SPECIAL GUEST EDITOR SECTION

The Protein Digestibility-Corrected Amino Acid Score (PDCAAS)—A Concept for Describing Protein Quality in Foods and Food Ingredients: A Critical Review

GERTJAN SCHAAFSMA
TNO Nutrition and Food Research,[1] PO Box 360, 3700 AJ Zeist, The Netherlands

Protein Digestibility-Corrected Amino Score (PDCAAS) is discussed. PDCAAS is now widely used as a routine assay for protein quality evaluation, replacing the more traditional biological methods [e.g., measurement of the Protein Efficiency Ratio (PER) in rats]. PDCAAS is based on comparison of the essential amino acid content of a test protein with that of a reference essential amino acid pattern and a correction for differences in protein digestibility as determined using a rat assay. Although PDCAAS is a rapid and useful method, it often shows discrepancies when compared to PER values. These discrepancies relate to the following issues: uncertainty about the validity of reference patterns, invalidity of correction for fecal (versus ileal) digestibility, truncation of PDCAAS values to 100%, failure to obtain full biological response after supplementation of the limiting essential amino acid, discrepancies between protein and amino acid digestibility, effects of processing on protein quality, and effects of the presence of antinutritional factors in the matrix containing the protein. Part of the discrepancy between PDCAAS and PER can be overcome by modifications of PDCAAS. This article describes some proposed modifications and puts forward the suggestion that the rat protein fecal digestibility assay be replaced by an in vitro ileal amino acid digestibility assay based on a computer-controlled gastrointestinal model.

It is well accepted that the nutritional requirement for dietary proteins consists of the following 3 components: indispensable (dietary essential) amino acids, conditionally indispensable amino acids, and nonspecific nitrogen required for the synthesis of dispensable (nonessential) amino acids and other important nitrogenous compounds (1). The amino acids that are indispensable under all conditions are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The amino acids that become indispensable under specific conditions are cystine, tyrosine, taurine, glycine, arginine, glutamine, and proline. Aspartic acid, asparagine, glutamic acid, alanine, and serine belong to the nutritionally dispensable amino acids.

The nutritional quality of a protein source for humans or animals can be considered as the power of that protein source to cover the requirements for nitrogen and amino acids of these organisms. It has become clear that the nutritional quality of proteins may differ widely, mainly depending on their (essential) amino acid composition and digestibility. For many years, bioassays, predominantly using growing rats, were the preferred approach to assessing the nutritional quality of proteins. Values were expressed in parameters such as the Protein Efficiency Ratio (PER), Net Protein Utilization , and Biological value. However, the only true measurement of protein quality for human use is the nitrogen balance evaluation in experiments with human volunteers, but such studies may not be performed for ethical reasons or are too expensive for routine use.

PER, the most widely used bioassay, was the first method adopted for routine assessment of protein quality of foods. It is a standardized method (2): weanling rats are fed a casein control diet or a test diet (both diets containing 10% of protein, w/w, N × 6.25) for a period of 4 weeks, and PER values are calculated as body weight gain (g)/g protein consumed. Outcomes are standardized to an assumed value for casein of 2.5. A disadvantage of PER is that the amino acid requirement pattern of the growing rat is not identical to that of humans. In humans, requirements are dominated by maintenance processes and not by growth. Another difference between rats and humans is the 50% higher requirement in growing rats for the sulfur-containing amino acids (to support the development of fur). These disadvantages, as well as the progress that has been had been made in the technology of amino acid analysis of foods and on amino acid requirements of humans, led a joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Consultation on Protein Quality Evaluation in 1989 (3) to conclude that protein quality could be assessed adequately by expressing the content of the first limiting essential amino acid in a test protein as a

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Corresponding author's e-mail: schaafsma@voeding.tno.nl.

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Table 1. The FAO/WHO/UNU-recommended amino acid requirement pattern based on amino acid requirements of preschool-age children[a]

Amino acid	Requirement, mg/g crude protein
Isoleucine	28
Leucine	66
Lysine	58
Total sulfur amino acids	25
Total aromatic amino acids	63
Threonine	34
Tryptophan	11
Valine	35
Total	320

[a] From FAO/WHO/UNU Expert Consultation (ref. 4).

percentage of the content of the same amino acid in a reference pattern of essential amino acids. This reference pattern was based on the essential amino acid requirements of preschool-age children as published in 1985 by FAO/WHO/United Nations University (UNU; 4). Subsequently, this percentage is corrected for true digestibility of the test protein as measured in a rat fecal-based assay. This scoring method, known as the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) appears to be generally accepted now as a routine procedure for protein quality evaluation. It is the aim of this article to discuss critically the PDCAAS method, its advantages, and its shortcomings. The article also discusses some proposals to improve the method.

PDCAAS, Principles and Assumptions

PDCAAS has been defined using the following formula:

$$\text{PDCAAS, \%} = (\text{mg of first limiting amino acid in 1 g test protein})/(\text{mg of the same amino acid in 1 g reference protein}) \times \text{TD (\%)}$$

where TD is true fecal digestibility of the test protein, as measured in a rat assay. True fecal protein digestibility is defined as the difference between intake of protein N and output of fecal N, expressed as a percentage of protein N intake, where fecal N is corrected for metabolic fecal N as measured using a protein-free diet. Proteins with PDCAAS values exceeding 100% are not considered to contribute additional benefit in humans and are truncated to 100%.

According to the formula, the PDCAAS method is based on 2 basic principles. The first one is that the content of the first limiting essential amino acid in a protein or in a mixture of proteins is a critical factor for the power of that protein or protein mixture to meet the nutritional amino acid requirements. The second sound principle of the method is that the protein can only meet the nutritional requirements when the amino acids can be absorbed from the diet and, thus, that protein digestibility should be taken into account. The main assumptions are that essential amino acid bioavailability is reflected correctly by true fecal protein digestibility and that the composition of the reference protein is valid. Since its introduction, the PDCAAS has been subject to criticism as, for instance, during the symposium on the Significance of Dietary Protein Sources in Humans held in San Francisco, CA, on October 4, 1999 (1). Similarly, the validity of the PDCAAS method in assessing the protein quality of foods and diets was recently assessed by FAO/WHO (5). The general consensus at these meetings was that, PDCAAS is a valuable tool for routine assessment of protein quality. However, several critical issues need to be addressed for improving the utility of the method. These include the suitability of the essential amino acid composition of the currently recommended reference proteins, the truncation to 100% of PDCAAS values that are higher than 100%, true fecal protein digestibility as a measure of amino acid bioavailability, impact of antinutritional factors, and biological efficiency of supplemental amino acids in improving protein quality.

Validity of the Essential Amino Acid Composition of the Reference Proteins

Because protein and essential amino acid requirements are dependent on age, it is important to realize that the power of a particular protein source to meet essential amino acid requirements may differ among age groups. Thus, in 1981, the Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements (4) proposed amino acid scoring patterns for adults, those of preschool-age children, and infants. The pattern of infants was based on the amino acid composition of human breast milk, and those preschool-age children and adults on a rather limited amount of research data. In the the years after 1981, it appeared from amino acid oxidation studies in adults that the 1981 FAO/WHO/UNU Expert Consultation had underestimated significantly the indispensable amino acid requirements of adults, and the 1989 FAO/WHO Expert Consultation on Protein Quality Evaluation (3) proposed, as an interim procedure, use of the 1985 FAO/WHO/UNU amino acid requirement pattern for preschool-age children to score dietary protein quality for all age groups except infants. This reference pattern is shown in Table 1. It was obtained by computing the ratios between the essential amino acid requirement values (mg/kg body weight/day) and the safe level of high-quality protein intake (g/kg body weight/day), thus resulting in values of mg/g of protein for each essential amino acid. This pattern is still the best that is available, although it is recognized that there is a need for further scientific evaluation and substantiation (1). The basis of the pattern given in Table 1 originates from amino acid balance studies performed by Torun et al. (6) and Pineda et al. (7) in a limited number of 2-year-old children. These children were recovering from malnutrition and, thus, not representative of normal healthy preschool-age children. In deriving the pattern given in Table 1, FAO/WHO/UNU

Table 2. Data showing that, unlike PER, PDCAAS does not recognize the additional value of high-quality proteins[a]

Product	PER (casein = 2.5)	PDCAAS, %
Casein + Met[b]	3.1	100
Whey protein concentrate	3.0	100
Egg-white solids	3.0	100
Lactalbumin	2.8	100
Skim milk powder	2.8	100
Milk protein isolate	2.8	100
Minced beef	2.7	100
Beef salami	2.6	100
Tuna	2.6	100

[a] As summarized by Sarwar (ref. 11).
[b] Met = Methionine.

assumed that the amino acid requirements (mg/kg body weight/day) include a margin of safety comparable to that of the FAO/WHO safe level of high-quality protein (meat, fish, egg, and milk) intake for this particular group of children. This assumption has, however, not yet been validated. Because in preschool-age children, like in adults, the maintenance component of essential amino acid requirements is dominant over the growth component, it is understandable that the essential amino acid requirements of children, when expressed per gram of protein requirement, do not differ much from those of adults. In other words, it may indeed appear that, at the adequate minimum intake level of protein, adults and children have similar dietary protein quality requirements, which would support the use of the preschool-age pattern for evaluating protein quality by PDCAAS for adults also. Estimations of essential amino acid requirements in adults from amino acid oxidation studies compare favorably with the FAO/WHO requirement pattern for preschool-age children, although the lysine requirement for the FAO/WHO pattern is 14% higher (8).

The current reference pattern is restricted to the indispensable amino acids and does not involve amino acids that become indispensable under specific physiological or pathological conditions, such as cystine, tyrosine, taurine, glycine, arginine, glutamine, and proline. This implies that these amino acids should also contribute to the nutritional value of a protein (9). This consideration also pleads for a critical contemplation of the current scoring pattern.

The Truncation of PDCAAS Values to 100%

According to the current PDCAAS method, values higher than 100% are truncated to 100%. This truncation procedure is valid when it comes to the evaluation of mixtures of proteins in total diets, or when a particular protein would be the only protein source in the diet. In those situations, digestible dietary essential amino acid concentrations in excess of those in the reference pattern of preschool-age children do not provide additional nutritional benefits. However, truncation of PDCAAS values of supplementary protein sources that could be used to improve the nutritional value of mixtures of proteins does not include a credit for the extra amino acids provided by the supplementary protein and does not provide any information about capacity for improvement. For many proteins, such as those from milk, meat, and egg, truncation causes a loss of useful information. This point was not adequately discussed at the FAO/WHO Expert Consultation (3). The power of high-quality proteins to balance the amino acid composition of a mixed diet is extremely relevant. A classic example is the combination of milk and wheat, in which the relatively high lysine concentration of milk proteins compensates for the low concentration of this essential amino acid in wheat. It has been shown (10) that 1.2 g casein can balance 1 g wheat protein, whereas 6.2 g soy protein would be needed to do so. This power of animal protein sources is not just relevant for compensating for the low content of lysine in cereals but also for the often-encountered low content of sulfur amino acids and/or threonine in many other plant protein sources. Thus, the PDCAAS method, unlike biological methods such as PER, fails to recognize the additional value of high-quality proteins (Table 2). The nontruncated PDCAAS values for milk, soy, pea, and wheat are 120, 99, 73, and 36% respectively (11). Interestingly, it has been shown in studies with humans given these isolated proteins (12) that postprandial nitrogen retention follows exactly the same order, stressing the need for revision of PDCAA regarding the truncation procedure. So it has been suggested (1) to identify the score of milk protein as 128 (lysine), 123 (threonine), and 120 (methionine + cystine). Such information, as compared to the truncated value of 100 for milk, is useful, taking into account that lysine, threonine, and the sulfur amino acids, in particular, are the limiting amino acids in vegetable proteins.

True Fecal Protein Digestibility as a Measure of Amino Acid Bioavailability

Correction for protein digestibility should take into account the loss of amino acids from the small intestine into the colon, and potential differences between protein and amino acid digestibility. Both of these issues have not been addressed adequately in the current PDCAAS method.

The FAO/WHO Expert Consultation on Protein Quality Evaluation (3) recognized that the intestinal flow of amino acids beyond the terminal ileum is an important route for metabolic consumption of amino acids by the intestinal flora, and these amino acids are most probably largely lost for body protein synthesis. This means that measurement of the fecal digestibility of proteins (determined in rats) may not provide an accurate correction for protein digestibility in the PDCAAS method. Ileal digestibility may be a preferred approach. Therefore, the Expert Consultation recommended studies to resolve uncertainties about the contribution and

Table 3. Data showing that unlike biological methods such as RPER and RNPR, PDCAAS does not reflect the adverse effects of antinutritional factors[a]

Product	PDCAAS	RPER[b]	RNPR	TPD[c]
Casein + Met[d]	100	100	100	100
Casein	100	80	84	99
Lactalbumin	100	89	91	99
Lactalbumin, treated[e]	67	0	0	73
Skim milk	100	77	82	94
Skim milk, heated	31	0	5	77
SPI[f]	100	56	64	96
SPI[f], treated	49	0	0	68
Soybean meal, raw	80	27	44	80
Soybean meal, heated	83	63	70	83
Black beans, raw	72	0	0	71
Black beans, heated	84	63	70	83
Mustard flour	92	0	0	92

[a] Abstracted from Sarwar (ref. 22).
[b] RPER = Relative Protein Efficiency Ratio.
[c] TPD = True Protein Digestibility.
[d] Met = Methionine.
[e] Alkaline/heat-treated.
[f] Soy protein isolate.

variation of endogenous amino acid losses at the terminal ileum before the determination of ileal digestibility could be recommended to replace fecal digestibility. Ileal-fistulated pigs appear to be a good model for the determination of ileal digestibility of proteins (13–15). Contrary to the rat, the pig is a meal-eating species and does not practice coprophagy as does the rat. Moreover, the gastrointestinal anatomy and physiology of the growing pig closely resembles that of adult humans. This supports the validity of using the pig as a model for the human in digestibility studies.

The assumption of the FAO/WHO Expert Consultation (3) that protein digestibility is an acceptable measure of amino acid bioavailability did not always appear to be correct. Often, large differences were found between digestibility values for proteins and individual amino acids (16). Thus, the accuracy of the PDCAAS would be improved by determining individual amino acid digestibility values as opposed to only that of protein.

Amino acid digestibility and/or bioavailability may be decreased by processing of foods. A well known example is the loss of bioavailable lysine by heat treatment and prolonged storage, causing so-called Maillard reactions. Under such circumstances, the measurement of true ileal reactive lysine digestibility may be inaccurate. (*See* paper by Moughan in this series). It has also been shown (17) that heat treatment under alkaline conditions may cause racemization of L- into biologically inactive D-amino acids. Because routine amino acid analysis used in the PDCAAS method does not distinguish between D- and L-forms of amino acids, special analyses of D-amino acids for some processed foods are needed to assess the importance of this problem. Processing may also cause oxidation of sulfur amino acids, and it has been shown that the oxidized forms are less bioavailable (18). The AOAC method for amino acid analysis (19) measures both available methionine and cystine and less-available oxidized methionine and cystine, and this causes an overestimation of the PDCAAS value whenever cases oxidation of the sulfur amino acids has occurred.

Antinutritional Factors in Protein Sources

True digestibility is a fundamental property of a food ingredient, and is a measure that is unaffected by the dietary conditions (20). Thus, true protein digestibility, by definition, is a measure that is independent of the matrix that contains the protein. It is, however, well known that matrix components, like dietary fiber, and antinutritional factors, such as trypsin inhibitors, may cause loss of essential amino acids from the terminal ileum to the colon and increase the essential amino acid requirement (21, 22). The increased flow of endogenous amino acids (from digestive secretions, mucosal cells, and bile) to the colon decreases apparent protein digestibility (22). The consequence of ingestion of antinutritional factors is, thus, a lowering of protein and amino acid utilization, and this is not taken into account in the PDCAAS values, which are based on true fecal digestibility. Antinutritional factors and other matrix components may, thus, cause a discrepancy between biological methods for protein quality evaluation,

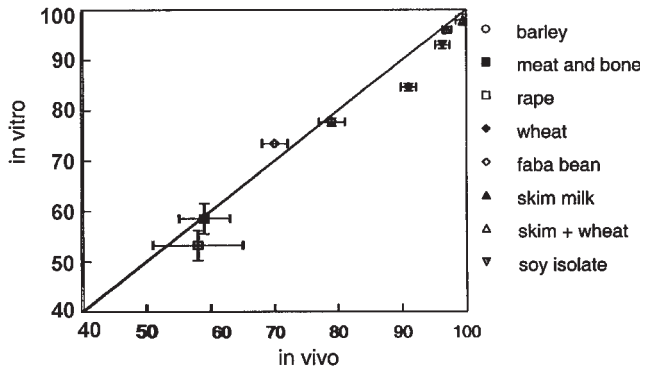

Figure 1. Relationship between true ileal protein digestibility in pigs and calves and the true digestibility in the TIM. The line presents the optimum relation.

like PER, and PDCAAS (23; Table 2). As a result, the PDCAAS method would overestimate the protein quality of products containing naturally occurring growth-depressing factors (e.g., glucosinolates and isothiocyanates in mustard flour, and trypsin inhibitors and hemagglutinins in soy protein and beans) or antinutritional factors formed during processing, such as Maillard compounds in heated milk and lysinoalanine in alkaline/heat-processed lactalbumin and soy protein isolate (Table 3).

To account for these additional endogenous losses of amino acids caused by the antinutritional factors, the term "real ileal amino acid digestibility" has been introduced (20). Real digestibility accounts for the total endogenous amino acid losses, including those caused by matrix components, whereas true digestibility only accounts for the basal endogenous amino acid loss as measured under standard conditions. In practice: apparent digestibility < true digestibility < real digestibility for most foods. Endogenous nitrogen flows in the ileal digesta of pigs fed skim milk, wheat, soy protein isolate, barley, and phaseolus beans were 1.3, 3.1, 3.3, 4.0, and 10.8 g/100 g protein (24). An increased endogenous essential amino acid loss will cause discrepancy between fecal and ileal digestibility values and, thus, reduces the validity of the use of true fecal digestibility in the PDCAAS method. However, it is likely that the difference between fecal and ileal amino acid digestibility in diets for humans is less important than that in animal feedstuffs, because only small differences (<5%) between ileal and fecal amino acid digestibility have been found in studies with ileostomy patients and normal subjects (13), although, in the latter study, relatively highly digestible diets were used.

Suitability of the PDCAAS in Predicting the Quality of Amino Acid-Supplemented Proteins

The PDCAAS method assumes complete biological efficiency of supplemental amino acids in improving protein quality, which may not be true, especially in the case of low-quality proteins (23). The PDCAAS and RNPR (Relative Net Protein ratio, based on rat growth plus an estimate of protein used for maintenance) values for zein (a protein of low digestibility and poor quality) were 9 and 11%, respectively. When the zein diet was supplemented with limiting amino acids such as lysine, tryptophan, and methionine, the PDCAAS and RNPR values were 81 and 30%, respectively (23). A marked difference between the PDCAAS and RNPR of the amino acid-supplemented zein would suggest incomplete biological efficiency of the supplemental amino acids. The poor biological response to amino acid-supplemented zein may have been due to the poor bioavailability of essential amino acid(s) other than those supplemented, which may not be 100%. In such cases, the assumed efficiency of supplementary amino acid(s) must be confirmed biologically.

Proposals for Improvement of the PDCAAS Measure

As described above, PDCAAS is a useful routine method for protein quality evaluation but, in its present form, it has several disadvantages. First, there is still the necessity for including an animal experiment to assess protein digestibility. Second, true fecal digestibility is invalid as a digestibility correction of the PDCAAS for animal feedstuffs and may also overestimate PDCAAS values in human diets. Third, protein digestibility may not correctly reflect essential amino acid digestibility. Fourth, in truncating PDCAAS to 100%, information is lost about the power of a protein to balance the amino acid composition of other proteins or protein mixtures. To solve this latter shortcoming, 3 PDCAAS values could be given for each protein, corresponding to the concentrations of the essential amino acids that are often limiting in plant protein diets (e.g., lysine, sulfur amino acids, and threonine). The application of the Tiny-TIM-AA in vitro system to predict digestibility of the limiting amino acids, required for the calculations of 3 PDCAAS values for each protein source, could be extremely useful.

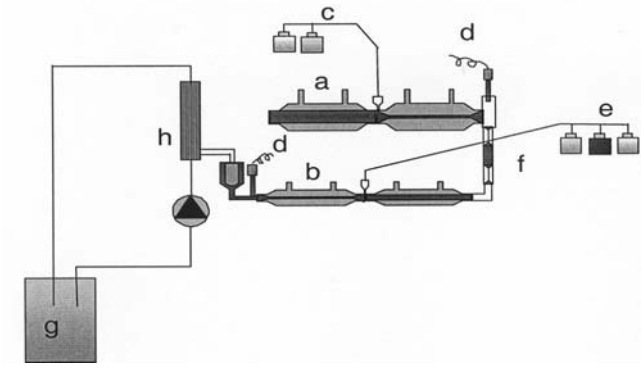

Figure 2. Schematic diagram of Tiny TIM-AA for protein quality testing, according to Minekus and Havenaar (ref. 27): a, gastric compartment; b, small intestinal compartment; c, gastric secretion pumps; d, pH electrodes; e, duodenal secretion pumps; f, peristaltic valve pump; g, dialysis fluid; h, hollow-fiber device.

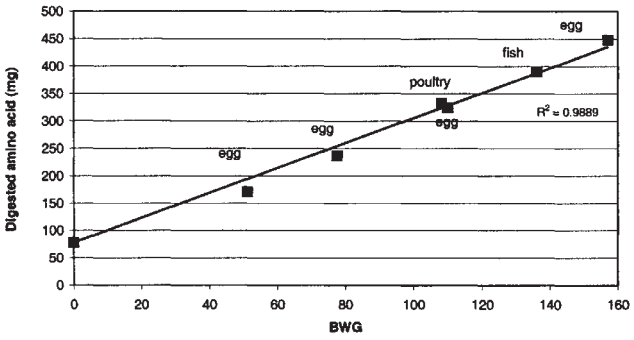

Figure 3. Relationship between the body weight gain (BWG) of broilers and the digested amounts of the first-limiting amino acids from different protein sources. The line represents the linear fit.

To overcome the other 3 disadvantages, the determination measurement of ileal digestibility to correct the amino acid score can be performed by using an advanced dynamic in vitro model system. TNO Quality of Life (Zeist, The Netherlands) has developed such an in vitro model system and has demonstrated that it can be applied efficiently for the determination of the true ileal digestibility of proteins and amino acids. TIM (25–28) simulates very closely the successive dynamic conditions in the gastrointestinal tract, such as the pH curves and concentrations of proenzymes in the stomach and small intestine, and concentrations of bile salts in the different parts of the gut. Gastric emptying, small intestinal passage, and secretion of digestive fluids are computer-controlled to produce realistic species- and meal-dependent conditions. Small molecules, such as products of protein digestion, are removed from the chyme with hollow fiber membranes. In this model, the availability for absorption of nutrients as well as the stability of specific ingredients (e.g., bioactive proteins and peptides) can be studied. Validation experiments with various types of food products have shown the reproducibility and reliability of the results for the digestibility and absorption of nutrients in comparison with in vivo experiments. The model has been tested to predict the true ileal digestibility of different proteins in pigs and calves (Figure 1).

Using a TIM system dedicated to study protein quality (Tiny TIM-AA, shown in Figure 2), the digestibility of limiting amino acids from various protein sources was compared to the body weight gain in broilers. A very high linear correlation was found between body weight gain and digestibility of the limiting amino acid of the test proteins (Figure 3). The determination of PDCAAS using the Tiny TIM-AA system is performed as follows: The test product is analyzed for amino acid and/or protein nitrogen. Products are tested by digesting a quantity of a meal that contains 5 g protein. After 5 h of digestion, the dialyzed fraction is sampled and analyzed for amino acids and/or protein nitrogen. A blank run is performed to determine the contribution of secreted proteins. The method allows the correction of the amino acid score for true ileal digestibility of the first limiting essential amino, which is much more relevant than correction for true fecal digestibility of the whole protein, as proposed by FAO/WHO. A further advantage of the method is that it is possible to install species-specific conditions for digestion. The Tiny TIM-AA thus offers a complete in vitro system to measure the true ileal digestibility of essential amino acids for test proteins.

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