Morphological development, herbage yield and quality of Italian ryegrass during primary growth and regrowth: Regression models and yield optimisation

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Abstract

The main aim of this research was to establish simple regression models for predicting herbage production parameters during uninterrupted growth and to contribute to forage optimisation of Italian ryegrass (*Lolium multiflorum* Lam.) cultivated as an overwinter catch crop. The field experiment in split-plot design with two block replicates consisted of two growth cycles: primary growth (C1) and regrowth (C2) as the whole plots, and twelve time series with five-day intervals as the sub-plots. For each time point, herbage dry matter yield, mean stage by weight (MSW) and contents of crude protein (CP) and net energy for lactation (NEL) were determined. Growth days for all production parameters and MSW for quality parameters were used as explanatory variables. Considering the practically relevant 47-day growth period, simple linear regression models explained from 84.9% to 94.0% of the variance of the investigated parameters. These models are better than those performed for the whole 67-day period, except for the model for MSW-based prediction of CP content. The comparison of the two predictors showed that growth days were at least as good as MSW in predicting CP and NEL contents determined during C1 and C2. The effect of growth cycle on the patterns of all investigated parameters was significant, indicating that growth conditions played an important role. Based on our results, CP and NEL yield potentials of Italian ryegrass cannot be completely exploited in a double catch crop system if the required forage quality for lactating cows is to be respected. It rather suggests getting the maximal single harvest in early May, which is justified from nutritional and economical standpoints.

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Key words: Crude protein; loess fitted lines; mean stage by weight; net energy for lactation; yield accumulation.

Funding: the research was financially supported by the Ministry of Education, Science and Sport and by the Slovenian Research Agency (Programme Sustainable Agriculture, P4-0133).

Acknowledgements: we thank Boštjan Medved Karničar for his excellent support in managing the field experiment and in botanical analysis.

Contributions: JV, JČ and PK conceived and designed the research; PK, JV and JČ performed the experiment; KE performed statistical analysis and graphed the data; JČ wrote the paper; KE, JV and JČ reviewed and edited the paper.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 6 July 2019.
Revision received: 24 September 2019.
Accepted for publication: 3 October 2019.

Italian Journal of Agronomy 2019; 14:1497
doi:10.4081/ija.2019.1497

Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is a short-lived perennial cultivated either as a component of grassland sward or as a forage crop. In the latter case, it promotes farming sustainability through improved soil fertility and crop rotation. Furthermore, as a catch crop, Italian ryegrass improves resource and energy use efficiencies in crop production systems (Fuksa *et al.*, 2013) and is often cultivated in double cropping with maize to provide livestock feed (Borrelli *et al.*, 2014). Italian ryegrass is used for forage production throughout the temperate regions of the world due to its vigorous growth, ensilability and feed quality for ruminants (Jung *et al.*, 1996; Humphreys *et al.*, 2010).

Italian ryegrass is regarded as one of the most productive temperate forage grasses, with an annual herbage dry matter yield of up to 20 t ha^{-1} in the first harvest year (Burns *et al.*, 2015) under favourable growth conditions. Considering this high yield potential, it should be noted that the reproductive growth habit of Italian ryegrass, which is exhibited during the season of long day-lengths (Cooper, 1950; Williamson, 2008), contributes to this potential through improved photosynthetic efficiency (Parsons, 1988). This phenomenon should also compensate the slow initial regrowth rate caused by slow leaf recovery from defoliation of elongated tillers, which precipitates their death (Behaeghe, 1986).

The nutritional value of young Italian ryegrass is high but decreases faster with progressing growth compared to that of herbage from productive grasslands (DLG, 1997). Its main quality advantages are a high content of water-soluble carbohydrates and net energy for lactation (NEL) in forage comprising tillers, which are developed up to the beginning of flowering. The morphological development of Italian ryegrass (such as herbage growth) considerably influences the forage quality, by stem elongation during reproductive growth.

The harvest time of grasses is critical for efficient forage production because crop ageing affects both herbage yield and quality. Generally, herbage accumulation shows a sigmoid time pattern, with a pronounced linear portion (Leafe *et al*., 1974). It can therefore be quite close to a linear pattern before the herbage accumulation approaches a plateau in the mature crop. Italian ryegrass growth follows the same rule (Wilman, 2004).

It is well known that the herbage quality of grasses decreases with progressing growth and that the morphological development of the crop plays an important role in this process (Beever *et al*., 2000). Nutrient content (*e.g*., crude protein and crude fibre) and digestibility often show linear patterns with growth or morphological progress, while some patterns are more curvilinear (*e.g*., Sanderson, Wedin, 1989; Valente *et al*., 1998, 2000; Nissinen *et al*., 2010). In this context, the NEL content in herbage has been investigated very rarely compared to other quality parameters.

Growth days and morphological development stages have often been used for herbage quality prediction, with the aim of establishing models of practical relevance. The development stage seems to be a better herbage quality predictor than growth days, due to a stronger relation to the plant physiological status (Solomon *et al*., 2017). To take into account the tiller morphological diversity of a grass crop at given time, the development index designated mean stage by weight (MSW) has been proposed for exploring relationships with quality parameters (Sanderson, 1992). It was devised by modification of the morphological development scale for forage grasses reported by Simon and Park (1983) and using the calculation method of Kalu and Fick (1981). Little information is available on Italian ryegrass growth processes relevant for optimisation of overwinter catch crop production and there is no information on the relationship between MSW and NEL content.

The objectives of the current study were therefore to ascertain: i) regression models for predicting herbage yield accumulation, MSW and nutritional quality of Italian ryegrass during growth; ii) a possible advantage of MSW over growth days as the explanatory variable for predicting nutritional quality; iii) the effect of growth cycle (which serves as a proxy for weather conditions) on growth processes; and iv) optimisation of forage production in a double catch crop system by taking into account the yield accumulation and nutritional value.

Materials and methods

Site and experimental design

A field experiment was carried out on a dairy farm near Ljubljana in the pre-Alpine region of Slovenia (46°7′ N, 14°26′ E; a.s.l. 325 m) in 2016. The climate of the region is moderate continental, with a mean annual air temperature of 10.9°C and mean annual precipitation of 1362 mm for the 1981-2010 period, both recorded at the Ljubljana meteorological station. In 2016, the mean air temperature and precipitation sum were 11.8°C and 1317 mm, respectively. Comparing the periods of growth cycles included, air temperature differed considerably in the second part, while precipitation differed considerably in the first part (Figure 1). However, we assume that only temperature might have a significant effect on the investigated parameters and not precipitation, deficiency of which did not appear at the beginning of spring due to the winter soil water reserve. The highest temperature difference between the two cycles occurred at the mid-point, due to the passage of a cold front in the region at the end of April.

The Dystric Cambisol (WRB, 2015) on shale and sandstone of the experimental site is 50 to 70 cm deep, with silt loam in the upper 25 cm layer. Before starting the experiment, the soil of the upper 6 cm layer had pH_{KCl} of 4.7, and the content of available P and K (extracted in ammonium lactate) was 25 mg and 116 mg kg^{-1} of dry soil, respectively. This pH value is accepted to be marginally suitable for Italian ryegrass growth. The low to moderate contents of phosphorus and potassium were compensated by the increased fertiliser rates used.

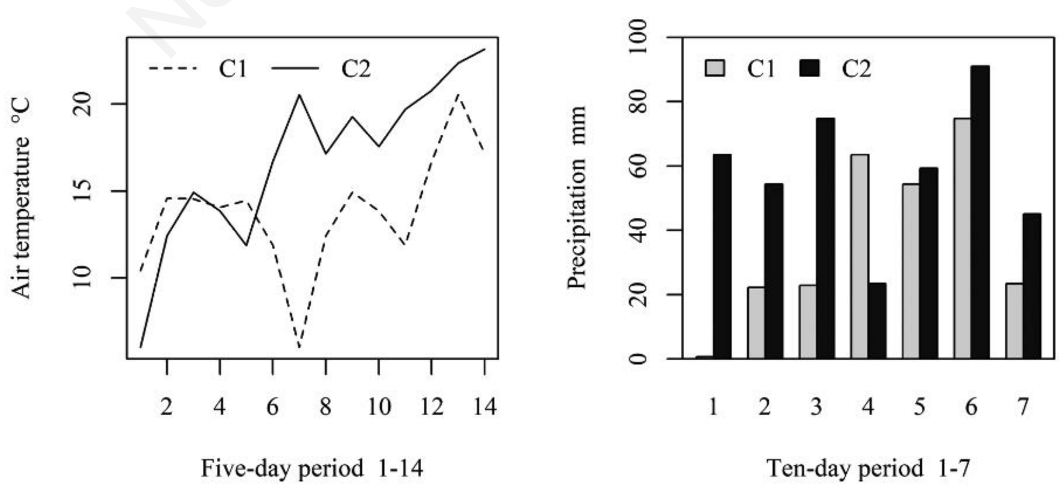

Figure 1. Five-day average air temperature and ten-day sum of precipitation during the primary growth (C1) and regrowth (C2) cycles of the experimental period. The starting date for C1 was 26 March and for C2 25 April 2016.

A field experiment was set up on a young, overwintered crop of the Italian ryegrass cultivar 'Tarandus' (2n = 4x = 28; Deutsche Saatveredelung AG) on 18 March 2016. Treatments were arranged in a split-plot design with two replicates. Main plots were assigned to two growth cycles, primary spring growth cycle (C1) and regrowth spring cycle (C2). Active growth in C1 started after the temperature threshold on 26 March, while C2 began on 25 April after the previous first harvest. Subplots were assigned to 12 time series of herbage measurements and herbage samplings with 5 days intervals. The subplot size was 2×5 m. In the previous autumn, the experimental field received 20 m^3 ha^{-1} of cattle slurry prior to ploughing. Early spring nutrient inputs were: 90 kg N, 39 kg P and 224 kg K ha^{-1}. For C2, the Italian ryegrass received an additional 80 kg N ha^{-1} immediately after the previous harvest. Nitrogen was applied as calcium ammonium nitrate (27% N) in both cycles. Phosphorus and potassium were applied as PK fertiliser (10% P_2O_5 and 30% K_2O).

Yield and mean stage by weight measurement

Mown herbage from the 1.2 m width central strip of each subplot, including tillers used for morphological analysis, was weighed. The fresh masses were converted to dry matter (DM) yields of herbage per ha using the data on DM contents. The stubble height was roughly 6 cm. Stand morphological development was determined as MSW for a tiller sample cut to ground level from two seeded rows of 50 cm length. Tillers were separated according to the quantitative scale for classification of tiller stage proposed by Sanderson (1992). Fresh tiller fractions were then weighed and the data were used to calculate the MSW with the equation:

$$MSW = \sum_{i=0.5}^{35} (i \cdot m_i) / \sum_{i=0.5}^{35} m_i,$$

(Kalu and Fick, 1981), where *i* is the number code for a particular development tiller stage (0.5-35) and m_i is the tiller mass of the *i-th* stage.

Chemical analysis

Samples of known mass were immediately taken from the mown herbage for chemical analysis. They were oven dried at 55°C to constant weight, ground to pass through a 1 mm sieve and packed into plastic bags to be kept in the dark at room temperature until analysis was performed. Crude protein (CP) content was calculated from the nitrogen (N) content in the herbage (N×6.25), determined by the Kjeldahl procedure (ISO 5983-2: 2009); crude fibre content was determined according to ISO 6865: 2000 (modified); crude lipids content was determined without preceding hydrolysis following the procedure prescribed in Commission Regulation (EC) No. 152/2009; crude ash content was determined according to ISO 5984: 2002/Cor. 1: 2005; acid detergent fibre content was determined according to Van Soest *et al*. (1991). *In vitro* gas production from herbage incubated in rumen liquor was determined using the modified Hohenheim gas test (Menke *et al*., 1979; Blümmel and Ørskov, 1993). A standard hay sample provided by the University of Hohenheim (Germany) was used to correct gas production values for variation in the activity of the rumen liquor. Net energy for lactation content was calculated using data of the aforementioned chemical analyses and the regression equations proposed by GfE (2001, 2008).

Data analysis

Local regression (loess) was used for graphic presentation of time (growth days) and MSW dependent patterns of the investigated parameters (yield, MSW, CP and NEL). Based on these figures and the design of the experiment, a linear regression model with three explanatory variables was chosen. The variables were: i) growth days within a cycle (time 0 was set to day 12; *i.e*., the first measurement in both cycles) or MSW as continuous variables; ii) growth cycle as a categorical variable; and iii) their interaction. Such a model provides an estimation of the model parameters with the relevant standard errors for each growth cycle and a coefficient of determination for the whole model. For each model, p-values for between-cycle differences in intercept and slope parameters were provided. The effects of growth days and cycle treatments and their interaction on CP and NEL yields were also tested by ANOVA. In this case, time (growth days) was used as a categorical factor due to the nonlinear response. Statistical analyses were performed in *R* environment (*R* Core Team, 2017) using the libraries *lattice*, *nlme* and *multcomp*.

Results and discussion

Loess curves and prediction models

The loess fitted curves of the investigated parameters in most cases showed roughly linear dependencies on growth days and MSW up to growth day 47 in each cycle (Figures 2 and 3). A nonlinear response was found for the growth days-dependent pattern of CP content, which started to curve at approximately day 37. Nevertheless, we took this 47-day period for each investigated parameter to perform the linear regression model analysis. This time period is long enough to satisfy practical requirements for Italian ryegrass silage or hay-making production. We also note here that all investigated parameters were affected significantly ($P<0.001$) by growth days, and that CP and NEL contents were affected significantly ($P<0.001$) by MSW.

Generally, all linear regression models predicted a large proportion of the variance of the data series for all investigated relationships (Table 1). The coefficient of determination ranged from 84.9% to 94.0%, which is similar to most values reported in the literature (Sanderson, 1992; Jeangros *et al*., 2001; Brueland *et al*., 2003). The prediction of CP and NEL patterns based on growth days is better than or similar, respectively, to those based on MSW. The advantage of growth days over the MSW predictor is unexpected if a stronger relation of the latter to the grass physiological status is perceived. This also contradicts findings in which codified morphological stages or crop age from 1 January were used to predict organic matter digestibility (Valente *et al*., 2000), which is inherently connected to NEL content. On the other hand, a very strong relationship (R^2=95%) between growth days and NEL content in Italian ryegrass was reported by Daccord *et al*. (2002) for spring growth, suggesting that an advantage of growth days over MSW is possible. An important reason for this may be the similar development of Italian ryegrass tillers in a stand. This tiller similarity was manifested in our experiment. A simple calculation of our mass data of individual stage categories determined in the MSW analysis showed that the two neighbour stage categories of the highest tiller mass accounted for 56% of the whole herbage

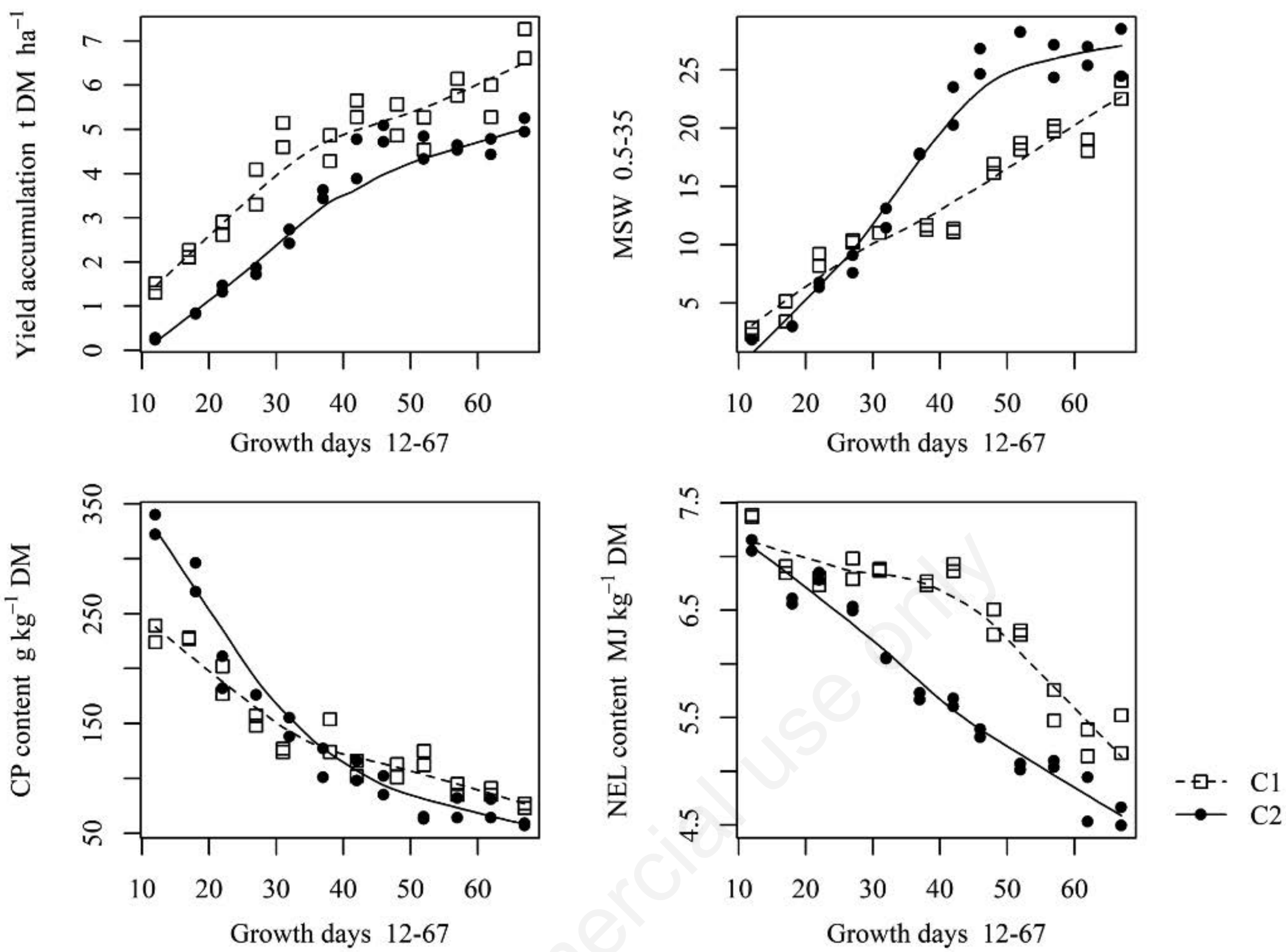

Figure 2. Loess fitted time curves for herbage yield accumulation, mean stage by weight (MSW) and contents of crude protein (CP) and net energy for lactation (NEL) in herbage dry matter (DM) of Italian ryegrass determined separately for uninterrupted primary growth (C1) and regrowth (C2).

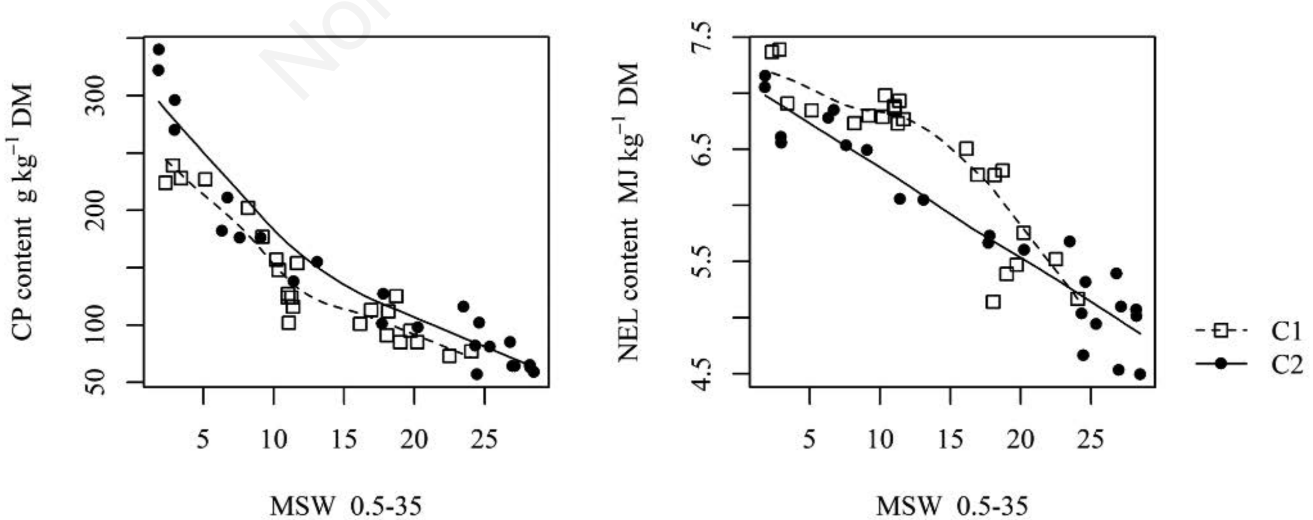

Figure 3. Loess fitted curves for crude protein (CP) and net energy for lactation (NEL) contents in herbage dry matter (DM) of Italian ryegrass plotted against mean stage by weight (MSW). The curves are determined separately for uninterrupted primary growth (C1) and regrowth (C2). The growth period was 12-67 days.

sample mass, averaged over all 48 samples. The average number of stage categories for all 48 herbage samples was 13 per sample.

Yield and mean stage by weight prediction models

In the linear regression model, growth days was a very good predictor of DM yield accumulation taking place during both cycles (Table 1). On average, it increased at a growth rate of 127 kg DM ha^{-1} per day, which is close to the maximal herbage growth of productive grasses from overlapping series of monthly cuts (Corrall and Fenlon, 1978; Finneran *et al*., 2011). The herbage accumulation in C2 increased marginally faster (P=0.066) but amounted to less over the whole tested period than that in C1 (Figure 4). The main reason for this is the slow recovery of Italian ryegrass after defoliation of elongated reproductive tillers, which occurs in all temperate grasses (Parsons, 1988).

MSW correlated strongly with growth days (R^2=94%) and increased significantly faster (P<0.001) during C2 than C1 (Figure 4), which resulted mainly from the higher air temperature in the second part of C2 (Figure 1). Day length and temperature are known to affect positively the rates of inflorescence development and stem elongation in temperate grasses, especially in the spring when the temperature is relatively low (Langer, 1979). The strong relationship between MSW and growth days resulted in similar prediction values for both explanatory variables in terms of CP and NEL contents in herbage.

Crude protein prediction models

Growth days were a slightly better predictor of CP content in herbage than MSW (R^2: 87.9% *vs* 84.9%), mainly due to the weaker correlation with MSW in C1 (Table 1), which can be partly ascribed to growth disturbance caused by the temperature drop at the end of April. Although the CP content patterns differ significantly between the growth cycles, it can be established that the majority of these differences result from the extremely high initial CP content in C2 (Figures 4 and 5). Such a high initial content of CP is less likely to occur in the primary growth, in which these values are usually relatively low (DLG, 1997; Jeangros *et al*., 2001). CP content decreased to 150 g kg^{-1} DM, the reference value for very good forage, at growth day 33 in both cycles. This crop age coincides with the optimal spring harvest time for producing high quality forage of important perennial grasses (Čop *et al*., 2009).

Net energy for lactation prediction models

In general, the linear model based either on growth days or MSW predicted NEL content in the herbage equally well during both growth cycles (R^2=91%; Figures 4 and 5). However, particular cycle standard errors showed that growth days are a better predictor than MSW in C1. In the first case, SE achieved 71% for intercept and 36% for slope of those in the second case (Table 1). Only one research paper was found dealing with time patterns of NEL content in grasses (Daccord *et al*., 2002) for comparing these data with others. The correlation that they found for Italian ryegrass was very strong (R^2=95%) in the primary growth cycle, while it was weak in regrowth (R^2=24%). In the latter case, several regrowth cycles were combined and then analysed, so comparison with our data is not appropriate. The time pattern of NEL content differed significantly between growth cycles in both coefficients (P<0.001), showing a persistent higher content in C1 than C2 (Figure 4). Lower air temperature during the second part of C1 was obviously the main reason for the higher NEL content. Air temperature is known negatively to affect herbage quality, resulting in higher proportions of supporting tissues and lignification in plants grown under warmer conditions (Akin *et al*., 1987). In addition, higher air temperature accelerates morphological development as well as cell wall digestibility decline (Akin, 1989; Groot *et al*., 2003). The former was also clearly manifested in our case. Due to the higher temperature in C2, an accelerated decline of NEL content was measured. Its content already decreased to 6.2 MJ kg^{-1} DM, the reference value for very good forage on growth day 30. On the other hand, in C1, due to the lower temperature, the NEL

Table 1. Estimated linear regression coefficients with standard errors (SE) for prediction of herbage yield accumulation (t DM ha^{-1}), mean stage by weight (MSW) and contents of crude protein (CP; g kg^{-1}) and net energy for lactation (NEL; MJ kg^{-1}) in herbage dry matter (DM) of Italian ryegrass during uninterrupted growth. The regressions were performed separately for primary growth (C1) and regrowth (C2). Coefficients of determination (R^2) are given for both cycles together. Intercepts denoted with the same letter are not significantly different within a particular parameter (P>0.05). The same holds for slopes.

		Explanatory variable: growth days (cycle period 12-47 days)			
Parameter	Cycle	Intercept*	SE	Slope	SE
Yield	C1	1.76^a	0.197	0.114^a	0.009
R^2=93.2%	C2	0.00^b	0.204	0.140^a	0.010
MSW	C1	3.72^a	0.753	0.329^a	0.036
R^2=94.0%	C2	−0.62^b	0.776	0.731^b	0.037
CP	C1	227^a	10.7	−3.78^a	0.508
R2=87.9%	C2	302^b	11.1	−6.91^b	0.534
NEL	C1	7.14^a	0.077	−0.016^a	0.004
R^2=90.8%	C2	7.11^b	0.079	−0.05^b	0.004
		Explanatory variable: MSW (cycle period 12-67 days for CP°, 12-47 days for NEL)			
Parameter	Cycle	Intercept	SE	Slope	SE
CP	C1	237^a	13.3	−7.61^a	0.925
R^2=84.9%	C2	283^b	11.4	−8.26^a	0.585
NEL	C1	7.35^a	0.109	−0.052^a	0.011
R^2=90.5%	C2	7.04^b	0.077	−0.067^a	0.005

*Intercept is set to growth day 12; °Extended series period improved the pattern prediction of CP content (R^2: 84.9% *vs.* 78.8%).

content stayed above this reference value until the end of sampling. At growth day 47, the NEL content in C1 was 6.4 MJ kg^{-1} DM. A similar high NEL content in Italian ryegrass during the first half of spring was reported by Daccord *et al*. (2002).

Crude protein and net energy for lactation yields time patterns

The time patterns of CP and NEL yields determined for uninterrupted primary growth and regrowth were significantly affected by both growth days and cycle (P<0.001). The interaction was marginal only for NEL yield (P=0.072). Both yields were higher in C1 than in C2 during the whole growth period. The cumulative yield of CP achieved a peak at a similar time (day 38 *vs.* 42) in both cycles and afterwards decreased (Figure 6). For NEL, the yield peak was achieved five days later in both cycles and the decrease was more gradual compared to CP yield. The loess fitted lines showed that the decrease of CP and NEL yields during advanced growth stages resulted mainly from their reduced content in the herbage (Figure 2).

In our case, the forage potential of Italian ryegrass can be completely exploited only in primary spring growth, if the herbage quality is taken into account. At maximum CP and NEL yields, their contents were close to or well above, respectively, the references for high quality forage (CP content 150 g kg^{-1} DM, NEL content 6.2 MJ kg^{-1} DM). In the regrowth cycle, the decrease of CP and NEL contents reached these references 9 and 16 days, respectively, before their maximum yields were achieved. If a more rigorous NEL quality criterion is taken into account, then the herbage yield with 6.2 MJ kg^{-1} DM amounts to only 2.5 t DM ha^{-1}. This is below the level of 3.5 t DM ha^{-1} recommended for silage making in intensive grass production (Dutch standard; Van Middelaar *et al*., 2014).

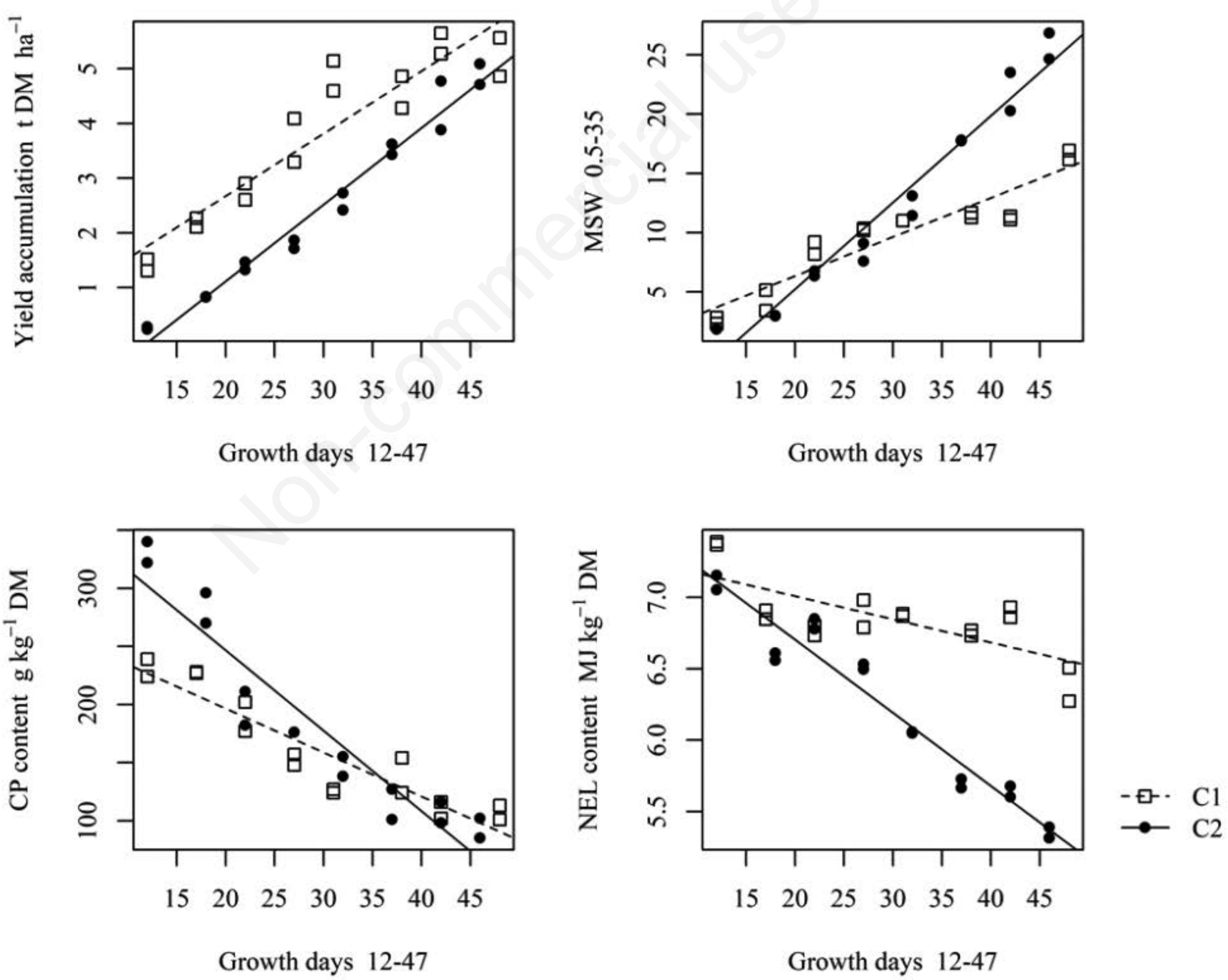

Figure 4. Linear regression relationships of growth days to herbage yield accumulation, mean stage by weight (MSW), contents of crude protein (CP) and net energy for lactation (NEL) in herbage dry matter (DM) of Italian ryegrass determined separately for uninterrupted primary growth (C1) and regrowth (C2).

Conclusions

It was established that simple linear regression models based on growth days can be justified for prediction of DM herbage yield accumulation, MSW and contents of CP and NEL in herbage of Italian ryegrass during uninterrupted growth. This holds for primary growth and spring regrowth, in both cases when the growth period is limited to its practical relevance.

As shown by our research, growth days can be at least as efficient as MSW in predicting CP and NEL contents. However, this finding needs to be confirmed in further investigations to be accepted more generally.

The growth cycle significantly affected all investigated parameters, suggesting that more than one model for each parameter may be needed for different growth cycles. The growth habit of Italian ryegrass stayed the same over the prevailing part of the season. Because of this, the growth cycle factor may be considered as a proxy for weather conditions. This suggests that the models may also differ between years with substantially different weather conditions. It would therefore be of interest to perform further studies lasting for several years to establish the robustness of the growth days-based and MSW-based models.

The study also showed that it is difficult to exploit the herbage yield potential of Italian ryegrass completely together with maximising nutritional quality under particular climatic conditions. It can therefore be recommended that Italian ryegrass is cultivated as a single catch crop instead of a double one in a crop rotation system in which Italian ryegrass is succeeded by maize.

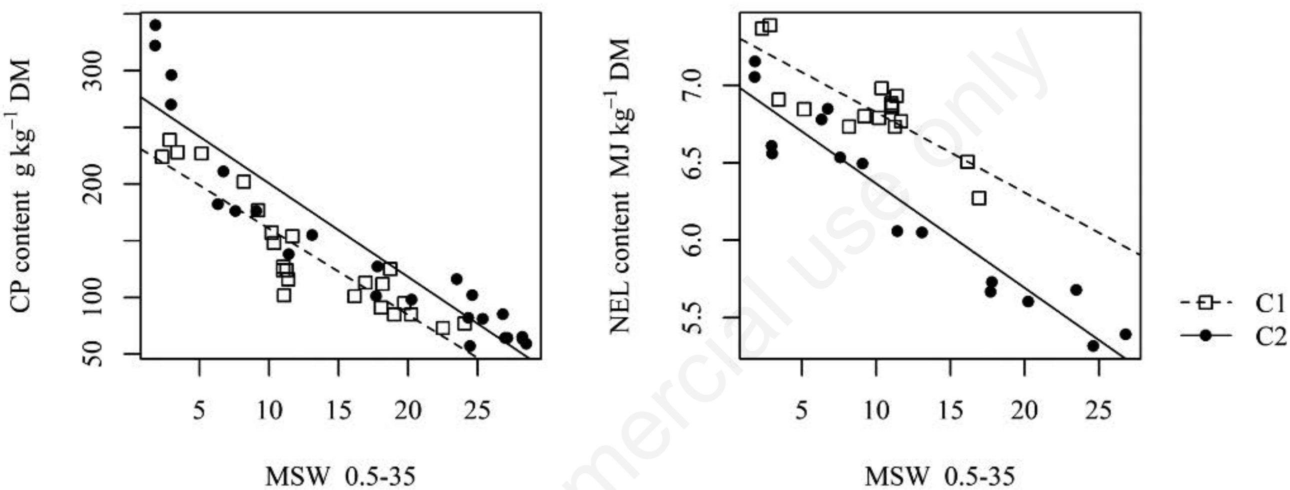

Figure 5. Linear regression relationships of mean stage by weight (MSW) to crude protein (CP) and net energy for lactation (NEL) contents in herbage dry matter (DM) of Italian ryegrass determined separately for uninterrupted primary growth (C1) and regrowth (C2). The growth periods are 12–67 days for the left graph and 12–47 days for the right graph.

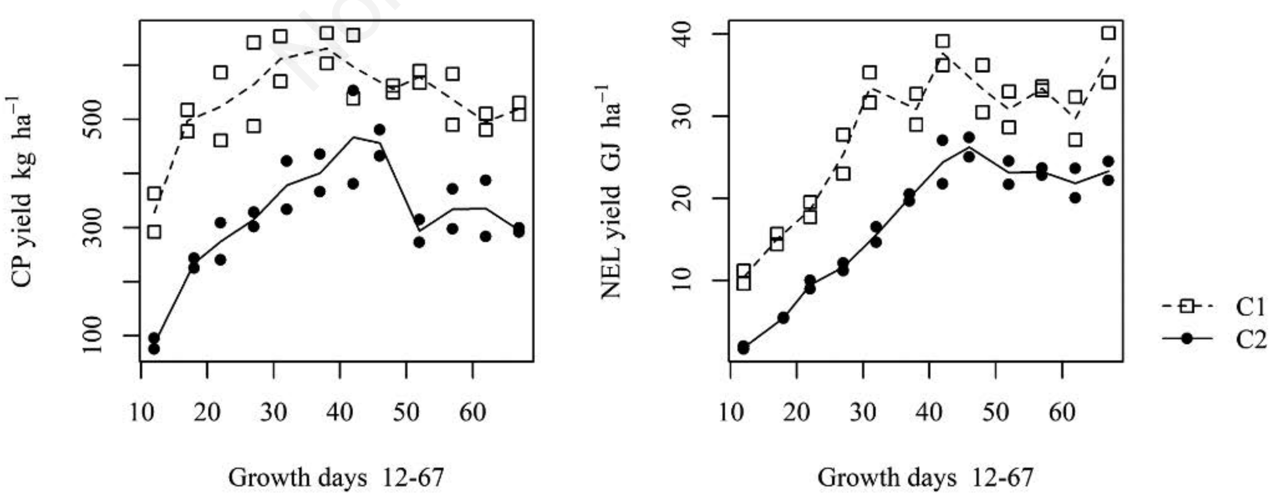

Figure 6. Loess fitted time patterns for crude protein (CP) and net energy for lactation (NEL) yields of Italian ryegrass determined separately for uninterrupted primary growth (C1) and regrowth (C2). The yields were calculated by multiplying CP or NEL content by the respective cumulative herbage dry matter yield.

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