

EFFECT OF MINERAL NITROGEN FERTILIZATION ON PROXIMATE COMPOSITION OF MAIZE GRAIN (*ZEA MAYS* L.)

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Abstract

Maize (*Zea mays* L.) is an important forage, food, and technical crop. Regarding climate change, the efforts of breeders are aimed at developing hybrids with high adaptive and productive potential. Appropriate agrotechnological practices are also important for successful maize cultivation. In intensive cultivation, a lot of mineral fertilizers are used, which are one of the most effective means to increase productivity. Mineral nitrogen fertilization is a prerequisite for higher maize yields. Poorly balanced fertilization is often the cause of severe yield reductions. The present work aimed to study the effect of two types of mineral nitrogen fertilizer (ammonium nitrate and urea) on the chemical composition of the grain of maize, Kneja 561 hybrid grown as a monoculture and after intercropping. The standard growing technology was applied under dry farming conditions (no irrigation). Doses of 60 and 120 kg/ha of nitrogen (active substance) applied as ammonium nitrate and urea at the 6-8 leaf stage of maize were tested, including an unfertilized control. Protein, starch, ash, and dry matter were determined using Near Infrared spectroscopy for analysis. The results showed the chemical composition was improved viz. oil and starch content under mineral nitrogen fertilization, with no statistically proven difference for the two mineral nitrogen fertilizers tested.

Key words: Maize; Nitrogen fertilization; Chemical composition

Introduction

Maize (*Zea mays* L.) is the most productive food crop grown worldwide and ranks third in distribution after wheat and rice (Anon., 2024). It is an important forage and industrial crop. Due to the high nutritional value of its grain, maize is primarily used as feed for farm animals and has strategic importance for livestock production (Szulc & Bocianowski, 2011; Júnior *et al.*, 2020). Grain quality is determined by its chemical composition – protein, oil, starch, ash, and fiber content, which are critical indicators of nutritional value (Singh *et al.*, 2014; Sharma *et al.*, 2022). One of the objectives of breeding is the development of genotypes with a yield and improved basic chemical composition (Božović *et al.*, 2018; Erenstein *et al.*, 2022; Ljubičić *et al.*, 2023). The chemical composition of the grain is important for animal productivity.

Among the most important factors influencing biochemical processes and plant productivity in general, and maize in particular, is mineral nitrogen fertilization (Mashele & Sebetha, 2024; Jiang *et al.*, 2025). Nitrogen fertilization affects both yield and grain quality traits, including protein content, starch accumulation, and nitrogen use efficiency (Yang & Li, 2024). Recent studies have highlighted that precision nitrogen management, genotype-specific responses, and integrated fertilization strategies can enhance NUE while improving maize grain quality (Jan *et al.*, 2025).

The forms of nitrogen (ammonium nitrate, amide) are absorbed by plants differently depending on agroecological conditions, technology of cultivation, and other factors (Popović *et al.*, 2013a, 2013b; Ludewig *et al.*, 2021; Rakašćan *et al.*, 2021; Rajičić *et al.*, 2020; Sharma *et al.*, 2022; Tewari, 2022).

The aim of the present study is to evaluate the effect of two types of mineral nitrogen fertilizer (ammonium nitrate and urea) on the chemical composition of maize grain grown as a monoculture and after intercropping.

Materials and Methods

The experimental work was carried out at the experimental field of the Maize Research Institute – Kneja, Bulgaria, on a typical chernozem soil type under dry farming conditions. Before the experiment, the soil had the following agrochemical characteristics (depth 0-20 cm): alkaline-hydrolyzable nitrogen, 31.2 mg/1000 g soil; phosphorus (P₂O₅), 2.3 g/100 g soil; potassium (K₂O), 4.98 g/100 g soil; pH, 5.98. Alkaline-hydrolyzable nitrogen was determined according to Kornfield (mg/1000 g soil); phosphorus - Specol 11, according to the Egner Reim method (% in 100 g soil), potassium - flame photometer (% in 100 g soil), pH - pH meter ORION 211.

The study was conducted with the hybrid Kneja 561, developed at the Institute through heterosis breeding by hybridization of genetically distant stabilized self-pollinated maize lines (Yordanov, 2019). A standard maize cultivation

technology was applied, both under monoculture and after intercropping. The plant density in both cultivation systems was 53000 plants per hectare.

For the intercropping treatments, a pea: oat mixture (3:1 ratio) was sown in August of the preceding year. The pea–oat mixture was used as a preceding cover crop to improve soil fertility and nutrient cycling before maize cultivation. Pea contributes biologically fixed nitrogen, while oat enhances biomass production and soil structure. The incorporation of residues was expected to influence nitrogen availability during maize growth, potentially affecting nitrogen uptake and grain protein and starch accumulation. Residues were incorporated before maize planting. Two mineral nitrogen fertilizers were tested: ammonium nitrate (NH_4NO_3) and urea ($\text{CH}_4\text{N}_2\text{O}$), applied at rates of 60 and 120 kg ha^{-1} of active substance, during the 6–8 leaf stage of maize. These rates represent local recommendations. The control treatment received no nitrogen fertilization.

The size of each experimental plot was 10 m^2 , with four replications per treatment. Randomized block design of trial was used.

Overall, weather conditions during the maize growing season were highly unfavorable, characterized by elevated average and maximum monthly temperatures and insufficient precipitation in June, July, and August. Nevertheless, rainfall in May and substantial precipitation events in late June and early July partially mitigated drought stress and supported grain formation, although final grain productivity remained low (Table 1).

Maize was harvested at full physiological maturity when grain moisture reached the standard level of 14%. Random plants were selected. Grain samples of 200 g were collected from each replication of every treatment.

Near Infrared spectroscopy (SupNIR-2700 Near-infrared Analyze, China) was used to determine the main chemical composition of the grain (protein content, starch, oil, ash, fiber) (% of dry matter). Kernel samples of 200 g were ground out with 0.5 mm sieve using a laboratory mill.

The analysis was performed on mature grain. Average values, standard deviation (SD) were determined. The coefficient of variation (CV%) was used to determine the variability of the indicators studied (Dimova & Marinkov, 1999). The experimental data were subjected to analysis of variance (ANOVA) to evaluate the effects of nitrogen form, nitrogen rate, and cultivation system on grain chemical composition. Statistical analyses were performed using SPSS 20.0 software.

Results and Discussion

The qualitative parameters of maize grain were evaluated, namely protein, oil, starch, fiber, ash, and dry matter content. NIR spectroscopy was used as a reliable method for rapid analysis of protein, oil, starch, and other components.

The analysis of the chemical composition data of maize grain grown under monoculture conditions revealed that, for all studied parameters except for oil and fiber, an increase in their values was observed under nitrogen fertilization. The protein content varied from 9.97 to 10.18% (Table 2).

In the studies of Pushpam *et al.*, (2015), Olowookere *et al.*, (2017), Pencheva & Sabeva (2019), the content of crude protein in maize was found between 9.0 to 12.93%, respectively. Abou-Deif *et al.* (2012) studied protein content in grains of the 14 maize inbred lines and found protein from 8.34% to 11.60%. In the studies of Koteva & Marcheva (2012) and Popović *et al.*, (2024), relatively lower values were reported for protein content (about 8.0%) and oil content (about 3.0%) in maize grain. Our results are consistent with those of Uarrotta *et al.*, (2013) and Mashele & Sebetha (2024). Maize is commonly regarded as energy fodder and contains less protein in comparison with basic cereal grains. However, because of high maize grain yield, protein yield per area unit may equal or even exceed protein yield of forage crops (Szmigiela *et al.*, 2013). Protein and starch content are considered key indicators of grain quality (Sharma *et al.*, 2022).

Szmigiela *et al.*, (2013) found N fertilization resulted in an increase in protein content. Protein concentrations in the Szmigiela *et al.*, (2013) own research were higher than the values reported in literature, which was due to cultivation in a very good soil (degraded chernozem formed from loess). These, as well as our results, indicate that although protein content depends on the genetic traits of the plant, it is also influenced by other factors, in this case, mineral nitrogen fertilization.

Table 1. Temperature and rainfall for the period of study.

Months	T max, °C	T min, °C	T av., °C	Rainfall, mm
April	22.3	7.0	14.3	35.0
May	22.4	13.3	16.3	82.7
June	32.5	16.9	24.9	25.6
July	34.9	17.3	26.1	42.3
August	34.4	16.5	26.0	2.1

Table 2. Basic chemical composition of maize grown as a monoculture, % of DM.

Variants kg ha^{-1}	Protein	Oil	Starch	Ash	Fiber	DM
	% of DM					
Control N 0	10.14	4.14	67.77	1.48	2.50	91.34
$\text{CH}_4\text{N}_2\text{O}$ 60	9.97	3.90	67.62	1.39	2.44	91.43
$\text{CH}_4\text{N}_2\text{O}$ 120	10.17	4.11	67.91	1.57	2.50	91.43
NH_4NO_3 60	10.18	3.87	68.29	1.47	2.45	91.34
NH_4NO_3 120	10.17	4.01	68.04	1.54	2.43	91.30
Average	10.13	4.01	67.93	1.49	2.46	91.37
CV%	0.87	3.02	0.38	4.67	1.36	0.06
St.dev	0.09	0.12	0.26	0.07	0.03	0.06

ns, Not significant at $p \leq 0.05$

Table 3. Basic chemical composition of maize grown in intercropping, % of DM.

Variants kg ha^{-1}	Protein	Oil	Starch	Ash	Fiber	DM
	% of DM					
Control N 0	9.88	4.36	68.29	1.42	2.51	91.25
$\text{CH}_4\text{N}_2\text{O}$ 60	9.86	4.23	67.96	1.44	2.42	91.09
$\text{CH}_4\text{N}_2\text{O}$ 120	10.19	3.95	68.03	1.43	2.41	91.02
NH_4NO_3 60	10.19	4.14	67.77	1.47	2.43	91.16
NH_4NO_3 120	9.64	3.99	68.81	1.49	2.44	91.43
Average	9.95	4.13	68.17	1.45	2.44	91.19
CV%	2.38	4.10	0.59	2.01	1.62	0.17
St.dev	0.24	0.17	0.40	0.03	0.04	0.16

ns, Not significant at $p \leq 0.05$

The oil content of the maize kernel comes mainly from the germ and depends on the hereditary predisposition of the plant and is little influenced by the growing conditions. Regarding oil content in our study, it was found from 3.87 to 4.14% and was not influenced by fertilization. Moreover, the coefficient of variation (CV% 3.02) was considerably higher compared to that of protein content (CV% 0.87). Pencheva & Sabeva (2019) found oil content ranging from 3.7 to 4.7%, which is confirmed by our study. Abou-Deif *et al.*, (2012) reported significantly higher oil content in maize grain. In the present study oil content in maize inbred line grains ranged from 7.67 to 11.56%. Higher content of oil in the maize grain was also reported by Sangeeta & Grewal (2018).

The major chemical component of the maize kernel is starch, which provides up to 72 to 73% of the kernel weight. Starch content is also primarily determined by genotype, but it can be influenced by environmental conditions (Terziev *et al.*, 2001a, 2001b). Starch is an extremely important indicator of the nutritional composition of maize grain. In our study, starch content varied from 67.62% to 68.29%. It was significantly higher than the control under mineral nitrogen fertilization, with no proven difference between the two experimental doses. The coefficient of variation was very low (CV% 0.38). Our results regarding changes in starch content were consistent with those reported by Egesel & Kahriman (2012).

Regarding ash and fiber content, at the higher experimental dose of both mineral nitrogen fertilizers (ammonium nitrate and urea), ash content was increased, with the highest coefficient of variation observed for this parameter (CV% 4.67).

Fiber content was not affected by fertilization and varied from 2.43 to 2.50%.

Koteva & Marcheva (2012) reported lower values for ash and fiber content in maize grain.

When ammonium nitrate was applied, there was a tendency for higher protein and starch content in the grain compared to urea. With urea application, protein content was lower, while oil and fiber content were relatively higher.

According to ANOVA, nitrogen fertilization significantly increased protein and starch content compared to the unfertilized control ($p \leq 0.05$). However, no statistically significant differences were found in type and dose of fertilizers, nor between the two nitrogen rates (60 and 120 kg ha⁻¹). The interaction between nitrogen form and dose was also not significant ($p > 0.05$). These results indicate comparable agronomic performance of the two nitrogen sources with respect to grain quality parameters under dry farming conditions.

In treatments with a cover crop, more pronounced differences were observed. Protein content in the grain varied from 9.64 to 10.19% and was influenced by mineral nitrogen fertilization (Table 3). On average, protein content was lower compared to maize grown in monoculture. Oil content ranged from 3.95 to 4.36%, being highest in the control treatment, with the coefficient of variation also the highest (CV% 4.100). Starch content (mean 68.17%) exceeded in monoculture (67.93%). The highest starch content was observed at the higher dose of ammonium nitrate (68.81%). A similar trend was noted for ash content (1.49%).

Fiber and oil content were not affected by mineral nitrogen fertilization, with the highest values observed in the control treatment.

When ammonium nitrate was applied, protein content reached up to 10.19%, and starch content exceeded 68%. When urea was used, particularly at the higher experimental dose, protein values were more favorable, but overall, the results exhibited greater variability. Starch, ash, and dry matter content were highest when ammonium nitrate was applied at a dose of 120 kg ha⁻¹.

Comparing the chemical composition of maize grain grown in monoculture and after a cover crop (Fig. 1), it is evident that variation in parameter values was higher under cover crop treatments, as follows: protein content (CV% 2.379 vs. CV% 0.874), oil (CV% 4.100 vs. CV% 3.019), starch (CV% 0.590 vs. CV% 0.378), fiber (CV% 4.674 vs. CV% 2.011), and dry matter (CV% 0.174 vs. CV% 0.064).

Two-way ANOVA (nitrogen form \times nitrogen rate) performed for maize grown in intercropping showed that nitrogen fertilization significantly increased grain protein content compared to the unfertilized control ($p \leq 0.05$). However, no statistically significant differences were observed between urea and ammonium nitrate treatments, nor between the two nitrogen rates (60 and 120 kg ha⁻¹). No significant interaction between nitrogen form and dose was detected ($p > 0.05$). Other grain quality parameters (fat, starch, ash, fiber and dry matter) were not significantly affected by nitrogen form or rate.

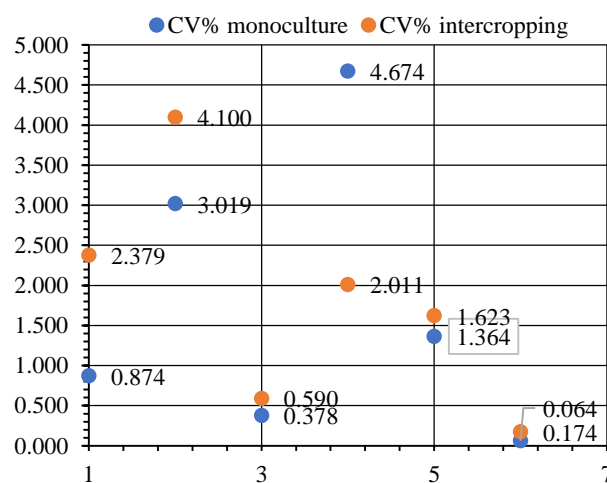


Fig. 1. Coefficient of variation for the basic chemical composition of maize grain grown in monoculture and in intercropping. (1– protein, 2– oil, 3– starch, 4– ash, 5– fiber, 6– dry matter).

In the control treatments without fertilization, under both cultivation systems, relatively lower protein content values were recorded, demonstrating the positive effect of mineral nitrogen fertilization on grain quality, as also reported in another study on maize for silage (Vasileva *et al.*, 2025).

The differences between the doses (60 and 120 kg ha⁻¹) were insignificant.

Although the differences between ammonium nitrate and urea were not statistically significant, the comparison remains agronomically relevant, as these fertilizers are the most widely used nitrogen sources in European maize production. Even small differences in performance may influence farmer

decisions under practical field conditions. From an economic perspective, urea often provides a lower cost per unit of nitrogen, whereas ammonium nitrate may offer more immediate nitrogen availability. Therefore, even small yield differences can have economic implications at farm scale.

Environmental considerations further justify the comparison. Urea is associated with higher ammonia volatilization losses under certain conditions, while ammonium nitrate may contribute to nitrate leaching. Understanding their relative performance supports sustainable nitrogen management strategies.

From a feed industry perspective, maize grain with protein content ranging between 9–10% is considered within the normal commercial quality range for energy-dominant feed ingredients. Therefore, the comparable protein levels observed across nitrogen forms and rates indicate that both urea and ammonium nitrate provide sufficient nitrogen supply to maintain standard feed quality.

Importantly, the absence of significant differences suggests that increasing nitrogen rate from 60 to 120 kg ha⁻¹ does not substantially improve grain nutritional value. This finding has practical implications, as lower nitrogen input may reduce production costs and environmental nitrogen losses without compromising feed quality.

Considering that feed maize is primarily valued for its energy contribution (starch content) rather than protein concentration, the stability of starch across treatments further confirms that nitrogen source selection had minimal influence on overall feed value.

Overall, the chemical composition of the grain was more favorable with ammonium nitrate fertilization, resulting in higher protein and starch content - key indicators of the nutritional value of maize.

Conclusions

Mineral nitrogen fertilization significantly improved maize grain protein and starch content compared with the unfertilized control under dry farming conditions. However, increasing nitrogen rate from 60 to 120 kg ha⁻¹ did not result in additional significant improvements in grain chemical composition, indicating that moderate nitrogen inputs are sufficient to maintain standard feed quality. No statistically significant differences were observed between ammonium nitrate and urea, although ammonium nitrate showed a slight tendency toward higher protein and starch values. The comparable performance of the two nitrogen sources suggests flexibility in fertilizer selection based on economic and environmental considerations. Intercropping increased variability in grain quality parameters, emphasizing the importance of considering cropping system effects in nitrogen management strategies. Overall, the study contributes field-based evidence that optimized nitrogen rates can sustain maize grain nutritional quality while potentially reducing unnecessary nitrogen inputs, production costs, and environmental risks.

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