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Short communication. Effects of endophyte infection on dry matter yield, persistence and nutritive value of perennial ryegrass in Galicia (north-west Spain)

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Abstract

Dry matter yield, persistence and three parameters of nutritive value were determined in two perennial ryegrass (*Lolium perenne* L.) genotypes either infected with a lolitrem B-free fungal endophyte or not infected, over a four-year period at two different locations in north-west Spain. The endophyte did not influence the productivity or persistence of perennial ryegrass in the four years of study. Insufficiently stressful conditions could explain these results. The nutritive value of the plants was not influenced by endophyte infection, except for organic matter digestibility and water-soluble carbohydrates on some occasions ($P < 0.05$). The organic matter digestibility mean value (2 significant comparisons out of 8) was slightly higher in infected ryegrass (72.4 and 83.4%) than in ryegrass free of endophyte (70.3 and 82.6%). The water-soluble carbohydrates mean content (2 significant comparisons out of 8) was higher for endophyte-infected (18.5 and 26.3%) than for endophyte-free ryegrass (17.3 and 24.6%). These differences were small and are insufficient to recommend endophyte-infected perennial ryegrass for forage production in the study areas.

Key words: Forage, *Lolium perenne*, *Neotyphodium lolii*.

Resumen

Nota corta. Efectos de la infección con un hongo endofito sobre la producción de materia seca, persistencia y valor nutritivo de raigrás inglés en Galicia (noroeste de España)

En dos localidades de Galicia, durante un periodo de cuatro años, se determinaron la producción de materia seca, la persistencia y tres parámetros de valor nutritivo en dos genotipos de raigrás inglés (*Lolium perenne* L.), bien infectados con un hongo endofito que no produce el alcaloide lolitreno B o bien libres de endofito. El endofito no influyó en la producción de materia seca ni en la persistencia del raigrás inglés en los cuatro años de estudio. La falta de un estrés importante en las dos zonas podría explicar estos resultados. El endofito no influyó en el valor nutritivo del raigrás inglés, salvo ocasionalmente en el caso de la digestibilidad de la materia orgánica y el contenido en carbohidratos solubles ($P < 0,05$). La digestibilidad de la materia orgánica (dos comparaciones significativas de ocho) fue ligeramente superior en el raigrás infectado (medias, 72,4 y 83,4%) que en el raigrás libre de endofito (medias, 70,3 y 82,6%). El contenido en carbohidratos solubles (dos comparaciones significativas de ocho) fue también más alto en el raigrás infectado (medias, 18,5 y 26,3%) que en el raigrás sin endofito (medias, 17,3 y 24,6%). Estas diferencias fueron pequeñas y no permiten recomendar el uso de raigrás inglés infectado con endofitos para producción de forraje en las localidades del estudio.

Palabras clave: forraje, *Lolium perenne, Neotyphodium lolii.*

Neotyphodium endophytes are reported to enhance the pest and stress tolerance and growth of its hosts (Hill, 1994). The endophyte lives completely within the plant, does not produce fruiting structures in the host, and is apparently only transmitted by infected seed. The relationship between fungus and plant is regarded as mutualistic (Clay, 1988).

Under field conditions, it is expected that endophyte-infected (EI) cultivars will show a better performance than endophyte-free (EF) cultivars. However, field comparisons between EI and EF cultivars

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have shown contrasting results. In a cool moist location in New Zealand, *Neotyphodium* endophyte had no effect on perennial ryegrass (*Lolium perenne* L.) (Eerens *et al*., 1992). In France (Ravel *et al*., 1995) and Spain (Oliveira *et al*., 1997), the effects of endophytes on agronomic traits depended on environmental conditions: the dryer they are, the more important the beneficial effects of the endophytes. Hesse *et al*. (2003) showed that the environmental conditions in the plant's original habitat may influence the symbiotic interaction between plant and fungus, probably through natural selection. It seems that the beneficial effects of endophytes are the result of specific interactions between plant and endophyte genotypes, and environmental conditions. Generally, the EI/EF pairs consist of a plant genotype with its native endophyte and the same genotype deprived of the fungus by selection or physical treatment. With this material, it can be difficult to separate the effects of the genotype of the plant and fungus. Although the perennial ryegrass germplasms from northern Spain were found to be highly infected since of 54 perennial ryegrass accessions examined, 39 (72%) were infected (Oliveira and Castro, 1998), little is known about the effects of endophyte infection on the agronomic performance of this host in Galician conditions. Studies of the effects of endophyte infection on the nutritive value of perennial ryegrass and tall fescue (*Festuca arundinacea* Schreb) have concentrated primarily on endophyte alkaloids, due to their large impact on health and productivity of grazing livestock. Two important alkaloids are produced by the ryegrass endophyte: lolitrem B and ergovaline. Lolitrem B causes ryegrass staggers and ergovaline is one cause of heat stress in warm, humid environments (Bouton, 2000; Fletcher and Easton, 2000; Oliveira *et al*., 2003). Few other quality parameters (protein content, digestibility, fibre, minerals) that may be affected by endophyte infection have been assessed (Belesky and Malinowski, 2000; Hume *et al*., 2000). This paper reports dry matter yield, persistence and nutritive value for endophyte-infected (*Neotyphodium lolii*) and endophyte-free perennial ryegrass herbage as assessed by NIRS (near infrared reflectance spectroscopy). These characters were measured in a field experiment in two locations of Galicia region (north-west Spain) over a 4-year period.

Plant materials used in this experiment were obtained from a characterisation of different accessions of perennial ryegrass. One endophyte-infected plant producing ergovaline but no lolitrem B was vegetatively propagated and retested for infection (Latch and Christensen, 1985). Independent crossing of two endophyte-free plants with the naturally endophyte-infected plant producing ergovaline but not lolitrem B created the two endophyte-infected (EI) genotypes. These crossings were made in two isolated fields with a cereal barrier. The seeds of these new infected genotypes were collected only on the endophyte-infected parent plants. One hundred seeds of each endophyte-infected genotype were microscopically examined for the presence of endophyte (Hinton and Bacon, 1985). The percentage of infected seeds was 81 and 90% for each infected genotype. Endophyte-free genotypes (EF) were obtained from seeds produced by endophyte-free parent plants. One hundred seeds of the each endophyte-free genotype were microscopically examined for endophyte infection. Infection levels were 0% in both cases.

In November 1998, each treatment, EI and EF was sown at two locations in Galicia region (Spain): Mabegondo in La Coruña province (43º 14' 50" N, 8º 15' 45" W, altitude = 100 m), and Puebla de Brollón in the Lugo province (42º 35' 40" N, 7º 24' 31" W, altitude = 400 m). Mabegondo is located on a silt loam, and Puebla de Brollón on a loamy sand soil. Soil samples were taken in September 1998 to a depth of 15 cm and analysed by the Galician Agrarian Laboratory to determine initial soil status. The Puebla de Brollón location is considered to have a higher stress level than the Mabegondo location because it is characterised by a higher mean maximum temperature, higher summer water deficits, and lower mean minimum temperatures than Mabegondo (30-year mean data: 28.9ºC *vs*. 24.3ºC, –144 mm *vs*. –124 mm, and 1.6ºC *vs.* 3.4ºC, respectively).

As both locations are characterised by low maximum temperatures and evapotranspiration and a medium to high Turc index (26 and 24 for Mabegondo and Puebla de Brollón, respectively), it can be assumed that environmental conditions at these locations are suitable for *Lolium perenne*. The Turc index, based on several data characterising environmental conditions (climatic and edaphic), permits the potential forage production to be estimated at each location.

Each treatment was sown in small plots (6.5 m^2), at a rate of 2.5 g seed m^{-2}, in a randomised block design with four replicates at Mabegondo and five at Puebla de Brollón. Fertiliser (150 kg ha^{-1} each of P_2O_5 and K_2O) was applied at each location before sowing, and 50 kg ha^{-1} N as calcium ammonium nitrate (20.5% of

N and with the same proportion of NO_3^- and NH_4^+) were applied after each mowing. Herbage was cut to a height of 20-25 mm above ground level, several times a year depending on plant growth: six and four times in the first year, five and three times in the second year, five and three times in the third year and four and three times in the fourth year at Mabegondo and Puebla de Brollón, respectively. The herbage samples consisted of the whole top of the plants, i.e. leaf blades and sheaths including stems and inflorescences in the late spring cut, or leaves and sheaths in the other cuts. Two sub-samples of 100 g per treatment and harvest were cut and oven-dried at 80ºC for 17 h in a Unitherm oven and reweighed to determine the dry matter content.

A visual estimate of persistence was made on each plot at the end of spring in the fifth year after sowing. This trait was scored on a scale from 1 (percentage of ground area covered with perennial ryegrass inferior to 10%, poor persistence of the plants in the plot) to 9 (percentage of ground area covered with perennial ryegrass superior to 90%, very good persistence).

Insects that could damage perennial ryegrass were absent at both locations in every year of the study.

In each genotype the level of endophyte infection was checked after sowing at each location by collecting at least 50 tillers per genotype growing in the plots and examining them for the presence of endophyte using the method described by Latch and Christensen (1985) at the end of every four years.

The dried yield samples were ground to pass through a 1 mm screen in a «Christy and Norris» hammer mill. Near infrared reflectance (NIRS) spectra of each sample were collected on a NIRS monochromator spectrophotometer NIRSystem 6500, from Foss NIRSystem, Inc., Silver Spring, MD 20904, USA. A subset of samples (72 samples) was selected on the basis of Mahalanobis distances to develop calibration NIRS equations. Calibration samples were analysed by wet chemistry to determine organic matter (OM), water-soluble carbohydrates (WSC) using the anthrone method, crude protein (CP), estimated as N * 6.25, and organic matter digestibility (OMD). All the determinations were expressed as % of dry matter except for OMD as % of organic matter. Another subset of samples (73 samples) was selected at random to validate NIRS calibrations and remaining samples were analysed by NIRS. Determination coefficients (R^2) ranged from 0.98 for protein to 0.75 for OMD. Standard errors of calibration and cross-validation of CP were lower than those of WSC and OMD.

Dry matter yield and nutritive value parameters were analysed as a randomised complete-block design, separately for each location and year because the number of harvests in each set of the study was unbalanced. Analysis of variance included the time of observation (sampling dates analysed as replicates over time) as an additional factor in the analysis, as described by Gómez and Gómez (1984). The endophyte status (endophyte-infected and endophyte-free genotypes) main effect was tested on the respective interaction with block, and the sampling date and endophyte status-sampling date interaction with the residual mean square error. Persistence data were analysed as a randomised complete-block design separately for each location with endophyte status as the main effect. The genotype effect was not included in the analysis because, previously, we checked that the two genotypes were similar for the characters studied. Analysis was performed by the GLM procedure of SAS (1989). Means were separated according to the Bonferroni T tests and considered to be significant at $P < 0.05$.

Soil results at Mabegondo showed a pH of 6.2, cation exchange capacity of 7.1 cmol(+)kg^{-1} and organic matter content of 5.7%. Potassium, Ca and Mg were present at levels of 0.2, 6.3 and 0.3 cmol(+)kg^{-1}, respectively. Olsen P content was 11 ppm, and ammonium acetate K content was 62 ppm. At Puebla de Brollón, soil had a pH of 5.1, cation exchange capacity of 5.6 cmol(+)kg^{-1} and organic matter content of 3.5%. Potassium, Ca and Mg were present at levels of 0.3, 4.2 and 0.4 cmol(+)kg^{-1}, respectively. Olsen P content was 101 ppm and ammonium acetate K content was 166 ppm.

The presence of endophyte did not affect ($P > 0.05$) dry matter yields in any of the four years following sowing (Tables 1 and 2), or persistence recorded at the end of the spring in the fifth year after sowing (EI = 6.8, EF = 6.4, SEM = 0.39 at Mabegondo and EI = 3.5, EF = 2.9, SEM = 0.35 at Puebla de Brollón).

Endophyte status did not affect crude protein content ($P > 0.05$) in any comparisons, whereas there were significant differences ($P < 0.05$), in organic matter digestibility and in water-soluble carbohydrates for some comparisons of endophyte-infected *versus* endophyte-free (Tables 1 and 2). In two comparisons out of eight, organic matter digestibility was significantly higher in infected ryegrass (means, 72.4 and 83.4%) than in ryegrass free of endophyte (means, 70.3 and 82.6%). Water-soluble carbohydrates (two significant comparisons out of eight) were higher for endophyte-

Table 1. Mean values for annual dry matter yields (DM) and nutritive values (NIRS-CP = NIRS predicted crude protein, NIRS-WSC = NIRS predicted water-soluble carbohydrates, NIRS-OMD = NIRS predicted organic matter digestibility) of two endophyte-infected (EI) and endophyte-free (EF) perennial ryegrass genotypes at Mabegondo (A Coruña)

Year	Endophyte	DM (Mg ha^{-1})	NIRS-CP (% DM)	NIRS-WSC (% DM)	NIRS-OMD (% organic matter)
1999	EI	13.9	14.5	17.7	74.9
	EF	13.6	14.7	17.4	74.9
	SEM	0.38	0.15	0.28	0.24
2000	EI	12.3	16.0	16.6	75.4
	EF	11.9	15.9	16.2	75.1
	SEM	0.31	0.22	0.14	0.17
2001	EI	11.4	13.0	20.8	76.9
	EF	10.6	13.5	20.1	74.7
	SEM	0.23	0.22	0.57	0.72
2002	EI	9.8	15.8	18.5a	72.4a
	EF	9.4	15.8	17.3b	70.3b
	SEM	0.30	0.07	0.24	0.23

Means followed by different letters in the column were significantly different at the 0.05 level according to Bonferroni T tests. SEM is the standard error of means.

infected (means, 18.5 and 26.3%) than for endophyte-free ryegrass (means, 17.3 and 24.6%).

It is frequently admitted that endophyte-infected perennial ryegrass is better adapted than uninfected perennial ryegrass to biotic (Latch, 1993) and abiotic stresses, including drought (Bacon, 1993). However, it is not always accepted that the endophytes of perennial ryegrass can improve the yield of their hosts (Ravel *et al*., 1995; Oliveira *et al*., 1997; Ravel *et al*., 1999). In the present study, endophyte infection did not influence the productivity or the persistence of perennial ryegrass over a 4-year period. According to several authors (Lewis, 1990; Eerens *et al*., 1992; Ravel *et al*., 1999), insufficiently stressful conditions could explain these results.

Perennial ryegrass is susceptible to drought and high temperature. As Mabegondo is characterised by a lower maximum temperature and evapotranspiration and a

Table 2. Mean values for annual dry matter yields (DM), and nutritive values (NIRS-CP = NIRS predicted crude protein, NIRS-WSC = NIRS predicted water-soluble carbohydrates, NIRS-OMD = NIRS predicted organic matter digestibility) of two endophyte-infected (EI) and endophyte-free (EF) perennial ryegrass genotypes at Puebla de Brollón (Lugo)

Year	Endophyte	DM (Mg ha^{-1})	NIRS-CP (% DM)	NIRS-WSC (% DM)	NIRS-OMD (% organic matter)
1999	EI	12.9	13.1	26.3a	85.6
	EF	13.2	13.5	24.6b	84.9
	SEM	0.18	0.18	0.42	0.21
2000	EI	5.6	10.5	24.3	80.9
	EF	5.0	10.7	24.2	80.4
	SEM	0.25	0.12	0.41	0.27
2001	EI	8.8	14.0	21.5	83.4a
	EF	8.7	14.1	21.7	82.6b
	SEM	0.24	0.31	0.47	4.44
2002	EI	7.7	15.9	15.1	76.5
	EF	7.6	15.6	15.4	76.4
	SEM	0.27	0.22	0.42	0.31

Means followed by different letters in the column were significantly different at the 0.05 level according to Bonferroni T tests. SEM is the standard error of means.

higher Turc index than the other location, it can be assumed that the environmental conditions at this location are suitable for this species. Puebla de Brollón is characterised by more stressful climatic conditions (high evapotranspiration), which are increased by soil characteristics (water stress was increased at Puebla de Brollón by light soils while it was decreased at Mabegondo by deep soils). The lower value of the Turc index at Puebla de Brollón than at Mabegondo confirms that that location can be considered as more stressful than Mabegondo, but not enough for endophyte infection to affect perennial ryegrass persistence. Thus, these locations can be characterised by negligible levels of stresses (insect predation or drought) and under these environmental conditions, the lolitrem B-free fungal endophyte did not influence the persistence of the two perennial ryegrass genotypes.

The nutritive value of forages is based on their nutritional level (energy, protein, etc.) and their availability to the digestive system of the animal. Small increases in digestibility are expected to have a significant impact on forage quality and animal productivity. A 1% increase in *in vitro* dry matter digestibility has led to an average 3.2% increase in mean live-weight gains (Casler and Vogel, 1999).

Endophyte infection only mildly affected organic matter digestibility (2.1% at Mabegondo and 0.8% at Puebla de Brollón) and water-soluble carbohydrates (1.2% at Mabegondo and 1.7% at Puebla de Brollón). Plant age within each forage class have a greater impact on digestibility than endophyte differences (Stone, 1994). It is known that dry matter digestibility of forage plants declines by more than 10% as the plant flowers and senesces.

Laboratory analyses of symbiotic and non-symbiotic perennial ryegrass indicate that they are identical in terms of crude protein, fibre components, dietary cation anion difference, or digestibility in normal environmental field conditions (Hume *et al*., 2000). Negative effects on animal performance, as reduced weight gain, lower milk production, lower fat and protein contents of the milk, etc., may result from the presence of endophytes in grasses (Prestidge, 1993).

On the basis of our results, we cannot recommend the use of endophyte-infected plants for forage production in the study areas due to the minimal positive effects obtained, and to the possible negative effects on animal performance due to the production of alkaloids. However, it would be interesting to study possible effects of the endophyte on increasing stand persistence of endophyte-infected turfgrasses in marginal areas of adaptation and their resistance to certain pests due to alkaloid production.

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