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Morphological, environmental and management factors affecting nutritive value of tall fescue (*Lolium arundinaceum*)

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Abstract. The aim of this study was to quantify the relative importance of leaf age and leaf length on the dynamics of neutral detergent fibre (NDF), and 24-h *in vitro* digestibility of NDF (NDFD) and dry matter (DMD) of tall fescue (*Lolium arundinaceum* (Schreb) Darbysh.). Mini-swards were conditioned and used to conduct two experiments, the first with 4-cm plant stubble height in spring–summer 2009 and autumn–winter 2011, and the second with 4-cm or 10-cm plant stubble height in spring–summer 2011. Plants were harvested at consecutive leaf-appearance intervals to measure nutritive value up to the four-leaf stage. In parallel, leaf morphogenetic traits (appearance, elongation and lifespan) and sheath length of the successive leaves produced on marked tillers were measured. Leaf NDF contents remained stable with increasing leaf age and length but showed a marked variation across seasons. Leaf NDFD and DMD showed a consistent decrease with increasing leaf age and length, and irrespective of growing season or residual pasture height. The negative effect of leaf age and length on digestibility was related to variations in sheath tube length and associated differences in leaf appearance and elongation rates. These findings highlight the relevance of monitoring the sheath tube length as a complementary measure to leaf stage for further management of the NDFD and DMD of grass forages. Although the focus of this study was tall fescue swards, the same morphogenetic implications on forage nutritive value could apply to other temperate and tropical grass species; however, the testing of this hypothesis warrants carefully controlled investigations.

Additional keywords: *Festuca arundinacea*, leaf morphogenesis, residual pasture height.

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Introduction

Efficient pasture-based animal production requires optimisation of both production and utilisation of forages with high nutritive value. In well-managed pasture-based systems, the nutritive-value dynamics during vegetative regrowth are driven by the continuous turnover of successive leaves, all of different ages and lengths (Chapman *et al*. 2014). When vegetative grazed pastures are defoliated at a similar stage of regrowth (based on days, growth rate or number of leaves per tiller), the dry matter digestibility (DMD) of forages (Burns *et al*. 2002; Donaghy *et al*. 2008; Raeside *et al*. 2012) and animal performance (Lattanzi *et al*. 2007) vary with changes in residual pasture (post-grazing) conditions or across seasons.

Further, a decrease in neutral detergent fibre digestibility (NDFD) of leaf blade tissue was reported as mainly responsible for declines of forage DMD during vegetative regrowth (MacAdam *et al*. 1996; Agnusdei *et al*. 2011; Di Marco *et al*. 2013). Researching tall fescue (*Lolium arundinaceum* (Schreb) Darbysh.) swards, Donaghy *et al*. (2008) reported that the decline in forage DMD during the regrowth cycle was exacerbated in warmer experimental conditions, and attributed the acceleration of leaf-tissue ageing to temperature. However, Insua *et al*. (2017), working with two cultivars of tall fescue, and Chapman *et al*. (2012), working with perennial ryegrass (*Lolium perenne* L.), reported that NDFD and DMD declined not only with the thermal time of leaf age, but also with the length of successive leaves.

Leaf length is a 'plastic' trait that is determined by the interaction between growing conditions (mainly temperature, mineral nutrition and water status), plant morphogenesis and defoliation management (Lemaire and Chapman 1996). Therefore, important questions here are: (*i*) how prevailing growing conditions and practices such as residual sward heights, which are known to influence leaf turnover and leaf length of tall fescue (Skinner and Nelson 1994; Duru and Ducrocq 2002), affect forage nutritive value indirectly; and (*ii*) whether these mediated effects on forage nutritive value are driven by increasing leaf age, leaf length or both. The answers to these questions improve the mechanistic understanding of key determinants of forage nutritive value in vegetative pasture regrowth, and provide day-to-day recommendations to maintain nutritive value when residual pasture structure is, intentionally or not, varied.

We conducted two field experiments using previously conditioned tall fescue swards to measure the relative contributions of leaf ageing and leaf length on declines in forage nutritive value of tall fescue regrowth across growing seasons and at different residual pasture heights. We hypothesised that, beyond the unavoidable leaf-ageing effect, the differences in nutritive value between regrowths at the same leaf stage are due to the (negative) effect of length on NDFD of leaf tissues.

Materials and methods

Experimental conditions

The study was carried out using mini-swards of a permanent tall fescue pasture established in Balcarce, in the province of Buenos Aires, Argentina (37°45′S, 58°18′W; 130 m a.m.s.l.). The soil at the study site was a typic Argiudol (Soil Survey Staff 2014) with an A horizon 25 cm deep, organic matter content 62 g kg^{-1} and pH 6.2. A monoculture of tall fescue pasture cv. El Palenque Plus[INTA] was sown in August 2009 in three replicated mini-swards (1 m by 2 m) arranged in a completely randomised design. Three weeks after sowing, seedlings were manually thinned to create a uniform stand of ~200 plants m^{-2}.

Experiment 1 compared two experimental periods, late spring–summer 2009–10 (‘warm season 2009’) and autumn–winter 2011 (‘cool season’), at a uniform residual pasture height of 4 cm (Table 1). (Some of the warm-season 2009 data have been presented previously (Insua *et al*. 2017) as part of a comparison of leaf nutritive value between two tall fescue cultivars.) Expt 2 was conducted during spring–summer 2011–12 (‘warm season 2011’), with two residual pasture-height treatments, 4 cm (‘Low’) and 10 cm (‘High’) of stubble (Table 1), chosen to generate sufficient contrast in plant structure traits, including leaf length.

Between experiments and periods, mini-swards were kept under common management, which included a monthly defoliation to 4-cm stubble height, and complementary irrigation as needed, with the aim of maintaining a leafy and uniform sward condition between experiments.

To ensure that nutrients and water were non-limiting, fertiliser and irrigation were applied as follows. Nitrogen (138 kg N ha^{-1}) was split evenly over three applications in each experiment. This was done to synchronise N supply and N uptake by plants and to mitigate unnecessary losses of N via volatilisation, runoff or leaching. Phosphorus (12 kg P ha^{-1}) was applied once at the beginning of each experiment. Irrigation was applied periodically (2–3-day intervals) to maintain soil moisture content above 0.6 of field capacity.

Both experiments used similar protocols as described by Insua *et al*. (2017). Briefly, for three months before an experiment (pre-experimental phase), monthly clippings of mini-swards were applied to obtain a dense and leafy plant stand. In Expt 1, the whole area in each mini-sward was clipped to 4-cm stubble height, whereas in Expt 2, two equal areas in each mini-sward were clipped to either 4- or 10-cm stubble height to establish the Low and High treatments, respectively. When the pre-experimental phase was completed, mini-swards were clipped once again to their corresponding stubble-height treatment (Table 1). Thereafter, mini-swards and plant tillers were marked, measured and sampled throughout the experimental period.

Table 1. Characterisation of tall fescue experiments

Expt	Year	Growing season	Residual height	Studied regrowth period	Mean daily temp. (°C, ± s.d)
1	2009	Warm	4 cm	8 Dec.–5 Mar.	21.0 ± 2.8
1	2011	Cool	4 cm	18 Apr.–19 Sept.	9.9 ± 3.1
2	2011	Warm	4 and 10 cm	27 Oct.–10 Feb.	19.7 ± 2.7

Temperature was monitored with four Watchdog data loggers (Spectrum Technologies, Aurora, IL, USA), two placed at the centre of the experimental site and the other two at the two outermost opposite points of the site. Data loggers were placed at ground level. Mean daily air temperature for each experiment is summarised in Table 1. The thermal time, expressed in accumulated growing degree-days (GDD), was calculated as the sum of daily mean air temperatures above a base temperature of 4°C (Peacock 1976; Hutchinson *et al*. 2000). GDD was used to express time of regrowth and leaf age.

Morphological and structural measurements

At the onset of each experiment, five randomly placed quadrats (0.2 m by 0.2 m) per mini-sward (total 15 quadrats) were left uncut for leaf morphogenetic and structural measurements, using methods adapted from Davies (1993). In Expt 1, one tiller per quadrat was randomly identified and permanently marked by a coloured plastic ring (total 15 marked tillers). In Expt 2, 15 tillers per treatment were randomly identified and permanently marked (total 30 marked tillers). All morphogenetic measurements were conducted every 2–4 days and up to the four successively formed leaves per marked tiller. Measurements included time of leaf and ligule appearance, length of leaf blade and sheath tube (pseudostem), and the onset of leaf senescence (decrease in green blade length). Leaf trait data were then used to estimate leaf elongation rate (LER, mm day^{-1}), leaf appearance interval (LAi), leaf lifespan (LLS), number of leaves per tiller (NLT), final leaf-blade length (i.e. leaf-blade length in cm at ligule appearance) and leaf senescence. LAi was calculated as the GDD accumulated between the appearance of two successive leaves, and LLS was the GDD accumulated between leaf appearance and the onset of leaf senescence.

Sample collection and tiller dissections

Tillers were destructively harvested following the methodology used by Groot and Neuteboom (1997), in which successive harvests are scheduled to coincide with the expansion of newly formed leaves per tiller, referred to here as the 1-, 2-, 3- and 4-leaf stages, respectively. On average, the interval between successive harvests was 14 ± 6 days for warm season 2009, 33 ± 9 days for cool season, and 25 ± 13 days for warm season 2011. At each harvest (eight in warm season 2009, three in cool season and six in warm season 2011), the herbage mass of ~1000 tillers was collected from one randomly placed quadrat (0.5 m by 0.5 m) per mini-sward cut to ground level. The collected material was frozen and stored at −20°C until analysis.

When the material was thawed, a subsample of ~300 tillers (or equivalent material sufficient for nutritive-value analysis) was randomly selected for further leaf measurements. Leaves of selected tillers were separated into leaf blades and leaf-sheath components for measurement of blade and sheath length and blade nutritive value. Leaf blades of successively formed leaves (referred as L1, L2, L3 and L4, respectively) were further classified into three defined leaf-age categories: (*i*) just expanded (ligule appearance), (*ii*) adult (between ligule appearance and pre-senescence), (*iii*) pre-senescent (leaf-blade tip senescent). All separated leaf-blade fractions were returned to the freezer awaiting laboratory availability for completion of leaf-blade nutritive-value analyses. In Expt 2, a subsample of leaf blades from Low and High treatments was also separated and weighed to evaluate both leaf-blade biomass accumulation and leaf-blade nutritive value at whole sward level.

Leaf nutritive-value analysis

Frozen leaf samples were freeze-dried to constant weight (Rificor L-A-B4 lyophiliser; Rificor SH, Buenos Aires), ground to pass through a 1-mm screen, and uniformly mixed for analysis of NDF, NDFD and apparent DMD. The NDF was determined following Van Soest *et al*. (1991), using a 500-mg DM sample treated with a heat-stable α-amylase and sodium sulfite solution (ANKOM^{200} fibre analyser; ANKOM Technology, Macedon, NY, USA). A second 500-mg DM sample was placed in filter bags (F1020, ANKOM Technology) and incubated *in vitro* for 24 h in a Daisy^{II} apparatus (ANKOM Technology), using rumen liquor from a donor steer fed a grass-based diet. After incubation, bags were boiled in neutral detergent solution (ANKOM^{200} fibre analyser) to measure residual NDF (NDFr). Digestibility of NDF (NDFD, calculated as: (NDFincubated – NDFr)/NDFincubated) was then estimated (Goering and Van Soest 1970) and expressed as percentage of DM. Apparent DMD was estimated according to Van Soest (1994) by subtracting the metabolic factor 119 mg kg^{-1} from the true DMD: 1 – (NDFr/weight of incubated DM) (Goering and Van Soest 1970).

To compare the forage nutritive value at the sward scale and between all study regrowth seasons (Expts 1 and 2), nutritive-value traits of leaf blades (NV.leaf), including NDF, NDFD and DMD, were used to estimate the pool nutritive value for all leaves per tiller (NV.pool) formed in a given regrowth period. This NV.pool of NDF (NDFpool), NDFD (NDFDpool) and DMD (DMDpool) was estimated following the approach described by Insua *et al*. (2017):

$$\text{NV.pool}_j = \sum_{i=1}^{3} [\text{NV.leaf}_{ij}(\text{LL}_{ij}/\text{TL}_{ij})]$$

where NV.leaf is NDF, NDFD or DMD of the individual leaf blades; LL is leaf-blade length; TL is total LL per tiller; *i* is the number of the successive leaf (L1, L2, L3, L4); and *j* is thermal time (GDD) from L1 emergence. For comparison and validation of the approach, the estimation of NV.pool values was tested against observed nutritive value data of Expt 2.

Statistical analyses

For each season, data were analysed by repeated-measures (morphogenetic and structural data) or ANOVA (nutritive value data) for a completely randomised design ($n = 3$), using the PROC MIXED procedure of SAS System 2000 (SAS Institute, Cary, NC, USA). For Expt 2, nutritive-value data from mini-swards were compared between the High and Low residual pasture treatments and among leaf stages (2-, 3- and 4-leaf stage). When significant effects ($P < 0.05$) were detected, means were separated by using a Tukey test. Nonlinear regressions of leaf-blade against sheath-tube lengths, and NDFD and DMD against leaf-blade length were fitted by using SigmaPlot (SPSS, Chicago, IL, USA). Linear regression of NDF, NDFD and DMD against leaf age was fitted by using the GLM procedure of SAS. Analysis of leaf age was performed by using the whole study dataset grouped by thirds of leaf-blade length (with ±15% of the mean group). Regression models also considered the dummy variable leaf length (arranged by thirds of leaf-blade length) to test (at $P = 0.05$) convergence (null hypothesis: intercepts are equal) and parallelism (null hypothesis: slopes are equal) among leaf-length groups.

Results

Morphological attributes of leaves

Leaf length

Final leaf-blade length of successive leaves increased ($P < 0.001$) with increasing thermal time in the warm seasons, but not ($P > 0.05$) in the cool season (Fig. 1). In Expt 2, reduced residual pasture height decreased the length of the sheath tube at the onset of the experiment (Low 3.5 ± 0.2 cm, High 9.0 ± 0.4 cm). Consequently, leaves appearing during regrowth were shorter ($P < 0.001$) in the Low than in the High treatment (Fig. 1). Both final leaf-blade length of individual leaves and time to reach that final leaf-blade length (i.e. visible ligule) differed among growing seasons (compare same symbols in Fig. 1).

Final leaf length and sheath-tube length were highly associated in a nonlinear manner (Fig. 2). Except in the cool

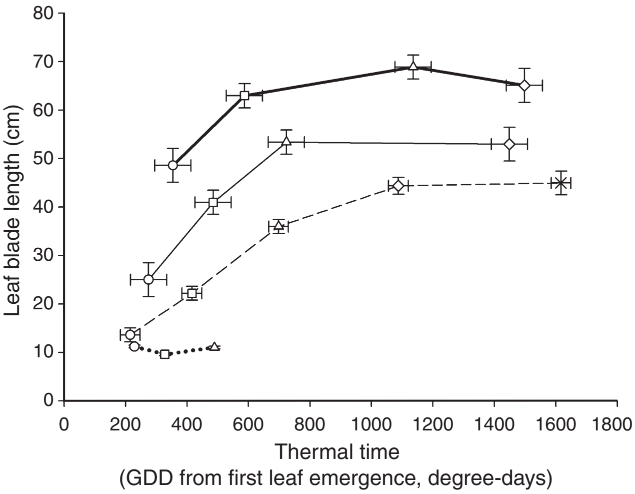

Fig. 1. Final leaf blade length for successive leaves appearing on a tiller (○, L1; □, L2; Δ, L3; ◇, L4; ×, L5) in the cool season (. . .), warm season 2009 (– – –), and warm season 2011 under Low (—) and High (**—**) residual pasture height. GDD, Growing degree-days. Capped bars (vertical, leaf blade length; horizontal, thermal time) indicate ±standard error of the mean.

season, when sheath-tube length did not vary much (3–6 cm), the final leaf-blade length strongly increased across successive leaves until reaching a plateau of ~55 cm (Fig. 2).

Leaf morphogenesis

Table 2 summarises main results for leaf morphogenesis. LER, LAi and NLT varied numerically among seasons, but a significant difference between Low and High treatments was detected for warm season 2011 (Expt 2). In Expt 2, LAi increased whereas NLT decreased with increased residual pasture height ($P < 0.0001$). The average LLS remained relatively stable at ~630 GDD during the warm seasons 2009 and 2011, and decreased by ~30% in cool season.

Nutritive value attributes of individual leaf blades

Leaf-blade nutritive value was highly variable across the study (Table 3). In each growing season, NDF, NDFD and DMD varied by ~10, 30 and 15 percentage units, respectively. Losses in DMD were strongly associated ($P < 0.0001$) with increases in NDF (DMD = 105 – 0.86NDF, $r^2 = 0.70$, $n = 75$) and with decreases in NDFD (DMD = 25 + 0.74NDFD, $r^2 = 0.83$, $n = 75$). NDF and NDFD were also associated ($P < 0.0001$), but

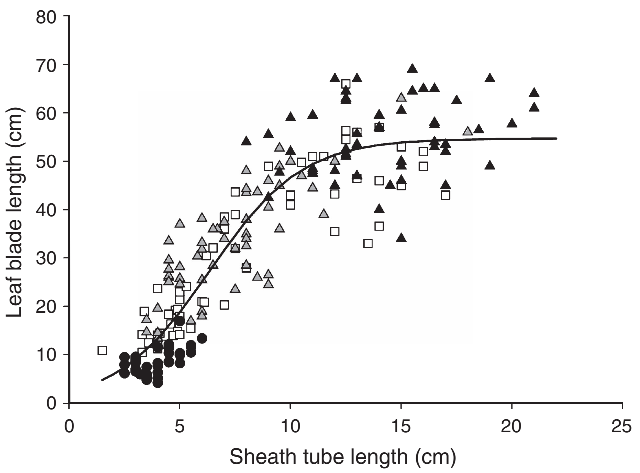

Fig. 2. Relationship between sheath-tube length and final leaf-blade length in tall fescue tillers in different growing seasons: cool season (circles), warm season 2009 (squares), and warm season 2011 (triangles) under two residual pasture heights: Low (light grey triangles) and High (dark grey triangles). Equation: $y = 54.7/(1 + e^{-(x - 64)/2.1})$; $R^2 = 0.85$; standard error 74.9; $n = 206$.

Table 2. Means of leaf elongation rate (LER), leaf lifespan (LLS), leaf appearance interval (LAi) and number of leaves per tiller (NLT) of tall fescue plants in different growing seasons and residual pasture-height treatments (Low and High)

Standard errors are in parentheses. Within a parameter, means followed by the same letter are not significantly different ($P > 0.05$)

Growing season	Treatment	LER (mm day^{-1})	LLS (degree-days)	LAi (degree-days)	NLT
Cool 2011	Low	3 (0.1)	438 (7)	152 (5)	3.1 (0.1)
Warm 2009	Low	16 (1.2)	632 (21)	192 (18)	3.3 (0.2)
Warm 2011	Low	14 (1.1)b	645 (37)a	231 (13)b	2.5 (0.1)a
	High	23 (1.1)a	600 (37)a	340 (13)a	2.0 (0.1)b

the coefficient of determination ($r^2 = 0.31$) between these variables was low.

When NDFD and DMD of the leaf blade at the just-expanded stage (ligule appearance) were plotted against its length for the whole dataset (Expts 1 and 2) (Fig. 3*b, c*), a single relationship was evident among data ($P < 0.0001$), independently of the leaf number (i.e. L1, L2, L3, L4), growing season or residual pasture height treatment. There was a weaker, yet significant ($P < 0.05$) relationship ($r^2 = 0.18$) between NDF and leaf blade length for warm seasons 2009 and 2011 (Fig. 3*a*).

In order to quantify the rate of decline of nutritive value with leaf age, regression analyses were performed on the whole dataset (Expts 1 and 2) grouped by leaf blade length. There was no association ($P > 0.05$) of NDF with leaf-age categories expressed in thermal time (Fig. 4*a*). However, negative associations ($P < 0.0001$) between NDFD and DMD with increasing leaf age were detected (Fig. 4*b, c*). There was a linear effect ($P < 0.0001$) of leaf ageing on declines in NDFD and DMD for the two leaf-length groups included in the analysis (Fig. 4*b, c*). Further, the regression analysis for both groups showed similar rates of decline (parallelism $P > 0.05$) but different intercept values ($P < 0.0001$).

Nutritive-value attributes of whole-sward

Effect of residual pasture height on whole-sward attributes

Changes in nutritive value at the whole-sward level for the Low and High residual pasture-height treatments are shown in Table 4. NDF increased ($P < 0.0001$) with increasing leaf stage (from 59.4% to 64.8%, average of residual pasture treatments), but without differences ($P > 0.05$) between residual pasture treatments. NDFD and DMD of whole-sward leaf-blade tissues declined ($P < 0.001$) from the 2-leaf to the 4-leaf stage (Table 4). Whole-sward NDFD and DMD were consistently higher ($P < 0.001$) for the Low treatment than the High treatment at the 2- and 3-leaf stages, but not at the 4-leaf stage. After the 2-leaf stage (nearly LLS), there was a progressive increase ($P < 0.0001$) in leaf senescent material in both Low and High treatments. Residual pasture height showed no significant effect ($P > 0.05$) on leaf-blade DM and senescent material accumulation (kg DM ha^{-1}) with increasing GDD of regrowth.

Table 3. Summary statistics for neutral detergent fibre (NDF) content, and digestibility of NDF (NDFD) and dry matter (DMD) of living leaf blades collected during vegetative regrowth of three different growing seasons

Leaf blade attribute	Season	No. of observations	Min.	Max.	Mean	s.d.
NDF (%)	Cool 2011	18	35.4	45.3	39.9	2.7
	Warm 2009	27	49.2	62.4	55.3	3.0
	Warm 2011	30	52.8	61.9	58.2	2.3
NDFD (%)	Cool 2011	18	37.8	66.7	55.7	8.3
	Warm 2009	27	30.0	62.7	46.8	8.9
	Warm 2011	30	24.4	49.7	39.5	6.4
DMD (%)	Cool 2011	18	63.3	76.1	70.5	3.4
	Warm 2009	27	49.9	68.4	58.7	5.1
	Warm 2011	30	44.0	58.9	52.9	3.9

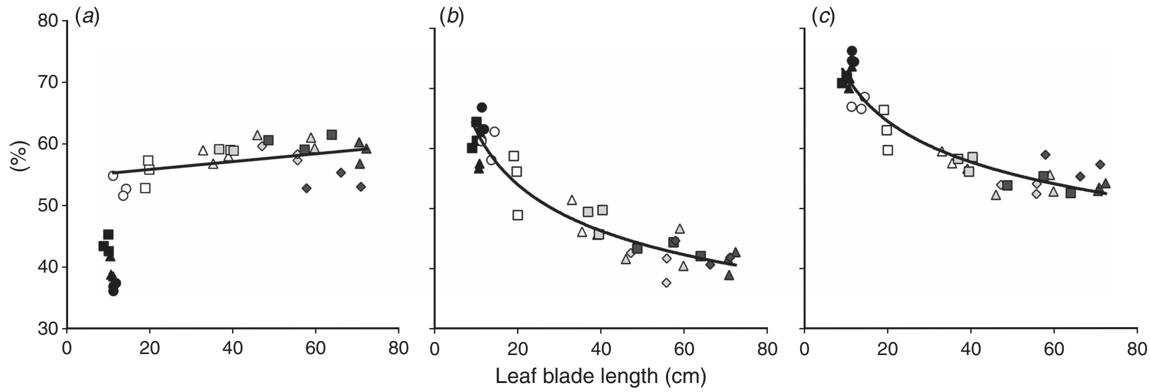

Fig. 3. Relationship between final leaf blade length and (*a*) leaf neutral detergent fibre (NDF) content, and digestibility of (*b*) leaf NDF and (*c*) leaf dry matter (DMD) of tall fescue under different growing seasons: cool season (black symbols), warm season 2009 (unfilled symbols), warm season 2011 under two residual pasture heights: Low (grey) and High (black). Symbols denote successive leaves: L1 (circles); L2 (squares); L3 (triangles); L4 (diamonds). Equations: (*a*) warm seasons 2009 and 2011, NDF = 55 + 0.06*x*, $P < 0.05$, $R^2 = 0.18$; (*b*) NDFD = 105*x* – 0.22, $P < 0.0001$, $R^2 = 0.90$; (*c*) DMD = 104*x* – 0.16, $P < 0.0001$, $R^2 = 0.90$.

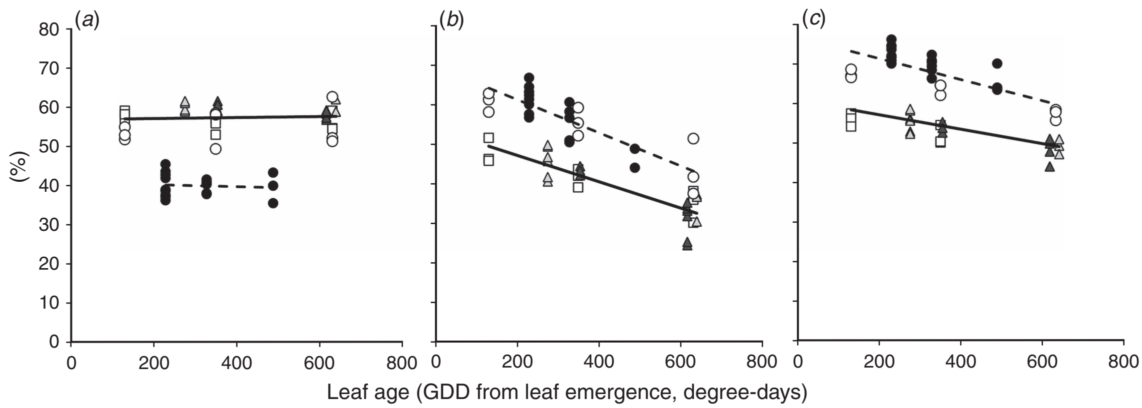

Fig. 4. Relationship between leaf age (accumulated thermal time since emergence, growing degree-days, GDD) and (*a*) leaf neutral detergent fibre (NDF) content, and digestibility of (*b*) leaf NDF and (*c*) leaf dry matter (DMD) for the shortest (- - -, 11.8 ± 1.7 cm) and longest (—, 57.5 ± 10.4 cm) thirds of leaves collected during the regrowth of tall fescue in warm season 2009 (unfilled symbols), cool season (black symbols) and warm season 2011 under two residual pasture heights: Low (light grey symbols) and High (dark grey symbols). Symbols denote successive leaves: L1 (circles); L2 (squares); L3 (triangles).

Whole-sward comparisons

In order to evaluate further the relative effects of leaf length and age on whole-sward nutritive value, NDFD values between all regrowths were compared at the 2-leaf stage (Table 5), when senescence was nil. There was a strong relationship ($R^2 = 0.97$) between observed (Table 4) and estimated (Table 5) values of NDF, NDFD and DMD in Expt 2 ($y = 1.06x - 2.30$, $n = 12$, RMSE = 1.8, where y is observed and x is estimated).

At the 2-leaf stage, greater whole-sward NDFD (~9 percentage units) for the Low residual pasture treatment (Table 4) was related to a shorter leaf-blade length (Fig. 1) and a shorter time to reach full expansion of the second leaf compared with the High treatment (485 vs 587 GDD, Table 4). According to the relationship presented in Fig. 4*b*, during the ~100 GDD of difference in regrowth time, leaf blades lost ~4 percentage units of NDFD through ageing. The remaining ~5 percentage units of the total difference could be attributed to a leaf-length effect. Following this mathematical reasoning for Expt 2, when comparing all experimental regrowths (Table 6), the isolated effect of leaf length accounted for 2–12 percentage units of differences in NDFD, or about half (40–66%) of the total differences in whole-sward NDFD (5–21 percentage units of NDFD), whereas the other half was explained by the effect of leaf ageing alone (Table 6).

Discussion

A large gradient of variation in leaf morphogenetic traits was generated with tall fescue swards grown under different seasons and sward heights. The goal of the study was to investigate the relative effects of the age and length of leaves on the forage nutritive value of tall fescue swards. Results indicated consistent decreases in NDFD as leaves become longer and aged, thereby providing insights into a new mechanistic perspective to understand and manage consistently the forage nutritive value in grazed swards.

Table 4. Whole-sward nutritive value and senescence of leaf blades at different leaf stages of tillers of tall fescue regrowth in warm season 2011 from two residual pasture heights, Low (4 cm) and High (10 cm)
NDF, Neutral detergent fibre content; NDFD, NDF digestibility; DMD, dry matter digestibility; s.e., standard error. Each leaf stage is defined as the time taken to full emergence of one new leaf (i.e. no. of leaves per tiller). Parameter means followed by the same letter are not significant different ($P > 0.05$) between treatments (lower case) and leaf stage of regrowth (upper case)

Leaf stage of regrowth	Residual pasture height treatment Low	High	s.e.
Thermal time of regrowth (degree-days)			
2-leaf stage	485	587	–
3-leaf stage	723	1136	–
4-leaf stage	1449	1498	–
Senescence (%)			
2-leaf stage	0.5aC	2.6aC	1.2
3-leaf stage	7.9bB	30aB	1.7
4-leaf stage	38aA	38aA	3.9
NDF (%)			
2-leaf stage	58.2B	60.7B	0.6
3-leaf stage	61.7A	63.8A	0.9
4-leaf stage	64.8A	64.8A	1.0
NDFD (%)			
2-leaf stage	47.9aA	39.3bA	2.4
3-leaf stage	42.3aB	31.7bB	1.4
4-leaf stage	29.6aC	32.0aB	1.9
DMD (%)			
2-leaf stage	57.8aA	51.3bA	1.1
3-leaf stage	52.5aB	44.5bB	1.2
4-leaf stage	42.5aC	44.1aB	1.4

Table 5. Estimated whole-sward nutritive value and observed sheath-tube length at the 2-leaf stage of regrowth in different growing seasons and residual pasture height treatments (Low and High)
Thermal time of regrowth at the 2-leaf stage is expressed in growing degree-days (GDD). NDF, Neutral detergent fibre content; NDFD, NDF digestibility; DMD, dry matter digestibility

Growing season	Treatment	GDD of regrowth	NDF	NDFD (%)	DMD	Sheath tube (cm)
Cool 2011	Low	328	42.3	60.1	71.2	4.7
Warm 2009	Low	418	55.2	54.9	63.2	6.4
Warm 2011	Low	485	57.8	45.4	56.6	8.1
	High	587	59.3	38.7	51.8	12.1

Leaf attributes

The nutritive value of tall fescue leaves declined with increasing leaf length (Fig. 3) and this effect was related to differences in sheath tube length (Fig. 2). Further, the results showed that with longer sheath tubes, both the appearance of new leaves (i.e. increased LAi) and the time required by those leaves to reach full length (Fig. 1) were significantly delayed; the significance of this for leaf nutritive value is discussed below. Longer leaf blades usually have greater proportions of less-digestible fractions (Wilson 1976), as our results suggest, which is also consistent with previous observations in other temperate and tropical plants species such as Rhodes grass (*Chloris gayana*) (Agnusdei *et al*. 2011) and tall wheatgrass (*Thinopyrum ponticum*) (Di Marco *et al*. 2013).

Declines in forage nutritive value with increasing leaf age (Groot and Neuteboom 1997; Duru and Ducrocq 2002) due to the well-known changes in fibre (Akin 1989; Jung and Allen 1995; Wilson and Mertens 1995; Buxton and Redfearn 1997) were also supported by this study. In addition, this inherent effect of tissue ageing was relatively constant alongside a large gradient in thermal time (Fig. 4*b*, *c*), and despite all experimental differences in growing–management conditions.

There was also important variation in the DMD of leaves that was not only dependent ($r^2 = 0.83$) on the above-mentioned variation in NDFD (Figs 3, 4), but also strongly related ($r^2 = 0.70$) to seasonal changes in NDF (Table 3). This seasonal effect of NDF (57 ± 3% and 40 ± 3% for warm and cold seasons, respectively) was likely explained by lower temperatures and growth rates (e.g. lower LER in cool season, Table 2), which might have favoured the accumulation of assimilates (Parent *et al*. 2010; Fatichi *et al*. 2014) and dilution of the NDF content (Van Soest 1994). Typically, prediction models rely on the use of NDF as the main predictor of DMD. Overall, our findings indicate that NDFD would be a much more reliable trait to understand changes in forage nutritive value independently of the environmental conditions.

Whole-sward attributes

Effects of leaf length and leaf age on whole-sward nutritive value (Table 6) appeared modulated by differences in leaf appearance and elongation rates, which in turn varied with changes in the sheath tube length. Because the effect of ageing in lowering nutritive value was relatively constant across different weather and management conditions (Fig. 4), variations in the nutritive value of vegetative pastures were mainly caused by the loss of fibre digestibility with the

Table 6. *In silico* analysis of leaf length and leaf age effects on differences in whole-sward neutral detergent fibre digestibility (NDFD) between growing seasons and residual pasture height treatments compared at the 2-leaf stage (corresponding to Table 5)
Leaf length and age effects are expressed in terms of NDFD % units and percentage of total differences (% in parentheses)

Growing season	Leaf length effect Cool 09	Warm 09	Warm 11, Low	Warm 11, High	Leaf age effect Cool 09	Warm 09	Warm 11, Low	Warm 11, High
Cool 09	–	2.1	6.7	11.7	–	3.2	5.5	9.1
Warm 09	(40)	–	4.6	9.7	(60)	–	2.3	5.9
Warm 11, Low	(55)	(66)	–	5.0	(45)	(34)	–	3.6
Warm 11, High	(56)	(62)	(59)	–	(44)	(38)	(41)	–

increment in leaf length (Fig. 3*b*). However, care should be taken with forage comparisons at same leaf stage (i.e. number of leaves) because, given marked differences in leaf-appearance intervals (Skinner and Nelson 1994; Lemaire and Chapman 1996; Rawnsley *et al*. 2010), there will be increasing differences in forage nutritive value due to variations in leaf age (Table 6).

This study reinforces the importance of leaf blade length as a very plastic variable with large effect on forage nutritive value variations (Table 6). It follows from this study that leaf length may help to explain 5–10 percentage units of acceptable variation in digestibility usually reported for carefully controlled trials conducted at same herbage mass (e.g. Nave *et al*. 2013) or leaf stages (e.g. Donaghy *et al*. 2008; Raeside *et al*. 2012). For example, NDFD differed in the order of 2–12 percentage units due to differences in the leaf-length effect alone (Table 6). Therefore, improvements in NDFD due to control of leaf length can represent significant improvements in animal intake and performance (Mertens 2009). For the present study, this improvement can represent increases of up to 3.0 kg day^{-1} of milk yield from a dairy cow, based on a conservative conversion of 0.25 kg milk per unit enhanced NDFD (Oba and Allen 1999). The corollary of this argument is that the negative effect of leaf length on nutritive value could be reduced through management practices that have better control over residual pasture height, as the results from Expt 2 suggest.

Previous experiments on contrasting environments and sward heights with either, tropical (Agnusdei *et al*. 2009; Avila *et al*. 2010, 2012) or temperate grass species (Di Marco *et al*. 2013; Insua *et al*. 2017) showed the same association between lower NDFD and increasing leaf length (Figs 5, 3*b*). Together, all findings suggest that leaf morphological traits would be more relevant to differences in nutritive value of vegetative forages than effects of genotype alone. The large variation in NDFD (>30 percentage units) of leaf blades confirms ample opportunity for increasing forage nutritive value and either meat and milk production through better forage management.

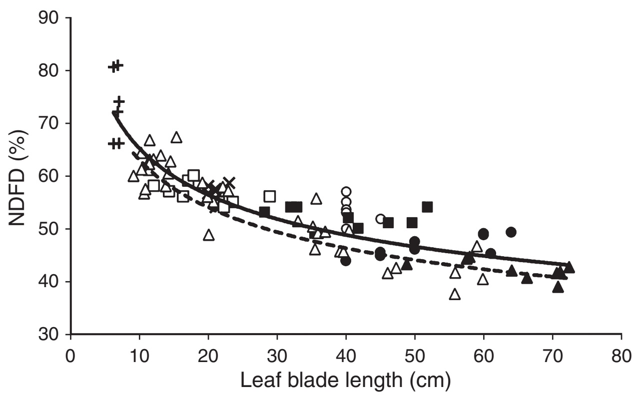

Fig. 5. Relationship between final leaf blade length and digestibility of leaf neutral detergent fibre (NDFD) for leaf blades at the moment of just-expanded leaf (ligule appearance) of different temperate and tropical forage grass species and residual pasture height. The general relationship is presented with a solid line: NDFD = 105*x* – 0.21, $P < 0.0001$, $R^2 = 0.80$, $n = 93$. The lineal relationship from Fig. 3*b* is shown as a dotted line: NDFD = 105*x* – 0.22, $P < 0.0001$, $R^2 = 0.90$, $n = 36$. Data are taken from: Agnusdei *et al*. (2009), + (*Digitaria decumbens*); Avila *et al*. (2010), × (*Cenchrus ciliaris*); Avila *et al*. (2012), ●○ (*Chloris gayana*); Di Marco *et al*. (2013), ■□ (*Thinopyrum ponticum*); Insua *et al*. (2017), ▲Δ (*Lolium arundinaceum*). Solid and open symbols denote Low and High residual pasture-height treatments, respectively.

Management implications

Criteria for pasture defoliation are often based on management rules that consider a fix number of resting days, desirable sward height, target herbage mass, or an ideal leaf stage. In addition, the present study stresses the opportunity to complement previous rules with the use of leaf age and leaf length. Setting grazing interval by these two morphogenetic-related criteria could allow managers to use forage resources with more consistent nutritive value. Further, differences in leaf length and age can be traced back to changes in sheath tube length, which could be used as biomarker for changes in nutritive value (Agnusdei *et al*. 2011) or as a link between pasture growth and nutritive value, as new models suggest (Insua 2018). More precisely, whenever the sheath tube is increased, intentionally or not, then the predefined defoliation target should be reduced in order to offset declines in fibre digestibility associated with modified leaf morphogenesis. Given the demonstrated importance of leaf length and age on forage nutritive value, it is advisable for producers to generate leafy, dense and short sward structures with sheath tubes no longer than ~10 cm.

Conclusions

The present study showed that ageing rates of tall fescue leaf tissues were constant across a gradient of growing and management conditions. On the other hand, observed variations in leaf length were strongly related to variations in forage nutritive value. Further, forage NDFD declined as leaf age and length increased, and both leaf age and length were structurally linked to differences in leaf morphogenesis and sheath tube length independently of growing seasons and residual pasture heights. The results suggest that the morphological linkage between longer sheath tubes with older and longer leaves and relationships with lower NDFD can be controlled through shorter residual pasture height. There is potential for the present results in tall fescue to be generalised to other temperate and tropical grass species, but confirmation of this hypothesis requires further investigation.

Conflicts of interest

The authors declare there is no conflict of interest.

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