# Study of Amino Acids in DDGS

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# Topics

1. AA testing: snap shots of AOCS collaborative study
2. Variations: crude protein and amino acids
3. CP and AA digestibility

# AA Testing
# Snap Shots of AOCS Collaborative Study

Part I

Introduction by Amy Johnson (AOCS)

Part II

Data

# Comparison of Testing Methods on Amino Acid Contents in Animal Feed

Joint Meeting of the
AAFCO Laboratory Methods and Service Committee
and the
AOAC Feed Additive and Contaminants Group of the Ag Community

**Wednesday, January 20, 2010**

**Crowne Plaza**
**Redondo Beach, California**

# Instrument and Derivatization Utilized

- Lab 1 - **HPLC Post-Column Ninhydrin Derivatization**
- Lab 2 - **HPLC Post-Column Ninhydrin Derivatization**
- Lab 3 - **HPLC Post-Column OPA Derivatization**
- Lab 4 - **HPLC Post-Column Ninhydrin Derivatization**
- Lab 5 - **HPLC Post-Column Ninhydrin Derivatization**

Lab 6 - **LC/MS/MS Pre-Column Derivatization**

# Protocol Used

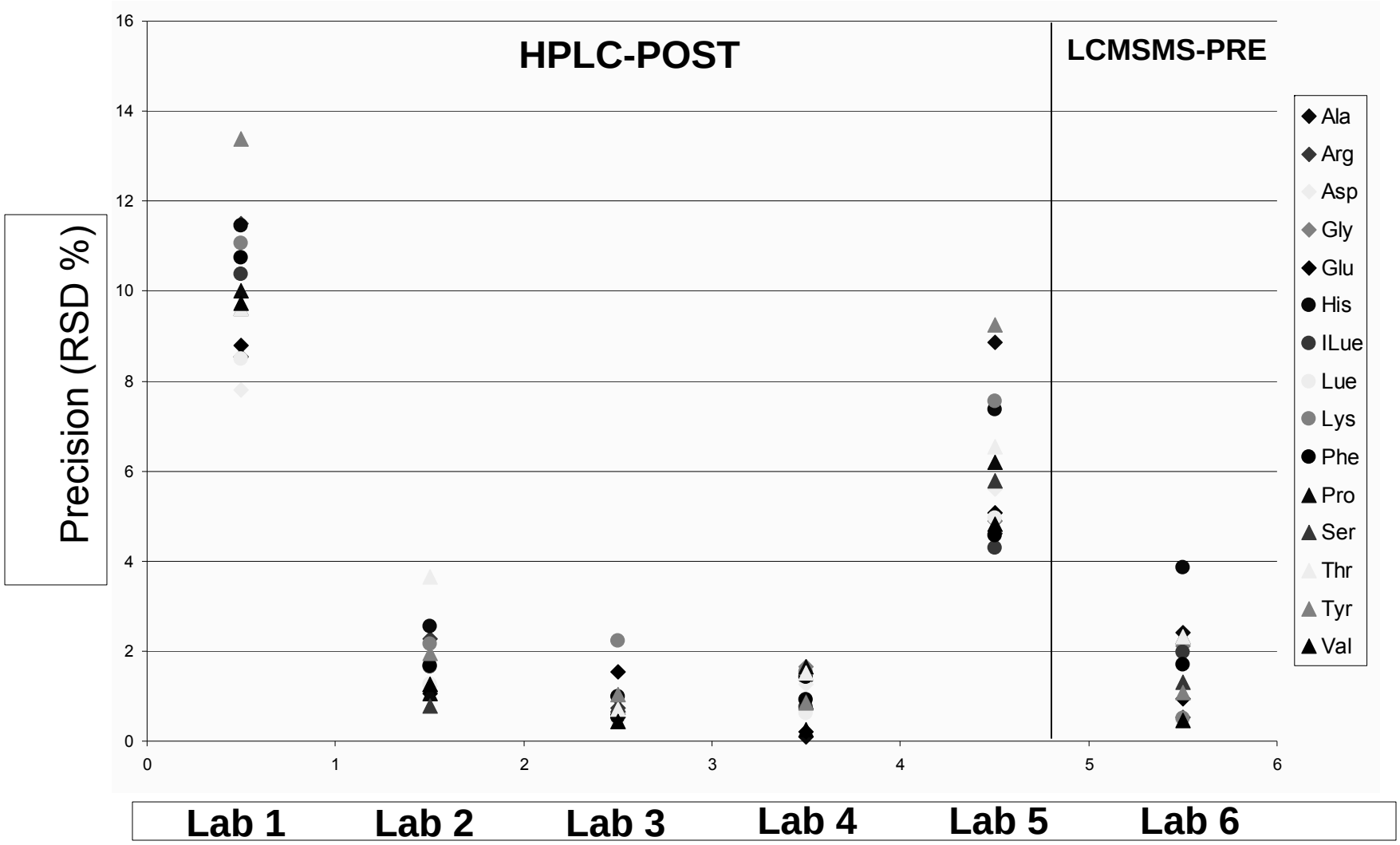
- Eighteen samples
- Hydrolysis procedure is based on the method of AOAC 994.12.
- Some of the samples will be spiked with Sigma amino acid hydrolysate standard.
- Four samples shall be analyzed once per week for 6 weeks, as part of repeatability assessment.

# Results

- 15 amino acids: alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine
- Report the final data in µg/mL (wt / vol)

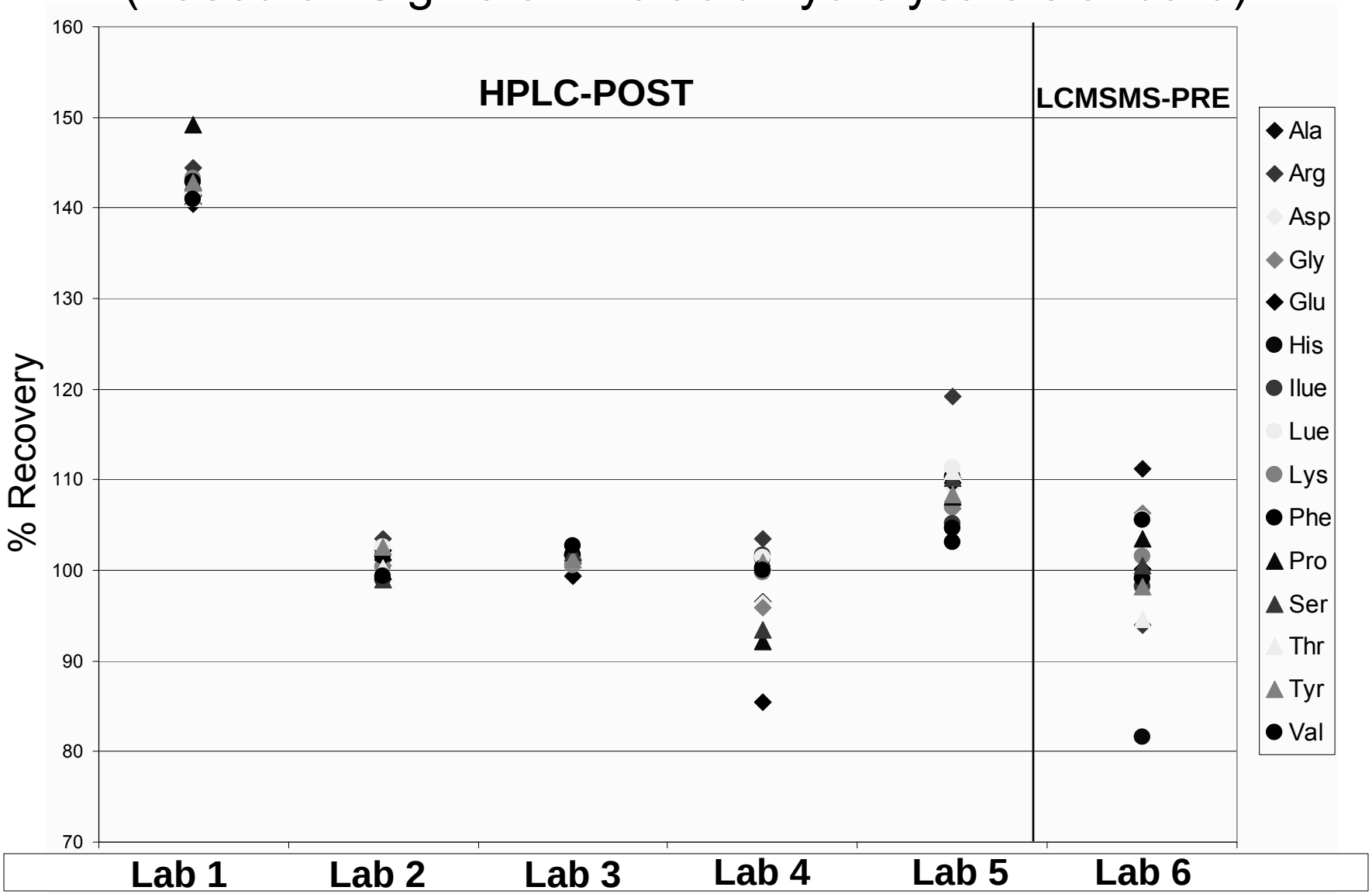
# Precision (RSD, %)

(Based on DDGS hydrolysate triplicate)

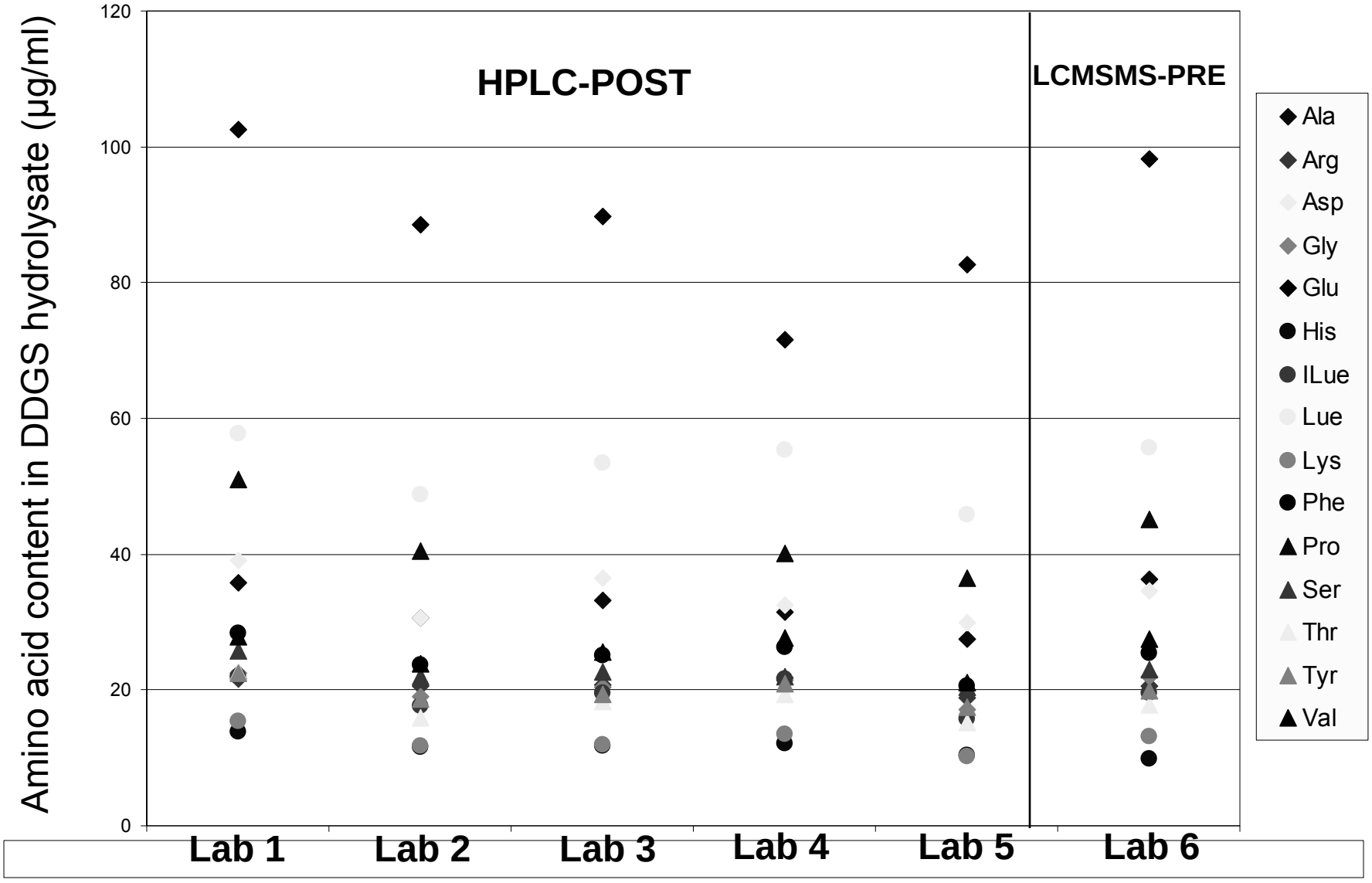


# Accuracy (Recovery, %)

(Based on Sigma amino acid hydrolysate standard)

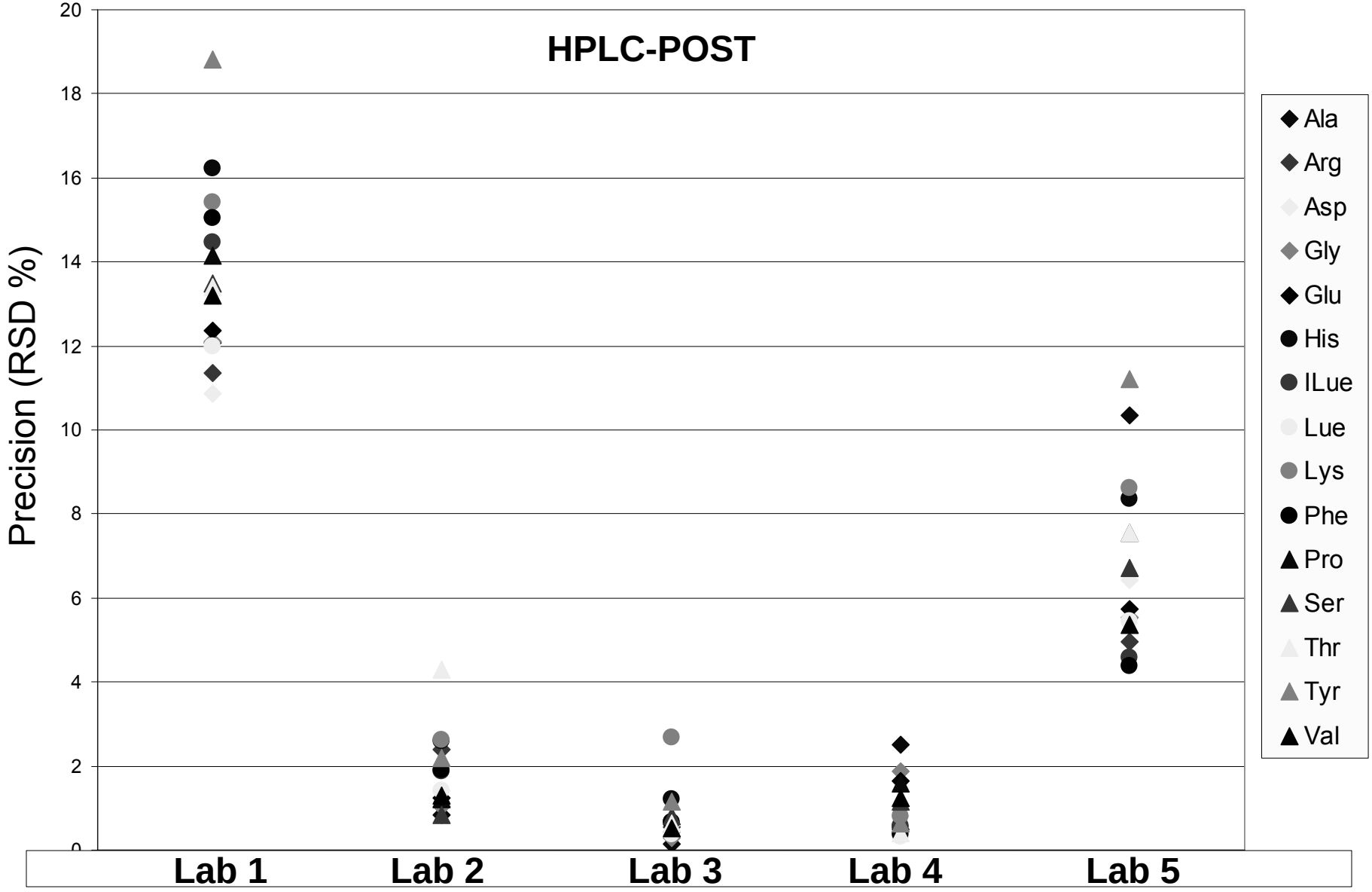


# Amino Acid Content in DDGS Hydrolysates



# Precision (RSD, %)

## (DDGS sample, tested in 6-week period)



# New Testing Method Development

Tryptophan in DDGS

- Microwave, basic hydrolysis
- Hydrolyze 12 samples in 2 hours, use LC/MS/MS for quantitation.
- Recovery of 85% for an NIST standard
- Look into the recoveries for other AAs

# Summary I

- HPLC-POS is a strong method
- Best performance of HPLC-POS:

  RSD < 2%

  Recovery between 95 and 105%
- There is need to develop a faster and more comprehensive hydrolysis method for AA testing

# Variations:

# Crude Protein and Amino Acids

## Data Sources

NCERC: **12 plants** (mainly from IL, IN, WI), **76 DDGS samples**
(http://www.value-added.org/renewableEnergy/ethanol/ddgs/)

MN: **49 plants** (mainly from MN, IA, IL, etc.), **49 DDGS samples**
http://www.ddgs.umn.edu/profiles/us_profile_comparison_march_2009.pdf)

# Variations between Plants

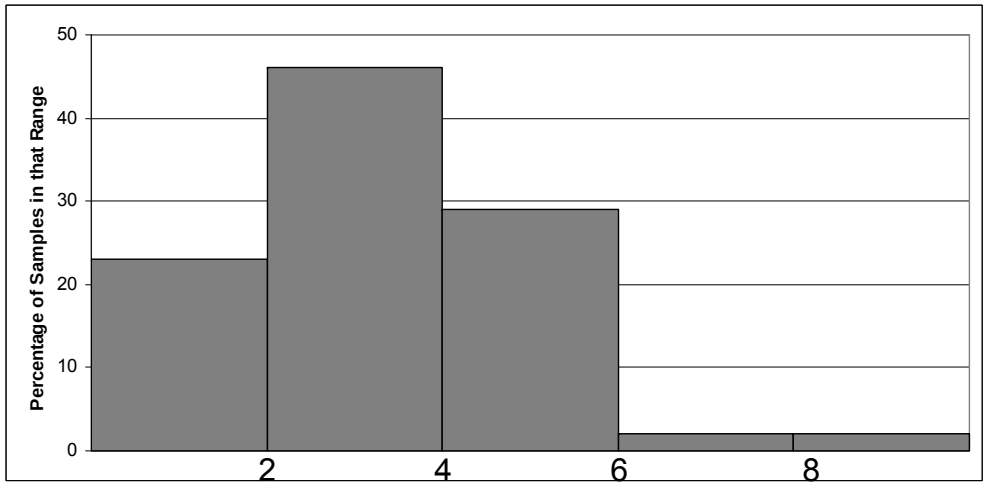
| In DDGS | NCERC (%, w/w dry) | MN (%, w/w dry) | NCERC (AA/ CP, g/kg) |
|---|---|---|---|
| **CP** | 29.3 (4%) | 30.8 (5%) | |
| **Arg** | 1.3 (7%) | 1.4 (9%) | 44 (5%) |
| **His** | 0.8 (10%) | 0.8 (6%) | 26 (9%) |
| **Ile** | 1.0 (4%) | 1.2 (7%) | 35 (4%) |
| **Leu** | 3.4 (5%) | 3.6 (7%) | 116 (4%) |
| **Lys** | 0.9 (9%) | 1.0 (13%) | 31 (9%) |
| **Met** | 0.6 (9%) | 0.6 (8%) | 20 (7%) |
| **Phe** | 1.4 (5%) | 1.4 (8%) | 46 (4%) |
| **Thr** | 1.1 (6%) | 1.1 (6%) | 37 (6%) |
| **Val** | 1.4 (4%) | 1.4 (7%) | 47 (4%) |

# Variations: Between vs. Within Plants
## (based on NCERC data)

| CV range | CV of between | CV of within |
|---|---|---|
| **CP** | 4% | 2% (2 – 4 %) |
| **Arg** | 7% | 4% (2 – 6 %) |
| **His** | 10% | 11% (3 – 19 %) |
| **Ile** | 4% | 4% (1 - 6%) |
| **Leu** | 5% | 4% (1 - 6%) |
| **Lys** | 9% | 10% (5 - 14%) |
| **Met** | 9% | 8% (6 -10%) |
| **Phe** | 5% | 4% (2 - 6%) |
| **Thr** | 6% | 8% (4 - 12%) |
| **Val** | 4% | 4% (2 - 5%) |

# Variations within Plants, CP

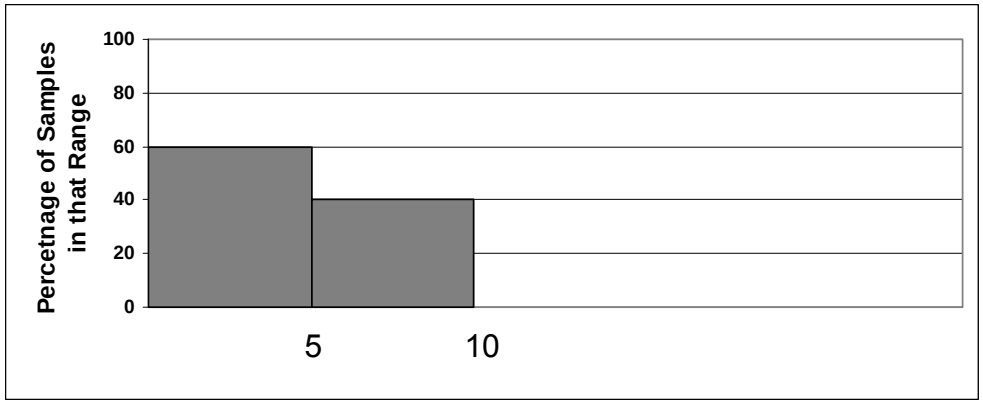
(from two earlier data sources)




**NCERC**

**40 plants**

**2 – 4 DDGS from each plant**




**MN**

**10 plants**

**12 DDGS from each plant**

**Within-plant coefficient of variance (%)**

# AA in DDGS / AA in Corn

(Corn data from Feedstuff, 2010)

| | NCERC (website) | NCERC Pilot Plant Trial | MN |
|---|---|---|---|
| Arg | 3.2 | 2.9 | 3.5 |
| His | 3.1 | 3.6 | 3.4 |
| Ile | 3.6 | 3.7 | 4.1 |
| Leu | 3.4 | 3.4 | 3.6 |
| Lys | 3.7 | 3.9 | 4.0 |
| Met | 3.4 | | 3.5 |
| Phe | 3.7 | 3.6 | 3.7 |
| Thr | 3.7 | 3.7 | 4.0 |
| Val | 3.7 | 4.0 | 3.8 |

# Summary II

- CP: most of the CVs are lower than 5% between and within plants.
- AA, variations between and within plants:

  Arg., Ile, Leu, Phe, Val, CV < 6%

  His., Lys, Met., Thr., CV between 6% and 19%
- The data set are representative of DDGS produced in the past 1 - 2 years

# CP and AA digestibility

# Amino acids / Lysine
# Animal Science Ideal Ratio

| AA / Lysine | Swine | Poultry | DDGS | Corn |
|---|---|---|---|---|
| **Arg** | | 105 | 147 | 167 |
| **His** | 32 | 36 | 87 | 104 |
| **Ile** | 60 | 69 | 117 | 121 |
| **Leu** | 111 | 124 | 386 | 417 |
| **Lys** | 100 | 100 | 100 | 100 |
| **Met** | 60 | 59 | 69 | 75 |
| **Phe** | 95 | 105 | 156 | 175 |
| **Thr** | 65 | 55 | 123 | 121 |
| **Val** | 68 | 76 | 158 | 175 |

# CP and AA Digestibility of DDGS in Swine Diet

**SID (%)**

(standardized ileal digestbility)

**=**

**AID (%)**

(apparent ileal digestbility)

**+**

**Basal $IAA_{end}$ /$AA_{diet}$ (%)**

(basal ileal endogenous loss)

# SID of DDGS in Swine Diet

| | **Mean of 33 DDGS** (Stein et al, JAFC, 2008) | **Mean of 14 DDGS** (Shurson website, 2009) | **One Corn Data** (Stein et al, JAS, 2006) |
|---|---|---|---|
| **CP** | 73 (8%) | 75 (6%) | 71 |
| **Arg** | 81 (7%) | 84 (6%) | 77 |
| **His** | 78 (6%) | 80 (4%) | 82 |
| **Ile** | 75 (6%) | 77 (5%) | 78 |
| **Leu** | 84 (5%) | 85 (3%) | 85 |
| **Lys** | 62 (12%) | 64 (9%) | 68 |
| **Met** | 82 (5%) | 84 (3%) | 84 |
| **Phe** | 81 (5%) | 83 (3%) | 83 |
| **Thr** | 71 (7%) | 71 (5%) | 74 |
| **Val** | 75 (6%) | 77 (4%) | 77 |

# Lysine and Furosine

- Maillard reaction: binding of lysine to reducing sugars produces unreactive lysine
- The unreactive lysine is not bioavailable for pigs, but cannot be detected by the usual testing method for total lysine, which involves acid hydrolysis of DDGS.
- Furosine, a lysine derivative, is produced during DDGS acid hydrolysis, and has been confirmed by food industry to be a quantitative indicator for unreactive lysine (unreactive lysine = 1.25 furosine)

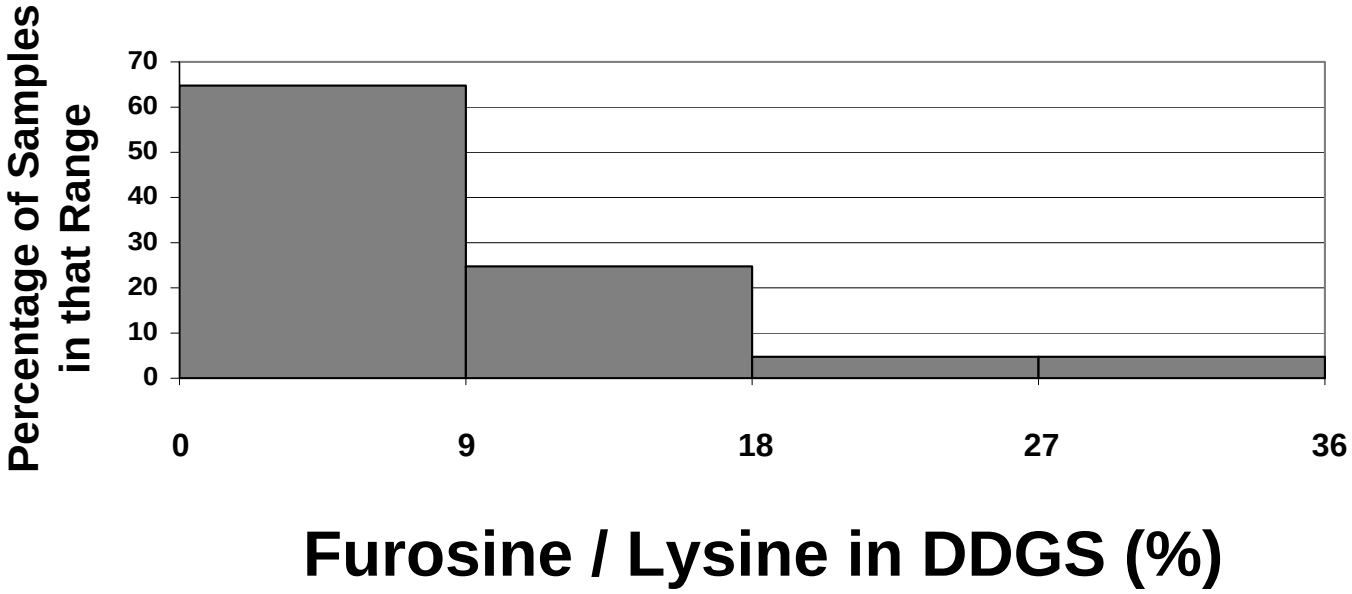
# Testing Furosine in DDGS by LC/MS/MS

- **System Suitability –** trace compound in aqueous acidic hydrolysate
- **Calibration Linearity -** $r^2$ = 0.999 for 5 external standards
- **Accuracy -** recovery between 95% and 105% for synthetic standard spiked in DDGS matrix
- **Precision -** triplicates, within batch (3%), between batches (5%)
- **Limit of Quantitation (LOD) –** 50 ppm in DDGS

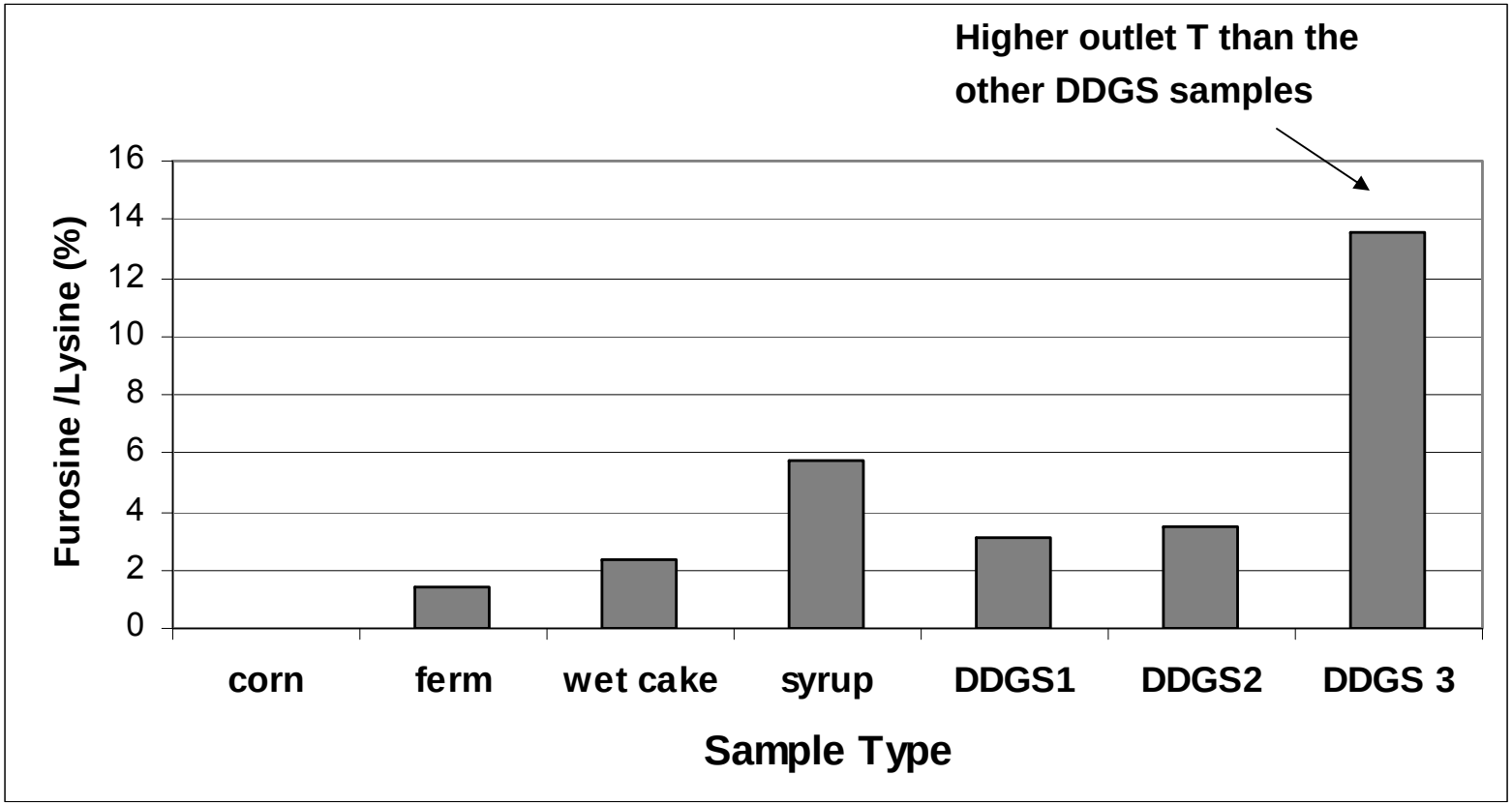
# Furosine in DDGS

| N = 55 (12/2008) | Furosine (%, w/w as-received) | Furosine / Lysine (%) |
|---|---|---|
| **Minimum** | 0.02 | 2 |
| **Maximum** | 0.20 | 37 |
| **N = 20 (06/2009)** | **Furosine (%, w/w as-received)** | **Furosine / Lysine (%)** |
| **Minimum** | 0.01 | 2 |
| **Maximum** | 0.24 | 30 |

# Furosine / Lysine in DDGS



# Furosine / Lysine in Intermediates from Corn to DDGS Production



# Animal Trial Study

(2009, U. of I)

- 20 DDGS sources
- SID of lysine: 63.3 ± 8.1%.

SID of lysine vs. reactive lysine, $r^2 = 0.90$

vs. CP, $r^2 = 0.02$

vs. color, $r^2 = 0.04$

## Any DDGS sample from a standard dry-grind plant in the US, likely (with 68% confidence level)

| | Content in DDGS (%, w/w dry) | Digestible Level (%, w/w dry) |
|---|---|---|
| **CP** | 28.1 – 30.5 | 18.9 – 24.0 |
| **Arg** | 1.2 - 1.4 | 0.9 – 1.2 |
| **His** | 0.7 – 0.9 | 0.5 – 0.7 |
| **Ile** | 0.9 – 1.1 | 0.7 – 0.9 |
| **Leu** | 3.1 – 3.7 | 2.5 – 3.2 |
| **Lys** | 0.8 – 1.0 | 0.4 – 0.7 (need furosine test to confirm) |
| **Met** | 0.5 – 0.7 | 0.4 – 0.6 |
| **Phe** | 1.2 – 1.5 | 1.0 – 1.2 |
| **Thr** | 1.0 – 1.2 | 0.6 – 0.9 |
| **Val** | 1.3 – 1.5 | 0.9 – 1.2 |

# Acknowledgement

AOCS

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