

Effects of inclusion of red clover silage on the carriage of gut pathogens in steers

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Abstract

Eight Hereford x Friesian steers prepared with rumen and duodenal cannulae were offered increasing proportions (increase of 33% each period) of red clover to grass over four periods with grass silage fed throughout to 4 steers as an experimental control. Numbers of *Escherichia coli* and *Listeria* were enumerated in silage from rumen, duodenal digesta and faecal samples at time points throughout each period. The results indicated that no silage contained *E.coli* whilst all silages had high populations of *Listeria*. Pathogen populations were lowest in the duodenum and highest in the rumen, although large populations were also found in faeces. The populations of *E.coli* were generally 10 fold lower than those of *Listeria*. Feeding red clover appeared to reduce pathogen loading but this was confounded by variation in the pathogen populations in the feed. Thus in conclusion, further research is required to elucidate the different effects of red clover and feed pathogen load on the gut and faecal pathogen populations.

Introduction

Despite recent public health scares associated with pathogenic bacteria such as *Listeria*, verotoxigenic *Escherichia coli* (VTEC's) plus numerous other potentially hazardous micro-organisms they are still often low in priority for control and remediation. Nevertheless, in the UK and USA several outbreaks of gastro-enteritis in humans have been sourced to livestock operations and the link between disease and livestock production is well documented (Pell, 1997).

In the case of *Escherichia coli* 0157:H7 less than 700 cells (Tuttle *et al.*, 1999) and as few as 10 are required to cause human disease which is frequently associated with food borne disease resulting from beef and milk products yet is rarely associated with animal disease. Little work has focused on the control and eradication of *E.coli* 0157:H7 in the animal on farm and this must be the primary point for combating the problem.

Listeria monocytogenes is a well documented pathogen as a main contaminant of forage (Fenlon *et al.*, 1989). It can survive and multiply during ensiling and storage, even where there is a very low oxygen concentration during the silage fermentation process (Donald *et al.*, 1995). At feed-out where silage is exposed to oxygen the rise in pH often causes a rise in the numbers of *Listeria*.

There are a number of factors that need to be investigated to assess the significance of pathogenic organisms and to elucidate critical points to break the cycle of contamination and thus reduce the pathogen load within the cycle. Waste manure and sewage sludge added to land to enrich the soil with nutrients and promote crop growth

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still contain pathogens in high numbers and these lead to contamination of soil and crops. The animals then consume contaminated grazed or ensiled forages. These pathogens then enter the gut, multiply and are deposited in faeces. Humans may then become infected by direct contact with the contaminated animal or by consuming infected meat and dairy products. It is this faecal shedding that results in environmental and food contamination and methods need to be found to reduce these processes.

Red clover and grass/clover-based forages are already widely used in feed rations for beef cattle and dairy cows. Red clover is a mainstay of organic rotations in many systems and its N fixation is an important source of N for low input and organic systems. There is limited evidence to suggest that Red Clover may impact negatively on pathogen growth (Garber *et al.*, 1995). Furthermore, components such as Formononetin in red clover have been shown to inhibit *E.coli* in laboratory cultures (Duncan *et al.*, 2000). This study aims to investigate this effect further.

Materials and methods

Eight Hereford x Friesian steers (300kg approx. weight) prepared with rumen and duodenal cannulae were offered either perennial ryegrass (*Lolium perenne*) silage throughout or an increasing mix of a red clover (*Trifolium pratense*) silage (RC): perennial ryegrass silage (G). The experiment was a continual design consisting of 4 periods of 21 days 4 animals were maintained on a grass silage diet throughout and a further 4 animals received grass silage in period 1 then G66:RC33, G33:RC66, RC100 in periods 2-4 respectively. Due to the simple nature of the diets, dietary treatments (silages) were switched from one to the other with immediate effect, with no gradual changeover. The animals were housed in individual pens and had access to fresh water and salt licks (Rockies) throughout. Animals received their daily food allocation in two equal meals at 08.30 and 15.30. Refusals if any were removed before early morning feed and dry-matter (DM) intake determined.

Microbial analysis

Rumen, faecal and duodenal digesta were collected post early morning feed on days 0,1,2,4,8,16 and 21 and enumerated for *Listeria monocytogenes* using Listeria Isolation Medium (LAB M) and total *Escherichia.coli* and *E.coli* 0157 using Sorbitol McConkey with B.C.I.G Agar (OXOID).

Results

Whilst the forage fed to the steers had no detectable *E. coli* at any time during the 12 week experimental period there was a large and variable population of *Listeria* associated with it. The grass silage over the 12 week period had a mean of 1.12×10^7 CFU/g DM (minimum 1.11×10^5 ; maximum 1.17×10^7 CFU/g DM), whilst the feed containing various proportions of red clover had at least 10 fold fewer *Listeria* at 1.2×10^6 CFU/g DM (minimum 3.16×10^4 ; maximum 1.05×10^7 CFU/g DM). The results also showed that greater the red clover inclusion level the lower the *Listeria* population with populations of 3.6×10^6 and 2.2×10^5 CFU/g DM respectively for diets containing 33:66 grass:red clover or 100 % red clover. Thus it is likely that the level of *Listeria* load on the forage fed will have had a confounding effect on the populations of *Listeria* present in the digesta and faeces of the animals consuming it.

The figures show the variation over time in rumen (Figs 1a and b) and faeces (Figs 2a and b) of populations of *E. coli* and *Listeria*. The results are shown for all 8 animals

used in the experiment and thus considerable time dependant variation within each animal can be seen. However in general all animals follow the same trend but it must be noted that by increasing the level of inclusion of red clover in the diet *Listeria* numbers tend to decline in all animals on this treatment.

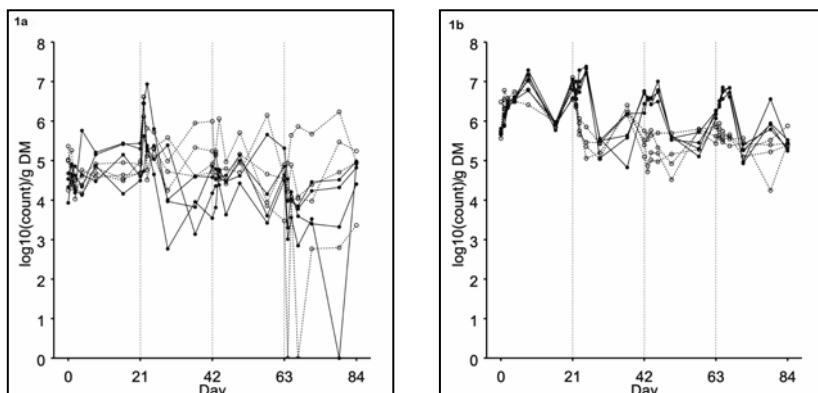


Figure 1 Variation in populations of *E. coli* (a) and *Listeria* (b) in the rumen of steers fed either grass silage (solid lines) or increasing proportions of red clover silage (dotted lines) with time over a 12 week period.

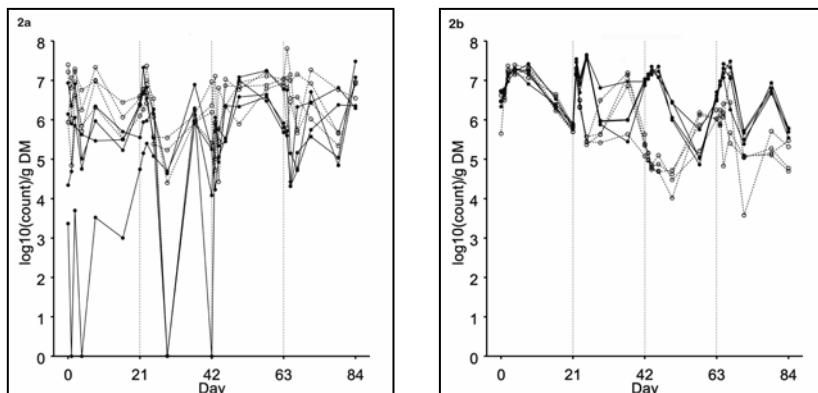


Figure 2 Variation in populations of *E. coli* (a) and *Listeria* (b) in the faeces of steers fed either grass silage (solid lines) or increasing proportions of red clover silage (dotted lines) with time over a 12 week period.

Discussion

The results indicate that there is considerable variation in shedding of pathogens with time over the 12 week experimental period. There appeared to be a reduction in the faecal shedding (Figure 2b) of *Listeria* with increased incorporation of red clover in the

diet, whereas the effect on *E.coli* was less marked. However, there was also considerable variation in the load of both *E. coli* and *Listeria* entering with the feed. It is highly probable that the burden of bacterial pathogens entering the mouth affects the magnitude of the pathogen populations present both in gut digesta and faeces.

Conclusions

The results outlined above do not give a clear indication of how diet may influence food borne pathogens in the gut of ruminants. Within this experiment two factors appear to be contributing to the pathogen load in gut digesta and faeces these are pathogen load of the feed as fed and in the case of *Listeria* the proportion of red clover in the diet.

Further work is required to elucidate which of these factors is exerting the biggest effect before practical on farm approaches to reduce pathogen carriage by ruminants can be introduced.

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