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Repeatability of the effects of dietary supplementation of yeast probiotic on gut fermentative metabolites and microbiota in dogs

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- I have the following disclosures\* related to my presentation
  - Employee: Gastrointestinal Laboratory, Texas A&M University
  - Grants/Research Contracts: Purina PetCare Research Excellence Fund, Royal Canin, AKC-CHF
  - Consulting: Purina, Nutramax Laboratories, IDEXX Laboratories, Phileo
  - Investments: none
- I will discuss results of clinical trial(s) for the following agents that are currently NOT approved for use in animals.

\*Disclosures include spouse and immediate family where relevant.



## **HEALTHY COLON**



Suchodolski JS - Assessing and Managing the Gut Microbiome in Canine and Feline Practice. In "Purina Institute - Canine and Feline Clinical Nutrition Handbook, 2023 edition"



Live microorganism (what we see as feces)

Mucus layer

Epithelium

Courtesy: Dr. Chi-Hsuan Sung, GI LAB, TAMU

#### Digestive health pillars involved in etio-pathogenic mechanisms and risk factors



Actisaf white book- Phileo by Lesaffre (2023)



Actisaf white book- Phileo by Lesaffre (2023)

#### **Objective**

 To evaluate the repeatability of the effect of yeast probiotic Saccharomyces cerevisiae on fermentative metabolites and gut microbiota composition and function in dogs undergoing abrupt dietary change.

## **Design & Diet**

- Animals: healthy adult Beagle dogs (1-5 years)
- Challenge type: abrupt nutritional diet transition from moderate to high protein & fiber content
- Study type & duration: longitudinal study repeated after 3 months period in dogs included in 2 groups: Actisaf<sup>®</sup>
   Sc50 PET for at least 28 days vs control
- Key read-out assessment: digestibility, fecal characteristic, immunity & microbiota

Actisaf<sup>®</sup> Sc50 PET yeast probiotic dose: 0.12g (1x 10<sup>10</sup>CFU/g) *S. Cerevisae* animal/day. 6 x 10<sup>9</sup>CFU/ kg of diet



# LPF DIET

**20%** CRUDE PROTEIN

**6%** TOTAL DIETARY FIBER

5% INSOLUBLE 1% SOLUBLE



**NO FUNCTIONAL ADDITIVES** 



HPF DIET 28% crude protein

**28%** TOTAL DIETARY FIBER

26% INSOLUBLE2% SOLUBLE



**NO FUNCTIONAL ADDITIVES** 



## Live Saccharomyces cerevisiae



Saccharomyces cerevisiae. CNCM I-5660) (Actisaf<sup>®</sup>, Phileo by Lesaffre, Marcq-en-Barœul, France)



## The Dysbiosis Index

RESEARCH ARTICLE

enteropathy

A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory

MK AlShawaqfeh<sup>1,2</sup>, B Wajid<sup>1,3</sup>, Y Minamoto<sup>1</sup>, M Markel<sup>1</sup>, JA Lidbury<sup>1</sup>, JM Steiner<sup>1</sup>, E Serpedin<sup>2</sup> and JS Suchodolski<sup>1,\*</sup>

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"Corresponding author: Gastrointestinal Laboratory Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, 72: 7784-744, USA. Tel: -978-480 3032: mail: Incideodial&Overn human edu One sentence summary: The aim was to develop a rayid PCR-based dyabiosis index to assess microbiota changes in dogs. Editor, Julian Marchesi

#### Beneficial





#### Properties

anti-inflammatory

Short chain fatty acids producer

Short chain fatty acids producer

Short chain fatty acids producer

Bile acid converter

Overgrowth in maldigestion Pro-inflammatory



## The Dysbiosis Index

Correlation with species diversity based on full 16S sequencing





#### **Statistics**

- One-way ANOVA was performed to assess the effects of supplementation on fecal pH, IgA, Ammonia, Biogenic amines, SCFA, BCFA, dysbiosis index and bacteria qPCR results.
- Normality and homoscedasticity assumptions were visually checked using QQ plots. Kruskal Wallis was used when if residual residuals were not uniformly distributed
- PCA was performed to assess the relationship between yeast probiotic supplementation and fecal variables in Trials 1 and 2. Comparisons among groups were performed by similarity analysis ANOSIM.

#### **Statistics**

- Statistical analyses performed using Minitab<sup>®</sup> (version 19.2020.1) software.
- Dog was the experimental unit
- Observations that were at least 1.5 times the interquartile range were considered as possible outliers. A Grubbs' test was performed for confirmation if only one possible outlier was detected. If more than one possible outlier were detected, a Rosner test was done using the function "rosnertest" from package "EnvStats" (Millard 2013) with software (version 4.0.2). Data are presented as means and SD.



Bastos et al. (2023)





Day (p < 0.001), S (p < 0.001), Day × S (p < 0.001) Day (p < 0.001), S (p < 0.001), Day × S (p < 0.001)

Bastos et al. (2023)



P<0,05

#### **Results. Fecal Characteristics**



Day (p < 0.001), S (p = 0.01), Day × S (p = 0.08) Day (p < 0.001), S (p < 0.001), Day × S (p = 0.01) Day (p < 0.001), S (p < 0.001), Day × S (p = 0.04)

#### **Results. Functional Genes**



Bastos et al. (2023)

The control group showed upregulation in genes related to virulence factors, antibiotic resistance, and osmotic stress

Virulence factor – *Streptococcus* 

**Activated in osmotic stress – nitrogen metabolites** 

Higher transportation of biogenic amines

**Greater methanogenesis** 



### **Results. DNA Shotgun Sequencing for probiotic**

#### SACCHAROMYCES CEREVISIAE



#### There was no cross-contamination among the groups in both experiments

# Results. Microbiome and Metabolome





Adib Lesaux et al. Under preparation

#### **Key Messages**

Daily supplementation with yeast probiotic in healthly dogs

- Safe and well tolerated by dogs (weight & body health condition maintained)
- Improves fecal quality through significant decrease of fecal putrefactive compounds (pH, ammonia & some biogenic amines) which may have a negative impact on gut health, impair intestinal functionality & worsen fecal odor.



#### **Key Messages**

Daily supplementation with yeast probiotic in healthly dogs

- Improves dysbiosis index and modulate gut microbiota composition and function
- The response to probiotic consumption was repeatable after 3 months period in dogs
- Promising results need to be confirmed in larger population with various life stages and/or disease models



# Thank You!

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Gastrointestinal Laboratory



