ZEOLITE AS A BINDING AGENT FOR AMMONIA IONS AND AS A SOIL ADDITIVE. II. EFFECT ON GRASS GROWTH AND QUALITY

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ABSTRACT

Since the organic fertilizers are very important for sustainable forage production, in this work, zeolitic tuff was used as a binding agent for ammonia released from cattle manure. It has been shown previously that the addition of 10 wt.% zeolite to fresh cattle manure increases retention of realized ammonia by 90% in comparison to the system without zeolite. Here, the experiments were established in pots, to study effect of different ammonia source on Italian ryegrass (*Lolium multiflorum* Lam.) growth and herbage quality. Preliminary tests indicate those fresh matter yields obtained in pots containing ammonia-loaded zeolite were higher compared to control pots. Herbage quality measured by protein content in Italian ryegrass shoots indicates that ammonia assimilation is not affected ryegrass quality.

Key words: cattle manure, herbage yield, Italian ryegrass, protein content, zeolite

INTRODUCTION

Nitrogen (N) is one of the most critical elements affecting grass growth and development. Herbage grasses respond favourably to high N fertilization by producing an abundance of vegetative matter. N uptake and distribution in plants involves many aspects of growth and development [1]; since the relationship between nitrogen and biomass accumulation in crops, relies on the interregulation of multiple crop physiological processes. Among these processes, N uptake and thus growth rate, N allocation between organs and between plants, play a particular role. Under sub-optimal N supply, N uptake of the crop depends on soil mineral N availability and distribution, and on root distribution. Under ample N supply, N uptake largely depends on growth rate via internal plant regulation.

There are reports of ryegrass preference for ammonium nutrition over nitrate [2]. Soil ammonium levels are regulated by numerous factors, such as soil temperate, pH, soil microbiology, fertilizer form, and moisture.

Interest in ammonia nutrition in herbage grasses arises from the following premises: 1) enhanced ammonia supply in cereals has been shown to increase tiller number and grain yield; 2) ammonia is often the nitrogen form most important in acidic soils; and 3) ammonia is not leached from soils as readily as nitrate, thus potentially increasing available nitrogen use efficiency and lessening nitrate ground water contamination [3].

Italian ryegrass is an important short duration grass in Serbia. High palatability and digestibility make this species highly valued for forage/livestock systems from early spring to late summer [4]. It is used in many environments where fast cover or quick feed is required. Italian ryegrass is preferred standardised grass species used as a model plant for nitrogen uptake. If all other elements are not limiting, nitrogen is the main nutritional determinant of ryegrass yield [5]. Applying N as fertilizer influences herbage N uptake and chlorophyll concentration in ryegrass [6]. Intensive fertilizer N management, high seasonal rainfall variability and an increasing awareness of the economic and environmental consequences of N loss have led to a growing interest in understanding how soil-plant system process and retain N.

The objective of this study was to investigate and launch the sustainable ammonia source for herbage grasses, which is based on natural zeolite mined from “Zlatokop” in south Serbia. Another goal of this work was to compare cattle manure as a fertilizer with NH_4^+-containing zeolite. Understanding the influence of ammonia from different sources on herbage yield and protein content can be used to improve N use efficiencies in the field. Since the Italian ryegrass is the grass of intensive growth and rapid spring development, especially after nitrogen application, the questions of nitrogen absorption dynamics and its transformation in the plant in the form of crude proteins are being posed.

EXPERIMENTAL

The ammonia-enriched zeolitic tuff (Zlatokop deposit, Vranjska Banja; containing 70 wt.% of clinoptilolite, grain size in the range 0.063-0.1 mm) prepared as described in details elsewhere [7] and denoted as NH_4-CLI was used as soil additive in this work. The experiments were performed in pots; in order to investigate the effects of enhanced ammonia supply on Italian ryegrass (*Lolium multiflorum* Lam.) tiller growth and quality, and to compare the impact of other nitrogen sources on the same parameters. In these pot experiments, ryegrass cultivar K-29t has been used with three different treatments:

- Control treatment - just pure soil was used;
- Soil mixed with manure - 25.55 g of manure was added in soil calculated on the basis of manure fertilization (20 t ha^{-1}) in the field conditions; and
- Soil mixed with zeolite - 25.55 g NH_4-CLI (containing 0,96 mg NH_4^+) formed by binding of ammonia ions from aqueous solution by zeolite and calculated on the same basis as above.

All other nutrients were adequately supplied, in the same way in all three treatments. As a first step of sowing procedure, a filter paper was placed at the bottom of each pot and 2.3 kg of soil/soil with additive was placed above. Further, ryegrass seed was sown with four replications at a rate of 50 seeds per pot, and finally, another 300 g of soil were placed on the top. The experiments started on 10 September 2012.

The pots were placed in an unheated glasshouse and were thoroughly watered, while the growth of the plants was monitored. Plants were cut back three times in all the individual experimental pots, each time when the stems were about 12 cm high, *id est*, about 2-3 cm above soil level. The foliage was collected for determination of fresh and dry weight per cut. The experiment was terminated 58 days after planting. The harvested material was left to dry at room temperature for a few days and then was dried in an oven at 60°C until constant mass. Plant tissue was examined for changes in fresh weight, dry weight and total nitrogen; data were analyzed by analysis of variance (ANOVA) and the treatment effect was determined according to Fischer's least significant difference procedure.

The protein contents were determined by measuring nitrogen content, using standardized methodology (ISO 1871-2009). The homogenized dry grass (moisture lower than 10 wt.%) samples were used; about 0.25 gram of sample was measured (with 0,1 mg accuracy). Digestion of samples was done using digestion unit (2000 Digestion System FOSS); while distillation and titration of released nitrogen was done using Kjeltec 2300 Analyzer Unit, FOSS. 6.25 was used as a factor for calculation of protein content. All measurements were done in duplicate. QC/QA – recovery for ammonium sulphate was 99%, minimum.

RESULTS AND DISCUSSION

Considering that every pot had the same number of seeds (50), the highest number of seedlings that grew was found in the zeolite-containing pots (about 42), while this number was lower in the controls (40) and in the manure-containing pots (39), but without significant effect of treatment (Table 1). Number of emerged seedlings per pot was similar among treatments. Addition of zeolite or manure doesn't have a negative effect on the germination of Italian ryegrass seeds.

After the first harvest, there was a marked increase in yield of fresh matter with zeolite, higher by about 20% compared to the pots with manure and control. But different N uptake in Italian ryegrass during plant development (Table 1) resulted in a somewhat diminished influence and finally in the equalization in dry matter yield among treatments. The equalization of the yield during three cuts of Italian ryegrass takes place due to more intensive mineralization and drawing (extraction) of nitrogen from relatively good soil reserves.

Table 1. Fresh matter, dry matter and protein content of Italian ryegrass grown in pots with different ammonia sources

	CONTROL	**ZEOLITE**	**MANURE**
		Seedlings	
Number pot^{-1}	39.8^{a*}	41.8^{a}	39.0^{a}
Harvest		**Fresh matter (g)**	
I	4.52	5.47	4.52
II	4.52	4.52	4.71
III	6.25	5.90	6.50
Total	15.29^{b}	15.89^{a}	15.73^{ab}
Harvest		**Dry matter (g)**	
I	0.43	0.46	0.39
II	0.43	0.46	0.39
III	0.57	0.56	0.58
Total	1.43^{a}	1.48^{a}	1.36^{a}
		Protein content (%)	
Total	30.8^{a}	31.6^{a}	30.8^{a}

$LSD_{0.05\ S}$=4; $LSD_{0.05\ FM}$=0.46; $LSD_{0.05\ DM}$=0.29; $LSD_{0.05\ PC}$=3.7

*Values followed by the same letter are not significantly different by Fisher's protected LSD values; $LSD_{0.05}$ – least significant difference at $P \leq 0.05$; S-seedlings; FM-fresh matter, DM-dry matter; PC-protein content

In enhanced ammonia supply by zeolite as carrier, the most pronounced effect of different nitrogen-source nutrition was on fresh matter yield. A marked increase in the fresh matter was seen for NH_4-CLI additive, compared to control, but this increase not occurred at the dried matter. Thus, the increased absorption of NH_4^+ from zeolite enriched by ammonia related to absorption from pure soil seems to be related primarily to the difference in N uptake, more accessible from zeolite as carrier than manure.

Total plant dry weight was not affected by nitrogen-source, as well as nitrogen content. There was same nitrogen status in DM, *id est*, quality expressed in protein content, irrespective of the fertilizer treatment at which plants were grown previously. Crude protein content was similar to content in intensive stem growth of Italian ryegrass (30.0%) in field conditions, previously reported by [5].

CONCLUSION

On the basis of results obtained in pot experiments on the crude protein content in Italian ryegrass under the influence of different N application, it can be concluded that this grass species reacts rapidly to increased N application increasing the yield of dry weight in relation to control. It appears that the greatest and most significant response of enhanced ammonia supply in Italian ryegrass, in terms of increasing fresh matter yield, results from the stimulation of vegetative development by satisfactory water supply. Finally, the herbage yield equalizes during vegetation as well as a final dry matter yield, due to weakening influence of applied nitrogen treatment.

It is hoped that continued research in enhanced ammonia supply by zeolite as carrier will result in better nitrogen/fertilization management through strategies best-suited to support vegetative development in herbage grasses. Further research is underway examining the effects of enhanced ammonia supply in herbage grasses grown under field conditions.

Based on the results presented here, natural zeolite can be recommended for agricultural purposes in terms of sustainable fertilizing and improving system cattle farm – manure - organic fertilizer for forage crops.

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