Journal of Ecological Engineering
Volume 16, Issue 3, July 2015, pages 189–197
DOI: 10.12911/22998993/2955

Research Article

THE EFFECT OF GROWTH REGULATOR ON STRUCTURAL AND NON-STRUCTURAL CARBOHYDRATES AND LIGNIN CONTENT IN SELECTED GRASS SPECIES AND CULTIVARS

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Received: 2015.02.18
Accepted: 2015.06.02
Published: 2015.07.01

ABSTRACT

The research was undertaken to determine the effect of the biostimulant Kelpak SL, derived from brown seaweed species Ecklonia maxima (kelp), on structural and non-structural carbohydrates, as well as lignin content in orchard grass and Braun's festulolium. The experiment was a split-plot arrangement with three replicates. It was set up at the experimental facility of the University of Natural Sciences and Humanities, Siedlce, in late April 2009. The following factors were examined: an application of the plant growth regulator Kelpak SL applied at the rate of 2 $dm^3 \cdot ha^{-1}$ vs an untreated control (0 $dm^3 \cdot ha^{-1}$), pure sown grass species and cultivars grown in monoculture: Dactylis glomerata, cv. Amila and Tukan, as well as Festulolium braunii cv. Felopa and Agula. This study revealed that an application of Kelpak significantly reduced cellulose, hemicellulose and lignin contents of the grasses but significantly increased non-structural carbohydrates, regardless of the remaining factors. Non-structural carbohydrates were the highest in Kelpak-treated Festulolium braunii (on average, 232.7 $g \cdot kg^{-1}$).

Keywords: *Dactylis glomerata, Festulolium braunii*, Kelpak, cellulose, hemicellulose.

INTRODUCTION

Grassland provides the cheapest and whole feed [Jankowska-Huflejt, Wróbel 2008], and structural and non-structural carbohydrates, as well as lignin content are, among others, one of criteria of assessing food value. Too high or too low contents in feed are not good for animals [Podkówka, Podkówka 2006]. Structural carbohydrates and lignin reduce feed digestibility [Chaves et al. 2002] and energy value whereas non-structural carbohydrates improve the nutritional value and flavour of plants and they determine feed utilisation direction and production technology. Concentration of the above components in individual grass species changes due to many factors, including agrotechnological practices, such as plant fertilisation. In recent years, sustainable agriculture has been increasingly interested in an application of seaweed extracts which may be used as biostimulants and liquid fertilisers because they contain plant hormones (auxins, cytokinins and gibberellins) [Durand et al. 2003, Strik et al. 2004], macroelements (e.g.: Ca, K, P) and microelements (Fe, Cu, Zn, B, Mn, Co and Mo) [Khan et al. 2009, Craigie 2011].

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 [Matysiak et al. 2012]. Kelpak SL, a seaweed (brown algae) Ecklonia Maxima extract, is one of such products. Research has confirmed a positive effect of Kelpak SL on crop plants. However, knowledge of its mode of action is still fragmentary.

The objective of the study was to determine the effect of the biostimulant Kelpak SL on structural and non-structural carbohydrates as well as lignin content in orchard grass and Braun's festulolium.

MATERIALS AND METHODS

A field experiment was arranged as a randomised sub-block design (split-split-plot) with three replicates at the Siedlce Experimental Unit of the Siedlce University of Natural Sciences and Humanities (Poland) 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 $mg \cdot kg^{-1}$, Mg – 84 $mg \cdot kg^{-1}$) and average total nitrogen and available potassium contents (N –1.8 $g \cdot kg^{-1}$ d.m., K_2O – 190 $mg \cdot kg^{-1}$). Soil chemical analysis was carried out at an accredited laboratory of the Chemical and Agricultural Research Laboratory in Warsaw (Poland). Available phosphorus and potassium in the soil were extracted by means of the Egner-Riehm method and available magnesium – using the Schachtschabel method [Staugaitis and Rutkauskiene 2010]. Phosphorus was determined by the colorimetric method, total nitrogen by the Kjeldahl method and potassium and magnesium by the atomic absorption spectrophotometry AAS.

The following factors were examined:

- biostimulant with the trade name Kelpak SL applied at 2 $dm^3 \cdot ha^{-1}$ and a control – no biostimulant,
- pure stands of grass species grown in monoculture
- orchard grass (Dactylis glomerata), cv. Amila and Tukan,
- Braun's festulolium (Festulolium braunii), cv. Felopa and Agula.

The growth stimulant applied in the experiment is an extract from the fastest growing seaweed (kelp) Ecklonia maxima harvested off the coast of South Africa. The extract contains, among others, the natural plant hormones auxins (11 $mg \cdot l^{-1}$) and cytokinins (0.03 $mg \cdot l^{-1}$). The commercial name of the stimulant is Kelpak SL and it is manufactured by Kelp Products (Pty) Ltd P.O. Box 325, Simon's Town, the Republic of South Africa.

The growth regulator was not applied in the year when the experiment was established (2009). The season was an introductory period when three weed-control cuttings were made. After the second cutting, mineral fertilisation was applied to all the plots at the rates of 30 $kg \cdot ha^{-1}$ N (ammonium nitrate) and 30 $kg \cdot ha^{-1}$ K_2O (potash salt). Phosphorus was not applied as the soil was rich in available forms of this element. Three cuts of grasses were harvested from 2010 to 2012. Ammonium nitrate was applied three times per year. The total nitrogen (100 $kg \cdot ha^{-1}$) amount was split into three equal rates which were applied before each cutting. Phosphorus and potassium needs of the grass 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 [1995] were used to convert the amounts of the nutrients taken up by grass yields into the rates of phosphorus and potassium fertilisers. P and K fertilisation was applied to all the plots. Phosphorus was applied once as triple superphosphate at a rate of 40 $kg \cdot ha^{-1}$ P_2O_5 in the spring. The amount of potassium (160 $kg \cdot ha^{-1}$ K_2O) was split into three equal portions and applied prior to each cutting as 60% potash salt. The seaweed extract was sprayed as an aqueous solution, the rate was 2 dm^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 two weeks after the first harvest, and the last three weeks after the second harvest. Plots with no biostimulant were water sprayed (400 $dm^3 \cdot ha^{-1}$).

During harvest of all the cuts, plants obtained from the whole plot (its whole area, that is 10 m^2) were mixed and 0.5 kg samples of green matter were taken from each plot to perform chemical analyses.

The samples were left to dry in a ventilated room. Airy dry matter was shredded and ground. The obtained material was subjected to chemical analysis to determine dry matter, cellulose, hemicellulose, lignin, total protein, crude ash and crude fat. The listed components were determined by near infrared spectroscopy (NIRS) using a NIRFlex N-500 spectrometer and ready-to-use INGOT® calibration applications. INGOT® is a set of Universal NIR calibrations (adapter to the NIRFlex N-500 data format) for the analysis of Raw materials and finished products e.g. grass.

Non-structural carbohydrates were calculated according to Virkajärvi et al. [2012]:

non-structural carbohydrates = 1000 – (cellulose + hemicelluloses + lignin + total protein + crude ash + crude fat).

Statistical analysis of the results included variance analysis of a four-factor repeated (biostimulant, N doses, species, cultivar, cut) experiment. The experimental design was split-split-split-plot. The statistical data were calculated by Excel software with the linear models as suggested by Trętowski and Wójcik [1991]. Significance of differences between means was checked using the Tukey test at the significance level of 0.05. Correlation coefficients were calculated with STATISTICA (data analysis software system), Version 10 (www.statsoft.com.).

Meteorological data for the study years (2010–2012) were obtained from the Siedlce Meteorological Station. The values of the Sielianinov's hydrothermal coefficient were calculated to determine the temporal and spatial variation in precipitation and air temperature as well as their effect on the chemical composition of the grasses tested [Skowera and Puła, 2004]. The values are presented in Table 1.

RESULTS AND DISCUSSION

Forage grass species are highly variable in relation to lignin content as well as structural and non-structural carbohydrates due to an effect of biological, ecological and anthropogenic factors, cultural practices in particular. The average cellulose and hemicellulose contents in plant dry matter, 287.5 and 186.2 $g \cdot kg^{-1}$, respectively, were significantly affected by all the experimental factors (Tables 2 and 3). A significant effect of Kelpak on structural carbohydrates was found in both species of grass. Regardless of the grass species, cultivar, study year or cut, the biostimulant significantly reduced structural carbohydrates in the grasses. Also, cellulose and hemicellulose contents (averaged across years) significantly decreased in grass harvested at each cut due to the application of Kelpak. These contents determined in the dry matter of the plants tested in each study year were significantly lower after treating with the seaweed extract.

According to Matysiak [2005], the effect of seaweed extract may be closely related to the plant genotype and depend on individual characteristics of a cultivar. The results reported here demonstrated that structural carbohydrates significantly decreased in the plants tested after the biostimulant was applied, regardless of the cut or study year. Also, the grass cultivars had significantly different amounts of structural carbohydrates, regardless of whether Kelpak was applied or not. Dactylis glomerata cv. Tukan and Festulolium braunii cv. Felopa contained more cellulose and hemicellulose than their species counterparts. Moreover, Dactylis glomerata had greater amounts of cellulose and hemicellulose than Festulolium braunii, the finding reported by Borowiecki [2002], too. Grass contents of both the components were significantly affected by the cut. Cellulose and hemicellulose contents were the highest in the first-cut (298.9 $g \cdot kg^{-1}$), and second-cut grass (197.5 $g \cdot kg^{-1}$), respectively. Cellulose content significantly decreased in successive cuts during the growing season, the finding confirmed in the study on reed canarygrass conducted by Golińska and Kozłowski [2006]. However, the results of this study demonstrated no significant differences in structural carbohydrates between the grasses harvested in successive study years. Statistical analysis of data for cellulose and hemicellulose contents in both the grass species revealed a significant correlation of all the experimental factors.

Average grass dry matter content of lignin in the tested plants amounted to 40.1 $g \cdot kg^{-1}$ (Table 4) and was significantly affected by the experimental factors. The lowest average lignin content was determined in Kelpak-treated Festulolium braunii cv. Agula and the highest in untreated Dactylis glomerata cv. Tukan (45.2 $g \cdot kg^{-1}$). An application of the seaweed extract significantly reduced lignin content in grasses, regardless of the species, cultivar, cut or study year. The work presented here indicated that Festulolium braunii is not as lignin-rich as Dactylis glomerata.

Table 1. Value of hydrothermal index by Sielianinow's in individual months of vegetation period and in study years

Year	Month					
	IV	V	VI	VII	VIII	IX
2010	0.40	2.15	1.20	1.19	1.79	3.10
2011	1.30	1.34	0.82	3.74	1.02	0.62
2012	1.49	1.33	2.41	0.68	1.19	0.72

≤ 0.4 – extreme dry; 0.41–0.70– very dry ; 0.71–1.00 – dry; 1.10–1.30 – rather dry; 1.31–1.60 – optima; 1.61–2.00 – rather humid; 2.10–2.5 – humid; 2.51–3.00 – very humid; >3.00 – extreme humid

Table 2. Content of cellulose in *Dactylis glomerata* i *Festulolium braunii* ($g\cdot kg^{-1}$ DM)

Specification		Dose of Klepak $dm^3\cdot ha^{-1}$ (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	0	314.6	327.6	296.9	289.3	321.1	293.3	307.2
		2	298.5	309.5	277.3	272.7	304.0	275.0	289.5
	2	0	308.0	308.9	288.2	274.5	308.4	281.3	294.9
		2	289.6	291.9	270.3	260.4	290.7	265.4	278.0
	3	0	296.0	302.4	277.9	268.4	299.2	273.2	286.2
		2	277.0	284.1	262.0	253.9	280.6	258.0	269.3
	1	Mean	306.6	318.6	287.1	281.2	312.6	284.1	298.3
	2		298.8	300.4	279.3	267.4	299.6	273.3	286.5
	3		286.5	293.3	270.0	261.1	289.9	265.6	277.7
Years (E) (mean from cuts)	2010	0	307.6	312.7	288.0	278.3	310.2	283.2	296.7
		2	289.9	296.0	271.2	262.4	293.0	266.8	279.9
	2011	0	310.0	318.1	288.5	279.0	314.0	283.8	298.9
		2	291.5	300.0	272.6	265.2	295.7	268.9	282.3
	2012	0	301.1	308.1	286.5	275.1	304.6	280.8	292.7
		2	283.8	289.5	265.9	259.3	286.7	262.6	274.6
	2010	Mean	298.8	304.6	279.6	270.4	301.6	275.0	288.3
	2011		300.7	309.0	280.6	272.1	304.9	276.3	290.6
	2012		292.4	298.8	276.2	267.2	295.6	271.7	283.7
Mean		0	306.2	312.9	287.7	277.5	309.6	282.6	296.1
		2	288.4	295.2	269.9	262.3	291.8	266.1	278.9
Mean			297.3	304.7	278.8	269.9	300.7	274.3	287.5
LSD p≤ 0,05 for: dose of Kelpak (A) – 4.9 cultivar (B) – 7.4 species of grass (C) – 7.2 cut (D) - 3,2 years (E) - n.s.			interaction: A x C – 6.3; A x D – 10.6; A x E – 11.8; B x C – 7.4; C x D – 8.3; C x E – 9.8; A x B x C – 9.5; A x C x D – 9.6; A x C x E – 12.5; B x C x D – 8.5; B x C x E – 8.2; A x B x C x D – 13.0; A x B x C x E – 13.0						

n.s. – not significant differences

Moreover, Kelpak significantly reduced lignin content in both the species tested. However, no significant differences were found between the cultivars of the same species. The analysis of data for successive cuts (averaged across years) revealed a significantly lower lignin content of grass in the third-cut grass compared with the remaining cuts. The highest lignin content was determined in the second-cut grass, which agrees with the findings reported by Golińskia and Kozłowski [2006]. The average lignin content was the highest in grass harvested in the second study year, compared with the remaining years. Also, an application of the biostimulant significantly reduced the grass content of lignin in all the study years.

Increased structural carbohydrates and lignin content cause poorer digestibility and reduce the energy value of feed. More frequent cutting of grass may restrict the amount of both the components [Ciepiela 2004]. Similar effect can be obtained after an application of a seaweed extract, as demonstrated in the study reported here.

Forage grass species display substantial variation in the amount of non-structural carbohydrates they contain. The average value for the species tested in this work was 205.2 $g\cdot kg^{-1}$ dry matter (Table 5). Non-structural carbohydrates increased by 7.7% following an application of the kelp extract, regardless of the remaining experimental factors. Similar findings have been reported by Ciepiela and Godlewska (2014). According to Joubert and Lefrance (2008), active ingredients of seaweed extracts act as phyto-activators, which may alter the chemical composition of the plants treated. Studies by Pise and Sabale [2010] as well as Zodape et. al. [2011] have demonstrated that seaweed-treated plants have

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

Specification		Dose of Kelpak dm^3·ha^{-1} (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	0	191.0	201.8	176.4	163.3	196.4	169.8	183.1
		2	171.5	178.7	164.1	154.7	175.1	159.4	167.3
	2	0	211.5	217.5	196.3	186.3	214.5	191.3	202.9
		2	201.5	205.1	189.4	172.0	203.3	180.7	192.0
	3	0	196.1	204.6	185.5	184.3	200.4	184.9	192.7
		2	182.8	190.5	175.0	168.9	186.7	171.9	179.3
	1	Mean	181.3	190.3	170.2	159.0	185.8	164.6	175.2
	2		206.5	211.3	192.9	179.2	208.9	186.0	197.5
	3		189.5	197.6	180.2	176.6	193.5	178.4	186.0
Years (E) (mean from cuts)	2010	0	199.7	211.8	186.3	176.7	205.8	181.5	193.6
		2	184.3	190.6	177.5	165.4	187.4	171.5	179.4
	2011	0	196.6	206.3	184.8	182.7	201.5	183.8	192.6
		2	187.9	188.1	171.7	165.8	188.0	168.8	178.4
	2012	0	202.4	205.8	187.1	174.5	204.1	180.8	192.4
		II	183.6	195.6	179.3	164.3	189.6	171.8	180.7
	2010	Mean	192.0	201.2	181.9	171.1	196.6	176.5	186.5
	2011		192.3	197.2	178.3	174.3	194.8	176.3	185.5
	2012		193.0	200.7	183.2	169.4	196.9	176.3	186.6
Mean		0	199,6	208.0	186.1	178.0	203.8	182.0	192.9
		2	185,3	191.4	176.2	165.2	188.4	170.7	179.5
Mean			192,4	199.7	181.1	171.6	196.1	176.3	186.2
LSD p≤ 0,05 for: dose of Kelpak (A) – 6.7 cultivar (B) – 6.7 species of grass (C) – 6,0 cut (D) – 5.7 years (E) – n.s.			interaction: A x C – 6.2; A x D – 8.4; A x E – 10.3; B x C – 4.7; C x D –6.9; C x E – 9.2; A x B x C – 9.1; A x C x D –8.1; A x C x E – 9.2; B x C x D – 5.6; B x C x E – 7.0; A x B x C x D – 9.8; A x B x C x E – 10.0						

n.s. – not significant differences

got higher carbohydrate contents. Dobrzański [2008] has reported a substantial increase in non-structural carbohydrates following an application of such an extract. Although there are no reports in international literature concerning the efficacy of natural biostimulants applied in grass, their effect has been found to be highly dependent on crop plant species and cultivar [Sultana et al., 2005].

In the study discussed here, Festulolium braunii contained by 16.7% more non-structural carbohydrates compared with Dactylis glomerata, the difference being statistically significant. Similar findings were reported by Downing and Gamroth [2007].

An application of Kelpak significantly increased the concentration of non-structural carbohydrates in both the grass species. According to Grzelak [2010], plant species is one of the factors affecting plant content of sugars. The lowest amount of non-structural carbohydrates was determined in the second-cut grass. It was the highest in the first-cut grass, the differences being statistically significant. An application of kelp extract significantly increased non-structural carbohydrates in the grass in successive cuts (averaged across years). Significant differences between the amounts of non-structural carbohydrates were found between the successive study years, the lowest amount being recorded in the second study year and the highest in the third study year, probably due to the weather conditions. Values of hydrothermal coefficient presented in table 1 indicate that the summer months in the third study year were either very dry or dry. According to many authors [Lu and Zhang, 1998; Olszewska et al. 2010], sugar contents of plants increase when water shortages oc-

Table 4. Content of lignin in *Dactylis glomerata* i *Festulolium braunii* (g·kg^{-1} DM)

Specification		Dose of Kelpak dm^3·ha^{-1} (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	0	45.1	45.3	39.6	37.8	45.2	38.7	41.9
		2	42.2	42.8	36.0	34.4	42.5	35.2	38.9
	2	0	46.3	47.4	40.2	39.9	46.9	40.1	43.5
		2	43.9	44.5	36.2	36.3	44.2	36.2	40.2
	3	0	42.3	42.7	36.7	35.7	42.5	36.2	39.4
		2	39.7	40.3	34.1	33.5	40.0	33.8	36.9
	1	Mean	43.7	44.1	37.8	36.1	43.8	37.0	40.4
	2		45.1	46.0	38.2	38.1	45.5	38.2	41.8
	3		41.0	41.5	35.4	34.6	41.3	35.0	38.1
Years (E) (mean from cuts)	2010	0	44.4	44.9	37.9	37.0	44.6	37.5	41.0
		2	41.7	42.5	34.9	34.7	42.1	34.8	38.4
	2011	0	44.1	46.8	40.3	40.6	45.5	40.5	43.0
		2	41.4	44.0	35.9	36.4	42.7	36.2	39.4
	2012	0	45.2	43.8	38.2	35.8	44.5	37.0	40.8
		2	42.7	41.1	35.6	33.1	41.9	34.3	38.1
	2010	Mean	43.0	43.7	36.4	35.8	43.4	36.1	39.7
	2011		42.7	45.4	38.1	38.5	44.1	38.3	41.2
	2012		44.0	42.4	36.9	34.4	43.2	35.7	39.4
Mean		0	44.6	45.2	38.8	37.8	44.9	38.3	41.6
		2	41.9	42.5	35.4	34.7	42.2	35.1	38.7
Mean			43.2	43.9	37.1	36.3	43.5	36.7	40.1
LSD p≤ 0.05 for: dose of Kelpak (A) – 1.1 cultivar (B) – 1.3 species of grass (C) – 0.7 cut (D) – 1.6 years (E) – 1.7			Interaction: A x C – 1.1; A x D – 2.4 ; A x E – 2.5; B x C – n.s.; C x D – 1.5; C x E – 1.7 ; A x B x C – 1.8; A x C x D – 1.7 ; A x C x E – 2.1; B x C x D – n.s.; B x C x E – n.s.; A x B x C x D – 2.5 ; A x B x C x E – 2.4						

cur in the soil probably because it is more difficult for a plant to transport assimilates from leaf blades to roots.

Linear correlation coefficients were calculated between biostimulant rates and the amounts of components in the plants tested (Table 6). The values indicate that non-structural carbohydrates and lignin content were negatively correlated with Kelpak rate in the successive study years, for individual species, and regardless of these factors. Non-structural carbohydrates were positively correlated, regardless of the study years or grass species. Correlation coefficients between the examined components of grasses (Table 7) indicate that structural carbohydrates were significantly negatively correlated with non-structural carbohydrates and positively associated with lignin content in the plants tested.

CONCLUSIONS

1. Kelpak-treated grasses had significantly reduced cellulose, hemicellulose and lignin contents. However, non-structural carbohydrates were higher in these plants regardless of the remaining factors.
2. The concentration of carbohydrates was significantly different in the plants tested. Festulolium braunii cv. Agula had the highest nutritional value.
3. The amounts of the examined components substantially differed during the growing season. Most non-structural carbohydrates and cellulose were determined in the first-cut grass and most hemicellulose in the third-cut grass.

Table 5. Content of non-structural carbohydrates in Dactylis glomerata i Festulolium braunii (g·kg^{-1} DM)

Specification		Dose of Kelpak dm^3·ha^{-1} (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	0	205.8	189.4	226.2	253.5	197.6	239.9	218.7
		2	224.5	205.5	250.0	265.8	215.0	257.9	236.5
	2	0	160.2	157.2	177.2	204.6	158.7	190.9	174.8
		2	180.2	165.4	201.7	213.2	172.8	207.4	190.1
	3	0	184.4	176.1	198.5	231.0	180.3	214.7	197.5
		2	200.0	188.8	224.1	241.3	194.4	232.7	213.6
	1	Mean	215.1	197.5	238.1	259.7	206.3	248.9	227.6
	2		170.2	161.3	189.5	208.9	165.8	199.2	182.5
	3		192.2	182.5	211.3	236.2	187.3	223.7	205.5
Years (E) (mean from cuts)	2010	0	181.9	173.4	200.8	227.5	177.6	214.2	195.9
		2	203.8	191.7	224.9	240.8	197.8	232.8	215.3
	2011	0	168.1	160.0	183.5	219.7	164.0	201.6	182.8
		2	185.3	169.7	210.6	226.4	177.5	218.5	198.0
	2012	0	200.5	189.4	217.6	241.9	195.0	229.7	212.3
		2	215.7	198.3	240.3	253.1	207.0	246.7	226.8
	2010	Mean	192.8	182.6	212.9	234.1	187.7	223.5	205.6
	2011		176.7	164.8	197.1	223.1	170.8	210,1	190.4
	2012		208.1	193.9	228.9	247.5	201.0	238,2	219.6
Mean		0	183,5	174.3	200.6	229.7	178.9	215.2	197.0
		2	201,6	186.6	225.3	240.1	194.1	232.7	213,4
Mean			192.5	180.4	213.0	234.9	186.5	224.0	205.2
LSD p≤ 0.05 for: dose of Kelpak (A) – 8.4 cultivar (B) – 12.4 species of grass (C) – 7.1 cut (D) – 10.4 years (E) – 11.8			Interaction: A x C – 12.5; A x D – 15.0 ; A x E – 15.5; B x C – 12.4.; C x D – 12.3; C x E – 15.3 ; A x B x C – 18.0; A x C x D – 14.0 ; A x C x E – 16.9; B x C x D – 16.7; B x C x E – 14; A x B x C x D – 15.3 ; A x B x C x E – 17.0						

Table 6. Correlation coefficients between Kelpak doses and the content of structural (cellulose and hemicellulose) and nonstructural carbohydrates and lignin

Year	Tested trait	Species		Independently of the species
		Dactylis glomerata	*Festulolium braunii*	
2010	Cellulose	-0.579*	-0.553*	-0.721*
	Hemicellulose	-0.584*	-0.398*	-0.407*
	Lignin	-0.501*	-0.575*	-0.300*
	Nonstructural carbohydrates	0.504*	0.423*	0.351*
2011	Cellulose	-0.550*	-0.553*	-0.399*
	Hemicellulose	-0.461*	-0.595*	-0.431*
	Lignin	-0.490*	-0.786*	-0.440*
	Nonstructural carbohydrates	0.305	0.270	0.227
2012	Cellulose	-0.581*	-0.612*	-0.468*
	Hemicellulose	-0.519*	-0.310	-0.334*
	Lignin	-0.523*	-0.534*	-0.293*
	Nonstructural carbohydrates	0.294	0.362*	0.252*
Independently of the years	Cellulose	-0.552*	-0.568*	-0.424*
	Hemicellulose	-0.521*	-0.427*	-0.390*
	Lignin	-0.498*	-0.446*	-0.335*
	Nonstructural carbohydrates	0.313*	0.309*	0.253*

* statistically significant coefficient (α = 0,05)

Table 7. Correlation coefficients between the tested traits

Tested trait	Tested trait			
	Cellulose	Hemicellulose	Lignin	Nonstructural carbohydrates
Dose of Kelpak – 0 $dm^3 \cdot ha^{-1}$				
Cellulose	–	0.831*	0.775*	-0.382*
Hemicellulose	0.831*	–	0.768*	-0.304*
Lignin	0.775*	0.768*	–	-0.626*
Nonstructural carbohydrates	-0.382*	-0.304*	-0.626*	–
Dose of Kelpak – 2 $dm^3 \cdot ha^{-1}$				
Cellulose	–	0.778*	0.782*	-0.393*
Hemicellulose	0.778*	–	0.673*	-0.206*
Lignin	0.782*	0.673*	–	-0.678*
Nonstructural carbohydrates	-0.393*	-0.206*	-0.678*	–

* statistically significant coefficient ($\alpha = 0.05$)

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