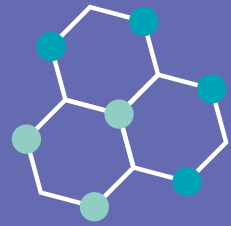


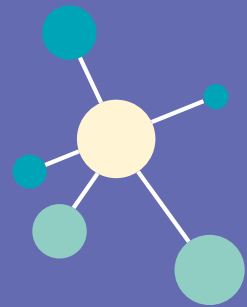
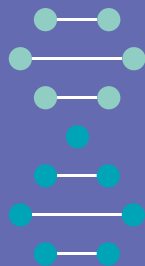
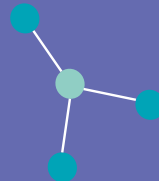


Food and Agriculture
Organization of the
United Nations



Protein Quality Assessment in Follow-up Formula for Young Children

Final Draft FAO Report



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Food and Agriculture Organization of the United Nations
Rome, 2021



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ABBREVIATIONS AND ACRONYMS

AAA Aromatic Amino Acids

AAS Amino Acid Score

FUF-YC Follow up Formula for Young Children

LAA Limiting amino acid

PDCAAS Protein Digestibility-Corrected Amino Acid Score

SAA Sulfur Amino Acids

GLOSSARY – DEFINITION OF TERMS USED IN THE GUIDELINE

A

Amino acid score or Chemical score:

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

The amino acid score is calculated as above and expressed either as a ratio to unity (recommended), or on a percentage scale (WHO, 1991).

B

Bioavailability: The term “bioavailability” encompasses three properties of foods that can alter the proportion of an amino acid that can be utilized; these are:

Digestibility, which describes the net absorption of an amino acid.

F

Fecal digestibility: Defined in terms of balance of amino acids or nitrogen measured from the mouth to anus.

I

Ileal digestibility: Defined in terms of balance of amino acids or nitrogen measured from the mouth to terminal ileum, which ends at the ileocaecal valve.

L

Limiting amino acid (LAA)

The essential amino acid of a dietary protein source present in the lowest proportion as compared to the same quantity of another protein (real or hypothetical) selected as a standard. The apparent limiting amino acid in a protein is thus dependent on the standard chosen. The true limiting amino acid in a protein is, however, the amino acid limiting growth in a biological experiment.

P

Protein requirement: The lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy and other nutrient balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health. It is acknowledged that this definition of the requirement in terms of nitrogen balance does not necessarily identify the optimal intake for health, which is less quantifiable.

Protein digestibility: Defined in terms of balance of amino acids or nitrogen across the small intestine. The difference between intake and losses provides a measure of the extent of digestion and absorption of food protein as amino acids by the gastrointestinal tract for use by the body.

EXECUTIVE SUMMARY

The objectives are to provide practical guidelines and assistance to members of the Codex Alimentarius Commission on how to determine protein quality in Follow-up formula for young children (FUF-YC) using the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) method. The report supplements guidance provided in the FAO 2018 Report on protein quality assessment in follow up formula for young children and ready to use therapeutic foods (FAO, 2018).

The FAO 2018 Report recommended the use of protein, amino acid requirements and reference scoring pattern for young children in the 1–2.9 year age group for determining protein quality for FUF-YC. The reference amino acid pattern is computed utilizing a protein requirement of 0.86 g/kg/day (0.66 g/kg/day for maintenance and 0.20 g/kg/day for growth) and the maintenance and tissue pattern of amino acids as summarized in Table 1.

Table 1: Protein and amino acid requirement and amino acid reference pattern proposed for FUF-YC (1–2.9 year)^b

Requirement	Protein (g/kg/d)	Amino acid (mg/kg/d)								
		His	Ile	Leu	Lys	SAA*	AAA*	Thr	Trp	Val
1-2.9 years	0.86	15	27	54	45	22	40	23	6.4	36
Amino acid reference pattern (mg/g Protein) ^a										
		His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
1-2.9 years		18	31	63	52	26	46	27	7.4	42

^a calculated as amino acid requirement in mg/kg/d divided by total protein requirement in g/kg/d

*SAA = sulphur amino acids (methionine + cysteine), AAA = aromatic amino acids (phenylalanine + tyrosine)

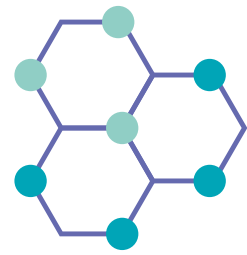
^b Adapted from FAO, 2018

A high-quality protein source will have a PDCAAS score of 1.0. However, a PDCAAS score of ≥ 0.9 can still be considered adequate for these formulations. In formulations with PDCAAS score of < 0.9 the quantity of protein should be adjusted to achieve the desired value.



1. INTRODUCTION

The objectives are to provide practical guidance on the calculation of protein quality in Follow-up formula for young children (FUF-YC) for children aged 12-36 months.



2. PROCEDURES FOR PROTEIN DIGESTIBILITY-CORRECTED AMINO ACID SCORE (PDCAAS) FOR FOLLOW-UP FORMULA IN YOUNG CHILDREN (FUF-YC)

The PDCAAS determines the effectiveness with which absorbed dietary amino acids meet the indispensable amino acid requirement at safe levels of protein intake. The method to compute PDCAAS has been outlined in detail with associated caveats in the 2007 protein requirements (FAO/WHO/UNU 2007).

- The “Chemical Amino Acid Score” is the ratio for each amino acid (mg/g protein) in the food ingredient or formulation on a reference pattern of amino acids (mg/g protein)

Chemical amino acid score % = 100 x [(mg of amino acid in 1 g test protein) / (mg of amino acid in reference pattern)] (WHO, 1991).

- The PDCAAS is computed by correcting the Chemical Amino Acid Score of the indispensable amino acids by the protein fecal or ileal digestibility

*PDCAAS% = weighted protein digestibility for the food formulation * chemical amino acid score.*

The recommendations are to use appropriate and available fecal or ileal digestibility values and the reference requirements and scoring patterns of children in the 1-2.9 years age group for determining protein quality of FUF-YC.

The following steps outline the PDCAAS computation procedure that can be applied to FUF-YC:

Step 1 - Each ingredient providing protein in a formulation is identified and the protein content of each of these ingredients is reported (analysed or extracted from appropriate food composition data if needed) (Table 2).

- a. The amino acid pattern (mg/g protein) of the nine indispensable amino acids in the protein of each of these ingredients (analysed or extracted from appropriate food composition data if needed) is reported.
- b. Data on the best available value of fecal or ileal digestibility of the protein from each of these ingredients is reported. The hierarchy of digestibility data is in the order ileal>fecal>in-vitro (FAO, 2018). A standard assumption is that digestibility of foods does not change when the foods are consumed in mixed diets. However, digestibility can be affected by processing and presence of anti-nutrient factors.

Table 2: Protein content, digestibility and amino acid profile of ingredient used in the formulation of FUF-YC

Ingredients	Protein content (g/100g)	Digestibility %	Amino Acid profile (mg/g protein)								
			His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Ingredient 1	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Ingredient 2	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Ingredient 3	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

(x) Value to be provided, extracted from a database or analysed

Step 2 - The content of each of these ingredients providing protein in the formulation is provided (g/100 ml or g/100g) (table 3).

- The quantity of protein and of each indispensable amino acid provided by each ingredient is calculated.
- Each amino acid is then multiplied by the digestibility value for that ingredient to calculate the amount of digestible amino acid present in that food item.

Table 3: Amount of each ingredient and calculation of the quantity of protein, amino acid and digestible amino acid provided by each ingredient in the formulation of FUF-YC

FUF-YC formulation	g/100ml or g/100g formula		Amino Acid content (mg/100ml or mg/100g formula) ^b								
	Amount	Protein ^a	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Ingredient 1	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Ingredient 2	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Ingredient 3	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
			Digestible Amino Acid content (mg/100ml or mg/100g formula) ^c								
			His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Ingredient 1	-	-	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Ingredient 2	-	-	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Ingredient 3	-	-	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Total	-	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

(x) Value to be provided or calculated

^a calculated from the protein content of the ingredient (table 2) and the amount in the formulation

^b calculated from the quantity of protein from the ingredient in the formulation and its amino acid profile (table 2)

^c calculated by correcting the content of each amino acid by protein digestibility (table 2)

Step 3 - The total protein content, the total digestible amino acid content and the amino acid score (AAS) for each amino acid in the food formulation are then calculated (Table 4).

- e. the amount of each digestible amino acid is converted to mg/g of protein. In other words, digestible amino acid pattern for the protein source is calculated (table 4).
- f. These values are divided by the recommended reference pattern to calculate the PDCAAS for each amino acid.

Table 4: Calculation of the digestible amino acid pattern and of PDCAAS value for each indispensable amino acid in the formulation of FUF-YC

	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Digestible Amino Acid pattern (mg/g Protein) ^a	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
1-2.9 years reference pattern (mg/g Protein) ^b	18	31	63	52	26	46	27	7.4	42
PDCAAS % ^c	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)

* SAA = sulphur amino acids (methionine + cysteine) ; ** AAA = aromatic amino acids (phenylalanine + tyrosine)

(x) Value to be provided or calculated

^a calculated by dividing the digestible amino acid content by total protein content in the formula (table 3)

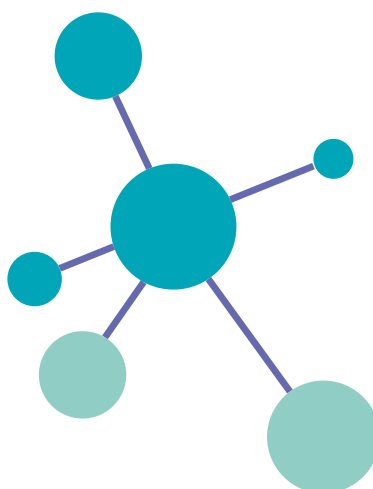
^b from table 1

^c Calculated by dividing for each amino acid the value in the Digestible amino acid pattern by the value in the reference pattern

Step 4 - The amino acid with the lowest score is the limiting amino acid (LAA).

- g. The PDCAAS of this amino acid is then used in computing PDCAAS of the protein.
- h. If the PDCAAS is over 1.0, it should be rounded down to 1.0.

The lowest PDCAAS value is related to the first LAA and is used as the PDCAAS of the protein in the food. Scores are truncated to 1.0. A PDCAAS score below 1.0 indicates that at least one amino acid is limiting in the food or diet and a score of 1.0 that there is no LAA in the food or diet (FAO, 2013). A high-quality protein source will have a PDCAAS score of 1.0 However, a PDCAAS score of ≥ 0.9 can still be considered adequate for these formulations. In formulations with PDCAAS score of < 0.9 the quantity of protein should be adjusted to achieve the desired value. Alternatively, the formulation can be fortified with the LAA(s) added in a free form at level(s) meeting the requirement(s).



3. EXAMPLES FOR COMPUTING PROTEIN DIGESTIBILITY-CORRECTED AMINO ACID SCORE (PDCAAS) FOR FOLLOW-UP FORMULA IN YOUNG CHILDREN (FUF-YC)

The protein sources used in the formulation of FUF-YC are mainly milk protein provided by skim milk, soluble milk whey protein concentrates, partially hydrolysed whey protein, and soy-protein isolates. As plant proteins have generally a lower indispensable amino acid content and have also generally a lower digestibility compared to milk protein, when protein sources of FUF-YC are plant proteins, including soy protein isolates, the minimum protein content may be increased. Protein sources other than milk and soy protein isolates are also being developed.

Table 5: Example of reported value of protein digestibility and amino acid profile of different protein sources used in the formulation of FUF-YC¹

Protein sources	Protein Digestibility %		Amino Acid profile (mg/g protein)								
	Fecal	Ileal	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Skim-milk	98	96	27	61	98	79	32	97	45	14	67
Milk whey protein	99	98	18	56	106	97	43	58	79	19	59
Soy protein isolate	99	96	26	48	77	60	27	55	36	13	47
Rice protein isolate	88	82	18	34	64	23	51	86	29	12	44

Values from : FAO, 2018; Sindayikengera and Xia, 2006; Kalman 2014; Rutherford et al. 2015; Mathai et al.2017; Gorissen et al., 2018; Macé et al., 2006; Gilani et al., 2011.

Three examples for computing PDCAAS for FUF-YC are outlined below. Each example is formulated using different sources of protein:

1. skim-milk with a final protein content in the range 1.3-1.6 g/100 ml (8 to 10 % energy); a mix between skim-milk and whey protein concentrate (60/40 % w/w) with a final protein content in the range 1.3-1.6 g/100ml (8 to 9.5 % energy).
2. partially hydrolysed whey protein with a protein content of about 1.5 g/100ml (9 % energy); soy protein isolate with a final protein content of about 2 g/100ml (12 % energy) fortified with methionine.
3. partially hydrolysed rice protein with a final protein content of about 2 g/100ml (12 % energy) fortified with lysine.

¹ This table summarises examples of published total indispensable amino acid digestibility values in pigs. Other published or analysed values may also be used. For digestibility, human true ileal digestibility values, growing pig true ileal digestibility values, rat true ileal digestibility values should be used in order of preference. If these are not available, human, pig, or rat fecal protein digestibility values should be used.

Example 1 – formulation of FUF-YC with a mix skim milk and whey protein concentrate with ratio of 60/40 w/w

Ingredients	Protein content (g/100g) ^{***}	Digestibility % ^{**}	Amino Acid profile (mg/g protein) ^{**}								
			His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Skim-milk	36.2 ^a	96	27	61	98	79	32	97	45	14	67
Milk whey protein	95	98	18	56	106	97	43	58	79	19	59

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

*** Value extracted from a database

^aFAO, 2018

To obtain a final protein content in the formula of 1.5 g protein /100ml with a ratio of 60/40 between skim milk protein and whey protein, 2.5 g/100ml and 0.63 g/100 ml of *skim milk and whey protein concentrate are used in the formula*. From the amino acid composition and the quantity of each protein, the quantity of each amino acid provided by each ingredient is deduced, and the values are corrected by the digestibility of the protein for each ingredient to calculate the digestible amino acid content.

FUF-YC formulation	g/100ml formula		Amino Acid content (mg/100ml formula) ^b								
	Amount	Protein ^a	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Skim-milk	2.5	0.9	24	55	88	71	29	87	41	13	60
Milk whey protein	0.63	0.6	11	34	64	58	26	35	47	11	35
			Digestible Amino Acid content (mg/100ml formula) ^c								
			His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Skim-milk	-	-	23	53	84	68	28	84	39	12	58
Milk whey protein	-	-	11	33	63	57	25	34	46	11	34
Total	-	1.5	34	86	147	125	53	118	85	23	92

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

(x) Value to be provided or calculated

^a calculated from the protein content of the ingredient (table 2) and the amount in the formulation

^b calculated from the quantity of protein from the ingredient in the formulation and its amino acid profile (table 2)

^c calculated by correcting the content of each amino acid by protein digestibility (table 2)

From the total digestible amino acid content and the total protein content, the digestible amino acid pattern of the formula is calculated. The ratio for each amino acid between this pattern and the reference pattern give the PDCAAS values.

	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Digestible Amino Acid pattern (mg/g Protein) ^a	23	57	98	83	36	79	57	16	61
1-2.9 years reference pattern (mg/g Protein) ^b	18	31	63	52	26	46	27	7,4	42
PDCAAS ^c	1.3	1.9	1.6	1.6	1.4	1.7	2.1	2.1	1.5

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

Value to be provided or calculated

^a calculated by dividing the digestible amino acid content by total protein content in the formula (table 3)

^b from table 1

^c Calculated by dividing for each amino acid the value in the Digestible amino acid pattern by the value in the reference pattern

In this formula, all the amino acids have a score above 1.0. Accordingly, the PDCAAS of the formula is 1.0 that indicates an adequate protein quality of the formula for the target population.

Example 2 – formulation of FUF-YC with a mix of soy protein isolate and partially hydrolysed rice protein isolate in a ratio of 50/50 w/w

This example 2 of computing PDCAAS for FUF-YC provided below stimulates a formulation of a FUF-YC containing as sources of protein soy protein isolate and partially hydrolysed rice protein isolate in a ratio of 50/50 w/w in the products, both ingredients having a protein content of 80%.

Ingredients	Protein content (g/100g) ^{***}	Digestibility % ^{***}	Amino Acid profile (mg/g protein) ^{***}								
			His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Soy protein isolate	80	96	26	48	77	60	27	55	36	13	47
Rice protein isolate	80	82	18	34	64	23	51	86	29	12	44

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

*** Value extracted from a database

To obtain a final protein content in the formula of 2g/100ml, 1.25 g/100ml of each ingredient is used. From the amino acid composition and the quantity of each protein, the quantity of each amino acid provided by each ingredient is deduced, and the values are corrected by the digestibility of the protein for each ingredient to calculate the digestible amino acid content.

FUF-YC formulation	g/100ml formula		Amino Acid content (mg/100ml formula) ^b								
	Amount	Protein ^a	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Soy protein isolate	1.2	1	26	48	77	60	27	55	36	13	47
Rice protein isolate	1.2	1	18	34	64	23	51	86	29	12	44
			Digestible Amino Acid content (mg/100ml formula) ^c								
			His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Soy protein isolate	-	-	25	46	74	58	26	53	35	12	45
Rice protein isolate	-	-	15	28	53	19	42	71	24	10	37
Total	-	2	40	74	127	77	68	124	59	22	82

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

(x) Value to be provided or calculated

^a calculated from the protein content of the ingredient (table 2) and the amount in the formulation

^b calculated from the quantity of protein from the ingredient in the formulation and its amino acid profile (table 2)

^c calculated by correcting the content of each amino acid by protein digestibility (table 2)

From the total digestible amino acid content and the total protein content, the digestible amino acid pattern of the formula is calculated. The ratio for each amino acid between this pattern and the reference pattern give the PDCAAS values.

	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Digestible Amino Acid pattern (mg/g Protein) ^a	20	37	64	38	34	62	29	11	41
1-2.9 years reference pattern (mg/g Protein) ^b	18	31	63	52	26	46	27	7.4	42
PDCAAS^c	1.1	1.2	1.0	0.7	1.3	1.3	1.1	1.5	1.0

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

Value to be provided or calculated

^a calculated by dividing the digestible amino acid content by total protein content in the formula (table 2)

^b from table 1

^c Calculated by dividing for each amino acid the value in the Digestible amino acid pattern by the value in the reference pattern

In this example the lower PDCAAS value is 0.7 obtained for lysine which is the LAA of the formulation. Accordingly, the formulation as a PDCAAS of 0.7, which indicates a low protein quality. The quality of the formulation can be corrected by lysine fortification, or by changing the ratio between the protein sources.

Example 3 – formulation of FUF-YC with a mix of soy protein isolate and partially hydrolysed rice protein isolate in a ratio of 75/25 w/w

In the example 3 below, the same sources of protein soy protein isolate and partially hydrolysed rice protein isolate are used but the ratio between the two protein sources is 75/25 w/w.

Ingredients	Protein content (g/100g)**	Digestibility %**	Amino Acid profile (mg/g protein) ***								
			His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Soy protein isolate	80	96	26	48	77	60	27	55	36	13	47
Rice protein isolate	80	82	18	34	64	23	51	86	29	12	44

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

*** Value extracted from a database

FUF-YC formulation	g/100ml formula		Amino Acid content (mg/100ml formula) ^b								
	Amount	Protein ^a	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Soy protein isolate	1.9	1.5	39	72	116	90	41	83	54	20	71
Rice protein isolate	0.6	0.5	9	17	32	12	26	43	15	6	22
			Digestible Amino Acid content (mg/100ml formula) ^c								
			His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Soy protein isolate	-	-	37	69	111	86	39	80	52	19	68
Rice protein isolate	-	-	7	14	27	10	22	36	12	5	18
Total	-	2	45	83	138	96	61	115	64	24	86

* SAA = sulphur amino acids (methionine + cysteine); ** AAA = aromatic amino acids (phenylalanine + tyrosine)

(x) Value to be provided or calculated

^a calculated from the protein content of the ingredient (table 2) and the amount in the formulation

^b calculated from the quantity of protein from the ingredient in the formulation and its amino acid profile (table 2)

^c calculated by correcting the content of each amino acid by protein digestibility (table 2)



	His	Ile	Leu	Lys	SAA*	AAA**	Thr	Trp	Val
Digestible Amino Acid pattern (mg/g Protein) ^a	22	42	69	48	30	58	32	12	43
1-2.9 years reference pattern (mg/g Protein) ^b	18	31	63	52	26	46	27	7.4	42
PDCAAS ^c	1.2	1.3	1.1	0.93	1.2	1.3	1.2	1.6	1.0

* SAA = sulphur amino acids (methionine + cysteine) ; ** AAA = aromatic amino acids (phenylalanine + tyrosine)

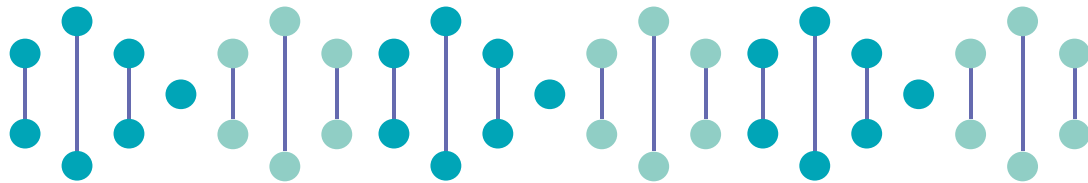
^a Value to be provided or calculated

^b calculated by dividing the digestible amino acid content by total protein content in the formula (table 2)

^c from table 1

^d Calculated by dividing for each amino acid the value in the Digestible amino acid pattern by the value in the reference pattern

In this case computing the PDCAAS value of the different amino acids indicate that the lower value is also obtained for lysine, but with a PDCAAS of 0.93. Accordingly, the PDCAAS value of the formulated FUF-YC is 0.93, indicating an acceptable protein quality.



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