

## The Effects of Various Fertilization and Seeding Patterns on Trace Minerals Content of Lucerne and Smooth Bromegrass Mixture in an Intercropping System for Livestock

<sup>1</sup>Halil Yolcu and <sup>2</sup>Metin Turan

<sup>1</sup>Kelkit Aydın Dogan Vocational Training School, Erzincan University, 29600, Kelkit, Gumushane, Turkey

<sup>2</sup>Department of Soil Science, Faculty of Agriculture, Atatürk University, 25240-Erzurum, Turkey

**Abstract:** The effects of various fertilization and seeding patterns on trace mineral contents of lucerne-smooth bromegrass mixture were evaluated for animal feeding. The lucerne and smooth bromegrass mixtures were established with different seeding pattern (mixed, alternative and cross-seeding), N fertilizer rates (0, 60 and 120 kg N ha<sup>-1</sup>) and P fertilizer rates (0, 40, 80 and 120 P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). Different seeding patterns, N and P fertilization affected the trace mineral contents of the lucerne-smooth bromegrass mixture under the intercropping system. Mn, Cu and Zn content of the mixture varied significantly depending on seeding patterns. However, the seeding patterns had no significant effect on the Fe contents of the mixture. N application decreased the content of Fe and increased the content of Cu, but had no effect on the Mn and Zn content; whereas, P application affected the Mn and Cu contents but had no effect on the contents of Fe and Zn.

**Key words:** Lucerne, smooth bromegrass, trace minerals, intercropping

### INTRODUCTION

In animal nutrition, minerals are considered essential as their deficiency or withdrawal causes some impairment of function or induces some abnormality (Sharma *et al.*, 2005). Essential trace minerals are necessary nutrients for normal function of the tissues and processes intimately related to reproduction (Kuhlman and Rompala, 1998). Trace minerals activate several enzymes responsible for various biochemical functions and some integral part of enzymes/hormones/vitamins (Prasad and Gowda, 2005).

The basic goal of many grazing programs is to provide high quality forage over the years to reduce the costs of storing and purchasing concentrate feeds (Mut *et al.*, 2006). But acute and chronic dietary deficiencies in macro and micro minerals have significant impacts on production efficiency of rangelands throughout the world (Pinchak *et al.*, 1989). In order to overcome this problem, it needs to add forage plants that are rich in terms of minerals to the rations of cattle foraging. High-yielding (Sollenberger *et al.*, 1984), high quality grass-legume mixtures play an important role in forage-animal systems (Haynes, 1980). They are commonly consumed in the diet of feeding animals.

It is important that the diet formulation for trace minerals considers the forage intake, the forage trace mineral content and animal's requirements (Corah, 1996). However, concentrations of dietary minerals can be estimated indirectly if mineral concentrations in forages consumed are known (Pinchak *et al.*, 1989). Trace mineral content in plants is governed by agro-climatic conditions, species of plants, the geochemical nature of the soil (Prasad and Gowda, 2005), use of fertilizers (Underwood and Suttle, 1999) and seeding patterns.

Herbage quality in grass and legume mixture is affected by N, P fertilizers (Nuttal, 1980; Aydın and Tosun, 1993; Karaca and Çimrin, 2001) and seeding patterns (Altın, 1982; Yolcu, 2005). The experiment presented here was designed to investigate the effects of seeding patterns and nitrogen and phosphorus fertilization rates on the trace mineral contents of lucerne and smooth bromegrass mixture for animal feeding.

### MATERIALS AND METHODS

An irrigated field experiment was carried out at the Atatürk University agricultural fields in Erzurum province (39°55'N, 41°61'E) of Turkey. The study area was

situated at an altitude at 1860 m above sea level in the eastern part of the Turkey. The climatic conditions in this location are characterized by drier summers, low humidity and cold-snowy and long winters. Mean annual temperature, precipitation and relative humidity in the region for 2003 are 5.2°C, 424.3 mm and 61.6%, respectively.

The experiment had a randomized complete block design with three Lucerne and smooth brome grass seeding patterns (mixed-rows, alternate-rows and cross-seeding), three nitrogen fertilizer doses (0, 60 and 120 kg N ha<sup>-1</sup>), 4 P application levels (0, 40, 80 and 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and 3 replications. The size of the plots was 3.0 m long by 1.8 m wide, with 30 cm row spacing. Individual plots were 1.8×3 = 5.4 m<sup>2</sup> in size. Binary mixtures of lucerne and smooth brome grass were sown in the spring of 2001. A legume-grass mixture of ‘Kayseri’ lucerne (*Medicago sativa* L.) and ‘Tohum Islah’ smooth brome grass (*Bromus inermis* Leyss.) was used in the experiment.

Nitrogen was broadcast at the rates of 0, 60 and 120 kg N ha<sup>-1</sup> on the plots early each spring in the form of ammonium sulphate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (20.5% N). Phosphorus was broadcast at the rates of 0, 40, 80 and 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> on the plots early each autumn as triple super phosphate (48% P<sub>2</sub>O<sub>5</sub>). Irrigation was made when the available soil moisture decreased to 50%, approximately 8-10 days, according to the instructions given by Çomaklı (1991). The soil of the experiment area is a silt loam. The pH level of the plot area was 6.9 and the area was rich in P and K levels (137 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 67.7 kg K<sub>2</sub>O ha<sup>-1</sup>), also its organic matter content was 0.79%. Fe, Cu, Mn and Zn contents of the soil were a 3.75, 1.41, 9.93 and 0.69 mg 1kg<sup>-1</sup> at 0-20 cm soil depth and 3.39, 1.47, 11.76 and 1.33 mg kg<sup>-1</sup> at 20-40 cm soil depth, respectively. In calcareous soils in eastern Turkey, lucerne production requires phosphorus addition (100-150 kg P ha<sup>-1</sup>) as the soils are naturally P deficient or very high P sorption isotherms (Özgül *et al.*, 2006).

The set of data was collected from the mixture that was harvested in the year 2003. Plots were harvested at

the early flower stage of lucerne (Manga, 1978). Forage samples were collected by clipping 1 meter squared areas in each plot. Forage species were separated as lucerne and smooth brome grass. These sub samples were dried 78°C for 24 h and ground to pass 1 mm. Fe, Mn, Zn and Cu were determined after wet digestion using a HNO<sub>3</sub>-HClO<sub>4</sub> acid mixture (4:1 v/v). In the diluted digests Fe, Mn, Zn and Cu analysis were determined by atomic absorption spectrometry (Perkin Elmer 3690) (AOAC, 2005). All of the results were calculated as weighted mean squares by taking botanical composition into consideration.

The statistical procedures of MSTAT-C were used for data analyses to test the effects of nitrogen and phosphorus fertilization rates, seeding methods and all interactions. All means were separated using the least significant differences (p<0.1).

## RESULTS AND DISCUSSION

The effects of the seeding patterns and N and P fertilization on the content of manganese, iron, zinc and copper in lucerne-smooth brome grass mixture crop are presented in Table 1-3. The results showed significant differences in Mn, Fe, Zn and Cu contents of lucerne-smooth brome grass mixture depend on N, P fertilizations and seeding patterns.

**The effects of seeding patterns on trace minerals:** When compare to different seeding patterns, the highest Mn (105.76 mg kg<sup>-1</sup>) contents of the intercrops were determined in alternative seeding patterns, but Zn (59.81 mg kg<sup>-1</sup>) and Cu (4.44 mg kg<sup>-1</sup>) contents of the intercrops were obtained from cross-seeding patterns (Table 2 and 3). The checkered pattern from cross-seeding keeps to better water in the soil than the other seeding patterns (Kilcher, 1982). Because Zn and Cu are also mobile elements in sludge (Pueyo *et al.*, 2003), it may have caused higher Zn and Cu contents in cross-seeding than the other seeding patterns.

Table 1: F-values for effects of various fertilization and seeding patterns on trace minerals content of lucerne and smooth brome grass mixture in intercropping system

Factors	Mn		Fe		Zn		Cu	
	F.V.	Sign.	F.V.	Sign.	F.V.	Sign.	F.V.	Sign.
S.P.	9.68	**	0.97	ns	2.68	+	3.18	*
N Rate	0.36	ns	5.96	**	0.62	ns	5.76	**
P Rate	2.87	*	0.93	ns	1.31	ns	2.37	+
SPXN	3.33	*	0.82	ns	0.65	ns	0.91	ns
SPXP	1.47	ns	2.29	*	1.25	ns	1.87	+
NXP	2.30	*	1.13	ns	0.83	ns	1.99	+
SPXNXP	1.79	+	2.73	**	1.20	ns	2.64	**

\*\* : (p<0.01), \* : (p<0.05), + : (p<0.1)

**Table 2: Mn and Fe contents of Lucerne and smooth brome grass in response to various sowing methods and fertilization in intercropping systems**

Sowing system	N level (kg N ha <sup>-1</sup> )	P (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )				Avarege
		0 (Control)	40	80	120	
2003		Mn (mg kg <sup>-1</sup> dw)				
Mixed	0 (Control)	96.87	82.51	83.40	114.44	98.02 B
	60	87.74	99.97	92.61	110.40	
	120	124.05	104.81	82.60	96.86	
Alternative	0 (Control)	102.67	115.91	105.74	126.08	105.76A
	60	104.69	123.93	108.02	89.88	
	120	102.80	100.74	82.93	105.78	
Cross-seeding	0 (Control)	81.78	81.23	87.02	79.85	90.62 C
	60	93.00	94.61	90.78	90.38	
	120	97.89	82.05	92.89	116.0	
Average	0 (Control)	93.77	93.22	92.05	106.79	96.46
	60	95.15	106.17	97.14	96.89	98.83
	120	108.25	95.87	86.14	106.21	99.12
General average		99.06ab	98.42ab	91.78b	103.30a	98.14
2003		Fe (mg kg <sup>-1</sup> dw)				
Mixed	0 (Control)	207.49	175.69	200.53	258.92	206.55
	60	193.91	218.14	178.26	228.01	
	120	207.71	210.51	200.70	198.72	
Alternative	0 (Control)	179.29	240.25	242.98	212.96	204.42
	60	214.05	207.24	192.77	200.20	
	120	198.89	166.52	205.91	191.98	
Cross-seeding	0 (Control)	222.50	213.95	220.93	211.51	204.58
	60	189.28	196.09	218.20	190.90	
	120	197.19	192.73	201.63	200.08	
Average	0 (Control)	203.09	209.96	221.48	227.80	215.58 A
	60	199.08	207.16	196.41	206.37	202.25 B
	120	201.26	189.92	202.75	196.93	197.71 B
General average		201.15	202.35	206.88	210.36	205.18

Values inside columns and rows with different letters differ significantly (Minuscule p<0.05, Capital letter p<0.01)

**Table 3: Zn and Cu contents of Lucerne and smooth brome grass in response to various sowing methods and fertilization in intercropping systems**

Sowing system	N level (kg N ha <sup>-1</sup> )	P (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )				Avarege
		0 (Control)	40	80	120	
2003		Zn (mg kg <sup>-1</sup> dw)				
Mixed	0 (Control)	54.58	54.38	49.08	58.91	55.48
	60	66.79	53.38	59.86	57.72	
	120	56.36	55.64	43.57	55.52	
Alternative	0 (Control)	61.70	45.00	61.02	60.15	53.87
	60	53.45	47.90	50.99	56.55	
	120	54.58	44.32	57.15	53.62	
Cross-seeding	0 (Control)	48.63	72.23	57.59	61.55	59.81
	60	65.97	52.85	66.88	56.36	
	120	68.19	51.08	66.51	49.82	
Average	0 (Control)	54.97	57.20	55.90	60.20	57.08
	60	62.07	51.38	59.24	56.88	57.39
	120	59.71	50.34	55.74	52.99	54.70
General average		58.92	52.98	56.96	56.69	56.39
2003		Cu (mg kg <sup>-1</sup> dw)				
Mixed	0 (Control)	3.76	3.65	4.32	3.03	4.14 ab
	60	4.41	3.75	3.81	4.47	
	120	4.49	5.46	4.96	3.57	
Alternative	0 (Control)	3.45	4.20	4.72	3.28	4.07 b
	60	3.88	4.31	3.76	4.40	
	120	3.95	4.28	3.90	4.70	
Cross-seeding	0 (Control)	3.93	4.58	4.14	4.45	4.44 a
	60	5.25	3.89	4.66	3.86	
	120	5.23	4.12	5.35	3.75	
Average	0 (Control)	3.71	4.14	4.39	3.59	3.96 B
	60	4.51	3.98	4.08	4.24	4.21 AB
	120	4.55	4.62	4.74	4.01	4.48 A
General average		4.26	4.25	4.40	3.95	4.22

Values inside columns and rows with different letters differ significantly (Minuscule p<0.05, Capital letter p<0.01)

**The effects of N and P applications on trace minerals:**

While no significant differences were observed with nitrogen fertilization on Mn and Zn contents of the intercrops, Fe and Cu contents of the intercrops was significantly affected by different nitrogen fertilization (Table 2 and 3). Increased doses of nitrogen fertilization decreased Fe content of lucerne and smooth brome grass intercrops ( $p < 0.01$ ). The highest Fe content of the intercrops was obtained at no nitrogen fertilization application treatments (215.58 Fe mg kg<sup>-1</sup>). This value was followed by 60 kg N ha<sup>-1</sup> (202.25 Fe mg kg<sup>-1</sup>) and 120 kg N ha<sup>-1</sup> (197.71 Fe mg kg<sup>-1</sup>) fertilization applications, respectively. N application increases forage yields in mixtures as in this the study at 2003 (Yolcu, 2005; Halvorson and Bauer, 1984; Aydin and Tosun, 1993; Çomaklı *et al.*, 1998; Karaca and Çimrin, 2001). Generally, both soil concentration ratio of Fe (Whitehead, 2000) and Fe distributions in sludge and soil samples are especially low (Pueyo *et al.*, 2003). Increasing forage yields depend on N application doses may have caused the decreasing of the Fe ratio of the mixture. But, Cu content of the lucerne and smooth brome grass intercrops was increased with increasing N fertilization doses. The highest Cu contents of the intercrops were obtained 120 kg N ha<sup>-1</sup> (4.48 Cu mg kg<sup>-1</sup>) fertilization doses (Table 3). This value was followed by 60 kg N ha<sup>-1</sup> (4.21 Cu mg kg<sup>-1</sup>) and 0 kg N ha<sup>-1</sup> (3.96 Cu mg kg<sup>-1</sup>) fertilization applications, respectively. Because Cu is more distribution element than Fe in the soil (Pueyo *et al.*, 2003), Cu uptake of the mixture plants may have increased with increasing N fertilization. These results are consistent with works (Sönmez and Yılmaz, 2000), who reported that nitrogen fertilization decreased Fe contents of Barley grain and Harapiak *et al.* (2000) suggested that N application increase Cu content of brome grass.

Mn contents of the lucerne and smooth brome grass intercrops were significantly affected by different phosphorus fertilization ( $p < 0.05$ , Table 1 and 2). The highest Mn content of the intercrops was obtained from 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> fertilization (103.30 Mn mg kg<sup>-1</sup> dw). 0 kg P ha<sup>-1</sup>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> fertilizations obtained 99.06 Mn mg kg<sup>-1</sup> dw, 98.42 Mn mg kg<sup>-1</sup> dw and 91.78 Mn mg kg<sup>-1</sup>, respectively. Similar findings were reported in previous studies showing that P fertilization increased Mn contents (Sönmez and Yılmaz, 2000; Adjei *et al.*, 2001; Jurković *et al.*, 2006). Cu content of the intercrops was marginally affected by different phosphorus fertilization (Table 1 and 3). No significant differences were determined in the Fe and Zn content of the intercrops. In some other studies, P application didn't affect Cu

concentration in legume and grass (Hendricksen *et al.*, 1992), Fe and Zn concentrations in limpgrass pasture (Adjei *et al.*, 1998) and Cu and Fe concentrations in maize (Komljenovic *et al.*, 2006; Jurkovic *et al.*, 2006).

Mn, Fe and Zn contents of the intercrops were above the requirements of growing and finishing cattle (20 Mn mg kg<sup>-1</sup>, 50 Fe mg kg<sup>-1</sup> and 30 Zn mg kg<sup>-1</sup>) as well as gestating and early lactation of cows (40 Mn mg kg<sup>-1</sup>, 50 Fe mg kg<sup>-1</sup> and 30 Zn mg kg<sup>-1</sup>). But Cu content was under the requirements of growing and finishing cattle besides these of gestating and early lactation cows (10 Cu mg kg<sup>-1</sup>) (NRC, 2000). The Mn, Fe, Zn and Cu contents were much lower than the maximum tolerable concentration of cattle (NRC, 2000).

Over all, it was determined that the lucerne and smooth brome grass mixture in terms of trace element except of Cu had important potential for animal feeding. Besides, nitrogen, phosphorus fertilizer and seeding pattern affected on micro element contents of the lucerne and smooth brome grass mixture. The highest Mn content of the intercrops was obtained from alternative seeding pattern, while the highest Cu and Zn content were obtained from cross seeding pattern. N application decreased the content of Fe and increased the content of Cu, but had no effect on the Mn and Zn content. P application affected the Mn and Cu content but had no effect on the contents of Fe and Zn.

These results demonstrates that the if producer get optimum Zn and Cu contents in lucerne and smooth brome grass mixture for trace minerals considers the forage trace mineral content and animal's requirements, they should be select cross-seeding treatments at 60 kg N ha<sup>-1</sup> in calcareous soil. If producer get optimum Mn contents, it should be used alternative patterns. But our results should be correlated with further years and study with other plants.

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