

The safety and microbiome impact of a novel single cell protein source in kennel and inhome trials.

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FeedKind Pet

- Calysta founded in 2012 to re-establish and refine technology developed in the 1990s
- Aim to close protein gap in feed production through use of cultured protein
- FeedKind Pet is grown at commercial scale in a closed loop fermenter, heat-killed, dried and bagged ready for use.
- Main bacteria in the consortia is *Methylococcus* capsulatus





Animal Studies





Modes of Action



Study Design Comparison

In-home Study

Dog breeds: Mixed

Duration: 12 weeks

Design: Crossover study (6 test + 6

control weeks)

Animals: 12 (10M, 2F)

Doses: One (8%)

Measures: Faecal score, BCS,

intake, welfare

Study purpose: Research, consumer understanding



Kennel Study

Dog breeds: Beagles

Duration: 8 months

Design: Safety study (6 test + 2

control months)

Animals: 32 (16M, 16F)

Doses: Three (4, 6, & 8%)

Measures: Faecal score, BCS, intake, welfare, clinical chemistry, urinalysis, hematology, digestibility Study purpose: Regulatory (GLP for GRASN submission)

Comparison of Diet Formulations

- Diets were not matched between trials, but proximates were similar:
 - Protein ~ 29%
 - Fat 12-14%
 - \circ Fiber < 4%
 - Ash < 8%
 - Moisture < 10%
- Both diets were grain inclusive
- In-home diets had higher inclusion of meat meals (chicken and fish) whereas kennel diets had higher vegetable-based protein (corn and soybean)



FeedKind is a safe ingredient for dog food formulations

- All diets showed good palatability and were well-received by the dogs
- There were no differences between the start and end of the trial for:
 - \circ faecal score
 - \circ feed intake
 - \circ urinalysis
 - hematology
- Digestibility of all diets was > 80%
- The rest of our focus today will be on the microbiome



What do these trials together tell us about FeedKind's impact on the GI microbiome?

Feedkind is well-tolerated by dogs and their microbiomes

Feedkind produces a consistently diverse and functional microbiome with traits consistent with microbiomes produced by high animal-source diets

 This is observed even across highly variable breeds and environments

Length of time on the diet is important! Some changes are visible after 6 weeks, but only significant when measured at 6 months



CALYSTA

FK diet promotes diversity and consistency in the microbiome

Microbial richness is strongly associated with homeostasis and **beneficial health** outcomes. Higher or more consistent diversity can be taken as an **indication of good health**.

Supplementation for 6 weeks numerically increased richness (8/12 dogs). After 6 months, there was a significant improvement in diversity in the MD diet group.





No major shifts in composition after 6 weeks or 6 months







FK produces modest differences in specific bacteria, but all are shifts in normal commensal bacteria



SIMA CALYSTA FirstMate

The Best Nutrition is Simple

Prevotella-Bacteroides ratios shift in favor of Bacteroides

The **Prevotella-Bacteroides ratio** is a useful **biomarker of dietary adaptation** in the gut.

Prevotella

Bacteroides

In dogs, the ratio can serve as a proxy for dietary composition, where high Prevotella/Bacteroides ratios are more typically associated with more fermentable carbohydrates (e.g., fiber-rich kibble, plant-derived treats). A low ratio (more Bacteroides) is associated with higher animal-source protein/fat diets (e.g., raw or high-meat formulations).

More Bacteroides \leftarrow 0 < log PB-ratio > 0 \rightarrow More Prevotella



Prevotella-Bacteroides ratios shift in favor of Bacteroides



The **FeedKind diet** in the in-home study produced a non-significant **lower PB ratio** than the baseline diet. We see in the kennel study that the PB ratio is also decreased at D178, after 6 months of feeding. This **difference is not maintained** 2 months after **cessation of the diet**.



Take home message

Good acceptance by the dogs in both trials

Lack of evidence of dysbiosis or negative GI outcomes

Lack of evidence of negative serum/urine outcomes

Shifts in the microbiome suggest that the protein stimulates a microbiome response similar to that of a high-animal-source diet

Suggestion of beneficial outcomes in the gut microbiome



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