*agronomy*

MDPI

*Article*

# Italian Ryegrass (*Lolium multiflorum* Lam.) Fiber Fraction Content and Dry Matter Digestibility Following Biostimulant Application against the Background of Varied Nitrogen Regime

**Agnieszka Godlewska * and Grażyna Anna Ciepiela**

Faculty of Medical and Health Sciences, Institute of Health Sciences, Siedlce University of Natural Sciences and Humanities, B. Prusa 14 st., 08-110 Siedlce, Poland; ciepielag@uph.edu.pl
* Correspondence: godlewskaa@uph.edu.pl

**Abstract:** An experiment was conducted to determine the effect of an application of biostimulants, against the background of varied nitrogen regime, on the share of neutral detergent fraction (NDF), acid detergent fraction (ADF), and acid detergent lignin (ADL) in the crude fiber fraction of Italian ryegrass as well as its digestibility. A field experiment was arranged as a randomized subblock design (split-plot) with three replicates at the Siedlce Experimental Unit of the University of Natural Sciences and Humanities in Poland in 2013. The following factors were examined: type of biostimulant: Algex, Tytanit, Asahi SL and a control; nitrogen application rate: 0 (control); 120 and 180 kg·ha$^{-1}$. There were confirmed positive effects resulting from an application of biostimulants in Italian ryegrass cultivation. There was confirmed the assumed hypothesis that an application of both natural and synthetic biostimulants will make it possible to improve the feeding value of grasses by reducing the fiber fraction. Particular attention should be paid to the biostimulant Algex whose application in Italian ryegrass cultivation produced the most beneficial response in terms of the share of NDF, ADF, and ADL fractions, which resulted in the greatest increase in the plant dry matter digestibility. Increasing nitrogen rates significantly reduced the quantity of analyzed fiber fractions, and increased grass digestibility.

**Keywords:** biostimulant; foliar application; NDF; ADF; ADL



**Citation:** Godlewska, A.; Ciepiela, G.A. Italian Ryegrass (*Lolium multiflorum* Lam.) Fiber Fraction Content and Dry Matter Digestibility Following Biostimulant Application against the Background of Varied Nitrogen Regime. *Agronomy* **2021**, *11*, 39. https://dx.doi.org/10.3390/agronomy11010039

Received: 28 November 2020
Accepted: 24 December 2020
Published: 27 December 2020



## 1. Introduction

Reduction of mineral fertilization has become one of crucial elements of ecosystem biological balance preservation [1]. Natural environment protection against contamination, coupled with production of high-quality healthy food, has become the main aim as well as a major challenge for science. Due to this, there have been more and more attempts made at designing new technologies employing, for example, unconventional fertilization which would supplement or even replace mineral fertilization [2].

Biostimulants, also called plant growth and development regulators, which represent this group of fertilization agents [3,4] have been increasingly applied in crop production [5]. In order to obtain such products, natural substances are used, e.g., algae, seaweeds (products whose commercial names are Algex and Kelpak SL), and humic and fulvic acids as well as synthetic compounds such as phenolic acids (the product whose commercial name is Asahi SL or Atonik) or titanite (the product whose commercial name is Tytanit) [6]. Frequently, biostimulants are multi-component products and they contain plant hormones or hormone-like substances, amino acids, betaines, peptides, proteins, sugars, aminopolysaccharides, lipids, vitamins, nucleotides, beneficial elements, phenolic compounds, sterols, etc. [7]. Many works have been published on the mechanism of activity of these compounds and a positive effect they have on yield and quality of various agricultural crops [8–10]. Attention should be paid to the fact that crop production should provide high yields of good quality [11], free of genetic modification, which is of particular importance in the era of unfavorable environmental conditions and global loss of ploughed land. Additionally, biostimulants influence the effectiveness of nutrient utilization by crop plants by affecting the biochemical, morphological, and physiological processes and ion uptake [12–15]; they increase water accumulation capacity as well as antioxidant content and chlorophyll production in plants [16,17]. Active compounds which biostimulants contain participate in the synthesis of other compounds in plants through participation in enzymatic processes [18]. Weather anomalies, which occur in Poland and worldwide with increasing frequency, weaken plant growth, and biostimulant application contributes to abiotic and biotic stress reduction in crop plants [19–22]. The plants' response includes increased resistance to drought and low temperatures, shortage of nutrients in the soil, and attack by pests and pathogens. However many authors point to the fact that the effect of biostimulants on crop plants goes in many directions and is often plant species- or even cultivar-specific [23,24]. According to literature [25,26], to a large degree, biostimulant effectiveness is also related to how well its choice meets agrotechnological needs as well as timing of application, application form, and rate.

Grasses represent the group of plants which has not been thoroughly researched so far as far as biostimulant application in their cultivation is concerned. Grass nutritive value is conditioned not only by total protein content but also structural and non-structural carbohydrates which are accompanied by lignins. Feed quality assessment is primarily based on the feed content of the following crude fiber fractions: NDF (neutral detergent fraction) and ADF (acid detergent fraction), both negatively influencing feed consumption, yield energy value and digestibility [27–29]. NDF and ADF contents in plants are determined by the amount of structural carbohydrates (cellulose and hemicellulose) and lignin. What is of importance is the fact that lignin, which is a non-digestible compound, when combined with cellulose is responsible for plants' mechanical resistance and, as a result, their ability to withstand the impact of negative biotic factors [30]. The concentration of these components is affected by a variety of factors. Literature on the subject [31,32] claims that biostimulants applied to crop plants may affect their chemical composition, including fiber content.

It was hypothesized in the present work that an application of natural and synthetic biostimulants will improve grass feeding value by fiber fraction reduction. An experiment was conducted to determine the effect of an application of biostimulants with different origins, against the background of varied nitrogen regime, on the share of NDF, ADF, and ADL (acid detergent lignin) in the crude fiber fraction of Italian ryegrass as well as its digestibility.

## 2. Materials and Methods

### *2.1. Experimental Design*

A field experiment was arranged as a randomized subblock design (split-plot) with three replicates at the Siedlce Experimental Unit of the University of Natural Sciences and Humanities in Poland (52.169° N, 22.280° E) in mid-August 2013. The plot area was 10 $m^2$ (2.5 m × 4.0 m). According to the Polish soil classification system [33], the soil used in the experiment was Hortic Anthrosol (WRB), developed from loamy sand. Based on the analysis performed at the Regional Chemical Station in Wesoła, it was found that the soil was of neutral reaction (pH in 1n KCL = 6.8), with a high humus content (3.78%), available phosphorus content ($H_2PO_4$—170 mg $kg^{-1}$), and magnesium content ($Mg^{2+}$—84 mg $kg^{-1}$), and average total nitrogen (N—1.3 g $kg^{-1}$) and available potassium ($K^+$—114 mg $kg^{-1}$) contents. The following factors were examined:

- type of biostimulant: Algex, Tytanit, Asahi SL, and a control (no biostimulant addition);
- nitrogen application rate: 0 (control); 120 and 180 kg·$ha^{-1}$.

Algex is an extract of the sea alga *Ascophyllum nodosum* which contains vitamins, amino acids, phytohormones (auxins, cytokinins, gibberellins), polysaccharides, betaine as well as macro- and microelements such as N—8%, P—3.6%, K—7%, B—0.036%, Zn—0.025%, Cu—0.009%, Fe—0.016%, Mn—0.036% and Mo—0.0036%. Algex is manufactured by Rosier, Moustier, Belgium.

Tytanit is produced by INTERMAG Ltd., Olkusz, Poland. Tytanit contains 8.5 g Ti per 1 $dm^3$ (0.8% m/m) in the form of Ti-ascorbate.

Asahi SL is produced by Arysta Life Science Ltd., Warsaw, Poland. The product contains the following phenolic compounds: sodium p-nitrophenolate—0.3%, sodium o-nitrophenolate—0.2%, and sodium 5-nitroguaiacolate—0.1%.

The grass (Italian ryegrass; *Lolium multiflorum* Lam.) sowing rate was 31 kg $ha^{-1}$ (TWG—thousand-grain weight—2.8 g, 11,071,429 kernels per hectare). In 2013, when seeds were sown, neither biostimulants nor nitrogen fertilizer was applied. In October 2013 only one cut was performed at the plant height of 6 cm. Over the study period (2014–2015), the cutting regime consisted of three harvests per year. Ammonium nitrate was applied three times per year. The total nitrogen amount was split into three equal rates which were applied to each regrowth (in spring after plants resumed their growth, 5 days after the first and second cutting was harvested). P and K fertilization was applied to all the plots. Phosphorus was applied once as triple superphosphate at the rate of 40 kg·$ha^{-1}$ $P_2O_5$ in the spring. The amount of potassium (160 kg·$ha^{-1}$ $K_2O$) was split into three equal rates and applied to each regrowth (in spring after plants resumed their growth, 5 days after the first and second cutting was harvested) as 60% potash salt. The biostimulants were sprayed as an aqueous solution. The spraying was performed before each cutting: the first application was three weeks before the first cutting, the second one two weeks after the first harvest and the last one three weeks after the second harvest. Biostimulant rates following each cut were applied as recommended by the manufacturer; that is, thinned with water to obtain the volume of 400 $dm^3$: Algex—6 $dm^3$ $ha^{-1}$, Tytanit—0.4 $dm^3$ $ha^{-1}$, Asahi SL—0.6 $dm^3$ $ha^{-1}$.

### *2.2. Weather Conditions*

Meteorological conditions during the study period were changeable and precipitation was very unevenly distributed (Table 1). The mean air temperature in the 2015 growing season was by 2 degrees lower than the mean across 2006–2015. By contract, unusual precipitation shortages occurred in September 2014, and April and August 2015.

**Table 1.** Meteorological condition in years 2014–2015 by Meteorological station in Siedlce.

| Years | Means Monthly Air Temperatures (°C) | | | | | | Means in Growing Season (IV–IX) (°C) |
|---|---|---|---|---|---|---|---|
| | Month | | | | | | |
| | IV | V | VI | VII | VIII | IX | |
| 2014 | 9.7 | 13.7 | 15.1 | 20.4 | 17.8 | 13.7 | 15.1 |
| 2015 | 8.1 | 12.3 | 16.5 | 14.3 | 21.1 | 8.8 | 13.5 |
| Mean of many years (2006–2015) | 9.6 | 14.0 | 17.2 | 19.9 | 18.4 | 13.6 | 15.5 |
| **Years** | **Monthly Precipitation (mm)** | | | | | | **Sum in Season (IV–IX) (mm)** |
| | **Month** | | | | | | |
| | **IV** | **V** | **VI** | **VII** | **VIII** | **IX** | |
| 2014 | 39.5 | 79.3 | 50.3 | 62.5 | 66.3 | 26.7 | 325 |
| 2015 | 29.7 | 100.6 | 41.1 | 68.3 | 12.0 | 77.5 | 329 |
| Mean of many years (2006–2015) | 26.9 | 68.9 | 64.6 | 55.8 | 65.3 | 44.3 | 326 |

### 2.3. Chemical Analysis

During each harvest, from each plot 0.5 kg green matter samples of grasses were taken from each plot to carry out chemical analyses. The samples were left to dry in a ventilated room. The airy dry matter was shredded and ground. The obtained material was subjected to chemical analysis to determine dry matter (by determining moisture content), crude fiber fractions of NDF, ADF, ADL, and dry matter digestibility. The method of determination was near-infrared spectroscopy (NIRS) using a NIRFlex N-500 spectrometer (Büchi Labortechnik AG., Flawil, Switzerland) and ready-to-use INGOT calibration applications.

### 2.4. Statistical Analysis

The statistical analysis of results was achieved by means of a three-way variance analysis run in the Statistica 13.3 Plus package (TIBCO Software, PaloAlto, CA, USA, 2017). Significance of differences between means was checked using the Tukey test at the significance level of $\alpha \leq 0.05$. Correlation coefficients were calculated using the program Statistica 13.3. TIBCO Software Inc. (2017). Statistica (data analysis software system), version 13. http://statistica.io.

Correlation coefficients were calculated using the program STATISTICA, version 10, (StatSoft Inc., Palo Alto, CA, USA, 2011).

## 3. Results and Discussion

It has been an increasingly frequent practice to take into account in the quality assessment of feed for ruminants the feed content of neutral detergent fraction (NDF) and acid detergent fraction (ADF). A high NDF level in feed, the fraction being the sum of hemicellulose, cellulose, and lignin, negatively affects its consumption whereas too high ADF level, the fraction being the sum of cellulose and lignin, reduces the total feed digestibility [27–29,34].

Results of the work reported here indicate that all the experimental factors significantly affected NDF and ADF contents in Italian ryegrass biomass (Tables 2 and 3). The effect of biostimulants on NDF and ADF levels in the grass biomass was consistent as they reduced NDF and ADF contents.

Algex proved to be the most effective biostimulant as it reduced both NDF and ADF contents by, respectively, 16.0 and 16.4% (when averaged across years). Moreover, Godlewska and Ciepiela [35] reported reduced contents of the fractions, by 6% on average, after an *Ecklonia maxima* extract was applied to grasses grown in pure stand. According to Joubert and Lefranc [32], active ingredients of seaweed extracts act as phytoactivators, which may alter the chemical composition of the plants treated. Literature reports mention the fact that an application of biostimulants may increase the plant accumulation of gibberellins, which, in turn, may contribute to an increase in the plant content of fiber [36]. Based on their research, Dayan [37] and Hedder [38] suggest that an application of biostimulants containing active compounds brings about an increase in the fiber content of plant cell walls. Further, Kocira et al. [39] observed an increase in NDF and ADF contents in bean seeds following an application of Kelpak which is a seaweed extract. Unfortunately, the research reported here does not seem to confirm this finding.

The effect of Asahi SL was seen as a decline in both fiber fractions, it being 9.59%. In their study, Szparaga et al. [26] carried out foliar applications of 0.1% Atonik (its other commercial name is Asahi SL) and observed a substantial decline in NDF content in soybean seeds. In turn, an application of 0.2% Atonik was followed by insignificant differences in NDF content in the tested plants. Such diverse findings may indicate that biostimulants have a varied effect on crop plants, it being often related to plant cultivar, biostimulant rate and application method.

**Table 2.** The effect of the biostimulants on content of neutral detergent (NDF) in Italian ryegrass by cut and nitrogen fertilization (g $kg^{-1}$ DM).

| Dose of N kg $ha^{-1}$ (B) | Biostimulant (A) | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cut (C) | | | Mean | Cut (C) | | | Mean |
| | | 1 | 2 | 3 | | 1 | 2 | 3 | |
| 0 | Control | 508 a | 542 a | 508 a | 519 a | 480 a | 496 a | 498 a | 491 a |
| | Algex | 409 b | 469 b | 423 b | 434 b | 398 b | 422 b | 409 b | 410 b |
| | Tytanit | 456 c | 499 c | 472 c | 476 c | 436 c | 460 c | 455 c | 450 c |
| | Asahi SL | 438 c | 493 c | 466 c | 466 d | 426 c | 445 d | 455 c | 442 d |
| 120 | Control | 489 a | 527 a | 497 a | 504 a | 455 a | 487 a | 484 a | 475 a |
| | Algex | 420 b | 441 b | 425 b | 429 b | 376 b | 422 b | 410 b | 403 b |
| | Tytanit | 450 c | 476 c | 455 c | 460 c | 429 c | 451 c | 444 c | 442 c |
| | Asahi SL | 444 c | 475 c | 452 c | 457 c | 412 d | 453 c | 438 c | 434 d |
| 180 | Control | 474 a | 520 a | 485 a | 493 a | 452 a | 482 a | 499 a | 478 a |
| | Algex | 388 b | 441 b | 413 b | 414 b | 386 b | 402 b | 402 b | 397 b |
| | Tytanit | 441 c | 477 c | 459 c | 459 c | 415 c | 453 c | 445 c | 437 c |
| | Asahi SL | 431 d | 477 c | 445 d | 451 d | 406 c | 447 c | 425 d | 426 d |
| Mean | Control | 490 a | 530 a | 497 a | 506 a | 462 a | 488 a | 494 a | 481 a |
| | Algex | 406 b | 450 b | 420 b | 426 b | 387 b | 415 b | 407 b | 403 b |
| | Tytanit | 449 c | 484 c | 462 c | 465 c | 427 c | 455 c | 448 c | 443 c |
| | Asahi SL | 438 d | 482 c | 454 d | 458 d | 415 d | 448 c | 439 d | 434 d |
| 0 | Mean | 453 a | 501 a | 467 a | 474 a | 435 a | 455 a | 454 a | 448 a |
| 120 | | 451 a | 480 b | 457 b | 463 b | 418 b | 453 a | 444 b | 438 b |
| 180 | | 433 b | 479 b | 451 c | 454 c | 414 b | 446 b | 443 b | 434 b |
| Mean | | 446 A | 486 B | 458 C | 464 | 423 A | 451 B | 447 C | 440 |
| $LSD_{0.05}$ | | A—2.92<br>B—2.30<br>C—2.30 | | AxB—5.05<br>AxC—5.05<br>BxC—3.98<br>AxBxC—8.75 | | A—4.13<br>B—3.26<br>C—3.26 | | AxB—7.16<br>AxC—7.16<br>BxC—5.64<br>AxBxC—12.39 | |

Different lower-case letters indicate that the values in the column for individual factors (Biostimulant and Dose N) and their interaction differ significantly. Different uppercase letters within line indicate significant differences.

The weakest effect was observed for Tytanit. Regardless of the remaining factors, an application of the biostimulants was followed by a decline in NDF and ADF contents (averaged across years) which amounted to 8.00 and 7.42%, respectively. Other workers [40] found that Tytanit caused reduction in NDF content in bean seeds compared with control samples. However, the product increased slightly ADF content, which corresponds to the results reported here.

Increasing nitrogen rates significantly reduced the amount of NDF and ADF in both study years, regardless of the remaining factors. The average drop in the amount of these fractions was 2.51%. Similarly, research by Szkutnik [41] revealed that high nitrogen rates contributed to a 5% decline in crude fiber amount in grasses, which is beneficial from the standpoint of feed value.

A significant relationship between nitrogen rates and biostimulant type should be highlighted. At all the levels of nitrogen fertilization, an application of biostimulants was followed by a decline in NDF and ADF contents in plants. Analysis of results indicated that the influence of biostimulants went in one direction and was very similar in both non-amended units and plots fertilized with 120 and 180 kg N $ha^{-1}$. It can be inferred that a beneficial reduction in NDF and ADF contents in plants due to an application of biostimulants occurred even in plots without nitrogen fertilization. Similar results were reported by Godlewska and Ciepiela [42] following an application of seaweed extract to Italian ryegrass. Du Jardin [43] and Malinowska et al. [44] mention the fact that an application of biostimulants reduces demand for fertilizers, which was confirmed in the study reported here. It is due to the fact that biostimulants contribute to modification of plant physiological functions [13,15] by providing them with potential benefits for their development [45]. Scientific works [16,46] have demonstrated a beneficial effect of biostimulants on rooting system development, water retention ability and photosynthesis extension, which is followed by increased nutrient uptake by crop plants.

**Table 3.** The effect of the biostimulants on content of acid detergent (ADF) in Italian ryegrass by cut and nitrogen fertilization (g $kg^{-1}$ DM).

| Dose of N kg $ha^{-1}$ (B) | Biostimulant (A) | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cut (C) | | | Mean | Cut (C) | | | Mean |
| | | 1 | 2 | 3 | | 1 | 2 | 3 | |
| 0 | Control | 328 a | 323 a | 349 a | 333 a | 303 a | 334 a | 311 a | 316 a |
| | Algex | 269 b | 275 b | 304 b | 282 b | 252 b | 298 b | 266 b | 272 b |
| | Tytanit | 298 c | 308 c | 327 c | 311 c | 287 c | 314 c | 287 c | 296 c |
| | Asahi SL | 293 d | 307 c | 324 c | 308 d | 278 d | 313 c | 280 d | 291 d |
| 120 | Control | 332 a | 319 a | 343 a | 332 a | 295 a | 338 a | 307 a | 313 a |
| | Algex | 274 b | 275 b | 279 b | 276 b | 245 b | 285 b | 264 b | 265 b |
| | Tytanit | 296 c | 295 c | 327 c | 306 c | 272 c | 321 c | 285 c | 293 c |
| | Asahi SL | 291 d | 294 c | 323 c | 303 d | 262 c | 311 d | 280 c | 285 d |
| 180 | Control | 318 a | 324 a | 343 a | 328 a | 297 a | 347 a | 303 a | 316 a |
| | Algex | 245 b | 265 b | 291 b | 267 b | 243 b | 277 b | 255 b | 258 b |
| | Tytanit | 286 c | 300 c | 309 c | 299 c | 274 c | 314 c | 285 c | 291 c |
| | Asahi SL | 277 d | 292 d | 306 c | 292 d | 259 d | 304 d | 278 d | 280 d |
| Mean | Control | 326 a | 322 a | 345 a | 331 a | 298 a | 339 a | 307 a | 315 a |
| | Algex | 263 b | 272 b | 292 b | 275 b | 247 b | 287 b | 262 b | 265 b |
| | Tytanit | 293 c | 301 c | 321 c | 305 c | 278 c | 316 c | 286 c | 293 c |
| | Asahi SL | 287 d | 298 d | 318 d | 301 d | 266 d | 310 d | 280 d | 285 d |
| 0 | Mean | 297 a | 303 a | 326 a | 309 a | 280 a | 315 a | 286 a | 294 a |
| 120 | | 298 a | 296 b | 318 b | 304 b | 268 b | 314 a | 284 a | 289 b |
| 180 | | 282 b | 295 b | 312 c | 297 c | 268 b | 311 a | 280 a | 286 c |
| Mean | | 292 A | 298 B | 319 C | 303 | 272 A | 313 B | 283 C | 290 |
| $LSD_{0.05}$ | | A—1.60<br>B—1.26<br>C—1.26 | | AxB—2.77<br>AxC—2.77<br>BxC—2.18<br>AxBxC—4.79 | | A—2.06<br>B—1.62<br>C—1.62 | | AxB—3.57<br>AxC—3.57<br>BxC—2.81<br>AxBxC—6.18 | |

Different lower-case letters indicate that the values in the column for individual factors (Biostimulant and Dose N) and their interaction differ significantly. Different uppercase letters within line indicate significant differences.

However, the mode of biostimulant action has not been fully established yet. Feitosa de Vasconcelos et al. [47] believe that biostimulants may affect plants in a direct and indirect way through soil response and plant microbiome. Moreover, Shahabivand et al. [2] and Bettoni et al. [48] mention the fact that an application of biostimulants in crop production results in lower demand for mineral fertilizers without threat to the natural environment.

According to Borowiecki [49] and Särkijärvi et al. [50], NDF content in grass biomass depends on the cut. The authors claim that the content is the highest in plants harvested at the second and third cut. The research reported here confirmed this thesis and demonstrated that, regardless of the experimental factors, NDF and ADF contents were the highest in grasses harvested at the final cuts. The amount of these fractions at the second and third cut was significantly higher compared with the first cut, which was confirmed by statistical analysis. Research by Ciepiela et al. [51] has demonstrated that increased hemicellulose and lignin contents in the first and second cut of Orchard grass and Braun's festulolium simultaneously increase NDF content in the plants.

The test Italian ryegrass was also compared in terms of lignin amount (ADL fraction). Lignin, together with cellulose and hemicellulose, is a component of the cell wall but it is not digested, thus reducing feed digestibility. Lignin increases plant resistance by forming lignin barriers which hinder pathogens [52]. Lignin content (ADL fraction) determined in the study reported here was significantly affected by all the experimental factors (Table 4). Statistical analysis revealed a significant interaction of all the factors analyzed An application of each biostimulant was followed by a significant drop in the plant content of lignin compared with control.

**Table 4.** The effect of the biostimulants on content of acid detergent lignin (ADL) in Italian ryegrass by cut and nitrogen fertilization (g kg$^{-1}$ DM).

| Dose of N kg ha$^{-1}$ (B) | Biostimulant (A) | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cut (C) | | | Mean | Cut (C) | | | Mean |
| | | 1 | 2 | 3 | | 1 | 2 | 3 | |
| 0 | Control | 42.9 a | 41.7 a | 44.2 a | 42.9 a | 36.9 a | 49.9 a | 42.2 a | 43.0 a |
| | Algex | 34.4 b | 33.5 b | 41.7 b | 36.5 b | 29.7 b | 41.7 b | 35.2 b | 35.5 b |
| | Tytanit | 36.3 c | 36.6 c | 44.2 c | 39.0 c | 33.9 c | 47.6 c | 38.4 c | 40.0 c |
| | Asahi SL | 36.1 c | 38.1 d | 44.1 c | 39.4 c | 33.8 c | 44.0 d | 38.4 c | 38.8 d |
| 120 | Control | 43.0 a | 41.6 a | 45.2 a | 43.3 a | 35.4 a | 50.1 a | 41.5 a | 42.3 a |
| | Algex | 31.5 b | 32.3 b | 34.7 b | 32.8 b | 28.7 b | 38.8 b | 34.5 b | 34.0 b |
| | Tytanit | 37.2 c | 38.2 c | 40.9 c | 38.8 c | 31.0 c | 46.2 c | 37.6 c | 38.3 c |
| | Asahi SL | 36.9 c | 35.2 d | 37.2 d | 36.4 d | 30.9 c | 47.3 d | 36.0 d | 38.1 c |
| 180 | Control | 39.6 a | 40.5 a | 44.2 a | 41.4 a | 35.9 a | 47.7 a | 40.2 a | 41.3 a |
| | Algex | 28.2 b | 33.6 b | 41.7 b | 34.5 b | 26.6 b | 35.6 b | 34.3 b | 32.2 b |
| | Tytanit | 33.7 c | 35.4 c | 44.2 c | 37.8 c | 31.2 c | 44.1 c | 37.4 c | 37.6 c |
| | Asahi SL | 33.2 c | 35.3 c | 44.1 c | 37.5 c | 28.8 d | 40.7 d | 37.4 c | 35.6 d |
| Mean | Control | 41.8 a | 41.3 a | 44.5 a | 42.6 a | 36.1 a | 49.2 a | 41.3 a | 42.2 a |
| | Algex | 31.4 b | 33.1 b | 39.4 b | 34.6 b | 28.3 b | 38.7 b | 34.7 b | 33.9 b |
| | Tytanit | 35.7 c | 36.8 c | 43.1 c | 38.5 c | 32.0 c | 45.9 c | 37.8 c | 38.6 c |
| | Asahi SL | 35.4 c | 36.2 c | 41.8 d | 37.8 d | 31.2 c | 44.0 d | 37.3 c | 37.5 d |
| 0 | Mean | 37.4 a | 37.5 a | 43.6 a | 39.5 a | 33.6 a | 45.8 a | 38.6 a | 39.3 a |
| 120 | | 37.1 b | 36.8 b | 39.5 b | 37.8 b | 31.5 b | 45.6 a | 37.4 b | 38.2 b |
| 180 | | 33.6 c | 36.2 c | 43.6 c | 37.8 b | 30.6 c | 42.0 b | 37.3 b | 36.7 c |
| Mean | | 36.1 A | 36.8 B | 42.2 C | 38.4 | 31.9 A | 44.5 | 37.8 | 38.0 |
| $LSD_{0.05}$ | | A—0.32<br>B—0.56<br>C—0.97 | | AxB—0.56<br>AxC—0.56<br>BxC—0.44<br>AxBxC—0.97 | | A—0.31<br>B—0.24<br>C—0.24 | | AxB—0.53<br>AxC—0.53<br>BxC—0.42<br>AxBxC—0.92 | |

Different lower-case letters indicate that the values in the column for individual factors (Biostimulant and Dose N) and their interaction differ significantly. Different uppercase letters within line indicate significant differences.

Moreover, it should be pointed out that the influence of Algex on the characteristic discussed was twice as pronounced as the effect of either Tytanit or Asahi (the respective declines in lignin content were 16% vs. 8.0 and 9.59% compared with the control unit). Further, in the study by Ciepiela et al. [51], an application of algae (*Ecklonia maxima*) extract contributed to a decline in ADL fraction in the grasses they cultivated, it being barely 8.77%. Asahi contains phenolic compounds which naturally occur in plants and are components of every living cell. According to Chen et al. [53], phenolic acids, particularly ferulic acid, are precursors for the production of other compounds such as lignin. Based on this theory, it can be surmised that the biostimulant Asahi should increase ADL content, which was not confirmed in the study reported here. Similarly, Szparaga et al. [26] observed a 3.5% decline in lignin content following an application of Atonik (also known as Asahi) solution compared with control. However, these same authors recorded an increase in ADL content in soybean seeds following an application of Tytanit, which is inconsistent with the findings of the present work. This fact is yet another proof that the impact of biostimulants may be closely related to crop plant species.

Nitrogen fertilizer regime significantly reduced ADL content in Italian ryegrass. In both the study years, the amount of ADL fraction in plants harvested from units fertilized with nitrogen applied at the rate of 120 and 180 kg ha$^{-1}$ was significantly lower compared with non-fertilized control. What is interesting is the fact that in the first study year lignin amount in plants was the same regardless of the rate of mineral fertilizer. The findings of this study concur with reports by Poisa et al. [54] whose work revealed a significant relationship between nitrogen fertilizer level and the amount of lignin in plants.

There were also recorded significant differences between ADL contents between cuts. In the first study year, lignin content in Italian ryegrass increased at successive cuts. In the following study year, plants harvested at the first cut contained the least lignin whereas the most extensive plant lignification was observed at the second cut.

In the study reported here, assessment of Italian ryegrass biomass digestibility was attempted and this characteristic was found to be significantly affected by all the experimental factors (Table 5). Italian ryegrass harvested in plots treated with biostimulants had significantly higher digestibility compared with plants harvested in non-treated plots. This was due to the fact that the biostimulants reduced NDF, ADF and ADL contents, the fractions known to hinder digestibility. Algex was the biostimulant whose contribution to improved digestibility was the greatest (on average, by 35.2% compared with control). Foliar application of Asahi and Tytanit had a less positive effect (means across years: 21.7 and 13.1%, respectively).

**Table 5.** The effect of the biostimulants on digestibility of Italian ryegrass (%) by cut and nitrogen fertilization.

| Dose of N kg ha$^{-1}$ (B) | Biostimulant (A) | 2014 | | | | 2015 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cut (C) | | | Mean | Cut (C) | | | Mean |
| | | 1 | 2 | 3 | | 1 | 2 | 3 | |
| 0 | Control | 40.3 a | 36.7 a | 39.8 a | 38.9 a | 48.3 a | 38.8 a | 45.0 a | 44.0 a |
| | Algex | 67.2 b | 48.8 b | 59.9 b | 58.6 b | 62.6 b | 48.4 b | 55.2 b | 55.4 b |
| | Tytanit | 57.4 c | 41.1 c | 47.4 c | 48.6 c | 55.6 c | 43.2 c | 47.6 c | 48.8 c |
| | Asahi SL | 62.4 d | 45.0 d | 51.5 d | 52.9 d | 58.1 d | 43.5 c | 52.6 d | 51.4 d |
| 120 | Control | 49.6 a | 36.6 a | 45.6 a | 44.0 a | 53.5 a | 37.6 a | 45.0 a | 45.4 a |
| | Algex | 64.4 b | 54.8 b | 60.0 b | 59.7 b | 70.2 b | 51.9 b | 58.1 b | 60.1 b |
| | Tytanit | 58.3 c | 42.9 c | 48.0 c | 49.7 c | 58.4 c | 42.0 c | 47.4 c | 49.3 c |
| | Asahi SL | 58.8 c | 50.9 d | 55.5 d | 55.1 d | 61.3 d | 46.4 d | 53.5 d | 53.7 d |
| 180 | Control | 54.5 a | 40.3 a | 42.8 a | 45.9 a | 54.8 a | 42.2 a | 47.3 a | 48.1 a |
| | Algex | 73.9 b | 55.0 b | 53.3 b | 60.7 b | 67.6 b | 59.7 b | 66.7 b | 64.7 b |
| | Tytanit | 61.6 c | 48.7 c | 46.4 c | 52.3 c | 60.0 c | 45.4 c | 50.4 c | 51.9 c |
| | Asahi SL | 64.2 c | 50.0 c | 48.8 c | 54.4 d | 64.2 d | 50.6 d | 53.5 d | 56.1 d |
| Mean | Control | 48.1 a | 37.9 a | 42.7 a | 42.9 a | 52.2 a | 39.5 a | 45.8 a | 45.8 a |
| | Algex | 68.5 b | 52.9 b | 57.7 b | 59.7 b | 66.8 b | 53.4 b | 60.0 b | 60.1 b |
| | Tytanit | 59.1 c | 44.3 c | 47.3 c | 50.2 c | 58.0 c | 43.5 c | 48.5 c | 50.0 c |
| | Asahi SL | 61.8 d | 48.6 d | 51.9 d | 54.1 d | 61.2 d | 46.8 d | 53.2 d | 53.7 d |
| 0 | Mean | 56.8 a | 42.9 a | 49.7 a | 49.8 a | 56.1 a | 43.5 a | 50.1 a | 49.9 a |
| 120 | | 57.8 b | 46.3 b | 52.3 b | 52.1 b | 60.9 b | 44.5 a | 51.0 a | 52.1 b |
| 180 | | 63.6 c | 48.5 c | 47.8 c | 53.3 c | 61.6 b | 49.5 b | 54.5 b | 55.2 c |
| Mean | | 59.4 A | 45.9 B | 49.9 C | 51.7 | 59.6 A | 45.8 B | 51.9 C | 52.4 |
| $LSD_{0.05}$ | | A—1.01<br>B—0.80<br>C—0.80 | | AxB—1.75<br>AxC—1.75<br>BxC—1.38<br>AxBxC—3.04 | | A—0.92<br>B—0.72<br>C—0.72 | | AxB—1.59<br>AxC—1.59<br>BxC—1.25<br>AxBxC—2.75 | |

Different lower-case letters indicate that the values in the column for individual factors (Biostimulant and Dose N) and their interaction differ significantly. Different uppercase letters within line indicate significant differences.

Statistical analysis demonstrated a significant interaction of biostimulants and nitrogen fertilizer regime. At each nitrogen fertilization level, biostimulant application significantly increased Italian ryegrass digestibility. The biostimulants/nitrogen regime interaction revealed that the digestibility of Italian ryegrass grown in plots without biostimulant application was the highest at the nitrogen rate of 180 kg ha$^{-1}$ and amounted to 47.0% (averaged across years). An application of Algex, Tytanit, and Asahi improved Italian ryegrass digestibility (respectively, 57.0, 48.7, and 52.2%) which was higher even in units without nitrogen fertilization compared with the rate of 180 kg N ha$^{-1}$ but without biostimulant application. The results indicate that it is possible to at least partially replace nitrogen fertilization with an application of biostimulants in Italian ryegrass cultivation. Statistical analysis demonstrated a significant interaction between biostimulants and nitrogen fertilizer regime. At each level of nitrogen fertilization, an application of each biostimulant significantly increased Italian ryegrass digestibility.

Based on the obtained results, the highest digestibility was found in plants harvested at the first cut, it being the lowest in plants of the second cut. Such relationships were present in both the study years. However, Stachowicz [55] claims that feed digestibility declines at successive cuts. Lack of literature on the issue renders its discussion impossible.

The correlation coefficient was used to analyze relationships between the content of individual fractions in Italian ryegrass harvested at individual cuts and its dry matter digestibility (Table 6). The obtained values of this coefficient demonstrated that there was a significant negative relationship between the NDF and ADF contents of the test grasses and their dry matter digestibility at each cut and regardless of the cuts. A decline in Italian ryegrass dry matter digestibility was probably due to an increase in the dry matter content of cellulose and hemicellulose, both included in NDF and ADF, and an increase in lignin content.

**Table 6.** Correlation coefficient between dry matter digestibility of Italian ryegrass and the content of crude fiber fraction depending on the cut.

| Cut | Crude Fiber Fractions | | |
|---|---|---|---|
| | NDF | ADF | ADL |
| 1 | −0.919103 * | −0.858000 * | −0.809516 * |
| 2 | −0.767312 * | −0.648491 * | −0.643842 * |
| 3 | −0.837756 * | −0.733603 * | −0.696548 * |
| Independently of cut | −0.844911 * | −0.739215 * | −0.791868 * |

* Statistically significant value.

## 4. Conclusions

There were confirmed positive effects resulting from an application of biostimulants in Italian ryegrass cultivation. There was confirmed the assumed hypothesis that an application of both natural and synthetic biostimulants will make it possible to improve the feeding value of grasses by reducing the fiber fraction. Particular attention should be paid to the biostimulant Algex whose application in Italian ryegrass cultivation produced the most beneficial response in terms of the share of NDF, ADF, and ADL fractions, which resulted in the greatest increase in the plant dry matter digestibility. It should be pointed out that Italian ryegrass digestibility following an application of all the experimental biostimulants was higher even in non-fertilized plots than the digestibility for non-treated units fertilized with 180 kg ha$^{-1}$.

Increasing nitrogen rates significantly reduced the quantity of analyzed fiber fractions and increased Italian ryegrass digestibility.

The amounts of the analyzed components in grasses was significantly affected by the cuts. NDF, ADF, and ADL contents were significantly lowest at the first cuts whereas their digestibility was the highest.

The findings of the study reported here indicate that further research is necessary including other plant species as well as varied dates and rates of an application of biostimulants. Such research would expand knowledge of mechanisms of biostimulant action followed by implementation in agricultural practice.

**Author Contributions:** Conceptualization, A.G. and G.A.C.; methodology, G.A.C; validation, A.G. and G.A.C.; formal analysis, A.G. and G.A.C.; investigation, A.G. and G.A.C.; resources, A.G. and G.A.C.; data curation, G.A.C.; writing—original draft preparation, A.G.; writing—review and editing, A.G., and G.A.C.; visualization, A.G. and G.A.C.; supervision, A.G. and G.A.C.; project administration, A.G. and G.A.C.; funding acquisition, A.G. and G.A.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** The results of the research carried out under the research theme No. 41/20/B were financed from the science grant granted by the Ministry of Science and Higher Education.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data sharing not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Pramanick, B.; Brahmachari, K.; Ghosh, A.; Zodape, S.T. Effect of seaweed saps on growth and yield improvement of trans-planted rice in old alluvial soil of West Bengal. *Bangladsh J. Bot.* **2014**, *43*, 53–58. [CrossRef]
2. Shahabivand, S.; Padash, A.; Aghaee, A.; Nasiri, Y.; Rezaei, P.F. Plant biostimulants (Funneliformis mosseae and humic substances) rather than chemical fertilizer improved biochemical responses in peppermint. *Iran. J. Plant Physiol.* **2018**, *8*, 2333–2344.
3. Colla, G.; Rouphael, Y. Biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 1–2. [CrossRef]
4. Kocira, S.; Szparaga, A.; Kocira, A.; Czerwińska, E.; Wójtowicz, A.; Bronowicka-Mielniczuk, U.; Koszel, M.; Findura, P. Modeling Biometric Traits, Yield and Nutritional and Antioxidant Properties of Seeds of Three Soybean Cultivars Through the Application of Biostimulant Containing Seaweed and Amino Acids. *Front. Plant Sci.* **2018**, *9*, 388. [CrossRef]
5. Brown, P.H.; Saa, S. Biostimulants in agriculture. *Front. Plant Sci.* **2015**, *6*, 671. [CrossRef]
6. Talar-Krasa, M.; Wolski, K.; Biernacik, M. Biostimulants and possibilities of their usage in grassland. *Grassl. Sci.* **2019**, *65*, 205–209. [CrossRef]
7. Yakhin, O.I.; Lubyanov, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in Plant Science: A Global Perspective. *Front. Plant Sci.* **2017**, *7*, 2049. [CrossRef]
8. Rouphael, Y.; Giordano, M.; Cardarelli, M.; Cozzolino, E.; Mori, M.; Kyriacou, M.C.; Bonini, P.; Colla, G. Plant and sea-weed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. *Agronomy* **2018**, *8*, 126. [CrossRef]
9. Di Mola, I.; Ottaiano, L.; Cozzolino, E.; Senatore, M.; Giordano, M.; El-Nakhel, C.; Sacco, A.; Rouphael, Y.; Colla, G.; Mori, M. Plant-Based Biostimulants Influence the Agronomical, Physiological, and Qualitative Responses of Baby Rocket Leaves under Diverse Nitrogen Conditions. *Plants* **2019**, *8*, 522. [CrossRef]
10. Drobek, M.; Frąc, M.; Cybulska, J. Plant biostimulants: Importance of the quality and yield of horticultural crops and the im-provement of plant tolerance to abiotic stress—A review. *Agronomy* **2019**, *9*, 335. [CrossRef]
11. Eckardt, N.A.; Cominelli, E.; Galbiati, M.; Tonelli, C. The Future of Science: Food and Water for Life. *Plant Cell* **2009**, *21*, 368–372. [CrossRef] [PubMed]
12. Rayorath, P.; Jithesh, M.N.; Farid, A.; Khan, W.; Palanisamy, R.; Hankins, S.D.; Critchley, A.T.; Prithiviraj, B. Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L.) Le Jol. using a model plant, *Arabidopsis thaliana* (L.) Heynh. *Environ. Boil. Fishes* **2007**, *20*, 423–429. [CrossRef]
13. Craigie, J.S. Seaweed extract stimuli in plant science and agriculture. *Environ. Boil. Fishes* **2011**, *23*, 371–393. [CrossRef]
14. Parađiković, N.; Vinković, T.; Vrček, I.V.; Žuntar, I.; Bojić, M.; Medić-Šarić, M. Effect of natural biostimulants on yield and nutritional quality: An example of sweet yellow pepper (*Capsicum annuum* L.) plants. *J. Sci. Food Agric.* **2011**, *91*, 2146–2152. [CrossRef] [PubMed]
15. Alam, M.Z.; Braun, G.; Norrie, J.; Hodges, D.M. Ascophyllum extract application can promote plant growth and root yield in carrot associated with increased root-zone soil microbial activity. *Can. J. Plant Sci.* **2014**, *94*, 337–348. [CrossRef]
16. Sharma, H.S.S.; Fleming, C.C.; Selby, C.; Rao, J.R.; Martin, T. Plant biostimulants: A review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *Environ. Boil. Fishes* **2013**, *26*, 465–490. [CrossRef]
17. Lyu, S.; Wei, X.; Chen, J.; Wang, C.; Wang, X.-M.; Pan, D. Titanium as a Beneficial Element for Crop Production. *Front. Plant Sci.* **2017**, *8*, 597. [CrossRef]
18. El-Nabarawy, M.A. Mitigation of dark induced senescence. 1—By some amino acids. *Ann. Agric. Sci. Moshtohor* **2001**, *39*, 225–232.
19. Spinelli, F.; Fiori, G.; Noferini, M.; Sprocatti, M.; Costa, G. A novel type of seaweed extract as a natural alternative to the use of iron chelates in strawberry production. *Sci. Hortic.* **2010**, *125*, 263–269. [CrossRef]
20. Haider, M.W.; Ayyub, C.H.M.; Pervez, M.A.; Asad, H.U.; Manan, A.; Raza, S.A.; Ashraf, I. Impact of foliar application of seaweed extract on growth, yield and quality of potato (*Solanum tuberosum* L.). *Soil. Environ.* **2012**, *31*, 157–162.
21. Povero, G.; Mejia, J.F.; Di Tommaso, D.; Piaggesi, A.; Warrior, P. A Systematic Approach to Discover and Characterize Natural Plant Biostimulants. *Front. Plant Sci.* **2016**, *7*, 435. [CrossRef] [PubMed]

22. Renuka, N.; Guldhe, A.; Prasanna, R.; Singh, P.; Bux, F. Microalgae as multi-functional options in modern agriculture: Current trends, prospects and challenges. *Biotechnol. Adv.* **2018**, *36*, 1255–1273. [CrossRef] [PubMed]
23. Sultana, V.; Ehteshamul-Haque, S.; Ara, J.; Athar, M. Comparative efficacy of Brown, Green and red Seaweeds in the control of Root infesting fungi and okra. *Int. J. Environ. Sci. Technol.* **2005**, *2*, 129–132. [CrossRef]
24. Matysiak, K. Kelpak—A natural regulator of plant growth and development. *Sel. Ecol. Issues Mod. Agric.* **2005**, *375*, 188–193.
25. Ertani, A.; Pizzeghello, D.; Baglieri, A.; Cadili, V.; Tambone, F.; Gennari, M.; Nardi, S. Humic-like substances from agro-industrial residues affect growth and nitrogen assimilation in maize (*Zea mays* L.) plantlets. *J. Geochem. Explor.* **2013**, *129*, 103–111. [CrossRef]
26. Szparaga, A.; Kocira, S.; Kocira, A.; Czerwińska, E.; Swieca, M.; Lorencowicz, E.; Kornas, R.; Koszel, M.; Oniszczuk, T. Modification of growth, yield, and the nutraceutical and antioxidative potential of soybean through the use of synthetic bi-ostimulants. *Front. Plant Sci.* **2018**, *9*, 1401. [CrossRef]
27. Brzóska, F.; Śliwiński, B. Jakość pasz objętościowych w żywieniu przeżuwaczy i metody jej oceny Cz. II. Metody analizy i oceny wartości pokarmowej pasz objętościowych. *Wiad. Zootech.* **2011**, *4*, 57–68.
28. Belanger, G.; Virkajarvi, P.; Duru, M.; Tremblay, G.F.; Saarijarvi, K. Herbage nutritive in less–favoured areas of cool regions. *Grassl. Sci. Eur.* **2013**, *18*, 57–70.
29. Baert, J.; Van Waes, C. Improvement of the digestibility of tall fescue (Festuca arundinacea Schreb.) inspired by perennial ryegrass (*Lolium perenne* L.). *Grassl. Sci. Eur.* **2014**, *19*, 172–174.
30. Bennett, R.N.; Wallsgrove, R.M. Secondary metabolites in plant defence mechanisms. *New Phytol.* **1994**, *127*, 617–633. [CrossRef]
31. Karr-Lilienthal, L.; Kadzere, C.; Grieshop, C.; Fahey, G. Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: A review. *Livest. Prod. Sci.* **2005**, *97*, 1–12. [CrossRef]
32. Joubert, J.M.; Lefranc, G. Seaweed biostimulants in agriculture: Recent studies on mode of action two types of products from alga: Growth and nutri-tion stimulants and stimulants of plant Demence reactions. Book of abstracts: Biostimulants in modern agriculture, Warsaw, Poland, 2008; p. 16.
33. Kabała, C.; Charzyński, P.; Chodorowski, J.; Drewnik, M.; Glina, B.; Greinert, A.; Hulisz, P.; Jankowski, M.; Jonczak, J.; Łabaz, B.; et al. Polish Soil Classification, 6th edition—principles, classification scheme and correlations. *Soil Sci. Ann.* **2019**, *70*, 71–97.
34. Van-Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
35. Godlewska, A.; Ciepiela, G.A. The effect of growth regulator on dry matter yield and some chemical components in selected grass species and cultivars. *Soil Sci. Plant Nutr.* **2016**, *62*, 297–302. [CrossRef]
36. Silva, R.D.A.; Santos, J.L.; Oliveira, L.S.; Soares, M.R.S.; Dos Santos, S.M.S. Biostimulants on mineral nutrition and fiber quality of cotton crop. *Rev. Bras. Eng. Agrícola Ambient.* **2016**, *20*, 1062–1066. [CrossRef]
37. Dayan, J.; Schwarzkopf, M.; Avni, A.; Aloni, R. Enhancing plant growth and fiber production by silencing GA 2-oxidase. *Plant Biotechnol. J.* **2010**, *8*, 425–435. [CrossRef]
38. Hedden, P.; Thomas, S.G. Gibberellin biosynthesis and its regulation. *Biochem. J.* **2012**, *444*, 11–25. [CrossRef]
39. Kocira, S.; Szparaga, A.; Findura, P.; Treder, K. Modification of Yield and Fiber Fractions Biosynthesis in *Phaseolus vulgaris* L. by Treatment with Biostimulants Containing Amino Acids and Seaweed Extract. *Agronomy* **2020**, *10*, 1338. [CrossRef]
40. Szparaga, A.; Kuboń, M.; Kocira, S.; Czerwińska, E.; Pawłowska, A.; Hara, P.; Kobus, Z.; Kwaśniewski, D. Towards Sustainable Agriculture—Agronomic and Economic Effects of Biostimulant Use in Common Bean Cultivation. *Sustainability* **2019**, *11*, 4575. [CrossRef]
41. Szkutnik, J.; Kacorzyk, P.; Szewczyk, W. The content change of total protein and crude fibre depending on the dose of ferti-lization and phonological phase of grasses. *Grassl. Sci. Pol.* **2012**, *15*, 185–191.
42. Godlewska, A.; Ciepiela, G. Carbohydrate and lignin contents in perennial ryegrass (*Lolium perenne* L.) treated with sea bamboo (*Ecklonia maxima*) extract against the background of nitrogen fertilisation regime. *Appl. Ecol. Environ. Res.* **2020**, *18*, 6087–6097. [CrossRef]
43. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
44. Malinowska, E.; Jankowski, K.; Kania, P.; Gałecka, M. The Effect on Tytanit Foliar Application on the Yield and Nutritional Value of Festulolium braunii. *Agronomy* **2020**, *10*, 848. [CrossRef]
45. Du Jardin, P. *The Science of Plant Biostimulants—A Bibliographic Analysis, European Commission (Contract 30–CE0455515/00–96, Ad hoc Study on Biostimulants Products)*; European Commission: Luxembourg, Germany, 2012.
46. Khan, W.; Rayirath, U.P.; Subramanian, S.; Jithesh, M.N.; Rayorath, P.; Hodges, D.M.; Critchley, A.T.; Craigie, J.S.; Norrie, J.; Prithiviraj, B. Seaweed Extracts as Biostimulants of Plant Growth and Development. *J. Plant Growth Regul.* **2009**, *28*, 386–399. [CrossRef]
47. De Vasconcelos, A.C.F.; Chaves, L.H.G. Biostimulants and Their Role in Improving Plant Growth under Abiotic Stresses. In *Biostimulants in Plant Science*; IntechOpen: London, UK, 2020.
48. Bettoni, M.M.; Mogor, A.F.; Pauletti, V.; Goicoechea, N.; Aranjuelo, I.; Garmendia, I. Nutritional quality and yield of onion as affected by different application methods and doses of humic substances. *J. Food Comp. Anal.* **2016**, *51*, 37–44. [CrossRef]
49. Borowiecki, J. The effect of nitrogen fertilization on fielding and feeding value of Festulolium braunii var. *Felopa. Pamiętnik Puławski* **2002**, *131*, 39–48.
50. Särkijärvi, S.; Niemeläinen, O.; Sormunen-Cristian, R. Changes in chemical composition of different grass species and -mixtures in equine pasture during grazing season. *Energy Protein Metab. Nutr.* **2012**, *132*, 45–48. [CrossRef]
51. Ciepiela, G.A.; Godlewska, A.; Jankowska, J. The effect of seaweed Ecklonia maxima extract and mineral nitrogen on fodder grass chemical composition. *Environ. Sci. Pollut. Res.* **2015**, *23*, 2301–2307. [CrossRef]

52. Karolewski, P.; Jagodziński, A.M. Share of carbon in defense compounds against biotic factors in woody plants. *Sylwan* **2013**, *157*, 831–841.
53. Chen, J.-Y.; Wen, P.-F.; Kong, W.-F.; Pan, Q.-H.; Zhan, J.; Li, J.-M.; Wan, S.-B.; Huang, W.-D. Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in harvested grape berries. *Postharvest Biol. Technol.* **2006**, *40*, 64–72. [CrossRef]
54. Poisa, L.; Adamovičs, A.; Platače, R.; Teirumnieka, Ē. Evaluationof the factors that affect the lignin kontent in the Reed canarygrass *Phalaris arundinacea* L.) in Latvia. In Proceedings of the Word Renewable Energy Congress, Linkőping, Sweden, 8–13 May 2011; pp. 224–231.
55. Stachowicz, T. *Racjonalne Wykorzystanie Użytków Zielonych w Gospodarstwie Ekologicznym*; Centrum Doradztwa Rolniczego w Brwinowie oddział w Radomiu: Radom, Poland, 2010; pp. 9–24.