

Future Protein: Nutritional Quality Assessment

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Declaration of Interests

- I hold the position of academic professor for Institute of Nutrition,
 Mahidol University
- The subject of this presentation is within the scope of the organizations' mandate
- I have no actual or potential conflict of interest in relation to this program/presentation

World Population is growing









The world faces a major challenge in food production and environmental sustainability over the next 30 years.

It is estimated that the world needs to produce 70% more food by 2050.

AND not just more food but **nutritionally** better food in an environmentally acceptable manner.

Novel alternative protein sources (e.g., legumes, insects, algae, fungal, cultured meat) have gained increasing popularity over the past decade





Protein: we need quality, not just quantity

Getting enough protein in our diets is essential for adequate nutrition. What is less well known is that protein represents a group of nutrients, the amino acids, each of which needs to be consumed in sufficient amounts. Here, we look at how we digest protein, the importance of amino acids, and show that protein quality, not just quantity, is vital.



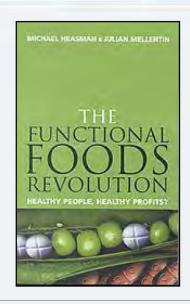




Crucially: People need good quality protein

Role of amino acids in:

- Satiety
- Body muscle metabolism (leucine)
- Maintenance of lean body mass (LBM) (food/health/wellness)
- Maximising lean body mass and muscle strength in sports and exercise.
- Also, estimates of protein/AA requirement being revised upwards (optimum function versus nitrogen balance).



Physiologic/metabolic responses

- · Absorption-digestibility
- Metabolic utilization
- Nitrogen balance
- · Lean mass/muscle/bone
- Tissue turnover
- Secretory proteins
- Host defences/Immunity
- · Growth & maturation
- Tissue repair

Epigenetic

Receptors

GENES PROTEIN
METABOLISM

Hormones

Genetic

Short-term outcomes

- Growth and tissue repair (wasting and stunting)
- Immune function and host defence system (prevalence and severity of infection)
- Muscle and skeletal mass (capacity for physical work and athletic performance)
- Mental performance, mood, sleep patterns
- Detoxication of chemical agents and anti-oxidant system

Long-term outcomes

 Life course events, linear growth, menarche, aging

Protein quality related health outcomes

- Age-related functional losses, muscle, bone strength, immunity, cognitive decline
- Nutrition related chronic diseases. CVDs, cancer, hypertension, oxidative damage, repair systems



Dietary protein quality evaluation in human nutrition

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Report of an **FAO Expert Consultation**



Quality of protein



Amino acid score

Sustainable Protein Sources

& Quality of Protein





Protein quality (PQ) is the capacity of a protein to meet the indispensable amino acid requirements of an individual.

- "Digestibility" refers to how well the proteases in the GI tract can breakdown an ingested protein into amino acids.
- "Bioavailability" refers to the fraction of the amino acids in an ingested protein that are actually absorbed by the body
- "Metabolic availability" refers to the digestibility, absorption, and utilization of an amino acid



Methodology related to protein quality



- Applying digestibility to measures of protein quality
 - Protein Digestible Corrected Amino Acid Score (PDCAAS)
 - Digestible Indispensable Amino Acid Scire (DIAAS)
 - in vitro methods for determining the digestibility of foods
- Isotopic methods for the determining whole-body use of amino acids
 - Dual isotope tracer method
 - Indicator Amino Acid Oxidation (IAAO) slope ratio method

Quality of Protein



- Protein quality refers to the ability of the amino acids in foods to adequately meet human requirements for indispensable amino acids (IAAs).
- The measurement of protein quality has <u>three components</u>:
 - IAAs content of food protein
 - Amino acid requirements vary specific age groups and physiological conditions
 - Digestibility

Amino acid scoring patterns for toddlers, children, adolescents and adults (amended values from the 2007 WHO/FAO/UNU report)

His He Lou Lus CAA AAA The Ten Vol



			His	lle	Leu	Lys	SAA	AAA	Thr	Trp	Val
Tissue amino acid pattern (mg/g protein) ¹ Maintenance amino acid pattern (mg/g protein) ²				35	75	73	35	73	42	12	49
				30	59	45	22	38	23	6	39
	Protein requireme	nts (g/kg/d)									
Age (yr)	Maintenance	Growth ³			amii	no acid i	requireme	ents (mg/l	kg/d)4		
0.5	0.66	0.46	22	36	73	63	31	59	35	9.5	48
1-2	0.66	0.20	15	27	54	44	22	40	24	6	36
3-10	0.66	0.07	12	22	44	35	17	30	18	4.8	29
11-14	0.66	0.07	12	22	44	35	17	30	18	4.8	29
15-18	0.66	0.04	11	21	42	33	16	28	17	4.4	28
>18	0.66	0.00	10	20	39	30	15	25	15	4.0	26
					scoring	pattern	mg/g pro	otein requ	irement	5	
0.5			20	32	66	57	27	52	31	8.5	43
1-2			18	31	63	52	25	46	27	7	41
3-10			16	30	61	48	23	41	25	6.6	40
11-14			16		. 52 . 52	. 1		mg	of an	ino a	cid i

amino acid score = $\frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern}}$

His, histidine; Ile, isoleucine; Leu, leucine; SAA, sulphur amino acids; AAA, aromatic amino acids, Thr, threonine, Trp, tryptophan; Val, valine

16

15

15-18

>18

PDCAAS & DIAAS



PDCAAS

Protein digestibility based on true fecal nitrogen digestibility ... Adopted by the Joint FAO /WHO Expert Consultation to determine protein quality in human foods since 1989

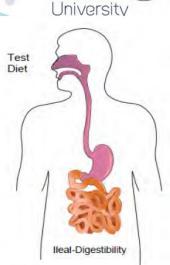
...is confounded by the absorption of nitrogen and microbial activity in the large intestine and discrepancies between AA and protein digestibility

- 1. AA Score_{each IAA} = $\frac{total\ IAA\ in\ food\ (mg/g\ of\ protein)}{reference\ pattern\ IAA\ (mg/g\ of\ protein)} \times 100$
- 2. PDCAAS_{food}= (lowest AA score) \times (weighted average true fecal nitrogen digestibility)

DIAAS

Amino acid digestibility based on specific true *ileal* digestibility of each amino acid Adopted by the Joint FAO WHO Expert Consultation to determine protein quality in human foods since 2011 & Recommended to replace PDCAAS (better reflect the amount of amino acid absorbed)

As dietary nitrogen and amino acid absorption essentially occurs in the small intestine, the ileal digestibility measured at the terminal ileum is considered to be a more accurate assay.

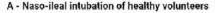


Mahido

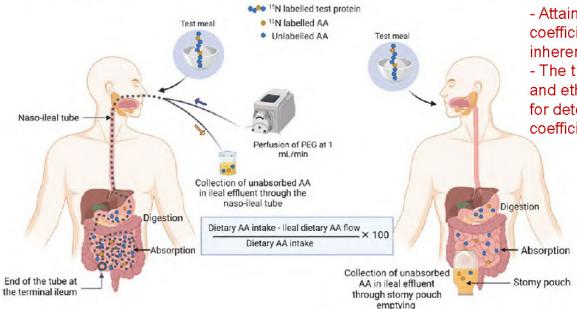
- Total Ileal digestible IAA (for each IAA):
 - = Total IAA in food $(g) \times ileal$ IAA digestibility coeficient
- 2. AA Score (for each IAA):
 - $= \frac{total \text{ ileal digestible } IAA \text{ in food } (mg/g \text{ of total protein})}{reference \text{ pattern } IAA \text{ } (mg/g \text{ of protein})}$
- 3. DIAAS_{food} = Lowest AA score × 100

DIAAS

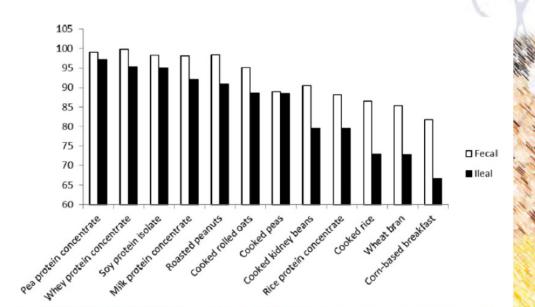




B - Ileostomized patients



- Attaining true ileal digestibility coefficients for humans is inherently difficult.
- The time cost, invasiveness, and ethical constraints required for determining ileal digestibility coefficients remains an issue.





Fecal and ilea digestibility (true nitrogen digestibility, %) of different humanconsumed ingredients determined in growing male rats fed a basal nitrogen-free diet supplemented with the test ingredients as the sole source of protein.

Adapted from Rutherfurd et al. (2015).

Digestible indispensable amino acid scores (DIAAS) and limiting amino acids for different plant and animal sources.

Food materials

DIAAS (%)

Limiting amino acid

Cooked kidney bean *	88	Lysine
Cooked mung bean a	86	Leucine
Cooked chickpeas ^a	76	Lysine
Cooked peas ^a	68	Lysine
Cooked adzuki bean ^a	64	Leucine
Cooked broad beans a	60	Leucine
Corn b	36	Lysine
Cooked Rice c	59	Lysine
Wheat h	48	Lysine
Hemp ^b	54	Lysine
Cooked Oat ^c	54	Lysine
Soy ^b	91	Methionine + Cysteine
Potato ^b	100	N/A
Milk b	116	N/A
Egg ^b	101	N/A
Pork ⁶	117	N/A
Chicken ⁶	108	N/A
Beef b	112	N/A
Insect protein d	75	Lysine + Tryptophan



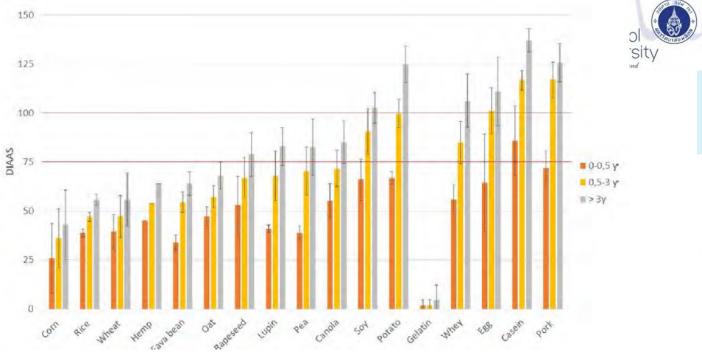
Han, Moughan, Li, and Pang (2020) *; McClements and Grossmann (2021) b; Loveday (2019) c Huang et al. (2018) d

Cantrad Lidney boon 3

The rows have been colour-coded, with green as the best protein sources (DIAAS ≥100) followed by light green (DIAAS < 100 ≥ 85), yellow

(DIAAS < 85 \geq 70), pink (DIAAS < 70 \geq 55) and orange (DIAAS < 55).

Alternative proteins vs animal proteins: The influence of structure and processing on their gastro-small intestinal digestion

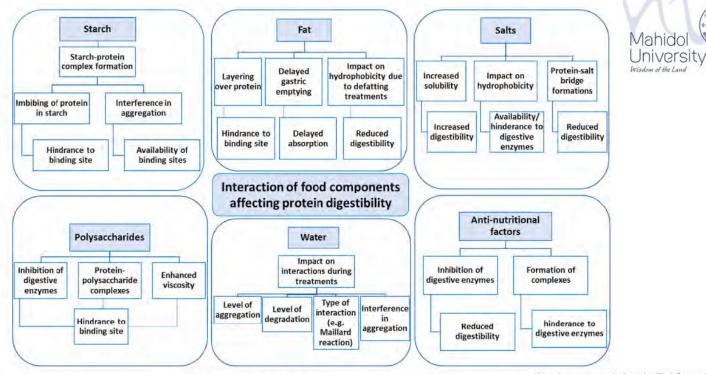


Average DIAAS of various protein sources according to the three reference pattern scores: infant (0-0.5 years), children (0.5-3 years), and children older than 3 years, adolescents, and adults.

Few studies have investigated protein digestibility after the blending and processing of ingredients derived from alternative protein sources or compared protein digestibility between animal-based and alternative food products.



- Multiple factors affect digestibility of proteins in a food system.
- The rate of protein digestion is important in addition to quality scores.
- The type and intensity of processing affects protein digestibility.
- Components of a food matrix also influence protein digestion.
- Protein modification has the scope to improve plant protein digestibility.



The effects of different food components on protein digestibility.

Alternative proteins vs animal proteins: The influence of structure and processing on their gastro-small intestinal digestion

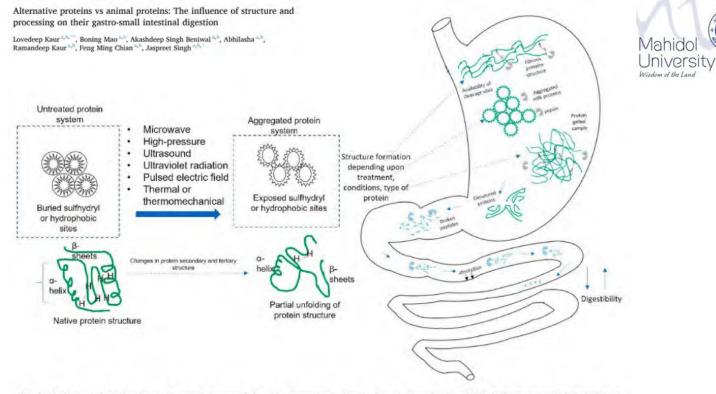
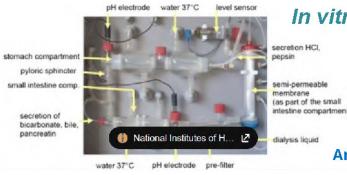


Fig. 2. Mechanisms depicting changes in protein structure induced by processing leading to changes in protein digestibility in the gastro-small intestinal tract.

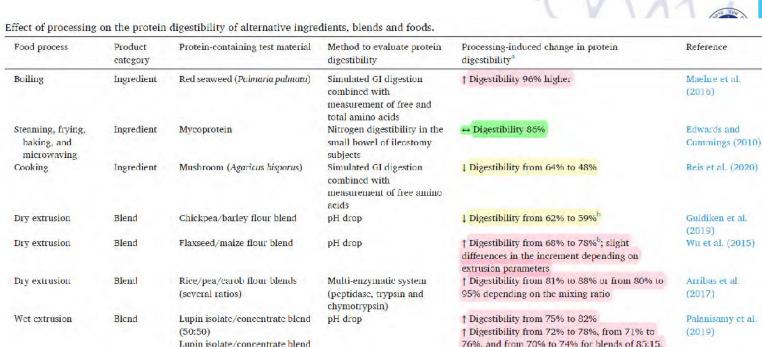


In vitro methods for determining Mahidol the digestibility of foods University



Artificial gut systems

- Havenaar and colleagues (2016) simulate similar gastric conditions as demonstrated in humans, and were successful at calculating DIAAS values.
- However, the dialysis membranes may not account for active transport.
 Moreover, control of pH and peristaltic movements, and immediate feedback from anti-nutritional components on digestion are difficult to mimic in vitro.
- Validate dynamic gut systems to ensure digestibility AA coefficients reflect the PQ of a food when consumed by humans is needed.



Lupin isolate/concentrate blend

and Spirulina (85:15, 70:30,

50:50)

Yogurt style quinoa drink

Fermentation

Food

measurement

combined with amino acid

Simulated GI digestion

70:30 and 50:50, respectively

† Digestibility from 71% to 80 86%

Lorusso et al.

(2018)

The nutritional quality of animal-alternative processed foods based on plant

or microbial proteins and the role of the food matrix J. Lappi , P. Silventoinen-Veijalainen, S. Vanhatalo, N. Rosa-Sibakov, N. Sozer

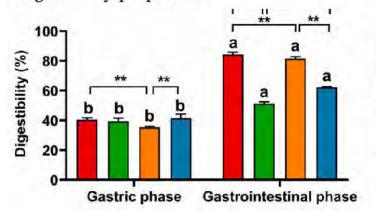
Food process	Product category	Protein-containing test material	Method to evaluate protein digestibility	Processing-induced change in protein digestibility ^a	Reference
Ultra-sound processing, microwaving	Food	Soy milk	Simulated GI digestion combined with protein measurement (colorimetric assay)	† Digestibility from 78–79% to 81–84% induced by ultrasonication for 4–16 min † Digestibility from 77% to 91 and 93% at 100 °C/ 6 min and at 85 °C/10 min, respectively, by microwaving	Vanga, Wang, and Raghavan (2020)
Ultra-sound processing	Food	Almond milk	Simulated GI digestion combined with protein measurement (colorimetric assay)	↔ Digestibility from 74% to 78%	Vanga, Wang, Orsat, and Raghavan (2020)
Fermentation	Food	Yogurt-style snack made from blended rice, lentil and chickpea flour	Simulated GI digestion combined with amino acid measurement	† Digestibility from 67% to 80%	Pontonio et al. (2020)
Fermentation combined with cooking	Food	Porridge made from sorghum/ cowpea flour blend (70:30). Tannin-rich and low-tannin sorghum varieties.	Pepsin digestion method	Digestibility from 76% for the raw blend to 61% for the fermented and cooked porridge, and to 57% for an unfermented and cooked porridge (tannin-rich sorghum variety). Digestibility from 86% for the raw blend to 77% for the fermented and cooked porridge, and to 72% for an unfermented and cooked porridge (low-tannin sorghum variety)	Anyango et al. (2011)
Fermentation combined with baking	Food	Bread made from faba bean flour/corn starch blend (50:50)	Simulated GI digestion combined with amino acid measurement	† Digestibility from 54% to 72%	Sozer et al. (2019)
Extrusion, baking	Blend	Buckwheat/pinto bean blend (50:50)	In vivo (rats): true protein digestibility In vitro: pH drop	³ Higher protein digestibility for the extruded blend (<i>in vitro</i> : 80%; <i>in vivo</i> : 82%) compared to the baked blend (<i>in vitro</i> : 73%; <i>in vivo</i> : 69%) ^b	Nosworthy et al. (2017)

J. Lappi , P. Silventoinen-Veijalainen, S. Vanhatalo, N. Rosa-Sibakov, N. Sozer

Effect of processing on the protein digestibility of alternative ingredients, blends and foods.

Real meat and plant-based meat analogues have different *in vitro* protein digestibility properties

Food Chemistry 387 (2022) 132917

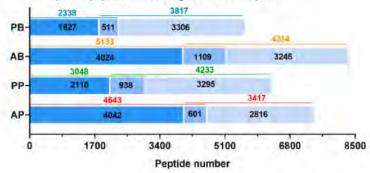


- Real meat had higher digestibility in intestinal phase
- Real meats released more peptides after intestinal digestion





- PP Plant-based Pork
- AB Real Beef
- PB Plant-based Beef
 - Specific peptide number in gastric phase
 - Common peptide number
 - Specific peptide number in gastrointestinal phase



ingredients		digestionity		
Soy-based ground beef patty	Ground beef patty (A)	INFOGEST model combined with measurement of free α-amino groups (OPA)	Lower protein digestibility for the soy-based beef patty (70%) compared to the reference beef patty (85%).	Zhou et al. (2021)
Commercial almond milk, oat milk, hemp milk, and soy milk	Cows' milk (A)	Simulated GI digestion combined with measurement of free α -amino groups (TNBS)	Similar protein digestibility for almond (25%) and oat (25%) milks and the reference (26%). Lower protein digestibility for hemp (22%) and soy (21%) milks compared to the reference.	Martínez-Padilla et al. (2020)
Wheat pasta enriched with faba bean (62%), lentil (65%), or split pea (79%)	Casein and soluble milk proteins (SMP) mixed with starch, cellulose and lipids (A)	INFOGEST model combined with measurement of free α -amino groups (OPA) and peptidome analysis	Lower protein digestibility for the legume- enriched pastas (50% for lentil and pea pastas, 58% for faba bean-pasta) compared to the references (66% for casein and 80% for SMP).	Berrazaga et al. (2020)
Wheat pasta enriched with faba bean (35%)	Wheat pasta (B)	Simulated GI digestion combined with amino acid measurement	Higher protein digestibility for the enriched pasta (46%) compared to the reference pasta (42%).	Laleg et al. (2016)
Wheat biscuit enriched with a mixture of pea protein and whey protein concentrates	Wheat biscuit (B)	TNO GI model combined with TCA measurement of soluble polypeptides (colorimetric)	Similar protein digestibility for the enriched (74%) and reference (78%) biscuits ¹ .	Villemejane et al. (2016)
Wheat breads enriched with 12% green microalgae	Wheat bread (B)	INFOGEST digestion model combined with measurements of protein solubility (combustion) and peptide size distributions	Lower protein digestibility (51–63%) for the enriched breads compared to the reference bread (69%).	Qazi et al. (2021)

Comparison of protein digestibility in foods composed of alternative protein ingredients and reference foods containing animal proteins (A) and in foods enriched with

Method to evaluate protein

digestibility

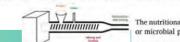
alternative protein ingredients and reference foods without enrichment with the alternative ingredients (B).

Reference food

Food made from or enriched

with alternative protein

No statistical significance available. INFOGEST, Cost Action aiming to harmonize in vitro protocols simulating human digestion; OPA, o-phthaldialdehyde; GI, gastrointestinal; TNBS, trinitrobenzene sulfonic acid; TNO, Netherlands Organisation for Applied Scientific Research; TCA, trichloroacetic acid.



The nutritional quality of animal-alternative processed foods based on plant or microbial proteins and the role of the food matrix J. Lappi , P. Silventoinen-Veijalainen, S. Vanhatalo, N. Rosa-Sibakov, N. Sozer

Difference in protein digestibility

Reference

INFLUENCE OF FOOD MATRIX ON PROTEIN DIGESTIBIL

- The molecular interactions and spatial organization
- Intact cell walls, macromolecular interactions,
 and dietary fiber content affect protein digestibility
- Food matrix can be altered by processing, resulting in changes in protein networks and structures
- Milling and boiling create more surface area and enzyme accessibility

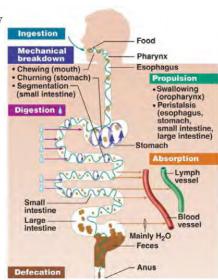


Credit: sweat.com/nutrients; what they are

EFFECT OF MASTICATION ON PROTEIN DIGESTIBILITY Mahido

Mastication affects the food matrix and protein digestibility

- Smaller particles generated influences nutrient release
- Initiation of starch hydrolysis
- Improves gluten accessibility
- Particle size persist during in vitro gastric digestion
- Correlations between bolus properties and digestibility can be challenging to establish



Credit: Physio.com/nutrients digestion

Dual isotope tracer method: measuring true AA digestibility

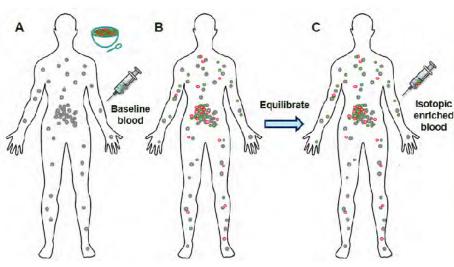
Comparing the concentration of amino acids found in the blood after consuming a test <u>University</u> meal to the concentration of a standard protein of known digestibility using isotopes, wisdom of the Land deuterium and carbon-13.



Phase 1: Bean amino acids are labelled with deuterium added to water during growth

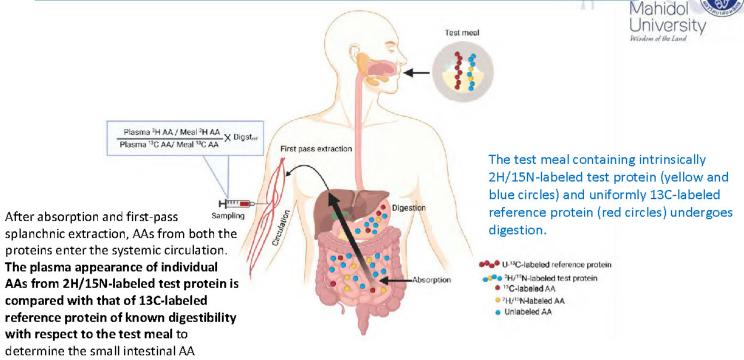


Phase 2:Deuterium-labelled beans used to prepare test meal consumed by study participants to determine protein digestibility



Dual Tracer Approach to Measuring DIAAS

Dual isotope tracer method: measuring true AA digestibility



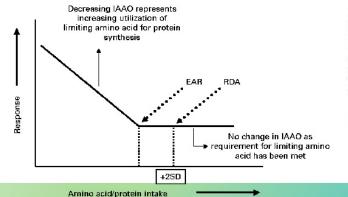
digestibility in the test proteins.

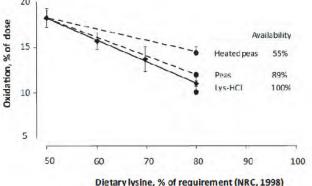
IAAO slope ratio method to estimate metabolic availability of limiting AAs in test proteins.



Indicator Amino Acid Oxidation (IAAO) method is based on the fact that when any single amino acid is limiting for protein synthesis that all other amino acids are in excess and thus must be oxidized. The indicator amino acid is maintained at a constant intake; therefore, the decline in IAAO is linear with incremental addition of the limiting amino acid below the requirement intake. Therefore, this portion of the response can also be used to test the change in net protein synthesis with increasing intake of a food ingredient in which an amino

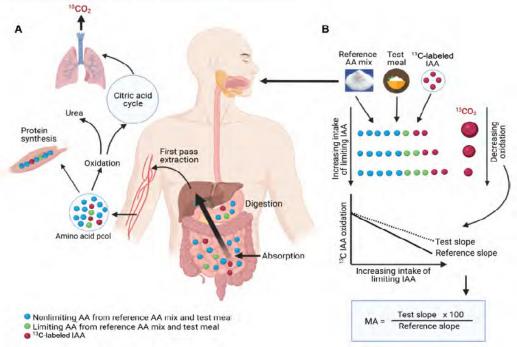
acid is limiting.





IAAO slope ratio method to estimate metabolic availability of limiting AAs in test proteins.





With increasing intake of limiting/test IAA (green circles), the incorporation of 13C-labeled indicator IAA (red circles) into tissue protein synthesis increases with the subsequent reduction in its oxidation, which is measured as 13CO2 in breath

AA digestibility/metabolic availability values using dual isotope tracer or IAAO slope ratio



Proteins and processing	Digestibility method	n						Digesti	bility (9	6) ¹				Study
method			Nitrogen	His	Met	Phe	Thr	Lys	Ile	Leu	Val	Trp	Mean	population
Cereal protein														
Rice, manually dehulled unpolished, pressure cooked for 20 min	Dual isotope tracer	4	-	1	79.7 ± 6.1	83.9 ± 3.4	73.4 ±4.5	78.3 ± 4.1	80.5 ±3.3	78.7 ±3.2	75.2 ± 2.9		78.5 ± 3.5	Children 1-3 y
Rice, polished, boiled for 23 mins	IAAO slope ratio ²	5	102	- 2.5	100	1-11	->1	97	97		00	-81	10	healthy young men
Rice, boiled for 15 mins	IAAO slope - ratio ²	6		-	-		8	97.5	-	-	-		-	School-Age Children
Rice, polished, oven baked at 188°C for 105 min and	IAAO slope ratio ²	3	1-	-	-	7.5	-	70	-	-		-	y = X	healthy youn men

											1	-)		
Proteins and	Digestibility	n						Digesti	ibility (9	%) ¹				Study
processing method	method		Nitrogen	His	Met	Phe	Thr	Lys	Ile	Leu	Val	Тгр	Mean IAA	population
Legume proteir	n													
Mung bean, whole, soaked for 12 h and pressure	Dual isotope tracer	6	1-1-1		52.2 ± 7.2	73.4 ± 6.3	42.5 ± 1.2	63.0 ± 5.4	75.8 ± 2.6	67.5 ± 3.2	67.8 ± 6.0	81	63.2 ± 1.5	Adult 18-45 y
cooked for 15 min														
Mung bean, whole, soaked for 12 h and pressure cooked for 15 min	Dual isotope tracer	4	*		54.0 ± 4.1	77.2 ± 3.6	61.6 ± 2.1	64.8 ± 6.0	63.0 ± 6.1	68.0 ± 1.5	68.0 ± 2.3		65.2 ± 7.1	Children 1-3 y
Mung bean, manually dehulled after soaking for 12 h, pressure cooked for 12 min	Dual isotope tracer	6	-		64.3 ± 4.7	75.1 ± 3.0	54.5 ± 2.4	63.4 ± 3.6	82.9 ± 3.0	76.3 ±3.2	80.0 ± 3.2		70.9 ± 2.1	Adult 18-45 y

Studies determining of true ileal N, AA digestibility/ metabolic availability using stable isotope technique Mahido

Type of food protein	No. of products studied	Study population
Milk protein	13	Adults
Egg protein	5	Children/adults
Meat protein	4	Adults
Cereal protein	12	Children/adults
Legume protein	16	Children/adults
Oil seed protein	1	Adults
Algae	1	Adults
Mixed meal	2	Adults

Summary



- There are several methods for determining PQ for human food, with varying advantages and disadvantages.
- The agreement between the methods has not been rigorously evaluated by measuring the digestibility of the same protein source across methods.
- More studies on AA digestibility or metabolic availability using stable isotope technique during the past decade; mainly studied in children and adults.
- Additional studies on the protein quality of alternative sustainable food sources are needed to create appropriate recommendations to improve the nutrition of individuals.

What do we need to understand better?



- Protein quality: metric in relation to function, not just digestibility and amino acids?
- Protein quantity: minimum vs optimum?
- Changes in need (both quantity and quality) over the life cycle
- Novel protein: adequate nutritionally, health impact, sustainability and reserve our environment





Optimizing bioavailability and physiological impact across life stages



Future Protein Food Digestibility

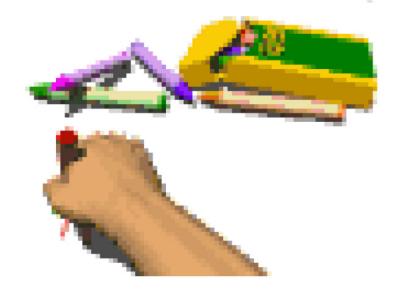
Bioavailability

Utilization

Sustainable! Healthier!

The sufficiency and appropriateness of amino acids and other nutrients for consumers in each age group should be considered.







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