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ORIGINAL ARTICLE

Consideration of utilizable amino acid content in the context of dietary protein quality assessment

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Abstract

Current evaluation systems for protein quality focus only on indispensable amino acids (IAAs) and do not consider the dispensable amino acids (DAAs). However, at excess levels of IAAs, DAAs may become limiting. Here, we used published ileal amino acid digestibility data determined in growing pigs and currently assumed protein and IAA recommendations to estimate utilizable, oxidized, and non-absorbed amino acids in different dietary protein and to compare these utilizable amino acid values to digestible indispensable amino acid score (DIAAS) values. For dietary protein sources with a DIAAS value <90, the amount of calculated utilizable amino acids increased proportionally with the DIAAS value according to "utilizable amino acids (mg/g protein) = DIAAS × 10"; for dietary protein sources with a DIAAS value >90, the amount of utilizable amino acid stays within the range 800–1000 mg/g protein. Cereals contained the lowest levels of utilizable amino acids and the highest amount of oxidized amino acids. Dairy and meat have the highest amount of utilizable amino acids followed by nuts and pulses. For many mixtures of protein sources, the highest value for utilizable amino acids, which considers both IAAs and DAAs, occurred at a different ratio of protein sources than the maximum DIAAS value, which only considers IAAs. From this theoretical study and based on the assumptions made, we conclude that estimation of utilizable amino acids from data typically reported in DIAAS studies further enables complementary insights next to DIAAS values. Careful consideration of protein and IAA requirements is critical in further work.

KEYWORDS
indispensable amino acids, protein complementarity, protein quality, utilizable amino acids

INTRODUCTION

Every human requires a daily quantity of proteins and amino acids that comes from dietary proteins sources. These dietary proteins do not only provide nitrogen, but also dispensable amino acids (DAAs) and indispensable amino acids (IAAs). IAAs are amino acids that cannot be synthesized in the body and hence need to be supplied via the diet. Although DAAs can be synthesized in the body, they are essential for the physiological functioning of the body (Reeds, 2000) and their precursors also need to be supplied via the diet. There are dietary recommendations for total protein (Rand et al., 2003) as well as IAA requirements (Joint WHO/FAO/UNU Expert Consultation, 2007; Nutrition and Panel on Dietetic Products, 2012). Dietary protein quality has received much attention. The most recent scoring system for dietary protein quality is the Digestible Indispensable Amino Acid Score (DIAAS), which is based on comparing the levels of ileal digestible IAAs (on a per gram protein basis) in a dietary protein source with a reference pattern for each of the IAAs. The lowest ratio of the concentration of an ileal digestible IAA compared to the reference pattern is the DIAAS value of a dietary protein source (FAO, 2013; Wolfe et al., 2016).

DIAAS values reported for different dietary protein sources differ widely, from 15 for a breakfast cereal (Hodgkinson et al., 2022) up to 144 for dry milk (Fanelli, Bailey, Guardiola et al., 2021b). DIAAS values <100 indicate that at least one digestible IAA is present at levels below those defined in the IAA reference pattern, but it is possible that this is the case for multiple IAAs in a dietary protein source (Adhikari et al., 2022). For example, yellow dent maize has a DIAAS value of 48 and the first limiting IAA is Lys, but ileal digestible levels for Trp, His and the SAA are also below the values of reference pattern (Cervantes-Pahm et al., 2014). For DIAAS values >100, all IAAs are present in digestible form at levels above the corresponding reference pattern values (Wolfe et al., 2016).

Current scoring systems for protein quality, such as DIAAS, but also the Protein Digestibility Corrected Amino Acid Score (PDCAAS), focus specifically on the IAAs, and do not consider the DAAs content of a protein. DAAs, however, also have important roles in physiological functions, such as building and maintaining bodily proteins, synthesis of metabolic compounds, and contribute to the nitrogen pool (Newsholme et al., 2003; Reeds, 2000; Tessari, 2019b; Wu, 2010). Despite the fact that DAAs can be synthesized by the human body, they can have limited availability in dietary protein sources, implying that adequate synthesis of DAAs from available precursors is not always assured (Hou et al., 2016). In pigs it has been shown that there is an optimal ratio between IAAs and DAAs, above which total nitrogen utilization notably decreases (Heger et al., 1998; Lenis et al., 1999). Similarly in humans, total IAA utilization in a meal that provided only IAAs was lower than in a meal that supplemented the IAAs with DAAs (Cooper et al., 2020). Hence, a DIAAS value >100 indicates that DAAs rather than the IAAs are actually limiting the optimal utilization of the amino acids. The sum of the IAAs in the recommended scoring pattern for the age group of 3 years and over is 291 mg of IAAs per gram protein (FAO, 2013), which implies that the remaining 709 mg of amino acids per gram protein are DAAs (Tessari, 2019a). Although ileal digestible DAAs levels are not considered in the calculation of DIAAS values, their levels are typically reported in publications determining DIAAS values of dietary protein sources (Bailey et al., 2020; Cervantes-Pahm et al., 2014; Fanelli, Bailey, Guardiola, et al., 2021b; Han et al., 2021) and these data can be applied to identify whether one of the IAAs or the DAAs are limiting in the utilization of absorbed amino acids.

When DAAs, rather than IAAs, are limiting in dietary protein utilization, nitrogen from the most IAAs, can be utilized in synthesis of DAAs in the body via transamination reactions (Lenis et al., 1999; Mann & Truswell, 2017); that is, some of excess IAAs can be utilized in the synthesis of DAAs. It has been reported that Lys and Thr do not undergo transamination due to the fact that the primary amino group on the side change of Lys and the hydroxyl group on the side chain of Thr prevent enzymatic transamination; hence, Lys and Thr cannot be used for synthesis of DAAs (Cooper et al., 2020; Sheppard et al., 2008). However, when one of the IAAs becomes limiting, excess IAAs and DAAs cannot be further utilized, and they are oxidized. Consideration of protein utilization is critical in the consideration of dietary protein quality. A previously study by Moughan and Smith (1984) modeled dietary amino acid utilization in pigs where the model included a linear programming component to calculate amino acid transamination and the conversion of excess IAAs to DAAs and the prediction of amounts of utilizable protein. They validated their mathematical model with pig experiment data to predict the total body protein deposition, taking into account the amount of ingested nitrogen and nitrogen free energy intake (Moughan & Smith, 1984). Such approaches would also be valuable for human dietary proteins. Hence, for this study we estimated the amount of crude amino acid in the dietary source that is absorbed and the amount and that can be utilized in body from available data. With the depth of data reported in most DIAAS studies, not only the DIAAS value, but also the amount of amino acids that can be utilized in the body can be estimated from digestible amino acid levels, and amino acid requirements, and thus provide notable complementary insights into dietary protein quality. In this study, we used published data to estimate utilizable amino acids in dietary protein sources and illustrate how different dietary protein sources do not only differ in DIAAS value, but also in levels of amino acids that can be utilized, as well as those that will be oxidized and non-absorbed. Furthermore, we investigated how these aspects affect complementarity of different dietary protein sources.

METHODS

The amino acid content and the standardized ileal digestibility (SID) of different dietary protein sources were collected through a literature search. All available data on the ileal digestibility of amino acids in dietary protein sources for human consumption determined in a growing pig model, were included. We calculated non-absorbed, utilizable and oxidized amino acids for a range of dietary protein sources based on available published data. Non-absorbed amino are hereby considered as those that have not been absorbed at the terminal ileum. Within the fraction of absorbed amino acids, distinction is made between utilizable and oxidized amino acids, with the distinction between these groups determined by the ratios at which the different amino acids are absorbed. Utilizable amino acids are considered those that, due to the proportions in which they are absorbed, can be utilized in protein synthesis in the body. Oxidized amino acids are those that are absorbed but are not utilizable due to an imbalance in amino acid composition in the absorbed amino acid fraction. The overview of these calculations is shown as flow diagram in Figure 1 where the amino acid composition and ileal digestibility available from reported DIAAS studies are used to calculate the total amount of utilizable amino acid. An overview of all dietary protein sources included in this study is shown in Table 1. The IAA:DAA ratio was calculated from the crude IAAs and DAAs levels for each dietary protein source. SID data for Pro are scarce; therefore, average SID data for total amino acid were used as proxy for SID for Pro for all dietary protein sources in this study. When average SID data not provided in the specific publications, average SID data were calculated from the SID data of the individual amino acids.

Concentrations of absorbed and non-absorbed amino acids for each amino acid were calculated according to:

$$\text{Absorbed AA concentration (mg/g protein)} = \text{total AA concentration (mg/g protein)} \times \text{SID} \quad (1)$$

$$\text{Non-absorbed AA concentration (mg/g protein)} = \text{total AA concentration (mg/g protein)} - \text{absorbed AA concentration (mg/g protein)} \quad (2)$$

For calculating the DIAAS value for the different dietary protein sources, levels of digestible IAAs were compared to the IAA scoring pattern for the age group of 3 years and over, as reported by FAO (FAO, 2013). The ratio for IAA with the smallest value from the comparison was identified as first limiting amino acid. For each dietary protein source, from the identified first limiting amino acid value, the DIAAS values were expressed as percentage. Based on the amount of absorbed amino acids compared to reference pattern two scenarios could be distinguished: (A) one or more of the IAAs in the absorbed fraction are below the values defined in the reference pattern, or (B) all IAAs in the absorbed fraction are above values in the reference pattern. Within scenario (B) we consider two sub-scenarios, that is, (B1) where excess IAAs cannot be utilized for DAAs synthesis and (B2) where excess IAAs can be utilized for DAAs synthesis.

In case of scenario A, where at least one of the IAAs was below the values defined in the reference pattern, the IAA with the lowest ratio relative to the reference pattern was identified. Subsequently, based hereon, the required amounts of other individual IAAs, as well as required amounts of DAAs were calculated

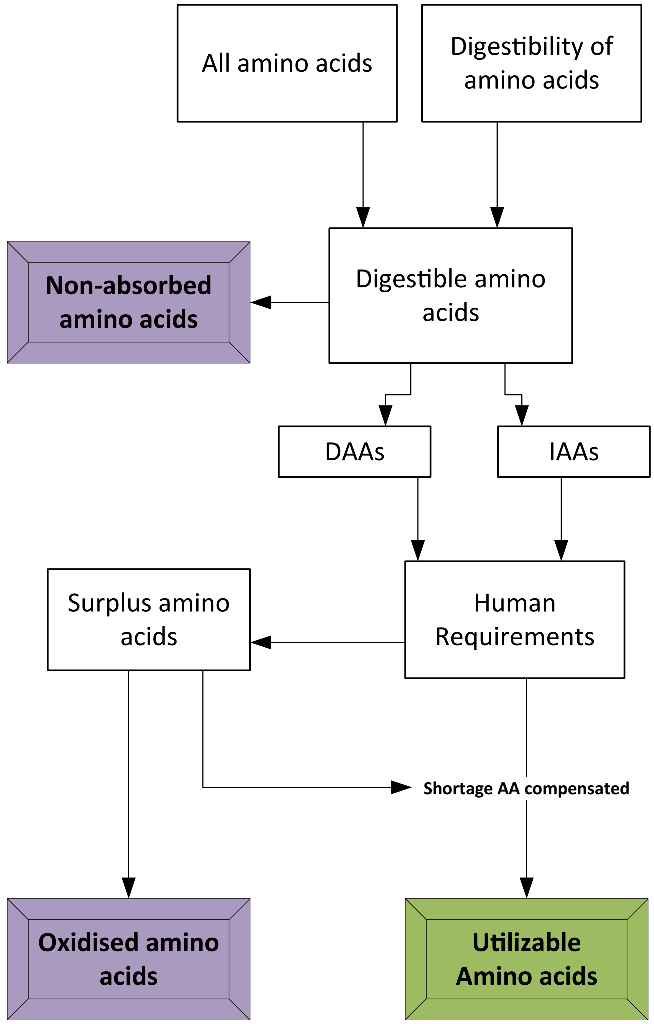

FIGURE 1 Structural outline for methodology. DAAs, dispensable amino acids; IAAs, indispensable amino acids.

based on the reference pattern to enable complete utilization of the first limiting IAA. For DAAs, this was done on the basis that the reference pattern for 1 g of protein contains 291 mg of IAAs and hence 709 mg of DAAs (FAO, 2013). The sum of these IAAs and DAAs was considered to be the utilizable amino acids. Any IAAs or DAAs present in excess relative to the limiting IAA based on the reference pattern were considered to become oxidized; that is,

$$\text{Oxidized amino acids (mg/g protein)} = \text{absorbed amino acids (mg/g protein)} - \text{utilizable amino acids (mg/g protein)} \quad (3)$$

The amount of non-specific nitrogen that is absorbed back into the amino acid pool following oxidation of amino acids, and form the non-absorbed fraction of amino acid (Mann & Truswell, 2017) was not taken into account as a nitrogen source of DAAs synthesis.

TABLE 1 Overview of digestible indispensable amino acid score (DIAAS) value including first limiting amino acid, the ratio of indispensable amino acid (IAA) to dispensable amino acid (DAA), amount of non-absorbed, utilizable and oxidized amino acid of different dietary protein sources.

Dietary protein source	IAA/DAA ratio	DIAAS	First limiting IAA	Amino acids (mg/g protein)			Reference
				Non-absorbed	Utilizable	Oxidized	
Wheat bran	0.58	15	Lys	320	149	531	(Hodgkinson et al., 2022)
Cornflakes	0.86	19	Lys	112	194	694	(Fanelli, Bailey, Guardiola, et al., 2021b)
Toasted wheat bread	0.58	24	Lys	70	237	693	(Hodgkinson et al., 2022)
Pigeon peas	1.67	29	SAA	179	430	391	(Hodgkinson et al., 2022)
Sorghum	0.78	29	Lys	282	294	425	(Cervantes-Pahm et al., 2014)
Burger bun	0.54	31	Lys	91	308	601	(Fanelli, Bailey, Thompson, et al., 2021a)
Black beans	0.85	41	SAA	258	406	336	(Hodgkinson et al., 2022)
Wheat	0.59	43	Lys	213	435	352	(Cervantes-Pahm et al., 2014)
Rey	0.60	47	Lys	267	473	260	(Cervantes-Pahm et al., 2014)
Yellow dent maize	0.81	48	Lys	222	476	302	(Cervantes-Pahm et al., 2014)
Dehulled barley	0.66	51	Lys	239	508	253	(Cervantes-Pahm et al., 2014)
Nutridense maize	0.83	54	Lys	171	542	287	(Cervantes-Pahm et al., 2014)
Oat protein concentration	0.78	64	Lys	154	639	207	(Abelilla et al., 2018)
Polished white rice	0.82	64	Lys	68	642	291	(Cervantes-Pahm et al., 2014)
Quick oats	0.91	67	Lys	173	673	154	(Fanelli, Bailey, Guardiola, et al., 2021b)
Chickpeas cooked	0.75	71	Val	159	709	132	(Han et al., 2020)
Pea protein concentrate	0.76	73	SAA	84	729	187	(Mathai et al., 2017)
Kidney beans cooked	0.88	74	SAA	257	713	30	(Han et al., 2020)
Dehulled oats	0.73	77	Lys	150	790	60	(Cervantes-Pahm et al., 2014)
Adzuki beans cooked	0.90	78	SAA	139	804	57	(Han et al., 2020)
Pistachio roasted	0.71	82	Lys	189	808	3	(Bailey & Stein, 2020)
Beyond burger patty	0.85	84	SAA	110	845	45	(Fanelli, Bailey, Thompson, et al., 2021a)
Peas cooked	0.69	84	Val	136	842	22	(Han et al., 2020)
Pistachio raw	0.72	86	Lys	96	872	32	(Bailey & Stein, 2020)
Broad beans cooked	0.73	87	Val	107	875	18	(Han et al., 2020)
Whey protein isolate	1.11	92	His	48	860	92	(Hodgkinson et al., 2022)
Mung beans cooked	0.82	94	Val	210	770	20	(Han et al., 2020)
Soya protein isolate	0.76	98	SAA	41	935	24	(Mathai et al., 2017)
Ground beef cooked	0.89	99	Leu	22	949	29	(Bailey et al., 2020)
Soya flour	0.79	105	SAA	85	890	26	(Mathai et al., 2017)
Ribeye (beef) well-done	1.02	107	Val	19	944	37	(Bailey et al., 2020)
Impossible burger patty	0.77	108	SAA	52	930	19	(Fanelli, Bailey, Thompson, et al., 2021a)
80% Lean beef burger patty	0.87	110	Val	92	868	40	(Fanelli, Bailey, Thompson, et al., 2021a)
Ribeye (beef) roast medium-rare	1.01	111	Val	14	941	45	(Bailey et al., 2020)
Bacon smoked	1.02	117	Val	39	907	55	(Bailey, 2018)
Loin (pork) medium-well-done	1.05	118	Val	43	899	59	(Bailey, 2018)
Loin (pork) well-done	1.03	118	Val	42	897	60	(Bailey, 2018)
Raw pork belly	1.00	118	Val	19	923	58	(Bailey, 2018)
Pork burger patty	0.87	119	Val	25	926	48	(Fanelli, Bailey, Thompson, et al., 2021a)

TABLE 1 (Continued)

Dietary protein source	IAA/DAA ratio	DIAAS	First limiting IAA	Amino acids (mg/g protein) Non-absorbed	Utilizable	Oxidized	Reference
93% Lean beef burger patty	0.94	119	Val	41	906	54	(Fanelli, Bailey, Thompson, et al., 2021a)
Salami (beef)	0.96	120	Leu	27	929	44	(Bailey et al., 2020)
Beef jerky	0.98	120	SAA	21	920	58	(Bailey et al., 2020)
Ground beef (raw)	0.86	121	Val	9	940	51	(Bailey et al., 2020)
Ham non-cured	1.07	123	Val	71	861	68	(Bailey, 2018)
Skimmed milk powder	1.01	124	SAA	98	853	49	(Mathai et al., 2017)
Ham conventional cured	1.05	125	Val	32	904	64	(Bailey, 2018)
Whey protein isolate (A)	1.12	125	His	6	890	104	(Mathai et al., 2017)
Bologna (beef)	0.95	129	Leu	22	922	56	(Bailey et al., 2020)
Ribeye (beef) roast-medium	1.02	130	Val	35	901	64	(Bailey et al., 2020)
Ham alternatively cured	1.05	132	Val	37	892	71	(Bailey, 2018)
Whey protein concentrate	1.14	134	His	30	879	91	(Mathai et al., 2017)
Milk protein concentrate	0.99	139	SAA	35	917	48	(Mathai et al., 2017)
Loin (pork) medium	1.06	NA	Val	43	874	82	(Bailey, 2018)
Bacon smoked-cooked	0.92	142	Val	41	884	75	(Bailey, 2018)
Dry milk	1.03	144	SAA	77	873	50	(Fanelli, Bailey, Guardiola, et al., 2021b)

Abbreviation: SAA, sulfur-containing amino acid.

In the case of scenario B, where all the IAAs in the absorbed fraction are in proportional excess relative to the reference pattern, it is actually the DAAs that are limiting for optimal utilization of the absorbed amino acid. For these protein sources with a shortfall of DAAs and an excess of IAAs in the absorbed fraction. For scenario B1, where the excess IAAs cannot be utilized for the synthesis of DAAs, similar to scenario A, all excess amino acids that remained after identifying the limiting amino acid or amino acid fraction, were considered oxidized. For scenario B2, where excess IAAs, can be utilized in the synthesis of DAAs, we calculated the amount of IAAs (excluding Lys and Thr) that could be used as nitrogen sources in the synthesis of DAAs by considering step wise conversion of these available IAAs in the absorbed fraction into DAAs and identifying the conversion rate at which utilizable amino acid levels of the absorbed fractions were highest and oxidized fractions were lowest.

A sample calculation is presented in Supplementary file S1. The two cases where the excess IAAs are considered as sources for DAAs synthesis and when IAAs are not considered as source for DAAs synthesis are used for comparison of utilizable, oxidized and non-absorbed amino acid.

To investigate the complementarity of protein sources in maximizing utilizable amino acids, the dietary protein sources maize, rice, peas, soy, pork and milk were combined in binary mixtures from 0 to 100% of each protein at 1% intervals. The amino acid profile and digestibility of individual protein source was used in the ratio of protein contribution in the mixture. Levels of non-absorbed, utilizable and oxidized amino acids were computed following the same method explained for individual dietary protein sources.

RESULTS

Table 1 shows the DIAAS values, the first limiting amino acid for DIAAS values, the IAA:DAA ratio and amounts of non-absorbed, utilizable and oxidized amino acids for the dietary protein sources considered in this study, which are arranged in ascending order of their calculated DIAAS values. The ratio of IAA/DAAs for all dietary protein sources included in Table 1 was notably higher than the ratio of 0.41 (0.291 mg IAAs/0.709 mg DAAs) in the reference amino acid pattern (FAO, 2013; Tessari, 2019b), indicating that IAAs were present in quantities higher and DAAs in quantities lower than in the reference amino acid pattern.

Figure 2 shows the calculated levels of utilizable amino acids for the different dietary protein sources in Table 1 as a function of DIAAS value based on two scenarios, that is, when excess IAAs do not serve as a nitrogen source for synthesis of DAAs (Scenario B1 described above; Figure 2a) and when excess IAAs, except for Lys and Thr, do serve as nitrogen sources for the synthesis of DAAs (Scenario B2 described above; Figure 2b). For both scenarios, levels of

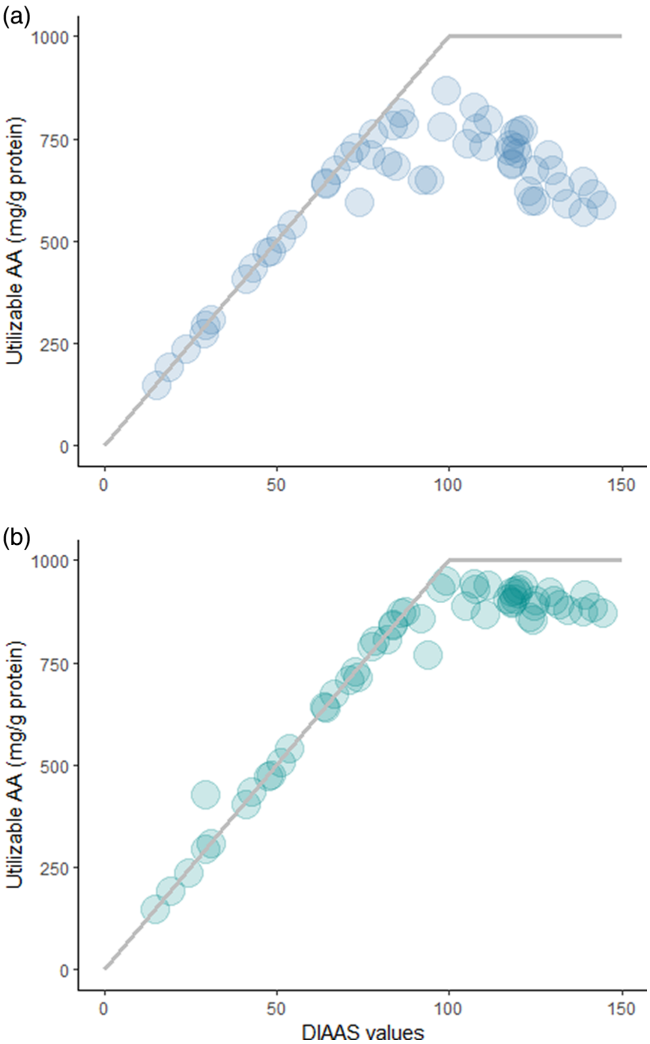

FIGURE 2 Amount of utilizable amino acid as a function of digestible indispensable amino acid score (DIAAS) value of dietary protein sources (a) without considering use excess indispensable amino acids (IAAs) for dispensable amino acid (DAA) synthesis or (b) when considering the use excess IAAs for DAAs synthesis.

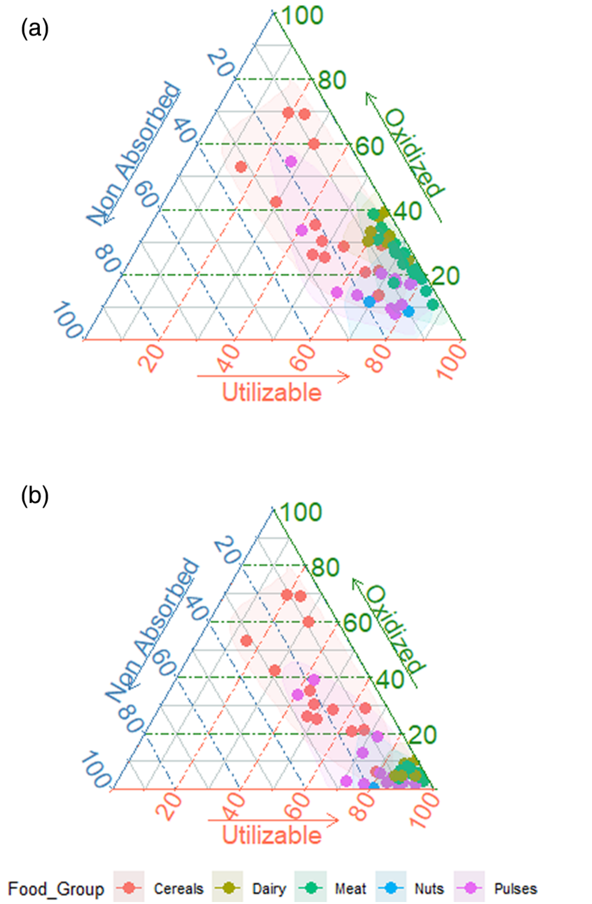

FIGURE 3 Proportion of utilizable, oxidized, and non-absorbed amino acid in different dietary protein sources when (a) utilization of excess indispensable amino acids in synthesis of dispensable amino acids is not considered or (b) when utilization of indispensable amino acids in synthesis of dispensable amino acids is considered.

utilizable amino acid increase linearly with DIAAS value up to ~90. For dietary protein sources with a DIAAS value >90, utilizable amino acid levels decreased with increasing DIAAS value when utilization of excess IAAs as a nitrogen source is not considered (Figure 2a), but plateaued around 900 mg/g protein when utilization of excess IAAs as a nitrogen source was considered (Figure 2b).

To illustrate the proportion of non-absorbed, utilizable and oxidized amino acids for different dietary protein sources, ternary plots are presented in Figure 3. When comparing Figure 3a,b, it is clear that the amount of oxidized amino acids was generally higher and the amount of utilizable amino acid was lower when the ability of excess IAAs to serve as nitrogen source for DAAs synthesis was not considered (Figure 3a) compared to when it was considered (Figure 3b). This, however, was not seen in cereals, for which the amount of oxidized and utilizable amino acid remained constant in both scenarios. Figure 3 illustrates that the amount of oxidized amino acids can be as high as 70% of the total amino acid content of dietary protein sources, which can occur when proteins have high digestibility but one of the IAAs is strongly lacking for utilization of the absorbed amino acids.

On food group level, meat products had the highest calculated average amount of utilizable amino acids (909 mg/g protein), followed by dairy (885 mg/g protein) (Figure 4). In contrast, on average, less than half of the amino acid in cereal products are considered utilizable when these products are considered as single protein sources (Figure 4). The amount of non-absorbed amino acids was notably higher in cereals, pulses and nuts than in meat and dairy (Figures 3 and 4).

To assess complementarity of dietary protein sources on a utilizable amino acid basis, we considered binary mixtures from six different dietary protein sources, that is, maize (Cervantes-Pahm et al., 2014), rice (Cervantes-Pahm et al., 2014), peas (Han et al., 2020),

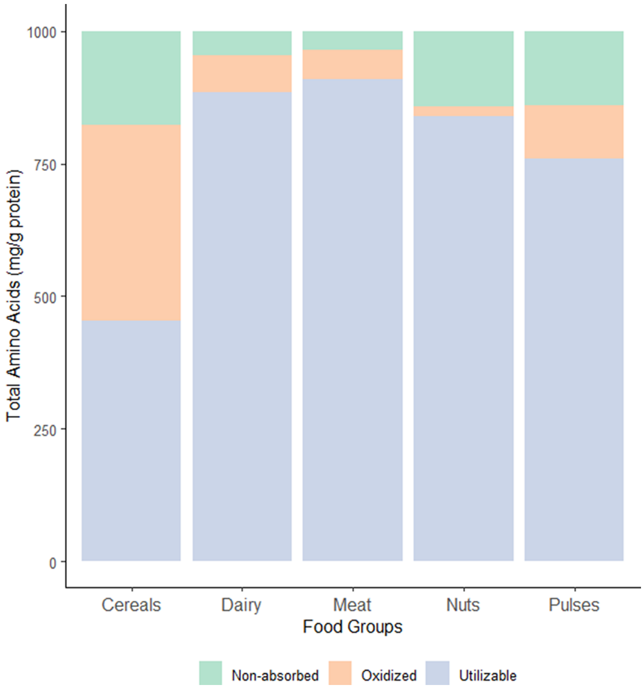

FIGURE 4 Average amount of absorbed, utilizable, oxidized amino acid in cereals ($n = 14$), dairy products ($n = 7$), meat ($n = 19$), nuts ($n = 2$), and pulses ($n = 13$).

soy (Mathai et al., 2017), pork (Bailey et al., 2020) and milk (Mathai et al., 2017), similar to those used previously when evaluating complementarity on the basis of DIAAS values (Adhikari et al., 2022). From these 15 pairs of dietary protein sources, we describe 4 specific cases of complementarity below and show these in Figure 5. The data of other combinations are shown in the Supplementary file S2.

Results from this complementarity analysis indicate that, with the exception for the combination of rice and maize protein (Figure 5c), the highest values for DIAAS and for utilizable amino acid levels occurred at different ratios of the two protein sources (Figure 5a,b,d). For example, for the combination of protein from rice and peas, both DIAAS value (99) and amount of utilizable amino acid (900 mg/g protein), showed a clear maximum as a function of the proportion of pea protein included, but this maximum occurred at a ratio of ~65% of protein from pea for the maximum DIAAS values and at ~50% of protein from pea for the maximum utilizable amino acid values (Figure 5a). For the combination of maize and milk, the highest value for DIAAS was found at ~75% milk protein, whereas utilizable protein increased with increasing proportion of milk protein and did not show a maximum at intermediate values (Figure 5b). For the combination of soy and pork, the DIAAS value progressively increased with increasing proportion of protein from pork, whereas for utilizable amino acids, a maximum was observed at ~95% protein from pork (Figure 5d). For all mixtures in Figure 5a–d, amount of non-absorbed amino acid was proportional to the weighted average of protein sources in the mixture. Overall, from the results presented in Figure 5, which were selected as examples to illustrate typical trends observed, it is clear that optimizing protein blends for highest utilizable amino acid content and highest DIAAS value can give very different trends.

Table 2 provides an overview of the maximum DIAAS values and maximum utilizable amino acid levels, as well the protein source ratios at which these maxima occur for the different mixtures of dietary protein sources. Similar to Figure 5, it can also be seen in Table 2 that the ratio which provides highest DIAAS value does not always have highest amount of utilizable amino acid possible for the pair at other ratios. Only three out of 15 combinations showed highest DIAAS value and utilizable amount of amino acid at the same ratio of the two protein sources. For seven out of 15 dietary protein combinations, the highest calculated DIAAS value occurred at a different ratio than the highest amount of utilizable amino acids. On the other hand, for some pairs, neither the DIAAS value nor the total amount of utilizable amino acid improved by mixing two dietary protein sources (Table 2).

DISCUSSION

We used available published data to estimate levels of utilizable, oxidized and non-absorbed amino acids from amino acid composition and SID for different dietary protein sources. In dietary protein sources with a DIAAS >100, the IAA:DAA ratio was higher than the ratio of 0.41 (291:709) in the amino acid reference pattern (Table 1). This indicates that DAAs can become limiting rather than IAAs. When the option of excess IAAs to be used as a nitrogen source in the synthesis of DAAs was not taken into account (Figure 2a), the excess IAAs cannot be utilizable due to the limiting DAAs. This, in turn, causes the excess amino acids to become oxidized and therefore the amount of utilizable amino acids gradually decrease when DIAAS >100 (Figure 2a). However, it is known that the IAAs, with the exception of Lys and Thr, can be used as nitrogen sources in the synthesis of DAAs (Cooper et al., 2020; Sheppard et al., 2008). When this was included in the calculation of utilizable amino acids, the amount of utilizable amino acid for dietary protein sources with DIAAS >100 was ~900 mg per gram protein (Figure 2b). In this case, the IAAs in these dietary protein sources, with the exception of Lys and Thr (Bhagavan & Ha, 2015), can be used as nitrogen source for DAAs synthesis until one of the IAAs becomes limiting. Therefore, more of the absorbed amino acids are utilizable and less become oxidized compared to when the ability of IAAs to serve as source

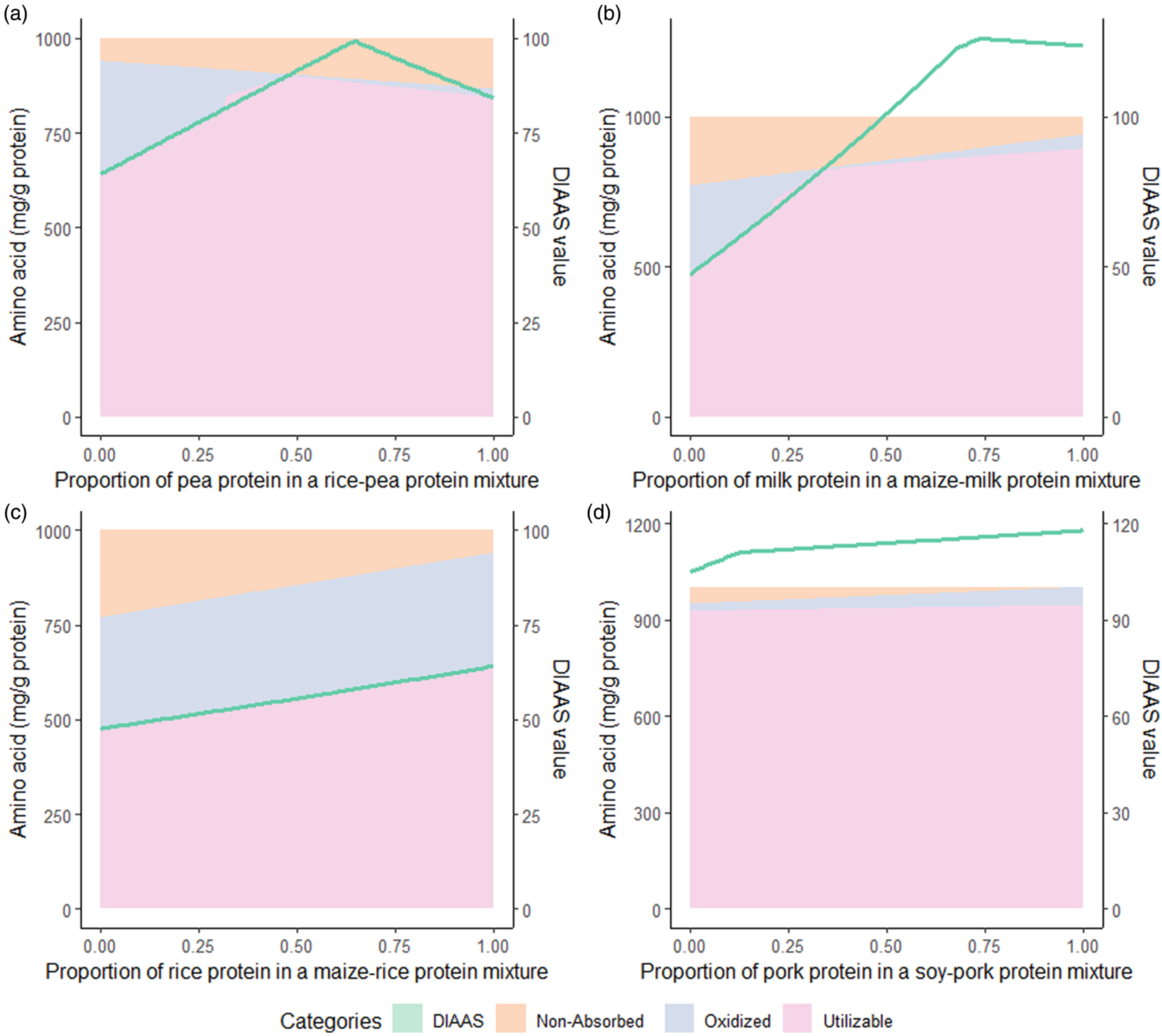

FIGURE 5 Amount of utilizable, oxidized and non-absorbed amino acid and DIAAS value for combination of different dietary protein sources for dietary protein from rice and peas (a) maize and milk (b), maize and rice (c), and soy and pork (d).

for DAAs synthesis is not considered (Table 1, Figure 2a vs. Figure 2b and Figure 3a vs. Figure 3b).

We observed, in general, that plant-derived dietary protein sources had lower amounts of total utilizable amino acids than animal-derived dietary protein sources (Table 1, Figures 3 and 4), which is in line with lower DIAAS values generally reported for the former than for the latter (Adhikari et al., 2022; Fanelli, Bailey, Thompson, et al., 2021a; Mathai et al., 2017). A higher level of non-absorbed amino acids in plant-derived dietary protein sources, especially from cereals (Table 1; Figures 3 and 4), is the result of a comparatively lower digestibility (<90%) in this group of dietary protein sources (Abelilla et al., 2018; Cervantes-Pahm et al., 2014). Furthermore, cereals had the highest levels of oxidized amino acids (Figures 3 and 4), which can be explained by the fact that the absorbed amino acid fraction of cereals has at least one strongly limiting IAA, mostly Lys (Table 1), as a result of which a large proportion of the absorbed amino acid fraction cannot be utilizable and becomes oxidized.

Similar to the previously reported opportunities for improving DIAAS values by creating mixtures of dietary protein sources (Han et al., 2021; Woolf et al., 2011), complementarity of different dietary protein sources can also be observed for utilizable amino acids in combinations of different dietary protein sources (Figure 5, Table 2). However, from the results shown in Figure 5 and Table 2, it is clear that the ratios of protein sources at which the highest DIAAS values are observed are

TABLE 2 The ratio of dietary protein sources in at which, in binary mixtures, the maximum value for Digestible Indispensable Amino Acid Score (DIAAS) value was reached (Ratio DIAAS Max), the maximum DIAAS value reached (DIAAS Max), and the ratio at which the maximum amount of utilizable amino acid was reached (Ratio utilizable Max) and the higher utilizable amino acid level (utilizable Max, in mg/g protein) reached.

Protein source 1	Protein source 2	Ratio DIAAS max	DIAAS max	Ratio utilizable max	Utilizable max
Maize	Milk	26:74	126	0:100	893
Maize	Rice	0:100	64	0:100	642
Milk	Pork	59:41	136	3:97	944
Milk	Peas	100:0	124	100:0	893
Milk	Soy	100:0	124	0:100	927
Milk	Rice	77:23	135	30:70	933
Pea	Soy	14:86	106	0:100	927
Pea	Maize	100:0	84	100:0	844
Pea	Pork	0:100	118	1:99	944
Pork	Maize	100:0	118	99:1	944
Rice	Pea	35:65	99	52:48	900
Rice	Pork	21:79	120	51:49	951
Rice	Soy	16:84	113	48:52	938
Soy	Maize	92:8	107	100:0	927
Soy	Pork	0:100	118	4:96	945

often not the same as ratios where the highest amount of utilizable amino acid is observed. We observed that mixing two protein sources with DIAAS < 100 and same limiting amino acid does not improve the DIAAS or the amount of utilizable amino acids (Figure 5c). However, mixing two protein sources with DIAAS < 100 but with different limiting amino acids increased both the DIAAS value as well as the level of utilizable amino acids (Figure 5a). This highlights that mixing two protein sources at random does not always assure maximum utilizable amino acid, rather requires mixing two complementing dietary protein sources in right ratio. Furthermore, our results also indicates that the DIAAS value provides adequate information of single protein source with DIAAS < 100, however, when looking at protein sources with DIAAS > 100 and mixtures of protein sources focusing at individual amino acids becomes of paramount importance.

Protein quality determination is often based on the availability of digestible individual IAAs per gram protein. These measurement methods focus on single most limiting IAA. However, DAAs are not included in assessing the quality of protein. In this study we show that DAAs play a major role in estimating the total amount of utilizable amino acids. It is especially important to estimate the total amount of utilizable amino acids of the dietary protein sources with DIAAS > 100. These protein sources have all IAAs in quantities higher than in the reference pattern and consequently have smaller quantities of DAAs. The type of IAA available in excess determines the amount of total utilizable amino acids in these protein sources. Therefore, taking DAAs into account highlights that not only the level of IAAs, but also the profile of the amino acids determines the efficiency of utilization of amino acids after absorption and needs to be confirmed experimentally.

Previously, the utilization of dietary protein for protein deposition in pigs has been mathematically modeled and verified by experimental data (Moughan & Smith, 1984). Compared to the study by Moughan and Smith (1984), this study does not aim to look at the protein deposition. This study is a theoretical approach taken to estimate the utilizable amino acid based on available data on digestibility of amino acids. Validation of this study with experimental data is currently not possible. However, upon availability of experimental data in the future, conclusion of this study can be validated. Furthermore, the accuracy of the reference pattern for IAAs recommended by FAO is still under debate as the IAAs and protein values are derived using disparate scientific approaches and therefore the estimate of DAAs requirement is likely incorrect (Szwiega et al., 2021; Wolfe et al., 2016). Any changes in the reference pattern of amino acids will affect the outcomes of this study. As such, we calculated that changing the IAA reference pattern from 3 years and older (used in this study) to 18 years and older recommended by (FAO, 2013) can result a 3% decrease on average in the amount of utilizable amino acids for the dietary protein sources included in this study.

The possibility of the use of non-amino acid nitrogen sources from amino acid break down (Mann & Truswell, 2017) or from the non-absorbed fraction of amino acid metabolized in the gut (van der Wielen et al., 2017) was not included in the study. Therefore, the amount of utilizable amino acids calculated in this calculation may be somewhat underestimated compared to the actual amount that can be utilized in the body using the non-amino acid nitrogen sources. The limitations imposed by availability and choice of data for the calculation in this study needs to be considered while interpreting the results and drawing conclusions.

CONCLUSIONS

The calculation of most commonly used protein quality scores, that is, DIAAS and PDCAAS, is based on the first limiting digestible IAA. This approach, however, overlooks the actual utilization of the absorbed amino acids in the body, because DAAs can be limiting the full utilization of absorbed amino acids. In this study, through mathematical calculation, we show the data typically reported in DIAAS studies enable estimation of amount of utilizable, as well as non-absorbed and oxidized amino acids. This approach provides complementary insights to DIAAS values in considering protein sources or the complementarity of blends thereof for optimal protein utilization in the body.

AUTHOR CONTRIBUTIONS

Shiksha Adhikari and Thom Huppertz conceived and designed the study. Shiksha Adhikari carried out the research and analyzed the data. Shiksha Adhikari wrote the first draft of the manuscript and revised the manuscript with support from Thom Huppertz, Imke J. M. de Boer and Ollie van Hal. All authors contributed to and approved the final draft of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

The authors did not directly use animal or human subjects. It was compiled from a literature source.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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