SUMMARY

Forage legumes have two advantages over other forage plants. They are high in protein and require little or no nitrogen fertiliser. However, there are also other benefits that some legumes can offer when fed to ruminant animals. Sainfoin and birdsfoot trefoil contain 'bioactive' tannin constituents that can i) ensure better protein use, ii) reduce methane gas emissions, iii) control parasitic worms (i.e. gastro-intestinal nematodes) and iv) improve the quality of ruminant-derived foods. Red clover contains polyphenol oxidase, which can also lead to better use of dietary protein by ruminants. LegumePlus has carried out ground-breaking research in all of these areas.

This report describes the key results of the LegumePlus project. It summarises the contributions from 17 fellows, 10 full partners, 5 associated partners from 6 European countries and two visiting researchers from New Zealand and the USA.

Project objectives

LegumePlus provided training for 15 Early Stage Researchers and 2 Experienced Researchers in professional skills in the context of research on bioactive legumes. Its objectives and contributions covered the mechanisms of bioactive constituents in forage legumes in order to

- Achieve better protein use efficiency (**Objective 1**)
- Reduce methane gas production (**Objective 2**)
- Enhance the quality of milk and meat products (**Objective 3**)
- Inhibit parasitic worms (**Objective 4**)
- Integrate and generate knowledge to improve selected European legumes (**Objective 5**).

Description of the work and the main results

The fellows' research progressed extremely well and they made many exciting discoveries. Fields containing sainfoin, birdsfoot trefoil and red clover were used for agronomic evaluations, molecular marker developments, laboratory studies and animal feeding trials.

In **Workpackage 1,** fields with three forage legumes, sainfoin, birdsfoot trefoil and red clover provided material for ensiling studies and for animal feeding trials. Silages were characterised by good fermentation quality and feeding trials revealed that sainfoin tannins could increase the proportions of beneficial 18:3n-3 and other polyunsaturated fatty acids in dairy cow milk and in Gruyere cheese. The intramuscular fat of sainfoin- and birdsfoot trefoil-fed lambs also contained less saturated and more of the beneficial polyunsaturated fatty acids; however growth performance was lower than with lucerne or red clover silages. Importantly, these animals also generated lower methane and urinary nitrogen emissions compared to standard control diets. Laboratory analysis revealed that ensiling and digestion increased tightly bound tannins, but lowered their reactivity and total contents. This might explain why parasitic nematodes in the intestine are especially difficult to control (see below). Of particular interest were the findings that sainfoin silage reduced metabolic stress, increased milk yield and shifted dairy cow metabolism towards synthesis of protein rather than body fat. Both sainfoin and red clover improved the synchronisation of nutrient use in the rumen in comparison to conventional diets.

In **Workpackage 2,** sainfoin pellets from MG2Mix, France, were tested against parasitic nematodes in cattle and lambs. The bioactive tannins reduced worm numbers, faecal egg counts and improved host resilience. In addition, the *in vitro* effects of a wide range of tannins (from Workpackage 3) were tested against *Ostertagia ostertagi, Cooperia oncophora, Haemonchus contortus* and *Trichostrongylus colubriformis* larvae. This established structure-function relationships for condensed tannins and for ellagitannins. Interestingly, synergistic effects were recorded for the first time between some tannins and flavonoid monomers, which is useful information for plant breeders. It is also of note that tannins showed a different mode of action to that of a conventional anthelmintic de-worming drug.

In **Workpackage 3,** fellows developed several tannin analysis methods based on thiolysis, mass spectrometry, nuclear magnetic resonance and near infrared spectroscopy in order to improve the detection and quantitation of tannins in these legumes. Analysis of >800 samples revealed that tannins varied greatly

between sainfoin accessions and that leaves had more tannins and prodelphinidins than stems. Pelleting and especially ensiling increased the proportion of bound tannins, which may impact on the utilisation of dietary protein. Levels of bound tannins varied along the digestive tract and could explain differences in activity against abomasal and intestinal parasites. Although, tannin structures did not change along the digestive tract, there were large tannin losses.

Workpackage 4 found that chicory was best as a companion species in the UK for sainfoin establishment but needed controlling in subsequent years. In Switzerland, *Lolium perenne* and *Festuca pratensis* were optimal companions for reducing weed and for increasing total stand yields. It could be shown that past breeding efforts achieved better sainfoin yields but lowered tannin concentrations. However, it could also be shown that it will be possible to breed new varieties with higher yields and with higher tannin contents. A particularly interesting finding was that two sainfoin accessions had higher tannin synthesis under drought conditions. Both of these accessions were also the only accessions, which did not lose biomass under drought.

Marker-trait associations were found in one population for plant height, flowering time and seed yield parameters. The SSR markers showed a high degree of polymorphism and the genetic differences separated sainfoin individuals into two groups based on geographic origins. These SSR markers can now be used for pedigree analysis in breeding programmes. Genomics analysis generated a sainfoin transcriptome library that allowed classification according to molecular function, location in the cell, and biological processes. This also provided quantitative information of expression levels of tannin biosynthesis genes. Over 75K SNPs are now available for genome wide association studies (GWAS) and for future genomics-assisted breeding programmes.

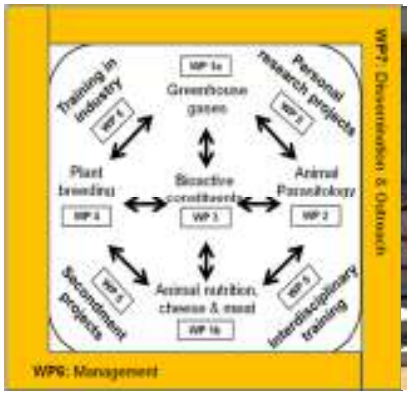
Potential impact and use

A French organic farmers' cooperative, Multifolia, in association with a feed pellet supplier, MG2Mix, have maintained close contact with our network throughout the EU HealthyHay and LegumePlus projects. The area sown by these farmers to sainfoin has increased rapidly ever since 2000 and now covers 500 ha. In the UK, there is also growing interest in forage legumes. The key reasons are that birdsfoot trefoil and especially sainfoin can be used to improve the quality of milk, cheese and meat and to lower damaging environmental nitrogen and methane emissions from ruminant production. However, red clover silages achieved higher animal growth rates.

Scientific impact will come from the new analytical tools that were developed for the rapid screening of tannins in sainfoin improvement programmes. Structure-activity relationships were discovered between tannins and antiparasitic effects, which together with newly identified molecular markers can now be used for breeding new European sainfoin varieties with better nutritional and de-worming properties.

Socio-economic impact and the wider societal implications of the project

LegumePlus trained 17 young researchers in a multi-disciplinary project, provided European farmers with a Sainfoin Growers' Guide, stakeholders with information on nutritional and environmental benefits that can be obtained from feeding sainfoin, birdsfoot trefoil or red clover, generated NIRS calibrations for feed producers and novel techniques for researchers and plant breeders.



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LegumePlus
Marie Curie Initial Training Network