

# VITAMIN A: METHOD EVALUATION

Heidi Hikes

# VITAMIN A EVALUATION

- Long history
- 1987 HPLC/UV method
- Bad performer: repeatability
- ISO 17025 Accreditation
- Influence of this committee
- Best performing method?

# 2015-2016 FEED SEASON

- ~ 345 feed samples
  - 93 pet foods
  - 252 animal feeds
- Total deficient samples: **110/345 (31.9 %)**
  - 14/93 (15.1%) deficient pet foods
  - 96/252 (38.1%) deficient animal feeds
- Some samples deficient for more than one component
- Vitamin A: worst performer

# BREAKDOWN OF FEED TYPES

## Feed Class

Beef

Poultry

Goat/Sheep

Swine

Dairy

Horse

Multiple Species

Exotic

## Form of Feed

Pellet

Meal

Granular

Texture

Cube

Milk

Crumble

Liquid

Compressed

Poured

Premix

Mineral

## Package Type

packaged

bagged

bulk

totes



# SAMPLES

- Samples arrive like this... very heterogeneous!
- Mass reduction to a subsample/split with a mechanical splitter
- Vitamin A Split - ~1/10
- Grind samples prior to testing
  - 0.75 mm grind
  - If a sample is being tested for Vitamin A or a drug - 2 mm grind

# DEFICIENT SAMPLE LAB PROCESSES

- If deficient a second split is obtained and tested
- Often include duplicate for second split
- Collect split and duplicate results
- Determine % RPD

# COMPILE DATA/DETERMINE %RPD

CLASS	FORM	CONTAINER	SPLIT	CLAIM	ANALYTE	RUN	RESULT	SPLIT AVG.	RPD %
beef	pellet	packaged	riffle	5000 IU/lb	VIT A-1	1	2962.000		
					VIT A-2	2	2501.000	2731.0000	
					reported		2700.000	2730.0000	16.9

1. The first split is tested during the initial run (run-1)
2. If deficient, a second split is obtained and tested (run-2)
3. An average of each split is calculated
4. The relative percent difference is calculated between the two split values

# AVERAGE RPD%

## Good Performers

Component	Average %RPD	N=
Calcium	1.66%	10
Fat	2.18%	11
Lysine	1.88%	8
Phosphorous	1.69%	13
Protein	1.51%	25
Salt	2.22%	17
Selenium	2.70%	6
Zinc	4.26%	5



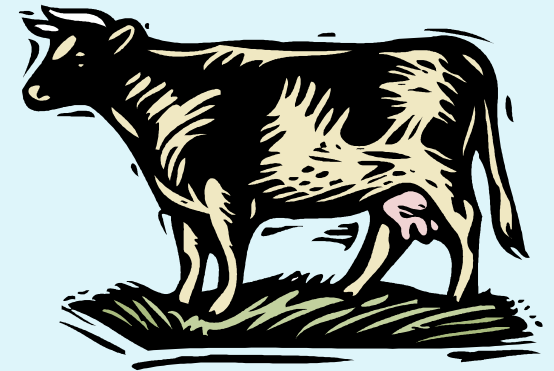
# AVERAGE RPD%

## Poor Performers

Component	Average %RPD	N=
Copper	11.0%	10
Vitamin A	14.5%	18

# VITAMIN A METHOD

- Vitamin A: worst performers
- SDSU method-LC/UV SPE
- Recognizing method inconsistencies
- What method is best choice
- Evaluate Indiana method



# VITAMIN A METHODS

## SDSU Method

- The Extraction of Vitamin A From Feeds, Premixes and Animal Remedies Using Solid Phase Extraction Disk Cartridges, pp. 1-6, Olson Biochemistry Laboratories, Department of Chemistry and Biochemistry, South Dakota State University, Box 1270, Brookings, SD 57007-1217

## Indiana Method

- Office of Indiana State Chemist Feed Chromatography Laboratory, Method 118-40: Analysis for Vitamin A in Feeds by HPLC Pyrene Internal Standard Overnight Shake Assay

# DIFFERENCES BETWEEN METHODS

## SDSU

- 2.0 mm sample grind
- Hot plate for 30-45 min
- SPE cleanup
- Methanol

## Indiana

- 0.75 mm sample grind
- Shake for 16-18 hours
- Neutralizing Solution
- ACN
- Pyrene Std

# %RPD COMPARISON

## SDSU Method

Sample	2015	2016	AVERAGE
Split	<b>13.3%</b> N=15	<b>20.4%</b> N=3	<b>14.5%</b> N=18
Duplicates	<b>15.5%</b> N=13	<b>18.0%</b> N=4	<b>16.1%</b> N=17

## Indiana Method

Sample 86-1	12.22.16	12.30.16
Duplicates	<b>3.0%</b> N=3	<b>9.4%</b> N=3

# LCS COMPARISON

## SDSU Method

- AAFCO 201591
- AAFCO average: 51.73
- MDA Average: 56.17 (N=11)
- STDEV: 7.73
- %RSD: 13.76

## Indiana Method

- AAFCO 201621
- AAFCO average: 42.85
- MDA Average: 46.545 (N=6)
- STDEV: 3.93
- %RSD: 8.44

# NIST COMPARISON

## SDSU Method

Date	NIST Value
11/22/16	15.21
11/28/16	15.14

## Indiana Method

Date	NIST Value
12/22/16	14.44
12/30/16	16.12
1/4/17	15.16

**NIST Range: 14.9-15.82**

# PROS/CONS OF INDIANA METHOD

## PROS

- No hot plate
- Increase samples tested
- Generate  $\frac{1}{2}$  waste
- Increase efficiency
- Pyrene std

## CONS

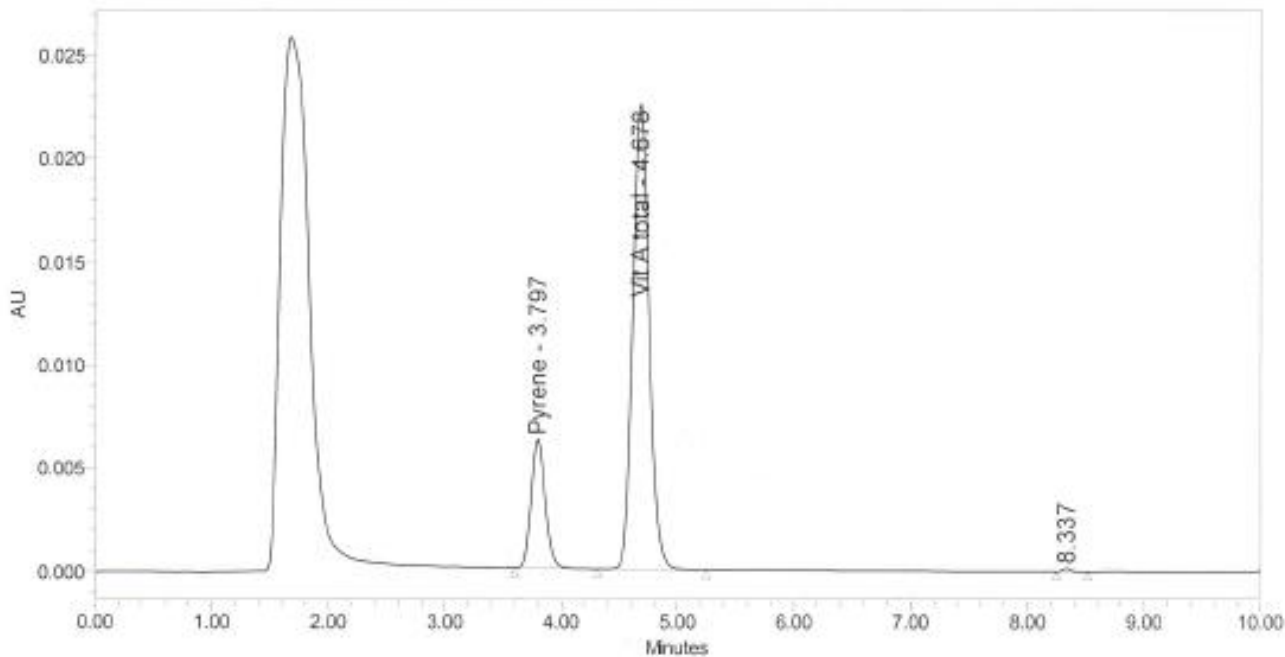
- Generates more plastic waste



## Vitamin A component summary

### SAMPLE INFORMATION

Sample Name:	0.724 ppm	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	122016 Vit A new meth
Vial:	29	Acq. Method Set:	Vitamin A
Injection #:	1	Processing Method:	Vitamin A_PM IS
Injection Volume:	30.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 325nm
Date Acquired: 12/20/2016 1:34:05 PM MST			
Date Processed: 12/22/2016 8:55:33 AM MST			



	Peak Name	RT	Area	Amount	Units
1	Pyrene	3.797	52376		
2	Vit A total	4.678	218163	0.724	ppm

**VITAMIN A  
STANDARD**

# CONCLUSIONS

- Prefer Indiana method
- More testing needed
- Verdict out on data quality
- ISO17025 Accreditation

# CREDITS

- **Study design team:**

- Heidi Hickers
- Robin Johnson

- **Presentation edits:**

- Nichole Chimbos