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Water-soluble carbohydrate and nitrogen concentrations after defoliation in perennial ryegrasses (*Lolium perenne* L.) in spring

Concentración de carbohidratos solubles en agua y nitrógeno posterior a la defoliación en ballicas perennes (*Lolium perenne* L.) en primavera

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A B S T R A C T

It is well documented that water-soluble carbohydrates (WSC) and nitrogen (N) are sources to start growth after defoliation, but there is little information about the concentration changes post-defoliation when normal and high sugar cultivars (CVS) are used under field conditions. The objective of this study was to evaluate, every three days, the WSC and N concentration change in stubble and regrowth of four perennial ryegrasses (AberAvon, AberDart AR1, Arrow AR1 and Jumbo). There was no interaction between CVS and day after defoliation on the WSC or N concentration for each tissue ($p > 0.05$). There were effects of time after defoliation in both tissues ($p < 0.05$). The WSC concentration on the stubble declined until day nine (148.9 g kg^{-1} DM), reaching initial levels on day 18 after defoliation (198.8 g kg^{-1} DM). In the regrowth the values changed from 114.4 to 159.5 g kg^{-1} DM, respectively. The N concentration in the stubble and regrowth increased up to day 9 (26.4 g kg^{-1} DM) and day 12 (38.2 g kg^{-1} DM), respectively. Later, there was a steady decline until the end of the regrowth period with N concentrations of 20.4 and 27.8 g kg^{-1} DM for stubble and regrowth, respectively. The results indicate that the use of CVS marketed as high sugar do not bestow any advantage in terms of higher concentrations of WSC in different tissues after defoliation.

RESUMEN

Se encuentra bien documentado que los carbohidratos solubles en agua (CSA) y nitrógeno (N) son fuentes para iniciar el rebrote posterior a la defoliación, pero hay poca información sobre sus cambios cuando cultivares normales, o comercializados por mayores niveles de azúcar son usados bajo condiciones de campo. El objetivo del estudio fue evaluar, cada tres días, la concentración de CSA y N en el residuo y el rebrote de cuatro ballicas perennes (AberAvon, AberDart AR1, Arrow AR1 y Jumbo). No se registraron interacciones entre cultivares y el tiempo posterior a la defoliación en la concentración de CSA y N para cada uno de los tejidos ($p > 0.05$). Hubo efectos del tiempo post-defoliación en ambos tejidos ($p < 0.05$). En el residuo, la concentración de CSA disminuyó hasta el día nueve post-defoliación (148.9 g kg^{-1} MS) con una recuperación a los niveles iniciales en el día 18 (198.8 g kg^{-1} MS). En el rebrote, estos valores cambiaron desde 114.4 a 159.5 g kg^{-1} MS respectivamente. La concentración de N en el residuo y en el rebrote aumentó hasta el día 9 (26.4 g kg^{-1} MS) y día 12 (38.2 g kg^{-1} MS) respectivamente. Posteriormente, una disminución constante hasta el final del periodo ocurrió, con una concentración de N de 20.4 y 27.8 g kg^{-1} MS para el residuo y el rebrote respectivamente. Los resultados indican que el uso de cultivares comercializados como altos en azúcar no confieren una ventaja en relación a mayores porcentajes de CSA en distintos tejidos posterior a la defoliación.

Palabras clave: hojas, reservas, rebrote, residuo.

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is distributed worldwide and is one of the most important forage species in temperate regions (Wilkins and Humphreys, 2003). However, the competitive advantage of the animal production in grazed based systems depends on increased and efficient grazed herbage and higher proportions in the annual feed intake (Dillon *et al.*, 2005). To this end, a way to increase herbage dry matter and animal production could be the use of improved cultivars (CVS) (Wilkins and Lovatt, 2011) in terms of seasonal growth, sward structure and nutritional value (Wims *et al.*, 2013). The use of perennial ryegrasses termed ´high sugar´, characterized by the accumulation of a greater amount of water-soluble carbohydrates (WSC) compared to CVS not selected for this trait (Wilkins and Humphreys, 2003) could be an alternative, although the magnitude of expression of this trait varies (Edwards *et al.*, 2007).

Feeding forage with higher levels of WSC in the foliage could confer a better synchrony between energy and protein availability, indicating that a greater expression of the trait would be valuable for improvements in animal production (Edwards *et al.*, 2007). In this way it is proposed that the use of these CVS could increase dry matter intake, milk yield and milk N production (Keim and Anrique, 2011), and in theory, increase N utilization by bovine (Hoekstra *et al.*, 2007a). To validate this statement, the high sugar trait should be expressed in higher amounts on the foliage.

From the plant's point of view, WSC in the stubble is the main source of energy to start the growth after defoliation (Fulkerson and Donaghy, 2001), and N concentration acts as a reserve during forage regrowth (Volenec *et al.*, 1996). Taking into account that a higher WSC and N concentration in plant stubble could give an advantage to the regrowth process, the objective of the present study was to evaluate, in normal and marketed high sugar perennial ryegrasses, the WSC and N concentrations in one regrowth period, both in stubble and regrowth tissue.

MATERIALS AND METHODS

Site and management

The study was conducted at the Santa Rosa Experimental Station (39°47´S, 73°13´W, 17 m.a.s.l.), at the Universidad Austral de Chile, Valdivia, Chile, from 28 October to 27 November 2009. The soil type corresponds to a Duric Hapludands, Valdivia series (CIREN, 2003), developed from volcanic ash. Maximum and minimum absolute daily temperatures and daily rainfall for the period are shown in Figure 1.

On 26 April 2008 plots of 5 m x 2 m were sown with a seeding rate of 25 kg ha^{-1}, and fertilizer application rate of 40 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1} at the experimental site. One month before sowing, 2,000 kg $CaCO_3$ ha^{-1} were applied. The chosen perennial ryegrasses were diploids, two marketed as having enhanced WSC levels (high sugar): AberAvon (late heading) and AberDart AR1 (intermediate heading), and two non-marketed as high sugars (normal sugar): Arrow AR1 (intermediate heading) and Jumbo (late heading). From the sowing date until 29 September 2009 (last yielding harvest before the beginning of the trial), the plots were harvested 9 times with a lawn mower (Grillo spa, Cesena, Italy) when plants reached an average height of 200 mm, leaving a stubble height of 50 mm. After each harvest a dose of 30 kg N ha^{-1} was applied except in summer and winter, with the last N dose applied on 18 August 2009, two harvests before the beginning of the study.

Sample procedure

The plots were harvested at 07:00 h, block by block, on 28 October 2009 (day zero) as previously explained. Forage samples (above 50 mm) were taken in order to have the initial WSC and N concentrations. Right after the harvest of day zero, each plot was divided into 11 equally sized strips (0.2 m x 2 m swaths) and sampling was undertaken with intervals of three days. Immediately after the day zero forage harvest, the first strip was harvested from ground level (only stubble) using hand scissors. After three days the second strip was harvested as before (stubble plus regrowth), and so on up to the 11th strip. Therefore each strip was harvested 3 days after the predecessor until the 27 November when the plants reached 200 mm height again. Samples were stored in a cooler box keeping them cold with ice to limit carbohydrate loss. To carry out WSC

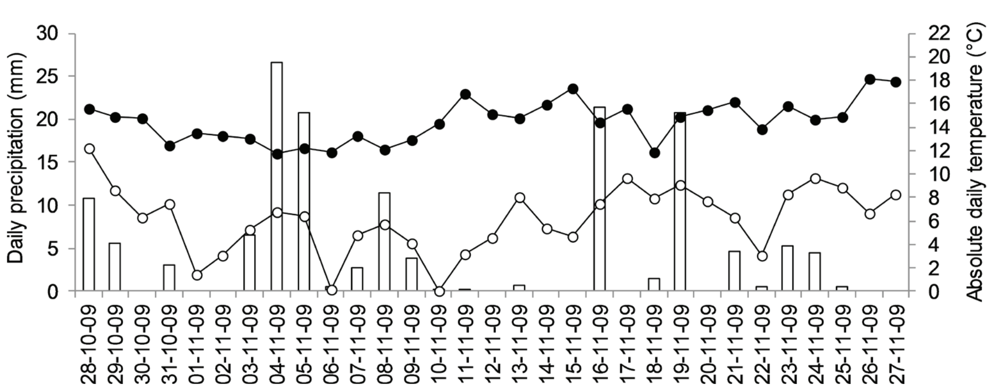

Figure 1. Daily precipitation (□) and absolute daily maximum (●) and minimum (○) temperatures at the trial site from 28 October to 27 November 2009.

Figura 1. Precipitación diaria (□) y temperaturas máxima (●) y mínima (○) absolutas en el sitio de ensayo desde el 28 de octubre al 27 de noviembre de 2009.

and N analysis, samples were sent to the Animal Nutrition Laboratory at the Universidad Austral de Chile. Dead tissue and weeds were removed and plants were cut at a height of 50 mm, dividing stubble (tissue under 50 mm) from regrowth (tissue above 50 mm). Samples were dried in a forced air oven at 105 °C for 2 h (Poff *et al.*, 2011) and were ground to pass through a 1 mm sieve (Thomas, Swedesboro, USA). By means of near infrared spectroscopy (Foss NIRSystem, Maryland, USA) WSC and N concentrations were determined in both stubble and regrowth on a dry matter basis (g kg^{-1} DM). The estimate comes from a previous calibration of wet chemistry following AOAC (1995) and MAFF (1986).

Experimental design and statistical analysis

A split-plot design was used with CVS as the main plot and sampling time as the sub-plot. ANOVA was applied to WSC and N concentrations on stubble and regrowth to identify significant effects (GLM Procedure) by means of SAS 9.2 software. To compare treatment means, Duncan's multiple range test comparison procedure was used ($p < 0.05$).

RESULTS

WSC in stubble and regrowth

There was no interaction ($p > 0.05$) between cultivars and day after defoliation on the WSC concentration of stubble and regrowth tissue. There was a significant effect of time on the stubble and regrowth WSC concentration ($p < 0.05$). In the stubble (Figure 2), the WSC concentration declined ($p < 0.05$) from day zero (204.5 g kg^{-1} DM) to day 9 (148.9 g kg^{-1} DM) after harvest. After this point the concentration increased ($p < 0.05$) until day 24 (227.9 g kg^{-1} DM), with no time effect ($p > 0.05$) after this date (mean of 235.3 g kg^{-1} DM). The replenishment of WSC in the stubble to initial concentration was observed on day 18 (198.8 g kg^{-1} DM) after defoliation. There was a significant effect of CVS. The mean WSC concentration for the whole sampling period for AberAvon and AberDart AR1 were 206.0 and 204.2 g kg^{-1} DM, higher ($p < 0.05$) than the concentration of Arrow AR1 (175.8 g kg^{-1} DM). The WSC concentration for Jumbo was 190.5 g kg^{-1} DM, and was similar to the other cultivars ($p > 0.05$).

In the regrowth (Figure 2), the WSC concentration after defoliation declined ($p < 0.05$) from day zero (159.4 g kg^{-1} DM) to day 9 (114.4 g kg^{-1} DM). After this date there was an increase ($p < 0.05$) until day 21(175.9 g kg^{-1} DM) with no effect afterwards (177.8 g kg^{-1} DM in average). WSC concentrations similar to the initial levels were found on day 18 (159.5 g kg^{-1} DM) after defoliation. In relation to WSC concentration of CVS, the regrowth followed the same pattern as the stubble, with the highest concentrations ($p < 0.05$) in AberAvon (166.3 g kg^{-1} DM) and AberDart AR1 (164.8 g kg^{-1} DM) and the lowest in Arrow AR1 (135.1 g kg^{-1} DM). The WSC concentration for Jumbo was similar ($p > 0.05$) to the other cultivars (145.8 g kg^{-1} DM).

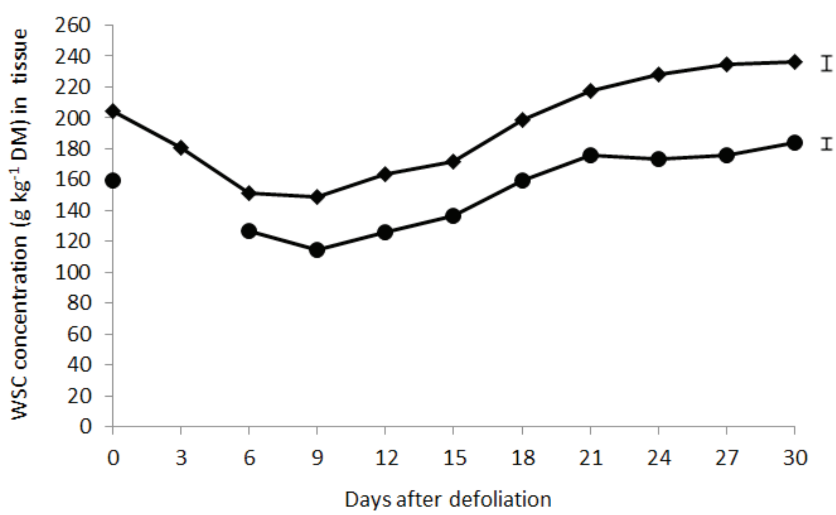

Figure 2. WSC concentration (g kg^{-1} DM) in stubble (♦) and regrowth (●) after defoliation in *Lolium perenne*. Error bars indicate standard error for each tissue over time. The amount of regrowth tissue available on the 3rd day after defoliation was insufficient to determine WSC concentration.

Figura 2. Concentración de CSA (g kg^{-1} MS) en el residuo (♦) y rebrote (●) posterior a la defoliación en *Lolium perenne*. Barras de error indican error estándar de cada tejido en el tiempo. La cantidad disponible de tejido de rebrote del tercer día post-defoliación fue inferior a la requerida para determinar la concentración de CSA.

N in stubble and regrowth

There was no interaction ($p > 0.05$) between cultivars and day after defoliation on the N concentration of stubble and regrowth tissue. In the stubble (Figure 3), N concentration did not vary ($p > 0.05$) in the first three days of regrowth (mean of 24.1 g kg^{-1} DM). From day six (26.0 g kg^{-1} DM) to day 15 (25.2 g kg^{-1} DM) after defoliation, the N concentration was higher compared with the rest of the period ($p < 0.05$), and after this point a decrease until day 21(21.6 g kg^{-1} DM) was found ($p < 0.05$). Afterwards there were no differences in N concentrations (21.0 g kg^{-1} DM in average). A similar concentration to that at the beginning of the study was observed on day 18 (22.9 g kg^{-1} DM) after defoliation. The mean N concentration for the whole sampling period for AberAvon, AberDart AR1 and Arrow AR1 were 24.6, 24.3 and 23.6 g kg^{-1} DM, respectively, all higher ($p < 0.05$) than the concentration of Jumbo (21.9 g kg^{-1} DM).

The N concentration in the regrowth (Figure 3) had a similar pattern as that shown by the stubble tissue. From day zero (30.9 g kg^{-1} DM) to day 12 (38.2 g kg^{-1} DM) after defoliation there was an increase in N concentration ($p < 0.05$). After day 15 (37.5 g kg^{-1} DM) there was a steady decline until the end of the regrowth period ($p < 0.05$), reaching the lowest level on day 30 (27.8 g kg^{-1} DM) after defoliation. A similar N concentration to that at the beginning of the study was observed on day 24 (30.9 g kg^{-1} DM) after defoliation. There were no differences ($p > 0.05$) between cultivars on the N concentration (33.3 g kg^{-1} DM on average) for the whole sampling period.

DISCUSSION

It is well documented that regrowth after defoliation relies firstly on WSC reserves, resulting in the depletion of WSC concentrations in the stubble, and later on when photosynthetic capacity is restored, the plant starts to replenish its WSC reserves again (Fulkerson and Donaghy, 2001). This U-shaped response, also seen in other studies (Morvan-Bertrand *et al.*, 1999; Lee *et al.*, 2010), was found in the present research, with no effect of CVS (some of them marketed as high sugar) in the WSC concentration in the stubble over time (Figure 2). The latter signifies that there were no differences in terms of a faster or lower restoration of WSC reserves among CVS, a fact that could change the use of pastures if WSC restoration criteria are used. This must be related to yielding forage, as forage production in the early stages of regrowth (between 1 and 2 leaves per tiller) is less productive compared with later stages (Lee *et al.*, 2010; Poff *et al.*, 2011). As before, the WSC concentration in the regrowth tissue (Figure 2) follows the same pattern of depletion and recovery that was reported by Rasmussen *et al.* (2009), where the lowest WSC concentrations were between days 3 and 6 after defoliation (depending on the cultivar) and nearly reaching initial contents on day 21 in a 20°C day and 10°C night treatment (18 days in the present study). Once again, in the present study there were no effects of cultivars on the WSC concentration in the regrowth. Here the WSC concentrations on day zero and 30 after defoliation were lower than those found in other works in the spring season, with diploid CVS and simulated grazing

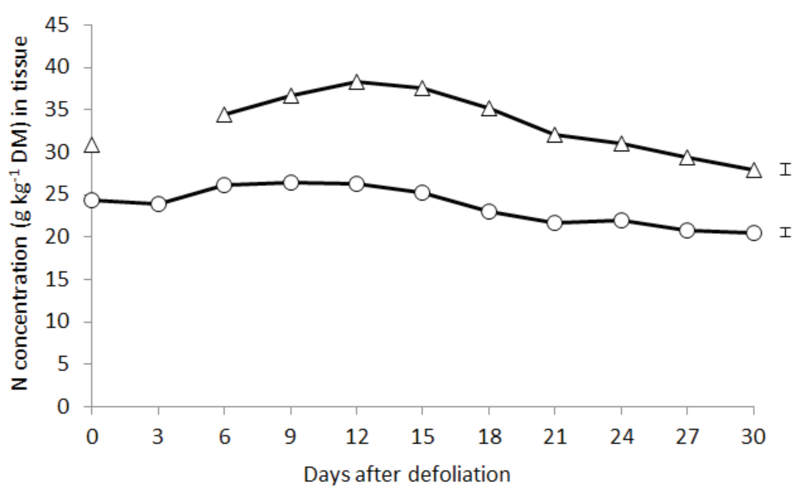

Figure 3. N concentration (g kg^{-1} DM) in stubble (○) and regrowth (Δ) after defoliation in *Lolium perenne*. Error bars indicate standard error for each tissue over time. The amount of regrowth tissue available on the 3rd day after defoliation was insufficient to determine N concentration.

Figura 3. Concentración de N (g kg^{-1} MS) en el residuo (○) y rebrote (Δ) posterior a la defoliación en *Lolium perenne*. Barras de error indican error estándar de cada tejido en el tiempo. La cantidad disponible de tejido de rebrote del tercer día post-defoliación fue inferior a la requerida para determinar la concentración de N.

management (Gilliland *et al.*, 2002; Burns *et al.*, 2013). Differences can be attributed to many reasons for instance a complex function of environmental factors such as irradiance, temperature, water availability (Humphreys *et al.*, 2006) and/or plant factors such as maturity and the inherent differences between CVS (Stewart and Hayes, 2011). Despite this, the higher WSC concentration in the marketed high sugar ryegrasses are consistent in some studies (Cosgrove *et al.*, 2007; Easton *et al.*, 2009; Wims *et al.*, 2013), but not in others (Parsons *et al.*, 2004; Hume *et al.*, 2010).

Using different CVS, there was no WSC trait expression in terms of a faster or lower recovery capacity in stubble or in a higher WSC concentration in the regrowth. However, taking into account the whole sampling period, there was a higher WSC expression in AberAvon and AberDart AR1 on stubble and regrowth compared to Arrow AR1, but not to Jumbo. This is important as the benefits of the termed high sugar cultivars for the ruminant depend firstly on the high WSC concentration, mainly in leaves.

The N concentrations in stubble and regrowth are in agreement with those of other studies. In the stubble they are lower than in the regrowth (Whitehead, 2000; Hoekstra *et al.*, 2007b), as the main role of the former tissue is structural and not photosynthetic as in the regrowth, which needs a greater level of N for its photosynthetic activity (Hoekstra *et al.*, 2007a). Also the higher N concentrations after the first days of defoliation have been reported before (Turner *et al.*, 2007), and could be explained by the negative relationship between N and WSC (Radojevic *et al.*, 1994; Cosgrove *et al.*, 2009). Additionally, the decrease after the peak was in line with the results of Turner *et al.* (2007) in prairie grass (*Bromus willdenowii* K.), with the highest N % in the stubble at the 1-leaf stage. The N concentrations in the regrowth at day zero and 30 are within the normal range for bovine production (Hoekstra *et al.*, 2007a), and similar to those found in spring by Balocchi and López (2009) and Hodgkinson *et al.* (2009) in southern Chile, who used the same defoliation criteria as this study, and similar to those found by Bryant *et al.* (2012) and Lee *et al.* (2008) when they harvested at the 3-leaves stage and at a stubble height of around 50 mm, a defoliation criteria that optimizes persistence and productivity of perennial ryegrass (Fulkerson and Donaghy, 2001). In addition, the highest N concentrations found in the first days on the regrowth (Figure 3) agree with the works of Fulkerson and Slack (1994) and Fulkerson *et al.* (1998), with the highest values at the 1-leaf stage and a steady decline of N concentrations thereafter. Poff *et al.* (2011) suggest that the relative decrease of N may be, in part, due to an increase on Neutral Fiber Detergent (NFD) and Acid Fiber Detergent (AFD) fractions but with a small or null absolute increase on N concentrations in plants when 1.5, 2.5 and 3.5 leaves stage defoliation criteria were used. This same relationship between N, NDF and ADF was found by Bryant *et al.* (2012), with a reduction of N % when extending the regrowth period from 2 to 4 leaves in three CVS of perennial ryegrasses.

CONCLUSIONS

There are no differences in the WSC or N concentrations of different CVS on the stubble and regrowth tissue over time. On average, for the experimental period, a higher WSC concentration in both tissues was found in AberAvon and AberDart AR1 compared to Arrow AR1, with the WSC concentration in Jumbo similar to all cultivars. Cultivar Jumbo had the lowest N concentration in the stubble tissue and between cultivars there were no differences in the N concentrations in the regrowth. The results indicate that the use of CVS marketed as high sugar do not present any advantage in terms of higher concentrations of WSC in different tissues after defoliation. Experiments are in progress with the new released high sugar ryegrasses, evaluating if there is a higher sugar trait expression in stubble and aftermath, and a faster WSC recovery on the stubble under the climate of the main grazing zone of southern Chile.

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