bernal (2008) reported that manure application decreased the content of Mg in oat plants, while the content of K and Ca decreased in corn shoots with zeolite application (Ahmed et al., 2010). In addition, studies with manure application showed no effect on the K, Mg and Ca content of cereals and common vetch (Yolcu, 2008; Yolcu et al., 2010) and also on K content of maize shoot (Iqbal et al, 2007). In our research, ryegrass concentration of most macro- and micro-minerals increased as the level of manure or leonardite applications increased, whereas only the Mn and Zn contents increased as the amount of zeolite increased. Similar results were reported by Akinremi et al. (2000) in canola with different levels of leonardite application, and in maize with zeolite application (Ahmed et al., 2010). In addition, Butler and Muir (2006) reported that P and K concentrations of wheatgrass increased linearly as rates of dairy manure compost increased.

Material and methods

Experiment Location

The experiment was carried out in the area of the Kelkit Aydin Dogan Vocational Training School of Gumushane University in the northeast of Turkey (40° 08' N, 39º 25' E) during 2008 and 2009. Soil samples (0-30 cm and 30-60 cm) were collected prior to crop planting and analyzed for selected chemical characteristics. The soils of the experimental field were clay-loamy with little alkali reaction (pH 7.7-7.8), rich in lime, potassium, magnesium and zinc, sufficient in phosphorous, calcium, iron and copper, poor in nitrogen and manganese and with low organic matter (Table 1). Climatic data of experimental site are presented in Table 2.

Field applications and experimental design

Sowing took place on 18 April of 2008, which is the optimal time for ryegrass sowing in northeast Turkey. Annual ryegrass (cv Peleton) was sown in rows (spaced 24 cm apart) at a seeding rate of 25 kg ha^{-1}. The experiment was arranged in a randomized complete-block design with nine treatments (fertilizers) replicated three times. Plot size was 1.68 by 3 m, with a 2 m buffer zone. The nine fertilizer applications were: two levels (M_1, M_2) of solid cattle manure (i.e., 20 and 40 t ha^{-1}, which contained 64 and 128 kg N ha^{-1}, respectively), three levels (Z_1, Z_2, Z_3) of zeolite (i.e., 250, 500 and 750 kg ha^{-1}, which contained 0.4, 0.8 and 1.2 kg N ha^{-1}, respectively), three levels (L_1, L_2, L_3) of leonardite (i.e., 250, 500 and 750 kg ha^{-1}, which contained 2.8, 5.6 and 8.4 kg N ha^{-1}, respectively), and no fertilizer application (control). Fertilizers were applied in the soil two days before seedbed preparation. Some characteristic properties of the fertilizers are presented in Table 3. The field was irrigated once in early stage in each year and was harvested twice at the beginning of anthesis of annual ryegrass in 2009.

Chemical analysis

Plants were harvested in a 1 m^2 area of each plot for forage yield determination. Hay samples (1 kg biomass from each plot) were oven-dried at 68 ^oC for 48 h and ground to pass through a 1 mm sieve. The samples were analyzed for N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu and B content. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner, 1996) in tissues of annual ryegrass. Macro and micro-elements of annual ryegrass were determined after wet digestion of dried and ground sub-samples using a HNO_3-H_2O_2 acid mixture (2:3 v/v) with three steps (first step: 145ºC, 75%RF, 5 min; second step: 180ºC, 90%RF, 10 min and third step: 100ºC, 40%RF, 10 min) in microwave (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens, 2005). The content of P, K, S, Ca, Mg, Fe, Mn, Zn, Cu and B in the samples was determined with Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005). Crude protein content was calculated by multiplying the N content by a coefficient of 6.25. ADF and NDF contents were determined according to Van Soest (1963).

Statistical analysis

Data of hay yield, crude protein, ADF, NDF and nutrients were subjected to analysis of variance (ANOVA). Furthermore, the means of the fertilizer groups (manure, zeolite, leonardite and control) were compared using single degree of freedom linear contrasts (F test). Treatment means were compared by Duncan's multiple range test method using SPSS software package (SPSS 11.5 for windows) (SPSS, Inc. 2004).

Conclusion

The results of this study clearly showed that in most cases manure, leonardite and zeolite applications positively affected the hay yield, the CP content and yield, and the macro- and micronutrients of annual ryegrass. Among fertilizers, zeolite applications were found more effective on the hay and crude protein yield of ryegrass, whereas manure application gave higher mineral contents than that of leonardite and zeolite. The results suggest that all fertilizers (especially zeolite) have potential for use in organic agriculture as with these treatments chemical fertilizers are avoided and hay production of annual ryegrass is improved in terms of yield, protein and mineral content.

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