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NITROGEN FRACTIONS IN TIMOTHY GRASS (*PHLEUM PRATENSE* L.) FERTILIZED WITH DIFFERENT DOSES OF MINERAL FERTILIZERS

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Abstract

The effect of mineral (NPK) fertilization on the content of nitrogen fractions in timothy grass was assessed from the results of a three-year field experiment conducted at the Elizówka Experimental Farm near Lublin, on podzolic soil developed from loess. The experiment was included 27 fertilized treatments, i.e. nine nitrogen and potassium variants tested, each in four replications. against the background of three increasing phosphorus fertilization doses. Nitrogen was applied as ammonium nitrate at a dose of 120 kg N ha^{-1} (N1) and at two-fold (N2) and three -fold (N3) higher doses. Phosphorus was applied in the form of granular triple superphosphate at a dose of 34.9 kg P ha^{-1} (P1) and at two-fold (P2) and three-fold (P3) higher doses. Potassium was used as potassium salt (47.3 %) at a dose of 83 kg K ha^{-1} (K1) and at two-fold (K2) and three-fold (K3) higher doses. After grass harvest, plant material was sampled and the content of total, protein, ammonium and nitrate nitrogen(V) was determined in duplicate. It was found that the content of the nitrogen fractions analysed in timothy grass, i.e. total, protein, mineral, ammonium and nitrate(V) nitrogen, was significantly positively correlated with the mineral fertilization, in particular, with nitrogen and phosphorus and, to a lesser extent, with potassium. The mean share of protein nitrogen in total nitrogen was 53.8%; mineral, ammonium, and nitrate nitrogen fractions constituted 4.0, 2.5, and 1.5%, respectively. Timothy grass fertilized with increasing doses of nitrogen, phosphorus, and potassium fertilizers contained the largest share of protein nitrogen in total nitrogen, did not contain excessive amounts of nitrates(V) and proved to be valuable bulk feed.

Key words: *Phleum pratense* L., nitrogen fractions, NPK fertilization.

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INTRODUCTION

Mineral fertilization exerts a strong effect on the yield and quality of crop plants. Among essential nutrients (N, P, K), nitrogen fertilization plays the key role. It has an impact on the biomass growth and plant chemical composition (Stuczyński 1969, Koter, Krauze 1978, Gąsior, Kaniuczak 1996, Havstad, Aamlid 2006, Bednarek 2011, Ashikaga et al. 2012). It also increases the content of this element in tissues and has an effect on its fractional composition (Koter, Krauze 1978, Czuba 1996, Bednarek 2005, Pelletier et al. 2007, Bednarek, Reszka 2009). Higher N doses typically lead to increased levels of total nitrogen and reduced amounts of protein nitrogen; additionally, there is a rise in the levels of mineral nitrogen, including nitrate nitrogen(V) (Nesić et al. 2008). The chemical composition of plants depends on agrotechnical practice (fertilization) and natural factors (soil, climate) (Bednarek et al. 2009). The content of nitrates in plants, particularly in forage crops, has an immediate effect on their quality (Tremblay et al. 2005, Havstad, Aamlid 2006, Pelletier et al. 2007, Ashikaga et al. 2012). In some animal organisms, deleterious effects are exerted by nitrates(V) as well as their derivatives, i.e. nitrates(III). Some authors argue that if forage is the only source of food, the content of nitrates(V) contained therein should not exceed 0.7 - 1.5 g $N\text{-}NO_3$ kg^{-1} d.w. Others claim that forage containing less than 3.5 - 4.5 g $N\text{-}NO_3$ kg^{-1} d.w. can be given to animals without harm, whereas forage with a content of 4.5 - 5.5 g $N\text{-}NO_3$ kg^{-1} d.w. sometimes causes poisoning, and animals should not be fed with forage containing > 6.5 g $N\text{-}NO_3$ kg^{-1} d.w. (Czuba 1996, Tremblay et al. 2005). A major role in nitrogen uptake and metabolism in plants is played by an optimal supply of other nutrients, including phosphorus, potassium and magnesium (Koter 1968, Mengel et al. 1976, Lee, Rudge 1986, Czuba 1996).

The aim of the study was to determine the effect of mineral (NPK) fertilization on the content of nitrogen fractions in timothy grass cultivated on podzolic soil developed from loess. Particularly, we tested the following hypothesis: the role of varied fertilization mineral doses of three major nutrients (N, P, K) on accumulation of total nitrogen, protein nitrogen, $N\text{-}NO_3$, and $N\text{-}NH_4$ in timothy grass.

MATERIAL AND METHODS

The present study is based on results of a three-year field experiment conducted at the Elizówka Experimental Farm near Lublin (2003-2005) and established on podzolic soil developed from loess. Before the experiment, the soil contained 77.2 mg P kg^{-1}, 187.0 mg K kg^{-1}, 44.0 mg Mg kg^{-1}, and 1.18% of humus, and the pH_{KCl} was 4.9. The experiment was comrpised 27 fertilized treatments, namely nine nitrogen-potassium combinations were tested against the background of three increasing phosphorus fertilization doses, each in four replications. The plot area was 50 m^2, of which 36 m^2 were harvested. Nitrogen was applied as ammonium nitrate (34% N) at a dose of 120 kg N ha^{-1} (N_1) and at two-fold (N_2) and three-fold (N_3) higher doses. Phosphorus was applied in the form of granular triple superphosphate (20.1% P) at a dose of 34.9 kg P ha^{-1} (P_1) and at two-fold (P_2) and three-fold (P_3) higher doses. Potassium was used as potassium salt (47.3 %) at a dose of 83 kg K ha^{-1} (K_1) and at two-fold (K_2) and three-fold (K_3) higher doses. The entire dose of phosphorus and half of the potassium dose were introduced into soil before sowing timothy grass var. Skrzeszowicka. In the second and third year, the fertilizers were applied after harvesting the third swath; the second dose of potassium was applied each year after the first swath. Nitrogen was added in three equal parts in early spring (immediately after the plant growth started) and after the first and second swath. Timothy grass mowed in the first swath was harvested in the third decade of May or the first decade of June; the second harvest took place in the third decade of July and the third grass harvesting was done in the third decade of September or the first decade of October. After harvest, plant material (from three swaths and three years) was sampled and the following parameters were determined in duplicate: total nitrogen with the Kjeldahl method; protein nitrogen using the Kjeldahl method with trichloroacetic acid after water extraction and protein precipitation; ammonium nitrogen using the Nessler reagent method; and nitrate nitrogen(V) with the phenoldisulphide acid method and mineral nitrogen ($N\text{-}NH_4$+$N\text{-}NO_3$). The content of nitrogen fractions determined in timothy grass was estimated using the *triple* test *cross analysis of variance with the* Tukey's confidence *semi-interval* ($p < 0.05$).

RESULTS AND DISCUSSION

Depending on the mineral fertilization applied, the content of total nitrogen in dry weight of timothy grass ranged from 19.1 to 28.1 g N kg^{-1} (Table 1). The biggest and significant impact on levels of this nitrogen form was exerted by fertilization with the increasing doses of this nutrient. Its mean content was 19.8 at a single nitrogen dose (N_1), 24.6 at a double dose (N_2), and 27.5 g N kg^{-1}d.w. at a triple dose (N_3). In comparison with the single dose, the double dose of ammonium nitrate increased the N content by 24.2%, and the three-fold higher nitrogen dose resulted in a 38.9% increase in the N content. The mean content of nitrogen was 23.4 at the single dose (P_1), 24.0 at the double dose (P_2), and 24.5 g N kg^{-1} d.w. at the triple dose (P_3) of phosphorus. The application of two-fold and three-fold higher doses of granular triple superphosphate increased the content of this nitrogen form by 2.6 % and 4.7%, respectively, in comparison with the single dose. Under

Table 1

Total nitrogen content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	19.1	19.1	19.5	19.3	21.4	18.4	19.5	20.6	21.0	19.8
N_2	24.1	24.4	23.4	23.9	25.4	24.4	26.3	23.4	25.9	24.6
N_3	27.1	27.5	26.6	27.5	28.0	27.4	27.6	28.1	28.0	27.5
PK Average	23.4	23.7	23.2	23.6	24.9	23.4	24.5	24.0	25.0	24.0
P Average	23.4			24.0			24.5			
K Average				23.8	24.2	23.8				

LSD$_{\leq 0.05}$: N, P, K – 0.2; NK, PK – 0.6; NPK – 1.2

the effect of the increasing potassium doses, the total nitrogen content in timothy grass did not exhibit vector trends and reached 23.8 at a single potassium dose (K_1), 24.2 at a double potassium dose (K_2), and 23.8 g N kg^{-1} d.w. at a triple potassium dose (K_3). In comparison to the single dose, the relative increase in the content of this form of nitrogen at the double dose was just about 1.7%. A similar effect of mineral fertilization, in particular with nitrogen, was reported by Gawęcki and Mikołajczak (1978), Dubiel et al. (1991) and Czuba (1996). Bednarek (2005) found that among the three experimental factors (N, P, K), nitrogen and phosphorus fertilization had a significant effect on the content of this nitrogen form, which was over two-fold lower in roots of *Dactylis glomerata* than in sward.

Depending on the form and dose of mineral fertilization, the content of protein nitrogen in timothy grass ranged from 10.7 ($N_1P_1K_1$) to 15.3 g N kg^{-1} d.w. ($N_3P_3K_1$) – Table 2. The application of single, double and triple N doses yielded 11.3, 12.9, and 14.4 g N kg^{-1} d.w. of this nitrogen fraction, respectively. The double nitrogen dose (relative to the single one) increased the content of protein nitrogen on average by *ca* 14.2%, while the triple dose increased this parameter by 27.4%. The increase was statistically significant.

Table 2

Protein nitrogen content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	10.7	11.2	12.1	11.9	11.8	11.4	11.4	10.6	10.7	11.3
N_2	12.6	13.2	12.8	13.0	13.1	13.1	13.3	12.4	12.5	12.9
N_3	13.7	15.0	14.4	14.7	13.7	14.5	15.3	14.2	14.0	14.4
PK Average	12.3	13.1	13.1	13.2	12.8	13.0	13.3	12.4	12.4	12.9
P Average	12.9			13.0			12.7			
K Average				13.0	12.8	12.9				

LSD$_{\leq 0.05}$: N, P, K – 0.1; NP, NK, PK – 0.2; NPK – 0.6

Phosphorus fertilization did not induce distinct changes in the content of this nitrogen fraction. After the application of a single dose of this nutrient, the mean content of protein nitrogen in the plant was 12.9 g N kg^{-1} d.w. The double and triple doses resulted in the protein nitrogen content of 13.0 and 12.7 g N kg^{-1} d.w., respectively. The double phosphorus dose raised the content of this fraction just 0.78% compared to the single dose. Potassium fertilization did not result in considerable, directional changes in the protein nitrogen content. Plants fertilized with the single dose of the element contained 13.0 g N kg^{-1} d.w., whereas those receiving the double and triple doses had 12.8 and 12.9 g N kg^{-1} d.w., respectively. The protein nitrogen content in timothy grass was not significantly correlated with the increasing phosphorus and potassium doses, possibly indicating a high content of the available forms of these compounds in the soil. KRZYWY et al. (1996) reported a steady increase ranging from 17.8 (N_0) to 18.7 g N kg^{-1} d.w. (N_3) in the protein nitrogen content induced by this nutrient (N) in meadow sward. Similar patterns were found by STUCZYŃSKI et al. (1970, 1971), STUCZYŃSKA (1973), and WIATER (1996). KRZYWY et al. (1996) reported that increasing doses of nitrogen caused a relatively steady increase in the content of protein nitrogen in grass, and potassium fertilization induced insignificant differences in that content. BEDNAREK (2005) found that the content of this nitrogen fraction was associated with nitrogen and phosphorus fertilization and was 66% higher in sward than in roots. WIATER (1996) reported a beneficial effect of fertilization with 240 kg N ha^{-1} on the share of non-protein and protein nitrogen in pasture sward, and an increase of a dose up to 360 kg N ha^{-1} more substantially elevated the level of non-protein rather than protein nitrogen. This corresponded with the results of KOTER (1973), KOTER and KRAWCZYK (1981). GĄSIOR and KANIUCZAK (1996) noted that the mean content of protein nitrogen in the first-swath hay was about 0.5% higher than that in hay harvested from the second regrowth. This was also reported by STUCZYŃSKI (1969).

The content of ammonium nitrogen in timothy grass depended on the form and dose of mineral fertilization and ranged from 0.42 to 0.83 g N kg^{-1} d.w. (Table 3). The nitrogen fertilization resulted in a significant increase in the

Table 3

Ammonium nitrogen content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	0.48	0.43	0.42	0.48	0.49	0.64	0.53	0.48	0.54	0.50
N_2	0.52	0.56	0.54	0.62	0.69	0.66	0.73	0.55	0.60	0.61
N_3	0.54	0.60	0.58	0.79	0.83	0.72	0.80	0.60	0.63	0.68
PK Average	0.51	0.53	0.51	0.63	0.67	0.67	0.69	0.54	0.59	0.60
P Average	0.52			0.66			0.61			
K Average				0.61	0.58	0.59				

$LSD_{\leq 0.05}$: N, P, K – 0.01; NP, NK, PK – 0.03; NPK – 0.07

content of this N fraction. The application of a single ammonium nitrate dose increased the average N-NH_4 content to the level of 0.50 g N kg^{-1} d.w.; double and triple doses yielded an increase to 0.61 and 0.68 g N kg^{-1} d.w., respectively. Compared with the single dose, the double and triple doses produced a 22% and 36% increase, respectively, in the content of this nitrogen fraction. Fertilization with the increasing phosphorus doses significantly increased the nitrogen fraction content in timothy grass. The single, double and triple phosphorus doses increased the nitrogen content to 0.52, 0.66, and 0.61 g N kg^{-1} d.w., respectively. In comparison with the single dose, double and triple doses yielded a 26.9 % and 17.3% increase in the element content, respectively. Potassium fertilization caused a slight but significant reduction in the N-NH_4 content. Single, double, and triple doses of this nutrient yielded 0.61, 0.58, and 0.59 g N kg^{-1} d.w. of protein nitrogen, respectively. Compared with the single dose, double and triple doses decreased the content by *ca* 4.9 and 3.3%, respectively. Bednarek (2005) reported that only nitrogen fertilization had a significant effect on the content of this N fraction in sward, which in aerial parts of *Dactylis glomerata* was 27% higher than in roots. Similar regularities were noted by Stuczyński (1969) and Gąsior and Kaniuczak (1996), who reported a slightly higher content of this nitrogen form in hay from swath II than from swath I. Czuba (1996) found that plants are considerably less tolerant to a high content of NH_4^+ rather than to NO_3^- ions, as toxicity symptoms appear in response to as littile as 0.2-0.4 g N-NH_4 kg^{-1} d.w. Potassium deficiency in plants fertilized intensively with nitrogen resulted in excessive accumulation of toxic amounts of amines and ammonium ions in tissues, which induced damage to aerial parts of plants, including plant death (Czuba 1996).

The content of nitrate nitrogen(V) in timothy grass ranged from 0.15 to 0.83 g N kg^{-1} d.w., depending on a dose and form of mineral fertilization (Table 4). Application of single, double, and triple ammonium nitrate doses yielded a content of 0.22, 0.29, and 0.56 g N kg^{-1} d.w., respectively. Compared with the single dose, the relative increase in this nitrogen form produced by the double and triple NH_4NO_3 doses reached 31.8 and as much

Table 4

Nitrate nitrogen(V) content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	0.16	0.16	0.15	0.23	0.48	0.23	0.23	0.18	0.20	0.22
N_2	0.18	0.19	0.22	0.39	0.31	0.26	0.36	0.37	0.29	0.29
N_3	0.39	0.27	0.54	0.64	0.76	0.68	0.66	0.48	0.65	0.56
PK Average	0.25	0.21	0.30	0.42	0.52	0.39	0.42	0.34	0.38	0.36
P Average	0.25			0.44			0.38			
K Average				0.36	0.36	0.36				

$LSD_{\leq 0.05}$: N, P, K – 0.05; NP, NK, PK – 0.09; NPK – 0.19

as 154.5%, respectively. Fertilization with single, double, and triple doses of granular triple superphosphate yielded the N-NO_3 content in timothy grass of 0.25, 0.44, and 0.38 g N kg^{-1} d.w., respectively. In comparison with the single dose, the increase resulting from the double and triple doses reached 76 and 52%, respectively. Fertilization with the increasing doses of potassium salt did not change significantly the content of this nitrogen fraction in plants. It remained on the same level of 0.36 g N kg^{-1} d.w., irrespective of a potassium dose applied. Krzywy et al. (1996) reported a significant increase from 0.62 ($N_0P_0K_0$) to 1.43 g N kg^{-1} d.w. ($N_3P_3K_2$) in this nitrogen fraction in sward. Similarly, Dubiel et al. (1991) found that an increase in nitrogen fertilizer doses was accompanied by an increase in N-$NO_{3,}$ which exceeded the admissible level of 2.2 g N kg^{-1} d.w. in objects fertilized with 480 kg N ha^{-1}. Bednarek (2005) reported that nitrogen fertilization only (and potassium fertilization in roots) exerted a significant effect on the content of this N fraction, exhibiting four-fold lower levels in *Dactylis glomerata* roots than in sward. Application of 80 kg N ha^{-1} $swath^{-1}$ did not made the content of this N fraction exceed the critical threshold (15 g NO_3 kg^{-1} d.w.), whereas after an application of 120 and 160 kg N ha^{-1} $swath^{-1}$ (in total, 600 and 1120 kg N ha^{-1} $year^{-1}$) the level of 15 g NO_3 kg^{-1} d.w. was exceeded by 19 and 47% of samples, respectively (Prins 1983). Accumulation of nitrates in plants was associated not only with the nitrogen content in soil, but also with meteorological factors or with molybdenum, manganese, phosphorus, potassium, and magnesium fertilization (Koter 1968, Czuba 1996, Bednarek, Reszka 2009, Bednarek et al. 2009). Phosphorus fertilization also enhanced the uptake of nitrate N from the environment, whereas elevated potassium fertilization lowered the percentage of nitrates(V) and decreased their share in the total nitrogen absorbed by plants (Koter 1968). Czuba (1996) found that plants could contain up to 15 g N-NO_3 kg^{-1} d.w., and a plant variety is to some extent important in nitrate accumulation. After application of 100 kg N ha^{-1}, different varieties of *Dactylis glomerata* contained from 0.68 to 1.30, meadow fescue from 0.74 to 1.06, and winter ryegrass from 0.56 to 1.11 g N-NO_3 kg^{-1} d.w.

In general, application of increasing doses of nitrogen fertilizers caused a very distinct and, in many cases, significant elevation of nitrate(V) levels in sward (Koter 1968, Stuczyński et al. 1970, 1971, Koter 1973, Stuczyńska 1973, Koter, Krauze 1978, Koter, Krawczyk 1981, Czuba 1996, Gąsior, Kaniuczak 1996, Krzywy et al. 1996, Wiater 1996).

The content of mineral nitrogen (the sum of ammonium nitrogen and nitrate nitrogen V) in timothy grass increased after nitrogen fertilization and reached 0.72 g N kg^{-1} d.w. (N_1); 0.90 g N kg^{-1} d.w. (N_2), and 1.24 g N kg^{-1} d.w. (N_3) – Table 5. The relative increase induced by the double and triple doses was 11.7 and 16.1%, respectively. The application of phosphorus also increased the content of this nitrogen fraction in plants: P_1 – 0.77 g N kg^{-1} d.w., P_2 – 1.10 g N kg^{-1} d.w., and P_3 – 0.99 g N kg^{-1} d.w. The relative increase was 14.3 (P_2) and 12.9% (P_3). The increasing potassium fertilization caused a slight decline in the mineral nitrogen content in timothy grass: K_1 – 0.97 g N

Table 5

Sum of ammonium nitrogen and nitrate nitrogen(V) content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	0.64	0.59	0.57	0.71	0.97	0.87	0.76	0.66	0.74	0.72
N_2	0.70	0.75	0.76	1.01	1.00	0.92	1.09	0.92	0.89	0.90
N_3	0.93	0.87	1.12	1.43	1.59	1.40	1.46	1.08	1.28	1.24
PK Average	0.76	0.74	0.81	1.05	1.19	1.06	1.11	0.88	0.97	0.96
P Average	0.77			1.10			0.99			
K Average				0.97	0.94	0.95				

$LSD_{\leq 0.05}$: N, P, K – 0.06; NP, NK, PK – 0.13; NPK – 0.27

kg^{-1} d.w., K_2 – 0.94 g kg^{-1} d.w. (a 3.1% decrease in the content in comparison with K_1) and K_3 – 0.95 g N kg^{-1} d.w. (a 2,1% decrease in the content).

The percentage of protein nitrogen in total nitrogen declined from 57.1 (N_1) to 52.4% (N_2 and N_3) after nitrogen fertilization. Phosphorus fertilization also resulted in a slight decrease in the share of this N fraction: 55.1 (P_1), 54.2 (P_2), and 51.8% (P_3). Potassium fertilization had an irregular effect on the relative content of protein nitrogen: 54.6 (K_1), 52.9 (K_2), and 54.2% (K_3). Nitrogen and phosphorus fertilization induced an increase while potassium fertilization did not change significantly the content of mineral nitrogen in timothy grass: 3.9 (N_1), 3.7 (N_2), 4.5% (N_3); 3.3 (P_1), 4.6 (P_2), 4.0% (P_3); 4.1 (K_1), 3.9 (K_2), and 4.0% (K_3).

The percentage of ammonium nitrogen in total nitrogen was relatively even and did not depend on the form and dose of mineral fertilization. It reached 2.47-2.52% (nitrogen fertilization), 2.22-2.75% (phosphorus fertilization), and 2.40-2.56% (potassium fertilization). Nitrogen and, to a lesser extent, phosphorus fertilization substantially increased the proportion of nitrate nitrogen(V) in total nitrogen: 1.11 (N_1), 1.18 (N_2), 2.04% (N_3); 1.07 (P_1), 1.83 (P_2), and 1.55% (P_3). Potassium fertilization did not induce significant changes in the proportion of this nitrogen fraction in total nitrogen: 1.51 (K_1), 1.49 (K_2), and 1.51% (K_3). Regardless of forms and doses of mineral fertilizers applied in the experiment, the average share of nitrogen fractions (protein, mineral, ammonium, and nitrate) in total nitrogen content in timothy grass was 53.8% (protein nitrogen), 4.0% (mineral nitrogen), 2.5% (ammonium nitrogen) and 1.5% (nitrate nitrogenV). The percentage of nitrate nitrogen V in mineral nitrogen was 37.5%. The content of the nitrogen fractions analysed indicates that timothy grass is a valuable bulk feed.

CONCLUSIONS

1. The content of nitrogen fractions assessed in timothy grass, i.e. total, protein, mineral, ammonium, and nitrate(V) nitrogen was significantly positively correlated with mineral fertilization, mostly with nitrogen and phosphorus, and to a lesser extent with potassium.

2. The average percentage of protein nitrogen in total nitrogen was 53.8; the values for mineral, ammonium, and nitrate nitrogen were 4.0; 2.5, and 1.5%, respectively.

3. Timothy grass fertilized with increasing doses of nitrogen, phosphorus and potassium contained the highest levels of protein nitrogen in total nitrogen, did not contain excessive amounts of nitrates(V) and proved to be valuable bulk feed.

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