Cultivar Differences and Seasonal Changes of Primary Metabolites and Flavor Constituents in Tall Fescue in Relation to Palatability

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Six tall fescue cultivars have been considered and investigated during the growing season to assess a possible relationship between palatability and chemical composition of the grass. The percentages of crude protein, fiber fractions, and water soluble carbohydrates have been determined in the dry matter, while the main flavor constituents of the fresh herbage have been obtained by steam distillation and analyzed by gas chromatography (GC) and GC–mass spectrometry. Slight variation has been found for primary metabolites comparing genotypes, whereas wider variations exist among harvests. Water soluble carbohydrate content seems to be highly related to preference rating estimated by leaf flexibility. The most abundant volatile constituents of the essential oil show wide variability among cultivars and cuts during the growing season. Relationships between primary metabolites and volatile compounds in tall fescue are presented and discussed, although no direct influence with palatability can be assessed.

Keywords: *Tall fescue; Festuca arundinacea; primary metabolites; essential oil; palatability*

INTRODUCTION

Feed choice by the animal is a highly sophisticated process, developed through evolution, genetically controlled, and related to factors regulating appetite, intake and diet selection (Owen, 1992). The assumption that flavors from feeds may stimulate appetite and preference of grazing animals has been studied for various forage crops with feeding trials and so-called "cafeteria tests" involving animals (Gillet et al., 1983; Burns et al., 1984; Reid et al., 1992). Scehovic et al. (1985) in particular reported an experiment on the attractive effect of volatile compounds of the juice pressed from the highly palatable species Italian ryegrass and sprayed over the low palatability tall fescue. A number of studies on volatile constituents of forage plants, affecting animal olfactory perceptions, have been carried out, focusing on chemical composition of the essential oil from several grasses and leguminous crops (Kami, 1975, 1978, 1983; Buttery et al., 1982, 1984; Aii et al., 1985; Morrison et al., 1986). Nevertheless, other factors have been considered to explain the low level of palatability and poor conversion efficiency of dry matter in some forage species. A lower organic matter degradation rate and a higher physical resistance of leaves to breakdown during animal chewing have been indicated as responsible for the poor palatability of tall fescue. Also, morphophysiological plant traits, such as vigor, leaf flexibility, and green appearance, have been considered. In addition, seasonal changes in the chemical composition of dry matter, particularly crude protein and soluble sugar content, have been reported as affecting preference and palatability in ruminants (Jadas-Hecart, 1982; Scehovic et al., 1985).

To examine the possible role as attractant of individual chemical components of the biomass in determining animal preference, a study has been undertaken with six tall fescue varieties, monitored at different seasonal cuts. Three of the selected cultivars, Magno, M. Kasba, and Tanit, have been previously studied for quality parameters related to palatability (Romani et al., 1991), and three other cultivars, Barcel, Clarine, and Sopline, have been evaluated in a cafeteria test for animal preference (J. Jadas-Hecart, personal communication). The varieties tested have been selected for their morphophysiological traits and geographical origin as representative of "continental types" and "Mediterranean types" of tall fescue, with different degrees of leaf flexibility or rigidity of the foliage. Tanit and M. Kasba are also characterized by their summer dormancy and drought resistance (Piano and Pusceddu, 1989). The occurrence of the endophytic fungus *Acremonium coenophialum*, checked in the six cultivars, has been found only in Tanit (40% seed infected), with no apparent effect on herbage yield or pest damage. On the basis of morphological traits, mainly leaf flexibility, three cultivars—Sopline, Barcel, and Tanit—have been classified as preferred and the remaining three as nonpreferred. Qualitative and quantitative analyses of the primary metabolites, as well as of the main volatile compounds of the essential oil extracted, are presented and discussed in their relation to palatability.

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MATERIALS AND METHODS

Plant Materials. Six tall fescue (*Festuca arundinacea* Schreb.) varieties have been studied: Barcel, Clarine, Magno, M. Kasba, Sopline, and Tanit, all referred to as middle-late maturing genotypes. Three of them, Sopline, Barcel, and Tanit, on the basis of plant softness and leaf flexibility, have been classified as preferred, the remaining three as poorly palatable. Plants were grown in a trial sown in March 1991 as randomized block design with three replicates at the Istituto Sperimentale Colture Foraggere farm in Lodi (MI, Italy). Samples of the aerial parts, stems and leaves, of each cultivar have been hand harvested from each plot three times, at the same growth stage (late tillering, vegetative, and vegetative, respectively), in July, August, and October 1991. Green materials, quick-frozen in liquid nitrogen, were then stored at −80 °C until chemical evaluation.

Chemical Analyses. Dry matter (DM) percentage and chemical composition of DM have been determined

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Table 1. Quality Parameters of the Fresh Herbage of Tall Fescue: Dry Matter (DM) Percentage, Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Water Soluble Carbohydrates (WSC) as Percent of DM, and Preference Index

cultivar or cut	DM %	CP	NDF	ADF	WSC	preference index[a]
A. Comparison of Six Varieties (Mean of Three Cuts)						
Barcel	22.8	16.3	60.9	34.1	13.6	+
Clarine	23.5	14.3	61.3	35.5	11.6	−
Magno	22.3	14.7	59.8	35.8	11.0	−
M. Kasba	22.7	16.8	59.5	35.2	9.8	−
Sopline	23.2	15.2	59.7	36.4	13.0	+
Tanit	24.7	14.3	59.9	36.7	13.4	+
lsd (5%)	ns[b]	1.9	ns	ns	1.0	
B. Seasonal Changes						
July	22.9	15.6	64.2	37.8	6.0	
August	23.6	14.1	68.5	38.3	10.5	
October	23.1	16.1	47.9	30.7	19.7	
mean	23.2	15.3	60.2	35.6	12.1	
lsd (5%)	ns	1.3	6.4	2.4	0.7	

[a](+) and (−) indicate preferred and nonpreferred cultivars, respectively, on the basis of leaf flexibility. [b]ns, not significant.

on subsamples dried at 65 °C to a constant weight and ground in a Cyclotec mill through a 1 mm sieve. Determinations of crude proteins (CP) were carried out according to the Dumas method (Kirsten, 1983); neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method of Goering and Van Soest (1976). Water soluble carbohydrates (WSC) have been determined by the anthrone method (Deriaz, 1961).

Extraction of Essential Oil. Frozen subsamples from the three replicates of each cultivar were mixed together, and then 90−120 g of sample was added to 1.5 μg of 3-methylcyclohexanone, 0.48 μg of *n*-tetradecane, and 0.59 μg of *n*-docosane (internal standards) and steam distilled with odor-free water to obtain 100 mL of distillate. The distillate was saturated with NaCl, extracted with diethyl ether (3 × 70 mL), and dried with Na_2SO_4 overnight. Ethereal solution was concentrated on the rotary evaporator and used as such for GC and GC−MS analysis.

GC and GC−MS Analysis. The essential oil was analyzed by gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS). Compounds were identified by comparison of their mass spectra to those of reference substances. For GC−MS analyses the capillary column was an OV-1 (25 m length, 0.25 mm i.d., 0.25 μm film thickness) and was directly introduced into the ion source. The electron impact mode (EI) was used. Samples were injected in splitless mode (1 μL) with start temperature of 40 °C for 5 min programmed at 4 °C min^{-1} to 270 °C and then held for 20 min at 270 °C. Injector temperature was 250 °C and interface temperature 280 °C. Helium was the carrier gas with a head pressure of 2.5−3.5 psi and 25 cm s^{-1} linear velocity. Mass spectra were acquired over 40−400 amu range at 1 scan s^{-1} with ionizing electron energy 70 eV, electron current 0.3 mA, and ion source 200 °C; the vacuum was 10^{-5} Torr. The quantitative composition of essential oil was determined by an internal standardization method on a gas−liquid chromatograph equipped with a flame ionization detector (FID). A DB-5 capillary column (J&W Scientific) (30 m length, 0.32 mm i.d., 0.25 μm film thickness) was used. The GC conditions were as above. Helium was the carrier gas with a head pressure of 12.0 psi.

Table 2. Representative Flavor Constituents (Micrograms per 100 g of Fresh Weight) of Tall Fescue (*Festuca arundinacea* Schreb.) and Relative Abundance (% RA)

compound	μg/100 g	% RA
aliphatic aldehydes		
hexanal	12.72	1.7
heptanal	4.68	0.6
octanal	5.00	0.7
nonanal	20.65	2.8
(*E*)-2-hexenal	79.18	10.7
(*E,E*)-2,4-hexadienal	8.80	1.2
aliphatic alcohols		
hexanol	4.58	0.6
octanol	38.45	5.2
(*Z*)-3-hexenol	355.35	48.2
(*E*)-2-hexenol	7.05	1.0
oct-1-en-3-ol	16.73	2.3
esters		
(*Z*)-3-hexenyl acetate	81.41	11.0
aromatic aldehydes		
benzaldehyde	4.22	0.6
phenylacetaldehyde	35.06	4.8
aromatic alcohols		
benzyl alcohol	2.62	0.4
phenols		
p-vinylguaiacol	41.75	5.7
terpenoids		
epoxy-β-ionone	2.98	0.4
β-ionone	15.38	2.1

Statistical Analysis. MSTAT (1990, Michigan State University, East Lansing, MI) was used to determine a two-way analysis of variance and separate means by lsd at the 5% level of probability.

RESULTS AND DISCUSSION

Mean dry matter percentage and chemical composition (on dry matter basis) of the six cultivars are reported in Table 1, in which primary metabolites such as protein, fiber fractions, and water soluble carbohydrates are presented. All of these compounds are considered of great importance in the mechanism of animal preference, conferring general feed attributes such as energy supply, texture, and sweetness (Owen, 1992). Quality parameters are shown separately, comparing varieties (Table 1A) and cuts (Table 1B). Leaf flexibility of the six cultivars, as an index of preference and palatability, is also indicated in the same table. Only minor differences were detected among varieties for dry matter percentage and fiber fractions, while significant differences exist for crude protein and chiefly for water soluble carbohydrate: sweetness in particular seems to be directly related to the preference rating based on leaf flexibility. Comparing the successive cuts, statistically significant differences were found for all traits studied, and seasonal changes, particularly at the end of the growing season in the fall, are mainly ascribed to lower fiber and higher water soluble carbohydrate content, as normally observed in our environmental conditions.

Concerning the volatile components of the fresh herbage, frozen samples were extracted by steam distillation immediately after each harvest. The essential oils were analyzed as such by GC and combined GC−MS. The identities of the components were based on mass spectral data in agreement with those reported in the literature (by HP Data Base) and/or direct comparison with data from authentic compounds. The GC−MS analyses of the whole plant extracts allowed the identification of the most abundant compounds, confirming the results of a previous investigation (Tava

Table 3. Total Volatiles and Major Flavor Constituents of the Essential Oil of Tall Fescue, Grouped According to the Functional Groups (Micrograms per 100 g of Fresh Weight)

cultivar or cut	aldehydes	alcohols	ester[a]	phenol[b]	terpenes	total volatiles
A. Comparison of Six Varieties (Mean of Three Cuts)						
Barcel	183.9	268.6	31.2	46.1	26.0	555.8
Clarine	116.0	300.4	107.4	51.7	13.8	589.3
Magno	138.6	324.2	83.2	35.4	14.5	595.8
M. Kasba	225.0	857.2	93.9	35.4	15.5	1227.0
Sopline	145.1	441.9	106.1	31.6	11.4	736.0
Tanit	213.1	356.5	66.6	50.3	28.6	715.1
lsd (5%)	75.7	ns[c]	ns	ns	ns	ns
B. Seasonal Changes						
July	154.2	657.4	128.4	13.5	9.0	962.5
August	167.2	407.2	27.6	74.2	27.3	703.5
October	189.4	209.8	88.2	37.5	18.6	543.6
mean	170.3	424.8	81.4	41.7	18.3	736.5
lsd (5%)	ns	366.3	78.6	22.6	ns	ns

[a] (*Z*)-3-Hexenyl acetate. [b] *p*-Vinylguaiacol. [c] ns, not significant.

et al., 1991). In this work we only considered the main volatile compounds reported as responsible of the "green leaf odor" characteristic of the species *F. arundinacea*. A complete list of the 18 compounds considered is shown in Table 2, in which yield and relative abundance are also reported. (*Z*)-3-Hexenol, commonly defined as "leaf alcohol", (*E*)-2-hexenal, the so-called "leaf aldehyde", and (*Z*)-3-hexenyl acetate were the most abundant flavor constituents of the fresh tall fescue herbage.

Leaf alcohol and leaf aldehyde are widely distributed in fresh leaves, vegetables and fruits and are responsible for the green leaf odor characteristic of grasses and trees (Hatanaka, 1983). They are formed by the enzymatic breakdown of linolenic acid, which produces a range of products and intermediates that contribute to characteristic odors of cut or damaged plant tissues (Hatanaka et al., 1987). Several lipid breakdown products, including C_6 volatiles, that are produced by the plant during plant defense responses may have a more direct effect on the pathogens. In fact, (*E*)-2-hexenal in particular is suggested to be involved in the mechanism of resistance to pests and diseases, showing antiprotozoal, antifungal, insecticidal, and acaricidal activity (Croft et al., 1993). C_6 volatiles, including (*E*)-2-hexenal, produced by wounded cotton leaves, have been reported to inhibit the growth of *Aspergillus flavus* in liquid cultures (Zeringue and McCormick, 1989), behaving like volatile phytoalexins.

The individual compounds identified in our material have been grouped into aldehydes (six aliphatic and two aromatic), alcohols (five aliphatic and one aromatic), esters [(*Z*)-3-hexenyl acetate], phenols (*p*-vinylguaiacol), and terpenoids (two compounds). In Table 3, the functional groups are reported separately for the six cultivars (Table 3A) and for the three cuts (Table 3B). The most abundant chemical group in all samples analyzed was alcohols, accounting, on average, for 58% of the total volatiles (70% in M. Kasba) (Table 3A). Slight but statistically significant differences between varieties were found for aldehydes, ranging from 225 μg/100 g of fresh weight in M. Kasba to 116 μg in Clarine. All remaining groups of volatiles showed no significant variations among cultivars. Volatile esters and phenols, which are considered attractant compounds in the herbs (Scehovic et al., 1985), are abundant in all varieties tested. The highest yield of volatile compounds considered in the essential oil (1227 μg) was obtained from M. Kasba, but no relationship was found in this experiment with other quality parameters or preference rating, whatever assessed.

Comparison of the successive cuts during the growing season (Table 3B) showed a decreasing trend by total volatiles from July to October. This was mainly due to alcohols, as the other chemical groups seemed to vary erratically. A decreasing yield of volatiles from spring–summer to autumn–winter is in accordance with results reported by other authors (Scehovic et al., 1985, in tall fescue; Hatanaka, 1983, in tea leaves; Court et al., 1993, in peppermint).

The most abundant individual components of the whole extract we considered belong to the classes of alcohols [(*Z*)-3-hexenol, octanol, oct-1-en-3-ol], aldehydes [(*E*)-2-hexenal, phenylacetaldehyde, nonanal, hexanal], esters [(*Z*)-3-hexenyl acetate], and phenols (*p*-vinylguaiacol). They are reported in decreasing order of abundance in Table 4, where (*Z*)-3-hexenyl acetate and *p*-vinylguaiacol, respectively the second and fifth most abundant compounds, are omitted. These compounds in fact have already been presented in Table 3, as representative of volatile esters and phenols. In our materials, the single largest volatile compound from all six cultivars in the three harvests was (*Z*)-3-hexenol,

Table 4. Major Volatiles[a] in the Essential Oil of Tall Fescue (Micrograms per 100 g of Fresh Weight)

cultivar or cut	(*Z*)-3-hexenol	(*E*)-2-hexenal	octanol	phenylacetaldehyde	nonanal	oct-1-en-3-ol	hexanal
A. Comparison of Six Varieties (Mean of Three Cuts)							
Barcel	213.0	74.8	35.4	49.8	21.0	10.2	13.0
Clarine	255.7	38.7	28.3	32.2	17.4	8.8	9.4
Magno	272.7	52.5	29.5	26.9	21.1	11.7	12.5
M. Kasba	770.0	151.7	43.4	20.9	18.2	16.4	13.5
Sopline	375.9	71.9	28.9	23.9	17.9	24.8	13.1
Tanit	244.7	85.4	65.1	56.6	28.2	28.5	14.8
lsd (5%)	ns	58.2	ns	ns	ns	ns	ns
B. Seasonal Changes							
July	607.1	67.8	5.1	32.5	19.5	28.8	10.9
August	269.5	50.3	104.2	53.7	24.8	17.0	16.1
October	189.5	119.5	6.0	19.0	17.7	4.3	11.2
mean	355.3	79.2	38.4	35.1	20.6	16.7	12.7
lsd (5%)	344.1	41.2	30.6	25.7	ns	13.1	3.5

[a] (*Z*)-3-Hexenyl acetate (mean value 81.4 μg) and *p*-vinylguaiacol (mean value 41.7 μg) have been reported in the previous table and are omitted here. [b] ns, not significant.

followed by (*E*)-2-hexenal. M. Kasba, which was the cultivar richest in total volatiles (Table 3A), was also the highest in terms of (*Z*)-3-hexenol, the leaf alcohol, and (*E*)-2-hexenal, the leaf aldehyde (Table 4A). No significant differences were shown by the individual compounds among cultivars, apart from (*E*)-2-hexenal, which ranged from 38.7 μg in Clarine to 151.7 μg in M. Kasba. Statistically significant seasonal changes, on the contrary, were observed for the single most abundant volatiles. Relative to the main compound, (*Z*)-3-hexenol, a clear, strong decrease was evident when the first cut was compared with the following cuts of the year. Concentrations of the other compounds observed among cuts did not seem to follow the same seasonal trend but are probably more regulated by the individual biosynthetic pathways. All of the C_6 compounds (aldehydes, alcohols, acetates), for example, are known to be produced by oxidative degradation of unsaturated fatty acids, particularly linoleic and linolenic acids, which are common constituents of plant membranes (Visser et al., 1979).

A precise relationship between grazing preference and chemical composition of tall fescue volatiles seems difficult to establish. The results presented here may only show a general trend in the relative abundance of several compounds derived from the same biochemical pathway. Apart from M. Kasba, the cultivar richest in terms of total volatiles, all variations detected among cultivars seem to display irregular patterns, with no apparent correlation between the compositional profile of dry matter and volatiles and the leaf flexibility as an index of animal preference. It can be easily concluded that palatability and herbage intake are very complex mechanisms regulated by a number of different factors, genetic, physical, and chemical, related to plant characteristics and acting on animal behavior.

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