

Changes in the bacterial populations in the equine hindgut following the addition of inulin to the diet

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Introduction Laminitis in the horse is associated with the over consumption of rapidly fermentable carbohydrate, in the form of simple sugars, fructans and/or starch. The fermentation of excessive carbohydrate in the hind-gut can result in the production of lactic acid and other toxins which in turn may act as "laminitis trigger factors." (Crawford *et al.*, 2007). However, whilst it is known that susceptibility to laminitis varies within the equine population, little is known about the factors governing this variation. We have previously shown that 16S *rRNA* t-RFLP profile from bacteria extracted from equine faeces clustered weakly into two clades representing normal versus laminitis-prone ponies suggesting a difference in gut microflora between the two groups (Newbold *et al.*, 2008). Here we have extended this observation using real time PCR to investigate changes in the number of *streptococci* and *lactobacilli* in the faeces of normal and laminitis-prone ponies following fructan administration.

Materials and methods Five normal and 6 laminitis-prone, native-breed ponies were acclimated to a basal hay diet before being fed a diet of inulin (3 g/kg of BW per day) hay plus chopped dried grass. No clinical problems occurred. Fresh faecal samples were collected at -4, -2, +2 and +5 days after diet change. DNA was extracted using the QIAamp[®] DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK). The quantification of 16S *rDNA* in the samples was performed by real time PCR on an Opticon system (GRI Ltd, Essex, Braintree, UK) as described previously (Duval *et al.*, 2007). *Streptococci* were determined using 5' ATG TTA GAT GCT TGA AAG (AG)AG 3' as the forward primer and 5' CGC C(AT)T GGT GAG CC(GT) TTA 3' as a reverse primer, *lactobacilli* were determined using 5'TGC CTA ATA CAT GCA AGT CGA 3' and 5'GTT TGG GCC GTG TCT CAG T 3' respectively. Data are expressed relative to quantification of the total bacterial population quantified using the primers described by Maeda *et al* (2003)

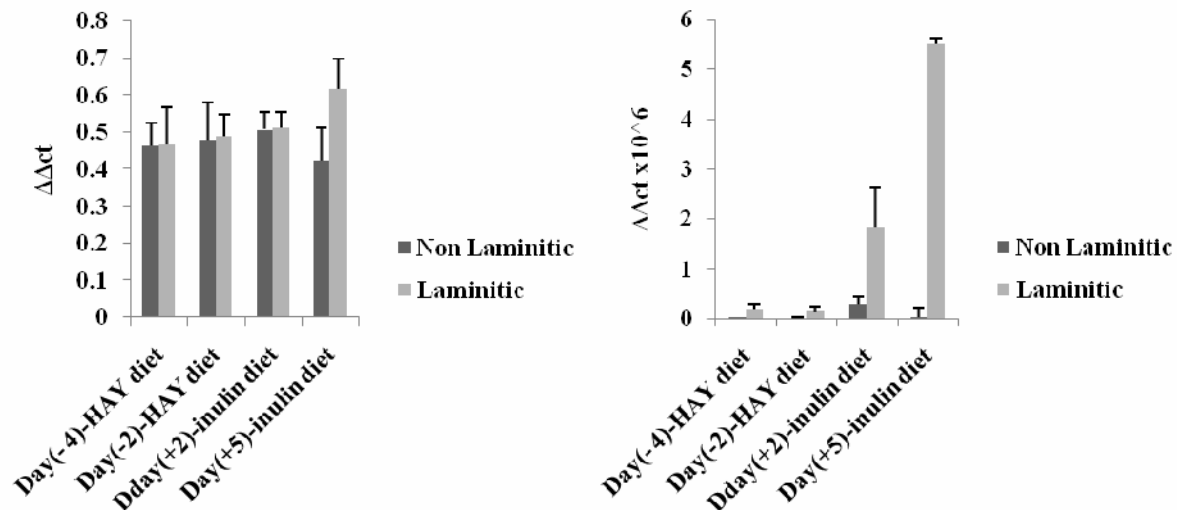


Figure 1 Relative numbers of *lactobacilli*

Figure 2 Relative numbers of *streptococci*

Results There was no clear effect of either inulin addition or susceptibility to laminitis on the numbers of *lactobacilli* that could be recovered from the faecal samples (Figure 1), however there was a dramatic increase in *streptococci* numbers recovered from the faeces of laminitis-prone ponies, but not non-laminitis prone ponies, following the addition of inulin (Figure 2).

Conclusions These results add further evidence that the bacterial population of ponies responds differently to the addition of inulin to the diet in laminitic and non laminitis prone animals. A clearer understanding of these differences may in time allow us to design strategies to avoid the changes in bacterial population in the hindgut of the horse fed diets high in rapidly fermentable carbohydrate that in time result in laminitis.

References

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