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Of Things to Come: DIAAS and how the world will measure protein quality

2014 Protein Trends & Technologies Seminar

Arlington Heights, Illinois, USA

9th April, 2014

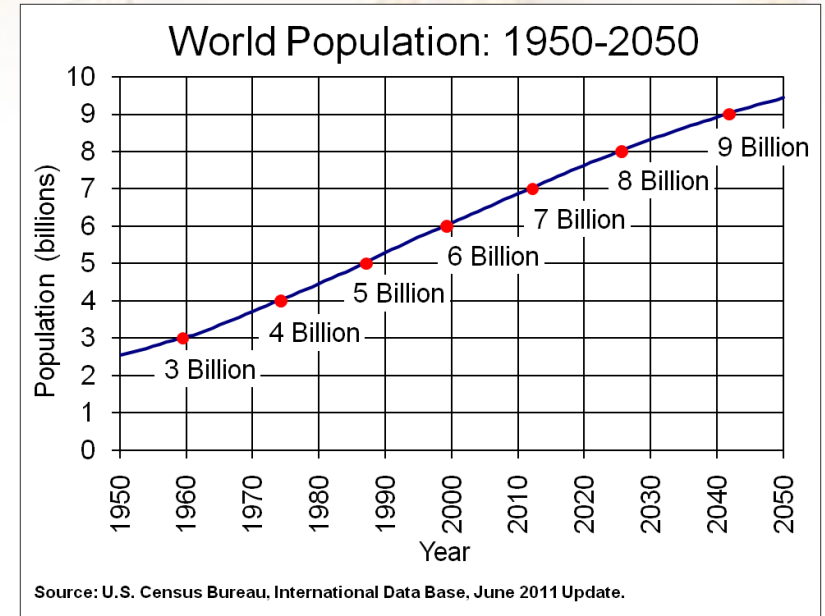
Joyce Boye, 2014 - Presentation



Canada

Why is Protein Quality Assessment Important?

- **Adequacy of global food supplies** (i.e., amount and quality of protein)
- **Informed decision-making** (i.e., food and nutrition policy)
- **Guidance for labelling, regulation and trade** (e.g., CODEX)
- **Health/clinical implications**



<http://www.census.gov>

Overview

- **PDCAAS summary**
 - **1991 FAO/WHO Report**
- **Recommended modifications**
 - **WHO/FAO/UNU 2007 Report**
- **Issues and concerns**
 - **Leading into 2011 FAO Expert Consultation**
- **DIAAS – Overview, applications and implications**
 - **2013 FAO Report**

Examples of Protein Quality Assessment Methods

METHOD	DESCRIPTION
Nitrogen Balance	Measure of daily N intake minus N excreted
Protein Efficiency Ratio	Gain in weight of rat per g of protein eaten
Net Protein Utilisation	Proportion of ingested protein retained
True Biological Value	Proportion of the absorbed N retained taking into consideration the metabolic N loss



In 1989 the Joint FAO/WHO Expert Consultation on Protein Quality Evaluation recommends use of PDCAAS

Protein Digestibility Corrected Amino Acid Score (PDCAAS)

- To calculate the PDCAAS, the **limiting amino acid score*** is multiplied by **protein digestibility**.

(**limiting amino acid score*** - ratio of first limiting amino acid in a gram of target food to that in a reference protein or requirement)

PDCAAS: 1991 FAO/WHO Report

PDCAAS = f (amino acid score, digestibility)

AAS = $\frac{\text{mg of IAA in 1g protein}}{\text{mg of same IAA in 1g of reference pattern}}$

mg of same IAA in 1g of reference pattern

 (**Lowest IAA ratio** is the **amino acid score**)

Amino Acids

“Indispensable” or “Essential”

- His
- Ile
- Leu
- Lys
- Met
- Phe
- Thr
- Trp
- Val

“Dispensable” or “Nonessential”

- Asp
- Asn
- Glu
- Ala
- Ser
- Cys
- Tyr
- Gly
- Arg
- Gln
- Pro

Example of reference AA scoring patterns

Recommended amino acid scoring patterns for infants, children and older children, adolescents and adults

Age Group	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
	<i>scoring pattern mg/g protein requirement</i>								
Infant (birth to 6 months) ¹	21	55	96	69	33	94	44	17	55
Child (6 months to 3 year) ²	20	32	66	57	27	52	31	8.5	43
Older child, adolescent, adult ³	16	30	61	48	23	41	25	6.6	40

¹ Infant is based on the gross amino acid content of human milk

² Child group is from the 6 month (0.5 y) values

³ Older child, adolescent, adult group is from the 3-10 y values

PDCAAS: 1991 FAO/WHO Report

PDCAAS = **f** (amino acid score, digestibility)

Digestibility => **True protein digestibility (TPD)**



(rat balance method)

$$\text{TPD} = 100 \times \frac{[\text{Protein intake} - (\text{Faecal protein} - \text{metabolic faecal protein})]}{\text{Protein intake}}$$

PDCAAS: 1991 FAO/WHO Report

PDCAAS = f (amino acid score, digestibility)

= mg of **limiting IAA** in **1g protein** x Digestibility

mg of **same IAA** in **1g of reference pattern**

 (Units can be expressed as a **decimal** or **percentage**)

PDCAAS: 1991 FAO/WHO Report

PDCAAS

Scores above **1.00** would be considered as **1.00 or 100%**

 (i.e., **PDCAAS** not AAS)

$$\text{PDCAAS} = \text{AAS} \times \text{TPD}$$

Other recommendations

- a) High moisture foods should be dried
(Effect of drying technique on AA?)

- b) High fat foods may require lipid extraction
prior to analysis
(Effect of technique used on AA?)

Recommendation

- a) **Same protocol** for calculation for individual protein sources can be applied to mixture

- b) **Weighted average** procedure can be used when data on AAS and TPD is available

Worked example

Analytical Data

Quantities in Mixture

Weight Protein Lys TSAA Thr Trp
(g) (g/100g) ----- mg/g-----

TPD

Protein Lys TSAA Thr Trp
(g) -----mg-----
 $\frac{A \times B = P}{100}$ PxC PxD PxE PxF

A B C D E F G

Wheat	350	13	25	35	30	11	0.85	45.5	1138	1593	1365	501
Chickpea	150	22	70	25	42	13	0.80	33.0	2310	825	1386	429
Milk Powder	50	34	80	30	37	12	0.95	17.0	1360	510	629	204
Totals								95.5	4808	2928	3380	1134

AA mg/g protein 50 31 35 12

Reference scoring pattern 58 25 34 11

AAS for mixture 0.86 1.24 1.03 1.09

Weighted Average Protein Digestibility 0.85

Score adjusted for digestibility (0.85x0.86) 0.73 (or 73%)

PDCAAS: 2007 WHO/FAO/UNU Report

Issue: Truncation (1991 Report)

Scores above 1.00 would be considered as 1.00 or 100%

?



(i.e., PDCAAS not AAS)

$$\text{PDCAAS} = \text{AAS} \times \text{TPD}$$

2007 Report

Example

Soy protein concentrate

$$\text{AAS} = 1.04$$

$$\text{TPD} = 95\%$$

$$\text{PDCAAS (1991 report)} = \underline{1.04} \times 0.95 = 0.99$$

$$\text{PDCAAS (2007 report)} = \underline{1} \times 0.95 = 0.95$$

NB: If AAS is truncated, then the additional benefit of the excess limiting IAA in compensating for any decrease in digestibility is lost.

Truncation: Other Issues

PDCAAS = **f** (AAS, digestibility)

- **To truncate or not to truncate**
(i.e., Value of excess IAA in mixed diets)
- **And if yes, which? (PDCAAS or AAS?)**

PDCAAS: 2007 WHO/FAO/UNU Report

Issue: Amino Acid Score (AAS) (1991 Report)

$$\text{PDCAAS} = \text{f (AAS, digestibility)}$$

- Reference requirement/pattern?
- Analytical method?
- Effect of processing?

PDCAAS: 2007 WHO/FAO/UNU Report

Issue: Digestibility (1991 Report)

PDCAAS = **f** (AAS, digestibility)

- True vs standardised vs apparent vs real?
- Ileal vs fecal ?
- *In vivo* vs *in vitro*?
- Protein vs AA digestibility?
- Digestibility vs bioavailability?
- Age and species of animals?
- Food quantity?
- Effect of processing, matrix, ANFs?

Amino Acid Bioavailability

The **bioavailability** of an amino acid is the proportion of ingested dietary AA that is absorbed in a chemical form suitable for it to be utilized for protein synthesis or metabolism

WHO/FAO/UNU (2007) Protein and amino acid requirements in human nutrition: report of a joint WHO/FAO/UNU expert consultation. WHO Technical Report Series 935. Geneva. FAO/WHO/UNU, 276.



- Digestibility
- Chemical integrity
- Matrix effects

2013

**Dietary protein quality
evaluation in human
nutrition**

ISSN 0254-4725

FAO
FOOD AND
NUTRITION
PAPER

92

**Report of an
FAO Expert Consultation**

**International FAO Expert Consultation Meeting on Protein
Quality in Human Nutrition**
(30th March – 1st April 2011, Auckland, New Zealand)

2013 FAO Report – Key Recommendations

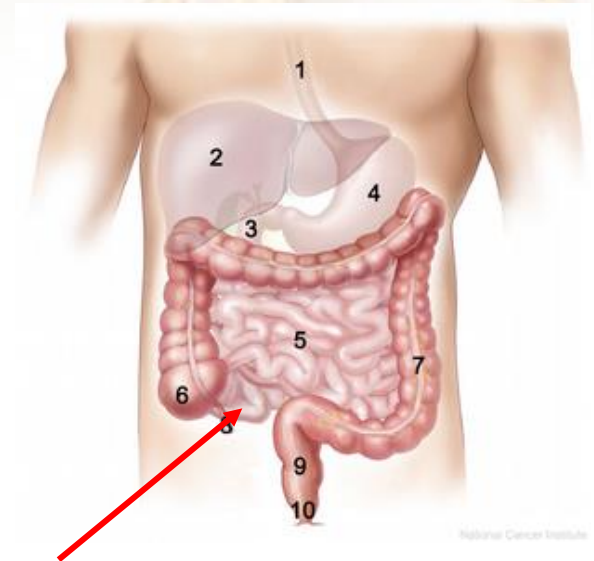
Amino Acids

- **Dietary amino acids** should be treated as **individual nutrients**
- Use digestible amino acids as opposed to digestible protein in calculating protein quality
- For lysine in processed foods, use available or reactive lysine

2013 FAO Report – Key Recommendations

Digestibility

- Use **true ileal** amino acid digestibility (i.e., at terminal ileum at the end of the small intestine as fecal digestibility data is confounded by microflora activity in lower GI tract)
- Preferably determined for each indispensable amino acid, using **humans**, if not then **pigs** or **rats**



1= [oesophagus](#) or [esophagus](#)
2= [liver](#) 3= [duodenum](#) 4= [stomach](#)
5= [small intestine](#) 6= [caecum](#) or
[cecum](#) 7= [colon](#) 8= [appendix](#)
9= [rectum](#) 10= [anus](#)

AA reference scoring patterns

- Infants (birth to 6 months), pattern of human milk
- Young children (6 months to 3 y), pattern for the 0.5 y old infant
- Older children, adolescents and adults, pattern for the 3 to 10 y old child

For regulatory purposes two scoring patterns recommended:

- For infant formulas - amino acid composition of human milk
- For all other foods and population groups - pattern for young children (6 months to 3 y)

2013 FAO Report – Key Recommendations

AA reference scoring patterns

Recommended amino acid scoring patterns for infants, children and older children, adolescents and adults

Age Group	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
	<i>scoring pattern mg/g protein requirement</i>								
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Older child, adolescent, adult ³	16	30	61	48	23	41	25	6.6	40

¹ Infant is based on the gross amino acid content of human milk

² Child group is from the 6 month (0.5 y) values

³ Older child, adolescent, adult group is from the 3-10 y values

DIAAS - Truncation

- Values above 100% should not be truncated for ingredients
- Values above 100% can be truncated when calculating protein quality for **mixed diets** and **sole source foods**

2013 FAO Report – Key Recommendations

A new method recommended to replace PDCAAS:

Digestible Indispensable Amino Acid Score (DIAAS)

DIAAS % = 100 x [(mg of **digestible dietary IAA in 1 g of the dietary protein**) / (mg of the same dietary IAA in 1g of the reference protein)]

(Note: value is calculated for each dietary IAA and lowest value is designated as the DIAAS)

DIAAS Calculation – Single Food Source

Calculation of DIAAS value for whole milk powder (WMP)

	Weight (g)	Composition data ¹					True ileal IAA Digestibility ¹				True ileal digestible IAA content in WMP ²			
		Protein (g/100g)	Lys	SAA	Thr	Trp	Lys	SAA ⁵	Thr	Trp	Lys	SAA	Thr	Trp
			(mg/g protein)									(mg/g protein)		
		A	B	C	D	E	F	G	H	I	J	CxG	DxH	ExI
Milk Powder	100	28	78	35	44	13	0.95	0.94	0.90	0.90	74	33	40	12

Age group (y)	IAA Reference pattern: mg/g protein (refer to Table 5 in this report)				³ Digestible IAA reference ratio				⁴ DIAAS for WMP (%)
	Lys	SAA	Thr	Trp	Lys	SAA	Thr	Trp	
Infant (birth to 6 mths)	69	33	44	17	1.07	1.00	0.91	0.69	69 (Trp)
Child (6 months to 3 yrs)	57	27	31	8.5	1.30	1.22	1.29	1.41	122 (SAA)
Older child, adolescent, adult	48	23	25	6.6	1.54	1.43	1.60	1.82	143 (SAA)

¹ Reference: CVB Feed Tables (2007). Chemical compositions and nutritional values of feed ingredients. Product Board Animal Feed, CVB, The Hague. True ileal indispensable amino acid (IAA) digestibility coefficients are based on predicted human values obtained from pig data.

² For the sake of example, calculation is shown for four amino acids, where possible all IAA should be included in the calculation.

³ Digestible IAA reference ratio (Digestible IAA in 1 g protein of whole milk powder /mg of the same dietary indispensable amino acid in 1g of the reference protein)

⁴ DIAAS for whole milk powder (Lowest value of the "digestible IAA reference ratio" expressed as % for each reference pattern; for infants WMP has a calculated DIAAS of 69; for children 122 and for older children, adolescents and adults 143).

⁵ This is the weighted average of the digestibility coefficients for methionine and cysteine.

Lys=lysine, SAA=sulphur amino acids (methionine + cysteine), Thr = threonine, Trp = tryptophan).

DIAAS Calculation – Mixed Diet

Calculation of DIAAS value for a mixture of wheat, peas and whole milk powder

	Composition ¹						True ileal IAA Digestibility ¹				Protein content in mixture	True ileal digestible IAA content in mixture ²				
	Weight	Protein	Lys	SAA	Thr	Trp	Lys	SAA	Thr	Trp		Lys	SAA ⁵	Thr	Trp	
	(g)	(g/100g)	(mg/g protein)									(g)	(mg)			
	A	B	C	D	E	F	G	H	I	J		AxB	(AxB)xCxG	(AxB)xDxH	(AxB)xExI	(AxB)xFxJ
Wheat	400	11	28	38	29	12	0.82	0.895	0.86	0.91	44	1 010	1488	1 097	480	
Pea	100	21	71	25	37	9	0.79	0.69	0.73	0.66	21	1 178	362	567	125	
Milk powder	35	28	78	35	44	13	0.95	0.94	0.90	0.90	10	726	322	388	115	
Totals	535										75	2 914	2 172	2 052	720	
Amino acids: mg/g protein (total for each amino acid/total protein)											38.9	29.0	27.4	9.6		
Age group		Reference pattern: mg/g protein (Refer to Table 5 in this report)								³ Digestible IAA reference ratio				⁴ DIAAS for mixture (%)		
		Lys	SAA	Thr	Trp					Lys	SAA	Thr	Trp			
Infant (birth to 6 months)		69	33	44	17					0.56	0.88	0.62	0.56	56 (Lys)		
Child (6 months to 3 yrs)		57	27	31	8.5					0.68	1.08	0.88	1.13	68 (Lys)		
Older child, adolescent, adult		48	23	25	6.6					0.82	1.26	1.10	1.45	82 (Lys)		

¹ Reference: CVB Feed Tables (2007). Chemical compositions and nutritional values of feed ingredients. Product Board Animal Feed, CVB, The Hague. True ileal indispensable amino acid (IAA) digestibility coefficients are based on the predicted human values obtained from pig data.

² For the sake of example, calculation is shown for four amino acids; where possible all IAA should be included in the calculation.

³ Digestible IAA reference ratio (Digestible IAA in 1 g protein of mixed diet /mg of the same dietary indispensable amino acid in 1g of the reference protein)

⁴ DIAAS for mixed diet (Lowest value of the "digestible IAA reference ratio" expressed as % for each reference pattern; for infants the mixed food has a calculated DIAAS of 56; for children 68 and for older children, adolescents and adults 82; NB: In this case as this is a mixed diet if the calculated DIAAS exceeded 100%, it would be truncated to 100%).

⁵ These are the weighted average of the digestibility coefficients for methionine and cysteine.

Lys=lysine, SAA=sulphur amino acids (methionine + cysteine), Thr = threonine, Trp = tryptophan.

Example of potential application

For CODEX, to qualify for the nutrition claim: “**source**” for protein, a food must meet the following criteria:

- 10% of NRV* per 100 g (solids)
- 5% of NRV per 100 ml (liquids)
- or 5% of NRV per 100 kcal (12% of NRV per 1 MJ*);
- or 10% of NRV per serving.

To qualify for: “**High**” for protein, the food must contain **two times** the values for “**source**”.

Example of potential application contd.

- When a food meets the criteria for protein quantity, then a quality measure should be applied.
- DIAAS cut-off values will be needed to distinguish between **excellent/high** (e.g. 100 or more), **good/source** (e.g. 75-99), and **no claim**.
- It is recommended that no nutrition claim be allowed for **source or high** protein for proteins with DIAAS less than a certain cut-off (**e.g. 75**).

NB: quality cannot be substituted for quantity

Example of potential application contd.

Example of the use of DIAAS for protein quality assessment in the context of making claims.

Food	Amount	Protein content (g/100g)	DIAAS ¹	Judged quality	Eligible for claim based on quantity	Eligible for claim based on quantity and quality
Wheat	100 g	11	40	Low	Yes, high	No, none
Peas	100 g	21	64	Low	Yes, high	No, none
Whole milk powder	100 g	28	122	High	Yes, high	Yes, High

¹ DIAAS calculated using true ileal indispensable amino acid digestibility values and reference amino acid pattern for child (6 months to 3 years).

Challenges

- **Lack of data on true ileal AA digestibility**
- **RECOMMENDATION** - Until more data becomes available
 - AA digestibility could be considered equivalent to crude protein digestibility
 - DIAAS should be calculated based on true ileal protein digestibility and when not available true faecal crude protein digestibility
 - Use protein digestibility to calculate digestible individual dietary amino acid values

Approaches to increase PDCAAS and DIAAS

- ❑ Develop formulations with ingredients containing **complementary IAAs** (*e.g., legumes and cereals, or protein sources with balanced AA profiles*)
- ❑ Consider the effect of **processing, storage** and **storage temperature** on digestibility and bioavailability of AA
- ❑ If possible exploit the use of **exogenous proteases** in processing
- ❑ Consider the use of **fermentation, germination, probiotics**, etc
- ❑ Remove or reduce **antinutritional** components
- ❑ Consider **matrix effects** on protein digestibility and bioavailability



NOTE: DIAAS more useful when measured on « food » not on grain (e.g., cooked chickpea not raw chickpea)

Examples of PDCAAS of some plant sources of protein

FOOD	PDCAAS* and LAA	REFERENCE
Buckwheat	80, Leu	Eggum et al., 1999
Quinoa (dried, milled)	109, Lys	FAO/WHO/UNU 1985*
Potato	88, Leu	Jørgensen et al., 2008*
Pea (soaked, dried)	48, Met + Cys	FAO/WHO 1973*
Pea (cooked)	91, Met + Cys	FAO/WHO 1973*
Pea protein (Nutralys®)	82, Met + Cys	Roquette, 2008
Canola protein (napin-r)	92, Phe + Tyr	FDA GRAS Notice 327*
Canola protein (cruciferin-r)	77, Phe + Tyr	FDA GRAS Notice 327*
Whole hempseed	49-53, Lys	House et al., 2010
Hempseed meal	46-51, Lys	House et al., 2010
Hempseed (dehulled)	63-66, Lys	House et al., 2010

Others: Almond - ~23 – 73%; Peanut - ~ 34-70%

*Calculated using reference pattern for **3-10 yr child**; LAA – Limiting amino acid

Impact of processing on PDCAAS

Protein digestibility-corrected amino acid scores (PDCAAS), relative protein efficiency ratio (RPER), and relative net protein ratio (RNPR) values for several protein products

Product	PDCAAS (rat)	PDCAAS (human)	RPER	RNPR
	%			
Casein + Met	100	100	100	100
Casein	85	100	80	84
Lactalbumin	100	100	89	91
Lactalbumin, treated	55	67	0	0
Skim milk	74	100	77	82
Skim milk, heated	29	31	0	5
SPI ¹	62	100	56	64
SPI, treated	44	49	0	0
SBM, ² raw	58	80	27	44
SBM, heated	58	83	63	70
Black beans, raw	45	72	0	0
Black beans, heated	51	84	63	70
Mustard flour	84	92	0	0
Zein	1	1	0	0
Zein + AA ³	63	71	3	44

¹ Soybean protein isolate.

² Soybean meal.

³ Lysine + methionine + threonine + tryptophan.

Complementation to improve protein quality

Table 1	Amino Acid Score ¹	True Protein Digestibility ² (%)	PDCAAS ³
Pea (yellow, split)	0.73	87.9	0.64
Pea (green, split)	0.59	85.2	0.50
Lentil (green, whole)	0.71	87.9	0.63
Lentil (red, split)	0.59	90.6	0.54
Chickpeas	0.61	85.0	0.52
Pinto Beans	0.77	76.2	0.59
Kidney Beans	0.70	78.6	0.55
Black Beans	0.76	70.0	0.53
Navy Beans	0.83	80.0	0.67
Soy Flour	0.92	83.5	0.77
Wheat Flour*	0.47	92.3	0.43
Rice Flour*	0.54	92.0	0.50
Lentil-Wheat (25:75) Blend*	0.78	91.0	0.71
Lentil-Rice (20:80) Blend*	0.82	90.0	0.74
Black Bean-Rice (25:75) Blend*	0.81	93.0	0.75
Pea-Wheat (30:70) Blend*	0.83	90.0	0.75
Casein	1.04	96.6	1.00

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¹ Amino acid score is limiting the amino acid with the lowest ratio relative to the established amino acid requirement values for humans, aged 2 to 5 years old.

² AOAC Method 991.29 (n = 10).

³ PDCAAS = Amino Acid Score x % True Protein Digestibility.

* Calculated data obtained from the 1989 WHO/FAO Report on Protein Quality.

Conclusion: Path Forward

- ❑ More data needed on true ileal amino acid digestibility of human foods (i.e., in human and animal models)
- ❑ Need for inter-species (human, pig, rat) true ileal amino acid digestibility comparisons
- ❑ Need for data on impact of processing, antinutritional factors, matrix effects, etc.
- ❑ Clear recommendations on practical applications of DIAAS and implications on food supply (e.g., CODEX applications)



Thank-you!

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