Carbohydrate to protein ratio in perennial ryegrass: effects of defoliation stage and nitrogen rate

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Introduction

In intensive grass-based systems, perennial ryegrass *(Lolium perenne L)* grazed at early stages has a high crude protein content (CP) and low water soluble carbohydrate (WSC) relative to animal requirements. This imbalance leads to low nitrogen (N) use efficiency and a large loss of N excreted to the environment (Dijkstra *et al.* 2011). Previous research has shown the importance of the WSC:PC ratio as a useful factor that determines the improvement of the animal's performance in terms of nitrogen utilization (Cosgrove *et al.* 2007). However, little is known about how the management of defoliation at specific growth stages can modify the WSC:CP ratio and other nutritive parameters related to N use efficiency. The objective of this study was to determine the changes of WSC:CP ratio and other nutritive parameters related to N use efficiency in response to nitrogen fertilizer rate and defoliation frequency based on leaf stage.

Methods

The study was located at the experimental research station of the Universidad Austral de Chile, Valdivia, Chile (39°47'S, 72°12'W) and undertaken between August 2010 and August 2011. The factorial combination of five fertilizer-N application rates and two defoliation frequencies was compared in a field experiment, using a complete randomized block design, replicated three times. The N levels used corresponded to the equivalent of an annual application of 0, 75, 150, 300, 450 kg N/ha/year.

The defoliation frequency was based on leaf stage (2 or 3 leaves per tiller) and the residual height was 4 cm. Each sampling was conducted at the same time of the day (09:00 AM) to avoid the effect of diurnal changes in WSC concentration. A target sample, (approximately 200g wet weight) was taken from each plot (2.5 x 5m), immediately frozen with liquid nitrogen and transported to freezer storage (-20°C). The frozen samples were freeze dried and ground to pass a 1mm sieve. The WSC content was estimated as the sum of the carbohydrate fractions which were analyzed with Waters Acquity ultra performance liquid chromatography (UPLC) according to Bertrand *et al.* (2007). Fractionation of CP was carried out according to the Cornell Net Carbohydrate and Protein System (CNCPS) into soluble nitrogen ($A+B_1$), true protein with intermediate (B_2) and slow (B_3) degradation rates and unavailable protein (C) (see Lanzas *et al.* 2008). For all variables plots were considered the experimental units.

The data were analyzed through ANOVA. The model included the fixed effect of the nitrogen fertilizer (N), leaf stage (L) and the random effect of blocks. The season (S) effect was introduced in the model as a repeated factor. Using Akaike´s information criterion, the unstructured residual covariance structure was determined to be the most appropriate for repeated measures on each plot over seasons within the year.

Results

Over the entire experimental period, the average of nitrogen fractions as proportions of total nitrogen were 388 g/kg CP for $A+B_1$, 511 g/kg CP , for B_2, 100 g/kg CP for B_3, and 15 g/kg CP for C. During early spring, a higher proportion (P<0.001) of $A+B_1$ was found which occurred at the expense of the B_3, C and to a lesser extent, B_2 fractions (Table 1). The opposite occurred during late spring and summer, where the lower $A+B_1$ fractions and the increase in B_3 and C fractions were consistent with increasing NDF and decline in CP content (Table 1).

Over the experimental period, extending the time of defoliation from the two-leaf to the three-leaf stage resulted in an increase of 19 and 7 g/kg CP for the $A+B_1$ and C fractions, respectively, and a decrease of 81 g/kg CP in the B_2 fraction (P<0.001). However, a two way S x L interaction (P<0.001) revealed the absence of leaf stage effect during early spring for $A+B_1$ and B_2 and during summer for B_3 (Table 1). The high CP content and lower WSC resulted in lower WSC:CP ratios during early spring and autumn (0.45 and 0.75, respectively) which was significantly higher (P<0.001) for defoliation at the three-leaf stage and declined with greater rates of nitrogen fertilizer in both seasons (P<0.001). However, a three way interaction (S x L x N) revealed the absence of a defoliation stage effect during summer and a marked decline in WSC:CP ratio from 3-leaf to 2-leaf stages, during early spring and autumn above 150 kg N/ha/year (Fig. 1).

Table 1. Effects of leaf stage, season of the year and their interaction on neutral detergent fibre (NDF), crude protein (CP) content (g/kg DM) and crude protein fractions as proportion of total crude protein (g/kg CP).

	Leaf stage	NDF	CP	A+B_1	B_2	B_3	C
		(g/kg DM)		(g/kg CP)			
Early spring	2-leaf	389	260	464	488	42	5
	3-leaf	383	214	456	482	52	9
	Sem	2.15	2.50	3.72	4.58	2.00	0.62
Late spring	2-leaf	424	181	275	652	60	12
	3-leaf	450	128	324	552	105	18
	Sem	3.66	3.19	8.98	9.91	8.42	0.72
Summer	2-leaf	446	152	303	518	161	19
	3-leaf	483	133	414	400	155	30
	Sem	5.46	2.86	5.50	7.63	6.71	0.71
Autumn	2-leaf	416	231	312	549	127	11
	3-leaf	416	182	437	448	99	17
	Sem	2.40	3.63	8.31	10.3	6.61	0.68
Effects	season (S)	***	***	***	***	n.s	***
	leaf (L)	***	***	***	***	***	***
	S x L	***	***	***	***	***	***

***: P<0.001; n.s. no significant. CP= crude protein; A+B_1 = buffer soluble protein; B_2 = buffer insoluble, neutral detergent soluble true protein; B_3 = neutral detergent insoluble, acid detergent soluble protein; C = acid detergent insoluble protein**;** sem = standard error of the mean.

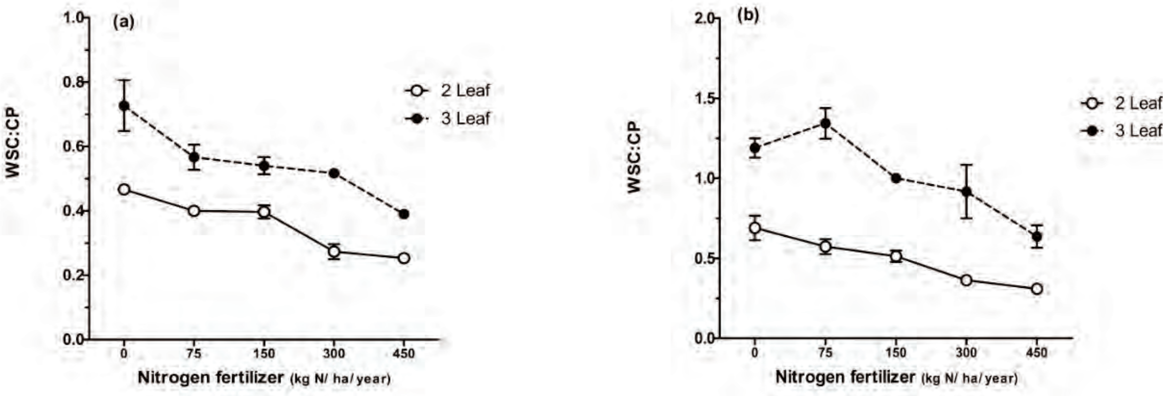

Figure 1. Effect of nitrogen level on soluble carbohydrate relative to crude protein ratio (WSC: CP) at two (○) or three (●) leaf stage, during early spring (a) and autumn (b). Vertical bars represent standard error of the mean (n=3).

Conclusion

The results indicate that increasing nitrogen application rate in combination with frequent defoliation leads to high crude protein and low carbohydrate fractions, which resulted in low WSC:CP ratios. However, the potential to manipulate WSC:CP by pasture management was dependent on the season of the year.

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