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Ensilability Characteristics and Silage Fermentation of Galega (*Galega officinalis* L.)

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Abstract: Galega (*Galega officinalis* L.) is one of the least investigated perennial leguminous herbs. It is usually present in natural pastures, grazed by animals, in Mediterranean areas and in the Italian Alps and Apennines below 1000 m. The aim of this study was to investigate the effect of the stage of maturity on the ensilability characteristics and to evaluate the fermentation quality of silage produced from first cut and regrowth of *G. officinalis* in the Po valley, northern Italy. The growth of *G. officinalis* was characterised by a rapid Dry Matter (DM) accumulation in the herbage during development and the DM content increased from 111-157 g kg^{-1} Fresh Matter (FM), while at the regrowth stage it was 180 g kg^{-1} FM. No significant changes were observed in the pH or Water Soluble Carbohydrates (WSC) content. The Buffering Capacity (BC) was lower at the regrowth than at the other three first cut stages, while Total Nitrogen (TN) and Soluble Nitrogen (SN) were low at the budding stage. Three types of silage without additives, wilted silage 1 day after wilting at the budding stage, wilted silage 2 days after wilting at the budding stage and wilted silage 1 day after wilting at the regrowth stage were prepared to investigate the effects of wilting on the chemical composition and characterstics of silage fermentation. The results of ensiling indicate that the fermentation of pure *G. officinalis* is characterized by the presence of alcohol and acetic acid and a lack of lactic acid and butyric acid. The good results obtained in lab-scale silos would seem to suggest that *G. officinalis* has the potential for large scale ensiling, if galega is harvested at the budding stage or during regrowth and then wilted to a DM level of >350 g kg^{-1} FM.

Key words: *Galega officinalis*, silage, water soluble carbohydrates, conservation quality, chemical composition

INTRODUCTION

Forage legumes play an important role in low-cost and sustainable agriculture because of their ability to fix nitrogen and their potential high feeding value for ruminants. Ensiling is the preferable method for preservation of green fodder, as its main advantages are the retention of the biological properties of green plants and the increase in the nutrient value of the finished silage (McDonald *et al*., 1991).

Galega (*Galega officinalis* L.) belongs to the group of legume crops. Legumes have a high nutritive value, but they are known to be difficult to ensile and often result in poorly fermented direct cut silage. This is mainly due to their high Buffering Capacity (BC) and low Water Soluble Carbohydrates (WSC) concentration (McDonald *et al*., 1991). Compared to other legumes, *G. officinalis* has even lower WSC concentrations and higher BC and ensiling is particularly problematical at direct cut. Furthermore, the conservation quality of galega silage could be positively influenced by the stage of development and Dry Matter (DM) content at ensiling.

The chemical composition and fermentation of legumes could be improved ensiling them in mixtures with graminaceous plants, for instance fodder galega (*Galega orientalis* Lam.), which is an excellent quality forage for all kinds of livestock and poultry (Baleþentienë *et al*., 2003), was used in fodder galega-grass mixtures to improve the fermentation properties of the silage material since the addition of grasses has solved the *G. orientalis* deficiency of WSC. Literature reports indicate that a satisfactory fermentation can be achieved both in different fodder galega-grass mixture silages (Lättemäe *et al*., 2005) and in pure *G. orientalis* with the use of an efficient additive that can considerably reduce clostridial fermentation and losses (Raig *et al*., 2001). The possibility of ensiling early flowering stage *G. orientalis* in mixtures with orchardgrass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), milk stage maize and sugar beet leaves was studied at Lithuanian University of Agriculture (Baleþentienë and Mikulioniené, 2006). This research showed that the quality of pure treated crop silages was low because of the dissbalanced rations of protein and WSC and the authors concluded that *G. orientalis* is suitable for ensiling with a grass component that has accumulated no <30% DM. The lowest losses (7.8%) of feed matter were determined in fodder galega-orchardgrass (1:1) silage (Baleþentienë and Mikulioniené, 2006).

Lättemäe *et al*. (2005) investigated the influence of different fodder galega-grass mixtures and the use of additives on fermentation quality and DM losses. Lactic fermentation of poorly ensilable *G. orientalis* was performed by introducing rifampin-resistant homofermenting representatives of the genus *Lactobacillus* (Shurkhno *et al*., 2005).

Root and Syrjälä-Quist (1993) showed that fodder galega can also be used for silage and but recommended that *G. orientalis* should be mixed with grass in swards for silage.

Only limited information is available on the chemical composition of *G. officinalis* during its growth cycle (Peiretti and Gai, 2006). At present, no data are available concerning the ensilability characteristics and silage fermentation of *G. officinalis* during its growth cycle.

The aim of this study was to determine the chemical composition and ensilability characteristics of *G. officinalis* at cutting during its first growth cycle at progressive morphological stages from the vegetative to the budding stage and in regrowth. The tendency of galega to be conserved as silage has been studied in crops harvested at the 2 wilted budding stage levels and at the regrowth stage.

MATERIALS AND METHODS

Crop and herbage sample preparation: The trials was carried out in 2004 in the western Po Valley. Stands of *G. officinalis* L. were seeded in the spring and no irrigation or fertiliser were applied after sowing. Herbage samples were collected with edging shears (0.1 m cutting width) at 3 progressive morphological stages from vegetative to budding, on subplots of 2 m^2 randomly located in plots of 4×10 m^2 with 3 replicates (each with 4 samples) cut to a 1-2 cm stubble height. Sampling ranged from June-July 2004 with a regrowth of *G. officinalis* also collected at the late vegetative stage in September 2004. Sampling for chemical analysis was not attempted on rainy days and only occurred in the morning after the disappearance of dew.

Ensiling and silage sample preparation: The wilting levels that were expected to be reached to allow ensiling in the same day as cutting was 360 g kg^{-1} for the budding stage and regrowth, or at least the day after, was 420 g kg^{-1} for the budding stage. The wilting levels were chosen as they include the range of DM content that is compatible with bunker silage under normal farm conditions. Weather conditions during the wilting and the harvesting of the herbage were always favourable for field drying and no rain fell during the drying period. The wilted herbage was chopped with a paper slicer to a length of 1-2 cm and ensiled in sterile 2 L laboratory glass silos equipped with a lid that only enables gas release. Two replications were performed at each DM level for the 2 budding stage wilting levels and for the regrowth, for a total of 6 laboratory glass silos. All the silages were preserved by spontaneous fermentation and stored at 10±2°C in a dark room for 180 days for the silages at the budding stage and 120 days for the silages at regrowth.

Crop and silage analysis: The herbage and silage samples were immediately analysed for DM content using a forced-draught oven (90°C) until constant weight was attained and for the Total N content (TN) according to the Dumas method, using a nitrogen analyser macro-N (Foss Heraeus Analysensysteme, Hanau, Germany). Wet samples were chopped and immediately frozen and kept at -30°C for qualitative analyses.

The frozen herbage was homogenised with water at 20°C for 5 min in a Lab Blender Stomacher 400 (Seward Lab. London) and filtered using Whatman 41 filter paper. The aqueous extracts were analysed to determine: the WSC content using the anthrone colorimetric method (Deriaz, 1961), the BC according to Playne and McDonald (1966), the pH using a pH-meter Crison Basic (Crison Instruments, SA, ES) and the Soluble N content (SN) according to the Kjeldahl method (AOAC, 1990).

The gross energy of the silages was measured with an adiabatic calorimeter bomb (IKA C7000, Staufen-Germany) according to Meineri and Peiretti (2005).

The volatile fatty acids, lactic acid and alcohol concentrations were determined on acid silage extracts: samples of chopped frozen silage were weighed (50 g) in a 400 mL poly-ethylene bag and extracted with 200 mL of 0.1 N H_2SO_4 at 20°C for 4 min in a Lab-blender Stomacher 400. The mixture was centrifuged for 5 min at 3000×g and then filtered through a Schleicher and Schull membrane filter (BA-83, 0.2 μm).

A 1 μL aliquot of the acid extracts was injected, using an on-column technique with an auto-sampler (Dani Instruments SpA, ALS 1000, Cologno Monzese, Italy), into a wide-bore capillary column (SGE BP21 2 5 m×0.53 mm internal diameter and 0.5 μm film thickness; P/N 054474, SGE International, Ringwood, Victoria, Australia) installed in a gas chromatograph (Dani GC 1000 DPC), running in a temperature-programmed mode and equipped with a flame ionization detector and a PTV

injection port, used in the split mode, with a split vent flow of 100 mL min^{-1}. The injector and detector ports were set at 230 and 240°C, respectively; helium was used as the carrier gas and the oven temperature was programmed from 60-200°C at 5°C per min and held for 2 min giving a run time of 30 min. The peak area was measured using a Dani Data Station DDS 1000. Each peak was identified and quantified according to pure standards (Sigma Chemical, St.Louis, MO, USA).

Statistical analysis: The chemical compositional data of herbages and silages were analysed for their statistical significance by one-way Analysis of Variance (ANOVA), using the Statistical Package for Social Science, v. 11.5 (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

The characteristics of the herbage quality at cutting of the 3 different stages of development and at regrowth are reported in Table 1. The growth of *G. officinalis* was characterised by a rapid DM accumulation in the herbage during development and the DM content increased from 111-157 g kg^{-1} FM, while at the regrowth it was 180 g kg^{-1} FM. The high moisture content at cutting in the early stages (vegetative and shooting) prolonged the field wilting, making the drying process difficult. Tamm *et al.* (1997) concluded that *G. orientalis* herbage cut at the budding stage is a good material for high-quality silage. Oshita *et al.* (2002) reported that, like alfalfa, suppression of clostridia activity and improvement in fermentation quality of silage are possible for *G. orientalis*, if fodder galega is wilted to a DM levels of >50%. Baleþentienë and Mikulioniené (2006) ensiled fresh masses of *G. orientalis* at the budding-early flowering stage, because, according to these authors, the forage reached an optimal amount of DM and of nutritious materials at this stage, even though the DM content of galega at ensiling was 221 g kg^{-1} FM, which is not sufficient to produce the best quality of silage as reported by Jeroch *et al.* (1999).

No significant changes were observed in the pH or WSC content. The sugar substrate, which ranged from 74-82 g kg^{-1} DM, could allow a good conservation quality of wilted silages. The Buffering Capacity (BC) was lower at the regrowth than at the other three first cut stages, while the Total Nitrogen (TN) and Soluble Nitrogen (SN) were low at the budding stage. Due to the high concentration of total nitrogen (from 20.2-33.5 g kg^{-1} DM) and some amino acids, such as aspartic and glutamic acids (Peiretti and Gai, 2006), *G. officinalis* fresh mass could be used in part as a partial substitute for soybean cakes, as was also reported for *G. orientalis* (Baleþentienë and Mikulioniené, 2006).

The fermentation characteristics of the galega silages at the budding stage and at regrowth are reported in Table 2. The wilting level affected silage fermentation both at the budding stage and at regrowth by restricting the microflora activity without lactic acid

Table 1: Galega herbage: chemical composition and ensilability characteristics at cutting at 3 morphological stages of growth and at regrowth

Stage (Cutting date)	Vegetative (26/06/04)	Shooting (03/07/04)	Budding (18/07/04)	Regrowth (18/09/04)	SEM	Stage effect
DM (g kg^{-1})	111.0	119.0	157.0	180.0	8.70	***
pH	5.7	5.7	5.7	5.6	0.02	ns
WSC (g kg^{-1} DM)	76.4	73.9	77.4	81.8	1.30	ns
BC (meq kg^{-1} DM)	511.0	541.0	463.0	367.0	21.00	***
TN (g kg^{-1} DM)	31.6	25.2	20.2	33.5	1.60	***
SN (g kg^{-1} DM)	5.9	5.8	4.8	5.4	0.15	*

*Significant response at a 0.05 probability level. ***Significant response at a 0.001 probability level

Table 2: Galega silages: chemical composition at 2 budding stage wilted levels and at wilted regrowth

Stage (Ensiling date, Ensiling days)	Budding (21/07/04) 180	Budding (22/07/04) 180	Regrowth (18/09/04) 120	SEM	Wilting effect
DM (g kg^{-1})	363	422	355	14.0	*
pH	5.5	5.4	5.5	0.1	ns[1]
TN (g kg^{-1} DM)	23.6	25.8	34.0	2.0	***
Gross energy (MJ kg^{-1} DM)	19.0	19.2	20.2	0.3	ns
Methanol (g kg^{-1} DM)	0.21	0.15	0.01	0.04	**
Ethanol (g kg^{-1} DM)	0.68	0.88	1.43	0.19	ns
Lactic acid (g kg^{-1} DM)	nd[2]	nd	0.78	-	-
Acetic acid (g kg^{-1} DM)	0.85	1.03	1.10	0.05	ns
Propionic acid (g kg^{-1} DM)	0.34	0.10	0.15	0.05	**
Butyric acid (g kg^{-1} DM)	nd	nd	nd	-	-
Isobutyric acid (g kg^{-1} DM)	0.96	0.30	0.38	0.13	**

[1]ns: not significant, [2]nd: not detected, *Significant response at a 0.05 probability level. **Significant response at a 0.01 probability level. ***Significant response at a 0.001 probability level

fermentation or differences in pH. All the silages were well preserved and were dominated by an alcohol and acetic acid fermentation with the absence of butyric acid. Lactic acid was only present in small quantities in the regrowth silages, which presented a higher total nitrogen and gross energy content than the other silages. Ensilaging plants that are protein-rich, but with a low WSC content, did not produce a sufficient amount of lactic acid and the acetic acid content increased, as reported by Baleþentienë and Mikulionienė (2006) for fodder galega.

Tamm *et al*. (1997) comparing the ensiling potential of fodder galega herbage harvested at the budding stage with red clover of the same stage, found that the silage quality was high for both species and that the silages did not contain butyric acid. The silages instead showed a good lactic acid fermentation since an additive was used in the treatment of the herbages at ensiling.

Baleþentienë and Mikulionienė (2006), ensiling fodder galega at a DM level of 270 g kg^{-1} FM and with or without a conservant, found that treated silage was characterised by a higher lactic acid fermentation than untreated silage, which was high in a butyric and acetic acid content.

Root and Syrjälä-Quist (1993) showed that *G. orientalis* could be used as raw material for silage, if harvested just before flowering during the primary growth stage and ensiled in clamp silos after treatment with formic acid at a DM level of 164 g kg^{-1} FM. Fodder galega treated silages presented lower lactic, acetic and butyric acid contents than the grass treated silages ensiled at a similar DM content.

Oshita *et al*. (2002) investigated the effects of wilting on the chemical composition and characteristics of silage fermentation of full-bloom fodder galega and alfalfa harvested on the same day. The quality of direct-cut silage with 0.5% formic acid and wilted silage with no additives 1 day after wilting of either alfalfa and fodder galega was characterised by high pH and high concentrations of butyric acid. The quality of wilted silage of both alfalfa and fodder galega with no additive 3 days after wilting was good, although the pH was high because of the restricted fermentation which derived from the high DM level at ensiling (488 g kg^{-1} FM).

CONCLUSION

The good results obtained in lab-scale silos would seem to suggest that galega has the potential for large scale ensiling if *G. officinalis* is harvested at the budding stage or during regrowth and wilted to a DM level of >350 g kg^{-1} FM. Further research is required to define the conservation quality in farm scale silos and animal performance.

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