

## Research Article

# Innovative Approaches to Managing the Mammalian Microbiome: Evidence for the Role of Anabiomics

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## Abstract

**Objective:** Mammals normally digest dietary polysaccharides in the upper gastrointestinal tract using amylase enzymes released from the pancreas. Should the process of polysaccharide digestion be incomplete, either because the carbohydrate load is too high or because the amylase activity is too low, then undigested residues may reach the lower bowel where they act as substrates for the growth of colonic bacteria. This cascade can generate an increase in pathogenic bacteria with the formation of toxic metabolites, increased inflammation, and greater gut wall permeability. Horses are often fed large amounts of starch-based feeds once or twice/day while dogs, which generally have low levels of pancreatic amylase, are frequently fed carbohydrate-rich diets. Both species are susceptible to gut dysfunction. This report explores the novel use of an enzyme-rich malt extract (ERME) to improve digestion and alter the gut microbiome in these two species.

**Methods:** Leisure horses and dogs were maintained on standard diets and fed ERME (0.7mL/kg/bw) for 8 weeks. Faecal samples were collected before the start of the study and then at the end. These were frozen at -80°C then analysed by specific ion flow tube mass spectrometry (SIFT/MS). The resulting data was used to perform principal components analysis for metabolomics with identification of volatile biomarkers of effect. Metagenomic analysis (16S) was used to identify bacteria in the microbiome, after isolation of DNA and analysis of the 16S RNA.

**Results:** In horses, the short chain fatty acids (SCFAs) were increased after supplementation with ERME, with overall decreases in dimethyl disulphide and ethanol, representing a decrease in toxicity. In dogs, all animals showed a reduction in at least one of the toxic compounds (ammonia, methanol, ethanol) while generally showing increases in SCFAs. Post supplementation with ERME, horses generally had lower levels of Spirochaetes with increased levels of Fibrobacter, Ruminococcaceae, Blautia and Oscillospira. Dogs showed reduced levels of Spirochaetes and Proteobacteria but higher levels of Blautia.

**Conclusion:** In both species, the use of additional digestive enzymes in a maltodextrin matrix supports an improved microbiome.

**Keywords:** enzyme-rich malt extract (ERME), gastrointestinal microbiome, metabolome, equine, canine

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## 1 INTRODUCTION

One of the major factors in gut dysbiosis is the presence in the lower bowel of undigested carbohydrate residues. These residues result either from low levels of the required hydrolytic enzymes such as amylase or from a starch overload. They act as a substrate for pathogenic bacteria which increase, in turn, the formation of toxic metabolites and produce an inflammatory state with increased permeability of the gut wall. Mammalian species respond similarly to carbohydrate overload.

The potential importance of such malabsorption is well shown in equines where pancreatic amylase secretion is very low and over-feeding with soluble starch may produce endotoxaemia, acidosis, colic, laminitis, and death<sup>[1]</sup>. This problem could potentially be avoided by supplying the required enzymes. When thoroughbred racehorses were given an energy-rich diet, delivery of starch to the microbiome was reduced by feeding malt extracts containing active digestive enzymes (ERME<sup>TM</sup>), with improvements in both health and the faecal microbiota<sup>[2,3]</sup>.

Dogs, which diverged from wolves, have low levels of salivary amylase. Many dogs also have relatively low levels of pancreatic amylase (AMY2B). This varies amongst breeds (those more closely related to wolves having lower copy numbers of the AMY2B gene and so lower enzyme activity) and amongst individuals<sup>[4]</sup>.

Dogs have been human companions for about 12,000 years<sup>[5]</sup>. Although the ancestors of modern dogs were almost entirely carnivorous, modern canines often share human meals such as toast and pizza and have a high dietary content of carbohydrates from commercial feedstuffs. In consequence, they may suffer a disordered microbiome, digestive issues<sup>[6,7]</sup> and allergies<sup>[8]</sup> as the increased permeability allows transport of toxins and protein fragments across the gut wall. Supplementing carbohydrate-rich diets with digestive enzymes has the potential to improve both gut health and general well-being in both horses and dogs.

Thus, despite varying dietary requirements, mammalian species respond similarly to carbohydrate overload, which results in changes to the microbiome. The bacteria involved are similar qualitatively if not quantitatively.

This observation has led to a novel hypothesis. Improvements in the microbiome might be obtained through supplementation with extrinsic digestive enzymes to increase the breakdown of food residues in the upper gut

and the presence of a polymeric carbohydrate matrix. This dual approach allows the enzymes to remain active after passing through the stomach and modulates the microbiome without the introduction of foreign microorganisms.

The term “anabiomics” has been adopted to describe approaches to the restoration of a healthy microbiome. In this paper, we discuss the potential of this dual system using supplementation with ERME as an example. Metagenomic analysis of DNA from faecal samples was used as a surrogate for the presence of specific bacteria. Metabolomic analyses provided a profile of the total metabolic products of the microbiome and so gave an overall picture of the response to dietary supplements.

## 2 EXPERIMENTAL METHODS

This report explores the novel use of an enzyme-rich malt extract (ERME) to improve digestion and alter the gut microbiome in equines and canines.

The experiment with horses was carried out in a stable yard near Cambridge, Cambridgeshire, UK while the experiment with dogs was carried out in a kennels in the same county.

### 2.1 Preparation of Malt Extract

Germinating barley generates hydrolytic enzymes to allow the growing plant access to the nutrients stored in the barley grain. These enzymes are normally destroyed by heating when malt is produced for human food, but extraction and evaporation at lower temperatures (45°C) allow the enzymes to remain active. These include  $\alpha$ - and  $\beta$ -amylases, fructanases and dextranases, phytase and others which break down cell-walls including cellulase,  $\beta$ -glucanases, and xylanases<sup>[9,10]</sup>. The organic matrix is largely composed of maltodextrins with smaller amounts of maltose polymers, maltose, and glucose. The product ERME<sup>TM</sup>, can easily be incorporated into diets or animal feeds; the enzymes remain active for at least 12 months when stored at room temperature in sealed containers (‘ERME’ Tharos Ltd London, UK).

### 2.2 Horses

Non-thoroughbred leisure horses (10) were kept on a standard diet. Faecal samples were collected before the start of the project and frozen at -80°C; the horses were then fed 150mL ERME (~0.7mL/kg body weight) with their diet twice daily for 8 weeks and the faecal samples were collected and frozen as before. The samples were analyzed by specific ion flow tube mass spectrometry (SIFT/MS) and by 16S metagenomic analysis.

### 2.3 Dogs

Adult dogs (10) were fed ERME (0.7mL/kg body weight) with their diet twice daily for 8 weeks; faecal samples were taken before the start of the study and after the study was completed, then frozen at -80°C and processed for SIFT/MS and 16S metagenomic analysis.

### 2.4 Metabolomic Analysis by SIFT/MS

Volatile organic compounds (VOCs) were studied in faeces or urine using SIFT/MS. Samples were stored frozen (-80°C) until the whole collection was transferred to the laboratory. The samples were taken and thoroughly defrosted. Exactly 5g of each sample was weighed out and placed in a sample bag of Nalophan tubing. The bag was filled with 0-grade (hydrocarbon-free) air before being sealed with a Swagelok fitting and then placed in the incubator at 45°C for 45 minutes to increase compound volatilization. After incubation, samples were then attached to the SIFT/MS via the heated sampling capillary. SIFT/MS is a real-time trace gas analyser where selected precursor ions ( $\text{H}_3\text{O}^+$ ,  $\text{NO}^+$  or  $\text{O}_2^+$ ) are generated in an air/water mixture via a microwave discharge and then selected via an upstream quadrupole mass filter, injected into helium carrier gas and passed along a flow tube into which the sample is introduced via the heated capillary. The cursor ions react with the sample compounds and the resulting product ions are separated in a downstream quadrupole mass filter before being detected and counted<sup>[2]</sup>

### 2.5 Data Analysis

Excel files were prepared from the SIFT/MS analysis and imported via Matlab which was employed to perform principal components analysis (PCA); outlying samples were not observed. Data analysis (script in R) was employed to perform data modelling via machine learning, using partial least squares discriminant analysis, random forest, and Bayesian additive regression trees. Biomarker Discovery was employed to interrogate optimum models from Data Analysis and suggest possible markers. This involved selecting the bootstrap iteration that produced the highest classification accuracy, regenerating the optimum model, and extracting the significant features. Box-and-Whisker plots were generated for each of the m/z ions from the SIFT/MS analysis and markers were identified by choosing plots that had median values where the pre-ERME and post-ERME results were significantly different.

### 2.6 Metagenomic Analysis

Faecal samples (8) were taken before and after supplementation with ERME. The genomic DNA was isolated according to protocol and 16S rRNA analysed using the Illumina platform. This provided identification of the Phyla, Class, Order, Family, Genus and Species present in the microbiome with estimates of the relative frequencies (percentage of total hits and total numbers of hits).

## 3 METABOLOMIC RESULTS

### 3.1 Horses

The gut metabolome of the horses was significantly affected. It was different before and after supplementation with ERME. Biomarker discovery of the respective optimum models attained via Random Forest and Bayesian additive regression trees agreed well. Pre- and post-ERME distinguishing biomarkers included ethanol, dimethyl disulphide and methanol, with a range of aldehydes and esters. As can be seen in Table 1, the short chain fatty acids (SCFAs) acetic, propionic and butyric acids were all increased after supplementation with ERME (total mean pre-ERME 1,376ppb; total mean post-ERME 2,077ppb). These are derived from breakdown of dietary fibre by bacteria in the lower gut; they provide nutrition to the cells in the gastrointestinal tract and are a major energy source in the horse. There was relatively little change in levels of acetone and ammonia but overall decreases in dimethyl disulphide and ethanol.

### 3.2 Dogs

The results in Table 2 show that all the dogs, which were healthy and without reported problems, had relatively low levels of toxic metabolites. The wide variation in metabolite levels reflects the differences between the components of the microbiome and makes statistical significance unobtainable. However, all dogs showed a reduction in at least one of the toxic compounds (ammonia, methanol, ethanol) while 9 out of the 10 dogs showed an increase in beneficial metabolites (acetone, acetic acid, propionic acid, and butyric acid).

## 4 METAGENOMIC RESULTS

### 4.1 Horses

The equine microbiome reflects diet, exercise and environmental conditions and is known to vary widely between horses. This was the finding in pre-ERME samples. However, after ERME supplementation, the values converged to a similar pattern. At the Order level, Clostridiales and Bacteroidales were the major components and there was an increased species richness. Post ERME, horses generally had lower levels of Spirochaetes (often pathogenic), increased Fibrobacter and Ruminococcaceae which increase fibre/cellulose breakdown, increased Blautia and Oscillospira which produce butyrate and, at the species level, increases in the abundance of Paraprevotella, which produces propionate (Table 3).

### 4.2 Dogs

The canine microbiome differed from the equine microbiome, reflecting the differences in diet and environmental inputs. Again however, after supplementation the microbiome profiles tended to converge (Table 4) and at the Order level, Bacteroidales were major components with

**Table 1. Analysis of VOCs from Equine Faecal Samples before (pre) and after (Post) Supplementation with ERME. Results are Given as ppb**

Horse	Acetic acid Pre/Post	Propionic acid Pre/Post	Butyric acid Pre/Post	Ammonia Pre/Post
1	178/374	303/340	105/204	681/809
2	264/403	403/596	254/243	531/231
3	1,346/2,076	1,266/1,831	587/895	600/779
4	293/630	268/716	205/222	169/459
5	587/458	677/545	423/405	373/555
6	542/1,294	638/1,193	525/661	356/1,038
7	420/479	331/581	196/322	404/406
8	449/329	453/473	340/306	407/242
9	650/1,002	746/2,795	440/655	754/433
10	325/323	274/309	217/133	613/247
Mean	505/737	533/938	335/405	489/519

  

Horse	Methanol Pre/Post	Ethanol Pre/Post	Acetone Pre/Post	Dimethyl disulphide Pre/Post
1	706/415	2,037/1,066	101/70	24/29
2	739/863	1,431/1,874	20/102	39/88
3	1,256/1,250	1,356/1,104	45/34	10/16
4	295/528	1,530/1,647	40/40	19/38
5	1,209/1,251	540/719	203/15	12/30
6	441/379	2,492/489	14/104	147/11
7	700/412	1,543/1,427	22/142	21/54
8	527/670	3,341/718	86/16	83/31
9	544/1,246	4,036/2,515	11/175	52/24
10	1,259/1,239	1,673/963	157/85	124/12
Mean	764/825	1,998/1,252	70/78	53/33

decreases in Clostridiales and Erysipelotrichales, There was increased species diversity with reduced levels of Spirochaetes and Proteobacteria. As with horses, there were higher levels of Blautia. Several of the dogs had increases in Clostridia hiranonis and Faecalibacterium prausnitzii, both of which are believed to be highly associated with improved gut function.

## 5 DISCUSSION

The metabolomic results in both species represent a decrease in toxicity after supplementation with ERME. The lower amounts of ethanol may represent a reduction in yeasts in the gut; these are known to form ethanol when the diet is high in sugars, especially fructose<sup>[11]</sup>, or high in carbohydrates such as starches. In humans, Candida albicans, Candida glabrata and Pichia kudriavzevii are thought to be involved<sup>[12]</sup>. Dogs generally had higher levels of ammonia than horses, probably because their diet contains more protein and hence more nitrogen. Faecal ammonia increased in dogs on high protein diets<sup>[13]</sup> while in cows, faecal ammonia increased linearly with increasing dietary protein concentration<sup>[14]</sup>, in agreement with the equine results.

In both species, the reduction in toxic chemicals in the

metabolome was associated with the major energy source for colonic cells) and better immune function with reduced inflammation<sup>[15]</sup>.

ERME significantly modified the gut microbiome in both the species studied. Starch break-down in the small bowel was increased, providing more sugars for absorption and thus increased energy while much less carbohydrate/polysaccharide reached the microbiome for fermentation. As the substrates reaching the lower bowel were altered, this changed the colonic microflora, reducing the abundance of pathogenic bacteria. The polysaccharide matrix surrounding the enzymes from the malted barley also affects the microbiome; studies using heat-denatured ERME have shown that this product too can alter the microbiome. These findings are in line with results from other workers who have found that carbohydrate polymers often modulate the gut microflora<sup>[16,17]</sup>.

The results seen for horses in this study were in agreement with earlier work with racehorses in training where faecal samples were collected before and after supplementation with ERME and were analysed for changes in the metabolome and microbiome<sup>[2]</sup> Racehorses on high-energy diets usually

**Table 2. Analysis of VOCs from Canine Faecal Samples before (Pre) and after (Post) Supplementation with ERME. Results are Given as Parts Per Billion (ppb)**

Dog	Acetic Acid Pre/Post	Propionic Acid Pre/Post	Butyric Acid Pre/Post	Ammonia Pre/Post
1	1,092/646	817/632	372/261	385/305
2	624/636	801/584	226/406	1,340/970
3	325/1,400	481/2,632	102/482	1,097/690
4	353/97	339/80	48/102	1,902/4,129
5	58/277	86/169	108/150	3127/543
6	286/612	583/1,047	27/370	2,107/1,368
7	301/754	271/822	346/384	868/758
8	422/480	679/670	151/682	1,503/1,106
9	108/392	501/953	238/240	1,706/1,654
10	564/681	396/568	129/287	657/682
Mean	413/598	495/816	166/288	1,559/1,220

  

Dog	Methanol Pre/Post	Ethanol Pre/Post	Acetone Pre/Post	Dimethyl disulphide Pre/Post
1	12,878/6,473	27,539/17,796	200/157	10/8
2	9,691/5,368	19,704/21,542	3,067/9,956	28/31
3	23,142/16,969	4,770/10,088	1,240/2,034	7/73
4	10,251/7,447	2,519/1,805	588/300	83/7
5	7,013/4,907	411/7,065	685/989	7/90
6	4,098/3,087	6,758/7,841	2,052/3,046	13/21
7	17,684/11,534	13,479/13,021	1,768/2,854	23/48
8	14,536/12,868	12,087/12,132	1,345/7,385	56/63
9	12,780/6,870	14,304/10,989	482/907	43/88
10	8,923/5,992	3,208/6,508	301/1,164	17/12
Mean	12,100/8,152	10,478/10,879	1,173/2,879	29/44

**Table 3. Analysis of Major Equine Gut Microbiome Changes Post Supplementation with ERME**

Horse	Phylum	Class	Family	Genus	Species
1	Spirochaetes decrease				Oscillospira increase
2		Fibrobacteria increase		Ruminococcus increase	Blautia increase
3				Ruminococcus increase	Fibrobacter increase
4		Fibrobacteria increase			Oscillospira increase
5	Spirochaetes decrease	Flavobacteria decrease	Ruminococcaceae increase	Ruminococcus increase Treponema decrease	Oscillospira, Paraprevotella increase
6	Spirochaetes decrease	Bacilli increase		Treponema decrease	Paraprevotella increase
7	Spirochaetes decrease Bacteroidetes increase	Fibrobacteria increase	Ruminococcaceae increase	Ruminococcus increase	Oscillospira , Fibrobacter succinogenes increase
8	Bacteroidetes increase	Fibrobacteria increase	Lachnospiraceae increase	Blautia, Prevotella increase	Paraprevotella increase

have irregular bowel function, but stools became quite regular within days of starting ERME. Increased energy uptake produced major improvements in condition and performance<sup>[3]</sup>. The faecal microbiome was considerably

different after ERME feeding, with increases by over 1,000-fold in certain species including Veillonaceae, Ruminococcus, Prevotella, Lawsonia and Bacteroidia, similar to the profiles seen in the present report.



**Table 4. Analysis of Major Canine Gut Microbiome Changes Post Supplementation with ERME**

Dog	Phylum	Class	Family	Genus	Species
1				Blautia increase	
2	Spirochaetes decrease	Beta proteobacteria decrease	Veillonellaceae increase		C.hiranonis increase
3	Spirochaetes decrease		Spirochaetes (Order) decrease	Streptococcus, Treponema decrease	Treponema decrease
4	Proteobacteria, Spirochaetes decrease	Proteobacteria, Spirochaetes decrease	Veillonellaceae, Ruminococcae increase	Blautia increase, Proteobacteria decrease	Proteobacteria decrease
5	Spirochaetes decrease		Ruminococcae increase	Faecalibacterium increase	F. prausnitzii increase
6	Proteobacteria decrease	Proteobacteria decrease	Ruminococcae increase	Faecalibacterium, Blautia increase	F.prausnitzii increase
7			Ruminococcae increase	Faecalibacterium, Blautia increase	F.prausnitzii , C.hiranonis increase
8	Spirochaetes, Proteobacteria decrease	Gamma-Proteobacteria decrease	Ruminococcae increase	Faecalibacterium increase	F. prausnitzii increase
9	Spirochaetes decrease	Erysipelotrichia decrease	Streptococcae eliminated	Streptococcus, Catenibacteria eliminated	C. hiranonis increase
10	Proteobacteria decrease	Proteobacteria decrease			F. prausnitzii increase

Many studies have shown that canine intestinal dysfunction is associated with an altered microbiome<sup>[18,19]</sup>; for example, canine irritable bowel disease is linked with increases in gamma-proteobacteria<sup>[20]</sup>. A ‘Dysbiosis index’ has been proposed<sup>[18]</sup> to give a semiquantitative estimate of diversion from a healthy state and in this, a panel was selected from seven bacterial groups. Raised levels of *Faecalibacterium* spp., *Blautia* spp., and *Clostridium hiranonis* were all associated with reduced inflammation and improved gut function. It is therefore of interest that these groups were all found at higher levels in animals where the diet had been supplemented with ERME. *F. prausnitzii* was increased in 5 of the 10 dogs; this is an important biomarker of an optimised microbiome when found in human faecal samples. *Escherichia coli*, *Streptococcus* spp., and Gamma-proteobacteria generally seem to have negative effects and these associations of bacteria with disease states have led to an interest in modifying the gut microbiome profile by supplementation with pre- and pro-biotics or faecal transplants<sup>[21]</sup>. However, results have been mixed and the benefit is often small and not sustained<sup>[22,23]</sup>. The use of a supplement such as ERME, which supports the beneficial host-derived micro-organisms, is more likely to lead to a stable microbiome.

## 6 CONCLUSIONS

ERME™ restores healthy gut flora without the administration of extraneous bacteria or prebiotic chemicals. It offers a dual anabiomic action. Additional enzymes to increase digestion and a maltodextrin matrix both support an improved microbiome. Like other dietary components that alter the colonic microflora, its use as a supplement may lead to improvements in the management of disease

states in both man and animals.

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## Conflicts of Interest

The authors declared no conflict of interest.

## Author Contribution

Waring RH and Hunter JO contributed to the design of the experiments and wrote the manuscript; Dagi TF revised and edited the manuscript.

## Abbreviation List

ERME, enzyme-rich malt extract  
SCFAs, short chain fatty acids  
SIFT/MS, specific ion flow tube mass spectrometry

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