Original Research

Changes in Protein Compounds and Monosaccharides in Select Grass Species Following Application of a Seaweed Extract

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Abstract

The objective of our study was to determine changes in protein and monosaccharide content in select grass species due to an application of the kelp species *Ecklonia maxima* extract. The field experiment was arranged as a randomized sub-block design (split-split-plot) with three replicates. The following factors were examined: growth stimulant with the trade name Kelpak SL, which was either applied at the rate of 2,000 $dm^3 \cdot ha^{-1}$ or not applied, pure-sown grass species, and cultivars (*Dactylis glomerata*, cv. Amila and Tukan; *Festulolium braunii*, cv. Felopa and Agula). Protein compounds and monosaccharides were determined in the dry matter of the tested grasses. The biostimulant Kelpak significantly increased both protein compounds and monosaccharides as well as the carbohydrate-protein ratio in the plants. The grass species and their cultivars had different levels of protein compounds and carbohydrates.

Keywords: *Dactylis glomerata*, *Festulolium braunii*, Kelpak, *Ecklonia maxima*

Introduction

Vast seaweed resources off the coast of South Africa, of which the kelp *Ecklonia maxima* is the dominant species, are a renewable source of raw material with a range of uses. Alginates and carrageen extracted from seaweeds are used worldwide in the food industry as stabilizers, thickeners, and emulsifiers [1]. Fresh seaweed leaves are fed to farmed snails [2] and over 10,000 tons of fresh matter per year are used in this way. Algae are applied as organic manure in the areas where they are harvested [3, 4] and used as a soil conditioner to improve the structure and water-holding characteristics of soil [5]. Despite the fact that seaweeds are so extensively used, only a small fraction of their yearly output is used. This fact provided the stimulus for more studies on these organisms.

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Many works have demonstrated that algae are able to bind heavy metals [6, 7] and can be used as biosorbents in e.g. sewage treatment plants [8, 9]. Scientists made a major breakthrough when they developed methods of obtaining stable extracts from seaweeds. Seaweed extracts, called liquid manures [10], contain growth-stimulating plant hormones, macro-elements such as Ca, K and P, micro-elements (Fe, Cu, Zn, Co, B, Mo, Mn) [11], vitamins, and amino acids [12]. Kelpak, an *Ecklonia maxima* extract, represents products based on natural plant substances. Research has proved a positive impact of this product on crop plants as it increases output and yield quality, and plant resistance to drought and soil deficiency of nutrients. There are a number of factors that contribute to the beneficial effect of seaweed extract application. The factors may act synergistically but the mode of their action has not yet been fully understood [13, 14]. Products containing plant growth and development regulators modifying physiological functions in the plant have

Table 1. Meteorological conditions in growing season 2010-12 by the meteorological station in Siedlce.

Year	Mean air temperatures (ºC)						Means in growing season
	IV	V	VI	VII	VIII	IX	
2010	8.9	14.0	17.4	21.6	19.8	11.8	15.6
2011	9.8	13.4	18.1	18.2	18.1	14.4	15.3
2012	9.0	14.5	16.4	20.4	18.0	14.2	15.4
Means of multiple years (2002-12)	7.7	10.0	16.1	19.3	18.0	13.0	14.0
Year	Monthly precipitation (mm)						Sum in growing season
	IV	V	VI	VII	VIII	IX	
2010	10.7	93.2	62.6	77.0	106.3	109.9	459.7
2011	38.1	55.6	44.3	204.2	55.4	26.6	424.2
2012	40.3	59.7	118.7	41.4	64.1	30.8	355.0
Means of multiple years (2002-12)	52.3	50.0	68.2	45.7	66.8	60.7	343.7

received increased interest in recent years. The activity of biostimulants obtained from seaweeds depends to a greater extent on the plant species or even cultivar and timing of application than the rate at which they are applied [15].

The objective of our study was to determine changes in protein and monosaccharide contents in select grass species due to the application of *Ecklonia maxima* extract.

Materials and Methods

A field experiment was arranged as a randomized sub-block design (split-split-plot) with three replicates at the Siedlce Experimental Unit of the University of Natural Sciences and Humanities in late April 2009. The plot area was 10 m^2. The soil of the experimental site represents average soils, Hortic Anthrosol (WRB). Prior to the experiment set-up the characteristics of the soil were as follows: neutral pH (pH in 1n KCl = 7.2), high humus content (3.78%), high available phosphorus and magnesium contents (P_2O_5 – 900 mg·kg^{-1}, Mg – 84 mg·kg^{-1}), and average total nitrogen and available potassium contents (N – 1.8 g·kg^{-1} d.m., K_2O – 19 mg·kg^{-1}). The following factors were examined:

- biostimulant with the trade name Kelpak SL applied at 2,000 dm^3·ha^{-1} and a control – no biostimulant,
- pure stands of grass species and cultivars grown in monoculture
- *Dactylis glomerata*, cv. Amila and Tukan,
- *Festulolium braunii*, cv. Felopa and Agula.

Kelpak is a growth stimulant unique on the Polish market that contains natural plant hormones: auxins (11 mg·l^{-1}) and cytokinins (0.03 mg·l^{-1}). It is an extract from the fastest growing seaweed (kelp), *Ecklonia maxima,* harvested off the coast of South Africa.

The sowing rate of the plants was calculated based on the standards drawn up by IMUZ (the Institute for Land Reclamation and Grassland Farming) in Falenty [16]. In the growing season when the experiment was set up the biostimulants were applied. The season was an introductory period when three weed-control cuttings were made. After the second cutting, mineral fertilization was applied to all the plots at rates of 30 kg·ha^{-1} N (ammonium nitrate) and 30 kg·ha^{-1} K_2O (potash salt). Phosphorus was not applied as the soil was rich in available forms of this element. Over the study period (2010-12), the cutting regime was 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 before each cutting. Phosphorus and potassium needs of the mixtures were calculated, taking into account the expected dry matter yields, the appropriate mineral (from the ruminant nutrition standpoint) contents of hay and soil P and K availability. Moreover, to determine phosphorus and potassium application rates, coefficients given by Fotyma and Mercik [17] were used to convert the amounts of the nutrients taken up by grass and clover yields into the rates of phosphorus and potassium fertilizers. P and K fertilization was applied to all the plots. Phosphorus was applied once as triple superphosphate at a 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 prior to each cutting as 60% potash salt. The biostimulant was sprayed as an aqueous solution, the rate was 2,000 cm^3 of biostimulant per hectare diluted in water to 400 dm^3. 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.

During each harvest, 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

Table 2. Content of protein compounds in *Dactylis glomerata* and *Festulolium braunii* (g·kg^{-1} DM).

Specification		Treatment (A)	*Dactylis glomerata* (C)		*Festulolium braunii* (C)		*Dactylis glomerata* (C)	*Festulolium braunii* (C)	Mean
			Cultivar (B)						
			Amila	Tukan	Felopa	Agula			
Cut (D) (mean from years)	1	I	80.2	75.1	89.0	83.7	77.7	86.4	82.0
		II	88.9	83.4	95.6	91.6	86.2	93.6	89.9
	2	I	94.4	91.5	103.9	96.3	93.0	100.1	96.5
		II	105.7	101.1	114.0	106.3	103.4	110.1	106.8
	3	I	105.1	99.3	114.9	109.8	102.2	112.4	107.3
		II	113.3	108.5	126.0	118.4	110.9	122.2	116.6
	1	Mean	84.6	79.3	92.3	87.6	81.9	90.0	85.9
	2		100.0	96.3	109.0	101.3	98.2	105.1	101.6
	3		109.2	103.9	120.5	114.1	106.6	117.3	111.9
Year (E) (mean from cuts)	2010	I	95.2	86.3	101.7	99.2	90.8	100.5	95.6
		II	103.6	95.3	110.8	106.2	99.4	108.5	103.9
	2011	I	91.5	89.8	101.2	94.3	90.7	97.8	94.2
		II	102.3	97.4	111.1	105.9	99.9	108.5	104.2
	2012	I	93.0	89.8	105.0	96.1	91.4	100.6	96.0
		II	101.9	100.4	113.8	104.2	101.2	109.0	105.1
	2010	Mean	99.4	90.8	106.2	102.7	95.1	104.5	99.8
	2011		96.9	93.6	106.1	100.1	95.3	103.1	99.2
	2012		97.5	95.1	109.4	100.2	96.3	104.8	100.5
Mean		I	93.2	88.6	102.6	96.6	90.9	99.6	95.3
		II	102.6	97.7	111.8	105.4	100.1	108.7	104.4
Mean			97.9	93.2	107.3	101.0	95.5	104.1	99.9
$NIR_{0.05}$: A – 3.4; C – 3.4; D – 3.2; E – n.s.; A×C – 3.9; A×D – 4.4; A×E – 8.2; B×C – 5.2; C×D – 4.5; C×E – 7.0; A×B×C – 8.0; A×C×D – 5.1; A×C×E – 8.0; B×C×D – 6.3; B×C×E – 6.0; A×B×C×D – 6.7; A×B ×C×E – 6.6									

I – control (without biostimulant), II – treatment with biostimulant
n.s. – not significant differences

dry matter (by determining moisture content), protein compounds, and monosaccharides. The method of determination was near-infrared spectroscopy (NIRS) using a NIRFlex N-500 spectrometer and ready-to-use INGOT calibration applications.

Statistical analysis of the results included:

- variance analysis of a four-factor repeated experiment (biostimulant, species, cultivar, cut). The experimental design was split-plot-split-block with the linear models as suggested by Trętowski and Wójcik [18]. Significance of differences between means was checked using the Tukey test at the significance level of $\alpha \leq 0.05$
- correlation coefficient, whose significance was checked using Student's-t test.

Weather conditions differed during the study period (Table 1). Average air temperatures and precipitation sums in all the growing seasons were higher than the long-term means and the precipitation was very unevenly distributed. In 2010 and 2011 rainfall was, respectively, by 115.3 and 80.5 mm higher than the long-term means. It is worth noting that in July 2010 the precipitation was 4.5-times higher than the long-term mean for July, and it constituted 48% rainfall of the whole growing season. By contrast, high rainfall shortages were recorded in April 2010 and September 2011.

Results and Discussion

The protein content in the dry matter of grasses ranged between 75.1 and 126.0 g·kg^{-1} (Table 2) and was affected by all the factors in the study. Regardless of the grass species, cuts, and study years, an application of the biostimulant

Table 3. Content of monosaccharides in *Dactylis glomerata* and *Festulolium braunii* (g·kg^{-1} DM).

Specification		Treatment (A)	*Dactylis glomerata* (C) Cultivar (B) Amila	*Dactylis glomerata* (C) Cultivar (B) Tukan	*Festulolium braunii* (C) Cultivar (B) Felopa	*Festulolium braunii* (C) Cultivar (B) Agula	*Dactylis glomerata* (C)	*Festulolium braunii* (C)	Mean
Cut (D) (mean from years)	1	I*	74.5	76.0	128.1	110.8	75.3	119.5	97.4
		II	99.2	91.0	162.3	140.6	95.1	151.4	123.3
	2	I	55.3	64.4	113.8	97.7	59.9	105.8	82.8
		II	81.6	75.4	147.1	127.5	78.5	137.3	107.9
	3	I	40.3	44.0	102.9	92.0	42.1	97.4	69.8
		II	55.9	54.4	136.3	115.3	55.2	125.8	90.5
	1	Mean	86.9	83.51	145.2	125.7	85.2	135.4	110.3
	2		68.4	69.9	130.4	112.6	69.2	121.5	95.4
	3		48.1	49.2	119.6	103.6	48.6	111.6	80.1
Year (E) (mean from cuts)	2010	I	59.1	62.3	107.9	88.3	60.7	98.1	79.4
		II	74.7	71.6	125.0	108.9	73.2	117.0	95.1
	2011	I	50.2	57.6	110.5	99.7	53.9	105.1	79.5
		II	79.9	69.1	163.5	132.6	74.5	148.1	111.3
	2012	I	60.7	64.5	126.3	112.5	62.6	119.4	91.0
		II	82.1	80.0	157.2	141.8	81.1	149.5	115.3
	2010	Mean	66.9	67.0	116.5	98.6	66.9	107.6	87.2
	2011		65.1	63.4	137.0	116.2	64.2	126.6	95.4
	2012		71.4	72.3	141.7	127.2	71.8	134.5	103.1
Mean		I	56.7	61.5	114.9	100.2	59.1	107.6	83.3
		II	78.9	73.6	148.6	127.8	76.3	138.2	107.2
Mean			67.8	67.5	131.8	114.0	67.7	122.9	95.3
NIR$_{0.05}$: A – 8.8; C –5.8; D –12.8; E – 13.5; A×C – 8.7; A×D – 19.8; A×E – 15.0; B×C – 10.2; C×D –11.3; C×E – 12.9; A×B×C – 12.7; A×C×D –12.0; A×C×E – 12.0; B×C×D – 13.0; B×C×E – 13.0; A×B×C×D – 14.8; A×B×C×E – 15.0									

*explanations see Table 2

significantly increased the average dry matter protein content, the increase being 9.56%. When protein content was determined for individual cuts of grass (means across years), we observed a significant influence of the growth regulator on the amount of protein in plants at each harvest. The greatest increase in protein content (by 10.7%), following an application of Kelpak, was recorded in the tested plants sampled from the second cut. Protein content of grass harvested in individual study years was significantly higher following the application of Kelpak. Regardless of the cuts and study years, protein content in both the grass species increased after Kelpak had been used. Natural growth regulators contain phytohormones, such as auxins and cytokinins, and amino acids. According to Szabo and Hrotko [19] as well as Szabo et al. [20], the above components increase plant physiological activity and influence protein synthesis, which was confirmed in the present study.

According to Falkowski et al. [21], chemical composition of plants is species-related, which was confirmed in this study, too. Regardless of an application of the biostimulant, cuts, and study years, *Festulolium braunii*, contained on average by 8.6 g·kg^{-1} d.m. more protein compounds compared with the protein content in *Dactylis glomerata*, the increase being statistically significant. Differences in protein concentration were also recorded between the cultivars tested in the experiment but they were statistically significant between *Festulolium braunii* cultivars only.

Least and most protein was determined in the tested plants harvested from, respectively, the first and the third cuts. The relationships were statistically significant. The findings of this study correspond with the results obtained by Rogalski et al. [22], who have reported that consecutive grass re-growth had increased protein contents. Such a low

Table 4. Value of carbohydrate-protein ratio in *Dactylis glomerata* and *Festulolium braunii*.

Specification		Treatment (A)	*Dactylis glomerata* (C)		*Festulolium braunii* (C)		*Dactylis glomerata* (C)	*Festulolium braunii* (C)	Mean
			Cultivar (B)						
			Amila	Tukan	Felopa	Agula			
Cut (D) (mean from years)	1	I*	0.93	1.01	1.44	1.32	0.97	1.38	1.18
		II	1.12	1.09	1.70	1.53	1.10	1.62	1.36
	2	I	0.59	0.70	1.10	1.01	0.64	1.05	0.85
		II	0.77	0.75	1.29	1.20	0.76	1.24	1.00
	3	I	0.38	0.44	0.90	0.84	0.41	0.87	0.64
		II	0.49	0.50	1.08	0.97	0.50	1.03	0.76
	1	Mean	1.02	1.05	1.57	1.43	1.04	1.50	1.27
	2		0.68	0.72	1.19	1.11	0.70	1.15	0.93
	3		0.44	0.47	0.99	0.91	0.46	0.95	0.70
Year (E) (mean from cuts)	2010	I	0.62	0.72	1.06	0.89	0.67	0.98	0.82
		II	0.72	0.75	1.13	1.03	0.74	1.08	0.91
	2011	I	0.55	0.64	1.09	1.06	0.60	1.07	0.83
		II	0.78	0.71	1.47	1.25	0.75	1.36	1.05
	2012	I	0.65	0.72	1.20	1.17	0.69	1.19	0.94
		II	0.81	0.80	1.38	1.36	0.80	1.37	1.09
	2010	Mean	0.67	0.74	1.09	0.96	0.70	1.03	0.86
	2011		0.66	0.68	1.28	1.15	0.67	1.22	0.94
	2012		0.73	0.76	1.29	1.27	0.74	1.28	1.01
Mean		I	0.63	0.72	1.14	1.06	0.68	1.10	0.89
		II	0.79	0.78	1.36	1.24	0.79	1.30	1.04
Mean			0.71	0.75	1.25	1.15	0.73	1.20	0.97
$NIR_{0.05}$: A – 0.10 ; C – 0.10 ; D – 0.11 ; E – 0.14; A×C – 0.13; A×D – 0.14 ; A×E – 0.12; B×C – n.s.; C×D – 0.29; C×E – 0.22 ; A×B×C – 0.11; A×C×D – 0.14 ; A×C×E – 0.14; B×C×D – n.s.; B×C×E – n.s.; A×B×C×D – 0.12 ; A×B×C×E – 0.13									

*explanations see Table 2

protein content in first-cut grass may result from poorer foliage compared with the next harvests.

Protein concentration in grass harvested in the third study year was the highest compared with the remaining years, but the differences were not statistically significant. Varied meteorological conditions, in particular precipitation (Table 1), did not affect the protein content in the plants tested.

Moreover, statistical analysis revealed a significant association of all the factors analyzed in the study.

Monosaccharides are an important feed component. Sugar content determined in the dry matter of grass fell into a broad range of 40.3 to 162.3 $g \cdot kg^{-1}$ (Table 3). Spraying of grasses with Kelpak significantly increased monosaccharide contents of plants, the increase amounting to 28.7%. The results correspond with the findings of other authors [3, 23, 24], who have reported that algae extracts contribute to increased sugar contents in plants, though no works concerning grass have been found. When individual cuts (means across years) were considered, we found an impact of the biostimulant on sugar content. An application of the algae extract significantly increased the concentration of monosaccharides in grass at each harvest, regardless of the grass species or cultivar. When average sugar contents from consecutive study years were analyzed (means of 3 cuts), significant increases in the contents were recorded due to an application of Kelpak. Matysiak [15] has reported that the effect of Kelpak may depend on the genotype, that is individual characteristics of a cultivar. The results discussed here demonstrate a clear beneficial effect of Kelpak on sugar content in the species and cultivars tested in the study. According to Grzelak [25], sugar content of plants is influenced by a wide range of factors, including plant species. The present results reveal that, regardless of whether the biostimulant was applied or not, *Festulolium braunii* was significantly richer in monosaccharides than *Dactylis glomerata*. The two *Festulolium braunii* cultivars tested in the study had different sugar contents, Felopa containing significantly more monosaccharides that Agula. On the contrary, *Dactylis glomerata* cultivars did not differ in respect to sugar concentration in plants.

Analysis of the study results for each cut (means across years) demonstrated that the concentration of monosaccharides was significantly higher in plants from the first rather than the remaining cuts. Significantly higher levels of sugars in the tested plants harvested in mid and late summer (the second and third cut) were probably the result of increased respiration of plants, which makes use of sugars and is more intense when temperatures are high. Similar seasonal changes in grass monosaccharides have been reported by other authors [26, 27]. Moreover, the present results indicated that the grasses tested were richer in sugars in the third study year compared with the first year, the finding confirmed by statistical analysis. A significant increase in grass monosaccharides in the third versus first study year may have been due to a much lower precipitation sum in 2012 (Table 1). According to many authors [28, 29], plant monosaccharides increase as a result of water deficiency in the soil. Insufficient amounts of moisture in the soil disrupt the transportation and distribution of photosynthesis products. Due to this the export of assimilates from leaf blades to roots decreases.

Many workers [30, 31] have confirmed that the reciprocal proportions of nutrients condition the nutritive and energy value and flavour attributes. According to Ciepiela [27], the value of feed does not depend directly on the amount of total protein and carbohydrates in grasses but on the ratio of these organic components. Other workers [27] have reported that the value of this ratio should not be less than 0.4, the optimum range being 0.8 to 1.5. The value of carbohydrate-protein ratio in the dry matter of the tested grasses fell within the range of 0.38 to 1.70 (Table 4), the mean value being 0.97%. The minimum and maximum values of this indicator only slightly differed from the optimum values. Non-sprayed *Dactylis glomerata* cv. Amila had the lowest sugar-protein ratio for the third cut, whereas sprayed *Festulolium braunii* had the highest ratio at the first harvest. An application of the biostimulant significantly increased the carbohydrate-protein ratio in grasses (by 16.9%), regardless of the remaining factors in the study. The mean value of the ratio increased in grass at each harvest following an application of Kelpak, but the differences were significant between the first and second cuts only. Mean values for consecutive study years were found to be higher in plants sprayed with the algae extract but the differences were significant for grasses grown in the second and third years only.

Festulolium braunii had a significantly higher carbohydrate-protein ratio compared with *Dactylis glomerata*. An application of the biostimulant significantly increased the ratio for both species. In the experiment, two cultivars of each grass species were chosen because they were likely to differently respond to the biostimulant. Kelpak significantly increased the sugar-protein ratio for *Dactylis glomerata* cv. Amila, whereas for cv. Tukan the increase was only slight and statistically insignificant. The response of both cultivars of *Festulolium braunii*, that is Felopa and Agula, was the same: the carbohydrate-protein ratio was significantly higher after an application of Kelpak.

Regardless of the presence/absence of the biostimulant, grass species, and cultivar, the significantly highest ratio was obtained for the first harvest and the lowest value for the last cut, which is associated with different protein and sugar contents in the dry matter of plants at these harvests. When mean ratios across years were considered, the significantly highest value was in the third study year.

Linear correlation coefficients between protein and monosaccharide contents as well as carbohydrate-protein ratios in *Dactylis glomerata* and *Festulolium braunii*, and Kelpak (Table 5) were calculated in this work. Their values indicated that there was a significant positive correlation between the contents in the tested plants and an application of Kelpak in the study years, for both the species, and regardless of these factors.

Table 5. Dependence of the content of protein and monosaccharides and the carbohydrate-protein ratio in *Dactylis glomerata* and *Festulolium braunii* of growth regulator (correlation coefficient).

Year	Species		Independently of the species
	Dactylis glomerata	*Festulolium braunii*	
Protein			
2010	0.925*	0.932*	0.932*
2011	0.979*	0.991*	0.832*
2012	0.977*	0.930*	0.955*
Independently of the year	0.967*	0.950*	0.961*
Monosaccharide			
2010	0.962*	0.957*	0.981*
2011	0.777*	0.876*	0.967*
2012	0.969*	0.978*	0.988*
Independently of the year	0.858*	0.841*	0.956*
Carbohydrate-protein ratio			
2010	0.970*	0.968*	0.980*
2011	0.923*	0.952*	0.970*
2012	0.982*	0.968*	0.976*
Independently of the year	0.927*	0.939*	0.960*

* significant coefficient at α = 0.05

Conclusions

1. The biostimulant Kelpak significantly increased protein and monosaccharide contents as well as carbohydrate-protein ratios in the tested plants regardless of their species, cuts, and study years.
2. Protein and sugar contents of grass depended on the cut. The amount of protein compounds was significantly highest in plants from the third cut and monosaccharides from the first cut.
3. The species and cultivars tested in the study had significantly different protein and carbohydrate contents. The highest levels were determined in *Festulolium braunii* cv. Felopa.
4. Carbohydrate-protein ratios in the dry matter of grasses ranged between 0.38 and 1.70. The minimum and maximum values of this indicator were close to the optimum values.

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