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Effects of Tytanit and Nitrogen on Cellulose and Hemicellulose Content of *Festulolium braunii* and on Its Digestibility

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Abstract: The aim of the experiment was to determine the effects of the foliar application of Tytanit, at the same time comparing it with the effects of mineral nitrogen, on the cellulose and hemicellulose content and its effect on *Festulolium braunii* digestibility. The experiment was founded in the spring of 2014 in the field of the University of Natural Sciences and Humanities in Siedlce, Poland. The plant used in the experiment was the Felopa variety of *Festulolium braunii*. The effects of Tytanit foliar application at a concentration of 0.2% and 1% and of mineral nitrogen at a dose of 80 and 160 kg/ha were studied in the experiment. During its full use (2015–2017), *Festulolium braunii* was harvested three times. The content of cellulose and hemicellulose was determined by near-infrared reflection spectroscopy (NIRS) using the NIRFlex N-500. The higher dose of 1% Tytanit contributed to an increase in cellulose content of *Festulolium braunii* (334.8 g kg^{-1}), at the same time decreasing hemicellulose content (175.0 g kg^{-1}), lignification degree (7.1%), dry matter digestibility (59.71%), and total digestible nutrient content (52.99%).

Keywords: *Festulolium*; structural carbohydrates; digestibility; digestible nutrients

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1. Introduction

In recent years, the use of products increasing plant resistance, soil fertility, and, consequently, the yield has become a new area of interest. Some of those products make it possible to limit the use of traditional mineral fertilizers or eliminate them [1–3]. They are applied to the soil as soil conditioners or to leaves. An example of one such product is Tytanit, containing titanium [4–7]. Tytanit contains 8.5 g dm-3 of the chelated form of titanium. Titanium belongs to the elements with the lowest phytoaccumulation index, with the exception of plants taking up a lot of silicon, i.e., nettle and some trees that accumulate up to 100 mg of Ti kg $^{-1}$. The action of titanium on plants is based on stimulating the activity of certain enzymes, e.g., catalase, peroxidase, lipoxidase, or nitrate reductase. In addition, this element increases chlorophyll content in the leaves, which has a positive effect on the yield of crops [8]. Pais [9] reports that despite the observed beneficial effect of water-soluble titanium compounds on vital functions, this element is not included in the components needed for the proper development of animal organisms. Titanium (Ti) has a beneficial effect on physiological and biochemical processes in plants, contributing to an increase in the yield [10]. According to Kolenčík et al. [11], it primarily affects quantitative and nutritional parameters, such as the oil content of sunflower seeds. According to Buettner and Valentine [12] and Radkowski and Radkowska [13], it increases the chlorophyll content of leaves, accelerates their growth and development, and reduces the sensitivity of plants to adverse environmental conditions. Studies on the reaction of plants to titanium have focused mainly on vegetables and some other crops [6,14,15]. The recommended concentration of Tytanit for use in agricultural practice is 0.02–0.04%. To cereals, it is applied during tillering, stem shooting, and flag leaf stages. The literature confirms its beneficial effect on the yield and its quality. However, there have been very few reports on the use of titanium as a stimulant in the cultivation of forage grass species, such as *Festulolium braunii* [16,17]. According to Borowiecki [18], this grass has a high yield potential and good nutritional value [19,20]. According to Sosnowski and Jankowski [21], *Festulolium* maintains good growth and development potential in successive years of use, and its characteristic feature is the high proportion of leaves in relation to generative shoots [22].

In the assessment of grass species, it is important to determine their chemical composition, including the content of cell wall components affecting forage intake and their digestibility and energy value [23,24]. The key components of cell walls are cellulose and hemicellulose, structural carbohydrates [25–27]. They make forage structure and fill the rumen as ballast matter. The levels of individual carbohydrates in plants vary considerably. The effect and direction of content changes are important from a nutritional point of view. As plants grow, the level of crude fibre increases, including the share of lignin, which reduces forage digestibility. Since some amounts of lignin, which is alkali-soluble, pass to nitrogen-free extracts, the digestibility of this fraction is much lower than other fibre fractions, which contain relatively well-digested hemicellulose. According to van Soest [28], hemicelluloses are better digested than cellulose. Their digestibility was estimated by the author at 79%, ranging from 72.3 to 85.7%. Cellulose is digested by ruminants at about 50%, ranging from 36.5 to 63.5%. Therefore, the aim of the experiment was to determine the effects of the foliar application of Tytanit, at the same time comparing it with the effects of mineral nitrogen, on the cellulose and hemicellulose content and its effect on *Festulolium braunii* digestibility.

2. Materials and Methods

2.1. Experimental Site and Design

The experiment was set up in the spring of 2014 in the experimental facilities of the University of Natural Sciences and Humanities in Siedlce, east-central Poland (52°17′ N, 22°28′ E). The anthropogenic soil had the granulometric composition of loamy sand [29]. Determined by the potentiometric method, its pH in 1 mol KCl dm^{-3} was 6.75. The content of organic carbon was 37.0 g kg^{-1}, and that of total nitrogen 1.75 g kg^{-1}. Organic carbon was determined by the oxidation-titration method, and total nitrogen was determined by the Kjeldahl method [30]. The macronutrient content of the soil was as follows (g kg^{-1}): P—1.05; K—1.00; Ca—2.40; Mg—1.25; S—0.508; Na—0.312. Determined by the Egner–Riehm method, the amounts of potassium and phosphorus absorbable forms were moderate, with 39.9 mg kg^{-1} and 128 mg kg^{-1}, respectively. The Felopa variety of *Festulolium brauni* (K. Richt.) A. Camus was used in the experiment. Its seeding rate was adopted according to the standard [31]. The grass seeds were provided by Danko, a plant breeding company [32]. The experimental field was divided into 1.5 m^2 plots, in a completely randomized design and replicated three times. There were 21 experimental plots in all. The following treatment combinations were arranged:

- control plot (no treatment);
- ammonium nitrate (N_1) at a dose of 80 kg N ha^{-1}, (24 g N per plot);
- ammonium nitrate (N_2) at a dose of 160 kg N ha^{-1}, (47 g N per plot);
- Tytanit® (Ti_1) at a concentration of 0.2% (1 cm^3 in 500 cm^3 of water);
- Tytanit® (Ti_2) at a concentration of 1% (5 cm^3 in 500 cm^3 of water);
- ammonium nitrate (N_1) + Tytanit® (Ti_1);
- ammonium nitrate (N_2) + Tytanit® (Ti_2).

2.2. Treatments and Data Colletion

Nitrogen treatment was divided into three annual doses, the first applied in early spring (before the start of the growing season) and two others after first and second harvests. All nitrogen doses were equal. Phosphorous and potassium were not applied due to satisfactory content of their absorbable forms in mineral soils of Poland. Tytanit was sprayed once on the plant leaves during the stem shooting stage. The amounts of water applied to treatment and control plots were the similar. Tytanit, a growth stimulant produced by the Intermag company, contains a form of titanium available to plants in a wide range of soil pH and is applied to soil and leaves. It contains 0.8% (mass percentage) of titanium (Ti), i.e., 8.5 g Ti dm^{-3} in the form of titanium ascorbate.

When it fully developed (2015–2017), *Festulolium braunii* was harvested three times a year. The first harvest was carried out in late May, the second in early July, and the third in mid-September. During each harvest, 0.5 kg of fresh matter was collected from each plot to determine dry matter content. Representative samples were collected randomly from many places in the plots. Samples were dried at 105 °C to constant weights in the SLN 32 drying oven produced by POL-EKO-APPARATURA. Then, the plant samples were ground in the WŻ-1S laboratory grinder from the Research Institute of the Bakery Industry in Bydgoszcz. The content of fibre fractions was determined by near-infrared reflection spectroscopy (NIRS) using the NIRFlex N-500 (PN-EN ISO 12099. 2013). Chemical analyses were carried out at the Institute of Technology and Life Sciences in Falenty. Cellulose, hemicellulose, lignification, dry matter digestibility (DMD), and amounts of total digestible nutrients (TDN) were calculated using the formulae developed by van Soest et al. [28].

$$
\begin{aligned}
&\text{DDM} = 88.9 - 0.779 \times \text{ADF} \\
&\text{DMI} = 120 : \text{NDF} \\
&\text{TDN} = 101.35 - (1.291 \times \text{ADF}) \\
&\text{Cellulose} = \text{ADF} - \text{ADL}
\end{aligned}
$$

Meteorological data for 2015–2017 were obtained from the Hydrological and Meteorological Station in Siedlce (air temperature, rainfall). The most favourable average temperature with its most favourable distribution across months was in the first year of research (2015), with 14.1 °C, comparable to the multi-annual average of 14.2 °C (Table 1). The third year of research with the average of 13.8 °C had the worst monthly temperature distribution. During the experimental period, the distribution of precipitation varied only slightly. Compared with the multi-annual average of 47.7 mm, the third year of research (2017) was the most favourable, with monthly rainfall of 54.1 mm.

Table 1. Average monthly temperature (°C) and rainfall (mm).

Year	Month							
	April	**May**	**June**	**July**	**August**	**September**	**October**	**Mean**
				Temperature (°C)				
2015	9.7	13.7	15.1	20.5	17.8	13.7	8.4	14.1
2016	8.2	12.3	16.5	18.7	21.0	14.5	6.5	14.0
2017	8.3	13.9	17.8	16.9	18.4	13.9	9.0	13.8
Mean	8.3	13.3	16.5	18.7	19.1	14.0	8.0	13.9
Mean of 2004–2014	8.5	14.0	17.4	19.8	18.9	13.2	7.9	14.2
				Rainfall (mm)				
2015	39.5	79.5	74.2	37.5	105.7	26.3	3.00	52.2
2016	30.0	100.2	43.3	62.6	11.9	77.1	39.0	52.0
2017	59.6	49.5	57.9	23.6	54.7	80.1	53.0	54.1
Mean	43.0	76.4	58.5	41.2	57.4	61.2	31.7	52.8
Mean of 2004–2014	33.0	52.0	52.0	65.0	56.0	48.0	28.0	47.7

2.3. Statystics

The results were statistically processed, with differences between means assessed using variance analysis. Statistica, Version 10.00 [33] was used for calculations. For significant differences, $LSD_{0.05}$ was determined with Tukey's test. To determine linear relationship between the characteristics, correlation coefficients were calculated. Variance analysis for a two-factor experiment in a split-plot design was used according to mathematical models:

1. Determination of the effect of treatment across growing seasons:

$$y_{ijl} = m + a_i + g_j + e^1{}_{ij} + b_l + ab_{il} + e^2{}_{ijl}$$

where: m—overall mean, a_1—the effect of growing seasons, g_j—the effect of replications, $e^1{}_{ij}$—error 1, b_1—the effect of treatment, ab_{il}—the effect of interaction between growing seasons and treatment, $e^2{}_{ijl}$—error 2.

2. Determination of the effect of treatment across *Festulolium braunii* harvests:

$$y_{ijl} = m + a_i + g_j + e^1{}_{ij} + b_l + ab_{il} + e^2{}_{ijl}$$

where: m—overall mean, a_i—the effect of treatment, g_j—the effect of replications, $e^1{}_{ij}$—error 1, b_l—the effect of harvests, ab_{il}—the effect of interaction between harvests and treatment, $e^2{}_{ijl}$—error 2.

In order to demonstrate the relationship between the variables, a linear correlation analysis (Pearson correlation analysis) was applied, on the basis of which correlation coefficients were calculated.

3. Results

3.1. Cellulose

In the plant material, cellulose content (Table 2), average across treatment combinations and growing seasons, was 318.0 g kg^{-1}. In each growing season, both nitrogen and Tytanit doses applied on their own increased cellulose content in *Festulolium* significantly. However, the combined application of the higher dose of nitrogen and Tytanit led to its decrease each growing season. The highest average cellulose content (Table 2) was in the first year (319.6 g kg^{-1}), and lowest was in the third (308.1 g kg^{-1}). According to statistical analysis, there were significant differences in cellulose amounts between the third and first growing seasons and the third and second. As an average over the years, the highest amounts of cellulose were recorded in *Festulolium* plants treated with the higher nitrogen dose (337.1 g kg^{-1}), the higher dose of Tytanit (334.8 g kg^{-1}), or the lower dose of both of them applied together (332.9 g kg^{-1}). These results differed significantly from the content of grass treated with other fertiliser combinations. By contrast, the lowest amounts were found in plants treated with the lower dose of Tytanit (298.7 g kg^{-1}).

Table 2. Cellulose content in *Festulolium braunii* dry matter (g kg^{-1}).

Treatments	Means Across Growing Seasons				Means Across Cuts			
	Years				Cuts			
	2015	2016	2017	Mean	I	II	III	Mean
0	325.4	301.5	286.9	304.6 BC	316.43	314.93	282.40	299.41 BC
N_1	324.7	286.7	318.6	310.0 B	310.90	318.63	300.53	305.71 B
N_2	329.9	353.0	328.5	337.1 A	355.66	325.36	310.36	333.01 A
Ti_1	302.0	319.4	274.6	298.7 C	311.70	304.00	280.30	296.00 C
Ti_2	336.9	335.5	332.1	334.8 A	343.90	341.96	318.63	331.26 A
$N_1 + Ti_1$	339.8	324.0	334.9	332.9 A	342.76	338.76	317.23	329.99 A
$N_2 + Ti_2$	278.3	320.9	324.5	307.9 BC	324.50	320.26	278.86	301.68 BC
mean	319.6 a	317.3 a	308.1 b	318.0	329.40 a	323.41 b	298.33 c	313.86

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments. 0—control; N_1—ammonium nitrate at a dose of 80 kg N ha^{-1}; N_2—ammonium nitrate at a dose of 160 kg N ha^{-1}; Ti_1—Tytanit at a concentration of 0.2%; Ti_2—Tytanit at a concentration of 1%.

Significant variation of cellulose content was also recorded across harvests (Table 2), with the highest in the first (329.40 g kg^{-1}) and the lowest in the third (298.33g kg^{-1}). In the first year, the most was noted in the first harvest (283.8 g kg^{-1}) and least was recorded in the third (268.2 g kg^{-1}). In the second year, the least cellulose was also produced during the third growth cycle.

Cellulose content increased from 305.71 in plants treated with the lower nitrogen dose to 333.01 g kg^{-1} when the higher amount was applied, with the same effect in the case of Tytanit, which increased it from 296.00 to 331.26 g kg^{-1}. The research also indicated that the combined higher dose of nitrogen and Tytanit resulted in a statistically significant decrease, from 329.99 to 301.68 g kg^{-1}.

3.2. Hemicellulose

The average hemicellulose content in *Festulolium* plants, across all experimental factors, was 183.4 g kg^{-1}, varying across both treatment combinations and years of research (Table 3). It increased in a statistically significant way in successive growing seasons, from 172.0 g kg^{-1} in the first to 197.9 g kg^{-1} in the third. In successive years, the higher dose of nitrogen did not affect this content in a consistent way. In turn, the higher dose of Tytanit, in all years, contributed to lower hemicellulose content. In the present studies, the combined use of nitrogen and Tytanit resulted in a reverse response. As an average for growing seasons, its higher dose resulted in a statistically significant increase, to 186.9 g kg^{-1}, compared to 159.2 for the lower one. Similarly, the three-year average (Table 3) showed that the higher dose of nitrogen used on its own resulted in a statistically significant increase from 180.9 to 186.8 g kg^{-1}. In turn, the higher dose of Tytanit used on its own contributed to a statistically significant decrease in hemicellulose content of *Festulolium* from 184.7 to 175.0 g kg^{-1}. Compared to the control plot (210.6 g kg^{-1}), all treatment combinations resulted in significantly lower hemicellulose content in *Festulolium*. As an average across treatments, it varied in a statistically significant way across harvests (Table 3), with the least amount found in the plants of the third growth cycle (200.99 g kg^{-1}) and the most of the second (325.71 g kg^{-1}).

Table 3. Hemicellulose content in *Festulolium braunii* dry matter (g kg^{-1}).

Object	Means Across Growing Seasons				Means Across Cuts			
	Years				Cuts			
	2015	2016	2017	Mean	I	II	III	Mean
0	180.6	225.8	225.3	210.6 A	205.9	174.1	250.2	210.1 A
N_1	177.6	195.8	169.2	180.9 C	174.7	183.3	171.0	176.3 C
N_2	189.5	159.7	214.7	186.8 B	164.9	208.2	207.6	193.6 B
Ti_1	169.5	171.7	212.8	184.7 BC	186.0	180.6	207.7	191.4 B
Ti_2	155.6	162.1	207.4	175.0 C	154.0	180.8	204.3	179.7 C
$N_1 + Ti_1$	137.4	166.9	173.4	159.2 D	144.3	154.3	162.9	153.8 D
$N_2 + Ti_2$	193.8	184.4	182.6	186.9 B	166.6	183.6	195.4	181.9 BC
mean	172.0 c	180.9 b	197.9 a	183.4	170.9 c	180.7 b	199.9 a	183.8

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments. 0—control; N_1—ammonium nitrate at a dose of 80 kg N ha^{-1}; N_2—ammonium nitrate at a dose of 160 kg N ha^{-1}; Ti_1—Tytanit at a concentration of 0.2%; Ti_2—Tytanit at a concentration of 1%.

3.3. Lignification Degree

Table 4 shows that the average degree of Festulolium lignification was 7.4% in the varying over growing seasons and treatments. In each growing season (Table 4), the higher dose of Tytanit (concentration 1%) contributed to a lower lignification rate of around 0.5–2.3%. In turn, the combined use of nitrogen and Tytanit resulted in an opposite effect. Thus, their higher dose, compared to the lower one, brought about an increase in lignification rates ranging from 0.2% to 3.0% in all years of research. As an average, it was the lowest in the second (7.0%) and the highest in the third (7.8%). On the other hand, it did not significantly vary across harvests (Table 4), ranging from 7.4 to 7.5%. It was decreased by the higher dose of nitrogen (from 8.1 to 7.6%) and the higher dose of Tytanit (from 8.0 to 7.1%). In turn, the combined use of nitrogen and Tytanit at the higher dose resulted in its increase from 6.7% to 7.7%.

Table 4. Lignification rate of *Festulolium braunii* dry matter (%).

Treatments	Means Across Growing Seasons				Means Across Cuts			
	Years				Cuts			
	2015	2016	2017	Mean	I	II	III	Mean
0	6.2	6.3	7.6	6.7 B	6.7	7.0	6.4	6.5 B
N_1	8.0	7.3	8.2	7.8 AB	8.2	7.4	8.0	8.1 A
N_2	6.4	7.8	8.4	7.5 AB	7.4	7.4	7.8	7.6 AB
Ti_1	8.9	7.2	8.5	8.2 A	7.8	8.5	8.2	8.0 A
Ti_2	8.3	6.7	6.2	7.1 AB	7.2	7.0	7.0	7.1 AB
$N_1 + Ti_1$	5.7	6.7	7.8	6.7 B	6.7	6.8	6.8	6.7 AB
$N_2 + Ti_2$	8.7	6.9	8.2	7.9 A	7.6	8.3	7.8	7.7 AB
mean	7.5 b	7.0 c	7.8 a	7.4	7.4 a	7.5 a	7.4 a	7.4

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments. 0—control; N_1—ammonium nitrate at a dose of 80 kg N ha^{-1}; N_2—ammonium nitrate at a dose of 160 kg N ha^{-1}; Ti_1—Tytanit at a concentration of 0.2%; Ti_2—Tytanit at a concentration of 1%.

3.4. Dry Matter Digestibility

In the present experiment it was found that dry matter digestibility was dependent on the treatment, growing season, and harvest (Table 5). The average value for *Festluloium braunii* was 62.61%. The highest value of this parameter was recorded in the third year (63.99%) for plants treated with Tytanit at the lower dose (concentration 0.2%). In the first year, slightly lower results (63.73%) were noted for grass treated with the combined higher dose of nitrogen and Tytanit. The difference between the effects of Tytanit used on its own and of the combined dose was statistically significant.

Table 5. Dry matter digestibility (DMD) of *Festulolium braunii* (%).

Treatments	Means Across Growing Seasons				Means Across Cuts			
	Years				Cuts			
	2015	2016	2017	Mean	I	II	III	Mean
0	60.96	62.67	63.27	62.12 A	60.77	61.11	64.44	62.61 A
N_1	60.19	63.59	60.69	60.44 C	60.36	59.61	61.34	60.85 AB
N_2	61.17	59.73	61.42	61.30 B	58.96	61.92	63.02	60.99 AB
Ti_1	61.46	61.18	63.99	62.73 A	60.85	62.44	64.89	62.87 A
Ti_2	59.17	59.75	60.25	59.71 D	58.67	59.31	61.14	59.91 B
$N_1 + Ti_1$	60.19	60.81	59.78	59.99 CD	59.21	59.57	61.18	59.99 B
$N_2 + Ti_2$	63.73	61.18	61.49	62.61 A	61.33	61.94	64.57	62.61 A
mean	62.35 a	61.93 b	62.38 a	62.61	61.05 b	61.52 b	64.51 a	62.36

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments. 0—control; N_1—ammonium nitrate at a dose of 80 kg N ha^{-1}; N_2—ammonium nitrate at a dose of 160 kg N ha^{-1}; Ti_1—Tytanit at a concentration of 0.2%; Ti_2—Tytanit at a concentration of 1%.

In the second year of research, the highest digestibility (63.59%) was noted for *Festulolium* treated with nitrogen at the lower dose (80 kg N ha^{-1}). As an average across growing seasons, its highest value was recorded in *Festulolium* treated with the lower dose of Tytanit (62.73%) and with the combined higher dose of nitrogen and Tytanit (62.61%). Even if the above values differed significantly from others, digestibility did not vary much across treatment combinations. The lowest was in the second year (61.93%), being significantly higher in the first (62.35%) and third (62.38%).

Additionally, treatments did not differentiate digestibility values across harvests much (Table 5). Although the higher dose of Tytanit reduced it from 62.87% to 59.91%, the higher dose of nitrogen and Tytanit increased it from 59.99% to 62.61%. The digestibility of *Festulolium* steadily improved in a statistically significant way in successive harvests, from 61.05% in the first to 64.51% in the third. The number of harvests during the growing season has a major impact on forage digestibility.

3.5. Total Digestible Nutrients (TDN)

As an average across treatments and growing seasons, total digestible nutrient percentage (TDN) was 55.53% (Table 6). It varied from year to year in a statistically significant way and was highest in the third (56.03%) and lowest in the first (55.0%). In the first year, the highest value was recorded in *Festulolium* treated with the combined higher dose of nitrogen and Tytanit (59.63%). In the second year, a similar percentage was recorded (59.41%) on the plot with the lower dose of nitrogen. In the third year, the highest value (60.08%) was in *Festulolium* treated with the lower dose of Tytanit. The three-year results indicated a small variation in the value of this parameter across fertiliser combinations. However, the higher doses of nitrogen and Tytanit, each applied on its own, decreased total digestible nutrient percentage from 55.92 to 54.37% and from 57.30 to 52.99%, respectively. On the other hand, when they were used together at the higher dose, it increased from 54.61 to 56.26%. Similarly, the parameter values increased in a statistically significant way in successive harvests (Table 6), from 53.37% in the first one to 58.33% in the third.

Table 6. Total digestible nutrient (TDN) percentage of *Festulolium braunii* (%).

Treatments	Means Across Growing Seasons				Means Across Cuts			
	Years				Cuts			
	2015	2016	2017	Mean	I	II	III	Mean
0	55.04	57.88	58.85	57.26 A	54.73	55.29	60.81	57.77 A
N_1	53.77	59.41	54.59	55.92 AB	54.05	55.68	55.67	54.86 B
N_2	54.30	53.01	55.80	54.37 BC	50.07	57.99	58.46	54.27 B
Ti_1	56.42	55.41	60.08	57.30 A	55.69	57.49	61.56	58.63 A
Ti_2	52.08	53.04	53.87	52.99 CD	51.26	52.31	55.35	53.31 B
$N_1 + Ti_1$	53.78	54.79	55.26	54.61 BC	52.15	53.76	57.65	54.90 B
$N_2 + Ti_2$	59.63	55.40	53.76	56.26 AB	55.66	55.64	58.80	57.23 A
mean	55.00 b	55.56 a	56.03 a	55.53	53.37 c	55.45 b	58.33 a	55.72

Different lowercase letters in the same row or different uppercase letters in the same column indicate significant differences between treatments. 0—control; N_1—ammonium nitrate at a dose of 80 kg N ha^{-1}; N_2—ammonium nitrate at a dose of 160 kg N ha^{-1}; Ti_1—Tytanit at a concentration of 0.2%; Ti_2—Tytanit at a concentration of 1%.

Correlation coefficients across the years of research (Table 7) indicated that when the cellulose content of *Festulolium braunii* increased, the amount of dry matter and digestible nutrients decreased. In turn, when the digestibility of dry matter increased, so did the amount of digestible nutrients with a very strong correlation. Moreover, linear correlation coefficients indicated (Table 8) that when the hemicellulose content of *Festulolium braunii* increased, so did the yield of dry matter and the amounts of digestible nutrients. In turn, when the digestibility of dry matter increased, so did the amounts of digestible nutrients, also with a very strong correlation.

Table 7. Linear correlation coefficients between cellulose content, lignification rate, DDM, and TDN.

Variable	Average from Growing Seasons			
	Cellulose	Lignification	DDM	TDN
Cellulose	1.00			
Lignification	−0.349	1.00		
DDM	−0.937 *	0.229	1.00	
TDN	−0.939 *	0.253	0.951 *	100

*—not significant difference.

Table 8. Linear correlation coefficients between hemicellulose content, lignification rate, DDM, and TDN.

Variable	Average from Growing Seasons			
	Hemicellulose	**Lignification**	**DDM**	**TDN**
Hemicellulose	1.00			
Lignification	0.092	1.00		
DDM	0.704 *	0.229	1.00	
TDN	0.678 *	0.253	0.951 *	100

*—significant difference.

4. Discussion

As the most significant component of plant cell walls [34], cellulose consists of interconnected glucose molecules [35]. According to Bach Knudsen [25], its structure prevents it from being penetrated by water molecules, which has a decisive influence on the fact that it is insoluble in water. Cellulose is found in large quantities in young plants, while in older ones its micelles are impregnated with lignin due to the hardening of plant tissue associated with aging. According to Jankowska-Huflejt and Wróbel [36], of all feedstuffs, pasture forage (249.8–271.0 g kg^{-1} DM) contains the least cellulose; in meadow plants it is larger, ranging from 287.7 to 299.7 g kg^{-1} DM, with the largest amounts in hay (294.4–302.0 g kg^{-1} DM). It should be emphasized that dairy cows digest only a third of cellulose [37]. The results showed that in the plant material, cellulose content, averaged across treatment combinations and growing seasons, was 318.0 g kg^{-1}, with Salama and Navar [38], Ciepiela [26], and Todorov et al. [37] recording similar amounts.

In this study, significant variation of cellulose content was also recorded across harvests, with the highest in the first (329.40 g kg^{-1}) and the lowest in the third (298.33g kg^{-1}). In the first year, a very uneven distribution of precipitation was recorded (Table 1), which could have affected the yield and nutritional value of the plant. This trend was confirmed by Ciepiela [26], who found the most cellulose in *Festulolium* grass of the first growth cycle (286.1 g kg^{-1}) and the least of the third (265.4 g kg^{-1}). The higher content of structural carbohydrates in the first harvest can be explained by a smaller number of leaves produced by the plants. Frankow-Lindberg and Olsson [22] found that the share of leaf blades in the first harvest of *Festulolium* was 42%, with 75% in the third. In the research of Wiśniewska-Kadżajan and Jankowski [39] on *Festulolium braunii*, cellulose content was slightly different.

Cellulose content in plants treated with the lower nitrogen dose increased from 305.71 to 333.01 g kg^{-1} when the higher amount was applied. Different results were recorded by Ciepiela [26], who found that an increase in nitrogen dose from 50 kg ha^{-1} to 150 kg ha^{-1} resulted in a statistically significant reduction in *Festulolium* cellulose amounts, from 288.1 to 268.4 g kg^{-1}DM. In the present experiment, however, the combined higher dose of nitrogen and Tytanit resulted in a statistically significant decrease, from 329.99 to 301.68 g kg^{-1}. Similarly, according to Ciepiela [26], plants treated with Tytanit and a higher dose of nitrogen contained less cellulose. This phenomenon may have been caused by the varying ratio of leaf blades to stems, which determines the nutritional value of forage plants. On the plots with Tytanit applied in combination with higher amounts of nitrogen, a much higher yield of *Festulolium* biomass was obtained than on those with Tytanit only or on those with traditional nitrogen fertilization [16]. An important component of plant cell walls is hemicellulose [39]. Consisting mainly of pentoses and hexoses, according to Bach Knudsen [25], it serves as a reserve material deposited in the structure of the cell wall together with cellulose fibres. Numerous studies [40] have shown that hemicellulose is digested completely a few hours after its intake by dairy cows. In successive years, the higher dose of nitrogen did not affect this content in a consistent way. In turn, the higher dose of Tytanit, in all years, contributed to lower hemicellulose content. Wadas and Kalinowski [41] noted an increase in Na content in potato tubers. Tytanit is used on many crops and many authors confirm its positive effect on the quantity and quality of plant yield [11,14,42]. Additionally, according to Ciepiela [26], the same biostimulant significantly reduced the amount of hemicellulose in *Festulolium*, but the effect of higher doses of nitrogen was not always statistically proven. The results showed that the higher dose of Tytanit used on its own contributed to a statistically significant decrease in hemicellulose content of *Festulolium* from 184.7 to 175.0 g kg^{-1}. Similar amounts were recorded by Wiśniewska-Kadżajan and Jankowski [39] in *Festulolium braunii* (167.8–206.9 g kg^{-1}), lower than those obtained by Ciepiela [26].

Lignification (i.e., tissue hardening) reduces forage digestibility, its energy value, and dry matter intake [25,37]. The research indicated that the average degree of *Festulolium* lignification was 7.4%, varying over growing seasons and treatments.

According to Stachowicz [43], grassland forage intended for ruminants should be at least 65% digestible. According to Podkówka and Podkówka [44], cellulose and excessive amounts of hemicellulose with lignin limit plant digestibility and energy value. The results showed that the average value of dry matter digestibility in *Festulolium* was 62.61% and did not fully meet the above standard set by Stachowicz [43]. Slightly lower digestibility of Tytanit-treated plants were recorded by Sosnowski et al. [6], with 57.7% for *Trifolium pratense* and 55.5% for hybrid alfalfa.

The difference between the effects of Tytanit used on its own and of the combined dose was statistically significant. Sosnowski [1] found that the digestibility of *Festulolium braunii* dry matter was 60%, with no change after soil conditioner application, while mineral fertilizers significantly reduced the parameter to 48.5%.

In his other research, Sosnowski [2] recorded higher digestibility (approximately 60.0%) of an alfalfa and *Festulolium braunii* mixture. The number of harvests has a significant impact on forage digestibility, but it is also related to the development phase and the rate of plant aging [45,46].

In the present study, the digestibility of *Festulolium* steadily improved in a statistically significant way in successive harvests, from 61.05% in the first to 64.51% in the third. These results were confirmed by Truba et al. [7]. Jankowski and Malinowska [3] recorded the highest digestibility in the forage of the second harvest (54.25%) and the lowest of the first (51.51%). The number of harvests during the growing season has a major impact on forage digestibility, which is also closely related to the developmental phase and the rate of plant aging of [45,46]. In the research of Gaweł and Żurek [45], they recorded the digestibility of dry matter of grass–legume mixtures harvested five times at the level of up to 80%.

5. Conclusions

The higher dose of Tytanit contributed to an increase in cellulose content, at the same time decreasing hemicellulose content, lignification degree, dry matter digestibility, and total digestible nutrient content of *Festulolium braunii*. The higher dose of nitrogen resulted in an increase in cellulose and hemicellulose content and in dry matter digestibility, decreasing lignification and total digestible nutrient content. The combined application of nitrogen and Tytanit to *Festulolium braunii* at the higher dose resulted in a decrease in cellulose content, at the same time increasing hemicellulose content, lignification, dry matter digestibility, and total digestible nutrient content. A similar trend persisted across harvests. Of the treatment combinations, the lower dose (0.2%) of Tytanit resulted in the most favourable effect on *Festulolium braunii* cellulose content, dry matter digestibility, and the amounts of total digestible nutrients. Taking into account hemicellulose content and the degree of digestibility, the best results were recorded in plants treated with the combined lower dose of Tytanit and nitrogen. Based on the research, the use of Tytanit combined with nitrogen fertilization is recommended. Foliar application of Tytanit in the cultivation of *Festulolium braunii* is justified in economic and environmental terms and also improves its nutritional value.

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