Communication

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Short Communication: Can Fecal pH Document Changes in the Intestinal Metabolome of Horses Receiving Enzyme Rich Malt Extract Feed Supplementation

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Simple Summary: Fecal pH is a simple potential tool that may reflect the metabolic functions of the intestinal microbiome; often referred to as the metabolome. Enzyme-rich malt extract (ERME) has been shown to alter the profile of volatile organic compounds and the intestinal microbiome. The aim of this pilot study was to determine whether fecal pH would provide evidence of changes in the intestinal microbiome and metabolome. Fecal pH increased significantly in a group of Thoroughbred racehorses when supplemented with ERME. Although there was no control population, this study provides an evidence base for the use of this simple tool.

Abstract: Manipulation of the intestinal microbiome is an emerging area of research, especially in hind-gut fermenters such as the horse. The aim of this study was to determine whether fecal pH can provide a simple and cost-effective tool to document changes in the intestinal metabolome and microbiome following supplementation of feed with an enzyme rich malt extract (ERME). Fecal pH was determined weekly in triplicate using a commercial soil pH meter in 72 Thoroughbred racehorses in training before and during supplementation with ERME (300mls per day divided into two feeds) alongside their regular feeding schedule. No control group was included. Fecal pH increased over the 4 weeks of treatment from 6.20 (6.15-6.24) to 6.40 (6.38-6.44; P<0.0001). Changes were not observed at one-week intervals but were apparent after 2 weeks There was a significant increase in the proportion of horses with a 'normal' pH at the end of the study (55%) compared to the start of the study (11%; p<0.001). These data support the further validation of the use of fecal pH in large scale microbiome/metabolome studies in the horse, although comparisons with these outcomes are now warranted.

Keywords: horse; intestinal microbiome; metabolome; dysbiosis

1. Introduction

The intestinal microbiome has an important aspect of health and disease in a wide range of species [1–8], including the horse [9–15]. As a hind-gut fermenter, the horse relies on the large intestinal microbiome for the breakdown of structural carbohydrates, including cellulose and fructans. The metabolism of these substances by the microbiome liberates energy substrates in the form of volatile fatty acids, predominantly butyrate, propionate and also lactate [16]. Recent investigations have proposed derangements in the normal microbiome in horses in a variety of clinical presentations that have been reviewed elsewhere [11,13,17]. While molecular techniques provide a unique insight into microbial populations, the role of these population changes is less clear, and causation is often hypothesized without evidence. There remains a lack of robust large-scale evidence base for therapeutic manipulation of the microbiome in any clinical presentation in the

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horse. Such studies are limited by the practicalities and expense of collection, storage and analysis [18].

The intestinal metabolome has been more recently investigated alongside the microbiome to attempt to ascribe a functional significance to such changes [19]. These rely upon the measurement of volatile fatty acids and other volatile organic compounds from the feces. Although sample collection is uncomplicated, the preservation requirements of such samples limit their usefulness in more extensive field trials of proposed interventions in the horse, where access to ultralow freezer capacity is not immediately accessible.

Fecal pH may represent a simple, cost-effective tool to document changes in the metabolome and can be measured patient side with limited equipment. Limited studies have evaluated fecal pH in horses undergoing attempts to manipulate the intestinal microbiome, although has been evaluated in responses to different diets [20–23]. However, if it can document the impact of manipulation of the microbiome, it would represent a valuable tool to generate large-scale data and thus be used to evaluate proposed therapeutic interventions.

Enzyme-rich malt-extract (ERME) is marketed as a pre-digestive supplement for a number of species and is proposed to promote pre-cecal digestion of starch, cellulose and fructans, to prevent excessive lactic acid production following their metabolism by the cecal microbiome [24,25]. ERME has been demonstrated to bring about changes in both the metabolome and microbiome of horses and this has been postulated to improve animal health and wellbeing, however investigations in larger populations of horses are limited by sample handling, storage and processing costs.

The hypothesis of this study was that ERME supplementation would bring about changes in the fecal metabolome, that would result in changes in fecal pH in racehorses being fed commercial diets. The aim was to determine both the rate and magnitude of change in fecal pH in Thoroughbred racehorses in training when supplemented with ERME.

2. Materials and Methods

2.1. Animal recruitment and data collection

Thoroughbred racehorses in flat race training were recruited from 9 training yards in Newmarket, UK. Horses were selected to enter the trial based on selection by the trainer, without any selection criteria. Fecal pH was determined at the start of the study and 2, 3 and 4 weeks after the initiation of supplementation, although the exact sampling period varied by 2-3 days based on availability and training / racing schedules. Fecal pH was determined by insertion of a handheld pH meter (HI-981030 soil pH tester; HANNA instruments, Woonsocket, RI. USA) into freshly produces feces. Recordings were made in triplicate from three areas of the feces and the average recorded for each animal at each time point. Where outliers were encountered, with individual recordings from a sample of feces differed by more than a pH of more than 0.2, further recordings were made and outliers excluded. The pH device was calibrated at the start of each day of data collection and washed between samples using the manufacturers instructions. All data were recorded using a digital record system (AppSheet; Solvebot Inc; WA, USA) and stored in a cloud database (Google Sheets; Google Inc, Mountain View, CA. USA).

2.2. Experimental design

All horses received enzyme-rich malt extract (Equinectar; Tharos, London. UK) in their normal feed at an inclusion rate of 150ml twice daily. There was no attempt to interfere with feeding patterns or to correct inclusion rates according to exact body weights. There was no control population. Horses that did not complete 4 weeks of supplementation from excluded from any subsequent analysis.

2.2. Statistical analysis

All pH data are presented as arithmetic mean (± 95% confidence intervals). Horses were classified into three categories of fecal pH, normal pH was considered above 6.40, subclinical acidosis was considered below 6.4 and severe acidosis below 6.0. Data were grouped to the nearest week

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period and grouped irrespective of actual day of sampling. Data were examined for normality using Shapiro-Wilk test and fecal pH were compared over the period of supplementation using mixed-effects analysis with the Geisser-Greenhouse correction. Tukey's multiple comparisons tests were calculated between each time period. Comparisons between classification (normal, acidosis, severe acidosis) were performed using Chi-squared analysis. Significant differences were assumed when $p<0.05$ and all comparisons were performed using GraphPad Prism version 9.5.1 for Windows, GraphPad Software, San Diego, CA. USA)

3. Results

78 horses were recruited into the study across 8 trainers, with each trainer selected between 8 and 10 horses. Data from 6 horses was excluded from analysis since they did not remain in training at the same trainer during the course of the study. 52 horses were sampled at week 2, and 60 horses were sampled at week 3. All 72 horses were samples both at week 0 and week 4. Fecal pH was normally distributed (W=0.9). Fecal pH at the commencement of the study was 6.20 (6.15-6.24) and increased to 6.40 (6.38-6.44) after 4 weeks of treatment ($p<0.0001$). There was no difference between fecal pH when compared at one-week intervals, such that there were no differences between pH at week 3 compared to week 2 or between week 4 and week 3 (Figure 1).

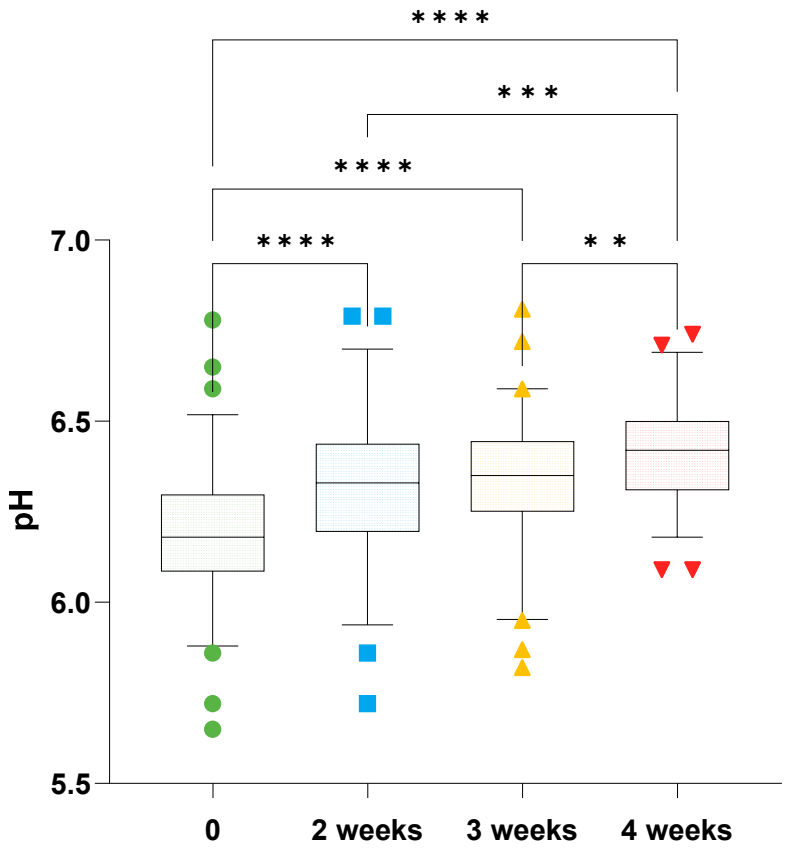

Figure 1. Box and whisker pot showing mean (line), 25th and 75th percentiles (box) and 5th and 95th percentiles (whisker) with outliers as individual datapoints of fecal pH in 72 Thoroughbred racehorses receiving an enzyme rich malt extract feed supplement. Significant differences are shown in the comparisons (**** = $p<0.0001$, *** = $p<0.001$, ** = $p<0.005$).

There were 8 horses (11%) with a normal fecal pH at the outset of the study, with a significant increase to 40 horses (56%) at the end of the study (Figure 2; $p<0.001$), although a considerable number remained in the subclinical acidosis category. There were 7 horses in the severe acidosis category at the beginning of the study, but none at the end of the study.

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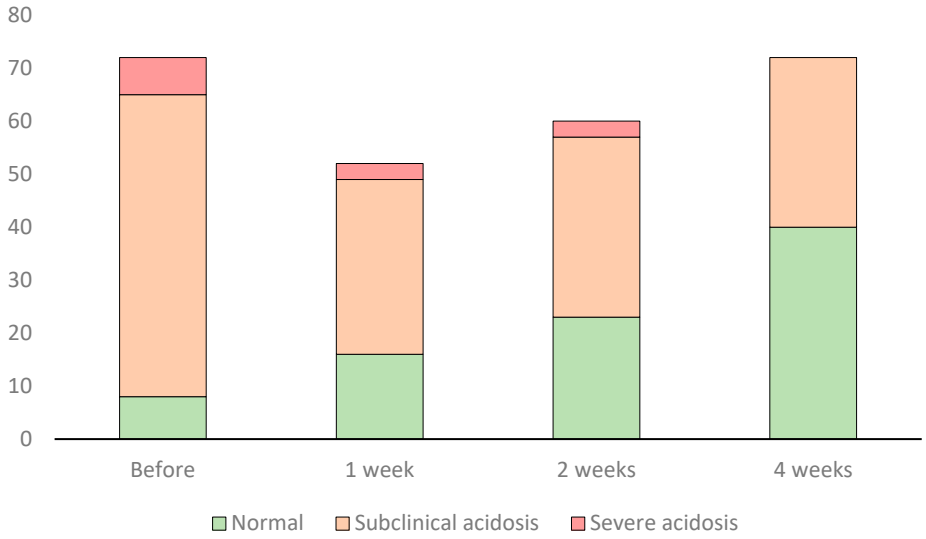

Figure 2. Bar chart showing numbers of horses classified by fecal pH into normal pH (>6.4; green), subclinical acidosis (6.0-6.4;orange) and severe acidosis (<6.0;red). There was a significant difference in the number of horses in the normal category between before supplementation and 4 weeks after supplementation.

4. Discussion

The study has demonstrated a significant reduction in fecal acidity in horses receiving enzyme rich malt extract. This demonstrates that fecal pH can be impacted by interventions that have been shown to impact the metabolome and microbiome. These changes correlate with the previous studies that have demonstrated changes in volatile organic compounds, including lactate [24] when this supplement was included. While changes in pH were relatively slow, with no difference from week to week, these differences were observed after 2 weeks. The study ended after 4 weeks, and it is not clear whether pH would have continued to increase to the normal range had the study been continued for longer.

Most horses had relatively acidic feces compared to those of grazing horses at the start of the study [22]. Previous studies have documented a normal pH in grazing horses in the range of 6.4-6.7 and that a pH between 6.0 and 6.4 of subclinical acidosis, while a pH below 6.0 has been associated with osmotic diarrhea[23]. Clinical records about the fecal consistency were not made and it would have been useful to correlate these with clinical signs and performance in these horses. Many horses developed a pH that would be comparable to grazing horses during the course of the study, without any changes in diet. Again, whether more horses would have moved into the normal category of fecal pH had the study continued for longer is not clear, but worthy of future study.

Feeding of starch-based diets is a common feature of the diets in high-performance competition horses, and these factors can lead to overflow of water-soluble carbohydrates. Furthermore, fructans are relatively resistant to digestion in the small intestine [26–28] and their breakdown to component parts within the large intestine. These factors can lead to change to rapid fermentation of these carbohydrates in the large intestine and accumulation of volatile organic compounds including lactate. Lactate is the primary driver of large intestinal acidosis that may subsequently impact the microbiome due to changes in the environment that alter bacterial proliferation [10,29]. It is assumed that the changes in fecal pH brought about by ERME dietary supplementation are related to these changes.

This pilot study did not consider a control population of horses, being fed a diet without supplementation and the validity of these conclusions would be greatly enhanced by such a study. Correlation between the pH changes and the microbiome and metabolome are warranted and a

longer duration of intervention would determine whether the effect of ERME would be more pronounced in longer duration of supplementation.

5. Conclusions

Fecal pH could be a valuable tool for investigating interventions of the intestinal microbiome, although further validation is necessary. Enzyme rich malt extract supplementation reduces the presence of subclinical fecal acidosis in horses and may prove to be a valuable tool to improve morbidities associated with feeding the high performance athletic horse.

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Institutional Review Board Statement: Ethical review and approval were not required for this study due to the non-invasive nature of the sampling and routine use of the intervention in routine management of horses.

Data Availability Statement Data available on request due to commercial restrictions

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Conflicts of Interest: The funders were involved in study design and the decision to publish the data including payment of APCs, but not in the analyses or interpretation of the data, nor in the preparation of the manuscript.

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