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Evaluation of the Effectiveness of NBPT and NPPT Application as a Urease Carrier in Fertilization of Maize (*Zea mays* L.) for Ensiling

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Abstract: The study presents the results of a 3-year field trial aimed at assessing the yield and quality of raw material for ensiling in the cultivation of three maize varieties differing in their agronomic and genetic profile, conditioned by the selection of nitrogen fertilizer. Maize cultivar ES Metronom showed a significant advantage over other cultivars when fertilized with UltraGrain stabile, or alternatively Super N-46. The application of nitrogen-stabilized fertilizers or urea + N-Lock significantly increased the yield of maize green fodder for ensiling. The "stay-green" maize cultivars were characterized by a higher content of non-structural carbohydrates, including starch and water-soluble sugars, and a lower content of structural carbohydrates, compared to the conventional cultivar, which increased their suitability for ensiling. The negative effect of maize fertilization with ammonium nitrate and ammonium nitrate + N-Lock on the chemical composition of green fodder was demonstrated by a reduced starch content and increased structural carbohydrate contents, including crude fiber and NDF. In turn, the positive effect of maize fertilization with urea and urea + N-Lock on the chemical composition of maize fodder was shown by increased starch content and reduced structural carbohydrate contents, including crude fiber and its NDF and ADF fractions. The analysis of the number and weight of leaves may indicate a highly effective utilization of nitrogen ("stay-green" maize hybrids), leading to the faster formation of leaves with a larger assimilation surface, which is the basis for the efficient absorption of solar radiation. The results obtained clearly show that only the correct choice of maize variety for silage cultivation, combined with nitrogen fertilizer guaranteeing access to N during the growing season, can guarantee a high yield for ensiling.

Keywords: maize; green fodder; urease inhibitors; fiber fractions; soluble sugars

1. Introduction

In many countries, the intensive expansion of dairy farming and dairy processing has resulted in a several-fold increase in silage production and a decrease in the amount of hay [1,2]. In the nutrition of dairy cows, silage is the basic volumetric component of the ration, constituting a factor limiting milk production and the health status of animals [3]. It enables the uniform feeding of animals throughout the year and is a source of readily available nutrients. Maize is most often used for the production of silage [4]. The quality and nutritional value of silage depends on many microbiological and agriculture factors that determine the course of the ensilage process [5]. The preservation effect depends, among other things, on the cultivar, dry matter content, soluble sugars, nitrogen compounds, pH and temperature, as well as microbial quantity and species composition [6]. Nitrogen is an essential nutrient for corn and a primary determinant of grain yield, especially because of its function in photosynthesis and many other biological processes [7]. In agricultural practice, mineral fertilizer doses, including nitrogen formulations, are determined according to the nutritional requirements of the plants, without considering the amount of assimilable nutrients present in the soil [8–10]. These result in an excess of soluble components in the soil and their increased leaching, which burdens the environment, but also reduces the efficiency of fertilization [11]. Therefore, the rationalization of nitrogen fertilizer application in corn cultivation is a crucial issue for sustainable agriculture, because it can limit the negative influence on the environment [12–14]. It is estimated that 50 per cent of the world's population uses nitrogen fertilizers for food production and about 60 per cent of the global nitrogen fertilizer pool is used for the production of three major crops, namely rice, wheat and maize [15]. Unfortunately, nitrogen from fertilizers is not used efficiently enough on a global scale, as in the soil–plant system, this use rarely exceeds 50% of the applied nitrogen [16]. Therefore, new agronomic solutions are constantly being sought to increase the use of nitrogen from the mineral fertilizer ration. To date, few studies have been carried out on nitrogen application in maize crops on the quantity and quality of maize raw material for ensiling [17–19]. Furthermore, also in other crop species, e.g., wheat, research is being carried out on the response of this species to differentiated nitrogen fertilization determining the biomass yield obtained [20]. According to Artyszak [21], currently, the maximization of food production must become the main objective of the European Union's agricultural production; reducing fertilizer use by 20% and increasing the proportion of organic production to 25%, as part of the "from farm to fork" strategy, do not help to achieve this goal. Based on current knowledge of the response of maize to the application of nitrogen fertilizers, the working hypothesis of the study was formulated as follows: classical and stabilized nitrogen fertilizers affect the quality and quantity of the raw material to be ensiled. The aim of this work is to present the assessment of fodder intended for whole plant silage on the basis of quantitative and qualitative criteria, and to indicate how selected elements of agricultural technology (cultivar, nitrogen fertilizer) can differentiate the yield intended for silage and its quality.

2. Materials and Methods

2.1. Experimental Field

Strict agricultural trials were carried out in three corn growing seasons (2017–2019) at the Experimental Station for Cultivar Testing in Chrząstowo, a field unit of the Central Research Center for Cultivated Plants in Słupia Wielka. The trial in each growing season was conducted in the same randomized design (split-plot) with two experimental factors in 3 field replicates. The following variables were tested: A—maize cultivar: A1—ES Bombastic (FAO 230-240)—single cross hybrid (SC), A2—ES Abakus (FAO 230-240)—three-way cross hybrid (TC, stay-green), and A3—ES Metronom (FAO 240)—single hybrid (SC, stay-green + roots power); B—type of fertilizer: B1—control (without N application), B2—ammonium nitrate, B3—urea, B4—ammonium nitrate + N-Lock, B5—urea + N-Lock, B6—Super N-46, and B7—UltraGran stabilo. Mineral fertilization was applied at the following doses: 150 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1}, and 130 kg K_2O ha^{-1}. Nitrogen fertilization was not applied in the control combination (B1—absolute control). On the experimental plots (B4 and B5), N-Lock nitrogen stabilizer was applied in the form of a spray on day 5 after nitrogen fertilizer application at a dose of 1.7 l ha^{-1}. It prevents the conversion of the bioavailable and stable ammonium form to the nitrate form in the soil, increasing its availability to plants [N-Lock label]. Phosphorus and potassium fertilization was applied before sowing maize on 2 dates: in the autumn of the previous year (for winter plowing) and in the spring, directly before sowing corn (before combined seed drill). On the first date, the compound fertilizer Lubofos 12 (P_2O_5—12%, K_2O—20%) was applied. The remaining dose of phosphorus and potassium was supplemented before sowing in the form of enriched superphosphate (40% P_2O_5) and potassium salt (60% K_2O). The application of two single-component mineral fertilizers aimed at correctly balancing the PK dose.

Characterization of the Nitrogen Fertilizers

Ammonium nitrate—nitrogen content 34%, nitrogen in the form of ammonium NH_4 (17%) and nitrate NO_3 (17%).

Urea—nitrogen content 46%, nitrogen in the amide form (N-NH_2).

Super N-46—nitrogen content 46%, nitrogen in the amide form (N-NH_2) with a coating of NBPT urease inhibitor and MDGE carrier. The coating during fertilizer hydrolysis largely eliminates nitrogen loss through oxidation and the leaching of ammonia, and also maintains nitrogen in amide form. This allows it to be transported to the capillary root system, where it undergoes conversion to the ammonium form and binds to the sorption complex, thus counteracting leaching.

UltraGran stabilo—nitrogen content 46%, nitrogen in the amide form (N-NH_2), which uses the nitrogen stabilizer Limus, a urease inhibitor manufactured by BASF. It contains two nitrogen-stabilizing forms in its composition—NBPT and NPPT. The mechanism of action of the fertilizer is based on active blocking of the urease enzyme, which increases the efficiency of nitrogen fertilization by up to 30% in comparison with a classical fertilizer containing nitrogen in the amide form.

2.2. Soil Conditions

The soils in the experimental fields belonged to quality class IVa, i.e., a very good rye complex. Regarding soil valuation, the upper horizons of the analyzed soils were classified as loamy sands, and the loam fraction content amounted to 4%, dust to 14%, and the sand fraction to 83%. The proportion of loam and dust fractions was slightly lower in the eluvial horizon. The enrichment (B) and bedrock levels were more compact. The pH was determined in the water extract at approx. 7.0, while in KCl it was about 0.5 units lower, i.e., slightly acidic. The content of organic carbon was around 1%, which translated into 1.7% humus. The C:N ratio was approximately 12:1, and the total nitrogen content amounted to 0.086% (Table 1). The content of assimilable forms of potassium was determined at 80.5 mg K kg^{-1}, and this result qualified the soils to the medium class of enrichment in this element. The levels of assimilable magnesium and phosphorus classified the studied soils as the highly abundant class, as the content of these components was as follows: 92.5 mg Mg kg^{-1} and 168.2 mg P kg^{-1}, respectively (Table 2).

Table 1. Chemical properties of the experimental field during the growing seasons adapted from [7].

Years	pH H_2O	pH KCl	% N	% C	% Humus	C:N
2017	7.01	6.52	0.086	1.037	1.79	12.1
2018	6.96	6.56	0.086	1.037	1.79	12.1
2019	7.07	6.45	0.085	0.987	1.70	11.6

Table 2. Macronutrient soil content in the growing seasons adapted from [7].

Years	Phosphorus mg P·kg^{-1}	Soil Fertility Class	Content Rating	Potassium mg K·kg^{-1}	Soil Fertility Class	Content Rating	Magnesium mg·kg^{-1}	Soil Fertility Class	Content Rating
2017	168.7	I	very high	79.5	III	medium	92.6	I	very high
2018	162.7	I	very high	87.5	III	medium	89.2	I	very high
2019	173.1	I	very high	74.5	III	medium	95.6	I	very high
Average	168.2	±5.2	-	80.5	±6.6	-	92.5	±3.2	-

2.3. Thermal and Moisture Conditions

During the study period (2017–2019), the lowest average daily temperature (13.8 °C) in the growing season was recorded in 2017 (Table 3). The average temperatures in all months of this year were lower than in 2018, but also compared to the 2007–2019 period (with the exception of May and October). The highest average daily temperature during the growing season was measured in 2018, and it was 2.7 °C higher than in the year 2017. The highest average daily temperatures in the years of the study were observed in 2018 in July (20.1 °C) and August (20.9 °C), while in 2019, they were recorded in June (21.7 °C) and August (20.6 °C). The measured total precipitation in the period from April to October 2017 was 617 mm which was the highest in years of the study; in addition, it was also 242 mm higher than the average of the long-term period of 2007–2019 (Table 3). The highest rainfall was noted in July (134 mm) and August (143 mm). The lowest rainfall, both compared to 2017 and the long-term period (2007–2019), was measured in 2018 (290 mm) and 2019 (277 mm). The lowest precipitation during the growing season of 2018 was recorded in May (5 mm) and August (14 mm), while the highest was in July (120 mm). In 2019, the lowest rainfall was recorded in April (3 mm), June (18 mm), and July at the corn flowering stage (25 mm). The highest monthly precipitation sums in the 2019 growing season were recorded in the months of May and September.

Table 3. Thermal and precipitation conditions during maize growing seasons [adapted from 7].

Years	IV	V	VI	VII	VIII	IX	X	Sum/Average
				Temperatures [°C]				
2017	6.9	15.0	16.8	17.4	18.0	13.0	9.8	13.8
2018	12.4	17.0	18.2	20.1	20.9	16.3	10.6	16.5
2019	9.8	12.1	21.7	18.8	20.6	14.4	10.6	15.4
Many years (2007–2019)	9.0	13.7	17.4	19.1	19.3	13.7	8.6	14.4
				Precipitation [mm]				
2017	30	85	62	134	143	64	99	617
2018	49	5	45	120	14	32	25	290
2019	3	72	18	25	44	84	31	277
Many years (2007–2019)	26	56	58	92	60	40	43	375

2.4. Plant Material

During the maize harvest, weight measurements of whole plants were carried out, followed by ears alone; total dry matter yield and yield structure were determined. The percentage of dry matter in the maize aerial parts was also determined in order to calculate the dry matter yield of stover, ears and whole plants (stover + ear) per unit area. Because the experimental factors examined did not significantly vary the dry matter content of maize biomass for ensiling, the average values for the years of the study are given. In 2017, the dry matter content of stover was 32.1%, while of ears, it was 70.1%. In turn, in 2018, the dry matter content of stover was 32.5%, and 66.8% for ears. In the last year of the study (2019), the dry matter content was as follows: stover—31.8%, ears—73.4%. The content of starch, soluble sugars, crude fiber, NDF, ADF, and ADL in maize green fodder was determined via the NIRS method using an NRR-Flex N-500 apparatus (Büchi Labortechnik AG, Switzerland) and ready-made calibration models for maize green fodder developed by INGOT.

2.5. Statistical Analysis

The statistical analyses, such as analysis of variance (ANOVA), Tukey HSD test for comparisons of pairs of means, were performed through the research years according to the model of data obtained from the experiments designed as a split-plot [7,22]. All calculations were carried out using the STATISTICA 13.3 software package (2017) and MS Excel. Statistical significance was set at the level $\alpha = 0.05$.

3. Results

3.1. Morphological Features of Maize Plants

The height of maize plants in our research was significantly affected by the cultivar, the type of nitrogen fertilizer (Table 4) and the interaction of the years with nitrogen fertilizer (Figure 1). It was shown that the cultivar ES Bombastic developed the lowest plants compared to the other two cultivars tested (Table 4). Considering the type of nitrogen fertilizer, it was found that maize developed the lowest plants after applying ammonium nitrate, while the highest was after the application of Super N-46 and UltraGran stabilo. In the case of the interaction of test year and type of nitrogen fertilizer, it was found that in the first test year (2017), by far the lowest maize plants were found on the control site (B1) after the application of ammonium nitrate (B2) compared to UltraGran stabilo fertilizer (B7). In 2018 and 2019, the effect of nitrogen fertilizer type on maize plant height is difficult to explain (Figure 1). In our study, the setting height of production ears on the plant was significantly influenced by the maize cultivar, the type of nitrogen fertilizer (Table 4), and the interaction of the cultivar with nitrogen fertilizer (Figure 2). It was shown that the cultivar ES Bombastic set the ears at the lowest height on the plant, while the hybrid ES Metronom was at the highest level. Considering the type of nitrogen fertilizer, it was found that maize on the control plot (B1), fertilized with ammonium nitrate (B2) and urea + N-Lock (B5), set the ears the lowest on the plant, while the highest located ears were found after Super N-46 application (B6) (Table 4). Considering the interaction between the cultivar and the type of nitrogen fertilizer, it was shown that the cultivar ES Bombastic fertilized with UltraGran stabile (B6) had the lowest set ears on the plant, while the greatest height of set ears was recorded for the hybrid ES Metronom fertilized with Super N-46 (B5) (Figure 2). In the present study, the number of leaves on a single plant and their dry weight were significantly affected only by the type of nitrogen fertilizer (Table 4). By far the fewest leaves on a single plant were developed by maize on the control plot (B1) and ammonium nitrate (B2), while the highest number of leaves was found in plants on the fertilizer Super N-46 (B5). Regarding their weight, the lowest value of this feature was observed in maize on the control plot (B1), and was by far the highest for the plants fertilized with urea +N-Lock (B5) and super N-46 (B6) (Table 4).

Table 4. Average values of morphological features of maize plants for cultivars (A) and fertilizer (B).

Factors	Levels of Factors	Plant Height [cm]	Height of Production Ears [cm]	Number of Leaves [pcs.]	Leaf Weight [g]
Maize cultivar	A1	185.10 b	79.67 b	10.46 ns	74.52 ns
	A2	206.05 a	84.57 ab	10.95 ns	78.21 ns
	A3	221.50 a	92.43 a	11.17 ns	80.89 ns
p-value		0.0014	0.0096	0.0670	0.3014
Type of N fertilizer	B1	197.72 bc	84.11 b	10.53 bc	70.00 b
	B2	195.61 c	84.06 b	10.24 c	73.89 ab
	B3	203.89 abc	85.28 ab	10.94 ab	75.14 ab
	B4	206.89 ab	85.28 ab	11.10 ab	76.25 ab
	B5	203.72 abc	83.56 b	11.03 ab	83.19 a
	B6	210.61 a	89.89 a	11.39 a	85.28 a
	B7	211.06 a	86.72 ab	10.82 ab	81.39 ab
p-value		0.0001	0.0227	0.0000	0.0003

Values in columns marked with the same letter do not differ significantly; ns—not significant.

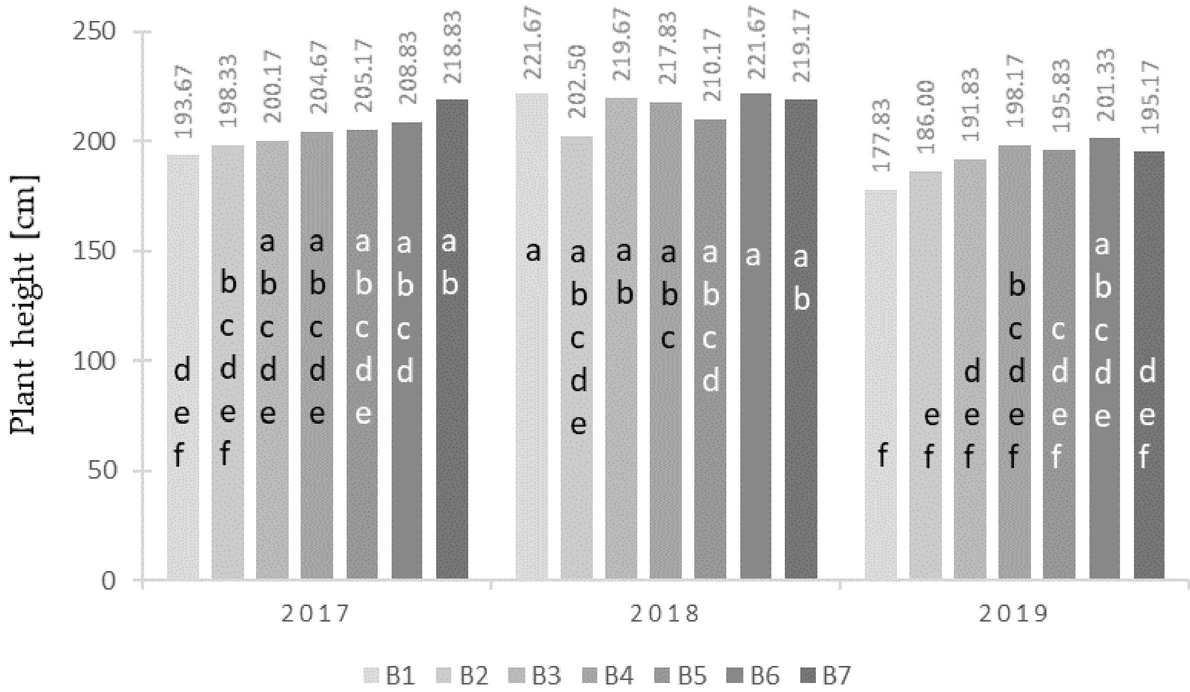

Figure 1. Average values of plant height [cm] for the combination of years (Y) and fertilizer (B). Values marked with the same letter do not differ significantly ($p = 0.0278$).

3.2. Yield of Stover, Ears, Whole Plants and Percentage of Ears in the Yield of Whole Plants

The yield of stover, ears, and whole plants was significantly dependent on the cultivar and the type of nitrogen fertilizer (Table 5). In addition, stover yield was shaped by the interaction of maize cultivar with the type of nitrogen fertilizer (Figure 3). By far the highest stover yield was recorded for the cultivar ES Metronom compared to the cultivars ES Bombastic and ES Abakus (Table 5). Analyzing the effect of nitrogen fertilizer on the value of the discussed trait, it was found that significantly the lowest stover yield was characteristic of maize on the control object (B1), and the highest after the application of urea + N-Lock (B5), super N-46 (B6), and UltraGran stabilo (B7). For the traditional cultivar ES Bombastic, none of the tested nitrogen fertilizers significantly modified the stover yield (Figure 3). On the other hand, the lowest stover yield for the hybrid ES Abakus was recorded for ammonium nitrate + N-Lock (B4), while the highest by far was for UltraGran stabilo (B7). For the hybrid ES Metronom, the lowest stover yield by far was characteristic of maize on the control plot (B1), and the highest on the fertilizer super N-46 (B6). The highest ear yield was found for the cultivars ES Abakus and ES Metronom compared to the traditional cultivar ES Bombastic (Table 5). With respect to nitrogen fertilizer, the lowest ear yield was found on the control object (B1), and the highest on the fertilizer UltraGran stabilo (B7). The hybrid ES Bombastic was characterized by the lowest mass yield of the whole plant, while ES Metronom was by far the highest (Table 5). With respect to nitrogen fertilizer, the lowest mass yield of the whole plant was found on the control object (B1), and the highest for the fertilizer UltraGran stabilo (B7).

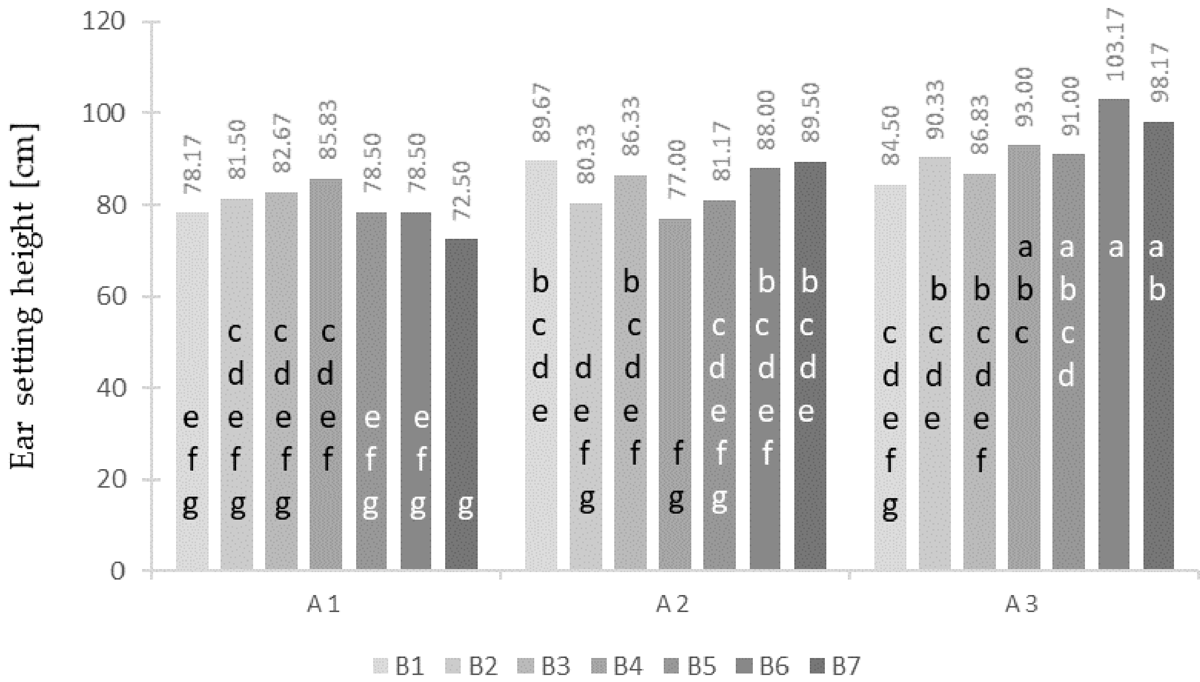

Figure 2. Average values of ear setting height [cm] for the combination of cultivar and (A) fertilizer (B). Values marked with the same letter do not differ significantly ($p = 0.0000$).

Table 5. Average yields of stover, ears, and whole plants and percentage of ears in the yield of whole plants for cultivars (A) and fertilizer (B).

Factors	Levels of Factors	Stover Yield [t.ha^{-1}]	Ears Yield [t.ha^{-1}]	Whole Plant Yield [t.ha^{-1}]	Ears Percentage [%]
Maize cultivar	A1	19.54 b	17.41 b	36.95 c	46.94 ns
	A2	20.84 b	19.47 a	40.31 b	48.36 ns
	A3	23.07 a	20.43 a	43.51 a	46.97 ns
p-value		0.0031	0.0025	0.0009	0.2175
Type of N fertilizer	B1	19.35 c	16.24 d	35.59 d	45.83 ns
	B2	20.81 abc	17.38 cd	38.19 cd	45.52 ns
	B3	20.41 bc	18.65 bcd	39.06 bc	47.62 ns
	B4	20.95 abc	19.36 bc	40.31 bc	48.19 ns
	B5	22.19 a	19.71 abc	41.90 ab	46.94 ns
	B6	21.84 ab	20.22 ab	42.05 ab	48.24 ns
	B7	22.51 a	22.17 a	44.68 a	49.63 ns
p-value		0.0000	0.0000	0.0000	0.0768

Values in columns marked with the same letter do not differ significantly; ns—not significant.

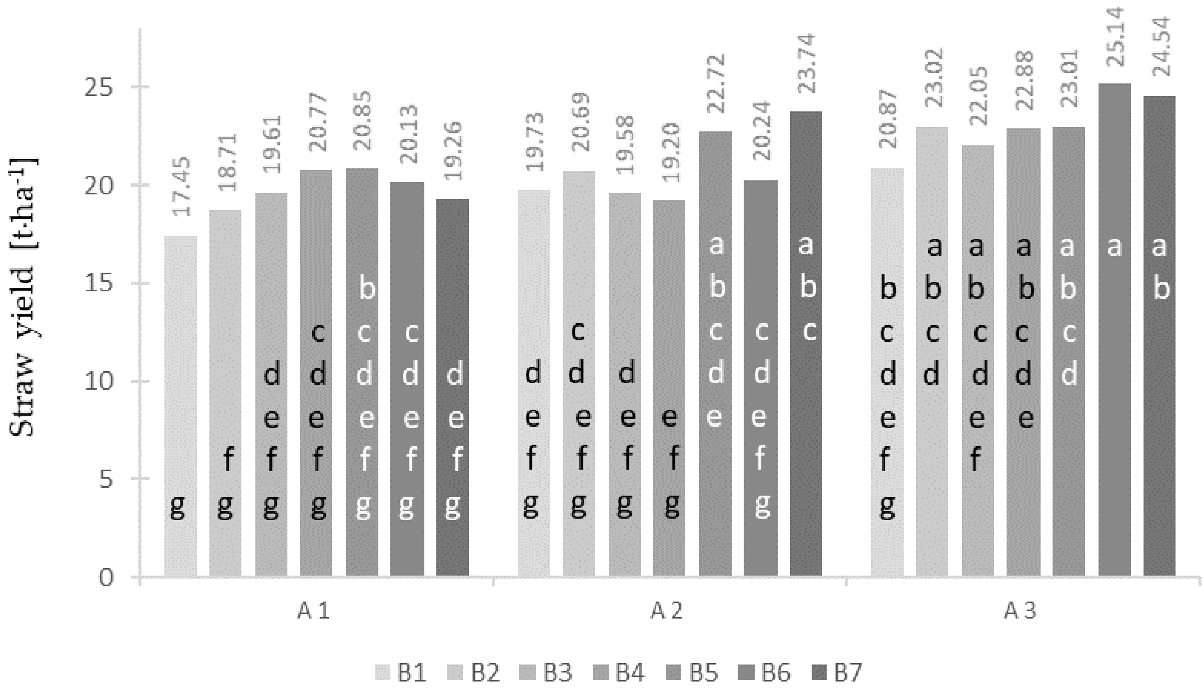

Figure 3. Average stover yield values [t·ha^{-1}] for the combination of cultivar (A) and fertilizer (B). Values marked with the same letter do not differ significantly (p = 0.0052).

3.3. Structural Carbohydrate Contents in Green Fodder for Ensiling

3.3.1. Non-Structural Carbohydrate Contents

The starch content in the raw material intended for ensiling was significantly influenced by the maize cultivar, the type of nitrogen fertilizer (Table 6), and the interaction of the cultivar with the type of nitrogen fertilizer (Table 7). By far the highest starch concentration was recorded for the cultivar ES Abaksu compared to the cultivars ES Bombastic and ES Metronom. Considering the type of nitrogen fertilizer, it was found that the highest starch content in maize green fodder was found in maize fertilized with urea + N-Lock (B5), while the lowest in maize on the control object (B1). For the cultivar ES Bombastic, by far the highest starch content was recorded on the nitrogen fertilizer Super N-46, and the lowest for ammonium nitrate. For the hybrid ES Abakus, by far the highest starch content was recorded on urea + fertilizer N-Lock (B5), and the lowest on the fertilizer Super N-46 (B6). In the case of the cultivar ES Metronom, by far the highest starch content was recorded for fertilizers B3 and B5, and the lowest for ammonium nitrate (B2). The content of soluble sugars in the present study significantly depended on the cultivar and the type of nitrogen fertilizer (Table 6), and the interaction of the cultivar with nitrogen fertilizer. By far the highest content of soluble sugars was recorded in the green fodder from the cultivar ES Metronom, and the lowest in ES Bombastic. Considering the type of nitrogen fertilizer, it was found that maize fertilized with UltraGran stabilo (B7) had the lowest content of soluble sugars compared to maize on the control plot (B1), ammonium nitrate (B2) and Super N-46 (B6) (Table 7). For the cultivar ES Bombastic, by far the highest amount of soluble sugars was recorded on the control object (B1), and the lowest on the objects with ammonium nitrate (B2), ammonium nitrate + N-Look and UltraGran stabilo (B7). In turn, for the hybrid ES Abakus, the highest content of soluble sugars was recorded for the fertilizer urea + N-Lock (B5), and the lowest for ammonium nitrate (B2). In the case of the cultivar ES Metronom, by far the highest content of soluble sugars was measured for ammonium nitrate (B2), and the lowest for the fertilizer UltraGran stabilo (B7) (Table 7).

Table 6. Average values of non-structural carbohydrates for cultivars (A) and fertilizer (B).

Factors	Levels of Factors	Starch [% DM]	Soluble Sugars [% DM]
Maize cultivar	A1	41.88 b	2.25 c
	A2	45.25 a	3.75 b
	A3	42.08 b	4.82 a
p-value		0.0000	0.0000
Type of N fertilizer	B1	42.69 b	4.94 a
	B2	38.75 c	4.89 a
	B3	45.20 ab	2.93 bc
	B4	39.42 c	3.16 b
	B5	45.81 a	2.45 cd
	B6	44.25 ab	4.54 a
	B7	45.38 ab	2.35 d
p-value		0.0000	0.0000

Values in columns marked with the same letter do not differ significantly.

Table 7. Average values of non-structural carbohydrates for the combination of cultivar (A) and fertilizer (B).

Maize Cultivar	Type of N Fertilizer	Starch [% DM]	Soluble Sugars [% DM]
A1	B1	39.47 de	5.78 b
	B2	37.58 ef	0.91 h
	B3	42.94 abcde	1.16 gh
	B4	39.57 de	0.83 h
	B5	43.41 abcd	1.46 gh
	B6	47.09 ab	4.92 bc
	B7	43.10 abcde	0.72 h
A2	B1	42.25 bcde	3.63 def
	B2	47.67 ab	2.03 g
	B3	45.07 abcd	4.49 cd
	B4	46.73 ab	3.41 ef
	B5	48.05 a	4.78 bc
	B6	40.45 cde	3.55 def
	B7	46.55 ab	4.38 cde
A3	B1	46.34 ab	5.40 bc
	B2	31.00 g	11.74 a
	B3	47.59 ab	3.16 f
	B4	31.96 fg	5.23 bc
	B5	45.96 abc	1.12 gh
	B6	45.20 abcd	5.15 bc
	B7	46.48 ab	1.93 g
p-value		0.0000	0.0000

Values in columns marked with the same letter do not differ significantly.

3.3.2. Structural Carbohydrate Contents

In our study, the content of crude fiber, and NDF, ADF and ADL fractions significantly depended on the cultivar, the type of nitrogen fertilizer (Table 8), and the interaction between the cultivar and nitrogen fertilizer (Table 9). The hybrid ES Bombastic contained the highest content of crude fiber, NDF, and ADF fractions, while the cultivars ES Abakus and ES Metronom had the lowest content of these fractions (Table 8). In terms of lignin (ADL), ES Bombastic and ES Abacus contained a significantly higher content compared to ES Metronom (Table 8). Considering the type of fertilizer, it was shown that the highest content of crude fiber and NDF fiber fraction was found for ammonium nitrate and ammonium nitrate + N-Lock, while the lowest was for the control object, urea, urea +N-Lock, Super N-46, and UltraGran stabilo. For the ADF fiber fraction, the highest amount was recorded for ammonium nitrate + N-Lock, while the smallest was for the control object, urea, urea + N-Lock, Super N-46, and UltraGran stabilo. Regarding lignin (ADL), the highest content of this fiber fraction was found for the fertilizer UltraGran stabilo, and was by far the lowest for the control object (Table 9). Considering the interaction of the cultivar with the type of nitrogen fertilizer, it was found that the highest crude fiber content for the cultivar ES Bombastic was obtained after the use of ammonium nitrate, and the lowest with the fertilizer Super N-46. For the cultivar ES Abakus, the highest content of crude fiber was found after application of the fertilizer Super N-46, while the lowest content was recorded for ammonium nitrate (Table 9). For the hybrid ES Metronom, the highest content of crude fiber was recorded after the application of ammonium nitrate, while the lowest was for the control object and urea. Considering the interaction of the cultivar with the type of nitrogen fertilizer, it was found that the highest NDF fiber fraction for the cultivar ES Bombastic was obtained after the use of ammonium nitrate, and the lowest with the fertilizer Super N-46. For the cultivar ES Abakus, the highest content of the NDF fiber fraction was found for the fertilizer Super N-46, while the lowest content was recorded for urea + N-Lock. For the cultivar ES Metronom, by far the highest content of the NDF fraction was obtained for ammonium nitrate + N-Lock fertilizer, while the lowest was for the control object, urea, and super N-46 (Table 9). Considering the interaction of the cultivar with the type of nitrogen fertilizer, it was found that the highest ADF fiber fraction for the cultivar ES Bombastic was obtained after the use of ammonium nitrate, and the lowest with the fertilizer Super N-46. For the cultivar ES Abakus, the highest content of the ADF fiber fraction was found for the fertilizer Super N-46, while the lowest content was recorded for the control object, ammonium nitrate, urea, ammonium nitrate + N-Lock, and UltraGran stabilo. For the cultivar ES Metronom, by far the highest content of the ADF fraction was obtained for ammonium nitrate + N-Lock fertilizer, while the lowest was for the control object, urea, and super N-46 (Table 9). Considering the interaction of the cultivar with the type of nitrogen fertilizer, it was found that the highest ADL fiber fraction for the cultivar ES Bombastic was obtained for ammonium nitrate, and the lowest for urea + N-lock and Super N-46. For the cultivar ES Abakus, the highest content of the ADL fiber fraction was found for the fertilizer Super N-46, while the lowest content was recorded for the control object. For the cultivar ES Metronom, by far the highest content of the ADL fraction was obtained for ammonium nitrate + N-Lock fertilizer and UltraGran stabilo, with the lowest for urea (Table 9).

Table 8. Average values of structural carbohydrates for cultivars (A) and fertilizer (B).

Factors	Levels of Factors	Crude Fiber [% DM]	NDF [% DM]	ADF [% DM]	ADL [% DM]
Mazie cultivar	A1	18.09 a	37.07 a	21.17 a	2.00 a
	A2	17.06 b	35.47 b	19.80 b	1.97 a
	A3	17.22 b	35.76 b	19.92 b	1.71 b
p-value		0.0005	0.0003	0.0000	0.0000
Type of N fertilizer	B1	17.20 b	35.64 b	20.14 c	1.76 b
	B2	18.64 a	38.23 a	21.32 b	1.97 ab
	B3	16.71 b	34.88 b	19.43 c	1.80 ab
	B4	19.47 a	39.31 a	22.47 a	1.94 ab
	B5	16.68 b	34.60 b	19.47 c	1.91 ab
	B6	16.65 b	34.85 b	19.34 c	1.83 ab
	B7	16.83 b	35.19 b	19.91 c	2.05 a
p-value		0.0000	0.0000	0.0000	0.0010

Values in columns marked with the same letter do not differ significantly.

Table 9. Average values of structural carbohydrates for the combination of cultivar (A) and fertilizer (B).

Maize Cultivar	Type of N Fertilizer	Crude Fiber [% DM]	NDF [% DM]	ADF [% DM]	ADL [% DM]
A1	B1	19.05 bc	38.76 bcd	22.32 bcd	1.86 abcd
	B2	21.28 a	41.84 ab	24.21 ab	2.23 a
	B3	17.22 cde	35.68 cdef	20.27 defgh	1.95 abcd
	B4	19.57 ab	39.00 bc	22.68 abc	2.13 ab
	B5	16.86 de	35.13 ef	19.87 efgh	1.92 abcd
	B6	15.47 e	33.37 f	18.44 gh	1.90 abcd
	B7	17.20 cde	35.71 cdef	20.40 defg	2.02 abc
A2	B1	17.27 cde	35.55 def	19.89 efgh	1.67 bcd
	B2	16.44 de	34.28 ef	18.90 efgh	1.98 abcd
	B3	17.09 cde	35.78 cdef	19.76 efgh	1.94 abcd
	B4	17.24 cde	36.02 cdef	20.17 efgh	1.93 abcd
	B5	16.24 de	33.69 f	18.84 fgh	1.95 abcd
	B6	18.07 bcd	37.48 cde	20.94 cde	2.06 ab
	B7	17.03 cde	35.47 def	20.10 efgh	2.25 a
A3	B1	15.29 e	32.61 f	18.21 h	1.77 abcd
	B2	18.22 bcd	38.57 bcd	20.87 cdef	1.69 bcd
	B3	15.81 e	33.17 f	18.25 h	1.51 d
	B4	21.60 a	42.92 a	24.56 a	1.76 abcd
	B5	16.94 de	34.98 ef	19.69 efgh	1.86 abcd
	B6	16.41 de	33.69 f	18.64 gh	1.53 cd
	B7	16.26 de	34.39 ef	19.23 efgh	1.88 abcd
p-value		0.0000	0.0000	0.0000	0.0040

Values in columns marked with the same letter do not differ significantly.

4. Discussion

In order to assess the photosynthetic potential of a species, leaf measurements were taken, as well as an analysis of the influence of environmental and agriculture factors (including fertilization, cultivar) on the number, area, and leaf weight. The area and number of leaves on a plant are important elements in assessing the efficiency of photosynthesis [23]. According to Pandey et al. [24], maize genotypes differ in growth rate and biomass production, as well as in the number of developed leaves under varying water and nitrogen conditions [25,26]. Szulc et al. [27] observed significant differences in the number and area of leaves in maize cultivars. According to Szulc et al. [27], one hectare of maize can have approx. 45,000 m^2 of sunlight-absorbing area (4.5 ha of leaves per ha of soil). By examining the reaction of two maize cultivars to different fertilization with nitrogen and magnesium, Szulc et al. [27] found that "stay-green" cultivars were characterized by a larger assimilation area; the results of Subedi and Ma [28] also confirmed this relationship. According to these authors, "stay-green" hybrids had a higher number of leaves and total area compared to traditional cultivars. In addition, according to Costa et al. [29], high leaf weight and higher biomass production resulted in higher nitrogen requirements of "stay-green" cultivars. In the present research, a higher number of leaves and their weight were observed in the "stay-green" cultivars; however, the differences compared to the traditional cultivar ES Bombastic were not statistically significant. In turn, a significant difference in the number of leaves on one plant was noted between the combinations where stabilized fertilizer Super N-46 (11.39 leaves) and ammonium nitrate (10.24 leaves) were applied. There was also a significant 22% increase in leaf weight relative to control for the combination with the fertilizer Super N-46, and a 19% increase for the combination of urea with N-Lock. Maize yield (vegetative biomass) was determined by both cultivar and fertilization factors. In the present study, the highest stover yield and the highest weight of set ears compared to other cultivars was found in the hybrid ES Metronom, which translated into the highest yield of whole plants. The best effects expressed in the yield of whole plants were obtained in the variant with the stabilized fertilizer UltraGran stabilo. In this combination, the highest stover yield (a significant result compared to control) and ear yield (a significant result compared to control, ammonium nitrate, urea, and ammonium nitrate + N-Lock) was obtained, which directly translated into the highest green mass yield of whole plants for this nitrogen carrier. The result obtained in our study has confirmed the previous literature reports that nitrogen is an essential component for the proper growth and development of maize. Its deficiency reduces the yield and deteriorates the quality of the harvested fodder [30]. Recent studies have also shown that the use of urea with an inhibitor increases crop yield and improves nitrogen utilization from the mineral fertilizer application rate (NUE) [31]. This is because urease inhibitors can delay urea hydrolysis, which is catalyzed by urease [32]. NBPT (N-(n-butyl) thiophosphoric triamide) is one of the most widely used urease inhibitors [33,34]. With the increasing nitrogen fertilization of maize, plant size, stem thickness, leaf area, and the leaf area index (LAI) also increase. This results in an increase in the plant's green mass yield [35]. A study of Budakli Çarpici et al. [36] showed that nitrogen fertilization affected the proportions of individual maize plant parts. As the nitrogen dose increases, the proportion of leaves and ears on the plant also raises, while the percentage of stems decreases. Ali et al. [30] reported that the leaves of maize fertilized with nitrogen contained more chlorophyll, which made them greener. According to Sheaffer et al. [37], maize dry matter yield increases quadratically to the rate of nitrogen fertilization applied. In addition, according to Cui et al. [38], nitrification inhibitors (NI) with ammonium sulphate can improve yield and nitrogen use efficiency in maize cultivation. According to these authors, soil pH and soil organic matter are the main factors influencing the effectiveness of NI. Harvesting at the optimum vegetative stage is when the concentration of nutrients is high in the plants and the content of water-soluble sugars reaches a maximum, allowing lactic acid bacteria to carry out proper fermentation [39]. The suitability of maize for ensiling is assessed on the basis of grain proportion [39]. The grain from the middle part of the ear should be in a milky-wax or glassy maturity, and the milk line should run halfway to 2/3 from the base of the kernel [40]. Green forage harvested late in development contain fewer soluble sugars that affect fermentation and more fiber, which makes crushing/pressing of the material more difficult and increases the risk of secondary fermentation. Such silages are characterized by poorer quality and mold as a result of air availability. Each day of delay in harvest results not only in the loss of nutrients and reduced digestibility, but also causes a slower decrease in the pH of the ensiled mass, thereby contributing to the development of undesirable microorganisms. An important factor determining the correct course of the ensiling process is the content of water-soluble sugars and buffer capacity. The amount of carbohydrates in the ensiled material is primarily determined by plant species, vegetation stage, dry weight, harvest date, fertilization, and time of day for crop harvest [41–44]. The buffering capacity of plants is determined by the content of protein and minerals and changes during their growth [45]. The fodder value largely determines the content of fiber and cellulose fractions that build the walls of plant cells. Fiber can be divided into acid detergent fiber (ADF, cellulose and lignins), acid detergent lignin (ADL, lignin) and neutral detergent fiber (NDF, cellulose, hemicellulose, lignin). The concept of determining NDF and ADF cell wall fractions was born in the US and was proposed by Van Soest and Robertson [46]. These authors assumed that feeds, including roughage, consisted of cell wall constituents (CWC) and cell contents (CC). CWC consist of cellulose, lignin, pectin, cutin, protein degradation products, wax, and silicon. CC include soluble proteins, lipids, N-protein, amides, alkaloids and tannins. In the proposed analytical system, the factors limiting feed intake, digestibility, and energy value are cell wall components designated as NDF and ADF. Hydrolysis in a neutral feed detergent yields the NDF component containing cellulose, hemicelluloses, and lignins. Further acid analysis of the feed separates the ADF component, containing lignin-bound cellulose. Modern cattle feeding systems make extensive use of both NDF and ADF in place of crude fiber [47].

5. Conclusions

The advantages of the UltraGrain stabilo formulation over ammonium nitrate and urea can become apparent when selecting a maize variety capable of the efficient uptake of nutrients in the pre-flowering period and their effective utilization during the grain filling stage (remobilization). Maize cultivar ES Metronom showed a significant advantage over other cultivars under fertilization with UltraGrain stabile, or alternatively Super N-46. The "stay-green" maize cultivars were characterized by a higher content of non-structural carbohydrates, including starch (ES Abakus) and water-soluble sugars (ES Metronom), and a lower content of structural carbohydrates compared to the traditional variety (ES Bombastic), which increased their suitability for ensiling. The negative effect of maize fertilization with ammonium nitrate and ammonium nitrate + N-Lock on the chemical composition of maize green fodder was demonstrated by a reduced starch content and increased structural carbohydrate contents, including crude fiber and NDF. The positive effect of maize fertilization with urea and urea + N-Lock on the chemical composition of maize fodder was shown by the increased starch content and reduced structural carbohydrate contents, including crude fiber and its NDF and ADF fractions. The analysis of the number and weight of leaves indicated highly effective nitrogen utilization, leading to the faster formation of leaves with a larger assimilation surface, i.e., the basis for the efficient absorption of solar radiation.

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