

Article

The Effect of Tytanit on Fibre Fraction Content in *Medicago x varia* T. Martyn and *Trifolium pratense* L. Cell Walls

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Abstract: The aim of the experiment was to determine the effect of foliar application of Tytanit, a stimulator based on titanium, on the content of fibrous fractions, cellulose, and hemicellulose in the cell walls of h. alfalfa and r. clover. The experimental factors were plant species and titanium doses. The content of cell wall fibrous fractions was determined with near-infrared spectroscopy, and Relative Feed Value (RFV), and cellulose and hemicellulose content were calculated based on acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL). The stimulator differentiated the content of fibrous fractions in plant cell walls. Its largest dose lowered ADF content to 2.3% in plant dry matter, and the smallest one increased accumulation of the ADL fraction by 1.6%. NDF content in the dry matter of h. alfalfa and r. clover was lower than the desired optimum for plants used as forage, and the stimulator additionally reduced it by 4.9%. Higher doses of titanium decreased carbohydrate content during unfavourable hydrothermal conditions. However, there was no significant effect of differentiated Tytanit doses on the RFV value.

Keywords: acid detergent fibre; acid detergent lignin; cellulose; *Fabaceae*; hemicellulose; neutral detergent fibre; relative feed value; titanium



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1. Introduction

Growth stimulators are becoming more important in modern plant growing technologies. In addition to their morphometric effects, they increase the resistance of plants to abiotic and biotic stress, which helps to make better use of their production potential, especially in difficult environmental conditions [1–4]. Titanium (Ti) is the active substance of Tytanit, a product considered to be one of such stimulators [5–9]. According to the manufacturer, Tytanit has been produced in Poland since 1989. Initially it was qualified as a mineral fertiliser, but since 2011 it has been classified as a mineral growth regulator [10]. In its composition, it contains 8.5 g of titanium per 1 dm³ in the form of Ti-ascorbate [11].

It is one of the nutrients necessary for plants as, in the form of ascorbate, citrate, or malate, titanium has a beneficial effect on physiological processes, stimulating chlorophyll biosynthesis and the activity of many enzymes such as catalase, peroxidase, lipoxigenase, and nitrate reductase [12,13]. In addition, it participates in the uptake of certain nutrients by plants. The result of these processes is a higher quality and quantity of crops [12,14–17]. Titanium applied to the rhizosphere or as a foliar spray stimulates plant growth, but its impact significantly depends on the species [18]. The current state of knowledge indicates that the response of plants deficient in nitrogen to titanium application is rather negligible. However, with an optimal supply of nitrogen to crops, its application can increase the yield from a dozen to several dozen percent [6,19]. A number of studies have proved that Ti affects the content of organic compounds in plant biomass, changing the concentration of protein and crude fibre in the dry matter of meadow plants [7,19]. Godlewska and Ciepiela [19] demonstrated the significant effects of this product on the content of monosaccharides in the aboveground parts of red clover and alfalfa, which are species that react very positively to the foliar application of growth regulators [20,21].

Nowadays, the level of scientific expertise makes it possible to determine neutral detergent fibre (NDF) content in plant dry matter, which, apart from protein content, should be a basic parameter to assess the nutritional value of plants [22]. The National Research Council does not specify optimal dietary NDF concentration but recommends a minimum of 250 to 280 g kg⁻¹ [23]. According to Beauchemin et al. [23], energy intake depends on the interaction between the NDF concentration of the diet and forage source. In addition, according to Kiraz [24], in modern systems of animal nutrition NDF content allows calculating the feed intake needed by animals. It is also an indicator of plant material quality; based on its concentration in dry matter, it is possible to define the relative feed value (RFV) [25]. According to Kawas et al. [26], the content of acid detergent fibre (ADF) in alfalfa hay for high producing dairy cows is 12–35%. The authors point out that an increase in its content deteriorates the feed value. According to Moore and Jung [27], lignin is considered an anti-quality component in forages due to its negative impact on the nutritional availability of plant fibre. High content of acid detergent lignin (ADL) indicates that the process of plant lignification is in progress. Lignin prevents bacteria from attaching to cell walls, which results in limited digestion of plant material. Therefore, as the ADL content increases, the digestibility of plant dry matter decreases. Cellulose is a polysaccharide, a structural carbohydrate that, together with hemicellulose, builds plant cell walls [28]. Hemicellulose is a pentose polymer responsible for storing plant energy. Like cellulose, it is an important component of cell walls [29].

In the light of the above, the aim of this paper is to determine the effects of Ti foliar application on the content of fibrous fractions, i.e., NDF, ADF, ADL, cellulose and hemicellulose in cell walls of hybrid alfalfa and red clover. In addition, RFV was calculated in order to determine the suitability of the plants for forage production.

2. Materials and Methods

The research was carried out in the experimental field in Siedlce (Poland) from 2015 to 2017. In the autumn of 2015, experimental plots with an area of 4.5 m² (1.5 m × 3 m) were set up, with a 1 m wide herbicide path between them.

The soil consisting of loose loamy sand (sieve analysis) was recognized as Technosols according to the Food and Agriculture Organization classification [30]. Before the experiment was set up the concentration of organic carbon was 13.50 g kg⁻¹ Dry Matter (DM), while total nitrogen concentration was 1.30 g kg⁻¹ DM. The soil pH of 6.8 was close to neutral. In addition, the soil had high content of absorbable forms of phosphorus and magnesium, but absorbable forms of potassium were within the limits of average content. The content of macro elements in soil was determined with an emission spectrometer, using the ICP-OES Optima 8300 (PerkinElmer, Waltham, MA, USA). The soil analysis was performed by the National Chemical and Agricultural Station in Warsaw. Due to the abundance of nutrients in the soil, mineral fertiliser was not applied either as pre-plant treatment or as topdressing. The seeds were planted in mid-September 2015, and the seeding rate was consistent with the standards for both species. The plants reached full maturity in the growing seasons of 2016 and 2017.

The experimental factors were as follows:

Plant species (legumes investigated separately):

h. alfalfa variety Comet;

r. clover variety Krynia.

Tytanit growth stimulator doses:

Ti_{0.0}—control with distilled water (without Ti);

Ti_{1.2}—0.2 dm³ ha⁻¹, (1.2 gTi ha⁻¹);

Ti_{2.4}—0.4 dm³ ha⁻¹, (2.4 gTi ha⁻¹);

Ti_{3.6}—0.6 dm³ ha⁻¹, (3.6 gTi ha⁻¹).

During each of the three growth cycles (growing seasons from April to October), the plants were treated twice in the growth and development stages according to the BBCh scales, separately for h. alfalfa [31] and r. clover [32]. The first Tytanit application to h.

alfalfa was during the first nod stage (BBCh 31), and the second when the first flower buds were visible outside leaves (BBCh 51), whereas the first application to r. clover was after forming the first lateral branch (BBCh 21), and the second when the first petals were visible (BBCh 51). The working fluid to be applied to plants ($200 \text{ cm}^3 \text{ ha}^{-1}$) was prepared by dissolving an adequate dose of Tytanit in water, while control plots were treated with the same amount of distilled water. The working fluid was applied to the plant using a spray bottle. In the growing seasons when plants reached full maturity (2016 and 2017), h. alfalfa and r. clover were harvested three times at the time when 30–40% of flowers were open.

During each harvest, samples of plant material were collected (an average of 1.0 kg) for chemical analysis. The content of cell wall fibrous fractions (g kg^{-1}), i.e., NDF, ADF, and ADL, in plant dry matter was determined with near-infrared spectroscopy (NIRS), using the NIRFlex N-500 spectrometer (BUCHI, Flawil, Switzerland).

The content of cellulose and hemicellulose was calculated from the formulas [33]:

$$\text{cellulose (\%)} = \text{ADF} - \text{ADL} \quad (1)$$

$$\text{hemicellulose (\%)} = \text{NDF} - \text{ADF} \quad (2)$$

Based on NDF and ADF fractions, RFV was determined. This parameter was used to evaluate the suitability of the forage to feed livestock [34], calculated using the formula:

$$\text{RFV} = \frac{\text{DDM} \times \text{DMI}}{1.29} \quad (3)$$

where:

RFV—relative feed value (dimensionless quantity),

DDM—digestible dry matter (%),

$$\text{DDM} = 88.9 - 0.779 \times \text{ADF} \quad (4)$$

DMI—dry matter intake (percentage of body weight).

$$\text{DMI} = \frac{120}{\text{NDF}} \quad (5)$$

Meteorological data, i.e., precipitation and temperature, were collected from the nearest hydrological and meteorological station, located about 3 km from the experimental field (Table 1). Based on the data, Sielianinov's hydrothermal coefficient was calculated [35].

Table 1. Average monthly air temperature ($^{\circ}\text{C}$), monthly total precipitation (mm), and Sielianinov's hydrothermal coefficient's values.

Year	Month							Means
	Apr.	May	June	July	Aug.	Sept.	Oct.	
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	6.9	13.9	17.8	16.9	18.4	13.9	9.0	13.8
Means	8.3	13.3	16.5	18.7	19.1	14.0	8.0	13.9
Multiannual means	8.5	14.0	17.4	19.8	18.9	13.2	7.9	14.2

Table 1. Cont.

Year	Month							Means
	Apr.	May	June	July	Aug.	Sept.	Oct.	
Precipitation (mm)								
2015	39.5	79.5	74.2	37.5	105.7	26.3	3.0	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
Means	43.0	76.4	58.5	41.2	57.4	61.2	31.7	52.8
Multiannual means	33.0	52.0	52.0	65.0	56.0	48.0	28.0	47.7
Sielianinov's hydrothermal coefficient (K)								
2015	1.36	1.87	1.64	0.59	1.92	0.64	0.12	-
2016	1.22	2.63	0.87	1.0	0.18	1.46	1.94	-
2017	2.88	1.15	1.08	0.45	0.96	1.92	1.90	-
Sielianinov's coefficient legend:								
Extremely dry	very dry	dry	fairy dry	optimal	fairy wet	wet	very wet	extremely wet

The results of the research were processed statistically using analysis of variance for multiannual experiments replicated three times, with a split-plot design, control plots, and with repeated measurements (for consecutive harvests). The Fisher–Snedecor F-test was used to check the significance of the effects of experimental factors on the results. The significance of differences between means was checked with Tukey's test at $HSD_{0.05}$. In the tables presenting the results, letters were used to show whether differences between means were significant. The calculations were performed, and correlation coefficients determined using the program STATISTICA, version 13 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

In the experiment, it was found that h. alfalfa, as the average response to all doses of the growth stimulator, had higher NDF content in dry matter, being 8.7% higher than r. clover (Table 2). In addition, a higher content of this fibre fraction was reported in h. alfalfa in the first year of the experiment compared to the second (about 2.4%). However, in r. clover biomass, the content of NDF remained at the same level in both growing seasons and amounted to 344 g kg^{-1} . According to Riviera et al. [36], the NDF content in legumes was due to differences in growth habits and species. Zhang et al. [37] compared the NDF content of various legumes and grasses. The results showed that NDF was greatest for *Lotus corniculatus* and *Astragalus cicer*, intermediate for *Medicago sativa*, *Onobrychis viciifolia* and *Sanguisorba minor* and least for *Bromus riparius*.

The experiment demonstrated that different Ti doses resulted in NDF content diversity (Table 2). As an average for both plant species and growing seasons, the highest content of NDF was in the dry matter of plants from the control plot. The titanium decreased NDF value in a statistically significant way. This decrease was from 2.5% for $Ti_{1,2}$ to 4.9% for $Ti_{3,6}$. In the years 2016–2017, about 50% of the growing season, the hydro-thermal conditions were dry or extremely dry. The lignin content in plant cell walls increased under drought conditions. The application of titanium slowed down the plant lignification process. Based on the above results, it was found that titanium reduced plant stress caused by unfavourable hydrothermal conditions.

The analysis of the results presented in Table 2 indicates that of all growth cycles the highest average NDF content across species and treatments was in plants in the second harvest (374 g kg^{-1}). The values of this feature in the first (346 g kg^{-1}) and third (356 g kg^{-1}) harvests were at a similar level and did not differ significantly. The NDF values obtained in the experiment were low [23]. Lower concentration of NDF in feed with legumes was observed by Brown et al. [38]. In addition, Genc-Lermi [39] obtained the lowest NDF values when using pure legume sowing.

Table 2. Effect of various growth stimulator doses on NDF concentration in h. alfalfa and r. clover dry matter in both growing seasons and all harvests (g kg^{-1}).

Year			Harvest			Means
Treatment	2016	2017	1	2	3	
Treatment means						
Ti _{0,0}	366 ± 4.15 Aa	373 ± 3.99 Aa	349 ± 6.01 Ca	392 ± 4.68 Aa	368 ± 4.27 Ba	369 ± 4.95 a
Ti _{1,2}	360 ± 5.90 Aa	358 ± 4.78 Ab	349 ± 5.78 Ba	374 ± 5.10 Aab	358 ± 4.38 ABa	360 ± 3.54 b
Ti _{2,4}	360 ± 4.11 Aab	348 ± 7.05 Ab	357 ± 5.20 Aa	370 ± 4.78 Ab	336 ± 5.17 Bb	354 ± 8.49 c
Ti _{3,6}	354 ± 5.12 Ab	347 ± 4.36 Ab	329 ± 4.17 Bb	359 ± 6.08 Ab	364 ± 6.29 Aa	351 ± 4.94 c
Species means						
h. alfalfa	378 ± 4.95 Aa	369 ± 4.95 Ba	357 ± 3.19 Ba	394 ± 4.11 Aa	369 ± 4.57 ABa	374 ± 6.36 a
r. clover	344 ± 4.95 Ab	344 ± 4.95 Ab	334 ± 5.27 Bb	353 ± 6.07 Ab	344 ± 4.46 ABb	344 ± 1.28 b
Means	361 ± 5.12 A	356 ± 6.12 A	346 ± 8.19 B	374 ± 6.28 A	356 ± 8.91 B	

Means in columns marked with small font letters do not differ significantly. Means in rows marked with large font letters do not differ significantly.

A similar tendency was observed for NDF content in both species across harvests. The highest content of NDF in h. alfalfa (394 g kg^{-1}) and r. clover (353 g kg^{-1}) was also in the second harvest. As is apparent from the distribution of the means in different harvests, application of different doses of Ti did not change this trend either. According to Truba et al. [40], the amount of fibre fraction in the feed is closely related to the applied fertilization, plant species and hydrothermal conditions. Probably, dynamically changing hydrothermal conditions caused significant fluctuations in the value of NDF fraction from different cuts. The second harvests were collected during dry periods in both years of the research. As a result, the application of different titanium doses to the plant did not result in significant differences in the fraction content (Table 2). On the other hand, in the first and third harvest, the hydrothermal conditions were changed from wet to dry in every year of the study. As a consequence, significant differences were obtained between the NDF fraction content on the objects fed with 2.4 and 3.6 titanite concentrations. According to Beauchemin et al. [23], energy intake depended on the interaction of NDF concentration of the diet and forage source. In addition, according to Kiraz [24], in modern systems of animal nutrition, NDF content allows calculating the feed intake needed by animals. It is also an indicator of plant material quality as, based on its concentration in dry matter, it is possible to define the relative feed value.

The present experiment (Table 3) showed that ADF content across treatments and growing seasons was larger in h. alfalfa dry matter (317 g kg^{-1}), 3.3% higher than in r. clover, where it was 307 g kg^{-1} . In addition, it turned out that higher ADF content was in dry matter accumulated in the first year than in the second year, in both h. alfalfa (321 g kg^{-1}) and r. clover (309 g kg^{-1}).

Application of different doses of Ti diversified ADF content (Table 3). On the control plots and on plots with a titanium dose of 1.2 g ha^{-1} the value of this feature remained the same (315 and 314 g kg^{-1}) and did not differ from each other. However, the smallest content of the ADF fraction was in plants treated with the greatest concentration of titanium, namely 308 g kg^{-1} ; its average decrease relative to control was 2.3%.

A positive effect of Ti applied at a dose of 3.6 g ha^{-1} on a significant reduction of ADF content in plant material should be pointed out. As Gawel and Żurek [41] indicated, the ADF concentration in alfalfa of different varieties range from 418 to 437 g kg^{-1} . It also needs to be stressed that the content of fibre fractions in plant dry matter vary from species to species. Tomic et al. [42] demonstrated that, as an average, some factors such as the harvest date and fertilizer treatment lead to differences among species, since they found higher accumulation of ADF fraction in cocksfoot dry matter than in perennial ryegrass dry matter.

Table 3. Effect of various growth stimulator doses on acid detergent fibre (ADF) concentration in h. alfalfa and r. clover dry matter in both growing seasons and all harvests (g kg^{−1}).

Year			Harvest			Means
Treatment	2016	2017	1	2	3	
Treatment means						
Ti _{0,0}	318 ± 5.78 Aa	311 ± 4.99 Ba	304 ± 6.04 Ba	334 ± 6.23 Aa	306 ± 4.75 Ba	315 ± 4.95 a
Ti _{1,2}	316 ± 4.04 Aa	311 ± 5.11 Ba	307 ± 5.66 Ba	323 ± 5.15 Aa	311 ± 5.33 Ba	314 ± 4.78 a
Ti _{2,4}	313 ± 3.98 Aa	308 ± 6.01 Ba	309 ± 5.17 ABa	318 ± 4.95 Ab	305 ± 4.85 Ba	311 ± 5.08 ab
Ti _{3,6}	311 ± 6.07 Aa	305 ± 5.28 Ba	297 ± 4.98 Ba	316 ± 5.21 Ab	311 ± 5.02 ABa	308 ± 4.25 b
Species means						
h. alfalfa	321 ± 3.87 Aa	313 ± 6.04 Ba	309 ± 4.01 Ba	333 ± 6.12 Aa	308 ± 5.44 Ba	317 ± 5.65 a
r. clover	309 ± 5.38 Ab	305 ± 6.44 Ab	299 ± 6.15 Bb	312 ± 7.00 Aa	309 ± 5.18 Ba	307 ± 2.83 b
Means	315 ± 3.10 A	309 ± 3.87 B	305 ± 5.25 B	323 ± 6.06 A	308 ± 3.20 B	

Means in columns marked with small font letters do not differ significantly. Means in rows marked with large font letters do not differ significantly.

In addition, the present experiment indicated that the largest ADF fraction content in the dry matter of plants across the species and different titanium concentrations was obtained in the second harvest (323 g kg^{−1}) (Table 3).

In the first and third harvests the values of this parameter were at a similar level and did not differ significantly. This tendency was not changed by the use of different concentrations of titanium, as can be seen from the distribution of the content in three different harvests. The higher values of ADF fraction in cell walls of second harvest were probably caused by unfavourable hydrothermal conditions. In both years of research, the second harvest was cut in dry period, which could have resulted in earlier lignification of plant cell walls.

The experiment showed that, as an average across different titanium concentrations, r. clover had higher content of ADL (58 g kg^{−1}) than h. alfalfa (Table 4). However, both plant species had similar content of ADL in the first and second year of research with an average of 57.5 g kg^{−1}.

Table 4. Effect of various growth stimulator doses on ADL concentration in h. alfalfa and r. clover dry matter in both growing seasons and all harvests (g kg^{−1}).

Treatment	Year		Harvest			Means
	2016	2017	1	2	3	
Treatment means						
Ti _{0,0}	57 ± 2.70 Ab	58 ± 1.78 Aa	57 ± 2.71 Aa	58 ± 2.11 Aa	57 ± 2.53 Aa	58 ± 1.07 a
Ti _{1,2}	59 ± 2.41 Aa	57 ± 1.01 Aa	55 ± 1.98 Ba	60 ± 2.12 Aa	59 ± 3.05 Aa	58 ± 1.40 a
Ti _{2,4}	59 ± 3.46 Aa	56 ± 2.67 Ba	56 ± 2.02 Ba	60 ± 2.61 Aa	55 ± 1.54 Ba	57 ± 2.21 b
Ti _{3,6}	59 ± 2.99 Aa	56 ± 3.12 Ba	54 ± 3.03 Ba	60 ± 2.44 Aa	57 ± 2.08 Ba	57 ± 2.18 b
Species means						
h. alfalfa	58 ± 2.65 Aa	56 ± 1.24 Ab	56 ± 2.22 Ba	61 ± 2.71 Aa	55 ± 2.81 Bb	57 ± 2.31 b
r. clover	58 ± 1.79 Aa	58 ± 1.90 Aa	55 ± 1.27 Ba	59 ± 2.39 Aa	60 ± 1.61 Aa	58 ± 0.87 a
Means	58 ± 0.78 A	57 ± 1.05 A	56 ± 1.29 B	60 ± 0.92 A	57 ± 1.63 B	

Means in columns marked with small font letters do not differ significantly. Means in rows marked with large font letters do not differ significantly.

Wróbel et al. [43] recorded lower values of the ADL fraction at the level of 45 g kg^{−1} in plant material. In assessing the value of feeding stuffs from meadows they suggested that diversity of plant species in fodder might be a factor lowering the share of lignin. Despite the occurrence of droughts in the first growing season, lignin content in the dry matter of the plants did not differ significantly from other growing seasons. Different results were

obtained by Jankowska [44]. This author found a significant effect of weather conditions on ADL concentration in plant biomass. The author noted higher content of the ADL fraction in meadow plants during a growing season with a drought. In the present experiment, the diversity of its content under the influence of different concentrations of titanium was also demonstrated.

Across the species and growing seasons, the highest biomass content of ADL was on the plots treated with the smallest concentration of titanium (58 g kg^{-1}). This value differed significantly from the results obtained from other experimental units and from the control series. In turn, the application of titanium in higher concentrations did not increase the ADL content in plant dry matter in relation to control (Table 4). At the same time, on the basis of the present research, it turned out that the greatest lignin content (60 g kg^{-1}) in the dry matter of h. alfalfa and r. clover, as an average for different concentrations of titanium, was recorded in the second harvest (Table 4). This value was significantly higher than those in the first and third harvests. This difference was 7.1% for first harvest and 5.3% for third harvest.

In addition, the analysis of the relationship between the concentration and the harvest, regardless of the species, showed that the impact of the applied concentrations of titanium in each harvest on ADL accumulation in biomass was insignificant (Table 4). The values ranged from 54 to 60 g kg^{-1} . However, a similar reaction to hydrothermal conditions can be noticed in all fractions of the fibre. The smallest discrepancies in parameters were obtained in the second harvest. The second harvest was cut in the dry period, which had a negative impact on the titanium uptake by plants.

The average cellulose content during the research was about 255 g kg^{-1} . Different doses of titanium did not significantly affect cellulose content in the dry matter of the grass species (Table 5). It was higher in h. alfalfa, but the difference between the species was not significant and amounted to approximately 5%.

Table 5. Effect of various growth stimulator doses on cellulose concentration in h. alfalfa and r. clover dry matter in both growing seasons and all harvests (g kg^{-1}).

Treatment	Year		Harvest			Means
	2016	2017	1	2	3	
Treatment means						
Ti _{0,0}	261 ± 9.98 Aa	253 ± 10.1 Aa	247 ± 10.2 Ba	276 ± 13.7 Aa	249 ± 10.1 Ba	257 ± 9.78 a
Ti _{1,2}	257 ± 7.90 Aa	254 ± 7.98 Aa	252 ± 13.0 Aa	263 ± 12.8 Aab	252 ± 12.0 Aa	256 ± 13.0 a
Ti _{2,4}	254 ± 11.9 Aa	252 ± 8.92 Aa	253 ± 10.1 Aa	258 ± 13.5 Aab	250 ± 11.7 Aa	253 ± 7.67 a
Ti _{3,6}	252 ± 9.91 Aa	249 ± 12.3 Aa	243 ± 9.08 Aa	256 ± 9.21 Ab	254 ± 12.0 Aa	251 ± 11.2 a
Species means						
h. alfalfa	263 ± 8.12 Aa	257 ± 12.3 Aa	253 ± 11.1 Ba	272 ± 13.9 Aa	253 ± 10.3 Aa	260 ± 10.8 a
r. clover	251 ± 6.99 Aa	247 ± 9.65 Aa	244 ± 10.8 Aa	253 ± 9.98 Ab	249 ± 9.92 Aa	249 ± 9.91 a
Means	257 ± 8.28 A	252 ± 7.97 A	249 ± 13.08 A	263 ± 12.10 A	251 ± 11.0 A	

Means in columns marked with small font letters do not differ significantly. Means in rows marked with large font letters do not differ significantly.

There were no significant differences between the average cellulose content across harvests (Table 5). Analysing the effect of titanium doses in different harvests, it was found that only forage from the second one contained varied amounts of cellulose. In the same harvest its content was the lowest in plants treated with the highest titanium concentration; about 8% lower than in control plants. According to the literature [29,45], young plants are richer in cellulose. With age, cellulose becomes lignified, and the content of lignin increases. In both years of research, the plants of the second harvest were affected by drought stress. The results showed that titanium did not have a positive effect on cellulose content under such conditions, and as a response to an increased dose, the cellulose content decreased.

Using the Kelpak bio stimulator, Sosnowski et al. [46] recorded a decrease in the content of cellulose and hemicellulose in *Lolium perenne* and *Festuca pratensis*.

The average hemicellulose content decreased with increased titanium doses, and the difference between control plants and those sprayed with Ti2.4 and Ti3.6 was 26% (Table 6). In addition, there was a significant difference in the content of hemicellulose between species, with h. alfalfa containing 54% more than r. clover. No significant differences across growing periods were recorded.

Table 6. Effect of various growth stimulator doses on hemicellulose concentration in h. alfalfa and r. clover dry matter in both growing seasons and all harvests (g kg^{−1}).

	Year		Harvest			Means
Treatment	2016	2017	1	2	3	
Treatment means						
Ti _{0,0}	48 ± 2.46 Ba	62 ± 3.11 Aa	45 ± 2.56 Bb	58 ± 3.17 Aa	62 ± 3.06 Aa	54 ± 2.40 a
Ti _{1,2}	44 ± 3.01 Aa	47 ± 1.96 Ab	42 ± 2.76 Bb	51 ± 3.16 Aab	47 ± 2.63 ABc	46 ± 2.12 ab
Ti _{2,4}	47 ± 1.96 Aa	40 ± 1.66 Ab	48 ± 3.01 ABa	52 ± 2.97 Aab	31 ± 1.89 Bd	43 ± 1.96 b
Ti _{3,6}	43 ± 2.23 Aa	42 ± 3.16 Ab	32 ± 1.96 Cc	43 ± 3.41 Bb	53 ± 3.07 Ab	43 ± 2.17 b
Species means						
h. alfalfa	57 ± 2.40 Aa	56 ± 3.30 Aa	48 ± 1.96 Ba	61 ± 3.08 Aa	61 ± 3.32 Aa	57 ± 2.21 a
r. clover	35 ± 3.10 Ab	39 ± 2.74 Ab	35 ± 2.06 Bb	41 ± 2.26 Ab	35 ± 2.01 Bb	37 ± 1.79 b
Means	46 ± 2.61 A	47 ± 2.45 A	41 ± 2.40 B	51 ± 3.10 A	48 ± 2.27 AB	

Means in columns marked with small font letters do not differ significantly. Means in rows marked with large font letters do not differ significantly.

Similar to the case of cellulose, the content of hemicellulose should decrease as the plant matures. The results (Table 6) showed that it was significantly higher in the second harvest, and it slightly decreased in the third (by 6%). Perhaps low hemicellulose content in the plants of the first growth cycle was due to the early harvest date, after which the plant rebuilt its biomass in the second harvest with higher hemicellulose content. There was also a visible tendency towards reducing the content of hemicellulose on plots where titanium was used. Sosnowski et al. [47] found that it decreased in alfalfa after spraying it with a mixture of auxin, cytokinin, and titanium chelate.

According to the standards [34], plant material with RFV above 151 should be used for feeding most productive dairy cows. The results showed that both h. alfalfa and r. clover were of high quality in both years of research and met the requirements for highly productive cattle, but the RFV value was about 9% higher for r. clover (Table 7). However, according to Markovic et al. [48], alfalfa retains high nutritional value from the first to third development stage, while RFV of r. clover during its growth and development decreases much faster. According to Table 7, there was a slight tendency towards increasing the RFV value as a response to increased doses of titanium, but statistical analysis did not show any significant differences.

The highest forage quality was recorded in the first cut (Table 7). For nearly all harvests, it was qualified as the first class, except for alfalfa in the second one. The second harvest in both years was in dry hydro-thermal conditions, which could have had a decisive influence on forage quality. Furthermore, RFV value depends on the stage of plant development [48,49]. According to Jeranyama and Garcia [49], in the initial stage of its growth, alfalfa had the RFV value of 164 and in the final one it was only 92.

The highest RFV value in the first and second harvests was recorded on the plot where the titanium dose of 3.6 g ha^{−1} was applied (Table 7). On the other hand, in the third harvest, the dose of 2.4 gTi ha^{−1} had the most beneficial effect on forage quality. The application of titanium and various weather conditions during plant growth significantly affected the components of the RFV index, differentiating it. The influence of titanium

was especially visible in the second harvest. The forage on the plots with titanium had a significantly higher RFV value than on the control plot.

Table 7. Effect of various growth stimulator doses on RFV in both growing seasons and all harvests.

Treatment	Year		Harvest			Means
	2016	2017	1	2	3	
Treatment means						
Ti _{0.0}	163 ± 4.08 Aa	161 ± 6.10 Aa	174 ± 5.18 Aab	149 ± 6.98 Cb	164 ± 7.01 Bb	162 ± 6.38 a
Ti _{1.2}	165 ± 6.01 Aa	168 ± 3.78 Aa	173 ± 6.11 Aab	159 ± 4.45 Bab	168 ± 4.48 Ab	167 ± 5.55 a
Ti _{2.4}	167 ± 3.98 Aa	174 ± 5.12 Aa	169 ± 5.23 Bb	161 ± 3.99 Ba	180 ± 5.58 Aa	170 ± 5.27 a
Ti _{3.6}	170 ± 5.12 Aa	175 ± 5.63 Aa	186 ± 4.88 Aa	167 ± 6.12 Ba	165 ± 5.01 Bb	172 ± 5.32 a
Species means						
h. alfalfa	157 ± 4.01 Ab	163 ± 6.02 Aa	169 ± 5.20 Ab	149 ± 5.11 Bb	164 ± 3.98 Aa	160 ± 4.08 b
r. clover	175 ± 5.38 Aa	176 ± 5.18 Aa	183 ± 6.74 Aa	170 ± 7.08 Ba	175 ± 4.18 Ba	176 ± 4.99 a
Means	166 ± 6.03 A	169 ± 5.11 A	175 ± 5.69 A	159 ± 4.87 B	170 ± 6.00 AB	

Means in columns marked with small font letters do not differ significantly. Means in rows marked with large font letters do not differ significantly.

The correlation coefficient was used to study the relationship between the content of cell wall components and the nutritional value (Table 8). There was a significant negative relationship between the content of NDF, ADF, ADL, cellulose and hemicellulose and the nutritional value of plants at each harvest. In their research, Godlewska i Ciepiela [50] recorded results proving that the fibre fractions of the cell walls are also negatively correlated with the digestibility of dry matter.

Table 8. Correlation coefficient for the relationship between RFV and the content of NDF, ADF, ADL, cellulose, and hemicellulose across harvests.

Harvest	RFV				
	x ₁ NDF Concentration	x ₂ ADF Concentration	x ₃ ADL Concentration	x ₄ Cellulose Concentration	x ₄ Hemicellulose Concentration
1	−0.72820 *	−0.85701 *	−0.83922 *	−0.77532 *	−0.67665 *
2	−0.78175 *	−0.74931 *	−0.85481 *	−0.65948 *	−0.77324 *
3	−0.69755 *	−0.67394 *	−0.74360 *	−0.66374 *	−0.69344 *

* Significant for $p \leq 0.05$.

4. Conclusions

The concentration of non-digestible fibre fractions of h. alfalfa and r. clover cell walls was typical for these species when they are harvested three times. Tytanit application in different ways differentiated fibre content. NDF content in the dry matter of h. alfalfa and r. clover was lower than the desired optimum for plants used as forage, and the stimulator reduced it further. Its largest dose lowered the ADF content in plants, and the smallest increased accumulation of the ADL fraction. The use of higher doses of titanium had a positive effect on the content of cellulose-lignin fractions in plant cell walls. This improved digestibility and forage quality. The content of structural carbohydrates decreased when higher doses of titanium interacted with unfavourable hydro-thermal conditions. The high RFV value of h. alfalfa and r. clover made them adequate for the most productive dairy cows, but no significant effect of varied Tytanit doses on this parameter was recorded.

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