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Composition and *in sacco* degradation characteristics of winter growth of *Lolium perenne*, *Lolium multiflorum* and *Lolium perenne* × *multiflorum* ryegrasses

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ABSTRACT

Nine ryegrass cultivars (3 perennial, 3 hybrid, 3 Italian types) were sampled during the winter of 2007 to measure chemical composition, degradation parameters and leaf morphology and to define differences that may be applicable to winter growth in dairy pastures. The grasses were harvested after seven and nine weeks of regrowth on June 18 and August 2. There were no differences between ryegrass types in the concentration (g/kg dry matter (DM)) of crude protein (227) but neutral detergent fibre was higher in perennial (468) than hybrid (435) or Italian (414) ryegrass types. Leaves were largest and widest for the Italian type, and shortest and narrowest for the perennials ($P < 0.001$). Italian ryegrasses had the highest concentration of soluble DM. There were differences in the degradation rate (h^{-1}) of insoluble DM; Italian (0.20), hybrid (0.18) and perennial (0.13) ryegrasses ($P = 0.06$). Ryegrass sampled after 9 weeks of regrowth, had a lower crude protein concentration than the earlier sampling (208 *vs*. 246) and a lower neutral detergent fibre concentration (392 *vs*. 486) but leaf characteristics, proportion of DM in the soluble fraction, and DM degradation rates were similar ($P > 0.05$). The identification of differences in composition and degradation will assist with planning grazing management over winter.

Keywords: ryegrass cultivars; autumn saved pasture; composition; *in sacco* degradation.

INTRODUCTION

Pasture composition affects the feeding value of a diet because it influences voluntary feed intake and the nutrients available for absorption (Waghorn & Clark, 2004; Waghorn *et al*., 2007). Changes in quality of ryegrass/clover swards over a growing season were reported by Johns (1955), and more recently Moller *et al*. (1996). They quantified changes in composition of pastures over 12 months on dairy farms in the Manawatu and Waikato regions. Litherland and Lambert (2007) summarised changes in pasture composition in several regions. The largest variations were in crude protein (CP) and neutral detergent fibre (NDF) concentrations (Moller *et al*., 1996). Rotational grazing practices are designed to minimise fluctuations in composition and avoid accumulation of dead matter in the sward (Hoogendoorn *et al*., 1992; Lambert *et al*., 2004).

Currently costs associated with regrassing, supplementary feeding and fertilizer account for approximately 50% of total on-farm production costs on New Zealand dairy farms (DairyBase, 2009). To maximise production and profitability, an understanding of pasture growth, composition, digestion and nutrient absorption by animals is essential so that pasture can be managed correctly and appropriate supplements fed to meet the cow's nutritional needs. Composition and *in sacco* degradation, as a representation of ruminal digestion, have been defined for perennial ryegrass (Grasslands Samson) from 14 to 105 days of regrowth (Chaves *et al*., 2006). More recently Sun *et al*. (2010) undertook similar measurements with three contrasting ryegrass cultivars. Their evaluations using vegetative ryegrasses demonstrated large effects of both regrowth age and cultivar on chemical composition and degradation. Chaves *et al*. (2006) showed initial cutting date in spring, had minimal effects on composition or ruminal degradation rate.

The present study focuses on autumn saved pasture, which is accumulated autumn growth that is fed to dairy cows during winter and early spring (Matthews *et al*., 1999). Cool conditions and slow growth enables an accumulation of dry matter (DM) with limited senescence. Pasture supply for both non-lactating cows and cows immediately post-calving may be less than is required and often has to be supplemented with silage or dry feeds until rapid growth in spring meets cow requirements. Ryegrass types evaluated here included cultivars of perennial (PRG, *Lolium perenne* L.), Italian (IRG, *Lolium multiflorum*) and hybrid (HRG, *Lolium perenne* × *multiflorum*) ryegrasses, all of which grow well in cool conditions (Hickey & Baxter, 1989; Hickey & Hume, 1994). Understanding pasture composition, and cultivar differences, will enable appropriate supplements to be offered to meet cow nutritional requirements.

The objective of this study was to define the composition and degradation characteristics of yegrass types and cultivars that represented autumn saved pasture for dairy cows, harvested during winter.

MATERIALS AND METHODS

Materials

The ryegrasses examined in this study are listed in Table 1.

Samples of nine ryegrass cultivars were taken from plots that had been established in October 2001 at the AgResearch Grasslands Campus, Palmerston North, New Zealand. The plots had been maintained by mowing at 1 to 2 month intervals. In Area 1, the plots were mowed on 3 May 2007 and harvested on 18 June 2007 with 46 days of re-growth. Area 2 was mowed on 1 June 2007 and harvested on 2 August 2007 with 62 days of re-growth. Harvesting was carried out using an electric handpiece, cutting at about 40 mm above the ground level.

About 200 g DM of material was harvested from each cultivar in May and June. From each harvest, about 50 leaves were stored on ice overnight between damp paper towels and then placed in a cold room (4°C) for morphological study and shear strength measurements the next day. The remainder was frozen (-16°C), with about 50 to 70 g freeze-dried for DM determination and chemical analyses using near infrared reflectance spectroscopy at FeedTECH, AgResearch, Palmerston North. The rest of each frozen ryegrass cultivar was minced for *in sacco* incubation (Sun *et al*., 2010).

Leaf morphology and shear strength

To measure leaf morphology and shear strength, 30 fully expanded young leaves of each cultivar were randomly grouped into three bundles of 10. Leaf length was measured for each bunch from ligule to tip and averaged (n = 3). Force to shear was measured for each bunch (n = 3), determined one fifth of the way up the blade from the ligule, based on the average leaf length, using a Warner-Bratzler meat tenderometer (General Electric Co., New York, USA) (Easton, 1989). Three leaves were randomly taken from each bunch with a total of nine leaves from each cultivar, adjacent to the site of shear force determination and the width and thickness measured using vernier callipers.

Sample preparation for incubation

The frozen forage was minced to achieve a particle size distribution similar to that of eaten and ruminated forage (Ulyatt *et al*., 1986). The mincing process used the method described by Chaves *et al*. (2006) with a Kreft Compact meat mincer R70 (Kreft GmbH, Gevelsberg, Germany). Mincing was carried out with frozen forage, after the mincer components had been placed in a freezer to ensure the forage did not thaw during mincing. Minced forage was stored in a sealed plastic bag in a freezer at -16°C.

In sacco incubations

In sacco incubations were conducted in a mature non-lactating Friesian × Jersey cow with a permanent ruminal fistula. The cow was given 5.2 kg DM of lucerne hay at 08:00 and at 17:00 hours daily. The hay contained 531 g NDF and 172 g CP/kg DM. The cow had free access to water. The incubation procedure was undertaken for all cultivars at both harvest dates using the procedure described by Sun *et al*. (2010). Briefly, two hours after morning feeding, lingerie bags containing 18 Dacron bags (5 cm × 10 cm, with 50 µm pore size) were placed into the ventral rumen and removed after 2, 4, 7, 9, 12, 24, and 72 hours of incubation. The "0 hour" bags were not incubated. Bags were duplicated at each incubation time and triplicated at 0 and 72 hours. The bags were washed and then dried at 65°C for 48 hours to determine residual DM.

TABLE 1: "Grasslands" ryegrasses used for evaluation.

Cultivar	Type	Scientific name	Ploidy	Characteristics
Samson	Perennial	*Lolium perenne*	2	With AR37 endophyte, high yielding, good summer growth and quality, low aftermath heading.
Impact		*Lolium perenne*	2	Late flowering, out-of-season growth, creeping stem.
Coronet		*Lolium perenne*	2	Turf ryegrass.
Supreme	Hybrid	*Lolium perenne* × *multiflorum*	2	Long rotation, fine-medium leaves.
Manawa		*Lolium perenne* × *multiflorum*	2	Erect growth, large wide-leafed tillers, persists for 6-8 years, good winter production.
Greenstone		*Lolium perenne* × (*perenne* x *multiflorum*)	4	Vigorously tillered, large tillers and erect growth.
Warrior	Italian	*Lolium multiflorum*	2	Extended spring/summer production, high winter growth, densely tillered, fine leaved.
Tama		*Lolium multiflorum*	4	Very high winter and early production.
Moata		*Lolium multiflorum*	4	Persists up to two years.

Source: www.agricom.co.nz; www.pi.csiro.au/ahpc/grasses/pdf/ryegrass_;
http:www3.meu.unimelb.edu.au%2fgrasslands%2fcultivar_search%2fFMPro%3f-db%3dcultivar_.fp5%26-format%3dsearch_results.htm%26-SortField%3dSpeciesCommonName%26-SortOrder%3dascend%26-SortField%3dCultivarName%26-max%3dall%26-findAll

Calculation of degradation kinetics

Degradation kinetics of dry matter were estimated from the time course of DM disappearance using the model of Ørskov and McDonald (1979) modified by López *et al*. (1999):

$$P = A + B\,(1 - e^{-k(t-L)})$$

where P is potential disappearance; A is the soluble fraction , being that proportion which can be washed out from incubation bags at 0 hours; B is the insoluble but degradable fraction; k is the fractional disappearance rate per hour of the degradable insoluble fraction; t is the incubation time (hours) and L is the lag time (hours). The parameters were estimated using the non-linear (NLIN) procedure of SAS (2003).

The effective degradability (E) was obtained from the following equation:

$$E = A + Bk/(k + k_p)$$

Where k_p is the fractional passage rate, assumed at 0.06/hour, based on a range from 0.041 to 0.067/hour in dairy cows fed fresh ryegrass (van Vuuren *et al*., 1993).

Statistical analyses

Forage chemical composition, leaf morphology and leaf shear strength data and degradation parameters were analysed using the ANOVA procedure of SAS (2003) with harvest date and cultivar, or harvest date and ryegrass type as experimental factors.

RESULTS

Chemical composition

The chemical composition of the evaluated ryegrasses is presented in Table 2.

The across harvest date mean concentrations of NDF were 468, 435 and 414 (g/kg DM) for PRG, HRG and IRG, respectively. The 13% difference

TABLE 2: Chemical composition of evaluated leafy ryegrasses (g/kg DM, unless indicated) estimated using near infrared reflectance spectroscopy. DM = Dry matter; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; CP = Crude protein; SSS = Soluble sugars and starch; ME = Metabolisable energy (MJ/kg DM); SED = Standard error of difference at P <0.05. June harvest cut on 3 May and harvested on 18 June 2007, August harvest cut 1 June and harvested on 2 August 2007.

Harvest date	Type	Cultivar	NDF	ADF	CP	SSS	Lipid	Ash	ME (MJ/kg DM)
June	Perennial	Samson	507	259	211	124	34	79	11.9
		Impact	521	274	223	94	29	75	11.2
		Coronet	576	302	204	86	32	65	9.9
		Mean	535	278	213	101	32	73	11.0
	Hybrid	Supreme	492	246	251	117	34	88	12.1
		Manawa	484	285	261	100	31	69	11.3
		Greenstone	468	232	253	128	38	89	12.6
		Mean	481	254	255	115	34	82	12.0
	Italian	Warrior	490	259	232	127	34	80	11.8
		Tama	423	222	290	108	38	90	13.0
		Moata	415	236	287	160	39	105	13.3
		Mean	443	239	270	132	37	92	12.7
		SED (Type)	27	19	16	17	2	9	0.7
August	Perennial	Samson	416	227	202	140	37	96	12.5
		Impact	399	210	233	125	42	105	12.6
		Coronet	390	230	239	122	43	107	12.0
		Mean	402	222	225	129	41	103	12.4
	Hybrid	Supreme	401	223	218	131	40	99	12.4
		Manawa	389	216	228	150	44	99	12.4
		Greenstone	381	213	213	147	41	97	12.8
		Mean	390	217	220	143	42	98	12.5
	Italian	Warrior	399	229	192	159	39	94	12.6
		Tama	375	212	180	202	37	84	13.3
		Moata	383	219	170	224	40	85	13.2
		Mean	386	220	181	195	39	88	13.0
		SED (Type)	10	7	11	17	2	4	0.3

between PRG and IRG was significant (P <0.05). Average NDF concentrations were higher (P <0.01) from all ryegrass cultivars harvested in June than August (486 *vs*. 392 g/kg DM, P <0.01). Acid detergent fibre (ADF) concentration in the three ryegrass types showed similar trends to NDF. All ryegrasses had similar CP contents in the August harvest (208 g/kg DM) (P >0.05), but in the June harvest PRG (213 g/kg DM) had a lower CP concentration than HRG (255 g/kg DM) and IRG (270 g/kg DM) (P <0.05). Soluble sugars and starch (SSS) concentration showed IRG (163 g/kg DM) had a higher concentration than PRG (115 g/kg DM) and HRG (129 g/kg DM) (P <0.01) and was lower in June (116 g/kg DM) than in August (156 g/kg DM). The metabolisable energy content (ME) was lower in June (11.9 MJ/kg DM) than in August (12.6 MJ/kg DM, P <0.05), and differed between ryegrass types (11.7, 12.3, 12.9 MJ/kg DM for PRG, HRG and IRG, respectively, P <0.05). There were no ryegrass type differences in lipid or ash concentrations (P >0.05).

Leaf morphology and shear strength

Leaf morphology and shear strength data, indicate the resistance to chewing are shown in (Table 3).

IRG leaves were longer by 22.6 cm, over both harvests, than PRG (14.2 cm) and HRG (15.4 cm) (P <0.01), but were similar for both harvest dates. Leaves of IRG cultivars were also wider (P <0.01) and thicker (P <0.05) than other types. The average leaf width was 5.74, 2.70 and 3.03 mm and the average leaf thickness 0.301, 0.233 and 0.240 mm for IRG, PRG and HRG, respectively. Consequently, cross sectional area was highest for IRG (1.82 mm^2) compared to 0.64 and 0.73 mm^2 for PRG and HRG, respectively. Leaf width and

TABLE 3: Leaf morphology and shear strength of evaluated ryegrasses. SED = Standard error of difference at P <0.05. June harvest cut on 3 May and harvested on 18 June 2007, August harvest cut 1 June and harvested on 2 August 2007.

Harvest date	Type	Cultivar	Leaf length (mm)	Leaf width (mm)	Leaf thickness (mm)	Cross sectional area (mm^2)	Shear force (kg/10 leaves)	Shear strength (kg/mm^2)
		Number of analyses per cultivar	3	9	9	9	3	1
June	Perennial	Samson	13.3	2.41	0.222	0.535	5.7	1.07
		Impact	19.3	2.43	0.217	0.526	5.4	1.03
		Coronet	10.7	1.90	0.205	0.389	3.3	0.85
		Mean	14.4	2.25	0.215	0.483	4.8	0.98
	Hybrid	Supreme	20.7	2.82	0.246	0.693	4.7	0.68
		Manawa	12.7	2.55	0.208	0.529	3.1	0.59
		Greenstone	19.3	2.90	0.264	0.766	4.8	0.63
		Mean	17.6	2.76	0.239	0.663	4.2	0.63
	Italian	Warrior	18.7	3.10	0.217	0.671	3.8	0.57
		Tama	30.3	8.01	0.354	2.836	4.3	0.15
		Moata	23.3	5.84	0.307	1.794	3.5	0.20
		Mean	24.1	5.65	0.293	1.767	3.9	0.31
		SED (Type)	2.0	0.42	0.016	0.195	0.5	
		SED (Cultivar)	4.0	1.17	0.036	0.515	0.8	0.12
August	Perennial	Samson	15.0	3.55	0.278	0.987	5.3	0.54
		Impact	15.5	3.15	0.239	0.752	5.6	0.74
		Coronet	11.2	2.80	0.237	0.662	4.9	0.74
		Mean	13.9	3.17	0.251	0.800	5.3	0.67
	Hybrid	Supreme	13.0	3.41	0.246	0.841	5.2	0.62
		Manawa	13.1	3.30	0.217	0.714	4.5	0.63
		Greenstone	13.7	3.23	0.260	0.840	4.3	0.51
		Mean	13.3	3.31	0.241	0.798	4.7	0.59
	Italian	Warrior	16.3	3.60	0.246	0.886	4.0	0.45
		Tama	25.7	7.65	0.341	2.608	5.5	0.21
		Moata	21.5	6.26	0.340	2.125	5.5	0.26
		Mean	21.2	5.84	0.309	1.873	5.0	0.31
		SED (Type)	0.7	0.31	0.017	0.005	0.3	
		SED (Cultivar)	2.5	0.99	0.030	0.428	0.5	0.09

thickness were not affected by harvest date. Shear force (kg/mm^2) was highest for PRG (0.83), least for IRG (0.31) and intermediate for HRG (0.61), (P <0.001).

In sacco degradation of DM

Dry matter distribution between soluble (*A*) and insoluble but degradable (*B*) fractions, and degradation kinetics differed greatly between ryegrass types and harvest dates (Table 4).

IRG had a significantly higher soluble "*A*" fraction (0.608) than PRG (0.443) and HRG (0.483) (P <0.01). Consequently the "*B*" fraction was smaller in IRG (0.360) than PRG (0.469) and HRG (0.455) (P <0.001). The indigestible "*C*" fractions were elevated in Coronet and Manawa harvested in June because of leaf senescence. Degradation rate tended to differ among ryegrass types (P = 0.06), with IRG degrading faster (0.196 h^{-1}) than HRG (0.176 h^{-1}) and PRG (0.150 h^{-1}). Harvest date did not affect degradation rate (P = 0.15).

DISCUSSION

Dairy cow transition at calving and the initiation of lactation involves major changes in physiology and nutritional requirements. The evaluation of ryegrass cultivars has indicated differences between ryegrasses that may have benefits for cows pre- and post-calving.

There were no significant differences in CP content among the three ryegrass types, averaging 227 g/kg DM, but NDF concentration was much higher in PRG than HRG and IRG, suggesting a higher structural carbohydrate and lower quality for PRG. Although non-structural carbohydrate content in pasture was lowest in winter (Machado *et al.*, 2005), SSS content was 163 g/kg DM in IRG and higher than for PRG and HRG. These structural and non-structural carbohydrate data support a higher ME (MJ/kg DM) for IRG (12.9) versus HRG (12.3) and PRG (11.7).

TABLE 4: Dry matter degradation kinetics parameters of evaluated ryegrasses. SED = Standard error of difference at P <0.05. June harvest cut on 3 May and harvested on 18 June 2007, August harvest cut 1 June and harvested on 2 August 2007.

Harvest date	Type	Cultivar	Degradation kinetics					
			Soluble fraction (%)	Insoluble degradable fraction (%)	Fractional disappearance rate (h^{-1})	Lag time (h)	Indigestible fraction (%)	Effective degradability (%)
June	Perennial	Samson	48.1	42.6	0.207	4.8	9.2	81.2
		Impact	45.9	44.8	0.134	3.4	9.2	76.9
		Coronet	28.1	48.9	0.095	3.3	23.0	58.0
		Mean	40.7	45.4	0.145	3.8	13.8	72.0
	Hybrid	Supreme	53.1	40.7	0.186	4.7	6.2	83.9
		Manawa	35.2	49.5	0.180	5.1	15.2	72.4
		Greenstone	51.9	43.0	0.200	4.7	5.1	85.0
		Mean	46.7	44.4	0.189	4.8	8.8	80.4
	Italian	Warrior	55.6	38.3	0.198	5.0	6.1	85.0
		Tama	68.4	29.0	0.214	3.7	2.6	91.1
		Moata	68.6	29.8	0.250	3.2	1.6	92.6
		Mean	64.2	32.4	0.221	4.0	3.4	89.6
		SED (Type)	7.8	3.6	0.030	0.6	4.7	7.0
August	Perennial	Samson	45.9	50.7	0.152	2.9	3.4	82.2
		Impact	49.0	48.0	0.157	2.8	2.9	83.8
		Coronet	48.7	46.1	0.157	3.0	5.2	82.0
		Mean	47.9	48.3	0.155	2.9	3.8	82.7
	Hybrid	Supreme	49.2	47.5	0.166	3.1	3.3	84.1
		Manawa	48.2	48.1	0.150	2.9	3.6	82.6
		Greenstone	51.9	44.5	0.170	2.8	3.6	84.8
		Mean	49.8	46.7	0.162	2.9	3.5	83.8
	Italian	Warrior	53.0	43.1	0.166	3.3	3.9	84.6
		Tama	60.0	38.0	0.144	2.6	2.1	86.8
		Moata	59.2	37.9	0.204	2.7	2.9	88.5
		Mean	57.4	39.7	0.171	2.9	3.0	86.6
		SED (Type)	2.2	2.0	0.015	0.2	0.7	1.2

Dairy farming utilises conserved pasture, usually with supplements, to sustain cows during winter for one to two months pre-calving, and at the commencement of lactation. Cows usually calve during late winter, well before peak pasture growth in spring, so that feed demand, which is highest about six weeks post-calving, coincides with maximum pasture growth (Holmes *et al*., 2002). This means the diet offered pre-calving may be a restricted mixture of autumn saved pasture, often with silage, but including brassicas and other crops. Immediately post-calving, a high proportion of autumn-saved pasture is fed, often with supplements (Holmes *et al*., 2002; Clark & Woodward, 2007). As the CP requirements four to six weeks pre-calving are only about 12% of the DM requirement (Holmes *et al*., 2002) the rumen will be relatively empty. After calving there is a two to three fold increase in demand for energy to sustain lactation. At this time dietary CP requirements will be about 20% of the DM requirement. A rapid transition is required to accommodate the increase in DM intake and absorbed nutrients.

Nutrient requirements of cows fed autumn saved pasture differ greatly pre- and post-calving. In an ideal situation, the diet will be altered to meet the specific requirements of each physiological state. The reticulo-rumen must also develop physically to accommodate the increased intake post-parturition. Feeding a high fibre ration pre-calving has on-going benefits during lactation (Beever, 2006), possibly because it stimulates chewing and development of rumen musculature enabling the cow to physically handle the increased post-calving intake. A pre-calving diet could have a high proportion of NDF such as PRG or straw (Drackley & Dann, 2005). On the other hand a post calving diet should be easily broken down, with high degradation rates and about 35% NDF (Waghorn *et al*., 2007) to avoid acidosis while maximising intake. Pasture CP should be high enough so that supplements with a lower CP content can be fed to provide additional energy. This data would suggest that the PRG ryegrass cultivars appear best suited to meet pre-calving requirements and IRG and HRG most suitable post-calving.

The suggestion that PRG be fed in pre-calving diets and IRG or HRG when cows are milking, is supported by differences in shear strength, proportions of DM in the insoluble "*B*" fraction and degradation rate. Shear strength is associated with ease of breakdown during chewing and rumination (Easton, 1989) and is related to feed intake and digestibility (MacKinnon *et al*., 1988). Variation in shear strength between cultivars has been demonstrated by Bryant *et al*. (2008). The current study suggested higher feed intakes with IRG compared to PRG, because the high proportion of soluble and immediately fermentable, DM in the "*A*" fraction in IRG (0.608) was complimented by the highest degradation rate (0.196 h^{-1}) of the insoluble "*B*" fraction, for the three types, confirming its suitability for high intakes by lactating cows.

The challenge faced by dairy farmers to maintain pasture quality and supply, is in part due to climatic variables, but also in part to changes in requirements associated with the cow's physiological state. Supplements are increasingly being used to fill pasture shortfalls. The range of supplements available has increased in response to increased dairy profitability. Best use of pasture and supplements requires knowledge of cow requirements, and the composition of available dietary inputs. Both chemical and physical attributes of dietary components affect cow health and nutrition. This is especially important during the transition to lactation. The data presented here indicate differences between ryegrass cultivars. This information can be used to optimise cow feeding over winter and early spring.

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