

# Novel dietary blends improve stool quality and alter fecal microbiota, metabolites, and immune markers of healthy adult cats

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## Introduction & Objective

- Functional ingredients are those that provide benefits beyond that of traditional nutrients and can improve overall health and well-being of the animal.
- Functional ingredients may be used in isolation or in a blend to support gastrointestinal and/or immune health. However, cats have been poorly studied in this area.
- Our objective was to test diets containing functional blends of dietary fibers, probiotics, probiotics and immune-modulating agents on gastrointestinal health outcomes in healthy adult cats.

## Methods

### Experimental Design, Animal and Diets:

- Replicated 3x3 Latin-square design.
- 12 adult domestic shorthair cats (age = 9.6±4.0 yr; BW = 3.9±1.0 kg).
- 3 dry extruded poultry- and fish meal-based experimental diets containing different types of functional ingredients.

Table 1: List of functional ingredients added to diets

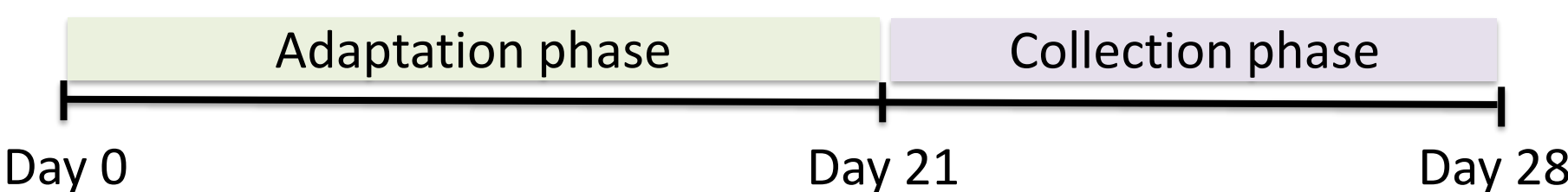
	Control	Test diet 1	Test diet 2
Dietary fiber (Brewer's rice, Pea fiber)	●	●	●
Additional fiber and prebiotic (Oat groats, Dried peas, Dried beet pulp, Flaxseed meal, Inulin)		●	●
Probiotic (Lacto-Sacc probiotic)		●	●
Immune-modulating agents (Yeast fermentation product, Spray-dried plasma)			●

## Methods

Table 2: Chemical composition of diets

	Control	Test Diet 1	Test Diet 2
Dry matter, %	90.6	94.3	95.9
%, Dry matter basis			
Ash	6.3	8.3	8.5
Crude protein	21.9	28.5	31.6
Acid-hydrolyzed fat	9.0	14.1	16.9
Total dietary fiber	7.8	12.0	16.0

### Experimental Timeline:



- Each treatment period consisted of 28 days
- Diet adaptation phase (D 1-21)
- Collection phase (D 22-28)
  - ✓ Fresh fecal collection (D 22-23)
  - ✓ Total fecal collection for nutrient digestibility (D 24-28)
  - ✓ Blood collection (D 28)

### Laboratory Analyses:

- Fresh fecal samples were used to measure fecal metabolites, microbiota and a measure of gut immune function (fecal IgA).
- Total fecal samples were collected to assess digestibility.
- Blood samples were collected to measure serum chemistry, complete blood count and immune cell numbers and function.

### Statistical Methods:

- Fecal microbiota data were analyzed using QIIME 2.
- All other data were analyzed using the Mixed Model procedure of SAS 9.4.

## Results

Table 3: Fecal characteristics and metabolite and IgA concentrations in cats fed experimental diets

Measure	Dietary treatment			SEM	P-value	
	Control	Test diet 1	Test diet 2		Treatment	Control vs. Test
pH	4.87 <sup>a</sup>	5.63 <sup>b</sup>	5.97 <sup>b</sup>	0.122	<.0001	<.0001
Fecal score <sup>1</sup>	3.62 <sup>b</sup>	3.18 <sup>ab</sup>	2.67 <sup>a</sup>	0.195	0.0001	0.0002
Fecal DM (%)	25.57 <sup>a</sup>	28.21 <sup>ab</sup>	31.62 <sup>b</sup>	1.264	0.0008	0.0013
Propionate, µmol/g	51.87 <sup>a</sup>	125.11 <sup>b</sup>	139.82 <sup>b</sup>	6.658	<.0001	<.0001
Butyrate, µmol/g	212.19 <sup>c</sup>	124.54 <sup>b</sup>	60.29 <sup>a</sup>	18.397	<.0001	<.0001
Total BCFA, µmol/g	36.26 <sup>a</sup>	50.54 <sup>b</sup>	41.03 <sup>ab</sup>	3.765	0.0417	0.0478
Isobutyrate, µmol/g	1.89 <sup>a</sup>	4.50 <sup>b</sup>	5.83 <sup>b</sup>	0.489	<.0001	<.0001
Isovalerate, µmol/g	3.73 <sup>a</sup>	6.57 <sup>b</sup>	8.45 <sup>b</sup>	0.696	0.0002	0.0001
Valerate, µmol/g	30.65 <sup>ab</sup>	39.47 <sup>b</sup>	26.76 <sup>a</sup>	3.364	0.0370	0.5508
Total P/I, µmol/g	0.05 <sup>a</sup>	1.18 <sup>b</sup>	1.42 <sup>b</sup>	0.211	<.0001	<.0001
Ammonia, µmol/g	65.09 <sup>a</sup>	105.63 <sup>b</sup>	127.27 <sup>b</sup>	8.313	<.0001	<.0001
Fecal IgA, mg/g	16.75 <sup>b</sup>	13.93 <sup>b</sup>	7.91 <sup>a</sup>	1.593	0.0002	0.0010

<sup>1</sup>Fecal score: 1 = hard, dry pellets; small hard mass; 2 = hard formed, dry stool; remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured.

### Fecal Microbial Analyses

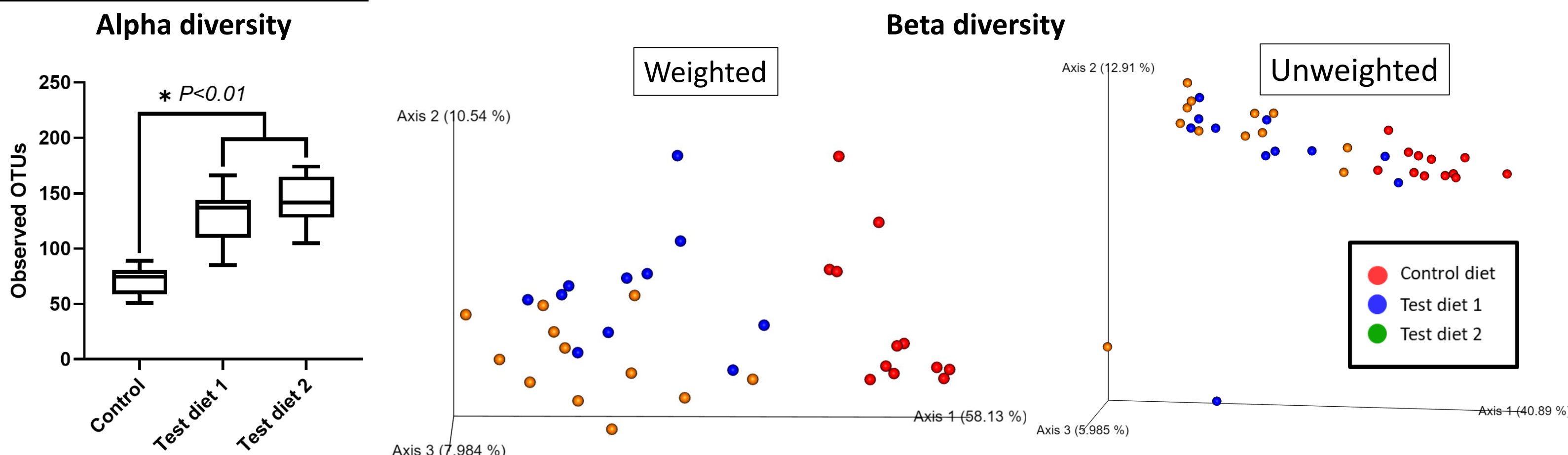


Figure 1. Alpha diversity measures (observed OTUs) suggest lower species richness in Control (P<0.05) vs. Test diets Principal coordinate analysis plots for weighted and unweighted UniFrac distances of fecal microbial communities were altered and differed in cats fed the Control diet compared to those fed the Test diets.

## Conclusion

- We observed mixed results of functional blends on gastrointestinal health markers. Changes to fecal pH, scores, and microbial richness were positive, but those to most fecal metabolites and immune markers were not.