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THE EFFECT OF NATURAL GROWTH REGULATORS OBTAINED FROM *Ecklonia maxima* AND MINERAL NITROGEN ON TRUE PROTEIN AND SIMPLE SUGAR CONTENTS OF *Dactylis glomerata* L. AND *Festulolium braunii* (K. richt.) A. camus

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ABSTRACT

The objective of the study was to evaluate the effect of the biostimulant Kelpak on true protein and simple sugar contents of *Dactylis glomerata* and *Festulolium braunii* at different nitrogen rates. A field experiment was set up as a randomised sub-block design (split-split-plot) with three replicates. The following factors were examined: the biostimulant with the trade name Kelpak SL, nitrogen rates: 0, 50 and 150 kg N·ha^{-1}, pure-sown grass species and cultivars (monoculture). Protein compounds and simple sugars were determined in the dry matter of plants, and a carbohydrate/protein ratio was calculated. The *Ecklonia maxima* extract significantly increased true protein and simple sugar contents as well as the sugar/protein ratio in the tested plants. Nitrogen rates significantly influenced protein and sugar contents in the dry matter of *Dactylis glomerata* and *Festulolium braunii*. The carbohydrate/protein ratio averaged 1.07 and was within the range of values which are believed to be optimal for ruminants.

Key words: Biostymulator, *Dactylis glomerata, Festulolium braunii*, Kelpak

INTRODUCTION

Sustainable agricultural production has to compromise between the positive effect of crops on the soil environment and high yields of good quality (Kitczak, Czyż 2006). So far, plant production intensification has been mainly associated with increasing rates of mineral fertilisers, as a result of which the chemical composition of feed has deteriorated, the sward has thinned out and some animal species have migrated from these habitats (Jankowska et al. 2008). Recently, intensification of agriculture has slowed down due to an introduction of pro-ecological models (Szoszkiewicz et al. 2003) as a result of which rates of mineral fertilisers applied to grassland have been reduced. Plant biomass obtained from organically farmed grassland tends to be of poor quality so attempts have been made to find new solutions to produce high quality yields and, at the same time, reduce e.g. soil contamination. One of such solutions, which has been receiving more and more attention in recent years, is offered by regulators of plant growth and development (Matysiak, Adamczewski 2006). Many studies have revealed a wide range of beneficial effects of plant growth regulator applications on plants (Güllüoglu 2011). When applied in small quantities, such substances modify physiological functions in plants. Sea algae extracts contain not only phytohormones such as auxins and cytokinins (Durand et al. 2003) but also macroelements: Ca, K and P (Stirk et al. 2004), and microelements: Fe, Cu, Zn, B, Mn, Co and Mo (Khan et. al. 2009). Kelpak is a biostimulant produced from the seaweed *Ecklonia maxima* which is harvested off the shores of South Africa. Research has demonstrated that Kelpak has a beneficial influence on crop plants but its effect depends rather on the crop plant species and cultivar than the rate at which Kelpak is applied (Sultana et al. 2005). The biostimulant is used worldwide in the production of vegetables and fruit as well as cereals, rape and maize. However, there is a paucity of reports on an application of natural biostimulants to grassland.

The present work is an attempt to evaluate the effect of the biostimulant Kelpak on true protein and simple sugar contents in *Dactylis glomerata* and *Festulolium braunii* at different nitrogen rates.

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 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 2000 dm^3 · ha^{-1} and a control – no biostimulant,

- nitrogen application rate: 50 and 150 kg · ha^{-1}, and a control (0 kg · ha^{-1}),

- 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 which 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 (Jankowski et al. 2005) . In the growing season when the experiment was set up, nor the biostimulant were applied. 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 · 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 (2010-2012), 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 (1995) were used to convert the amounts of the nutrients taken up by grass and clover 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 · 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 2000 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 dry matter (by determining moisture content), protein compounds and simple sugars. 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 (biostimulant, N doses, species, cultivar, cut) experiment. The experimental design was split-plot-split-block 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 $\alpha \leq 0.05$,

- correlation coefficient whose significance was checked using Student's t test.

RESULTS AND DISCUSSION

True protein content in the dry matter of the grass species tested averaged 94.6 kg·ha^{-1} (table 1). Statistical analysis of the results demonstrated that the biostimulant significantly affected true protein and simple sugar contents in the grasses examined depending on the nitrogen rate, cultivar, cut and study year. The average true protein content in plants increased by 11.3% following an application of the growth regulator regardless of the remaining factors examined in the experiment. According to Szabo et al. (2011), components in biostimulants such as auxins, gibberellic acid, cytokinins and amino acids increase the physiological activity of plants, for example protein synthesis. There are many reports in the world literature of the positive effect of sea algae extracts on protein content in various plant species (Sivasankari et al. 2006, Matysiak et al. 2012). However, no papers have been found concerning grasses. Both *Dactylis glomerata* and *Festulolium braunii* contained significantly more true protein when sprayed with Kelpak. Analysis of plant material showed that the biostimulant applied at different nitrogen rates significantly increased the concentration of true protein although the greatest increase, 12.4%, was recorded in plots which had not been N-fertilised. Statistically significant differences between true protein contents were found for each cut. The greatest accumulation of protein was in grass from the first harvest and it increased at consecutive cuts. Similar findings have been reported by Łyszczarz and Dembek (2003) as well as Jankowska et al. (2008). According to these authors, protein content in grass increases in successive re-growths due to poorer grass foliage at the first harvest compared with the following cuts. An application of Kelpak significantly increased the quantity of protein in grass at all the harvests. Statistical analysis revealed no significant effect of study years on protein amount in plants. In turn, the biostimulant significantly increased true protein content in the plant species tested in each study year.

Table 1. The effect of biostymulant on the true protein content and simple sugars in *Dactylis glomerata* and *Festulolium braunii* depending on nitrogen doses, cultivar, cut and years of study

Factor		True protein ($g \cdot kg^{-1}$ s.m.)			Simple sugars ($g \cdot kg^{-1}$ s.m.)		
		Treatment (A)		Mean	Treatment (A)		Mean
		I	II		I	II	
N dose ($kg \cdot ha^{-1}$) (B)	0	75.6	85.0	80.3	94.7	134.9	114.8
	50	88.3	97.1	92.7	89.0	120.2	104.6
	150	104.8	117.0	110.9	74.5	100.3	87.4
Dactylis glomerata (D)	Amila (C)	87.8	98.1	92.9	62.3	87.3	74.8
	Tukan (C)	81.7	91.6	86.6	62.7	82.4	72.6
Festulolium braunii (D)	Felopa (C)	97.8	107.4	102.6	117.7	155.4	136.5
	Agula (C)	90.9	101.7	96.3	101.6	148.8	125.2
Dactylis glomerata (D)	Mean	84.8	94.8	89.8	62.5	84.9	73.7
Festulolium braunii (D)	Mean	94.3	104.6	99.5	109.6	152.1	130.9
Cut (E)	1	76.3	84.1	80.2	100.7	136.4	118.5
	2	91.2	102.4	96.8	86.6	117.9	102.2
	3	101.2	112.6	106.9	71.0	101.2	86.1
Years (F)	2010	90.0	100.6	95.3	80.4	108.8	94.6
	2011	88.5	98.0	93.3	84.0	124.6	104.3
	2012	90.1	100.5	95.3	93.7	122.1	107.9
Mean		89.6	99.7	94.6	86.1	118.5	102.2
$LSD_{0.05}$		A – 2.8; B – 4.5; D – 4.1; E – 3.5; F – n.s.; A x B – 5.0; A x C x D – 7.9; A x D – 5.1 ; A x E – 5.5; A x F – 6.7 ; C x D – 4.2			A – 5.6; B – 8.6; D – 4.3; E – 8.5; F – 8.9; A x B – 12.8; A x C x D – 9.6; A x D – 6.2 ; A x E – 12.6; A x F – 13.3 ; C x D – 7.8		

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

The average simple sugar content in grasses amounted to 102.2 $g \cdot kg^{-1}$ d.m. Joubert and Lefranc (2008) have stated that active substances in sea algae extracts are similar to physiological activators in their mode of action because they may change the chemical composition of plants sprayed with such extracts. The biostimulant applied in the present study was found to influence the concentration of sugars in plants. Kelpak increased monosaccharide concentration in grasses by 37.6%, regardless of the remaining factors investigated in the study. Similar findings have been reported by many authors (Dobrzański et al. 2008, Szymczak-Nowak 2009, Pacholczak et al. 2012) who have stressed that sea algae extracts contribute to increased sugar contents in plants but, as with protein content, no literature data for grasses have been found. The effect of the biostimulant on sugar concentration was significant at each nitrogen level. At 0 N $kg \cdot ha^{-1}$, sugars increased by as much as 42.4% following an application of Kelpak. Just like in the study by Kozłowski et al. (2001), seasonal changes in the carbohydrate content of grasses were observed. More simple sugars were determined in the grass of the first cut, which was most likely due to increased respiration of plants, induced by high temperatures, which makes use of sugars. Moreover, the three-year data of this study showed that significantly least sugars were determined in plants in the first study year, regardless of the remaining factors examined in the study. Moreover, statistical analysis revealed that the biostimulant contributed to significantly increased sugar contents determined for each cut and study year. The chemical composition of plants is species- and even cultivar-related (Falkowski et al. 2000), the finding confirmed in this study. Significantly more sugars were determined in *Festulolium braunii* regardless of the remaining factors. What is more, an application of the growth regulator significantly increased monosaccharide levels in both the grass species.

The true protein content in the dry matter of grasses significantly depended on the nitrogen rate (table 2). When the rate was increased from 0 to 50 $kg \cdot ha^{-1}$, protein amount in *Dactylis glomerata* and *Festulolium braunii* increased by 18 and 12.9%, respectively, whereas at the rate of 150 kg $N \cdot ha^{-1}$ the respective increases were 41.7 and 34.8%. Also Ciepiela et al. (2008) have reported a significant increase in the protein content of *Dactylis glomerata* at the nitrogen rates of 60 and 90 $kg \cdot ha^{-1}$ compared with the rate of 0 kg $N \cdot ha^{-1}$. Moreover, they found a significant association of nitrogen rates with the concentration of protein in the grass herbage at each harvest and for consecutive study years.

The nitrogen fertilisation regime significantly influenced the sugar content of each grass cultivar. Increasing nitrogen rates significantly reduced sugar content determined in the grass dry matter, which agrees with results reported by other authors (Lemaire and Salette 1982, Ciepiela 2004). The nitrogen fertilisation regime x cuts interaction and nitrogen fertilisation regime x study years interaction were found. During the three-year study period, increasing N rates significantly reduced the concentration of sugars in the grass dry matter from each cut and study year.

Table 2. The effect of nitrogen dose on the true protein content and simple sugars in *Dactylis glomerata* and *Festulolium braunii* in each cultivar, cut and study year

Factor		True protein ($g \cdot kg^{-1}$s.m.)			Simple sugars ($g \cdot kg^{-1}$s.m.)		
		N dose ($kg \cdot ha^{-1}$) (B)			N dose ($kg \cdot ha^{-1}$) (B)		
		0	50	150	0	50	150
Dactylis glomerata (D)	Amila (C)	79.4	90.3	109.1	83.6	76.8	64.0
	Tukan (C)	70.3	86.6	103.0	82.2	71.4	64.0
Festulolium braunii (D)	Felopa (C)	89.1	99.8	119.0	150.6	140.9	118.1
	Agula (C)	82.5	94.0	112.4	142.7	129.3	103.6
Dactylis glomerata (D)	Mean	74.9	88.4	106.1	82.9	74.1	64.0
Festulolium braunii (D)	Mean	85.8	96.9	115.7	146.7	135.1	110.8
Cut (E)	1	67.6	78.0	94.9	132.4	120.3	102.8
	2	82.4	95.3	112.8	114.5	105.1	87.0
	3	90.9	104.8	124.9	97.4	88.4	72.4
Years (F)	2010	82.4	92.5	111.0	107.1	96.5	80.1
	2011	77.8	91.5	110.5	118.4	108.3	86.2
	2012	80.7	94.0	111.1	118.8	109.0	95.9
$LSD_{0.05}$		B x C x D – 7.7; B x D – 4.9; B x E – 4.3; B x F – 6.9			B x C x D – 9.5; B x D – 9.3; B x E – 9.3; B x F – 10.0		

Festulolium braunii, a *Festuce pratensis* and *Lolium multiflorum* cross (Zwierzykowski et al. 1998), is of substantial nutritional value. It has got high protein and sugar levels (Borowiecki 2005), and in this respect is better than *Dactylis glomerata*. The results discussed here (table 3) showed a significantly higher true protein and simple sugar contents of *Festulolium braunii* compared with *Dactylis glomerata* harvested at each cut and in each study year. *Festulolium braunii* cv. Agula proved to be the best in terms of chemical composition.

Table 3. Content of true protein and simple sugars in two cultivar *Dactylis glomerata* and *Festulolium braunii* in each cut and study year

Factor		True protein content ($g \cdot kg^{-1}$d.m.)						Simple sugars ($g \cdot kg^{-1}$d.m.)					
		Cut (E)			Years (F)			Cut (E)			Years (F)		
		1	2	3	2010	2011	2012	1	2	3	2010	2011	2012
Dactylis glomerata (D)	Amila (C)	78 .2	95.6	105.0	94.1	91.4	93.4	97.0	75.2	52.3	72.4	76.0	75.9
	Tukan (C)	73 .7	88.6	97.5	86.2	85.8	87.9	89.1	75.1	53.5	71.2	71.6	74.9
Festulolium braunii (D)	Felopa (C)	87 .4	104.9	115.5	102.9	101.0	104.0	149.9	134.9	124.8	124.1	142.6	142.9
	Agula (C)	81 .3	98.2	109.3	98.1	94.9	95.9	138.2	123.7	113.6	110.7	127.1	137.9
Dactylis glomerata (D)	Mean	76 .0	92.1	101.3	90.2	88.6	90.6	93.0	75.1	52.9	71.9	73.8	75.4
Festulolium braunii (D)	Mean	84 .4	101.5	112.4	100.5	97.9	99.9	144.0	129.3	119.2	117.3	134.8	140.4
$LSD_{0.05}$		C x D x E – 6.0; C x D x F – 5.3; D x E – 5.5; D x F – 4.9						C x D x E – 10.3; C x D x F – 13.0; D x E – 8.9; D x F – 10.0					

The forage value of grassland herbage is often determined based on its carbohydrate/protein ratio which should not drop below 0.4, the optimal range for ruminants being 0.8 to 1.5. The average value of this ratio in the grass cultivars tested was 1.07 (table 4) and thus was within the above-mentioned limit. The most favourable value (1.59) was recorded for the plots which had not received nitrogen fertilisation but had been sprayed with Kelpak. The *Ecklonia maxima* extract clearly influenced the value of this indicator regardless of the nitrogen rate, cultivar, cut or study year. The highest values were determined for Kelpak-sprayed plants. Moreover, there was found an interaction between the biostimulant and the remaining factors. The ratios which were significantly highest were calculated for plants harvested from the Kelpak-sprayed plots which received the nitrogen rates of 0 and 50 kg·ha^{-1}. However, the increase was insignificant when the rate was increased to 150 kg N·ha^{-1}. When both grass species were compared, a higher sugar/protein ratio was calculated for *Festulolium braunii* although Kelpak significantly increased the value of this indicator for both the species. The sugar/protein ratio was also different between harvests. Ratios calculated for consecutive re-growths were significantly lower whereas increasing values were obtained for consecutive cuts when Kelpak had been applied. Analysis of the results showed that carbohydrate/protein ratios in the tested plants decreased in consecutive study years, the differences being statistically significant. The nitrogen rates/study years interaction was found in the first and second study year. The carbohydrate/protein ratio was not influenced by Kelpak application in the last study year. Nitrogen rates significantly affected the values of this indicator (table 5). The response of both the grass species was the same: the higher N ratio was calculated, the lower carbohydrate/protein ratio was obtained. The same tendency was observed for a nitrogen rates x cuts interaction and a nitrogen rates x study years interaction. Increased nitrogen rates contributed to significantly lower values of carbohydrate/protein ratio for each cut and study year.

Table 4. The effect of biostymulant on the value of carbohydrate/protein rations in *Dactylis glomerata* and *Festulolium braunii* in each nitrogen doses, cultivar, cut and study year

Factor		Treatment (A) I	Treatment (A) II	Mean
N dose (kg · ha^{-1}) (B)	0	1.25	1.59	1.42
	50	1.01	1.24	1.12
	150	0.71	0.86	0.78
Dactylis glomerata (D)	Amila (C)	0.71	0.89	0.80
	Tukan (C)	0.77	0.90	0.83
Festulolium braunii (D)	Felopa (C)	1.20	1.45	1.33
	Agula (C)	1.12	1.46	1.29
Dactylis glomerata (D)	Mean	0.74	0.90	0.82
Festulolium braunii (D)	Mean	1.16	1.45	1.31
Cut (E)	1	1.32	1.62	1.47
	2	0.95	1.15	1.05
	3	0.70	0.90	0.80
Years (F)	2010	0.89	1.08	0.99
	2011	0.95	1.27	1.11
	2012	1.04	1.21	1.13
Mean		0.96	1.19	1.07

$LSD_{0.05}$: A – 0.09; B – 0.10; D – 0.07; E – 0.10; F – 0.11.; A x B – 0.16; A x C x D – 0.17; A x D – 0.13 ; A x E – 0.15; A x F – 0.18 ; C x D – n.s.

I – control (without biostimulant); II – treatment with biostimulant

n.s. – not significant differences

Values of sugar/protein ratio calculated for both the cultivars of *Dactylis glomerata* and *Festulolium braunii* remained mostly unaffected by cuts and study years (table 6). Significant differences were determined for the grass species only. Compared with *Dactylis glomerata*, *Festulolium braunii* had higher values of the sugar/protein ratio for each cut over the whole study period. The values for *Festulolium braunii* were higher in each study year but the differences were not statistically significant.

Coefficients of linear correlation were calculated between true protein content, simple sugar content as well as carbohydrate/protein ratio for *Dactylis glomerata* and *Festulolium braunii* and the growth stimulant (table 7). The results indicated that the contents were significantly and positively correlated with an application of the stimulant for both the individual nitrogen rates and grass species, and regardless of these factors.

Table 5. The effect of N dose on the carbohydrate/protein ratios in *Dactylis glomerata* and *Festulolium brauni* in each cultivar, cut and study year

Factor		N dose (kg · ha^{-1}) (B) 0	50	150
Dactylis glomerata (D)	Amila (C)	1.05	0.85	0.59
	Tukan (C)	1.17	0.82	0.62
Festulolium braunii (D)	Felopa (C)	1.69	1.41	0.99
	Agula (C)	1.73	1.38	0.92
Dactylis glomerata (D)	Mean	1.11	0.84	0.60
Festulolium braunii (D)	Mean	1.71	1.39	0.96
Cut (E)	1	1.96	1.54	1.08
	2	1.39	1.10	0.77
	3	1.07	0.84	0.58
Lata (F)	2010	1.30	1.04	0.72
	2011	1.52	1.18	0.78
	2012	1.47	1.16	0.86

$LSD_{0.05}$:B x C x D – 0.20; B x D – 0.14; B x E – 0.16;B x F – 0.21

Table 6. Carbohydrate/protein ratios for two cultivars of *Dactylis glomerata* and *Festulolium braunii* in each cut and study year

Factor		Cut (E)			Years (F)		
		1	2	3	2010	2011	2012
Dactylis glomerata (D)	Amila (C)	1.24	0.79	0.50	0.77	0.83	0.81
	Tukan (C)	1.21	0.85	0.55	0.83	0.83	0.85
Festulolium braunii (D)	Felopa (C)	1.72	1.29	1.08	1.21	1.41	1.37
	Agula (C)	1.70	1.26	1.04	1.13	1.34	1.44
Dactylis glomerata (D)	Mean	1.22	0.82	0.52	0.80	0.83	0.83
Festulolium braunii (D)	Mean	1.71	1.27	1.06	1.17	1.38	1.41

$LSD_{0.05}$: C x D x E – n.s.; C x D x F – n.s.; D x E – 0 .13; D x F – 0.17

n.s. – not significant differences

Table 7. Association of the true protein content, simple sugars content and carbohydrate/protein ratio in *Dactylis glomerata* and *Festulolium braunii* with the biostimulant (correlation coefficient)

N dose (kg · ha^{-1})	Species		Independently of the species
	Dactylis glomerata	*Festulolium braunii*	
	Biostimulant	Biostimulant	Biostimulant
	True protein		
0	0.867*	0.974*	0.933*
50	0.941*	0.974*	0.949*
150	0.937*	0.844*	0.901*
Independently of N dose	0.960*	0.888*	0.956*
	Simple sugars		
0	0.821*	0.489*	0.909*
50	0.777*	0.580*	0.915*
150	0.819*	0.937*	0.895*
Independently of N dose	0.811*	0.789*	0.925*
	Carbohydrate/protein ratio		
0	0.914*	0.865*	0.926*
50	0.901*	0.829*	0.918*
150	0.906*	0.939*	0.941*
Independently of N dose	0.931*	0.925*	0.945*

* significant coefficient at $\alpha = 0.05$

CONCLUSIONS

1. The *Ecklonia maxima* extract significantly increased true protein content, simple sugar content and the sugar/protein ratio in the tested plants, regardless of their cultivar, nitrogen rates, cuts and study years.

2. Nitrogen rates significantly affected the protein and sugar contents in the dry matter of both *Dactylis glomerata* and *Festulolium braunii*. Higher nitrogen rates increased protein levels but also reduced sugar levels in the grass species tested.

3. The value of carbohydrate/protein ratio averaged 1.07 and fell within the range of values which are believed to be optimal for ruminants. The most favourable ratio (1.59) was calculated for plants harvested from non-fertilised Kelpak-sprayed plots.

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