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INFLUENCE ON THE PROTEIN AND STARCH CONTENT OF MAIZE (*Zea Mays* L.) OF INTEGRATED FERTILIZATION WITH GREEN MANURE AND MINERAL FERTILIZERS

Ionel Alin GHIORGHE[1,2], Adrian TUREK-RAHOVEANU[1]

[1]University of Agricultural Sciences and Veterinary Medicine Bucharest, 59 Marasti, District 1, 11464, Bucharest, Romania; E-mail: turek.adrian@managusamv.ro
[2]Agricultural Research and Development Station of Braila, Viziru Street km 9, Brăila. Romania; E-mail: alin.ghiorghe@scdabraila.ro

Corresponding author: turek.adrian@managusamv.ro

Abstract

Through this study, we aimed to test the variability of protein and starch content of maize when applying an integrated fertilization system using green manure and mineral fertilizer. The research was conducted under specific conditions at the Agricultural Development Research Station of Braila, where a bifactorial experiment was set up to investigate the effects of cultivation and incorporation of green manures and nitrogen fertilizers on the quality traits of maize yield. The green manure species were carefully chosen and were represented by species such as winter pea, rye, white mustard, rapeseed, and control without green manure. Nitrogen fertilizers were applied at rates of 60 kg, 90 kg, and 120 kg nitrogen per hectare. Based on the results obtained in this study, we can state that integrated fertilization with green and mineral fertilizers influences the protein and starch content of maize. The results revealed that the dose of 120 kg/ha nitrogen together with green winter pea fertilizer had the highest influence on protein content, the maximum level being 12.28%, and in the case of starch, the highest content was recorded at the application of 90 kg/ha nitrogen and green winter pea fertilizer with a percentage of 72.83%. The results reported in this study are the average results obtained in two years of research.

Key words**:** *maize, green manure crops, mineral fertilization, protein content, starch content*

INTRODUCTION

Maize is a nutrient-intensive crop due to its high dry matter and grain production. One of the most important factors contributing to increased production is fertilization. Fertilization influences the morphological and physiological characteristics of maize plants. The application of mineral fertilizers, especially slow-release and organic fertilizers can improve all characteristics and performance of the maize crop [10].

The use of organic and mineral fertilizers aims at sustainable nutrient management, practicing sustainable agriculture all with the aim of meeting human needs and also protecting soil and environmental resources. [9].

The combined use of organic and mineral fertilizers aims to improve the yield and better absorption of nutrients from the soil and also to increase the protein and oil content of maize kernels and basically through this fertilization practice an optimization of maize yield is achieved, with influences on the nutrient composition and levels of protein, total soluble sugars, starch and total carbohydrates in the kernels [6].

Present strategies for global food security are deceiving farmers into utilizing excessive inputs for crop productivity. The indiscriminate application of synthetic fertilizers, herbicides, and insecticides is degrading soil properties and yield-related factors. Green manuring has developed as a cost-effective alternative for farmers to address this issue, replacing artificially synthesized chemical substances. Green manure can fulfil all the requirements (physical, chemical, biological, pathological) of plants from germination to fruiting [3].

Integrated fertility management technologies are effective in increasing maize yield under low rainfall conditions. Higher yields can be obtained if more fertilizer is used, but there is

not a directly proportional relationship between fertilization and yield [14].

Fertilization has a significant influence on grain yield and quality, starch and protein content, and the health of the maize crop. Moreover, it maximizes the yield potential of the tested genotypes but in favorable years [2]. A practical strategy for optimizing both yield and quality may be to select hybrids with the best agronomic suitability and then use precise nitrogen management practices to optimize grain yield. Studies are needed to evaluate the potential of variable nitrogen application for corn quality parameters in fields with different soil and field conditions and the potential of variable hybrid selection [12].

Green manuring is a method that can be employed to improve the soil's characteristics, leading to increased productivity. Green manuring is a method used to introduce organic matter into the soil. Recently, it has been hypothesized that the main impact of green manures on improving soil quality is their potential to raise levels of soil organic matter and enhance nutrient availability. Green manuring is an excellent choice for improving soil health and meeting the nutritional requirements of future crops. Integrating green manure crops into the soil can effectively prevent nitrogen loss [8].

Growing green manure is a promising way to protect N in off-seasons and reduce fertilizer input in corn production. Due to high levels of organic and inorganic N dissolved through incorporation and increased nutrient uptake into the soil, recommended fertilizer inputs for corn could be reduced by 15-30%. Green manure-corn rotation could be very promising in replacing some inorganic fertilizers without sacrificing crop yield [17].

Different combinations of green manures with legumes have been shown to influence the chemical composition of maize grains. The nitrogen accumulation capacity of each genotype depends on the efficiency of nitrogen utilization and accumulation by the plant. The application of legume biomass increased the content of most minerals. Fiber and starch showed low values, which were compensated by increased protein content and maize grain quality improve [5].

Another essential role of green manure use is the change in the composition and functionality of soil microbial activity which positively influences nitrogen assimilation by the main crops and increases their yield [11].

The application of N between 50-100 kg N ha has the potential to boost the yield. This suggests that N treatment can result in increased grain yield, as well as higher protein and starch contents. Moreover, by applying an appropriate quantity of nitrogen fertilizer, it is possible to enhance the overall protein and starch levels in maize, hence increasing its nutritional value and elevating its significance in agriculture [13].

Nitrogen fertilizer application increases the protein content of maize seeds. Nitrogen application also increases the crude protein content of maize seeds up to 100 kg N ha. Maize seed protein and starch depend on the conditions of the crop year, which is influenced by weather changes [15].

Széles et al. (2018) [16], through their research carried out between 2011-2016, revealed that different levels of nutrient deficiency or excess nutrient supply, inadequate nitrogen, potassium, and phosphorus balance, and environmental stress factors cause disturbances in nutrient supply in plants. As a result, yield decreases and quality declines. A 73% increase in yield can be obtained with an adequate nutrient supply (120:92:108 kg NPK ha) compared to the non-fertilized variant. Adequate protein content resulted from the 150:115:135 NPK ha treatment, and the growth rate was 17.7% [16].

Research conducted by Chitu et al. from 2018 to 2023 in the field of emerging agro-technical technologies concluded that efficient nitrogen management can significantly improve crop yields. Balancing nitrogen application and actual crop requirements, taking into account soil, weather conditions, and crop variety, is essential to maximize nitrogen use efficiency [4].

Therefore, this study aims to evaluate the efficacy of several types of green manures and

nitrogen fertilizer levels on maize yield quality, especially protein and starch content as a result of tests carried out under the conditions at ARDS Braila in the North-East Baragan.

MATERIALS AND METHODS

Field experiment was carried out in the 2022 and 2023 growing seasons on vermic chernozem soil with a medium humus content of 2.4 - 3.1% in the upper horizons and only 1.6% in the transition horizon, 0.14-0.25 % total nitrogen content at the trial site of Agricultural Research and Development Station (ARDS) Braila - Chiscani Experimental Center.

The experiment was designed in fully randomized blocks with 4 replicates.

The experimental factors are as follows:

Factor A- Green manure crops

a_1– control – without green mure crop

a_2 - winter pea (*Pisum sativum* L. var.arvense.)

a_3- white mustard (*Sinapis alba* L)

a_4- winter rye (*Secale cereale* L.)

a_5- white mustard (*Sinapis alba* L) + rapeseed (*Brassica napus* L.)

a_6- rapeseed (*Brassica napus* L.)

Factor B – Mineral fertilization

b_1– N_0 unfertilized

b_2- N_{60} (60 kg/ ha of N)

b_3- N_{90} (90 kg/ ha of N)

b_4- N_{120} (120 kg/ ha of N).

The size of each test plot was 42 m^2 and the total surface area of the research plot was 4,032 m^2.

Green manure cultivation was conducted in early September for both years of study. The green manure was severed and integrated into the soil based on species: mustard before the commencement of winter, and winter pea and rye species were severed and integrated in the spring, approximately one month before corn sowing.

Mineral fertilization involved the application of a 15:15:15 complex NPK fertilizer concurrently with seedbed preparation, while fractional dosages of urea were administered throughout the maize growing season. Consequently, N doses of 60, 90, and 120 kg/ha, along with an agro-foundation of 40 kg/ha of phosphorus and 40 kg/ha of potassium, were administered for all experimental variants.

Maize was planted on May 4, 2022, and May 5, 2023, using the F423 hybrid at a density of 65,000 plants per hectare, with harvesting conducted in the second decade of October in both 2022 and 2023 [7]. Throughout the growing season, treatments for weed and pest management were administered, and the maize was irrigated throughout the two-year experiment..

For this paper, corn yield quality results were followed, and protein and starch contents were evaluated. A granolyser grain analyzer from PFEUFFER was used to determine protein and starch indices. The statistical analyses included analysis of variance and Fisher's least significant differences test (LSD), using the Polifact statistical software [1].

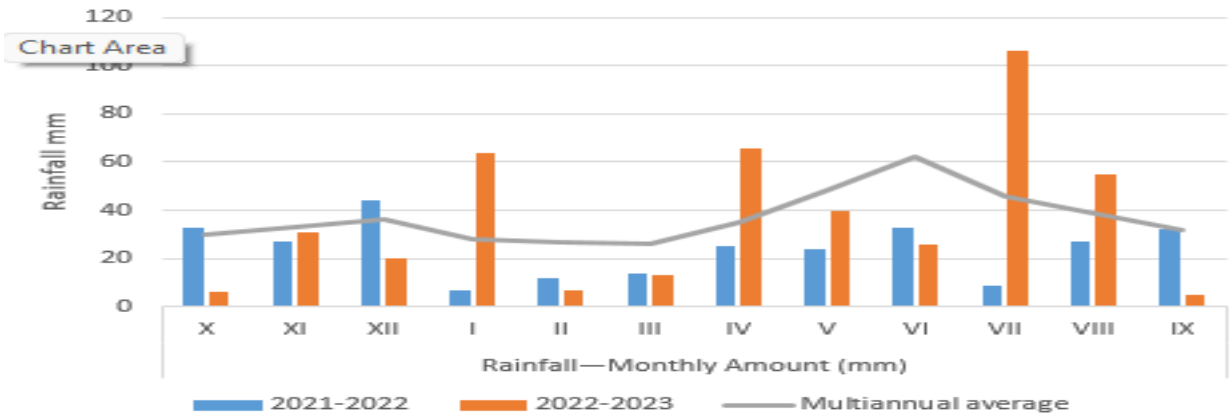

Fig. 1. Rainfall regime from 2021 to 2023 at the ARDS Braila
Source: Meteorological Stations Braila [18].

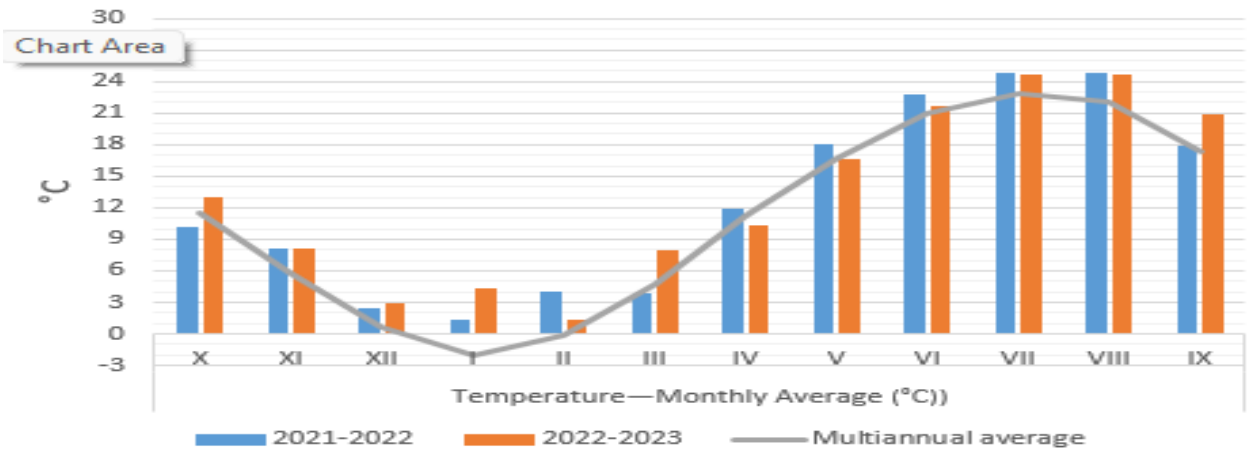

Fig. 2. Thermal regime from 2021 to 2023 at the ARDS Braila
Source: Meteorological Stations Braila [18].

RESULTS AND DISCUSSIONS

ARDS Brăila is situated in the eastern region of Northern Bărăgan, within one of Romania's driest agricultural zones. The region of Northern Bărăganului de Nord, like the entire Romanian Plain, exhibits a temperate continental climate. Summers are characterized by high temperatures and aridity; precipitation is minimal, intense, and irregularly dispersed. The climatic data presented in this research is sourced from the Meteorological Station of Brăila, situated near the Experimental Center Chiscani. The multiannual temperature average is 10.9°C, while the annual precipitation average is 442 mm.

During the agricultural year 2021-2022, the mean temperature documented was 12.5°C. This year experienced a mean annual temperature difference of +1.6°C from the multi-year average. Moreover, it was an extraordinarily arid year, exhibiting a negative divergence of 155 mm from the normative precipitation levels. Summer 2022, with an average temperature of 24.1^{0}C exceeded the multi-seasonal multi-year average by 2.1^{0}C being very warm. June recorded a positive deviation of 1.8^{0}C, July a positive deviation of 1.9^{0}C and August a positive deviation of 2.8^{0}C . In terms of rainfall, summer 2022 started with June providing 33.3 mm of water, 28.7 mm below the multi-year average of 62 mm, July recorded only 8.9 mm with a deficit of 37.1 mm and August recorded 26.9 mm and a deficit of 12.1 mm. September was normally supplied with precipitation (32 mm).

The 2022-2023 crop year experienced elevated temperatures, with an average of 13.1°C, above the multi-year normal by 2.2°C. The rainfall was nearly average, totalling 439 mm, while the distribution of precipitation was varied. The air temperature conditions in the summer of 2023 were quite challenging. The monthly mean temperature values recorded positive deviations from the normal averages for this period, ranging from 0.7 to 2.6 °C. September was also extremely warm, with a positive deviation of 3.6°C.

In terms of precipitation, the summer of 2023 started with a deficit of 36 mm in June but followed a period of good water supply from precipitation, with July providing a water supply of 106 mm, 60 mm above the multi-year monthly average. August provided 55 mm of precipitation.

Protein content

The Romanian maize hybrid Fundulea 423 was the subject of this research, which has very good grain quality, as follows: protein content: 11.0-11.8% and starch content: 70.5-72.0% [16].

Table 1 shows the average results obtained in 2021-2022 and 2022-2023 on the influence of green manure fertilization on protein content. The level of protein content in maize grains ranged from 11.03 to 11.74 % the highest value was recorded by incorporation of mustard as green manure. The differences were statistically ensured against the control for the mustard and winter pea variant..

Table 1. Protein content under the influence of fertilization with green manure. Average results 2022-2023

Green manure crop	Protein		Differences		Significance
	%	%	%	%	
a1- control	11.03	100.0	Mt.	-	
a2- winter pea	11.71	106.1	0.67	6.1	**
a3- white mustard	11.74	106.4	0.71	6.4	**
a4- winter rye	11.38	103.2	0.35	3.2	-
a5 white mustard + rapeseed	11.31	102.5	0.27	2.5	-
a6- rapeseed	11.36	103.2	0.33	3.0	-
LSD (5%)= 0.45 %; LSD (1%)=0.62 %; LSD (0.1%)=0.85 %					

Source: Results of the own experiments.

The results on mineral fertilization (Table 2) show the influence of nitrogen fertilization on protein content, which increased progressively with increasing nitrogen doses. The highest level of protein content was obtained when applying mineral fertilization with 120 kg/ha nitrogen.

Table 2. Protein content under the influence of fertilization with mineral fertilizers. Average results 2022-2023

Mineral fertilization	Protein		Differences		Significance
	%	%	%	%	
b1– $\boldsymbol{N_0}$ Unfertilized	11.00	100.0	Mt.	-	
b2- $\boldsymbol{N_{60}}$ (60 kg/ ha s.a. N)	11.27	102.5	0.27	2.5	*
b3- $\boldsymbol{N_{90}}$ (90 kg/ ha s.a. N)	11.56	105.0	0.55	5.0	***
b4- $\boldsymbol{N_{120}}$ (120 kg/ ha s.a N)	11.86	107.8	0.86	7.8	***
LSD (5%)= 0.24 %; LSD (1%)=0.32 %; LSD (0.1%)=0.41%					

Source: Results of the own experiments.

From the analysis of the interaction of the two studied factors (Table 3), it can be observed that the interaction of nitrogen dose gradations on green manure agrofounds is not very high.

Table 3. Protein content under the influence of integrated fertilization. Average results 2022-2023

Mineral fertilization	Green manure crop								
	a1- control			a2- winter pea			a3- white mustard		
	Prot. %	Diff.	Sign.	Prot. %	Diff.	Sign.	Prot. %	Diff.	Sign.
b1– $\boldsymbol{N_0}$	10.40	Mt.	-	11.38	Mt.	-	11.25	Mt.	-
b2- $\boldsymbol{N_{60}}$	10.93	0.52		11.50	0.13		11.65	0.40	
b3- $\boldsymbol{N_{90}}$	11.03	0.63	*	11.68	0.30		11.78	0.52	
b4- $\boldsymbol{N_{120}}$	11.78	1.38	***	12.28	0.90	**	12.28	1.03	***
	a4- winter rye			a5 white mustard + rapeseed			a6- rapeseed		
	Prot. %	Diff.	Sign.	Prot. %	Diff.	Sign.	Prot. %	Diff.	Sign.
b1– $\boldsymbol{N_0}$	10.80	Mt.	-	11.05	Mt.	-	11.13	Mt.	-
b2- $\boldsymbol{N_{60}}$	11.28	0.48		11.20	0.15		11.08	-0.05	
b3- $\boldsymbol{N_{90}}$	11.50	0.70	*	11.58	0.53		11.78	0.65	*
b4- $\boldsymbol{N_{120}}$	11.95	1.15	***	11.40	0.35		11.48	0.35	
LSD (5%)= 0.59 %; LSD (1%)=0.78 %; LSD (0.1%)=1.02 %									

Source: Results of the own experiments.

For mineral fertilization level b1- N_0 the interaction with the a2- winter pea variant recorded the highest protein content level, 11.38%.For the mineral fertilization level b2- N_{60} the interaction with a3- white mustard had the highest protein content level, 11.65%.

For the mineral fertilization level b3- N_{90} the interaction with a3- white mustard a6- rapeseed had the highest protein content level, 11.78%.

For mineral fertilization level b4- N_{120} the interaction with a2- winter pea and a3- white mustard had the highest protein content level, 12.28%.

Starch content

Table 4 shows the average results recorded in the crop years 2021-2022 and 2022-2023 on the influence of green manure fertilization on starch content. The values of starch content ranged from 70.86% in the control variant to 72.37% in variant a2. Differences from the control were highly significant for variant a2, distinctly significant for variant a4.

Table 4. Starch content under the influence of fertilization with green manure. Average results 2022-2023

Green manure crop	Starch		Differences		Signifiance
	%	%	%	%	
a1- control	70.86	100.0	Mt.	-	-
a2- winter pea	72.37	102.1	1.51	2.1	***
a3- white mustard	71.22	100.5	0.36	0.5	
a4- winter rye	71.95	101.5	1.09	1.5	**
a5 white mustard + rapeseed	71.03	100.2	0.17	0.2	
a6- rapeseed	71.64	101.1	0.79	1.1	*
LSD (5%)= 0.76 %; LSD (1%)=1.05 %; LSD (0.1%)=1.45 %					

Source: Results of the own experiments.

From the analysis of the interaction of the two studied factors (Table 5), it can be observed that the interaction of nitrogen dose gradations on green manure agrofoundations under the conditions of crop years 2021-2022 and 2022-2023 are higher when 120 kg/ha nitrogen dose is used.

Table 5. Starch content under the influence of integrated fertilization. Average results 2022-2023

Mineral fertilization	Green manure crop								
	a1- control			a2- winter pea			a3- white mustard		
	Starch. %	Diff.	Sign.	Starch %	Diff.	Sign.	Starch %	Diff.	Sign.
b1– N_0	70.13	Mt.	-	71.93	Mt.	-	70.77	Mt.	-
b2- N_{60}	70.90	0.78		72.15	0.22		71.03	0.25	
b3- N_{90}	70.85	0.72		72.83	0.90		71.23	0.45	
b4- N_{120}	71.55	1.42	**	72.58	0.65		71.85	1.08	*
	a4- winter rye			a5 white mustard + rapeseed			a6- rapeseed		
	Starch %	Diff.	Sign.	Starch %	Diff.	Sign.	Starch %	Diff.	Sign.
b1– N_0	71.35	Mt.	-	70.50	Mt.	-	70.65	Mt.	-
b2- N_{60}	71.83	0.47		70.98	0.47		71.93	1.28	*
b3- N_{90}	72.33	0.97		71.13	0.63		71.73	1.08	*
b4- N_{120}	72.30	0.95		71.50	1.00		72.25	1.60	***
LSD (5%)= 0.98 %; LSD (1%)=1.31 %; LSD (0.1%)=1.71 %									

Source: Results of the own experiments.

The other gradations influenced less the starch content. For the b1- N_0 mineral fertilization grade the interaction with the a2- green manure variant a2- green manure fall pea had the highest starch content level, 71.93%.
For mineral fertilization level b2 - N_{60} the interaction with a2- green manure variant green manure autumn pea had the highest starch content level, 72.15%.
For the mineral fertilization level b3- N_{90} the interaction with variant a2- green manure green manure autumn pea had the highest starch content level, 72.83%.
For the mineral fertilization level b4- N_{120} the interaction with variant a2- green manure green manure autumn pea had the highest protein content level, 72.58% (Table 5).

Table 6. Starch content under the influence of fertilization with mineral fertilizers. Average results 2022-2023

Mineral fertilization	Starch		Differences		Signifiance
	%	%	%	%	
b1– N_0 nefertilizat mineral	70.89	100.0	Mt.	-	
b2- N_{60} (60 kg/ ha s.a. N)	71.47	100.8	0.58	0.8	**
b3- N_{90} (90 kg/ ha s.a. N)	71.68	101.1	0.79	1.1	***
b4- N_{120} (120 kg/ ha s.a N)	72.00	101.6	1.12	1.6	***
LSD (5%)= 0.40 %; LSD (1%)=0.54 %; LSD (0.1%)=0.70%					

Source: Results of the own experiments.

The results on mineral fertilization (Table 6) attest to the influence of nitrogen fertilization on starch content, which increased progressively with the nitrogen doses. The highest level of starch content was obtained when applying mineral fertilization with 120 kg/ha nitrogen.

CONCLUSIONS

The study conducted and the results obtained confirm that integrated fertilization contributes to the improvement of maize yield quality. Thus in terms of protein content of maize yield it can be concluded that the level of mineral fertilization b4- in interaction with variant a2- winter pea green manure and a3- white mustard green manure recorded the highest level of 12.28%. From the analysis of the influence of integrated fertilization with green and mineral fertilizers on the starch content of maize yield, it can be concluded that the level of mineral fertilization b3- N_{90} interaction with variant a2- green manure winter pea green manure recorded the highest level of starch content, 72.83%.

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