Short summary of:

DETERMINATION OF PDCAAS AND DIAAS VALUES IN A PROTEIN HYDROLYSATE DERIVED FROM ANTARCTIC KRILL

Natalia S. Fanelli and Prof. Hans H. Stein
Department of Animal Sciences
University of Illinois
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Summarized by: Katina Handeland, MSc, PhD.

understory

INTRODUCTION

In human nutrition, protein is usually supplied by either plant-based or animal-based proteins. To evaluate the protein quality in foods, the protein digestibility-corrected amino acid score (PDCAAS), or the digestible indispensable amino acid score (DIAAS) methods are used. PDCAAS reflects the total tract digestibility of the Amino acids (AA) of the protein, whereas DIAAS reflects the digestibility only in the small intestine, which makes it the recommended method by the FAO, with the pig accepted as the best animal model (FAO, 2013).

Understory®, a novel protein hydrolysate derived from Antarctic krill (Euphausia superba), was recently developed for human health and nutrition. Although data for PDCAAS and net protein utilization measured in rats are available for different krill proteins (Gigliotti et al., 2008; Suzuki and Shibata, 1990), no PDCAAS or DIAAS values measured in pigs for protein hydrolysate of krill have been reported. Furthermore, different processing methods can change the amino acid profile and the digestibility and therefore also the PDCAAS and DIAAS. It was, therefore, the objective of this experiment to assess the protein quality with PDCAAS and DIAAS calculations for this krill protein hydrolysate measured in growing pigs.

MATERIALS AND METHODS

Preparation of experimental diets
The krill protein hydrolysate was delivered to the University of Illinois from Aker BioMarine, Oslo, Norway. Two diets were prepared. The krill protein was included in one diet as the only source of crude protein (CP) and amino acids (AA). The second diet was a nitrogen-free diet that was used to measure basal endogenous losses of CP and AA.

Experimental design and digestibility trial
Eight growing gilts [average body weight (BW): 26.6 ± 2.5 kg] were equipped with a T-cannula in the distal ileum (Stein et al., 1998) and randomly allotted to a 2-period switch back design and 4 replicated pigs per diet in each period. Each period had 9 days and no pig received the same diet more than once during the experiment, therefore, there were 8 replicate pigs per treatment. All pigs were fed their assigned diets daily and fecal samples and ileal digesta were collected using standard operating procedures (Hodgkinson et al., 2020; Stein et al., 1998).

Chemical analysis
Samples of krill, diets, fecals, and ileal digesta were analyzed for dry matter (DM) (Method 930.15; AOAC Int., 2019) and for nitrogen by combustion (Method 990.03; AOAC Int., 2019) using a LECO FP628 Nitrogen analyzer (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as N × 6.25. The krill, diets and ileal digesta samples were analyzed for AA [Method 982.30 E (a, b, c); AOAC Int., 2019] on a Hitachi Amino Acid analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA, USA).

Calculations
Values for standardized ileal digestibility (SID) of CP and AA was used to calculate DIAAS values using the following equation (FAO, 2013):
DIAAS (%) = 100 × lowest value of digestible indispensable AA reference ratio.

Values for standardized total tract digestibility (STTD) of CP were used to calculate PDCAAS values using the following equation (Schaafsma, 2000):
PDCAAS (%) = mg of limiting AA in 1g of test protein/mg of the same AA in 1 g of reference protein × STTD (%) × 100.

STATISTICAL ANALYSES

Normality of data was verified. To compare values for protein quality, the (DIAAS or PDCAAS) were set as fixed effect and pig, period, and square as random effects. Data were analyzed by ANOVA using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with pig as the experimental unit. The LSMEANS was used to calculate means, which were then separated using the PDIFF option if they were significant. An alpha of 0.05 was judged statistically significant.

RESULTS

Ingredient composition and digestibility values

The specific krill protein hydrolysate tested contained all essential amino acids (Table 1). Protein quality is determined not only by the indispensable AA composition of the food ingredient, but also by these AAs' digestibility in the intestine. The standardized ileal digestibility (SID) values were close to 100% for almost all AA, including most of the indispensable AA, indicating good digestibility (Table 2).

PDCAAS and DIAAS

PDCAAS and DIAAS is, among other things, influenced by the specific contents of the AAs in a product.
The krill protein hydrolysate had a truncated PDCAAS value for preschool children of 100, which is in agreement with published PDCAAS measured for krill protein concentrate in rats (Gigliotti et al., 2008). The krill protein hydrolysate had untruncated PDCAAS values of >100 for children, older children, adolescents, and adults, indicating excellent protein quality (Table 3)

The krill protein hydrolysate had DIAAS ≥ 100 qualifying as an "excellent" source of protein for children, older children, adolescents, and adults (Table 3).

Untruncated PDCAAS values were greater ($P < 0.05$) than DIAAS values calculated for infants from birth to 6 months and children from 6 months to 3 years, whereas no differences were observed for individuals > 3 years (Table 3). This was expected due to colonic bacteria fermentation of CP in the large intestine, which influences PDCAAS scores but not DIAAS (FAO, 2014; Rutherfurd et al., 2015).

The rate-limiting AAs are calculated based on the indispensable amino acid reference patterns for different age-groups. For infants, the rate-limiting AA for this specific krill protein hydrolysate was Tryptophan, while for the group of children and older children it was either Tryptophan, Histidine or Leucin.

CONCLUSION

According to the DIAAS results, the specific contents and intestinal digestibility of the indispensable AA in this product is sufficient to meet AA requirements for human individuals older than 6 months, indicating that UnderstoryTM is an excellent quality protein for human consumption.

REFERENCES

Food and Agriculture Organization of the United Nations – FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Rome.

Gigliotti, J.C., Jaczynski, J., and Tou, J.C. 2008. Determination of the nutritional value, protein quality and safety of krill protein concentrate isolated using an isoelectric solubilization/precipitation technique. Food Chem. 111:209-214. doi:10.1016/j.foodchem.2008.03.030.

Hodgkinson, S.M., Stein, H.H., de Vries, S., Hendriks, W.H., and Moughan, P.J. 2020. Determination of true ileal amino acid digestibility in the growing pig for calculation of digestible indispensable amino acid score (DIAAS). J. Nutr. 150:2621-2623. doi:10.1093/jn/nxaa210.

Rutherfurd, S.M., Fanning, A.C., Miller, B.J. and Moughan, P.J. 2015. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J. Nutr. 145:372-379. doi: 10.3945/jn.114.195438

Schaafsma, G. 2000. The protein digestibility–corrected amino acid score. J. Nut. 130:1865S-1867S. doi:10.1093/jn/130.7.1865S.

Stein, H. H., Seve, B., Fuller, M. F., Moughan, P. J., and de Lange, C. F. M. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180. doi:10.2527/jas.2005-742.

Suzuki, T., and Shibata, N. 1990. The utilization of Antarctic krill for human food. Food Rev. Int. 6:119-147. doi:10.1080/87559129009540863.

TABLE 1.
Analyzed AA composition of the krill protein hydrolysate, as-fed basis

ITEM, %	EXPERIMENTAL PROTEIN
Dry matter	96.50
Crude protein	93.38
Indispensable amino acids	
Arg	6.06
His	2.34
Ile	5.50
Leu	7.58
Lys	8.37
Met	2.79
Phe	4.15
Thr	4.32
Trp	1.30
Val	5.54
Dispensable amino acids	
Ala	5.54
Asp	11.04
Cys	0.52
Glu	14.73
Gly	4.22
Pro	3.50
Ser	3.68
Tyr	4.22
Total	95.40

TABLE 2.
Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in krill protein hydrolyzate[1,2]

	AID		SID	
ITEM, %	AVERAGE	SD	AVERAGE	SD
Crude protein	74.5	2.57	88.5	2.57
Indispensable amino acids				
Arg	86.0	1.49	96.7	1.49
His	77.8	2.57	84.8	2.57
Ile	87.5	1.42	91.9	1.42
Leu	88.7	1.54	93.8	1.54
Lys	85.0	1.73	89.7	1.73
Met	87.9	1.27	90.0	1.27
Phe	87.3	1.71	92.7	1.71
Thr	75.6	2.81	85.4	2.81
Trp	82.5	2.01	90.6	2.01
Val	83.3	2.01	91.0	2.01
Mean	84.9	1.65	91.3	1.65
Dispensable amino acids				
Ala	83.3	1.67	91.7	1.67
Asp	59.6	4.08	65.1	4.08
Cys	34.3	7.85	71.4	7.85
Glu	77.0	2.33	81.8	2.33
Gly	55.5	4.27	89.3	4.27
Ser	70.6	2.92	82.2	2.92
Tyr	87.5	1.58	93.2	1.58
Mean	71.1	2.63	80.4	2.63
Total amino acids	78.3	2.10	86.1	2.10

[1] Data are means of 7 observations per treatment.
[2] Values for SID were calculated by correcting the values for AID for basal ileal endogenous losses.
Basal ileal endogenous losses were determined (g/kg of DM intake) as CP, 17.63; Arg, 0.81; His, 0.21; Ile, 0.32; Leu, 0.52; Lys, 0.53; Met, 0.08; Phe, 0.31; Thr, 0.57; Trp, 0.13; Val, 0.54; Ala, 0.61; Asp, 0.81; Cys, 0.24; Glu, 0.94; Gly, 1.91; Ser, 0.57; and Tyr, 0.27.

TABLE 3.
Comparison of protein digestibility amino acid scores (PDCAAS) and digestible indispensable amino acid scores (DIAAS) in krill protein hydrolysate based on different requirement patterns[1]

	PDCAAS[2]		PDCAAS[3]			DIAAS[3]			SEM	P-value
	Truncated 2 – 5yr	Untruncated 2 – 5yr	Infant 0 – 6 mo	Child 6mo – 3yr	Older than 3 yr	Infant 0 – 6mo	Child 6mo – 3yr	Older than 3 yr		
ITEM, %	100[d]	116[b]	77[e] (Trp)	116[b]	126[a]	74[f] (Trp)	106[c]	125[a]	0.73	<0.001
Reference ratio, mg/g										
His	1.00	1.25	1.13	1.19	1.48	1.01	1.06	1.33		
Ile	1.00	1.99	1.01	1.74	1.86	0.98	1.69	1.80		
Leu	1.00	1.16	0.80	1.16	1.26	0.79	1.15	1.25		
Lys	1.00	1.46	1.23	1.49	1.77	1.17	1.41	1.68		
SAA	1.00	1.34	1.02	1.24	1.46	0.94	1.14	1.34		
AAA	1.00	1.35	0.90	1.63	2.07	0.89	1.60	2.03		
Thr	1.00	1.29	0.99	1.41	1.75	0.90	1.28	1.58		
Trp	1.00	1.20	0.77	1.55	2.00	0.74	1.48	1.91		
Val	1.00	1.60	1.02	1.31	1.40	0.98	1.26	1.35		

[1] Data are means of 7 observations per treatment.
[2] Values for SID were calculated by correcting the values for AID for basal ileal endogenous losses.
Basal ileal endogenous losses were determined (g/kg of DM intake) as CP, 17.63; Arg, 0.81; His, 0.21; Ile, 0.32; Leu, 0.52; Lys, 0.53; Met, 0.08; Phe, 0.31; Thr, 0.57; Trp, 0.13; Val, 0.54; Ala, 0.61; Asp, 0.81; Cys, 0.24; Glu, 0.94; Gly, 1.91; Ser, 0.57; and Tyr, 0.27.
[3]Values for PDCAAS and DIAAS were calculated using the recommended AA scoring pattern for three age groups. The indispensable amino acid reference patterns are expressed as mg AA/g protein: Infant (birth to 6 months) - His, 21; Ile, 55; Leu, 96; Lys, 69; SAA, 33; AAA, 94; Thr, 44; Trp, 17; Val, 55. Child (6 months to 3 years) - His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 43. Older child, adolescent, and adult - His, 16; Ile, 30; Leu, 61; Lys, 48; SAA, 23; AAA, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

APPENDIX

After the initial experiment was performed, Aker BioMarine's technicians performed a method adjustment/optimization which affected the AA composition, including a reduction in Tryptophan levels. Since the method adjustment was not assumed to change the intestinal digestibility of the protein hydrolysate, there was no need to perform a new animal experiment. Table S1 shows the AA composition of the new protein hydrolysate. Prof. Hans Stein updated the DIAAS calculations based on the new AA composition and protein concentration, and the results are shown in Table S2.

TABLE S1.
Analyzed AA composition of the krill protein hydrolysate, after method adjustment.

ITEM, %	EXPERIMENTAL PROTEIN
Arg	6.43
His	2.42
Ile	5.14
Leu	7.58
Lys	8.37
Met	2.99
Phe	4.15
Thr	4.77
Trp	0.89
Val	5.46
Dispensable amino acids	
Ala	6.02
Asp	12.59
Cys	0.57
Glu	16.29
Gly	4.44
Pro	3.48
Ser	4.39
Tyr	4.22

RESULTS

Table S2 shows the DIAAS of the old and new version of the krill protein hydrolysate, assuming the digestibility of AA is the same in both products. The DIAAS for infants is quite low in the new product and also decreased a little bit in the 6-to-36-month group. This is due to the lower tryptophan content in the new product. The reference ratio for some of the other AA went up for the new product, but because DIAAS is determined by the first limiting AA, the lower tryptophan concentration in the new product resulted in reduced DIAAS for these two age groups. For the older age group (3 years and above), there was no change in DIAAS. This is because tryptophan is not limiting for this age group due to lower requirement for tryptophan for older children and adults. For these age groups, leucine was the limiting AA in the new krill protein hydrolysate.

TABLE S2.
Comparison of digestible indispensable amino acid scores (DIAAS) in two sources of krill protein hydrolysate based on different requirement patterns[1]

	DIAAS, old product			DIAAS, new product		
ITEM, %	Infant 0 – 6 mo	Child 6mo – 3yr	Older than 3 yr	Infant 0 – 6mo	Child 6mo – 3yr	Older than 3 yr
Reference ratio, mg/g						
His	1.01	1.06	1.33	1.06	1.11	1.39
Ile	0.98	1.69	1.80	0.93	1.60	1.71
Leu	0.79	1.15	1.25	0.79	1.15	1.25
Lys	1.17	1.41	1.68	1.29	1.57	1.86
SAA	0.94	1.14	1.34	0.95	1.16	1.36
AAA	0.89	1.60	2.03	0.82	1.48	1.88
Thr	0.90	1.28	1.58	1.00	1.43	1.77
Trp	0.74	1.48	1.91	0.52	1.03	1.33
Val	0.98	1.26	1.35	0.98	1.25	1.35
DIAAS (Limiting AA)	74 (Trp)	106 (His)	125 (Leu)	52 (Trp)	103 (Trp)	125 (Leu)

[1]Values for DIAAS were calculated using the recommended AA scoring pattern for three age groups. The indispensable amino acid reference patterns are expressed as mg AA/g protein: Infant (birth to 6 months) - His, 21; Ile, 55; Leu, 96; Lys, 69; SAA, 33; AAA, 94; Thr, 44; Trp, 17; Val, 55. Child (6 months to 3 years) - His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 43. Older child, adolescent, and adult - His, 16; Ile, 30; Leu, 61; Lys, 48; SAA, 23; AAA, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).