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ABSTRACT

A disadvantage of tall fescue (*Festuca arundinacea* Schreb.) is its low voluntary intake, resulting in suboptimal performance under grazing. Ideally selection for this trait is done using grazing animals, but their use in plant breeding programmes is costly and laborious. Repeatable, stable and quantifiable traits linked to animal preference could ease tall fescue breeding. We established a trial to find relationships between the grazing preference of sheep and sward- and plant-related traits. Seventeen genotypes were studied in swards. Sheep grazing preference, pre-grazing sward height (SH), leaf softness, leaf blade length, width, colour and shear strength, and concentration of fibre, silica, digestible organic matter (DOM) and water soluble carbohydrate (WSC) were quantified throughout the growing season. The traits with the strongest correlation with sheep preference were DOM, SH, leaf colour, leaf width and WSC. Leaf softness, silica content and leaf shear strength were not correlated with sheep preference. We conclude that DOM is the trait that offers the best prospects for contributing to progress in tall fescue plant breeding for both intake and feeding value.

Keywords: *Festuca* spp, Plant breeding, Digestibility, Palatability

1. INTRODUCTION

Grassland production in NW Europe is expected to face some challenges arising from the effects of climate change in the decades to come, including increased frequency of summer droughts (Ergon et al., 2016). To counter this, a shift in the species used for grassland production is often suggested (Volaire et al., 2014). Owing to its good drought resistance and high yield potential, tall fescue (*Festuca arundinacea* Schreb.; syn *Schedonorus arundinaceus* (Schreb.) Dumort.) may address current needs and future threats for forage production in NW Europe (Reheul et al., 2017). The currently available tall fescue varieties, however, have a lower feeding quality compared to, e.g. perennial ryegrass (*Lolium perenne* L.), which is due to two characteristics. First, the digestibility of the organic matter (DOM) of tall fescue is on average circa 8 percentage points lower than that of perennial ryegrass cut with the same frequency (Cougnon et al., 2014). Second, the voluntary intake of stabled animals fed with freshly cut tall fescue is, on average, circa 7 % lower than the intake of ryegrasses, when both species are harvested at similar heights (Luten and Remmelink, 1984). Even though this difference in animal intake decreases substantially after ensiling (Luten and Remmelink, 1984), tall fescue varieties with an improved intake are still desired for grazing or for situations when freshly cut grass is fed to livestock.

While selection for higher digestibility of the organic matter (DOM) has been reported by several authors (e.g. Johnston and MacAneney, 1994), the issue of reduced animal intake of tall fescue has received less attention due to methodological difficulties. Total grass intake by ruminants is influenced by pre- and post-ingestion factors. Pre-ingestion factors influence the short-term intake of grass. Many authors previously used the term “palatability” to describe short-term intake (Aderibigbe et al., 1982; Buckner and Burrus, 1962) but as this term is imprecise and confounded with “preference” it is not a recommended term (Allen et al., 2011). Examples of leaf and biomass characteristics affecting short-term intake are leaf blade length and sward height (Barre et al., 2006). In the longer term, the retention time of the grass in the rumen, which is determined by characteristics like digestibility and shear strength of grass leaves (Inoué et al., 1994a), limits further intake (Baumont, 1996). The term “preference” is a measure of relative intake of different forages, where access to the forage is unrestricted (Allen et al., 2011). It integrates the effects of both pre-and post-ingestion factors on the intake. Research indicates that ruminants gather experience on which food and in which quantities they should graze to fulfil their needs through post-ingestive feedback (Provenza, 1995); as a result both the pre- and post-ingestive factors are eligible to influence short-term preference.

The early work of Buckner and Burrus (1962) and Buckner and Fergus (1960) showed that it is possible to improve intake of tall fescue through breeding for increased preference. Hence, in early tall fescue breeding programmes, grazing animals were used in the breeding process to quantify the preference of different genotypes or varieties. This was done using cattle or sheep on the plant breeding nurseries (Jadas-Hécart, 1982; Petersen et al., 1958) or by the use of so-called "cafeteria experiments" (Gillet et al., 1983), in which cut grass of different varieties (or genotypes) was presented to sheep. Nevertheless, animal trials are not easy to manage and have a low repeatability as the preference of grazing animals is difficult to quantify. In grass breeding programmes, where observations are performed at a small spatial scale (e.g. single plants or small plots) and the number of genotypes to be tested is high, the use of animals is even more complicated. For these reasons, both breeders and researchers have been looking for (quantifiable) measurements and leaf and biomass characteristics that correlate well with grazing preference. Most studies that have attempted to explain animal preference have focused only on one or a few plant quantifiable variables (further referred to as "traits"), yet their explanatory value is not always large and far from consistent between different studies. The studied traits can be grouped into three categories: mechanical, morphological and chemical traits. Below follows an overview of studies for each of the three groups of traits.

Early studies linking mechanical properties to animal production were these of Kneebone (1960) and Evans (1967). The hypothesis was that mechanical properties reflect the ease with which the forage can be ingested by the animal and can be reduced to a particle size that facilitates passage through the rumen (Henry et al., 1997; Mackinnon et al., 1988). In perennial ryegrass, there was no evidence that selection for a lower shear strength led to an increased voluntary intake or animal performance (Inoué et al., 1994b). In tall fescue on the other hand, MacAdam and Mayland (2003) found a negative relationship between leaf shear strength and cattle preference in a trial comparing eight varieties.

Leaf softness, as determined by touching plants or swards, correlates well with preference according to breeding researchers in France (Gillet and Jadas-Hécart, 1965; Jadas-Hécart, 1982). The potential progress in tall fescue intake and quality by means of selection for leaf softness is, however, limited to the first cycle(s) of any breeding programme. First, perceiving differences in leaf softness becomes more difficult as breeding progresses: the more the breeding material improves the less variation for these traits remains. Second, the correlation between leaf softness and animal preference is low in elite breeding material of tall fescue (Rognli et al., 2010).

Closely related to the leaf mechanical characteristics, the morphological characteristics of grass leaves and the sward canopy structure also influence the intake of grass. They influence the relative ease with which biomass can be grazed. In studies with sheep in grazing cages on grass swards with different heights and densities, Burlinson et al. (1991) found that mean bite weight was positively correlated to surface height. Also, in grazed perennial ryegrass, leaf blade length explained most of the variation present in short term intake rates between different varieties: the varieties with the longest leaves having the highest intake rate (Barre et al., 2006).

Finally, herbage chemical composition also has an important influence on intake. Fibre and lignin concentrations are generally negatively correlated while digestibility of the organic matter and sugar concentration are most often positively correlated with preference and intake (Barre et al., 2006; MacAdam and Mayland, 2003; Tas et al., 2005). Moreover, in elite material of perennial ryegrass the correlation between digestibility, intake and animal performance was found to be low (Orr et al., 2003; Tas et al., 2005). Evidence for these relationships in tall fescue is scarce. Mayland et al. (2000) found a significant positive relationship between non-structural carbohydrate concentration and cattle grazing preference in eight tall fescue varieties. Tightly linked to lignin is plant silica concentration (Schoelynck and Struyf, 2016). The presence of silica in leaves can also negatively affect herbivorous vertebrates and invertebrate crop pests (Cooke and Leishman, 2011). This was evidenced for grass species by Massey et al. (2009): in sheep preference trials, sheep grazed longer, took more bites and had a higher bite rate on grass species with low silica concentration. Cougnon et al. (2016) found variation in the silica concentration of tall fescue genotypes: in a study of 19 tall fescue genotypes the silica concentration varied between 5 mg (g dry matter (DM))$^{-1}$ and 12.3 mg (g DM)$^{-1}$. Hence, it is probable that the silica concentration of tall fescue genotypes plays a role in their preference. Moreover, Hartley et al. (2015) found that soft-leaved tall fescue varieties contain less silica and have less sharp silicified dentations (trichomes) on the leaf margins and midribs. Hence, the trichome presence might also influence the preference through its effect on leaf softness and silica content.

Clearly, single mechanical, morphological or chemical traits cannot fully predict the preference of grazing animals. Yet, most of the studies to date have focused exactly on one-to-one relationships between tall fescue preference and one of these traits. In the current study, we address this knowledge gap and assessed herbivore preference (using sheep as a model species) of tall fescue genotypes with different quantifiable traits simultaneously. Following traits that proved to influence preference and that could be (easily) assessed in a breeding programme, were measured on different genotypes of tall fescue the following: sheep grazing preference; pre-grazing sward height and softness; leaf blade length, width, colour and shear strength; concentration of fibre, silica, digestible organic matter and water soluble carbohydrates.

We specifically addressed the following research questions: (i) Are there differences in herbivore (in this case by sheep) preference between elite tall fescue genotypes? and (ii) Which chemical, morphological and physical traits can explain the difference in sheep preference between different genotypes of tall fescue?

2. MATERIAL AND METHODS

2.1 Site

A grazing trial was established in March 2013 at the experimental farm of Ghent University in Melle, Belgium (50.9°N, 3.8° E; 11 m above sea level). The climate of the study site is characterized by an annual mean air temperature of 10.5 °C and an annual mean precipitation of 825 mm (average 1981-2010). The warmest and coldest months are July (mean air temperature 18.1°C) and January (mean air temperature 3.4°C) respectively. The soil on the site is sandy loam with clay (< 2 µm), silt (2-20 µm), fine sand (20-200 µm) and coarse sand (200-2000 µm) concentrations of 86, 116, 758 and 40 g kg^{-1} respectively.

2.2 Study species and trial establishment

From a clonal nursery, part of the tall fescue breeding programme of Ghent University, 20 genotypes with vigorous growth, no signs of re-heading in summer and resistance to crown rust (*Puccinia coronata*) and bacterial wilt (*Xanthomonas campestris*) were selected. Although all the material in this clonal nursery had gone through at least one cycle of selection in which the harshest genotypes were eliminated, there was still some variation for leaf softness in the nursery. Attention was paid to select genotypes with contrasting morphogenetic traits: broad *versus* fine leaves, dark green *versus* light green leaves, soft *versus* harsh leaves. Plant material of the 20 selected genotypes was sampled in the nursery and divided into 200 ramets of 3 to 5 tillers. Plots were planted with ramets of a single genotype spaced 0.20 m between and within the rows, resulting in plots of 1.2 × 2.0 m with genetically identical plants. The trial was planted as a randomized complete block design with three replicates. Between blocks, and between blocks and fences, 2 m wide corridors were sown with an amenity type of tall fescue (4 g m^{-2}).

In the establishment year 2013, plots were mown *circa* every month during the growing season with a lawn mower to a residual height of 5 cm. Fertilization in 2013 was 50 kg N ha^{-1}, 42.5 kg K ha^{-1} and 21.5 kg P ha^{-1}. The plots were kept free of monocot weeds by hand weeding and of dicot weeds using a herbicide treatment (clopyralid + MCPA + fluroxypyr). In March 2014 the grass was fertilized with 300 kg ha^{-1} of calcium cyanamide (19.8 % N) (Perlka®, AlzChem, Trostberg, Germany).

On three occasions in 2014 (12 June, 29 July, 14 September) the measurements described below were performed on the plots, grass samples were taken and the trial was stocked with sheep. To exclude the effect of differences in heading date between the genotypes, the trial was cut after the start of the heading of all genotypes and measurements started after six weeks of regrowth. Three of the 20 genotypes (numbers 9, 10 and 11) were excluded from the experiment: in genotypes 10 and 11 symptoms of bacterial wilt were present, whereas genotype 9 had a different growth pattern and was identified as an amphiploid. Thus, in total 17 genotypes were used in the experiment.

The developmental stage of the grass, measured on 10 tillers per genotype using the developmental scale described in Gustavsson (2011), was similar in the three stocking periods and the differences between the genotypes were limited: between 11.8 - 12.8, 12.0 - 13.2, 11.7 - 12.9 in the first, second and third period respectively. This means that the harvested tillers were all in the vegetative stage and had on average 1 (stage 11) or 2 (stage 12) fully expanded leaf blades. No extreme weather events (summer storms, periods of heavy rainfall, extreme heat) occurred during the stocking periods.

2.3 Measurements

Pre-grazing sward height was measured with a falling plate meter, built according to the description of Bransby et al. (1977), on five randomly selected spots per plot. Leaf softness was scored by two plant breeders on an ordinal scale from 1 (very soft leaf blades) to 5 (very harsh leaf blades). Ten tillers were collected per genotype. The youngest adult leaf blade of each tiller was clipped with scissors at the collar and the length from the ligule to the tip was measured using a ruler. Leaf blade width was measured using vernier callipers at circa 1/3 of the distance between the ligule and the tip, based on the finding of MacAdam and Mayland (2003) that leaf width was constant in this part of the leaf blade. Shear force, the maximum load needed to cause breakage at a 90° breaking angle to the length of the leaf, was measured using a texture analyser (Lloyd Instruments Ltd, Leicester, UK) equipped with a square cutting blade with a thickness of 1.02 mm. The leaf was mounted on the slotted testing table with paper clips so that the leaf was sheared at 1/3 of the distance between ligule and tip. The blade was moving through the leaf at a rate of 50 mm min^{-1}. Leaf shear strength [N mm^{-1}] was calculated by dividing leaf shear force [N] by leaf width [mm]. Chlorophyll concentration of each leaf blade was measured using a SPAD meter (Konica Minolta SPAD- 502plus): it determined the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions. In the present trial, chlorophyll concentration was used as a proxy for leaf colour.

On the same occasions, a sample of circa 125 g of fresh grass was clipped from each plot using an electric hedge trimmer (Stihl HLA 65, Stihl, Waiblingen, Germany) (clipping height of 5 cm). The samples were washed with tap water, dried at 75°C during 16 h and ground to pass through a 1 mm sieve. Biogenic silica (BSi) content was determined according to DeMaster (1981): 25 mg subsamples were incubated in 25 mL 0.5 M NaOH at 80 °C for 5 h. The extracted and dissolved silica was analysed colorimetrically on a segmented flow analyser (SAN++, Skalar, Breda, The Netherlands). The extraction in 0.1 M NaOH at 80° has been well established and tested; it is capable of fully dissolving the BSi from plant phytoliths at the solid-solution ratios and extraction time we applied (Saccone et al., 2007). Digestibility of the organic matter (DOM) and water soluble carbohydrate concentration (WSC) were determined using near infrared reflectance spectroscopy (NIRS). The NIRS equation for DOM (SEC = 1.84 % , R^2 = 0.89) was based on 668 grass samples analysed according to Tilley and Terry (1963). The NIRS equation for the WSC (SEC = 10.4 g kg^{-1} dry matter, R^2 = 0.98) was based on 1572 grass samples analysed according to Wiseman et al. (1960). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) concentrations were determined according to Van Soest (1963) and Van Soest and Wine (1968) respectively.

Trichome density on the leaf surface and the proportion of sclerenchyma in the cross-sectional area were studied to gain more insight in the relation between these traits brought in relation with preference. Trichome density was determined on three of the harvested leaf blades of four genotypes (genotyes 3 and 13 with high preference and 12 and 19 with low preference). For each leaf, a section of 3 mm was cut from the middle of each leaf blade. Material for scanning electron microscopy (SEM) was washed in ethanol 70% for 20 minutes, transferred to 100% dimethoxymethane for 2 times 20 minutes and sonicated a few times for approximately 2 seconds. The material was critical point dried using liquid CO_2 with a critical point dryer (CPD 030, Bal-tec AG, Balzers, Germany). The dried samples were mounted on aluminium stubs using carbon adhesive tape and coated with gold with a sputter coater (SCD 020, Bal-tec AG, Balzers, Germany). SEM images were obtained with a scanning electron microscope (JSM5800-LV, JEOL, Peabody, USA). A series of pictures was taken that spanned the whole surface of the leaf sample at a magnification of 50x. The number of trichomes on the pictures was counted and the exact surface of the scanned leaf sample determined using the image analysis software Image J (Schneider et al., 2012).

The proportion of sclerenchyma in the cross-sectional area was determined in the second sampling period only on three adult leaf blades of eight of the genotypes (genotypes 3, 8, 12, 13, 14, 16, 19, 20). Leaf sections of circa 5 mm were cut at circa 1/3 of the total length measured from the collard to the tip of the leaf. The samples were fixed in FAA (formaldehyde - acetic acid - ethanol) solution for 24 hours and dehydrated afterwards in a series of tertiar-butanol solutions in ethanol (70%) in five steps (15%, 35%, 55%, 75% and 100% butanol). Finally they were embedded in pure paraffin to cut slides using a microtome (R. Jung AG, Heidelberg, Germany). The slides were stained with astra blue 1%, acridine red 0.5% and chrysoidine 0.5%. The slides were studied under a light microscope (Olympus BX51) at magnification of 100×. Pictures of the slides were taken and Image J (Schneider et al., 2012) was used to measure the proportion of sclerenchyma tissue on the section.

After sampling, the trial was stocked with sheep that had been grazing for at least two weeks on tall fescue pastures adjacent to the trial. Stocking density was regulated to allow complete grazing of the trial in *circa* 4 days. The trial area of 4.5 a was stocked with 3 ewes of the Belgian "Houtland" breed (typical average adult weight of 60 kg) and their 6 lambs (4 males, 2 females) in the first two stocking periods and with the same 3 ewes and two of their lambs (2 females) in the last stocking round. In accordance with Shewmaker et al. (1997), grazing preference was scored every morning on a scale from 0 (no grazing at all), 1 (between 0% and 10% of standing biomass eaten), 2 (between 10 and 20% of standing biomass eaten)…. to 9 (between 80 and 90% of standing biomass eaten). The stocking period ended as soon as one of the genotypes reached a score of 9; this took between 6 days in period 1 and 7 days in period 3. The observation that discriminated most between the genotypes with the highest and the lowest preference was withheld; this was after 5 days in periods 1 and 2 and after 7 days in period 3. After each stocking period, the whole trial area was mown with a lawn mower at 5 cm height to remove the non-grazed herbage and fertilized with 300 kg ha^{-1} of a 15-4-15 N-P-K compound fertilizer.

2.4 Data analyses

In all statistical analyses, the sheep preference was our response variable, whereas the other measured characteristics were treated as predictor variables. Statistical analyses were performed using the software package R (R core team, 2014).

To assess the effects of each genotype and grazing period on each of the studied traits, analysis of variance (ANOVA) was performed using the aov() function. Period was treated as a random effect, genotype as a fixed effect. The F-value for genotype ($F_{genotype}$)was calculated as $MS_{genotype}/MS_{genotype\ x\ period}$.

To assess the effects of each of the measured morphological, chemical and mechanical leaf variables, and as collinearity was expected between the measured variables that have an influence on sheep preference, first a principal component analysis (PCA) was performed using the *princomp()* function.

To exclude the effect of the different units of the measured parameters, the PCA was based on the correlation matrix. As a second step, a linear regression was then performed between the first PC axis of the PCA analysis and the sheep preference using the *lm()* function. Finally, the relationship between the preference and each particular variable was studied using a general linear model including the grazing period as factor. To cope with the different units of the variables, the PCA and these regressions were performed on standardized data (from each data point for each of the traits, the mean was subtracted and divided by the standard deviation).

3. RESULTS

Genotype had a significant effect (p<0.001) on the sheep preference. Genotypes with consistently high preference (average > 6 in each period) were genotypes number 3, 6, 16, 20; those with a consistently low preference (average < 3 in each period) were genotypes number 8, 14 and 19 (Figure 1). The effects of period and the period × genotype interaction were not significant (p=0.805 and p=0.338) (Table S1). An overview of the effects of genotype and period on the other measured traits are given as supplementary material (Figure S1-S11 and Table S1).

[Fig1]

In a PCA of all measured morphological, chemical and mechanical leaf characteristics, the first two principal components explained 36.5% and 19.7% of the variation found in the data (Figure 2). The first principal component (PC1) showed a contrast between WSC (Pearson correlation between PC1 and WSC: r=-0.92) and DOM (r=-0.90) on one hand and ADF (r=0.93), NDF (r=0.85) and sward height (r=0.67) on the other hand. As WSC, NDF and ADF are components of the DOM, PC1 means that the genotypes in the experiment differed mainly in their digestibility. PC1 contrasted genotypes with high DOM, low NDF and high WSC (negative values for PC1) (e.g. genotypes 4, 7, 20) and genotypes with low DOM, high NDF and low WSC (e.g. genotypes 2, 8, 14 and 19). PC1 also revealed a contrast between the sampling periods: higher DOM in period 1, lower DOM in period 2. The second principal component (PC2) discriminated between genotypes with dark and harsh leaves (e.g. genotype 19) and genotypes that had softer and lighter leaves (e.g., genotype 12).

[Fig2]

A significant linear effect of the digestibility axis (PC1) on sheep preference was found (p<0.001), indicating that the DOM and the related characteristics are important determinants of sheep preference. The linear effect of the harshness axis (PC2) on sheep preference was not significant (*p*=0.354), indicating that physical characteristics like harshness (HAR) and colour (SPAD) had a smaller impact on the preference than the digestibility-linked characteristics (PC1).

A significant linear effect and strong correlations were found between preference and DOM (*p*= <0.0001; r=0.63), sward height (*p*= 0.0007; r=-0.59) (Figure 3), leaf colour (*p*=0.01; r=0.41), leaf width (*p*= 0.007; r=0.42) and WSC (p= 0.008; r=0.35). No significant correlations were found between preference and silica-concentration (*p*=0.46; r=-0.21), shear strength (*p*=0.83; r=-0.14) and harshness (*p*=0.43; r=0.01). No significant relationships were found between preference and the fibre fractions NDF (*p*=0.17; r=-0.25) and lignin (*p*=0.65; r=-0.061), but there was a significant negative effect of ADF (*p*=0.008; r=-0.39). There was a significant positive effect of NDF (*p*<0.001, r=0.53), ADF (*p*<0.001, r=0.53) and leaf blade length (*p*=0.037) on sward height.

The period only had a significant effect on the intercepts of the regressions between preference and the other studied traits, and no difference in slopes was observed. More specifically, the intercept for period 2 differed from that of period 1 and 3 in the regressions between preference and pre-grazing sward height (p= 0.0007) (Figure 3), DOM (*p*= 0.0002) (Figure 3), WSC (*p*= 0.010) and ADF (*p*= 0.0013). The slopes were the same for all periods, meaning that the effect of the studied traits on preference were the same in each period.

[Fig3]

Concerning trichome presence, there was no significant effect of the genotype on the trichome density; the effect of period on trichome density, however, was significant (*p* = 0.002) (Figure 4). In the third period, the trichome density on the leaves was lower but the variation within the genotypes increased. For example, In genotype 13, leaves with densities between 288 trichomes 10 mm^{-2} and 10 trichomes 10mm^{-2} were found in the third stocking period. There was no effect of trichome density on the sheep preference (*p*=0.604) neither was there a significant effect of trichome density on leaf harshness (*p*=0.075).

[Fig4]

No significant difference was found in the proportion of sclerenchyma in the cross-sectional area of the different genotypes (*p*=0.23) and there was no relationship with preference (*p*=0.11). Neither was there a significant relationship between the proportion of sclerenchyma and the closely related characteristics shear strength (*p*=0.35), DOM (*p*=0.53) and pre-grazing sward height (*p*=0.13).

4. DISCUSSION

DOM and WSC were the traits with the best prospects for improving preference in tall fescue. This is not surprising as a positive effect of DOM on preference had been established in previous studies with several grass species: for cut tall fescue by Gillet et al. (1983) and for grazed smooth bromegrass (*Bromus inermis* Leyss.) by Falkner and Casler (1998). Mayland et al. (2000) found a positive effect of the total non-structural carbohydrates (which includes WSC) concentration on cattle grazing preference. Interestingly, DOM also proved to have a clear positive effect on the intake of grass (Peyraud et al., 1996); e.g., in an analysis of data from intake trials with dairy cows fed with fresh grass, wilted grass or grass silage performed in Belgium between 1970 and 1985 there was an increase of 0.17 kg DM of voluntary intake for a 1% point increase in DOM (De Boever and De Campeneere, 2016). Closely related to the DOM are the fibre fractions NDF (total cell walls) (r = -0.79), ADF (Total cell walls – hemicellulose = cellulose + lignin) (-0.69) and ADL (Lignin) (r = 0.10). ADF was the only fibre fraction with a significant negative correlation with preference (r=-0.39). The genotypes with the highest preference were the grasses that had high DOM in all grazing periods. The strong relationship found between DOM and WSC, suggests that the high DOM comes from a high WSC concentration rather than from high digestible NDF concentration.

The NDF and ADF concentration and the leaf blade length were related to the pre-grazing sward height, which had an important negative effect on sheep preference. Genotypes with an erect growth architecture had a relatively high pre-grazing sward height and were not preferred by the sheep (e.g. genotype 19). Apart from the higher fibre concentration in erect growing genotypes, another factor explaining the low preference could be that erect growing genotypes may prickle the muzzle of the grazing animal. In experiments with perennial ryegrass, high sward height and long leaf blades generally had a positive effect on preference and intake rates (Barre et al., 2006; Peyraud et al., 1996).

There was no effect of leaf softness, silica concentration or of leaf blade shear strength on the preference of tall fescue genotypes by sheep. In a similar experiment prior to the present study (Cougnon, 2013), in which the effect of leaf softness on sheep preference was tested on 16 tall fescue genotypes (previously not selected for softness) there was a significant positive effect of leaf softness on the sheep preference. These conflicting results confirm the statement of Rognli et al. (2010) that correlation between softness and animal preference is lost in elite breeding material. Interestingly, in the present trial, genotype 19, a harsh ecotype with erect leaves, and genotype 8, a soft genotype with narrow and flexible leaves, were both among the least-preferred genotypes. From these observations, one might conclude that harsh genotypes have a low preference, but that there is no guarantee that soft genotypes have a good preference. Moreover, tall fescue varieties with the highest digestibility are not necessarily varieties with the softest leaves, as indicated by the Swiss official variety trials with tall fescue (Suter et al., 2009). Thus, (subjectively measured) leaf softness is a trait with limited value in recurrent breeding programmes with tall fescue. Although it is very useful to eliminate the harshest genotypes at an initial stage, a further selection against harshness has no further priority in later cycles of the recurrent breeding programme. The absence of an objective and repeatable method to measure harshness explain this observation. If we were able to develop, for example, a device with sensors allowing an accurate measurement of softness, that could enable an improved correlation between softness and preferences.

Trichome density on the leaves cannot serve as proxy for softness: there was no significant correlation between these characteristics. Neither was trichome density related to preference. From the findings of the present study, therefore, there would seem to be no interest in counting trichome densities to improve the preference of tall fescue genotypes.

While the literature suggests silica concentration to be a qualifying characteristic for forage preference of herbivores (Coocke and Leishmann 2011; Massey et al. 2009) our study found no such correlation. The restricted range of silica concentrations in the tested genotypes might be the reason for the absence of such an effect. Indeed, the range of the silica concentrations measured in the present experiment was 03.8 – 8.2 mg $(gDM)^{-1}$, whereas in the study of Massey et al. (2009) much larger ranges of silica concentration were present, 2.7 – 17.4 mg $(gDM)^{-1}$. Although it was not the aim of this study to test the relation between leaf harshness and silica concentration, there was no good correlation between both traits. The harshest genotype (genotype 19) was not among the highest in silica concentration. Inversely, genotype 18, the genotype with the highest silica concentration, was found to be soft rather than harsh. Neither was there a relationship between the lignin and silica concentration as suggested in Schoelynck and Struyf (2016)

The positive effect of leaf blade width on preference in this trial was consistent with the findings of MacAdam and Mayland (2003). The range of leaf widths measured in the present trial (3.6 mm – 6.9 mm) were in the same range as those measured by MacAdam and Mayland (2003) (4.7 mm – 7.3 mm). Based on the observation that there was a negative correlation between leaf width and shear strength, MacAdam and Mayland (2003) explained this positive effect of leaf width on animal preference by a higher ratio of mesophyll to structural tissue in wider leaves. Contrary to MacAdam and Mayland (2003) there was no effect of shear strength on preference and the correlation between width and shear strength was very low in the present trial. This means that the shear strength (shear breaking load/width) was not decreasing with increasing width and thus that the distance between fibres or the strength of the fibres in the leaf blades was independent of width.

Measuring the structural tissue directly by means of measuring the sclerenchyma proportion on cross-sectional area was revealed to be difficult: many transverse sections did not span the whole width of the leaf blade, especially for the genotypes with wider leaves. Moreover the proportion of sclerenchyma in the measured genotypes gave no information regarding the preference and it was not correlated with the NDF concentration nor with shear strength. Hence, based on the results of the present trial, measuring the sclerenchyma offers no prospects to improve the preference of tall fescue.

Colour, measured by SPAD (determining the relative chlorophyll concentration), showed a significant positive effect on preference. In repeated preference experiments, Jadas-Hecart (1982) also found this positive effect, but not in all years and all experiments. Cougnon et al. (2013) found no significant effect of colour on sheep preference. As there is a positive relationship between leaf colour (as measured by SPAD) and the N content of grass leaves (Gáborčík, 2003) the higher preference of the darker genotypes could be an indication that the sheep appreciate the higher crude protein content of the dark genotypes. This hypothesis needs further research.

Visual estimation of preferences has certainly the disadvantage of a low repeatability. Other methods to compare preference, with a higher repeatability, are available but are more labour intensive and result mostly in comparable results. Buckner and Burrus (1962) calculated the preference of 22 tall fescue populations by measuring sward biomass before and after grazing. They found correlation coefficients between 0.75 and 0.85 between the physical measurement and the visual estimation. Also in "trough-cafeteria" experiments (Gillet et al., 1983) where cut grass of different genotypes is presented to stabled sheep and the remaining grass and relative consumption calculated after a fixed time interval, strong correlations with the visual estimation were found. In repeated experiments with 5 to 14 tall fescue varieties, correlation coefficients between the trough-cafeteria method and the visual preference estimation in field plots were between 0.77 and 0.99. Other, more sophisticated methods to measure intake by grazing animals include the use of sensors that measure jaw movement that allow researchers to compare grazing and rumination time on different varieties (Orr et al., 2005) or the use of cameras to observe grazing time on different swards (Penning and Rutter, 2004).

Using NIRS-techniques, it is possible to make good breeding progress in DOM and WSC in forage grasses (Robins et al., 2016). The results of the present trial show that improving DOM and WSC should be the first concern in tall fescue breeding; breeding for leaf softness, low shear strength, low silica concentration or less trichomes does seem not useful for improving the preference of tall fescue. The next step to study is whether a higher preference really results in a higher intake and consequentially in a higher production. Parsons et al. (2011), for instance, clearly show that scientifically based breeding progress often fails to result in enhanced production in practice.

5. CONCLUSIONS

Sheep are able to differentiate between tall fescue genotypes when grown in small plots. In the elite breeding material that was tested, traits like leaf softness, shear strength and silica concentration were not related to sheep preference in the present trial. The digestibility of the organic material, the water soluble carbohydrate concentration and the acid detergent fibre concentration were highly correlated to preference and offered the best prospects for use in breeding programmes. Measuring the trichome densities on the leaf blades or the sclerenchyma concentration on leaf sections could not explain differences in sheep preference.

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Figure Captions

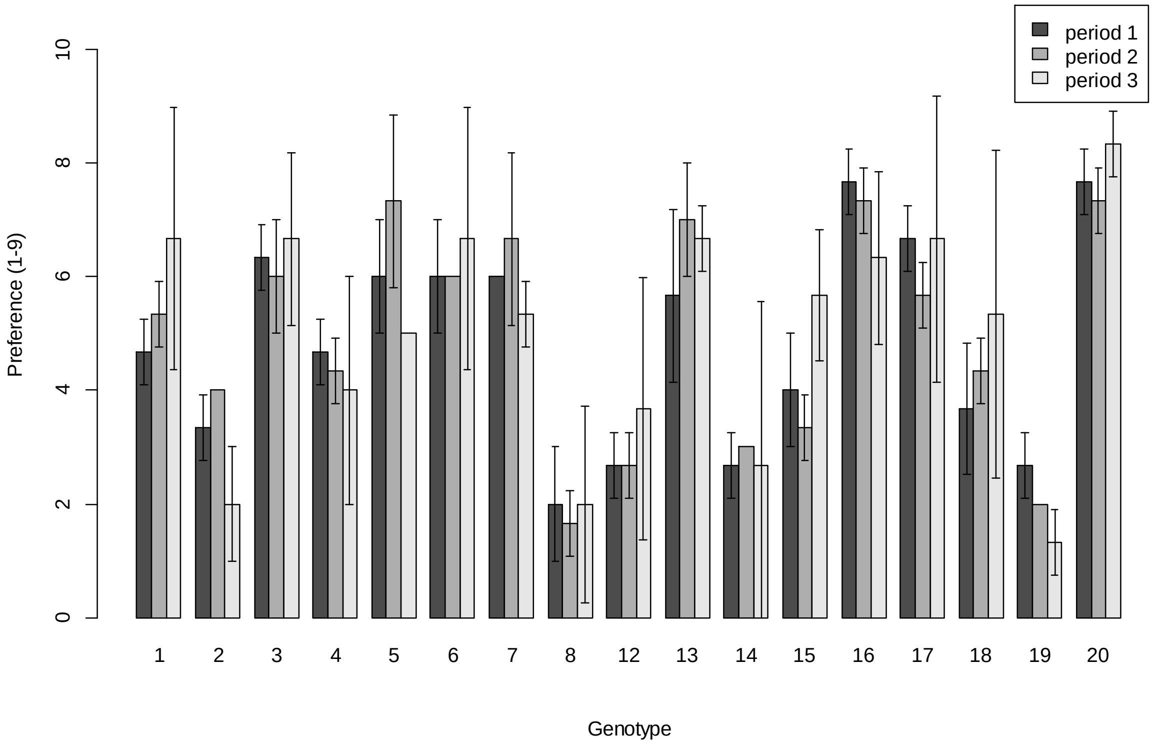

Figure 1 Sheep preference of 17 tall fescue genotypes estimated in three successive grazing periods in 2014. Preference was scored on an ordinal scale from 0 (lowest preference) to 9 (highest preference). Data are averages of three blocks; bars represent standard deviation.

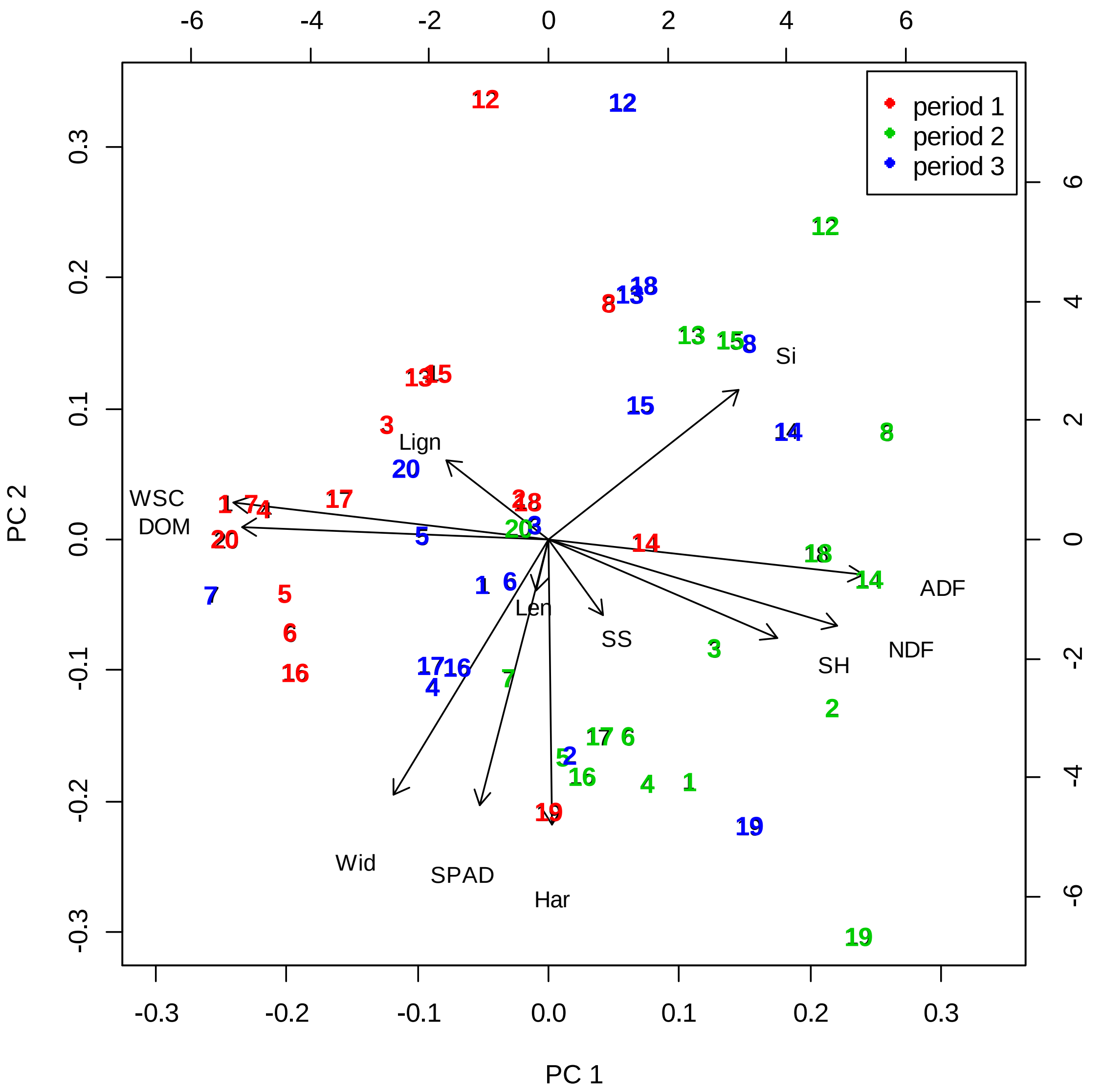

Figure 2 Biplot of the principal component analysis of the mechanical, morphological, and chemical traits measured on 17 tall fescue genotypes in three successive grazing periods in 2014. Wid = leaf blade width, SPAD=leaf chlorophyll concentration, Har = harshness, Len = leaf blade length, SS = leaf blade shear strength, SH = pre-grazing sward height, NDF = neutral detergent fibre concentration, ADF = acid detergent fibre concentration, Si = silica concentration, Lign = lignin concentration, WSC = water soluble carbohydrate concentration, DOM = digestibility of the organic matter.

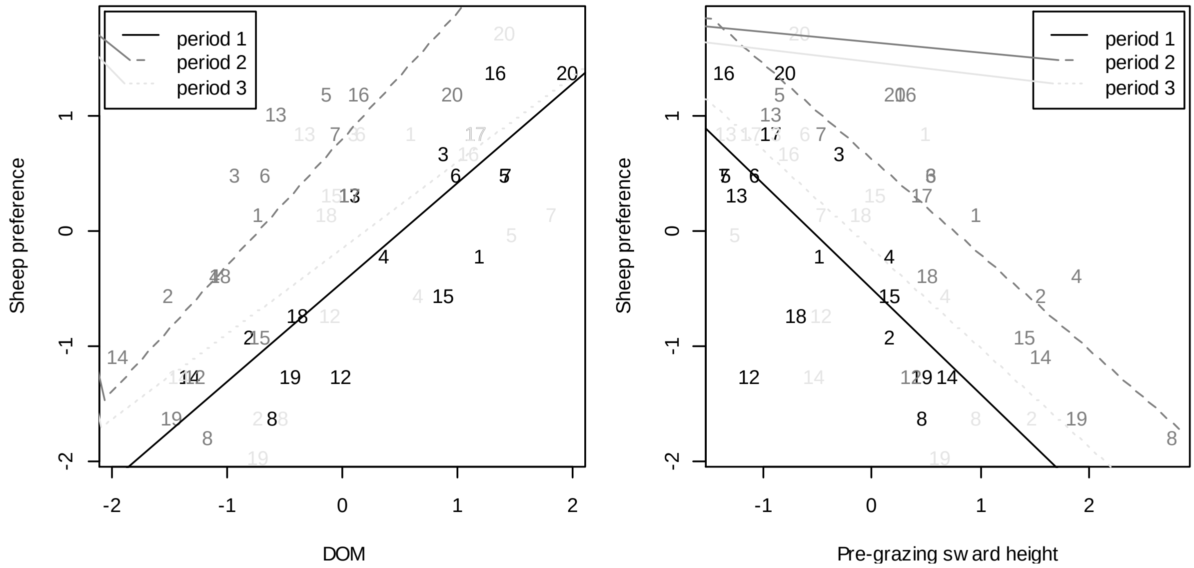

Figure 3 Relationship between sheep preference and digestibility of the organic matter (DOM) (left) and pre-grazing sward height (right) for 17 genotypes of tall fescue in three grazing periods in 2014 Regressions were performed on standardized data to eliminate the effect of the different units.

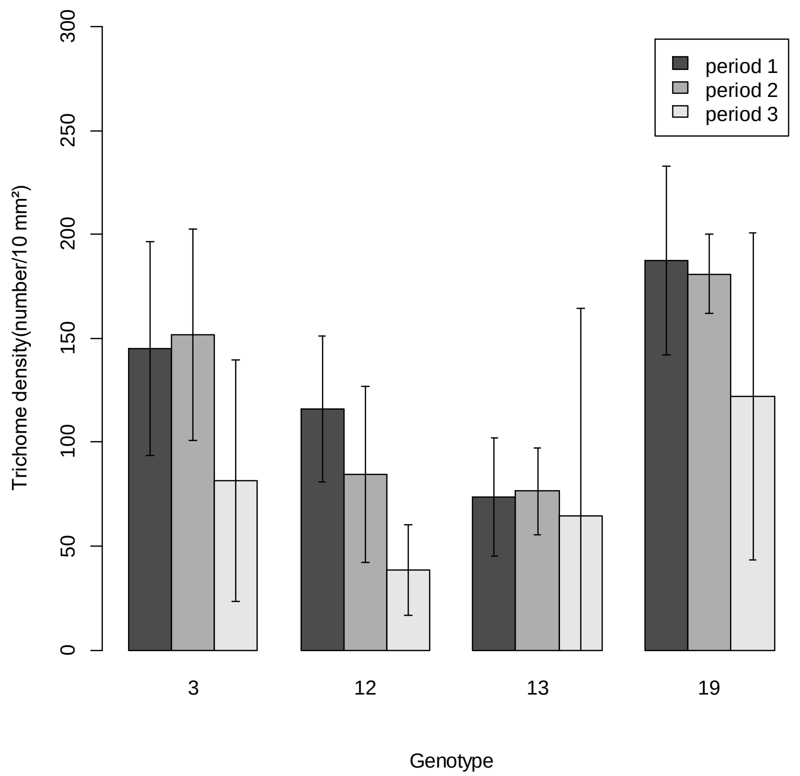

Figure 4 Trichome density on the leaf blades of four tall fescue genotypes in three grazing periods in 2014. Data are averages of three leaves; bars represent standard deviation.