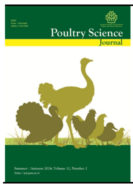

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Determine Prediction Equations of the Total and Standardized Ileal Digestible Amino Acids of Fish Meal From its Chemical Characteristics in Broilers

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Abstract

This experiment was conducted to determine total amino acid (AA) content and standardized ileal digestible (SID) amino acid content of 6 different origins of fish meal (FM) and subsequently determine equations that estimate total and SID amino acid contents from chemical composition as well as SID amino acid contents from its total AA. In order to conduct this study, a total of 210 one-day-old male broiler chicks were randomly assigned to 7 dietary treatments consisting of 6 semi-purified diets containing each of FM as the only source of dietary protein (200 mg of crude protein/g diet) and one nitrogen-free diet in order to determine ileal endogenous amino acids (IEAA) flow. Birds were allowed *ad libitum* access to a corn-soybean meal starter diet until ten days, a grower diet from 11 to 23 days, and then experimental diets from 24 to 28 days of age. The total content of Lys and Met among the various samples significantly differed from 1.52 to 2 and 0.59 to 0.77%, respectively ($P < 0.05$). This difference was observed among the other total and SID amino acids. In this study the accuracy and precision of the models were tested by the adjusted coefficient of determination (R^2) value, P-value regression coefficients, and standard error of prediction (SEP). The SEP of the developed regression equations for the predicting SID amino acids of FM were from 0.009 (for Met) to 0.056 (for Arg).

Introduction

Soybean meal is commonly used as the first source of amino acids (AA) and protein in poultry nutrition, but some countries need to import soybean meal because domestic soybean production cannot meet the demand (Sheikhhasan *et al.*, 2020). Fish meal (FM) is a valuable feed source of protein and has a balanced amino acid pattern that can improve the growth performance of poultry (Karimi, 2006). FM contains long-chain n-3 fatty acids, which are beneficial for the health of poultry and meat consumers, but these fatty acids (n-2, n-3) are susceptible to peroxidation, leading to increased dietary content of oxidative agents (Amaral *et al.*, 2018; Alagawany *et al.*, 2019). Manhden is a common FM that has 63% protein and 2% methionine. Despite being a suitable nutrient for poultry, it may produce harmful compounds such as biogenic amines due to improper protection. (Feng *et al.*, 2016).

Protein quality (PQ) is a key criterion for evaluating the bioavailability of amino acids in ingredients (Mansilla *et al.*, 2020). Standardized ileal digestibility (SID) of amino acids is the gold standard method for evaluating PQ in poultry feedstuffs (National Research Council, 1994; Adedokun *et al.*, 2008). Nutritionists are always looking to meet the amino acid requirements of fast-growing broilers. Fish meal is a protein source with an excellent amino acid profile that is recommended for poultry.

Feed amino acids have different availability, especially the amino acids that are present in processed feed or animal by-products (Sheikhhasan *et al.*, 2020). A large number of data have been published on the amino acid digestibility of raw materials in poultry, but different techniques have been shown to lead to great differences in the estimate of endogenous losses (Donkoh and Moughan, 1994; Ravindran and Bryden, 1999;

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Jansman *et al.*, 2002; Rodehutscord *et al.*, 2004; Adedokun *et al.*, 2007; Adedokun *et al.*, 2008), and all the existing methods and techniques have certain limitations and criticisms (Adedokun *et al.*, 2011). Thus, approaches and methods that do not depend on a separate determination of endogenous losses seem useful for feed checking (Rodehutscord *et al.*, 2004).

Reducing feed costs, feed safety margins, and nitrogen excretion into the environment are the advantages of formulation of feed ration based on amino acids (Classen and Stevens, 1995). Although there are several ways to estimate the total and SID amino acid content of feedstuffs, the use of linear regression equations is a rapid method to evaluate the total and SID amino acid contents of feedstuffs. Ebadi *et al.* (2005); Roudi *et al.* (2012) stated that the digestible amino acids of feedstuffs have a positive correlation with their chemical characteristics. Recently, prediction equations have been developed to predict the SID amino acids of soybean meal and sorghum from their chemical characteristics (Ebadi *et al.*, 2011; Sheikhhasan *et al.*, 2020). The main objectives of this study were to develop linear regression equations based on the chemical characteristic in order to estimate the total and SID amino acids content of fish meal.

Materials and Methods

Test Samples

Six samples of fish meal (FM) from different origins were obtained from several fish meal processing factories. Sardine (SA), Jonob (JO), Microfeede (MF), Salehi (SH), Talesh (TA), and Bandarabbas (BA) were obtained from different factories.

Birds and Treatments

This experiment was approved by the Animal Care Committee of the Tehran University, Tehran, Iran, which considered standards for the protection of animals used for scientific purposes. A total of 210 one-day-old male (Ross 308) broiler chicks were obtained from a commercial hatchery and then were randomly divided into 35 experimental pens with five replications of 6 birds in each. After dividing the experimental treatments, chickens were vaccinated against Newcastle disease (7, 18 days old), and Bursitis infection (1 day old). The diet based on soybean meal included a starter (1 to 10 days of age), and a grower (11 to 23 days of age) were formulated according to Ross 308 (Aviagen, 2019) strain catalog recommendations (Table 1).

Table 1. Composition of starter and grower diets were fed to chicks

Ingredients (g/kg as fed)	Starter (0-10 D)	Grower (11-23 D)
Corn	563.5	600.5
Soybean meal (44% CP)	380	345
Soybean oil	14	16
Limestone	12	11
Dicalcium phosphate	17	15
Salt	3.8	3.4
DL-Methionine	2.6	2.4
L- Lysine	1.4	1.2
L- Threonine	0.7	0.5
Vit/ Min Premix [a]	5	5
Calculated nutrient content		
Dry matter (%)	89	88
Crude Protein (%)	21.5	20.2
Metabolizable Energy (Kcal/Kg)	2850	2920
Total Met (%)	0.58	0.54
Total Met + Cys (%)	0.92	0.87
Total Lys (%)	1.27	1.17
Total Thr (%)	0.85	0.80
Calcium (%)	0.91	0.81
Available P (%)	0.45	0.40

[a] Vitamin/ Mineral premix provided the per Kg of complete diet: vitamin A, 10,000 IU; vitamin D_3, 3000 IU; vitamin E, 35 IU; menadione, 2.2 mg; D-pantothenic acid, 15 mg; riboflavin, 6.0 mg; folic acid, 1.0 mg; niacin, 60 mg; thiamine, 2.2 mg; pyridoxine, 4 mg; vitamin B12, 0.015 mg; biotin, 0.2 mg; iodine, 0.5 mg; manganese, 70 mg; copper, 10 mg; zinc, 80 mg; selenium, 0.2 mg; iron, 50 mg and Provided 100 mg of choline per Kg of complete diet.

During the experimental period, the feed and water were offered *ad libitum*. The lighting program followed the recommendations outlined in the Ross 308 broiler management handbook (Aviagen, 2019. The temperature of the room was set at 32°C for the first three days and then reduced until it reached 21°C. On day 24, after an overnight fast, chicks were given *ad libitum* access to the experimental diets (Table 2). There were seven dietary treatments that consisted of six semi-purified diets containing one of the FM samples as the only source of dietary protein and one nitrogen-free diet for determination of basal endogenous AA losses. The diets were based on the corn starch, dextrose, and FM samples; FM were included at 32.4 to 39.7 % of diets based on their protein contents. All diets contained 200 mg/g crude protein (CP). Corn starch and dextrose were in the N-free die as an energy source and there were no protein sources. Diets were balanced in terms of calcium and phosphorus, and vitamins and minerals were the same in all diets.

Table 2. Composition of experimental diets was fed to chicks from 24- 28 days old in order to determine of SID of amino acids (g/kg as-fed)

Ingredients	Diets [a]						
	FM-1	FM-2	FM-3	FM-4	FM-5	FM-6	N-Free
Corn starch	340	340	340	340	340	340	358
Dextrose	150	150	150	150	150	150	430
Oil	50	50	50	50	50	50	50
Salt	0	0	0	0	0	0	3
Dicalcium phosphate	0	0	0	0	0	0	25
Limestone	0	0	0	0	0	0	11
Vit and Min premix [b]	7	7	7	7	7	7	9
Sodium bicarbonate	0	0	0	0	0	0	4
Celite	10	10	10	10	10	10	10
Sand [c]	46	117	65	89	119	83	100
FM	397	326	378	354	324	360	0
SUM	1000	1000	1000	1000	1000	1000	1000
Calculated nutrients content							
Dry matter (%)	96	96	96	96	96	96	94
Crude protein (%)	20	20	20	20	20	20	-
AMEn (Kcal/kg)	3200	3250	3200	3220	3140	3250	3190
Calcium (%)	1.2	1.1	1.14	1.05	0.96	1.08	0.94
Available Phosphor (%)	0.68	0.55	0.65	0.60	0.54	0.61	0.44

Abbreviation: SID, standardized ileal digestibility

[a] the fish meals (FM) were obtained from different origins Sardine (SA), Jonob (JO), Microfeede (MF), Salehi (SH), Talesh (TA), and Bandarabbas (BA), respectively from sample 1 to 6.

[b] Vitamin/ Mineral premix provided the per Kg of complete diet: vitamin A, 10,000 IU; vitamin D_3, 3000 IU; vitamin E, 35 IU; menadione, 2.2 mg; D-pantothenic acid, 15 mg; riboflavin, 6.0 mg; folic acid, 1.0 mg; niacin, 60 mg; thiamine, 2.2 mg; pyridoxine, 4 mg; vitamin B12, 0.015 mg; biotin, 0.2 mg; iodine, 0.5 mg; manganese, 70 mg; copper, 10 mg; zinc, 80 mg; selenium, 0.2 mg; iron, 50 mg and Provided 100 mg of choline per Kg of complete diet.

[c] Particle size was to 2-3 millimeter©

To estimate the digestibility of ileal amino acids, 1% of celite was added to all experimental diets and an N-free diet as an indigestible ash marker. The physical form of all rations was mesh. On day 28, all of the birds were euthanized by CO_2 asphyxiation, and ileal digesta were collected from the last two-thirds of the ileum (part of the small intestine from Meckel's diverticulum to approximately 1 cm anterior to the ileocecal junction) by flushing with distilled water (Kluth and Rodehutscord, 2005). To analyses of acid insoluble ash (AIA) and AA Collected ileal digesta from 6 birds within a cage were pooled and stored at -20° C. Frozen digesta samples were thawed, lyophilized, and ground using an electric coffee grinder (Moulimex, PRC) to obtain finely ground samples while avoiding significant losses.

Chemical Analysis

AOAC International (2000) analytical methods (930.15, 920.39, 990.03, 978.10 and 942.05, respectively) was used to analyze dry matter (DM), ash, crude protein (CP), crude fiber (CF), and ether extract (EE) of all FM samples neutral detergent fiber (NDF) by Van Soest *et al.* (1991) and acid detergent fiber (ADF) by Robertson (Robertsonet al., 1981) was analyzed. The gross energy (GE) of samples was measured by an adiabatic calorimetric bomb (Ika-Kalorimeter; C400 adiabatisch, Germany). The nitrogen content of all diets was determined in combustion with an automatic nitrogen analyzer (Kjeltec Aouto 1030 analyzer, Sweden; method 968.06) (AOAC International, 2005).

These were with three duplicates. Mathematical calculations were used to determine the nitrogen-free extract (NFE). For AA analysis, the samples (fish meals, diets, and ileal digesta) were prepared by 6 N HCL hydrolysis for 24 h at 110° C, afterward neutralized with 15 mL of 9.8 N NaOH, cooled to room temperature, and then sodium citrate buffer was added. Finally, the mixture was equalized to 100-mL volume (AOAC International, 2000). Methionine and cystine (sulfur-containing AA) were analyzed by performic acid oxidation at 0°C and then hydrolyzed by 6 N HCL (Moore, 1963). The hydrolyzed AA were determined by high-pressure liquid chromatography (Knauer, Germany) with 3.5 μm Agilent ZORBAX Eclipse AAA column (4.6 mm ×

150 mm, 3.5 µm column, PN 993400-902, 963400-902) using reverse phase chromatography with precolumn derivation with ortho-phthalaldehyde with two replicates. After burning the samples and then boiling the ash in NHCL in duplicate the acid-insoluble ash content of diets and ileal digesta was determined according to the procedure of (Van Keulen and Young, 1977). Apparent ileal AA digestibility (AIAAD) was calculated using the following equation (Lemme *et al.*, 2004).

AIAAD, % = [1- (AIA diet / AIA ileal digesta) × (AA ileal digesta / AA diet)] × 100.

Ileal endogenous AA (IEAA) flow in broilers fed the N-free diet was calculated as milligrams of AA flow per kilogram of DM intake (DMI) using the following equation (Adedokun *et al.*, 2008). IEAA, mg/kg of DMI = ileal AA, mg/kg × [(AIA) diet / (AIA) digesta]. Apparent ileal AA digestibility coefficients were standardized base on the determined IEAA flows using the following equation. SIAAD, % = AIAAD, % + [(IEAA flow g/kg DMI) / (AA content of the raw material, g/kg DM)] × 100.

Statistical Analysis

Data (chemical compositions, Total AA, SID amino acids, and SID amino acids coefficients) were analyzed by the GLM procedure of SAS (SAS Institute, 2003) with a randomized complete design. To predict each individual total and SID amino acids content of FM samples, the Simple and multiple linear regression was used by SPSS software version 19 with the following model (Statistic, 2011). In the equations, the dependent variable was each of the individual total and SID amino acids, and the independent variables were CP, DM, CF, NDF, ADF, EE and ASH:

$$y_i = \beta_0 + \beta_{1\times 1} + \beta_{2\times 2} + \ldots + \varepsilon_i$$

where y_i is the predicted concentration of each of individual total and SID amino acids, β_0 is the intercept of the regression equation, β_j is the regression coefficient, x_j is the independent variable (contains: CP, DM, CF, NDF, ADF, EE and ASH) and ε_i is the random error of the regression model. In order to define the equation with the best fit of the independent variable, the coefficient of determination (R^2), adjusted R^2, *P*-value regression, *P*-value coefficients, and standard error of prediction (SEP) were used. Statistical significance was considered at $P \leq 0.05$. The SEP was calculated according to the following equation (Yegani *et al.*, 2013):

$$SEP = \sqrt{\frac{\sum(y - y')^2}{N}}$$

Where y is the total AA content and concentration of SID amino acids determined in the chick's bioassay, y' is the predicted total AA and SID amino acids value based on the prediction equation, and N is the number of test samples.

Results and Discussion

The determined CP contents of the experimental diets was 20% (Table 3) which were close to calculated values (considered 20.0% CP for all experimental diets). These results are in agreement with other studies (Sheikhhasan *et al.* 2020). In the present study, because inclusion levels of FM varied among the diets (32.4 to 39.7 % for SA and TA, respectively), the total AA content of experimental diets was different.

Table 3. Analyzed total AA and CP content of semi purified diets were fed to chicks from 24 to 28 days old (%, as fed) [a]

Item	Diets [b]						
	FM-1	FM-2	FM-3	FM-4	FM-5	FM-6	N-Free
CP	20.0	20.0	20.0	20.0	20.0	20.0	0.18
Lys	1.52	1.60	1.71	1.65	2.00	1.59	0.003
Met	0.59	0.62	0.66	0.64	0.77	0.61	0.001
Cys	0.20	0.21	0.22	0.21	0.26	0.20	0.000
Thr	0.85	0.89	0.95	0.92	1.12	0.89	0.001
Val	1.13	1.15	1.17	1.13	1.37	1.10	0.002
Arg	1.19	1.20	1.28	1.24	1.51	1.20	0.002
Ileu	0.48	0.50	0.54	0.52	0.63	0.50	0.005
Leu	1.49	1.57	1.68	1.62	1.97	1.56	0.007
Phe	0.84	0.87	0.93	0.90	1.09	0.86	0.004
His	0.48	0.50	0.54	0.52	0.63	0.50	0.001

Abbreviation: TAA, total amino acid; CP, crude protein.

[a] All means were obtained from an average of 3 replicates.

[b] The fish meals (FM) were obtained from different origins origins Sardine (SA), Jonob (JO), Microfeede (MF), Salehi (SH), Talesh (TA), and Bandarabbas (BA), respectively, from sample 1 to 6.

The coefficient of standardized ileal digestibility of amino acids with their means is shown in (Table 4). The standardized ileal amino acids digestibility coefficients (SIAADC) of Lys, Thr, Val, Arg and Leu among the different samples significantly differed ($P < 0.05$). The SIAADC of Lys were 87.76% and 82.80% for TA and

SH, respectively. For Met, the greatest and least values of SIAADC were 87.09% and 84.24% for TA and SH, respectively. TA showed the highest level of Thr digestibility (79.91%), and SH had the lowest level of Thr digestibility (76.54%) with an average of 78.31%.

Table 4. Coefficient of standardized ileal amino acid digestibility of broilers in 28 D of age (%) [a]

Item	Fish Meal [b]						*P*-value	*SEM*	Mean
	FM-1	FM-2	FM-3	FM-4	FM-5	FM-6			
Lys	85.18[b]	85.93[b]	82.96[c]	82.80[c]	87.76[a]	85.46[b]	0.0001	0.506	85.02
Met	85.81[abc]	86.78[ab]	84.56[bc]	84.24[c]	87.09[a]	85.14[abc]	0.1106	0.678	85.61
Cys	58.04[ab]	60.38[a]	57.95[ab]	56.38[b]	60.63[a]	58.01[ab]	0.0954	0.912	58.56
Thr	78.57[a]	79.15[b]	76.82[b]	76.54[b]	79.91[a]	78.86[a]	0.0141	0.486	78.31
Val	78.12[ab]	78.92[a]	76.46[bc]	74.99[c]	79.91[a]	78.16[ab]	0.0034	0.531	77.76
Arg	82.46[b]	83.10[ab]	80.76[c]	80.59[c]	83.57[a]	82.17[b]	0.0001	0.299	82.11
Ileu	83.70[ab]	83.88[ab]	84.43[a]	82.71[b]	84.98[a]	83.48[ab]	0.1208	0.467	83.86
Leu	80.96[a]	81.17[a]	78.64[c]	78.28[c]	79.81[abc]	80.04[ab]	0.0143	0.426	79.81
His	79.51[ab]	81.67[a]	79.61[ab]	78.90[b]	81.90[a]	80.25[ab]	0.0998	0.693	80.31
Phe	81.29[ab]	82.61[a]	82.31[a]	80.06[b]	82.16[ab]	81.59[ab]	0.1467	0.583	81.67

Means within a row with no common superscript letters (a-d) differ significantly ($P \leq 0.05$).
[a] There were 5 cages of 6 birds per each treatment.
[b] The fish meals (FM) were obtained from different origins: Sardine (SA), Jonob (JO), Microfeede (MF), Salehi (SH), Talesh (TA), and Bandarabbas (BA), respectively, from samples 1 to 6.

Suthama and Wibawa (2018) reported that the digestibility of Met, Lys, Thr microparticle protein derived from fish meal were 75%, 77.7% and 78 respectively. In some studies, it has been shown that amino acid digestibility is similar to protein pattern, but in some cases, higher protein digestibility is not equal to a similar level of single amino acid digestibility. Also, Ravindran (2013) reported that a given substrate or nutrient of a certain feedstuff was not entirely similar found in other ingredients. So, alike nutrients of the dissimilar ingredients showed unlike reactions to digestive enzymes. The findings of Moughan *et al.* (2014) also confirmed that the diversity of individual amino acid digestibility was further down between grains or their by-products. A clear example of this case is the comparison of individual amino acid digestibility between fish meal and blood sources as well as animal protein. FM has less amino acid digestibility and more variety than blood meal. The result is that there is a lot of variability in the digestibility of individual amino acids in FM (Suthama and Wibawa, 2018).

The determined chemical composition of FM samples is shown in (Table 5). In terms of chemical composition, different samples were significantly differed. The CP content varied from 66.79% to 49.15% for SA and TA, respectively, with a mean of 53.9%. The average EE content was 9.59 %. The maximum value of EE was 18.34 % for TA, and the minimum value was 6.55 % for SA samples.

Table 5. Determined chemical composition of fish meal samples (as fed %)

Sample	DM	CP	EE	Ash	CF	NDF	ADF
FM-1	92.23[b]	66.79[a]	6.55[d]	18.20[b]	1.65 [bc]	10.60 [b]	3.97 [d]
FM-2	91.08[c]	62.85[c]	11.07[b]	16.22[c]	1.45 [bc]	8.35[c]	1.35 [f]
FM-3	86.88[e]	59.53[e]	7.24[c]	18.17[b]	1.03 [c]	7.06 [d]	2.73 [e]
FM-4	95.07[a]	61.42[d]	7.14[c]	23.96[a]	4.05 [a]	14.76 [a]	11.07 [a]
FM-5	92.51[b]	49.15[f]	18.34[a]	15.40[c]	1.97 [b]	9.42 [bc]	8.38 [b]
FM-6	90.27[d]	63.65[b]	7.24[c]	18.41[b]	1.59 [bc]	9.48 [bc]	5.40 [c]
Mean	91.34	53.90	9.59	18.39	1.96	9.94	5.48
SEM	0.11	0.18	0.10	0.19	0.18	0.37	0.19
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Abbreviation; DM, dry matter; CP, crude protein, EE, ether extract; CF, crude fiber; NDF, neutral detergent fiber; ADF, Acid detergent fiber
Means within a row with no common superscript letters (a-d) differ significantly ($P \leq 0.05$).
[a] All means were obtained from the average of 3 replicates.
[b] The fish meals (FM) were obtained from different origins: Sardine (SA), Jonob (JO), Microfeede (MF), Salehi (SH), Talesh (TA), and Bandarabbas (BA), respectively, from sample 1 to 6. Values within columns not sharing the superscripts are significantly different at $P < 0.05$

Moreover, the average CF content was 1.96 %, ranging from 1.03 for MF to 4.05 % for SH in the present study. The average ash content was 18.39%, ranging from 15.40% (TA) to 23.96% (SH). This result indicates that the chemical compositions of FM from different origins are widely different.

Table 6. Total AA content of FM samples [a] (%, DM)

Item	Fish Meal [b]						*P*-value	*SEM*	Mean
	FM-1	FM-2	FM-3	FM-4	FM-5	FM-6			
Lys	4.93 [a]	3.97 [c]	4.66 [ab]	4.52 [b]	4.05 [c]	4.68 [ab]	0.0001	0.108	4.47
Met	1.29 [ab]	1.24 [b]	1.31 [ab]	1.38 [a]	0.95 [c]	1.26 [b]	0.0001	0.030	1.24
Cys	0.69 [b]	0.58 [dc]	0.80 [a]	0.54 [d]	0.43 [e]	0.63 [c]	0.0001	0.015	0.61
Met + Cys	1.98 [ab]	1.82 [b]	2.11 [a]	1.92 [b]	1.38 [c]	1.89 [b]	0.0023	0.044	1.85
Thr	2.62 [a]	2.17 [c]	1.97 [d]	2.41 [b]	2.11 [cd]	2.26 [bc]	0.0003	0.054	2.26
Val	3.50 [a]	2.70 [b]	2.41 [c]	2.21 [c]	2.26 [c]	2.76 [b]	0.0001	0.064	2.64
Arg	5.32 [c]	5.94 [ab]	5.65 [bc]	6.15 [a]	3.99 [d]	6.15 [a]	0.0002	0.132	5.53
Ileu	2.73 [a]	1.79 [b]	1.21 [e]	1.29 [e]	1.43 [d]	1.59 [c]	0.0001	0.042	1.67
Leu	5.89 [a]	5.19 [b]	3.77 [c]	3.29 [d]	2.59 [e]	2.89 [a]	0.0001	0.098	3.94
Phe	2.35 [d]	3.34 [a]	2.73 [c]	2.92 [bc]	2.70 [c]	3.00 [b]	0.0001	0.067	2.84
His	1.97 [a]	1.82 [b]	1.59 [c]	1.24 [d]	0.95 [e]	1.19 [d]	0.0001	0.036	1.46

Means within a row with no common superscript letters (a-d) differ significantly ($P \leq 0.05$).
Abbreviation: TAA, total amino acid; FM, fish meal: DM, dry matter
[a] All means was obtained from average of 3 replicate.
[b] The fish meals (FM) were obtained from different origins: Sardine (SA), Jonob (JO), Microfeede (MF), Salehi (SH), Talesh (TA), and Bandarabbas (BA), respectively from sample 1 to 6.

Table 7. SID amino acid content of FM samples [a] (%, DM)

Item	Fish Meal [b]						*P*-value	*SEM*	Mean
	FM-1	FM-2	FM-3	FM-4	FM-5	FM-6			
Lys	4.43 [a]	3.66 [d]	4.33 [b]	3.83 [c]	3.74 [d]	4.27 [b]	0.0001	0.0247	4.050
Met	1.16 [bc]	1.15 [c]	1.24 [a]	1.19 [b]	0.87 [d]	1.16 [bc]	0.0001	0.0094	1.133
Cys	0.42 [b]	0.37 [c]	0.51 [a]	0.31 [d]	0.27 [e]	0.39 [c]	0.0001	0.0058	0.381
Met + Cys	1.58 [b]	1.52 [cd]	1.75 [a]	1.50 [e]	1.14 [e]	1.56 [bc]	0.0001	0.0087	2.311
Thr	2.16 [a]	1.84 [c]	1.69 [e]	1.89 [b]	1.78 [d]	1.93 [b]	0.0001	0.0136	1.644
Val	2.89 [a]	2.28 [c]	2.06 [d]	1.70 [f]	1.90 [e]	2.34 [b]	0.0001	0.0153	2.190
Arg	4.63[d]	5.31 [b]	5.12 [c]	5.07 [c]	3.51 [e]	5.48 [a]	0.0001	0.0166	4.857
Ileu	2.41 [a]	1.61 [b]	1.13 [e]	1.09 [f]	1.28 [d]	1.44 [c]	0.0001	0.0105	1.499
Leu	5.02 [a]	4.53 [b]	3.31 [c]	2.63 [d]	2.17 [f]	2.50 [a]	0.0001	0.0170	3.367
Phe	2.01 [e]	2.97 [a]	2.52 [c]	2.39 [d]	2.33 [d]	2.66 [b]	0.0001	0.0127	2.484
His	1.65 [a]	1.60 [b]	1.41 [c]	1.00 [d]	0.81 [e]	1.03 [d]	0.0001	0.0120	1.254

Means within a row with no common superscript letters (a-d) differ significantly ($P \leq 0.05$).
Abbreviation: SID, standardized ileal digestible; FM, fish meal: DM, dry matter
[a] All means were obtained from an average of 3 replicates.
[b] The fish meals (FM) were obtained from different origins: Sardine (SA), Jonob (JO), Microfeede (MF), Salehi (SH), Talesh (TA), and Bandarabbas (BA), respectively, from sample 1 to 6.

The total and SID amino acid content of FM samples are shown in (Tables 6) and (Tables 7), respectively. The contents of total and SID amino acids among the different samples were significantly different. The means of total Lys was 4.47%, which was the highest level for SA (4.93%) and the lowest level for the JO sample (3.97%). The total Met contents varied from 0.95% for TA to 1.38% for SH. The total Thr content ranged from 2.62% (SA) to 1.97% (MF), with a mean of 2.26%. In general, the levels of total AA profiles have a positive relationship with the CP content of FM samples such that SA samples with the highest CP content (66.79%) had the highest level of total AA profile. In contrast, TA samples with the lowest content of CP (49.15%) had the lowest level of total AA profile. The total AA values (%DM) of FM in the National Research Council (1994) were slightly different from the means of total AA profile determined in this present study; the average of Lys and Met levels in this study were less than ones in National Research Council (1994) (5.07%Vs 4.47%. and 1.95% Vs. 1.27 %, respectively), whereas in the Phe and Arg determined in the current study were higher than ones in National Research Council (1994) (3.81% vs. 5.53%; 2.75% vs. 2.84%, respectively). These discrepancies can be attributed to the variety in animal by-products and processing condition of fish meals.

To predict the total amino acid content of FM based on its chemical components, linear regression equations were developed by SPSS software (Table 8). In terms of the total AA content, there is a positive relationship between all of the amino acids and CP. Moreover, in the linear equation developed to predict the total Met from CP content (T Met = 0.02 × CP), the adjusted R2, regression, P-Value, and SEP are 0.996, 0.001 and 0.081, respectively.

Table 8. Linear Regression equations for predicting the total amino acids (TAA) content of FM from its chemical characteristics (DM basis)[1]

Amino acids	Basis	Prediction equations	Statistical parameters [2]					
			R^2	Adjusted R^2	P-Value Regression	P-Value Coefficients		SEP
T Met	CP	T Met = 0.02 × CP	0.996	0.996	0.001	CP	0.001	0.081
	Ash	T Met = 0.066 × ASH	0.990	0.988	0.001	ASH	0.001	0.134
	Cons CP	T Met = 0.003 + 0.02 × CP	0.710	0.637	0.035	Cons CP	0.995 0.035	0.081
	DM	T Met = 0.015 × DM	0.985	0.982	0.001	DM	0.001	0.171
T Cys	CP	T Cys = 0.01 × CP	0.977	0.972	0.001	CP	0.001	0.102
	ASH	T Cys = 0.032 × ASH	0.950	0.940	0.001	ASH	0.001	0.150
	DM	T Cys = 0.007 × DM	0.984	0.937	0.001	DM	0.001	0.155
T Met + Cys	CP	T Met + Cys = 0.03 × CP	0.944	0.993	0.001	CP	0.001	0.151
	ASH CF ADF	T Met + Cys = 0.132 × ASH – 0.22 × CF – 0.033 × ADF	0.999	0.999	0.001	ASH CF ADF	0.001 0.010 0.043	0.031
	Cons EE DM	T Met + Cys = 7.206 – 0.049 × EE – 0.052 × DM	0.963	0.938	0.007	Cons EE DM	0.008 0.006 0.025	0.070
T Lys	CP	T Lys = 0.072 × CP	0.994	0.993	0.001	CP	0.001	0.358
	ASH	T Lys = 0.235 × ASH	0.983	0.980	0.001	ASH	0.001	0.622
	DM	T Lys = 0.052 × DM	0.990	0.988	0.001	DM	0.001	0.490
T Thr	CP	T Thr = 0.036 × CP	0.993	0.992	0.001	CP	0.001	0.200
	ASH	T Thr = 0.118 × ASH	0.982	0.978	0.001	ASH	0.001	0.325
	DM	T Thr = 0.026 × DM	0.995	0.994	0.001	DM	0.001	0.174
T Val	CP	T Val = 0.043 × CP	0.986	0.983	0.001	CP	0.001	0.337
	ASH	T Val = 0.137 × ASH	0.945	0.934	0.001	ASH	0.001	0.670
	DM	T Val = 0.031× DM	0.971	0.965	0.001	DM	0.001	0.491

Continued **Table 8**. Linear Regression equations for predicting the total amino acids (TAA) content of FM from its chemical characteristics (DM basis)[1]

Amino acids	Basis	Prediction equations	Statistical parameters [2]					
			R^2	Adjusted R^2	P-Value Regression	P-Value Coefficients		SEP
T Arg	CP	T Arg = 0.089 × CP	0.993	0.991	0.001	CP	0.001	0.508
	ASH	T Arg = 0.292 × ASH	0.983	0.980	0.001	ASH	0.001	0.770
	DM	T Arg = 0.065 × DM	0.978	0.973	0.001	DM	0.001	0.894
T His	CP	T His = 0.024 × CP	0.966	0.959	0.001	CP	0.001	0.299
	DM ADF	T His = 0.024 × DM – 0.105 × ADF	0.982	0.973	0.001	DM ADF	0.001 0.032	0.217
	NDF CF	T His = 0.315 × NDF – 0.872 × CF	0.959	0.939	0.002	NDF CF	0.007 0.038	0.326
T Ileu	CP	T Ileu = 0.027 × CP	0.937	0.924	0.001	CP	0.001	0.470
	ASH	T Ileu = 0.087 × ASH	0.881	0.858	0.002	ASH	0.002	0.644
	DM	T Ileu = 0.02 × DM	0.919	0.903	0.001	DM	0.001	0.531
T Leu	CP	T Leu = 0.064 × CP	0.941	0.930	0.001	CP	0.001	1.067
	ASH	T Leu = 0.204 × ASH	0.886	0.863	0.002	ASH	0.002	1.487
	DM	T Leu = 0.046 × DM	0.912	0.894	0.001	DM	0.001	1.308
T Phe	CP	T Phe = 0.046 × CP	0.981	0.977	0.001	CP	0.001	0.422
	EE	T Phe = 0.246 × EE	0.839	0.807	0.004	EE	0.004	1.228
	NDF	T Phe = 0.266 × NDF	0.928	0.914	0.001	NDF	0.001	0.820

Abbreviation; FM, fish meal; DM, dry matter; CP, crude protein.

[1] Analyzed using SPSS statistical software and stepwise procedure.

[2] R^2 is the coefficient of determination; adjusted R^2 for the number of predictors in the model, *P*-value<0.05, is statistically significant (Sheikhhasan *et al.*, 2020).

The current study showed that this equation can predict the total Met content of FM easily and quickly. However, in the National Research Council (1994), there are no equations for FM that predict total AA based on the CP content. In some cases, the inclusion of other chemical components into the linear regression equation increased the accuracy and precision of the total AA value prediction while it decreased SEP. Inclusion of Ash, CF and ADF into the total Met + Cys prediction equation (T Met + Cys = 0.132 × ASH – 0.22 × CF – 0.033 × ADF) decreased the SEP (0.031%) compared to the equation that contained only CP (T Met + Cys = 0.03 × CP, SEP: 0.151).

In previous studies, the use of chemical composition to predict total amino acid content through linear regression has been reported (National Research Council, 1994; Cravener and Roush, 2001; Sheikhhasan *et al.*, 2020). The National Research Council (1994), in order to predict the total AA content of FM (Met, Met + Cys, Lys, Thr, Trp and Agr), offered equations, but the accuracy of these regression equations was inconsistent. In addition, for predicting the total Met + Cys of FM, the National Research Council (1994) proposed an equation: T Met + Cys = 5.0029 - 0.0651 × Moisture -0.0702 EE × -0.0754 × Ash. In a study, multiple linear regression and artificial neural network models were used to predict amino acid content in feed ingredients based on chemical analysis and reported that the amino acid content of feed ingredients is strongly correlated with chemical compounds (Cravener and Roush, 2001).

The linear regression equations to predict the SID amino acid content of FM from its chemical composition is shown in (Table 9). Due to the cost and long time to determine the amino acid concentration before formulation, mathematical equations are one of the candidates to solve the problem (Sheikhhasan *et al.*, 2020). The equations obtained in the present study can be used by poultry nutritionists to predict the total and SID amino acid contents of FM. The adjusted R2 values for the SID amino acid prediction equations based on the CP content ranged for SID Met, SID Cys and SID Lys were 99.7%, 97.8% and 99.4% respectively.The inclusion of CP and other chemical components together in equations decreased the SEP. Roudi *et al.* (2012) used multiple linear regression in order to predict the apparent ileal digestible amino acids content of wheat grain from its CP content and they showed that using this method reduces the risks of unbalanced levels of energy and amino acids in feed formulation. In the past years, protein content was used to predict amino acid digestibility coefficients (Angkanaporn *et al.*, 1996; Short *et al.*, 1999; Bryden *et al.*, 2009).

Table 9. Linear Regression equations for predicting the standardized ileal digestible amino acids (SIDAA) content of FM from its chemical characteristics (DM basis)[1]

Amino acids	Basis	Prediction equations	Statistical parameters [2] R^2	Adjusted R^2	P-Value Regression	P-Value Coefficients		SEP
SID Met	CP	SID Met = 0.017 × CP	0.997	0.997	0.001	CP	0.001	0.060
	ASH	SID Met = 0.056 × ASH	0.989	0.987	0.001	ASH	0.001	0.118
	ASH ADF	SID Met = 0.066 × ASH – 0.034 × ADF	0.998	0.997	0.001	ASH ADF	0.001 0.011	0.048
	Cons CP	SID Met = 0.045 + 0.016 × CP	0.749	0.686	0.026	Cons CP	0.894 0.026	0.066
SID Cys	CP	SID Cys = 0.006 × CP	0.978	0.973	0.001	CP	0.001	0.059
	Cons DM	SID Cys = 2.759 – 0.026 × DM	0.681	0.601	0.043	Cons DM	0.028 0.043	0.044
	Cons DM EE	SID Cys = 2.663 – 0.024 × DM – 0.009 × EE	0.933	0.888	0.017	Cons DM EE	0.009 0.015 0.044	0.021
SID Met + Cys	CP	SID Met + Cys = 0.023 × CP	0.996	0.996	0.001	CP	0.001	0.093
	ASH CF	SID Met + Cys = 0.1 × ASH – 0.234 × CF	0.998	0.997	0.001	ASH CF	0.001 0.004	0.062
	Cons EE DM	SID Met + Cys = 4.942 – 0.033 × EE – 0.034 × DM	0.950	0.917	0.011	Cons EE DM	0.011 0.008 0.040	0.048
SID Lys	CP	SID Lys = 0.061 × CP	0.994	0.993	0.001	CP	0.001	0.312
	ASH	SID Lys = 0.198 × ASH	0.980	0.976	0.001	ASH	0.001	0.568
	DM	SID Lys = 0.044 × DM	0.992	0.990	0.001	DM	0.001	0.374
SID Thr	CP	SID Thr = 0.028 × CP	0.992	0.991	0.001	CP	0.001	0.169
	ASH	SID Thr = 0.092 × ASH	0.978	0.974	0.001	ASH	0.001	0.279
	DM	SID Thr = 0.021 × DM	0.995	0.994	0.001	DM	0.001	0.137

Continued **Table 9**. Linear Regression equations for predicting the standardized ileal digestible amino acids (SIDAA) content of FM from its chemical characteristics (DM basis)[1]

Amino acids	Basis	Prediction equations	Statistical parameters [2]					
			R^2	Adjusted R^2	P-Value Regression	P-Value Coefficients		SEP
SID Val	CP	SID Val = 0.033 × CP	0.983	0.980	0.001	CP	0.001	0.287
	ASH	SID Val = 0.107 × ASH	0.939	0.927	0.001	ASH	0.001	0.549
	DM	SID Val = 0.024 × DM	0.969	0.962	0.001	DM	0.001	0.395
SID Arg	CP	SID Arg = 0.073 × CP	0.994	0.993	0.001	CP	0.001	0.386
	ASH	SID Arg = 0.239 × ASH	0.982	0.978	0.001	ASH	0.001	0.661
	Cons CP	SID Arg = -0.72 + 0.084 × CP	0.665	0.582	0.048	Cons CP	0.735 0.048	0.379
	DM	SID Arg = 0.053 × DM	0.979	0.975	0.001	DM	0.001	0.707
SID His	CP	SID His = 0.019 × CP	0.965	0.958	0.001	CP	0.001	0.242
	ASH	SID His = 0.061 × ASH	0.915	0.898	0.001	ASH	0.001	0.377
	NDF CF	SID His = 0.253 × NDF – 0.705 × CF	0.959	0.938	0.002	NDF CF	0.007 0.038	0.263
SID Ilu	CP	SID Ileu = 0.023 × CP	0.936	0.923	0.001	CP	0.001	0.397
	DM	SID Ileu = 0.016 × DM	0.919	0.903	0.001	DM	0.001	0.447
SID Leu	CP	SID Leu = 0.051 × CP	0.937	0.924	0.001	CP	0.001	0.889
	ASH	SID Leu = 0.163 × ASH	0.879	0.854	0.002	ASH	0.002	1.230
	DM	SID Leu = 0.037 × DM	0.907	0.888	0.001	DM	0.001	1.080
SID Phe	CP	SID Phe = 0.037 × CP	0.980	0.976	0.001	CP	0.001	0.354
	EE	SID Phe = 0.201× EE	0.841	0.810	0.004	EE	0.004	0.996
	NDF	SID Phe = 0.217 × NDF	0.923	0.907	0.001	NDF	0.001	0.694

Abbreviation; FM, fish meal; DM, dry matter; CP, crude protein; Cons, intercept.
[1] Analyzed using SPSS statistical software and stepwise procedure.
[2] R^2 is the coefficient of determination, adjusted R^2 for the number of predictors in the model, *P*-value<0.05, is statistically significant (Sheikhhasan *et al.*, 2020).

Table 10. Regression equations for predicting the SIDAA concentration from its TAA value (DM basis)[1]

Amino Acids	Prediction Equations	Statistical Parameters[2]				
		R^2	Adjusted R^2	*P*-Value Regression	P-Value Coefficients	SEP
Met	SID = 0.085 + 0.791 × T Met	0.994	0.992	0.001	0.001	0.009
Cys	SID = 0.015 + 0.562 × T Cys	0.991	0.988	0.001	0.001	0.007
Met + Cys	SID = 0.764 × T MetCys	0.999	0.999	0.001	0.001	0.028
Lys	SID = 0.506 + 0.743 × T Lys	0.953	0.941	0.001	0.001	0.026
Thr	SID = 0.011 + 0.778 × T Thr	0.962	0.953	0.001	0.001	0.028
Val	SID = -0.068 + 0.802 × T Val	0.990	0.988	0.001	0.001	0.037
Arg	SID = 0.209 + 0.785 × T Arg	0.993	0.991	0.001	0.001	0.056
His	SID = 0.802 × T His	0.999	0.999	0.001	0.001	0.016
Ilu	SID = 0.006 + 0.835 × T Ilu	0.999	0.999	0.001	0.001	0.010
Leu	SID = -0.105 + 0.825× T Leu	0.998	0.997	0.001	0.001	0.035
Phe	SID = 0.818 × T Phe	0.998	0.996	0.001	0.001	0.026

Abbreviation: DM, dry matter; R^2, adjusted coefficient of determination; SEP, standard error of prediction; SID, standardized ileal digestibility; SIDAA, standardized ileal digestible amino acids; TAA, total amino acids.
[1] Analyzed using SPSS statistical software and stepwise procedures.
[2] R^2 is the coefficient of determination, adjusted R^2 adjusted for the number of predictors in the model, *P*-value < 0.05 is statistically significant (Yegani *et al.*, 2013).

In the study, regression equations were used to determine the digestible amino acid content of sorghum grain from chemical composition and reported that chemical composition is a suitable parameter for predicting amino acids (e.g., Met = 0.3885 – 0.2454 × total phenols – 0.0109 × CP –

0.0336 × EE – 0.0158 × CF + 0.0830 × Ash, R^2 = 72%) (Ebadi *et al.*, 2011). Linear regression equations were also developed to determine the SID amino acid content of FM from its total AA concentration (Table 10). The adjusted R^2 and SEP values for these equations ranged from 95.3% (Lys) to 99.9% (Met + Cys, His, Ilu) and 0.007% (Cys) to 0.056% (Arg). The SID amino acid concentration of Lys was predicted using the following equation: % SID = 0.506 + 0.743 × T Lys (adjusted R^2 = 95.3% and SEP = 0.026 %). The SEP values for these equations were relatively lower than the equations that included CP and other chemical components. Use of total AA content in order to predict the SID amino acid concentration of soybean meal reported by Sheikhhasan *et al.* (2020): soybean meal SID Met (%) = 0.080 + 0.788 × Total Met (adjusted R^2 = 79.5% and SEP = 0.018 %). They reported that the SEP values of equations based on the total AA were lower than the equations that included chemical composition.

Conclusion

According to the results of this study, it is concluded that the total AA content and amino acids digestibility of FMs were variable. Therefore, it is not feasible to consider a fixed value for amino acid content and its digestibility in diet formulation. On the other hand, it is difficult for poultry nutritionists to measure the total and SID amino acid contents of different FMs. The prediction equations obtained from this study can be used by poultry nutritionists to predict the total and SID amino acid contents of FM easily and fast with high accuracy.

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