WRSS Food & Nutrition Insights®

Clean Label Conference

May 23, 2023

Ethical Analyses and Labeling for Protein Claims

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Driven to Discover℠

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Overview

- Beyond Meat Class Action Lawsuit.
- How to spot errors in protein labeling and claims.
- Why protein labeling is difficult for food manufacturers.
- Development of a new, in vitro enzyme digestion method for regulatory compliance.
- Standardization and distribution for regulatory approval.

Beyond Meat Protein Content Class Action

Class-action lawsuits alleging Beyond Meat deceived consumers about protein content will be combined in Chicago

Beyond Meat announced last fall it would lay off 200 workers. That was on top of a 4% reduction in its workforce it announced in August.

NEWS

Feb. 2, 2023, 11:18 AM CST

By Rob Wile

Issues with Beyond Meat Protein Labeling

- Labeled as 20g/serving vs 19 g/serving found in Class Action.
- Labeled as 40% DV Protein vs 7% DV Protein found in Class Action.

Water, pea protein*, expeller-pressed canola oil, refined coconut oil, rice protein, natural flavors, dried yeast, cocoa butter, methylcellulose, contains 1% or less: potato starch, salt, potassium chloride, beet powder color, apple extract, pomegranate concentrate, sunflower lecithin, vinegar, lemon juice concentrate, vitamins and minerals (zinc sulfate, niacinamide [vitamin B3], pyridoxine hydrochloride [vitamin B6], cyanocobalamin [vitamin B12], calcium pantothenate).

Beyond Meat: 20 grams Crude Protein vs 19 grams

Nutrition Facts

2 servings per container
Serving size **1 Patty (113g)**

	Per serving		Per container	
Calories	**230**		**450**	
		% DV*		% DV*
Total Fat	14g	**18%**	28g	**36%**
Saturated Fat	5g	**25%**	10g	**50%**
Trans Fat	0g		0g	
Polyunsaturated Fat	3g		5g	
Monounsaturated Fat	6g		11g	
Cholesterol	0mg	**0%**	0mg	**0%**
Sodium	390mg	**17%**	780mg	**35%**
Total Carb.	7g	**3%**	14g	**5%**
Dietary Fiber	2g	**7%**	4g	**14%**
Total Sugars	0g		<1g	
Incl.Added Sugars	0g	**0%**	0g	**0%**
Protein	20g	**40%**	40g	**80%**
Vitamin D	0mcg	0%	0mcg	0%
Calcium	100mg	8%	200mg	15%
Iron	4mg	20%	8.1mg	45%
Potassium	330mg	6%	650mg	15%
Niacin	4.7mg NE	30%	9.4mg NE	60%
Vitamin B6	0.3mg	15%	0.6mg	35%
Vitamin B12	2.4mcg	100%	4.8mcg	200%
Pantothenic Acid	0.5mg	10%	1mg	20%
Zinc	4.6mg	40%	9.2mg	80%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

INGREDIENTS: Water, Pea Protein[1], Expeller-Pressed Canola Oil, Refined Coconut Oil, Rice Protein, Natural Flavors, Dried Yeast, Cocoa Butter, Methylcellulose, Contains 7% or less: Potato Starch, Salt, Potassium Chloride, Beet Juice Color, Apple Extract, Pomegranate Concentrate, Sunflower Lecithin, Vinegar, Lemon Juice Concentrate, Vitamins and Minerals [zinc sulfate, niacinamide (vitamin B3), pyridoxine hydrochloride (vitamin B6), cyanocobalamin (vitamin B12), calcium pantothenate].

[1]Peas are legumes. People with severe allergies to legumes like peanuts should be cautious when introducing pea protein into their diet because of the possibility of a pea allergy. Contains no peanuts or tree nuts.

- Crude protein is what is labeled for total protein (nitrogen x conversion factor).
- Beyond Meat is fortified with protein and is making a protein claim. It must have 100% of the protein content claimed (20 g).*

*Class I nutrients are those added in fortified or fabricated foods. These nutrients are vitamins, minerals, protein, dietary fiber, or potassium. Class I nutrients must be present at 100% or more of the value declared on the label ; in other words, the nutrient content identified by the laboratory analysis must be at least equal to the label value. - 21 CFR 101.9(g)(3)

Beyond Meat: 40 % Daily Value vs 7%

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Polyunsaturated Fat	3g		5g	
Monounsaturated Fat	6g		11g	
Cholesterol	0mg	**0%**	0mg	**0%**
Sodium	390mg	**17%**	780mg	**35%**
Total Carb.	7g	**3%**	14g	**5%**
Dietary Fiber	2g	**7%**	4g	**14%**
Total Sugars	0g		<1g	
Incl.Added Sugars	0g	**0%**	0g	**0%**
Protein	20g	**40%**	40g	**80%**
Vitamin D	0mcg	0%	0mcg	0%
Calcium	100mg	8%	200mg	15%
Iron	4mg	20%	8.1mg	45%
Potassium	330mg	6%	650mg	15%
Niacin	4.7mg NE	30%	9.4mg NE	60%
Vitamin B6	0.3mg	15%	0.6mg	35%
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*Peas are legumes. People with severe allergies to legumes like peanuts should be cautious when introducing pea protein into their diet because of the possibility of a pea allergy. Contains no peanuts or tree nuts.

20g crude protein/50g quality protein = 40%

- The %DV is calculated based on quality protein content – not crude.
- Labeled as 40% DV Protein vs 7% DV Protein found in Class Action.
  - Likely used crude protein instead of quality protein for calculation.
  - 7% DV does not even meet a good source of protein.

U.S. Regulations for Protein Claims

- Protein claimed = Crude Protein
  - Nitrogen by Dumas combustion or Kjeldahl x [6.25 or Jones Factor]

- Daily value (DV) of protein = 50 g **Quality Protein**/serving
  - Quality Protein must be determined by **PDCAAS** method
    - **P**rotein **D**igestibility **C**orrected **A**mino **A**cid **S**core

U.S. Regulations for Protein Claims (cont.)

- Labeling Protein Content outside of Nutrition Facts Label
  - Consensus: Constitutes claim of good source of protein in consumer mind.
  - Some manufacturers disagree.

- Good source of protein = 5 g **Quality Protein**/serving (10% DV)
  - Requires %DV protein in Nutrition Facts Label

Identifying Mislabeled Protein Products (Oat)

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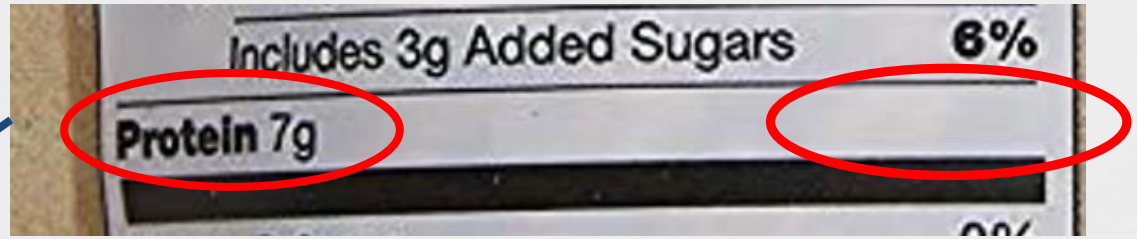
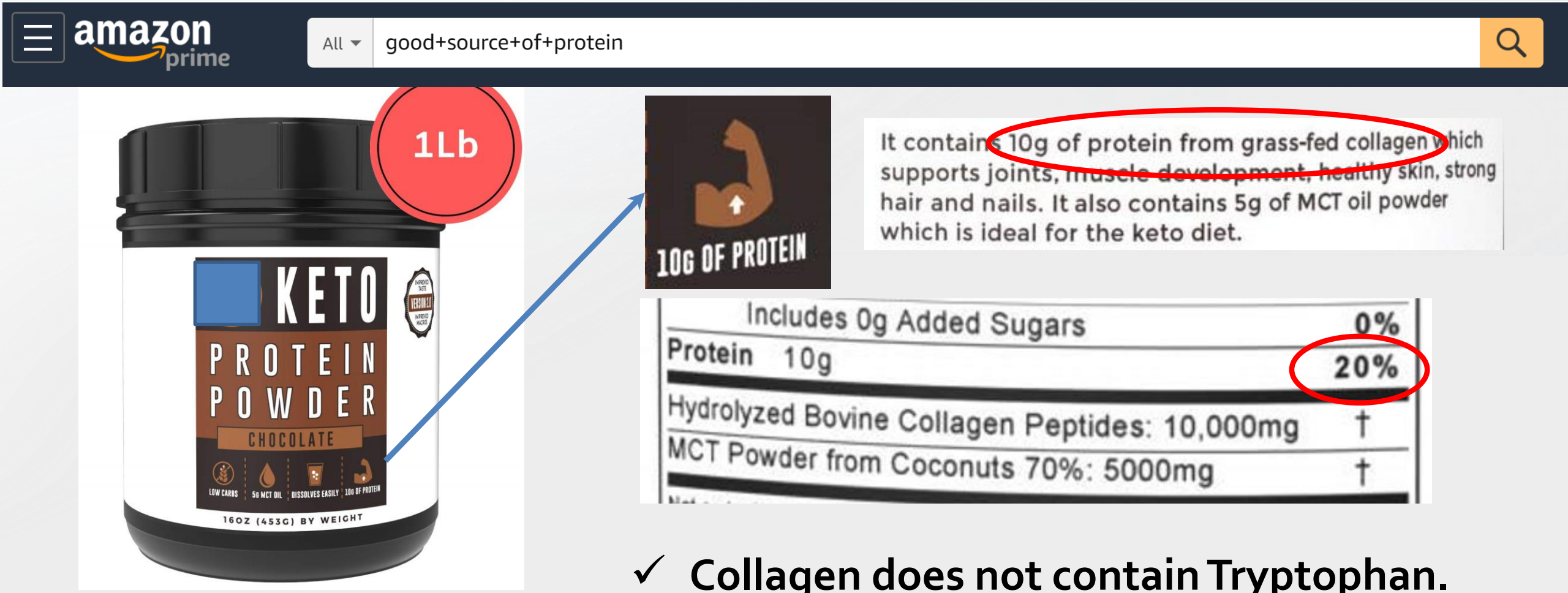
- ✓ Good Source of Protein Claim
- ✓ No %DV listed
- ✓ Mostly oats – PDCAAS typically <0.6
- ✓ Likely less than 5g Quality Protein.



Protein claims for Collagen Protein Sources



- ✓ **Collagen does not contain Tryptophan.**
- ✓ **PDCAAS Value = Zero.**

Why are protein labeling errors so common?

- Requirements are not well understood
- Manufacturers follow competitor labeling mistakes
- True compliance is:
  - Expensive
  - Time consuming
  - Contrary to ethics of some manufacturers

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Impact of Food Processing on Protein Digestibility

Sample	Amino Acid Score	True Protein Digestibility (%)	PDCAAS	Protein Efficiency Ratio (PER)
Casein	1.03	97.31	100	2.5
Black				
Extruded	0.85	**82.01 aA**	69.74	1.27
Cooked	0.83	81.66 aAC	67.54	1.42
Baked	0.91	**63.55 bA**	57.52	0.43
Navy				
Extruded	0.70	**87.41 aB**	60.82	1.24
Cooked	0.71	86.02 aAB	61.23	1.52
Baked	0.78	**69.08 bC**	53.62	0.65
Pinto				
Extruded	0.80	**82.53 aA**	66.21	1.26
Cooked	0.92	82.07 aAC	75.10	1.45
Baked	0.83	**57.58 bD**	47.75	0.64
Red Kidney				
Extruded	0.78	**83.21 aAB**	64.98	1.23
Cooked	0.77	80.67 aC	62.40	1.47
Baked	0.72	**69.12 bC**	50.10	0.65

Nosworthy, MG et al. (2018) Effect of Processing on the In Vitro and In Vivo Protein Quality of Beans (Phaseolus vulgaris and Vicia Faba). Nutrients (10) 671; doi:10.3390/nu10060671

True Protein Digestibility was analyzed via Two-Way ANOVA with Tukey's post-hoc test. Means followed by different letters (small in the same pulse class and large in the same treatment) indicate a significant difference between samples ($p < 0.05$).

Elements of PDCAAS Protein Quality Method

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- Amino Acid Analysis
  - Acid hydrolysis for majority of amino acids.
  - Performic acid oxidation for cysteine and methionine.
  - —Base hydrolysis for tryptophan.

- Determination of crude protein.
- Calculation of limiting AA relative to 1991 FAO Complete Protein.
- Determination of protein digestibility in **rats**.

PDCAAS Value = Limiting Amino Acid Value x Digestibility

Rat PDCAAS Digestibility Method

Sample
Food or Ingredient

Measure
Total Nitrogen, Amino Acid Profile, Fat, Ash, Moisture, Sugars, and Fiber for feed calculation

Feed

Measure
Food intake, body weights, Fecal N_2

Sacrifice

Drawbacks to Rat PDCAAS method

Method	Use of animals for product testing	
Timing	2-3 month turnaround	X
Cost	$5,000+ per sample	X
Sample size	1 to 1.5 kg	X
Nutritional analysis	Full analysis of all nutrients ($1,200/sample)	X
Over-fortification	— Processing effects on score unpredictable — Significant over-use of expensive ingredients	X

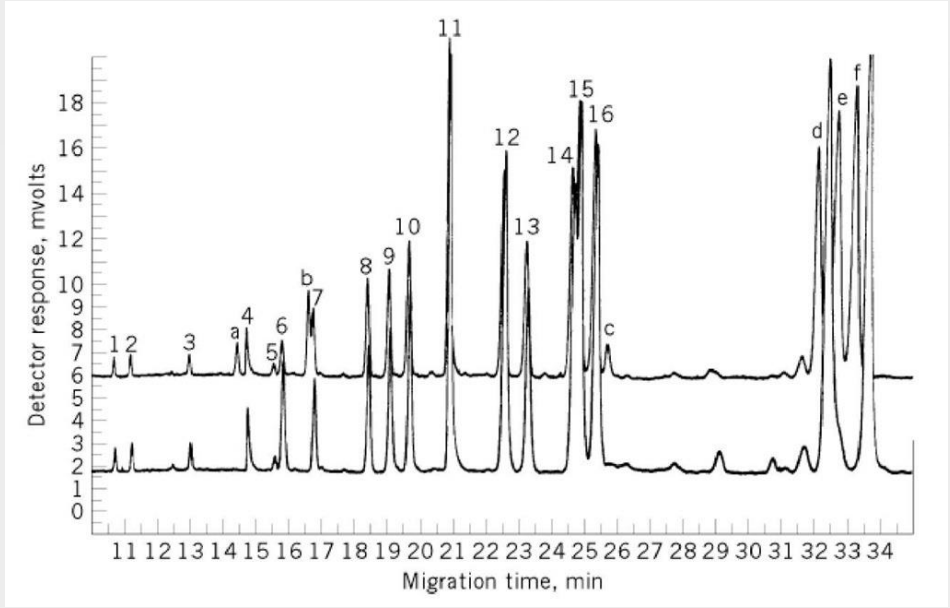
~$6,200 USD Total Cost per sample

How can compliance with protein labeling regulations be improved?

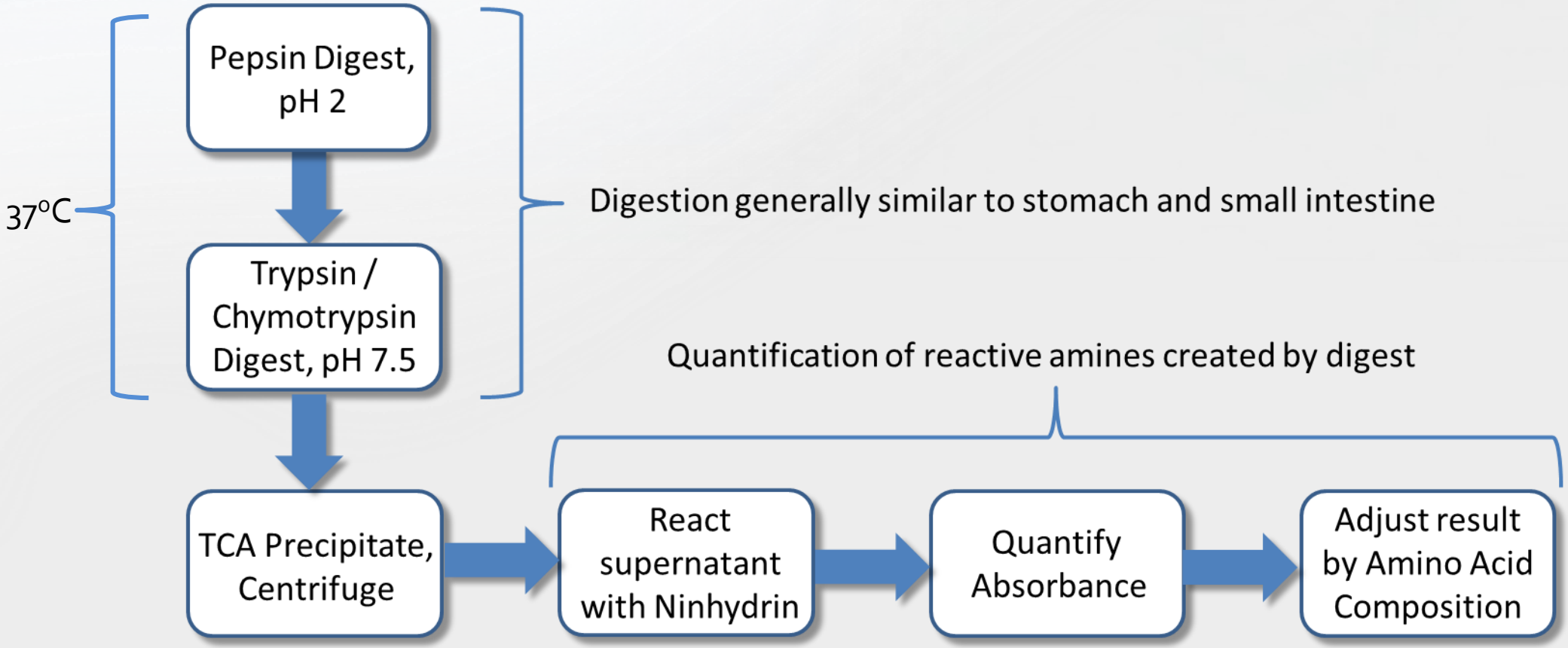
- Lower barriers to compliance.
  - Cost
  - Time
  - Amount of sample
  - Amount of testing
  - Eliminate the use of animals
- Develop *in vitro* digestibility method with respect to above needs.

Considerations for an enzymatic *in vitro* digestion method

- Compensate for individual amino acid reactivity
- Enzymes for digestion cannot interfere with measurement
- Model after enzyme digests for peptide mapping protocols
  - Keep enzyme levels less than 2% of substrate/sample
  - Avoid self-digestion of proteases
    - Make-up enzymes just prior to digestion
    - Maintain Trypsin and Chymotrypsin at pH 2 prior to digest



ASAP-Quality score digestion overview



Method Reference – US Pat No. 9,738,920, IN VITRO METHOD FOR ESTIMATING IN VIVO PROTEIN DIGESTIBILITY, Plank, DW. (2017)

Amino Acids with Exceptional Ninhydrin Reactions

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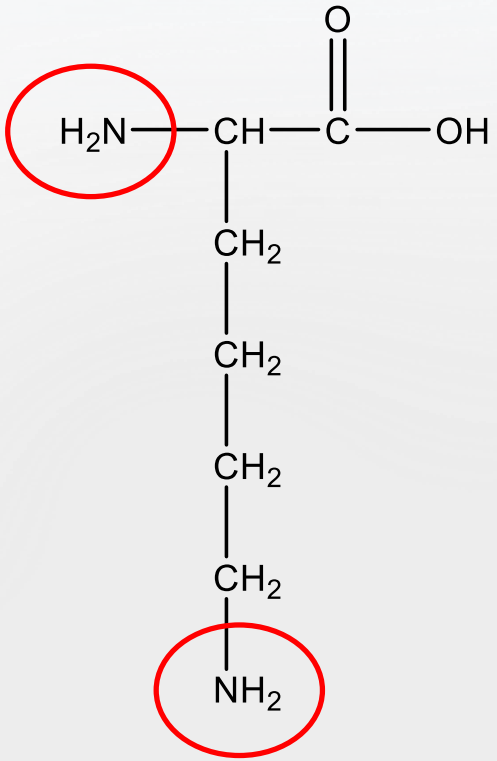
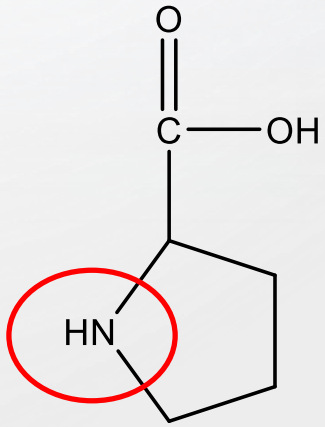
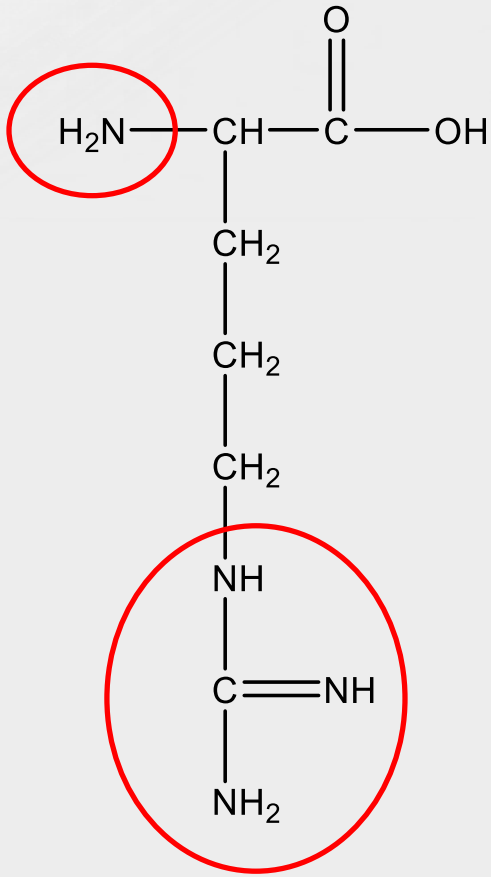
Lysine (~2X)



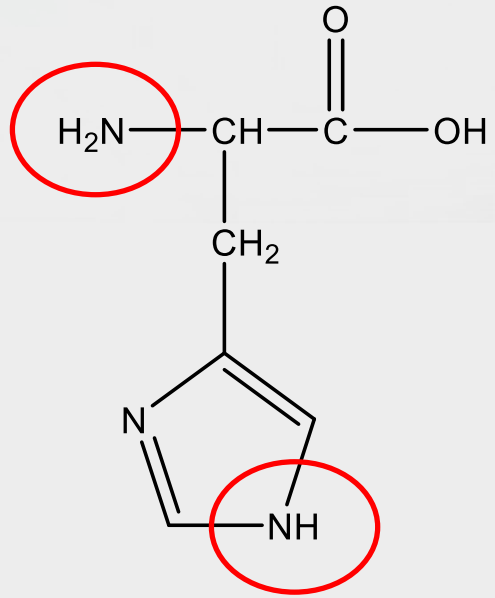
Proline (~0.5X)



Arginine (~1.2X)



Histidine (~1.2X)



Correlation of ASAP-Digestibility Score to the Rat Digestibility Score

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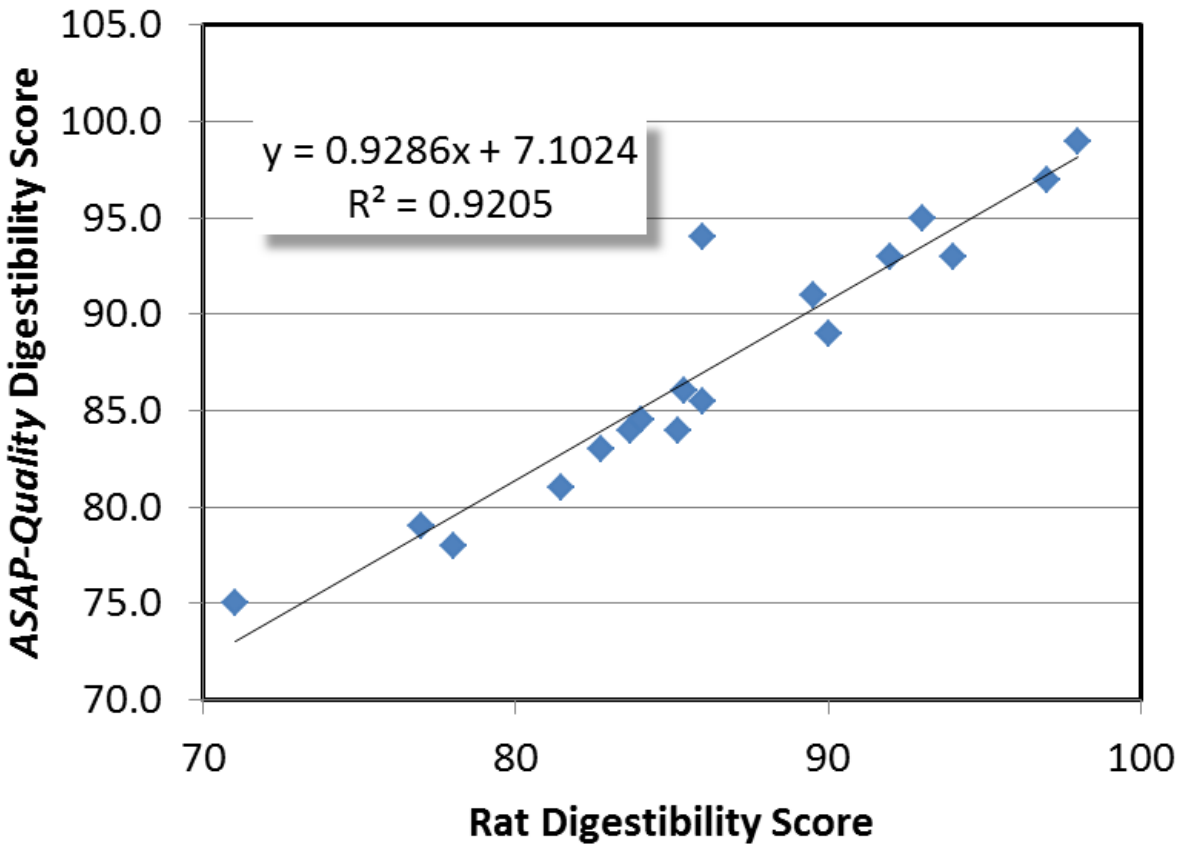
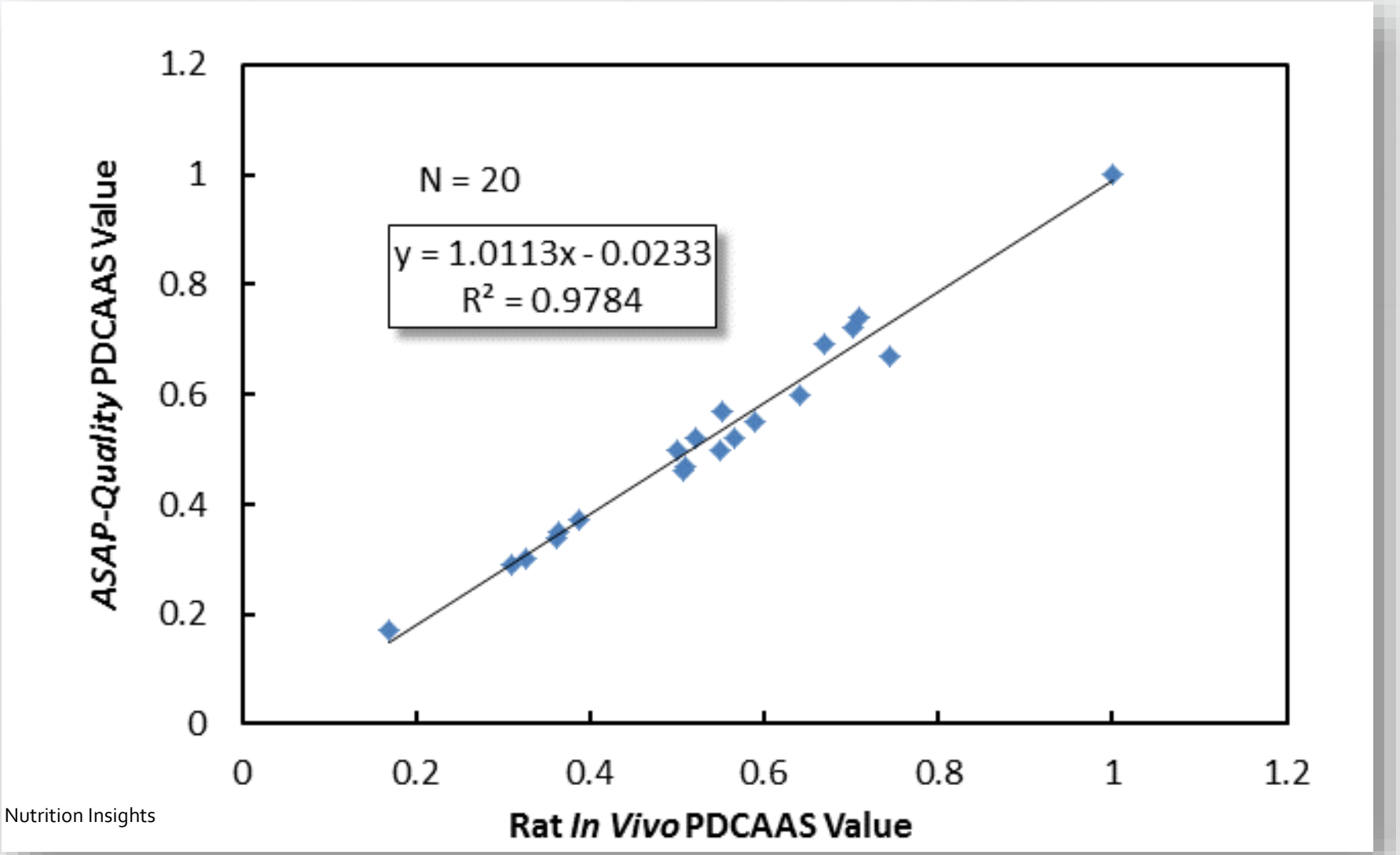
Matrix	ASAP-Quality Digestibility Score	Rat Digestibility Score
Casein	100.2	100.0 [1]
Rolled Oats	82.7	83.0 [1]
Lentils	85.4	86.0 [1]
Wheat	89.5	91.0 [1]
Split Pea	85.2	84.0 [1]
Sunflowers Seeds	86.0	94.0 [1]
Black-eyed peas	83.7	84.0 [1]
Kidney Beans	81.5	81.0 [1]
Peanuts Roasted High Oleic	71.0	75.0 [2]
Sunflower Kernels Roasted No Salt SL80	77.0	79.0 [2]
Peanut Butter#7	92.0	93.0 [2]
Pea Protein Bar Fruit and Nut	90.0	89.0 [2]
Oats Rolled #15	93.0	95.0 [2]
High Pro Nutty Granola Cluster	97.0	97.0 [2]
Granola Base #7 Sucrose/Canola Natural	98.0	99.0 [2]
Granola Bar #1	78.0	78.0 [2]
Granola Bar #2	84.0	84.5 [2]
Chicken Stock Concentrate Salt	86.0	85.5 [2]
Granola base #5	94.0	93.0 [2]

[1] based on published literature values

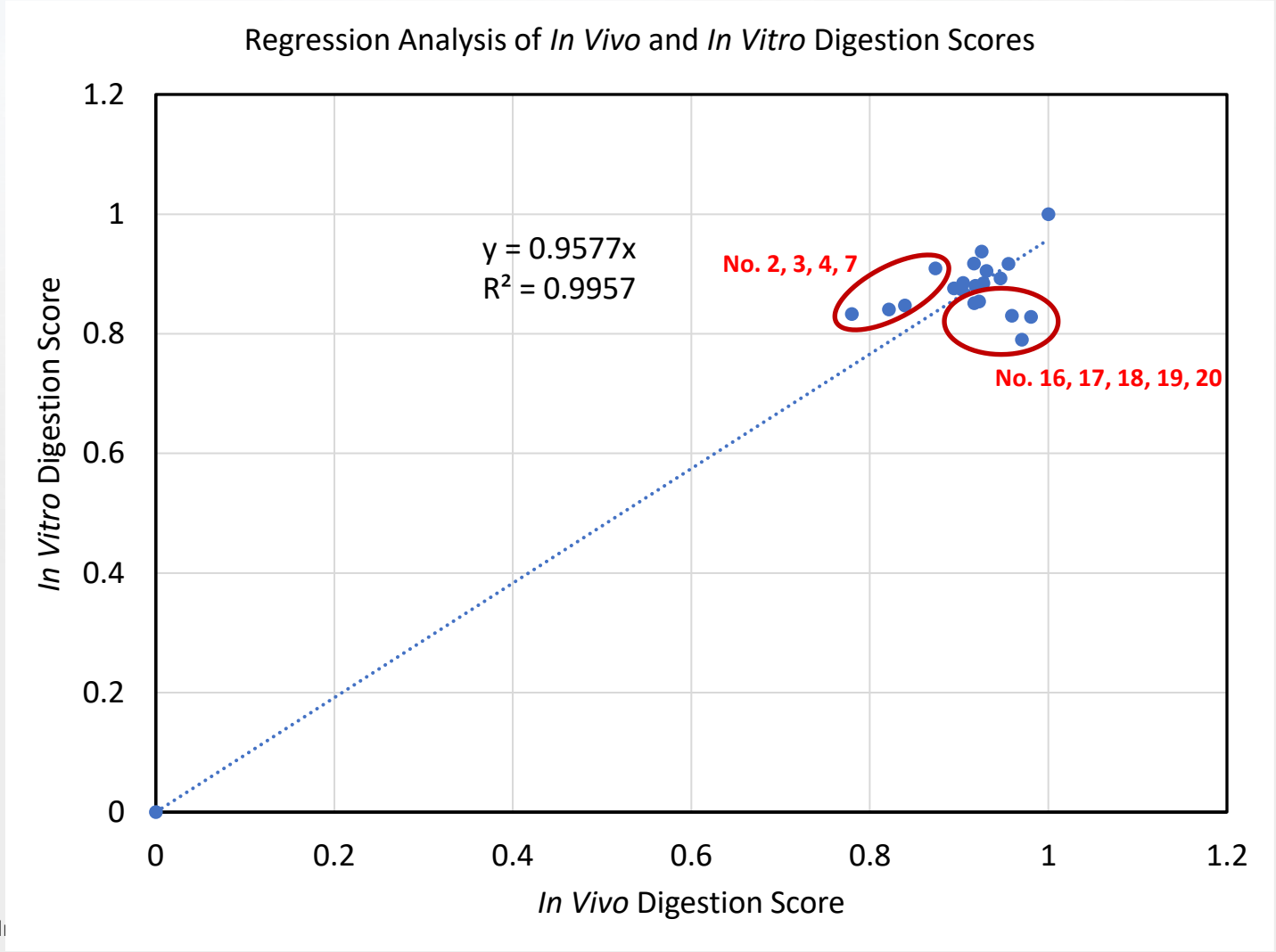
[2] based on direct analysis of the same sample by both methods



Correlation of ASAP-Quality Score to the Final Rat PDCAAS Value



Method Updates: Outlier Samples Resolved



Low Protein Outliers Group Together

PDCAAS Method only validated with Post-column Derivatization Amino Acid Analysis

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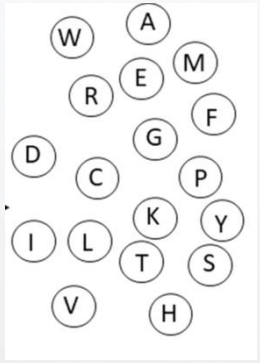
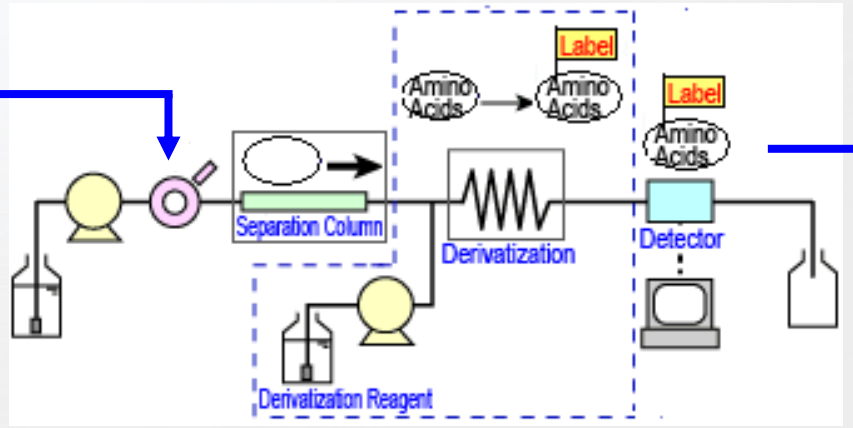
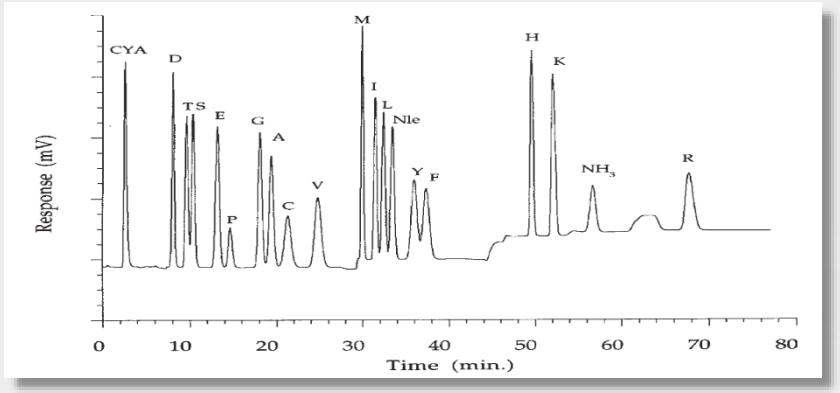
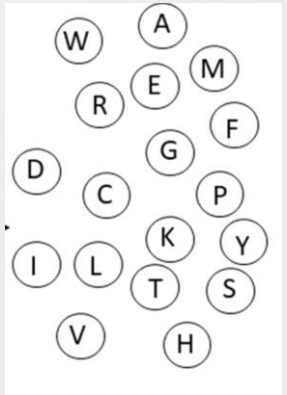
Post-column ninhydrin-derivatization



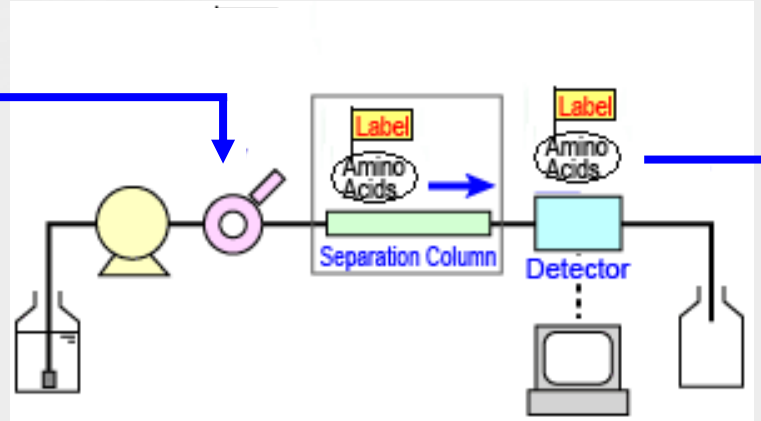


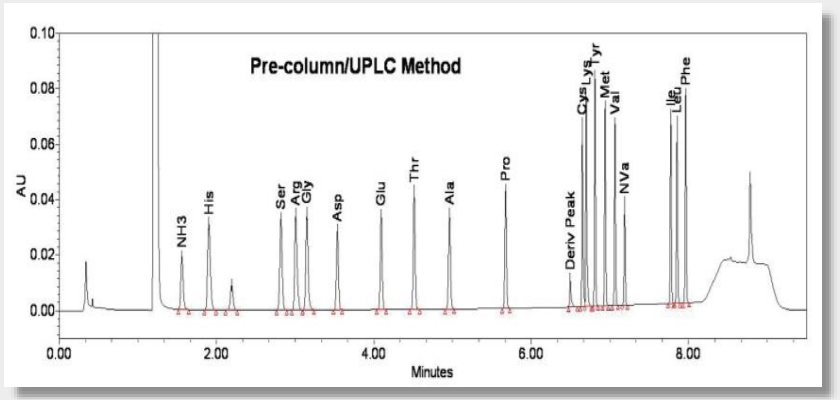


Pre-column derivatization



\+ Label





Reference - https://www.shimadzu.com/an/hplc/support/lib/lctalk/53/53intro.html

Advantages of ASAP-Quality Score

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Cost Reduction per Sample

$6,200

$1,500

	Rat PDCAAS	ASAP-Quality	
Method	Use of animals for product testing	No Animals	✓
Timing	2-3 month turnaround	15-days	✓
Cost	$5,000+ per sample	$1,500 per sample	✓
Sample size	1 to 1.5 kg	20 grams	✓
Nutritional analysis	Full analysis of all nutrients ($1,200/sample)	Not Required	✓
Over-fortification (Ingredient Waste)	— Processing effects on score unpredictable — Significant over-use of expensive ingredients	Significantly reduced	✓

Next steps to advance FDA regulatory approval

- Launch of improved equation
- AOAC collaborative study
  - Recruit participating labs internationally from
    - Academia
    - Industry
    - Governmental agencies
- AOAC First action status
- Citizen Petition to FDA

Who is using the in-vitro test now?

- Companies with animal testing bans.
- Companies who want to reduce class action risk at low cost.
- Companies who want to comply with the law using an ethical test for clean label.

Summary

Protein Labeling Regulations –

- Currently in the US
  - Low-levels of compliance by industry.
  - Requires use of animal testing (considered unethical).
  - High-expense and product development delays.
  - Class action and regulatory risk.

- Potential solution
  - In vitro PDCAAS alternative
    - Low-cost
    - Rapid
    - High correlation to rat

Thank you!

CLEAN LABEL CONFERENCE

Appendix

Reporting of Protein Quality Results

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Megazyme ASAP-Quality
PDCAAS Analysis Report

Date: July 31, 2019
Sample Number:
Sample Name: 2019-WRSSFNI-XXXX

Amino Acid	Hydrated Amino Acid Content (g/100g sample)	Hydrated Amino Acid Content (g/100 g crude protein)	Hydrated Amino Acid Content (mg/g crude protein)	1991 Reference Protein[2] (mg/g crude protein)	Ratio	
Cysteine + Methionine*	0.50	2.92	29.19	25.00	**1.167**	1
Tryptophan*	0.24	1.40	13.95	11.00	1.268	
HydroxyProline	ND					
Aspartic acid	2.04					
Threonine*	0.68	3.98	39.82	34.00	1.171	
Serine	0.85					
Glutamic Acid	2.67					
Proline	0.68					
Glycine	0.70					
Alanine	0.75					
Valine*	0.77	4.50	44.97	35.00	1.285	
Isoleucine*	0.82	4.78	47.75	28.00	1.705	
Leucine*	1.37	7.98	79.85	66.00	1.210	
Tyrosine + Phenylalanine*	1.53	8.91	89.07	63.00	1.414	
Lysine*	1.18	6.83	68.34	58.00	1.178	
Histidine*	0.44	2.58	25.83	19.00	1.359	
Arginine	1.36					
Total =	16.60					

*essential amino acid for nutrition

[1] limiting amino acid for sample

Reporting of Protein Quality Results

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Crude Protein by Dumas Combustion (g/100g) =	17.2
In Vitro Digestibility =	0.85
First Limiting Amino Acid =	Cys + Met*
Amino Acid Score =	1.000
PDCAAS Value =	0.85
Total Quality Protein (g/100g) =	14.62
Serving Size (g) =	28.00
Total Quality Protein per Serving (g/serving) =	4.09
Total Crude Protein per Serving (g/serving) =	4.82
% Daily Value =	8.19

[2]The 1991 Reference Protein Values are required by current US Federal regulations. If you would like to evaluate against an alternative reference protein composition, please inquire.

Reporting of Protein Quality Results

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July 31, 2019

Amino Acid Analysis Results

Library Number: 2019-WRSSFNI-XXXXX

Sample Name:

Serving Size (g) = 28

Amino Acid	Dehydrated molecular Weight (g/mol)	Hydrated Molecular Weight (g/mol)	Dehydrated Amino Acid Content (g/100g)	Hydrated Amino Acid Content (g/100g)	Hydrated Amino Acid Content (mg/serving)
HydroxyProline	113.12	131.13	**ND**	ND	ND
Aspartic Acid	115.09	133.10	**1.765**	2.041	571
Threonine	101.10	119.12	**0.581**	0.685	192
Serine	87.08	105.09	**0.702**	0.847	237
Glutamic Acid	129.11	147.13	**2.346**	2.673	749
Proline	97.12	115.13	**0.575**	0.681	191
Glycine	57.05	75.07	**0.529**	0.697	195
Alanine	71.08	89.09	**0.600**	0.752	210
Valine	99.13	117.15	**0.655**	0.774	217
Isoleucine	113.16	131.17	**0.709**	0.821	230
Leucine	113.16	131.17	**1.185**	1.373	385
Tyrosine	163.17	181.19	**0.455**	0.505	142
Phenylalanine	147.17	165.19	**0.915**	1.027	288
Lysine	128.17	146.19	**1.031**	1.175	329
Histidine	137.14	155.16	**0.393**	0.444	124
Arginine	156.19	174.20	**1.217**	1.358	380
Cysteine	103.20	121.16	**0.215**	0.253	71
Methionine	131.20	149.21	**0.219**	0.249	70
Tryptophan	186.22	204.23	**0.220**	0.241	68
		Total =	14.310	16.597	4,647

Sample hydrated amino acid content label

Due diligence review by customer regulatory and labeling experts required.

Typical Amino Acid Profile

(example) (per serving)[1]

Essential Amino Acids	mg per 28 g serving
Cysteine + Methionine	140
Threonine	192
Tyrosine + Phenylalanine	429
Lysine	329
Tryptophan	68
Valine[2]	217
Leucine[2]	385
Isoleucine[2]	230
Total BCAAs	831
Non-Essential Amino Acids	
Aspartic Acid + Asparagine	571
Serine	237
Glutamic Acid + Glutamine	749
Proline	191
Glycine	195
Alanine	210
Histidine	124
Arginine	380

[1]subject to natural variability

[2]Branched Chain Amino Acids (BCAAs)

***Note**: When condensed into a protein polypeptide chain, amino acids would not contain the additional molecular weight of water. It should also be noted that FAO/WHO expert panels have advised that protein determined by amino acid analysis should be calculated with amino acid molecular weights minus the molecular weight of water. However, the availability of amino acids in the PDCAAS analysis assumes amino acids digested from food proteins are in the free or hydrated amino acid form when absorbed. The appropriateness of using the results calculated using either hydrated or dehydrated amino acid molecular weights is left solely to the discretion of the end user and whether they are labeling based on whole protein content or amino acids following digestion.*

Speaker Contact Information

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Contact Information	
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