WELCOME TO PETFOOD FORUM 2023

PETFOOD FORUM

May 1-3, 2023

#petfoodforum • www.PetfoodForumEvents.com

Measuring the Impact of Chicken Meal and Chicken By-product Meal Oxidation on Canine Palatability, Enose Chromatograms, Metabolomics and Caco-2 Cell Viability

Fiona B. Mccracken, Lili Towa, Jason W. Fowler, Jessica Varney, Craig N. Coon

PETFOOD FORUM

FOUR RIVERS KENNEL, LLC

#petfoodforum

COTTEY COLLEGE

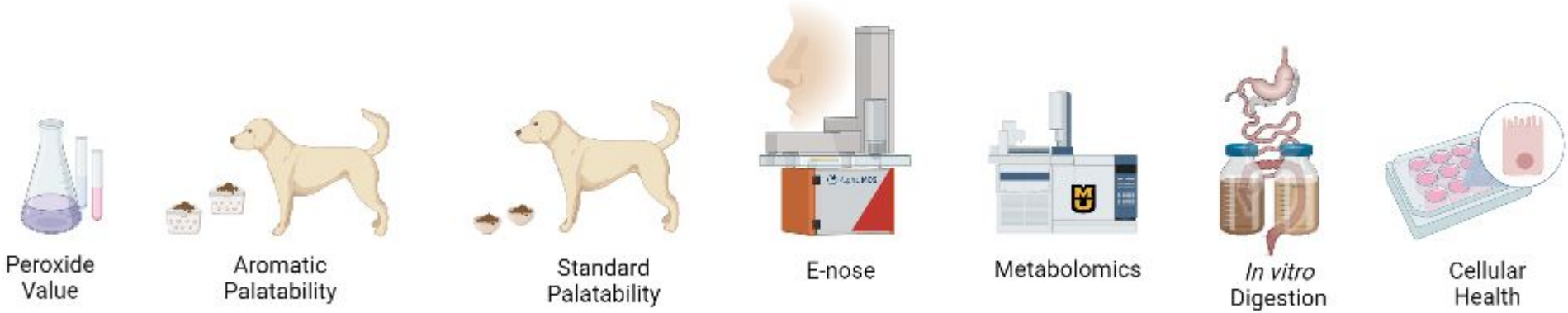
Background

- Rancidity, oxidation, and spoilage are a major source of dissatisfaction with products, industry waste, and financial losses
- Understanding the effects of oxidation can allow the industry to utilize more ingredients, improve quality of products, and create a more palatable product
- Advances in sensory technology reduce the reliance on sensory panels/consumer panels, provide a higher sample throughput, and create high quality data
- Recently researchers have begun to utilize chromatography methods to evaluate the sensory and palatability properties of dog foods[1-3]
- This previous research has focused primarily on dog food attractants, rather than off-odors and palatability reducers

[1] Koppel et al., 2014; [2] Chen et al., 2017; [3] Yin et al., 2020

Study Overview

- Four Rivers Kennel embarked on a substantial project to take a comprehensive look at how lipid oxidation in rendered chicken meals affects quality of companion animal feeds
- The project is funded by the Fats and Proteins Research foundation and investigates many aspects of lipid oxidation



PETFOOD FORUM #petfoodforum

FOUR RIVERS KENNEL, LLC

COTTEY COLLEGE

Methods

PETFOOD FORUM

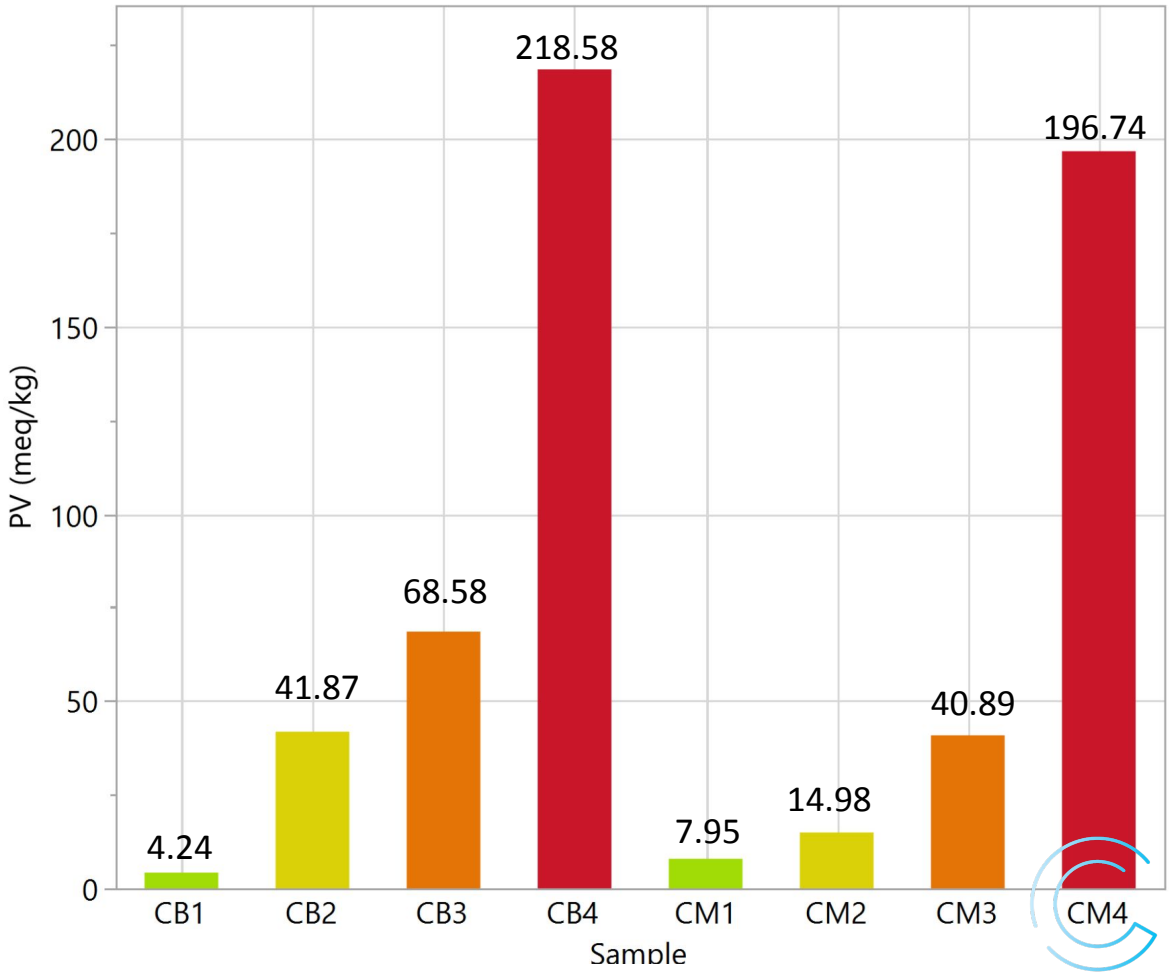
FOUR RIVERS KENNEL, LLC

#petfoodforum

COTTEY COLLEGE

Samples

- Samples of rendered chicken meal with varying levels of oxidation
  - Chicken by-product meal (CB) and chicken meal (CM) acquired from a commercial poultry producer
  - PV determined through iodometric titration
  - Samples were stored in airtight containers at 4C



Aromatic Palatability

A two pan aromatic palatability trial was designed to evaluate the effect of aromatic changes on palatability

Aromatic Palatability

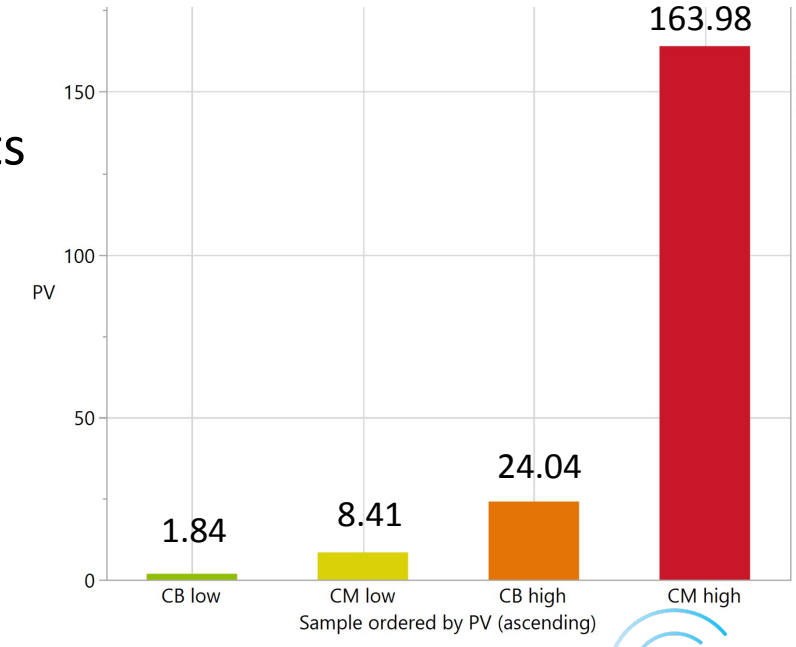
- 20 dogs (10M; 10F)
- Aromatic sample boxes designed for interaction without consumption
- 2 comparisons per interaction test
- Each PV level was tested against the other for both CB and CM
- 60 sec time limit per dog/interaction test
- First approach, and interaction time was recorded

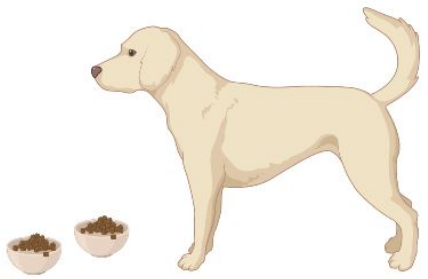
Standard Palatability

A standard two pan palatability trial was also completed to validate the trends observed

Standard Palatability

- Additional samples were acquired for traditional 2-pan tests
- Standard 2-pan methods used
- 10 dogs (5M/5F)
- 2 comparisons per interaction test
- Intake ratio, first approach, first bite recorded





PETFOOD FORUM #petfoodforum

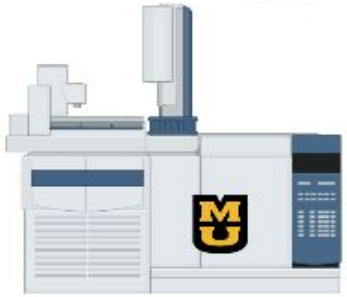
FOUR RIVERS KENNEL, LLC

COTTEY COLLEGE

Metabolomics

Polar, nonpolar and volatile compounds were tentatively identified through broad spectrum GC-MS at the University of Missouri's Metabolomics Center

- Derivatization: methoxylated in pyridine with methoxyamine hydrochloride, and then trimethylsilylated with MSTFA (N-methyl -N-(trimethyl-silyl) trifluoroacetamide) + 1%TMCS (chlorotrimethylsilane) reagent
- GC-MS with scan range from m/z 50 to 650
  - 60 m DB-5MS column
    - 0.25 mm ID, 0.25 mm film thickness
  - Split ratio of 1:1
  - 80C for 2 minutes, ramped at 5C /min to 315 ºC, hold at 315 ºC for 12 minutes
  - Constant flow of 1.0 mL/minute of helium gas



E-nose

Electronic nose technology at Alpha MOS was utilized to evaluate the odors present in the samples

Heracles Neo UFGC electronic nose
- PAL RSI autosampler
- Flame Ionization Detectors

Column
- 10m length and 0.18mm ID
- MXT-5 column for non-polar compounds
- MXT-1701 for slightly polar compounds

Calibration
- n-pentane to n-hexadecane alkane solution
- retention time converted to Kovats indices
- AroChemBase V7 database

Procedure
- 2g of sample in 20 mL vial
- 250 s acquisition duration
- Incubated for 20 min at 60C
- 5 mL injection volume

In vitro Digestion

Samples were introduced to an in vitro digestion process

- Gastric phase (pH 3.0):
  - 5 grams sample meal
  - 2,000 U/mL pepsin
  - 0.15 mM calcium chloride
  - Incubated at 37C for two hours
- Intestinal phase (pH 7.0):
  - 10 mM bile
  - 100 U/mL pancreatin
  - 2,000 U/ml lipase
  - 0.6 mM calcium chloride
  - Incubated at 37C for two hours
- Processing:
  - samples were heat shocked at 100C
  - separated and frozen

Cell

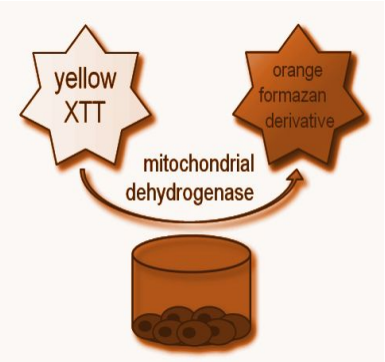
Sample digest were used in cellular health assays to investigate the relationship between PV and cellular damage in the intestines

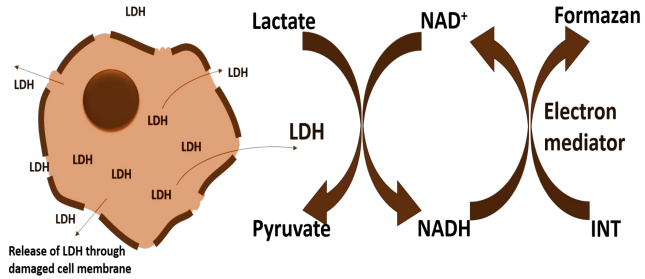
Culture conditions

- Caco-2 cells between passages 14 and 20
- Cultured in DMEM
- with 10% FBS, 1X anti-anti, 1% NEAA, and 10% L-Glutamate
- Incubated at 37C and 5% CO2
- Seeded in 96 well plates at 1x10^6 cells/ml

Assays

- 100 ul of filter sterilized digesta added to wells
- Incubated for 20 hours
- LDH release assay and XTT viability assay
  - completed according to manufacturer's instructions





PETFOOD FORUM #petfoodforum

FOUR RIVERS KENNEL, LLC

COTTEY COLLEGE

Statistics

All statistics were completed in JMP Pro 16

Statistical Significance

- Metabolomics, cell viability, and palatability analyzed with ANOVAs and Tukey's post-hoc tests
- Statistical significance was defined as $P < 0.05$

PCA

- The PCA of e-nose chromatograms were ran in the unscaled area peaks

PLSR

- Primary least squares regression models were run to determine relationship between odor and palatability
- Average peak area of each MXT5 KI as the X variable
- Average PV * average aggregated interaction time as the response variable (Y)
- SIMPL with automatic scaling and two factors

Results

PETFOOD FORUM

FOUR RIVERS KENNEL, LLC

#petfoodforum

COTTEY COLLEGE

Metabolomics Results

Composition between samples did not vary significantly.

- 431 volatile compounds
- 340 polar compounds
- 177 nonpolar compounds

	Volatile SPME GC-MS Analysis	Nonpolar GC-MS Analysis	Polar GC-MS Analysis
Functional group (# of compound)	Acid (24)	Acid (41)	Acid (51)
	Alcohol (21)	Alcohol (17)	Alcohol (27)
	Aldehyde (27)	Aldehyde (1)	Amino Acid (48)
	Amino Acid (2)	Amide (1)	Ester (11)
	Ester (13)	Amino Acid (8)	Hydrocarbon (11)
	Silicate (4)	Ester (11)	Ketone (5)
	Hydrocarbon (67)	Ether (2)	N Organic (31)
	Ketone (27)	Hydrocarbon (9)	N and S Organic (2)
	N Organic (5)	Ketone (3)	Unknown (160)
	S Organic (1)	N Organic (11)	
	Unknown (244)	Unknown (78)	
Total	431	177	340

PETFOOD FORUM

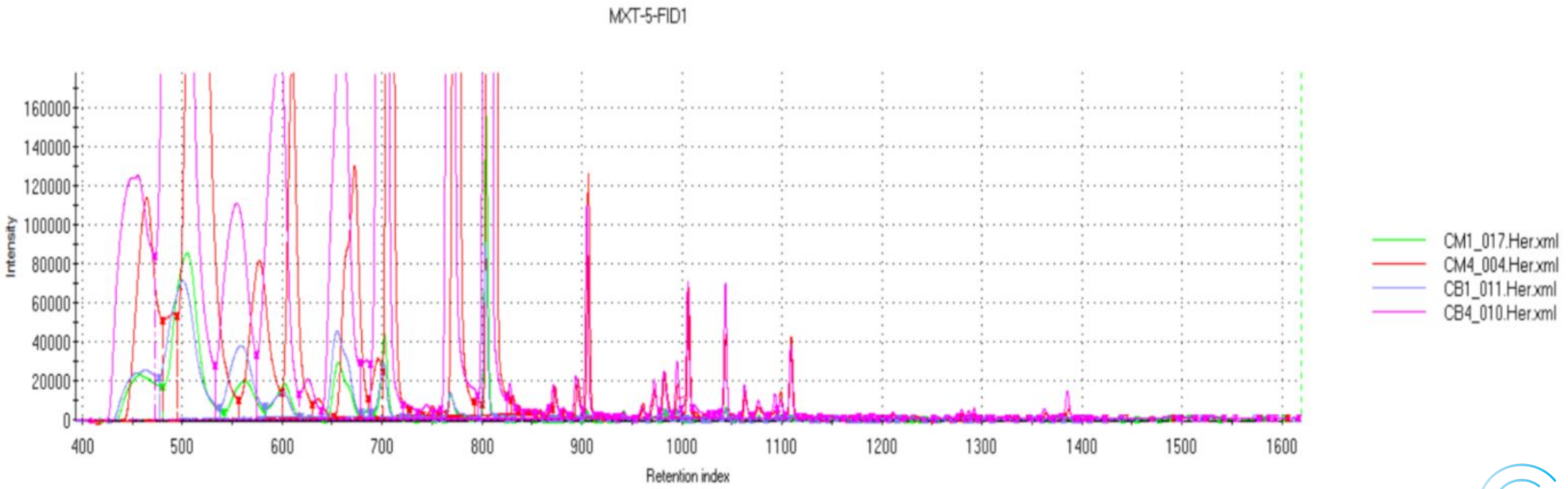
FOUR RIVERS KENNEL, LLC

#petfoodforum

COTTEY COLLEGE

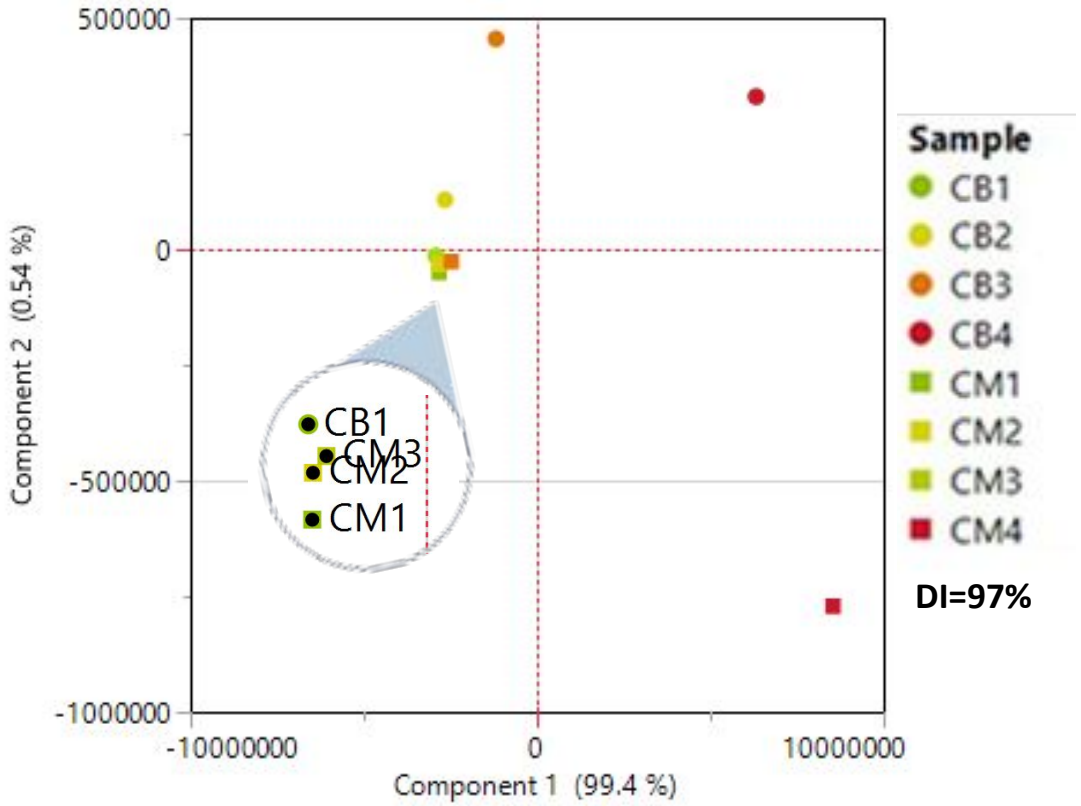
Enose Results

There were differences in enose chromatograms between samples



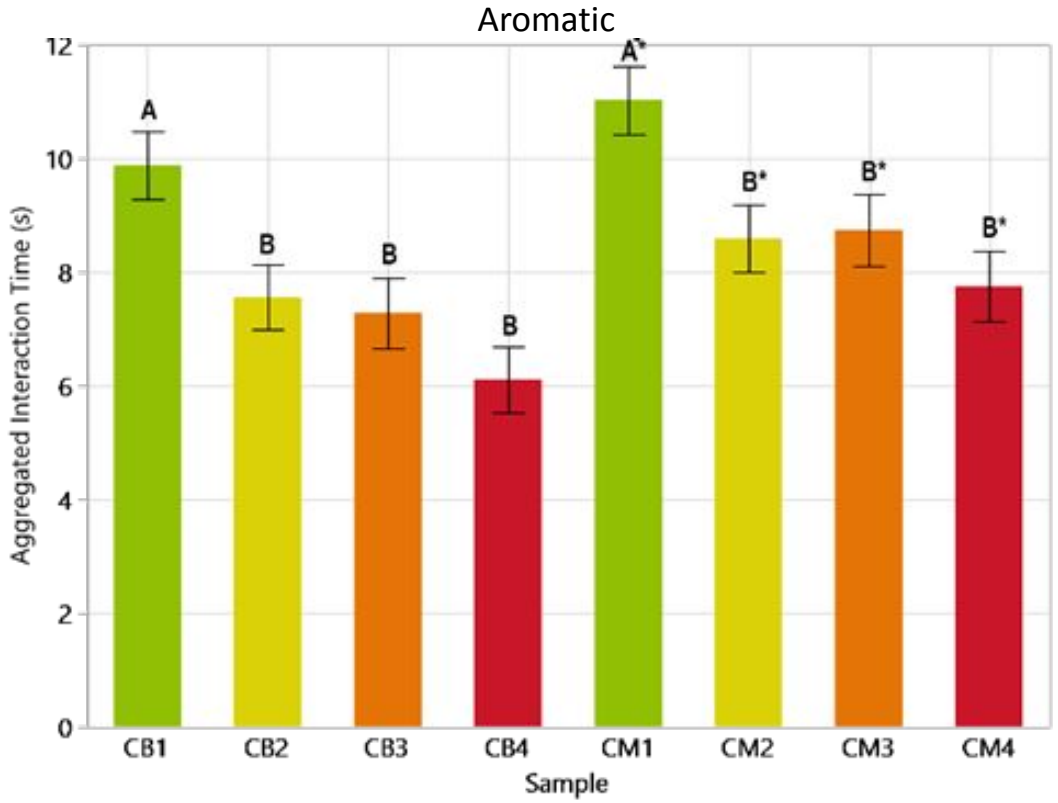
Enose Results

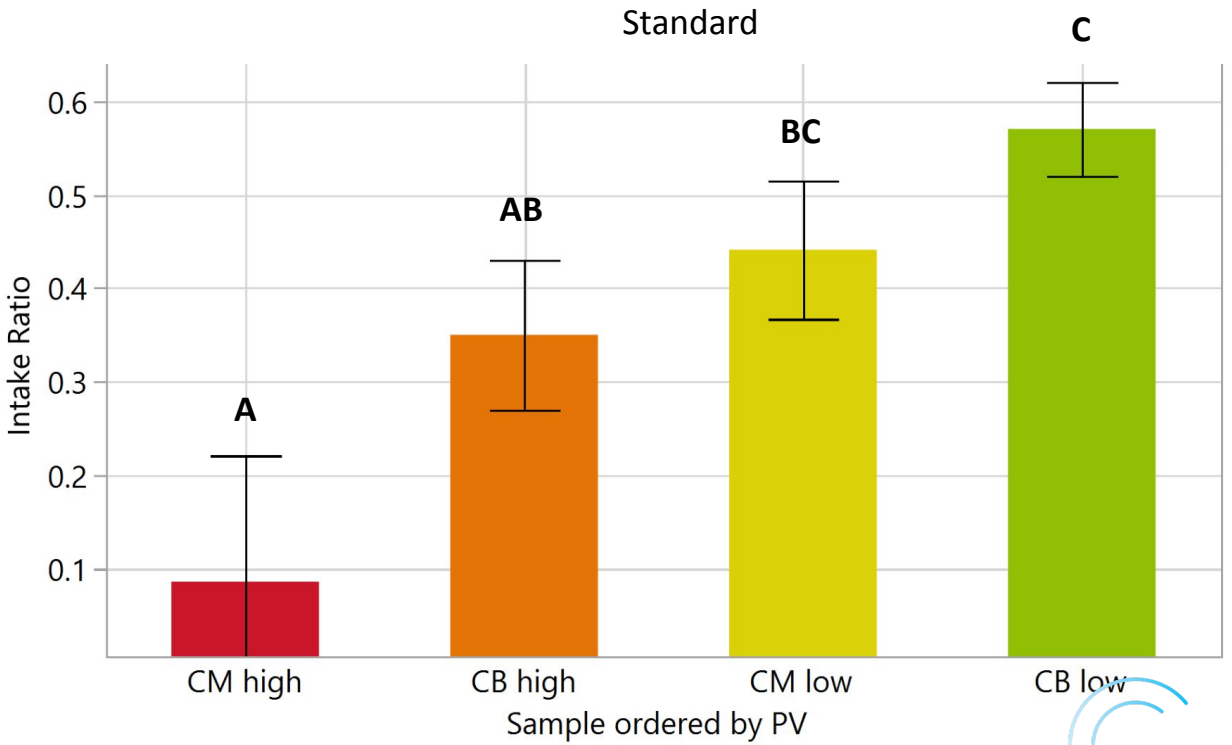
Primary Component Analysis created from the e-nose area peaks showed that differences in odors was correlated with increasing oxidation



Palatability Results

Increasing oxidation decreased both the aromatic and standard palatability





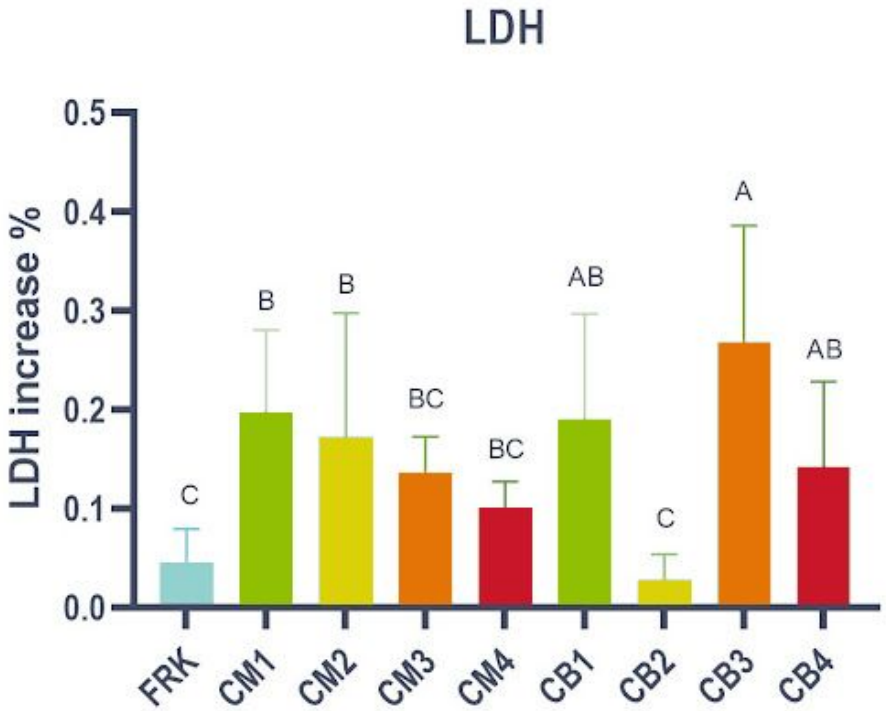
PETFOOD FORUM #petfoodforum

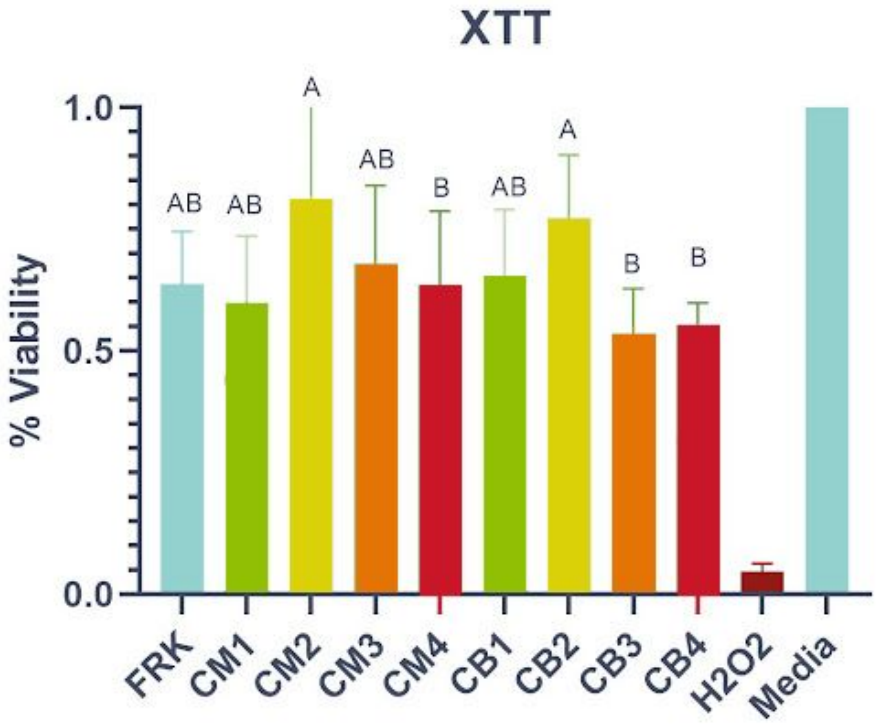
FOUR RIVERS KENNEL, LLC

COTTEY COLLEGE

Cellular health

Neither cellular health assay showed trends associated with the meal's PV, perhaps due to compositional differences or degradation of cytotoxic compounds due to the digestion process





PETFOOD FORUM #petfoodforum

FOUR RIVERS KENNEL, LLC

COTTEY COLLEGE

Discussion

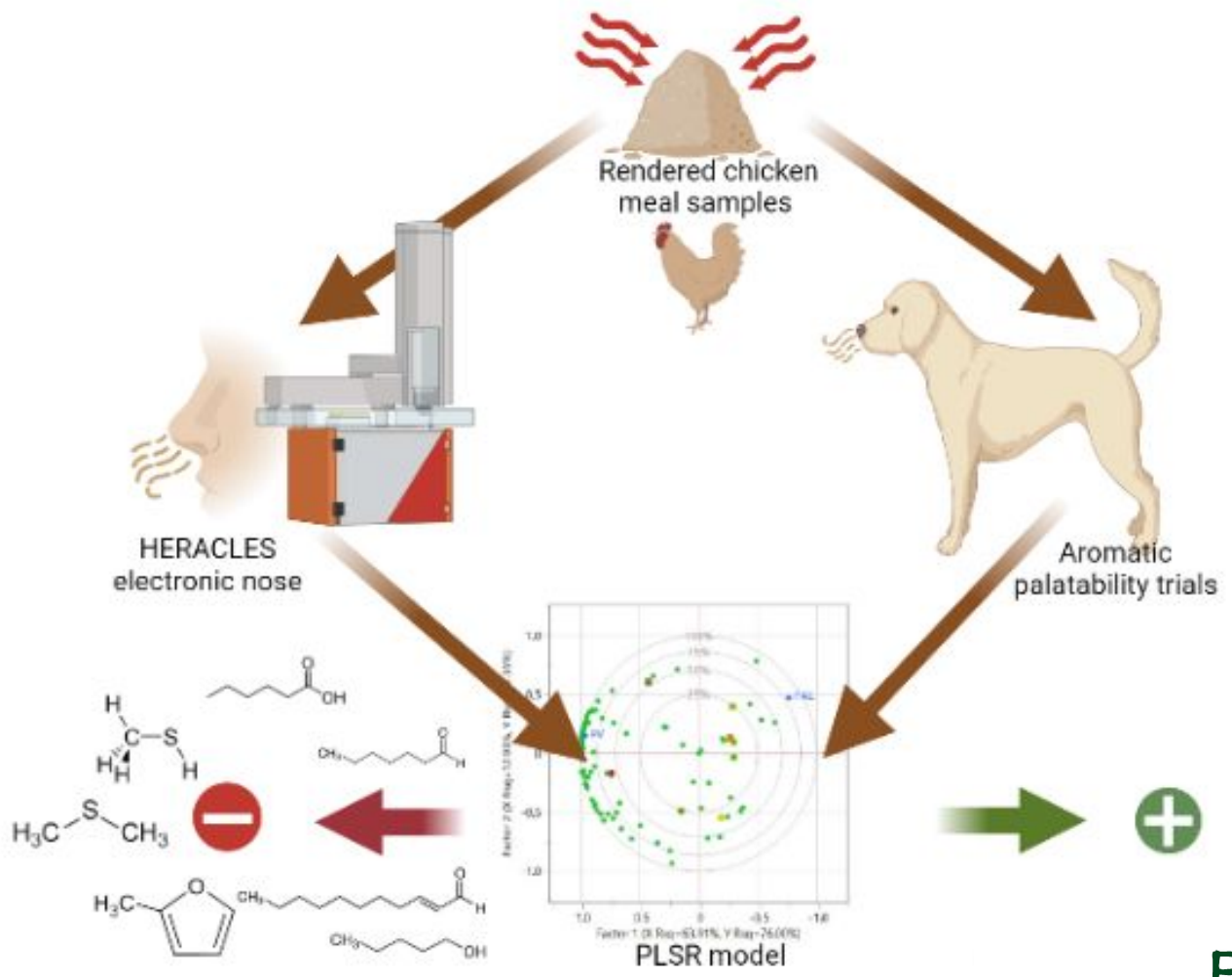
PETFOOD FORUM

FOUR RIVERS KENNEL, LLC

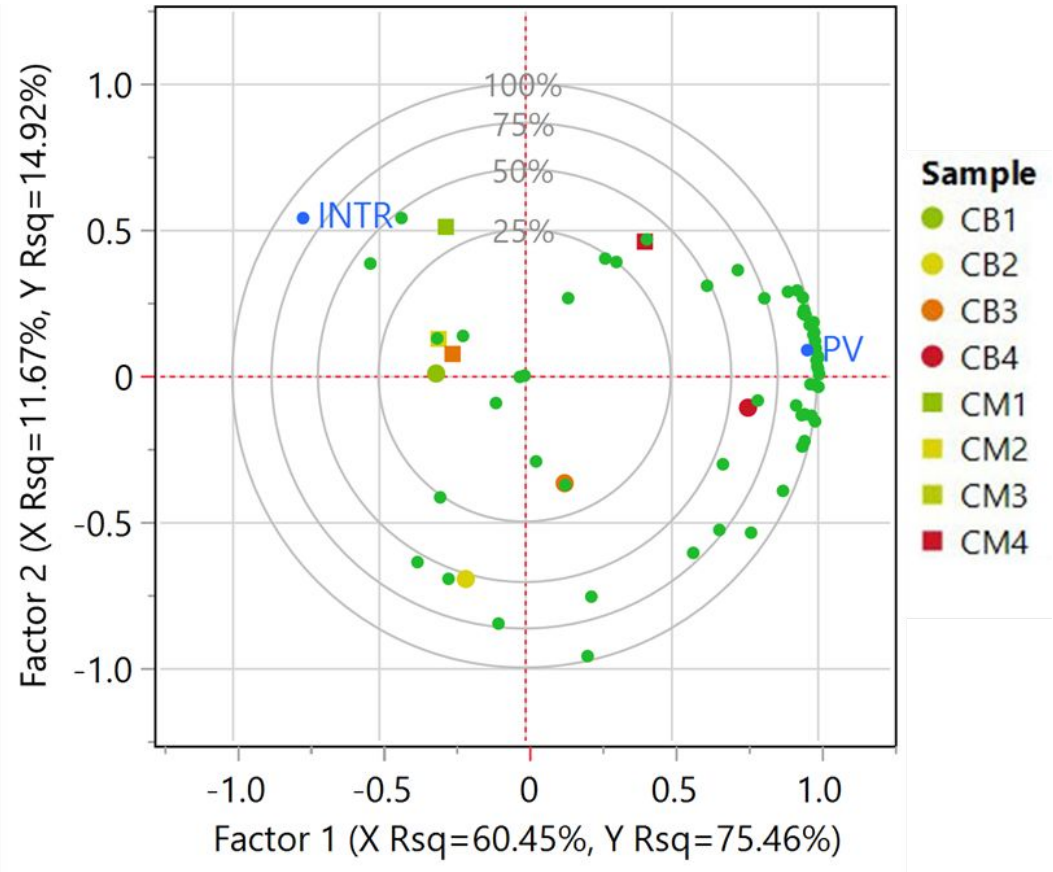
#petfoodforum

COTTEY COLLEGE

Correlations



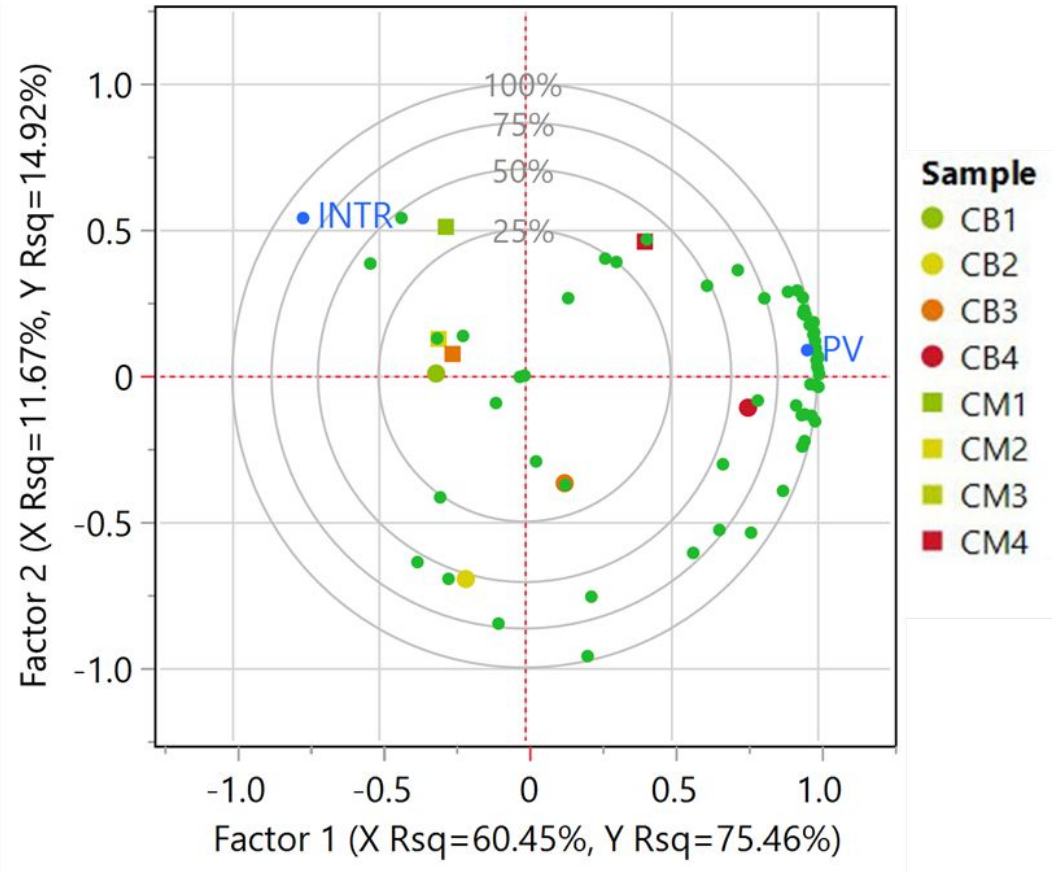
Correlations



The PLSR model showed a strong fit

- The model explained 72% of the variation in the e-nose peak area data (X) and 90% of the variation in the PV*palatability (Y)
- The majority of compounds clustered with PV
- Palatability (INTR) showed a negative spatial correlation with oxidation (PV)

Correlations

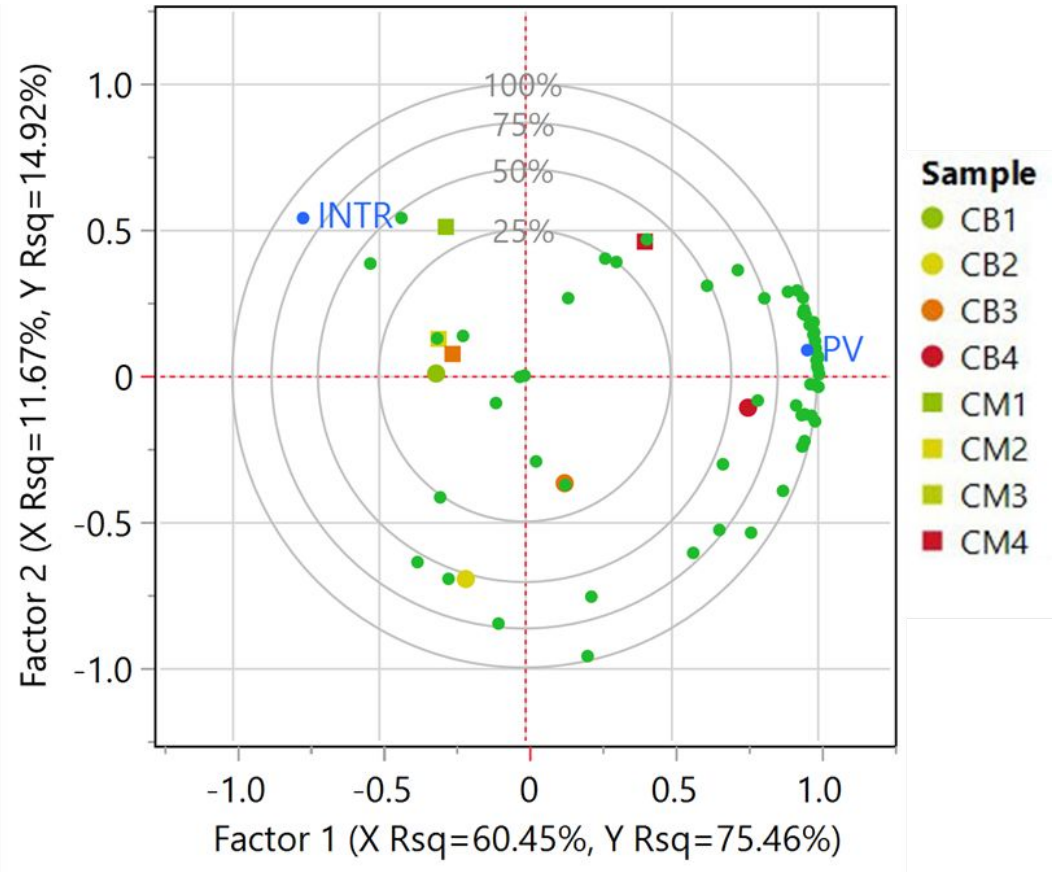


36 compounds with a VIP score > 1.0

The top drivers of reduced palatability included:

- methanethiol (1.3)
- dimethyl sulfide (1.3)
- 2-methylfuran (1.3)
- pentanol (1.3)
- propanoic acid (1.3)
- 1-hexanol (1.3)
- heptanal (1.3)
- pentanoic acid (1.3)
- hexanoic acid (1.3)

Correlations



- Multiple other compounds had a significant effect on palatability
- Scaling controls for the magnitude of components such as hexanal
  - allows the detection of less abundant compounds that still have a strong influence on palatability.
- Observed compounds associated with chicken meat's off odors after heat processing
  - Maillard reaction products
  - Oxidation products
- The identified compounds may be utilized as quality control markers, or in development of more palatable dog foods

PETFOOD FORUM #petfoodforum

FOUR RIVERS KENNEL, LLC

COTTEY COLLEGE

Conclusion

PETFOOD FORUM

FOUR RIVERS KENNEL, LLC

#petfoodforum

COTTEY COLLEGE

Conclusions

- Oxidation did not result in overall changes to a rendered meat meals' chemical composition
  - But likely effects the concentration of some compounds
- Oxidation strongly affected a meal's odor
  - particularly by increasing hexanal concentrations
- The creation of off odors in meat meals negatively influences palatability in Labrador retrievers
  - both aromatic and oral palatability
- PLSR models can be used to identify specific drivers of reduced palatability
  - These drivers are not necessarily the strongest odors
- Further research is needed to clarify the potential of adverse consequences on intestinal cellular health

Acknowledgments

Special thanks to Dr. Sumner's lab, Lili Towa at AlphaMOS, and BJ Bench from Tyson.

This project was generously funded by grants from the Fats and Protein Research Foundation.

Fats and Proteins Research Foundation

Tyson

University of Missouri

Alpha MOS

PETFOOD FORUM

#petfoodforum

FOUR RIVERS KENNEL, LLC

COTTEY COLLEGE

Questions?

PETFOOD FORUM

FOUR RIVERS KENNEL, LLC

#petfoodforum

COTTEY COLLEGE