

# Negative Fecal Characteristics Are Associated with pH and Fecal Flora Alterations During Dietary Change in Dogs

Joseph J. Wakshlag

Kenneth W. Simpson

Angela M. Struble

Scot E. Dowd\*

*Cornell University College of Veterinary Medicine,  
Department of Clinical Sciences, Ithaca NY;  
\*Research and Testing Labs, Lubbock TX*

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## ABSTRACT

Many dietary factors influence fecal out-put and consistency including digestibility, fiber, and resistant starches. To better understand how dietary change can influence the microbiome and fecal characteristics we performed a 4 week feeding trial using two different foods in six dogs. While feeding to maintain each dog's present body weight; fecal scoring and defecations per day were recorded for each food. At the end of each time period fresh fecal samples were collected and then partitioned for pH analysis and bacterial tag-encoded FLX 16S rDNA amplicon pyrosequencing to evaluate the microbiome. Food A resulted in a significant decrease in fecal pH and fecal quality, and an increase in daily defecations. Fecal flora analysis revealed significant increases in Lactobacillales, and Clostridiales VII and decreases in Erysipelotrichales and Coriobacteriales family microorganisms when the dogs were fed Food A. While we have yet to determine the specific component in food A responsible for this dramatic shift in fecal pH and change in fecal microflora, the

proximate analysis suggests the potential for reduced digestibility, higher fiber (soluble and insoluble) content, and/or resistant starch as potential causes.

## INTRODUCTION

The simple act of changing dog foods has been associated with gastrointestinal disturbances that can lead to diarrhea and fecal inconsistency (Davenport et al, 2009). This change in the fecal ecology often plagues veterinarians leading to suggestions of gradual food adaptations to prevent these maladies. Of equal interest is that certain foods tend to produce better fecal consistency than others, with very little evidence as to the reasons for better assimilation of certain feeds. There are many variables that can affect fecal consistency including resistant starches, substrate digestibility, insoluble and soluble fiber content, fat tolerance and overall dry matter intake.<sup>1</sup> Dry matter intake may adversely affect total fecal volume as digestibility decreases with higher overall intake, and can also play a significant role in fecal consistency.<sup>2</sup> Insoluble fiber content can decrease digestibility of foods and increases fecal volume and frequency of defecation.<sup>2,3</sup> Soluble fiber sources on the other hand play a role in fecal consistency : Diet and fecal microbiome in dogs

**Table 1:** Range of culturable *Lactobacillales* spp, *Escherichia coli*, and *Enterococcus faecium* during food change across all dogs.

Bacterial Morphology	Population (CFU/g) Dog Food A	Population (CFU/g) Dog Food B	Genus species
Long rod, non motile	$2 \times 10^8 - 7 \times 10^9$	ND – $7 \times 10^8$	<i>Lactobacillus acidophilus</i>
Very long thin rod, non motile	$5 \times 10^6 - 5 \times 10^8$	$2 \times 10^7 - 5 \times 10^8$	<i>Lactobacillus johnsonii</i>
Fat, medium rod, short chains	$6 \times 10^7 - 2 \times 10^9$	$5 \times 10^7 - 3 \times 10^9$	<i>Lactobacillus animalis</i>
Very fat medium-large rod	ND – $2 \times 10^8$	ND – $5 \times 10^8$	<i>Lactobacillus salivarius</i>
Long skinny rod	ND – $3 \times 10^8$	ND – $1 \times 10^9$	<i>Lactobacillus reuterii</i>
Coccus, in chains	ND	ND – $1.5 \times 10^9$	<i>Enterococcus faecium</i>
Short fat rod, motile	ND – $2 \times 10^6$	$4 \times 10^4 - 1 \times 10^7$	<i>Escherichia coli</i>

as well as large colon volatile fatty acid production, which directly affects fecal pH.<sup>4-6</sup> Furthermore, resistant starch, a product of excessive heat during cooking and extrusion, may act similarly to soluble fiber allowing rapid fermentation by the colonic microbiome leading to a significant alteration in the colonic flora.<sup>7</sup>

Carbohydrate sources may also play a role as various carbohydrate sources have variable fiber contents.<sup>8</sup> For instance, carbohydrate sources like barley can be the major carbohydrate substrate in pet food. However, barley creates unfavorable fecal consistency, suggesting that barley cannot be used as a sole carbohydrate in pet foods.<sup>9</sup> Therefore, our hypothesis was that a canine diet lower in digestibility and higher in soluble fiber and resistant starch would cause changes in the microbiome that corresponded with alterations in fecal pH and fecal consistency.

**MATERIALS AND METHODS**

Six kenneled dogs on a Cornell University approved institutional care and use committee protocol, between the ages of 7 and 10 years, including three neutered males and three neutered females, were used in the study. The dogs were fed based on a cross-over design where dogs were placed onto one of two foods. Dogs were transitioned to dog food A a or dog food B b over a 4-day period and then fed for 28 days. Dogs were then switched to the other food using the same protocol for 4 weeks.

Fecal consistency (based on a 1-9 scale with 1 being hard and crumbly to 9 being liquid consistency) and frequency of defecation was recorded for 28 days.<sup>10</sup> At the end of the 28-day period, fresh feces were collected from each dog at the time of defecation, mixed well, and cultured on selective media for selected total bacterial counts (CFU/g; Microbios Labs Inc) and assessed for fecal pH (Mettler Toledo SevenEasy, Schwerzenback, Switzerland).

Another portion of the fecal sample was immediately frozen at -80°C for further tag-encoded FLX 16S rDNA amplicon pyrosequencing (bTEFAP) analysis according to previously published methods.<sup>11</sup> Fecal pH, consistency, and frequency as well as percent of change in the fecal flora species between the two foods were analyzed using Wilcoxon Ranked Sum testing. Both dog foods were analyzed for protein, fat, moisture, and NFE by Dairy One analytical services and sample of the foods were also sent to Nestle-Purina Analytic labs to assess resistant starch and soluble fiber content. Ingredient lists for both pet foods are as listed in appendix A. Additionally, food A, which contained bacterial fermentation products was, solublized in distilled water and cultured for viable microbial growth for the identified species added to the commercial product.

**RESULTS**

**Food Analysis**

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All results are presented on a dry matter basis. Food A contained 23.3% crude protein, 14.4% crude fat, 38.5% non-fiber extract, 6% ash, 14.1% insoluble fiber, 3.7% soluble fiber, 10.8% resistant starch, and contained approximately 3.7 kcals/g. Food B contained 35% crude protein, 23.2% crude fat, 32.2% non-fiber extract, 5.9% ash, 7.5% insoluble fiber, 0.5% soluble fiber, 3.7% resistant starch, and contained approximately 4.4 kcals per gram. Microbial culturing of Food A resulted in no viable growth of the organisms identified on the label.

### Fecal Assessment

There was a significant difference in fecal pH, consistency, and number of defecations per day. Fecal pH was significantly lower for food A (5.9 + 0.6) than for food B (pH 7.1 + 0.3 ; p<0.01). Fecal consistency scoring was significantly different between groups, with Food A showing a higher consistency score (5.4+ 0.5) than food B (3.3 + 0.6; p <0.01). The frequency of defecation experienced daily was significantly higher while on food A (3.1 + 0.6) than food B (2.2 + 0.5; p<0.01).

### Microbial Analysis

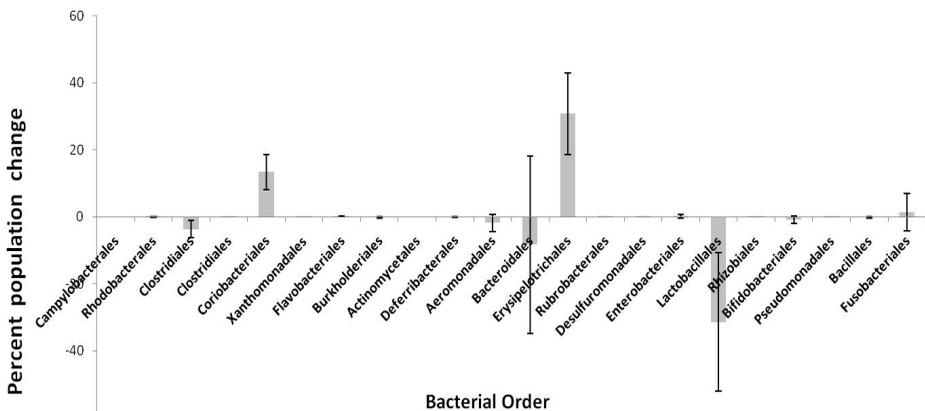
bTEFAP results showed a significant proportional increases in *Lactobacillus* spp, and *Clostridiales* VII spp, and decreases in *Erysipelotrichales* spp and *Coriobacteriales*

spp when the dogs were fed Food A (Figure 1; p < 0.05), and a rise in culturable *Lactobacillus acidophilus* numbers when dogs were fed Food A suggesting that the rise in *Lactobacillus* spp was primarily *Lactobacillus acidophilus*, and not from DNA contamination from Food A. When double dendrograms were generated based on microbial populations five of the six dogs grouped in a hierarchy based on food consumed rather than individual dog, suggesting a relatively homogeneous microbial population among the dogs and significant shifts due to type of food consumed (Figure 2).

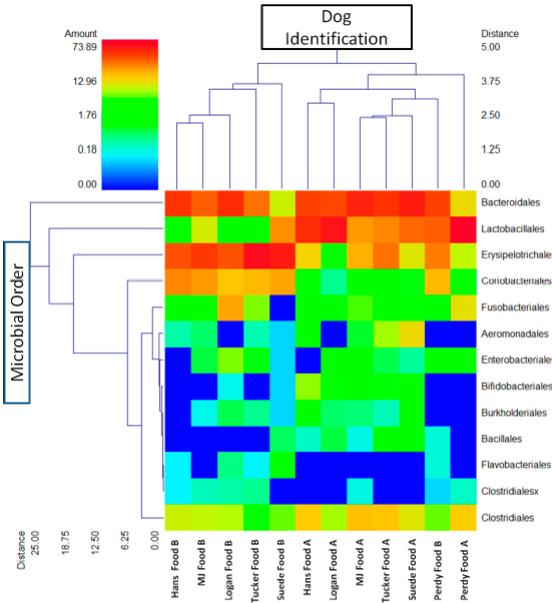
### DISCUSSION

The simple act of changing diets can have significant impact on many parameters involved in lower gastrointestinal system ecology. One of the most important parameters to overall food performance is fecal consistency, and there was a noticeable increase in fecal volume and looser consistency when dogs were fed Food A. The higher overall fiber content in food A is the likely contributor the higher fecal volume, as this food contained more insoluble fiber, soluble fiber and resistant starch. Additionally, while the dogs were consuming Food A, the fecal pH was significantly lower which may disturb large colon function. It is well known that soluble fiber components such as lactulose are used

**Figure 1:** Percent change in fecal microbial orders during switch from Food A to Food B. p < 0.05 for Lactobacillales, Erysipelotrichales, Coriobacteriales and Clostridiales VII only.



**Figure 2:** Double Dendrogram of relative order percentages represented in the feces of each dog on both diets A and B. Figure depicts microbiome changes are more closely related to food characteristics than individual dog microbiome differences.



therapeutically in human and veterinary medicine to produce a lowering of fecal pH, both the lower pH and osmotic effect of lactulose produce less efficient colonic water absorption.<sup>12</sup> Our study produced similar decreases in fecal pH that were associated with overall increased *Lactobacillales* spp. and *Clostridiales* spp. flora and a decrease in *Erysipelotrichales* spp. and *Coriobacteriales* spp. when compared to Food B. Both *Erysipelotrichales* and *Lactobacillales* are acid producing fermenters with *Lactobacillus acidophilus* being nearly a log fold higher in dogs while on Food A. This increase in *Lactobacillales* spp. is the likely cause for the drop in fecal pH, and may be playing a role in diminished fecal quality while dogs were fed Food A versus Food B.

Previous studies using various forms of soluble fiber have been shown to alter fecal microflora and induce changes in fecal pH, and *Lactobacillales* spp. are often impli-

cated in producing a healthful benefit.<sup>13,14</sup> However, many of these studies using prebiotic or probiotic treatment have not shown the dramatic increase in *Lactobacillales* spp. that we observed.<sup>13-16</sup> This may be due to the relatively low concentrations of soluble prebiotics used in previous studies which are usually at less than 2% of the dry matter of the feed used.<sup>4,13,14</sup> Furthermore, in some of these studies, there were negative changes in fecal consistency with higher concentrations of soluble fiber.<sup>4,6</sup> Food A has roughly 3.7% soluble fiber and over 10% resistant starch that can act similarly to soluble fiber, which is far more than recommended when trying to enhance fecal quality and improve colon health.<sup>12,17</sup>

Our study suggests that when assessing pet food for fecal quality and consistency, there is little information regarding soluble

fiber content, as it is not part of the crude fiber component measured and displayed on the label. Of course, fecal quality is also a direct reflection of overall dry matter intake, and the two dogs on the highest dry matter intake consistently had the poorest fecal quality scores and ingested more of the dietary soluble and insoluble fiber. Additionally, the processing of raw ingredients and extrusion processes involved in manufacturing feeds can create resistant starches. Studies in dogs have shown a moderate change in fecal quality and increased short chain fatty acids when resistant starches were introduced into the diet.<sup>7</sup> These resistant starches were far higher in diet A and might also influence the colonic microbial ecology and alter fecal characteristics.

Regardless of whether resistant starch or soluble fiber are the primary causes for the dramatic rise in *Lactobacillales* spp.,

addition of a *Lactobacillus* based probiotic supplement to alleviate poor fecal quality would likely be of limited benefit. Additionally, the use of soluble fiber to alleviate poor fecal quality might be detrimental when used with diet A. The increasing use of novel grains such as barley, oats, and sorghum rather than rice and corn may contribute to higher soluble fiber content.<sup>8</sup> Examination of a barley based diet by Murray et al suggested that barley could not be used as the primary carbohydrate source in food due to poor fecal characteristics, even though the carbohydrate absorption was no different than rice and corn.<sup>9</sup> Therefore, the use of pre- and probiotics to alter fecal characteristics need to be weighed against the characteristics and ingredients of the dog food being fed.

## CONCLUSIONS

In today's pet food market, changing brands of dog food based on "healthful" ingredients is a rising trend. Veterinary recommendations regarding food transition often recommend a gradual switch due to the potential of gastrointestinal disturbances which our data suggests are real concerns, particularly in light of the trend towards using more complex ingredient mixtures in dog foods. Consumers and veterinarians alike lack important information regarding digestibility and total dietary fiber amounts, making selection of pet foods difficult, particularly when gastrointestinal signs are involved in selection.

The use of synbiotics and probiotics to ameliorate inconsistent stool quality is commonly used in veterinary medicine, and results from our study suggest that these approaches would be inappropriate and potentially detrimental. Therefore, veterinary practitioners and consumers alike need to be better informed regarding total dietary fiber and digestibility as major determinants in selecting pet foods.

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## Appendix A

Food A : Chicken, Chicken Meal, Brown Rice, Mendenham Fish Meal, Pearled Barley, Oat Groats, Chicken Fat, Flaxseed, Dried Egg Product, Apples, Carrots, Spinach, Sweet Potatoes, Blueberries, Cranberries, Kelp Meal, Glucosamine, Dehydrated Chicken Cartilage, Green Lipped Mussel Powder, L-Carnitine, DL-Methionine, Marigold Extract, Taurine, *Lactobacillus acidophilus* dehydrated fermentation product, *Bifidobacterium thermophilum* dehydrated fermentation product, *Bifidobacterium pseudolongum* dehydrated fermentation product, *Enterococcus faecium* fermentation dehydrated fermentation product, Vitamin and Mineral Premix

Food B : Chicken, Corn Gluten Meal, Brewer's Rice, Animal Fat, Poultry Byproduct Meal, Whole Grain Corn, Corn Bran, Fish Meal, Animal Digest, Fish Oil, Dried Egg Product, Vitamin and Mineral Premix