A simple method for quantifying the phenological development of perennial ryegrass swards (*Lolium perenne L.*) and determining its relationship with some quality parameters

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Introduction

Accurate identification of the growth stage of a grass sward is critical to many forage breeding and management decisions. The quantity and quality of forage grasses is affected greatly by plant morphology (Moore and Moser, 1995). Many forage quality traits such as crude protein and fibre change unfavorably with advancing maturity (Simon and Park, 1983). Several previous studies were conducted with a common goal to quantify the developmental stages of cool-season (Haun, 1973; Simon and Park, 1983; Sweet et al., 1991) and warm-season (Moore et al., 1991; Sanderson, 1992) grasses. In the early 1990s Moore et al. (1991) recommended using the mean stage by count (MSC) as a numerical maturity index for quantifying the developmental morphology of a population of tillers. However, the time it takes to classify a herbage sample is relatively long. In addition, the decisions involved in classifying individual tillers are relatively subjective. Despite of that, MSC appears to have a high correlation to the nutritive value of perennial forage grasses (Van Soest, 1994). The objective of the present study was to develop a new comprehensive numerical index for quantifying the phenological development of perennial grasses, which is less time consuming and can be applied routinely, easily in the field, and provides similar correlations to the prominent quality traits as the commonly used MSC.

Materials and methods

The study was conducted through a 2-year field experiment (2006-2007) at the experimental station Hohenschulen of the Christian-Albrechts University, Kiel. Twenty diploid intermediate-heading perennial ryegrass (*Lolium perenne L.*) genotypes, which provide the range of phenological variation found in the corresponding maturity group, were evaluated with respect to their quality performance. Three replicated 3- by 6.5-m plots per genotype were sown in a randomized complete block design. The plots were managed with four cuts, the first and second were included in the present study and involved three sampling dates within each cut. Forage quality was estimated by NIRS, based on the following wet chemical analysis: Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) using the semiautomatic ANKOM apparatus (Van Soest *et al*., 1991). DOM (g kg^{-1} DM) was calculated according to Weißbach *et al*. (1999) based on the cellulase method (De Boever et al., 1988). The phenological stage of the plants was quantitatively monitored at each sampling date by classifying a representative sample of around 50 tillers from each plot according to the 17 stages of development described by Park (1980). In both years, only 10 stages, namely 3 to 12, were detected in the experimental plots. The Mean Stage by Count (MSC), representing the reference method, was calculated as the average of the individual stage categories present in the herbage sample, weighted for the number of tiller at each stage (Moore et al., 1991). The percentage of tillers above a given developmental stage *i* was calculated, where *i* varied between the first and the last of the observed stages, resulting in a total of 10 different new maturity indices (NMI_i). MSC and NMI_i, and forage quality traits (NDF, ADF, ADL, DOM) were computed using Spearman's rank correlation coefficient in SAS 9.1 PROC CORR (SAS Institute, 2000) as the data were not normally distributed.

Results and Discussion

The correlation between MSC and the tested quality parameters reached the level of significance for the three sampling dates within the first cut, in addition to the first and second sampling dates within the second cut. While, in the third sampling date within the second cut, MSC was significantly correlated only to ADL and DOM (g kg^{-1}) contents (Table 1). Correlation analyses between percentage of tillers above each developmental stage and the investigated parameters revealed that considering the percentage of tillers above stage B (denoted as NMI8), which is defined as swelling of the upper leaf sheath indicating presence of the inflorescence inside it proved to show as significant correlation to the parameters as the MSC. Moreover, the magnitude of correlation was highly comparable to that produced from the MSC for both cuts and all sampling dates. This was in good accordance with the findings of Ansquer et al. (2009), who identified three phenological stages, namely; the start of stem elongation, flowering, and seed ripening, as the key to managing the dynamics of growth and the demography of temperate grassland species. Furthermore, the authors stated that making observations at the flowering stage gives relevant information on the other stages. Similarly, Mika (1983) found that varietal differences in timothy were more pronounced at ear emergence than at later sampling dates suggesting that a sampling date at or near ear emergence is to be preferred for routine evaluation. Correlation coefficients clearly fluctuated for the quality parameters and reached mostly the highest values in case of the DOM compared to the fibre fractions. Generally, higher correlation coefficients were observed for 1st rather than 2nd cut. Because the reproductive tillers are the main component of the NMI8, its correlation with the studied quality parameters will become weaker and less distinguishable as the number of reproductive tillers decrease which was clearly observed in the 2nd cut.

Conclusion

In the present research, a new maturity index (NMI8) for quantifying the morphological development of perennial forage grasses has been developed. The NMI8, expressed as percentage of tillers beginning the reproductive stage, provided similar correlations to the yield and studied quality attributes as the mean stage by count (MSC), but it was less time consuming and can be applied routinely and easily in the field. Correlations of the new maturity index with the studied quality parameters were more pronounced in the first cut than in the second cut.

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Table 1. Spearman's rank correlation coefficients between percentage of tillers beginning each developmental stage and the different quality parameters, pooled over year and replicate. Maturity stages S2 to OH represent the new maturity indices NMI_i (i = 3-12)

Cut	Sampling Date	Parameter	MSC	NMI_i									
				3	4	5	6	7	8	9	10	11	12
				S2	S3	K1	K2	K3	B	G1.1	G1.5	G1.9	OH
1	1	NDF	0.32**	0.04	0.10	0.36**	0.31*	0.21*	0.22*	0.17			
1	1	ADF	0.34**	0.06	0.14	0.37**	0.32*	0.20*	0.24*	0.22*			
1	1	ADL	0.54**	0.03	0.18	0.48**	0.55**	0.48**	0.44**	0.40**			
1	1	DOM	-0.84**	-0.03	-0.49**	-0.85**	-0.82**	-0.69**	-0.74**	-0.59**			
1	2	NDF	0.57**	0.06	0.54**	0.48**	0.59**	0.52**	0.62**	0.58**	0.57**		
1	2	ADF	0.67**	0.11	0.62**	0.56**	0.68**	0.62**	0.68**	0.59**	0.61**		
1	2	ADL	0.51**	0.05	0.44**	0.42**	0.52**	0.47**	0.63**	0.55**	0.58**		
1	2	DOM	-0.69**	-0.08	-0.59**	-0.53**	-0.72**	-0.65**	-0.68**	-0.64**	-0.69**		
1	3	NDF	0.78**	0.22*	0.25*	0.48**	0.70**	0.76**	0.81**	0.67**	0.64**	0.39**	
1	3	ADF	0.72**	0.31*	0.32**	0.53**	0.72**	0.74**	0.74**	0.55**	0.56**	0.59**	
1	3	ADL	0.83**	0.21*	0.19	0.37**	0.59**	0.70**	0.83**	0.79**	0.77**	0.55**	
1	3	DOM	-	-0.23*	-0.21*	-	-	-	-	-	-	-	

			0.81**			0.39**	0.63**	0.75**	0.81**	0.73**	0.73**	0.54**	
2	1	NDF	0.47**	0.14	0.21*	0.43**	0.39**	0.33**	0.39**	0.49**	0.55**	0.56**	0.40**
2	1	ADF	0.39**	0.17	0.21*	0.32**	0.27*	0.23*	0.32**	0.43**	0.50**	0.49**	0.32**
2	1	ADL	0.48**	0.17	0.31*	0.54**	0.49**	0.40**	0.38**	0.41**	0.46**	0.46**	0.31*
2	1	DOM	-0.51**	-0.12	-0.26*	-0.44**	-0.41**	-0.37**	-0.41**	-0.56**	-0.66**	-0.65**	-0.51**
2	2	NDF	0.31*	0.04	0.037	0.20*	0.22*	0.26*	0.28*	0.36**	0.40**	0.21*	0.19
2	2	ADF	0.33**	0.18	0.08	0.17	0.18	0.25*	0.34**	0.37**	0.38**	0.20*	0.21*
2	2	ADL	0.47**	0.12	0.11	0.34**	0.36**	0.37**	0.31*	0.39**	0.49**	0.36**	0.31*
2	2	DOM	-0.35**	-0.10	-0.05	-0.28*	-0.28*	-0.30*	-0.26*	-0.33**	-0.42**	-0.24*	-0.23*
2	3	NDF	0.18	0.03	0.20*	0.09	0.13	0.17	0.18	0.26*	0.29*	0.16	0.09
2	3	ADF	0.18	0.001	0.20*	0.09	0.16	0.18	0.20*	0.27*	0.30*	0.17	0.09
2	3	ADL	0.21*	0.0006	0.24*	0.16	0.20*	0.23*	0.23*	0.28*	0.33**	0.22*	0.15
2	3	DOM	-0.40**	-0.01	-0.39**	-0.28*	-0.37**	-0.41**	-0.43**	-0.50**	-0.54**	-0.41**	-0.24*