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Feeding clover seed to improve permanent grasslands

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Executive summary

Around three-quarters of Wales' agricultural land is classified as permanent pasture. The vast majority of these sown swards are now 30 - 50 years old, and over time the grasses and legumes originally planted have been replaced by unsown grass and other species, leading to a marked decline in both pasture and stock performance. The introduction of modern varieties of clover into such swards can lead to substantial improvements in both nutrient supply and nutrient use efficiency; improving growth rates and reducing time to finish. In turn this can reduce GHG emissions intensity at a time when the environmental costs of livestock production are under considerable scrutiny. An alternative approach to introducing legumes that was popular with some farmers historically was to feed clover seeds to sheep. Having passed through the digestive tract the seeds are deposited within faeces. The overall aim of this project was to evaluate the effectiveness of feeding red and white clover seed to grazing stock as a means of increasing the legume component of permanent grassland. An initial experiment aiming to isolate clover seeds from faeces following feeding failed and so a revised approach with seeds incubated in the rumen using the Dacron bag technique and subsequently tested for viability was adopted. Seventeen different varieties of red and white clover were tested. The majority of seeds of all varieties germinated within the rumen. The percentage of seeds showing evidence of continued growth was recorded after 10 d. For red clover, only seeds from the variety Sangria continued to grow. In contrast, more of the white clover seeds continued to grow; notably the varieties AberAce and AberDai and Riesling. Variability in germination, particularly of white clover seeds, appeared to be linked to seed colouring: white seeds readily germinated; yellow, orange and brown were slower; and dark brown seeds appeared impermeable. Further experimental work is planned to explore the extent to which emerging seedlings are damaged or lost as the seeds pass through the remainder of the gastro-intestinal tract. A third trial tested the approach of feeding clover seed in practice. Replicate small plots were fenced off and grazed by 4 sheep supplemented with sugar beet with or without clover seeds added. The seeds fed were a mixture of white and red clover seeds of different varieties. The intention was to carry out sward measurements at the start of the 2020 growing season to compare forage yield and the contribution of legumes to the overall sward competition in the different plots. However, lockdown restrictions put in place in the wake of the coronavirus outbreak have meant research has been on hold since March. The intention is to carry out the pasture measurements once restrictions are lifted.



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Introduction

The concept of sustainable intensification (defined as “producing more output from the same area of land while reducing the negative environmental impacts and at the same time increasing contributions to natural capital and the flow of environmental services”) has particular resonance for upland areas. Around three-quarters of Wales’ agricultural land is classified as permanent pasture. The vast majority of these sown swards are now 30 - 50 years old, and over time the grasses and legumes originally planted have been replaced by unsown grass and other species, leading to a marked decline in both pasture and stock performance. Since the species and varieties of grass originally used were heavily reliant upon substantial nutrient inputs to remain competitive, this process has been exacerbated by reductions in application rates of inorganic fertilisers in response to rising costs and environmental concerns. The introduction of modern varieties of clover into such swards can lead to substantial improvements in both nutrient supply and nutrient use efficiency; improving growth rates and reducing time to finish. In turn this will reduce GHG emissions intensity at a time when the environmental costs of livestock production are under considerable scrutiny. However, reseeding of permanent pastures is costly and risks soil and carbon loss. Over-seeding by direct drilling or broadcast sowing is more commonly undertaken today, but related costs can still be high and establishment rates variable.

An alternative approach to introducing legumes that was popular with some farmers in times gone by was to feed clover seeds to sheep. Having passed through the digestive tract the seeds are deposited within faeces. The faeces not only supply additional nutrients to the seeds as they germinate, they also offer some protection from grazing as the seedlings establish. If this approach were effective it could facilitate annual sward improvement (e.g. through seed as an ingredient in a feed block), giving a more stable sward composition. However, there is little information available about the relative success of this approach. Very few studies have been published in the scientific literature, and those that have relate to species of clover and growing conditions that are not common to the UK (Cocks et al., 1998; Cosyns et al., 2005; Lehrer & Tisdale, 1956; Fortune & Ru, 2001). They do, however, report that there can be considerable variation in recovery rates for different varieties of the same legume species.

The overall aim of this project was to evaluate the effectiveness of feeding red and white clover seed to grazing stock as a means of increasing the legume component of permanent grassland.

Methods

In total, three separate experiments were carried out, due to the first (planned) experiment failing.

Experiment 1: This was intended as an initial screening trial. Eight Welsh Mountain wether sheep were housed in individual pens and fed a standard basal diet of sugar beet pellets at maintenance (based on UK feed guidelines (AFRC) and including a 5% safety margin). Seed



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from eight different clover varieties were fed as a supplement (1 variety per sheep): 1) AberChianti (red clover), 2) Merviot (red clover), 3) AberClaret (red clover), 4) AberAce (white clover), 5) AberDai (white clover), 6) AberLasting (white clover), 7) AberSwan (white clover), and 8) Alice (white clover). A feeding rate of 14g/d was chosen, based on a broadcast seeding rate of 5 t/ha and an anticipated stocking rate in practice of 20 sheep/ha. Clover seed was added to the diet eight days before the collection period began to ensure complete passage by the time samples were taken. During a 3-day collection period the animals were housed on rubber mats and all faeces were collected twice daily. The faeces were weighed, bulked for each day, and stored at 4 °C prior to germination tests. Subsamples of 50 g of faeces were examined under a magnifying lens. The intention had been to identify and count the clover seeds present, but it proved extremely difficult to accurately identify the seeds. Given that this would undermine all confidence in the results obtained, the trial had to be abandoned.

Experiment 2: In an alternative approach to investigating the digestibility of different species and varieties of clover, seeds were tested for survival following incubation in the rumen using the Dacron bag technique. The varieties tested were:

- 1) AberChianti (red clover)
- 2) Merviot (red clover)
- 3) AberClaret (red clover)
- 4) AberAce (white clover)
- 5) AberDai (white clover)
- 6) AberLasting (white clover)
- 7) AberSwan (white clover)
- 8) Alice (white clover)
- 9) Aran (white clover)
- 10) Atlantis (red clover)
- 11) Iona (white clover)
- 12) Klondike (white clover)
- 13) Reisling (white clover)
- 14) Magellan (red clover)
- 15) Coolfin (white clover)
- 16) Amos (red clover)
- 17) Sangria (red clover)

The study used 5 rumen-fistulated cows fed on their standard dry cow winter forage mix, which consisted primarily of grass silage and straw. Replicate (n = 5) samples of 10 g of each variety of clover seed was weighed out and placed in Dacron bags with a pore size of 40 micron. All 17 varieties were then incubated in each animal (giving a total of 85 bags for the experiment). Following 24 h of incubation the bags were removed. Each bag was soaked in water for 5 min, then rinsed under a running tap for around 1 min, until any rumen liquor and



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forage particles had been removed. The bags were dried for 30 min at room temperature (approx. 20 °C), before 10 seeds were selected at random for germination testing. The remainder of the sample was dried at room temperature to constant weight without light with the dry weight of seed remaining recorded.

The germination tests were carried out using trays with 25 wells arranged in a 5 x 5 design. One plate was used for each variety. A total of 10 seeds were taken from each Dacron bag and plated out in 5 wells (2 seeds per well). Each row of 5 wells corresponded to one cow. In total 50 seeds per variety were plated out. Approximately 1 ml of tap water was then added. Once prepared the plates were covered to reduce evaporation and placed in a location where they received around 10 h of light per day at a temperature of approx. 20 °C. The number of germinated seeds after 3 days was recorded. This process was then repeated after 7 and 10 days. The number of seeds showing evidence of further growth (i.e. lengthening of the radicle and emergence of the plumule) was also recorded at this time.

Experiment 3: A field trial was set up in autumn 2019 to test the approach of feeding clover seed in practice. An area was prepared by topping and mob grazing for 7 d to minimise existing grassland cover and expose any bare ground. Replicate 12 x 7 plots (n = 3 per treatment) were fenced off and each grazed by 4 sheep supplemented with sugar beet either with or without clover seeds added. The feeding rate of the clover was calculated to be equivalent to the standard overseeding rate of 5 kg/ha. The seeds fed were a mixture of white and red clover seeds of different varieties. The animals were fed and grazed on the plots for a total of 7 days. The intention was to carry out sward measurements at the start of the 2020 growing season to compare forage yield and the contribution of legumes to the overall sward competition in the different plots. However, lockdown restrictions put in place in the wake of the coronavirus outbreak have meant research has been on hold since March. The plots have been maintained by short periods of mob grazing to keep the pasture in a vegetative state and reduce the risk of the clover component being outcompeted whilst avoiding prolonged selective grazing, and the intention is to carry out the pasture measurements once restrictions are lifted.

Results / Discussion

Experiment 1: Sugar beet pellets were chosen as the basal diet for Experiment 1 (rather than grass, hay or silage) to minimise the risk of contamination of the faecal samples by seeds other than the clover seeds fed. However, it is possible that it would have been easier to identify seeds in the faeces had the sheep been fed a more fibrous, forage-based diet. The study that the methodology was based on had tested weed seed transmission through goats, and the plant species involved had larger seeds.

Experiment 2: When the Dacron bags were removed after 24 h it was found the vast majority of seeds of varieties of both white and red clover had already started to germinate within the rumen (exact conditions not known but the temperature would be expected to be 30 – 37 °C with constant moisture available) (Table 1), and in most cases there was little difference in



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germination percentage after 10 d compared to 3 d post removal and plating out.

Table 1: Germination rates of different varieties of white and red clover following incubation within the rumen for 24 h, plus percentage showing continued growth after 10 d.

	Percentage of seeds germinated			Percentage of seeds growing
	After 3 days	After 7 days	After 10 days	
<i>Trifolium pratense</i>				
AberChianti	82	85	85	0
AberClaret	89	91	91	0
Amos	93	94	94	0
Atlantis	92	92	92	0
Magellan	95	95	95	0
Merviot	77	80	80	0
Sangria	83	83	83	22
<i>Trifolium repens</i>				
AberAce	98	98	98	60
AberDai	96	98	98	46
AberLasting	90	91	91	10
AberSwan	92	94	94	18
Alice	90	90	90	0
Aran	96	98	98	6
Coolfin	66	66	66	14
Iona	76	76	76	30
Klondike	91	91	91	10

In a simple test to explore this finding further, seeds were incubated in plain water within an oven at 37 °C. No germination occurred until the seeds were plated out on a lab bench at 20 °C, suggesting that it was the presence of nutrients within rumen liquor which triggered germination.

As well as noting germination rates for the seeds that had been incubated in the rumen, the percentage of seeds showing evidence of continued growth was recorded after 10 d (Table 1). Although the majority of the red clover seeds had germinated, only seeds from the variety Sangria continued to grow. In contrast, more of the white clover seeds continued to grow; notably AberAce and AberDai and Riesling. Variability in germination, particularly of white clover seeds, appeared to be linked to seed colouring: white seeds readily germinated; yellow, orange and brown were slower; and dark brown seeds appeared impermeable (Fig 1). While

it is likely that the process of being handled, washed and plated will have had an impact on seed viability to some extent, it seems there are substantial between-variety differences in response to exposure to the rumen environment.



Figure 1: Germinated seed of *T. pratense* cv Atlantis next to unresponsive hard brown seed.

During this experiment, rumen-fistulated cows were used to allow as many varieties as possible to be simultaneously tested (due to the considerably greater rumen capacity of cattle). A follow up sheep-based experiment has been planned to predict the extent to which emerging seedlings are damaged or lost as the seeds pass through the remainder of the gastro-intestinal tract. Selected varieties of clover (i.e. those that performed well in Expt 1) will be incubated in rumen-fistulated sheep before being subjected to emersion in an acid solution to mimic potentially damaging pH changes in the abomasum. We will also incubate seeds in rumen liquor or water within an oven at a temp of 37 °C to further test whether it is warmth and moisture alone triggering the germination, or whether substrates in the rumen liquor are a factor. We plan to proceed with these experiments once lockdown has lifted and the necessary research equipment and facilities can be accessed once more.

Experiment 3: The methodology used to introduce the seeds through feeding was straightforward and could be easily adopted on farm should the results be positive. It was envisaged that reseedling using this approach could perhaps be done by feeding yearling replacement ewe lambs in April/May. By rotating the areas grazed over different years a continuous programme of clover maintenance seeding could be introduced.

Summary

This study has established that there are species and varietal differences in the response of clover seeds to exposure to rumen conditions. Based on the results to date there is potential for selected varieties to be fed as a means of introducing clover seeds into existing pasture.



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However, further research is required to explore varietal performance after exposure to the entire gastro-intestinal tract.

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