Development of a Rugged Sugar Method for Animal Feeds

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Agenda

- Lessons learned from mini-collaborative

- Method Development

- Single Laboratory Validation

- Path forward

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Method Needs Statement

6 Mono and Disaccharides: Sucrose, Lactose, Maltose, Glucose, Fructose, Galactose

Operational Range: 0.1 to 100%

Accuracy and Precision:

Content	Accuracy	Repeatability (CV$_r$)	Reproducibility (CV$_R$)
0.1 to 1%	90 – 108%	<5 %	<10 %
1 to 10%	92 – 105%	<4 %	<8 %
10 to 100%	95 – 102%	<3 %	<6 %

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Lessons learned from mini-collaborative

Mini Collaborative

Using same 50% Ethanol extraction and HPAEC-PAD detection

3 Laboratories
Tested on 2 days
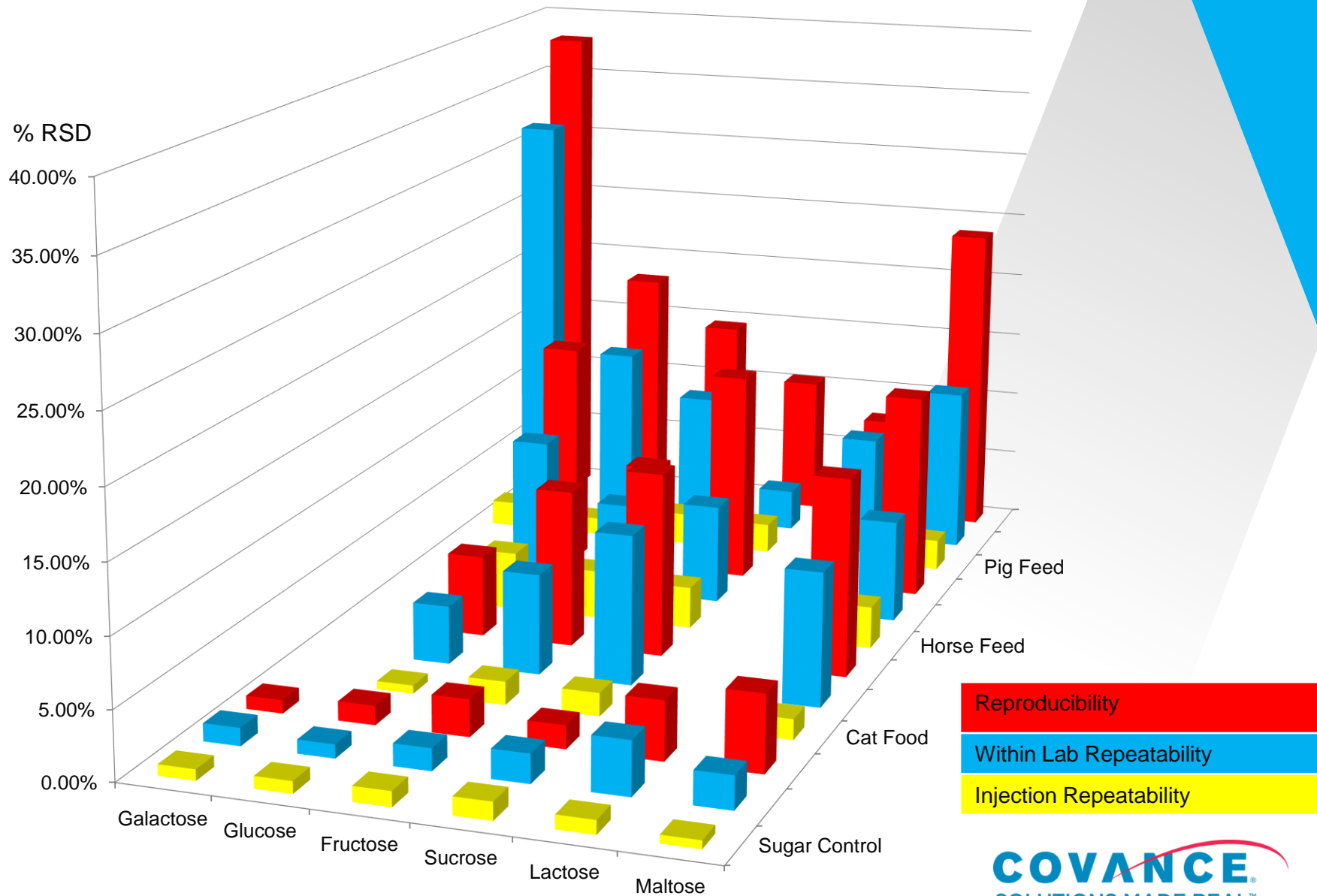Duplicate injections

Total of all sugars	Mean	Range	RSD of all data
Sugar standard	3.13	3.04 – 3.23	1.9%
Cat food	2.07	1.67 – 2.29	11.1%
Horse feed	2.75	2.33 – 3.24	12.9%
Pig feed	5.45	4.62 – 5.99	9.0%

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Repeatability and Reproducibility

Show Pig Primer
AAFCO Check Sample 201425

12 labs reported values for Total sugars

1 lab 2.2% excluded

	Mean	Range	Multi lab Reproducibility (CV_R)
AAFCO data from 11 labs	6.85	4.87 – 8.31	18.8%
Mini-collaborative	5.45	4.62 – 5.99	9.0%

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Method Development

Milk replacer comparison to Check Sample Data

7 labs reported values for Total sugars

Mean 28.709%

Range 20.695 – 34.490%

RS_D 21.3%

From SLV

    30.7% Lactose

    1.1% Glucose

    0.5% Galactose

    1.4% RSD

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Column evaluation

Previous work on a PA-1 Column with gradient
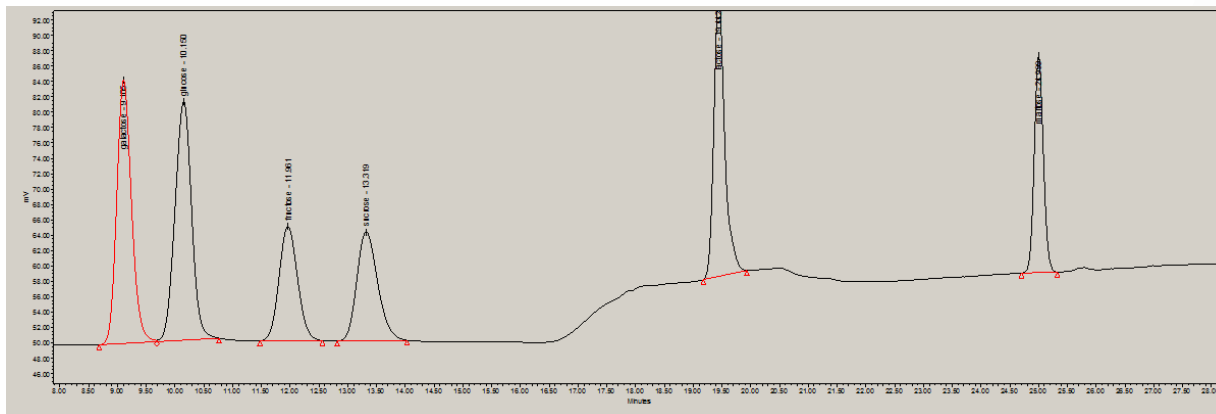
SA-10 Column

   Isocratic separation to improve ruggedness between labs

   Greater sensitivity allows for dilution of interferences

   Result: coellution of stachyose and raffinose with measured peaks

PA-20 Column

   Isocratic through 4 peaks then gradient. Passed specificity

Intermediate Precision

Day to Day and Interlab variance points to possible sample non-homogeneity

Increase sample size to 5 g and extract into 200 mL solvent

Single Laboratory Validation method

Sample Prep
        5g ground sample
        Extract into 100ml 50% EtOH for 1hr at room temp
        Bring to 200ml

Extract handling
        Filter and centrifuge through 10k Da Molecular Weight Filter tube
        Dry under nitrogen at 55 C
        Reconstitute into 5ml of Ulta Pure Water.
        Dilute 1/10 with UPW or further if needed

Analysis

        Dionex ICS-5000 HPAEC-PAD
        10 uL injection 30° C column
        CarboPac PA20 analytical column (3mm X 150mm)
        hold at 10 mM NaOH, then gradient to 200 mM NaOH
        Gold Electrode using triple potential waveform

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Single Lab Validation Results

Feed	Day 1 Total Sugar	Day 1 RSD	Day 2 Total Sugar	Day 2 RSD	Day 3 Total Sugar	Day 3 RSD	Overall RSD
Dry Dog Food	2.36%	4.2%	2.52%	1.5%	2.45%	4.7%	4.3%
Dry Cat Food	1.95%	3.0%	1.95%	1.1%	1.59%	3.9%	10.1%
Wet Cat Food	2.56%	9.6%	2.35%	16.0%	3.02%	6.5%	14.7%
Horse Feed	2.54%	1.6%	2.65%	2.6%	2.57%	0.6%	2.4%
Pig Feed	12.8%	8.7%	13.5%	0.8%	12.8%	3.5%	5.5%

4 replicates each day, spans multiple analysts and instruments

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Confidential

Path forward

Finalize Single Laboratory Validation

Publish in JAOAC

Acceptance by Board for use in claims

Initiate Collaborative study and official method status

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