Journal of Veterinary Science & Animal Husbandry
Volume 11 | Issue 1
ISSN: 2348-9790

Research Article Open Access

Total and Standardized Ileal Digestible Amino Acids Regression Equations Base on Chemical Composition for Canola Meal in Broilers

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Citation: Mohammad Latifi, Hossein Moravej, Fatemeh Ghaziani, Woo Kyun Kim (2023) Total and Standardized Ileal Digestible Amino Acids Regression Equations Base on Chemical Composition for Canola Meal in Broilers. J Vet Sci Ani Husb 11(1): 103

Abstract

This experiment was conducted to determine the contents of total amino acids (TAAs) and standardized ileal digestible amino acids (SIDAAs) and chemical composition of canola meal (CM) samples, and to develop regression equations for predicting the TAAs and SIDAAs content of CM for broilers. Eight samples of CM were obtained from different origins. A total of 180 one-day old male broiler (Ross 308) were randomly assigned to nine treatments with four replicate, and five birds for each replicate. Birds were fed a corn- soybean meal starter diet until 10 days old and then a corn-soybean meal grower diet was fed until 23 days old. On day 24, after overnight fasting, the experimental diets were fed. The experimental diets consisted of eight semi-purified diets that contained each of CM as only source of protein (contained 20 mg protein/kg of diet, as fed) and one nitrogen free diet for determine basal endogenous AA losses. The crude protein content of CM samples varied from 32.21 to 36.18%, and the gross energy level was from 3680 to 4162 kcal/kg DM. The digestibility of amino acids (Lys, Met, Cys, Thre, Val, Arg, Ileu, Phe, and His) among the samples were significantly different ($P < 0.05$). The standard error of prediction (SEP) and adjusted R^2 of linear regression equations base on crude protein content showed that these equations can be use in order to predict the content of TAAs and SIDAAs (e.g., SID Met = 0.017 × CP, SEP 0.019, Adj R^2 0.996; SID Lys = -3.338 + 0.13 × CP, SEP 0.128, Adj R^2 0.656). Inclusion of other chemical compositions into regression equations increased the Adj R^2 and decreased SEP (e.g., SID Lys = 0.066 × CP – 0.109 × ASH, SEP 0.109, Adj R^2 0.994).

Keywords: Broiler; Amino acid; Regression equation; Canola; Predict

List of Abbreviations: TAA: Total Amino Acid; SIDAA: standardized ileal digestible Amino Acids; CM: Canola meal; SID: Standardized Ileal Digestible; AA: Amino Acid;

Introduction

There are many sources of protein and amino acids for poultry diets. The most common one is soybean meal [1], but many countries do not have suitable conditions for planting soybean. One of the protein sources that can replace soybean meals in poultry diets is canola meals. Canola meal is valuable byproducts of oil refining factories and canola is the most widely grown oilseed crop of the brassica family [2], and its oil can be extracted by solvent extraction, expeller pressing, or expeller cold pressing [3]. It has relatively high CP content (38%; NRC, 1994) but a somewhat low ME value (2070 kcal/ kg; NRC, 1994). It has lower amino acid (AA) digestibility and protein content than soybean meal, and its high fiber content limits inclusion into poultry diets so canola meal can be used up to 8% of poultry diets [4]. The availability of amino acids in feedstuffs is vastly different, especially for those in processed feed or by-products [1]. Knowledge of digestibility coefficient (DC) for individual AAs in feed ingredients and the requirement of digestible AAs for a defined production target enable the formulation of diets more closely to the precise requirement of the bird. Diets based on digestible amino acids will allow the use of alternative protein sources with low DC, because such formulations will improve the precision of least cost diets and reduce nitrogen output from poultry operations. Finally, diets formulated on a digestible AA basis offer economic benefits [5]. The advantage of the digestible AA system is recognized and in order to determine AA availability for poultry, there are several ways such as *in vitro* methods (enzymatic, chemical, and microbiological assays), indirect methods (plasma amino acids assays), and direct methods (digestibility and growth assays) [6]. Digestibility studies using live animal have become the most common method for estimating amino acids digestibility but are expensive and time consuming [7]. Therefore, poultry nutritionists' interest to finding rapid, inexpensive, and accurate methods for determination of TAA and SIDAA content of ingredients. Use of regression equations in order to predicting the nutritive value of feed ingredients from its chemical characteristics has been attempted for many years (NRC, 1994) [1]. Therefore, the aim of this experiment was to determine the TAA and standardized ileal digestible amino acids (SIDAA) content of different samples of CM by the biological method using growing broiler chicks and the chemical composition of CM by analytical methods and to then develop linear regression equations to predict the SIDAA and TAA contents of CM from its chemical composition.

Material and Methods

Test Samples

Eight samples of canola meal (CM) from different origins were obtained: Behpak (Be), Negin Nahavand (NN), Talaye Sephide Gonbad (TSG), Roghan Talayie Neyshabor (RTN), Olva (OL), Danehaye Roghanye Khorasan (DRK), Shokofeh Baharane Tehran (SBT) and Yeganeh Khazar (YK).

Birds and Treatments

This experiment was approved by the animal care committee of the Tehran university, Tehran, Iran. In order to conduct this study, a total of 180 one-day-old Ross 308 male chicks were obtained from a commercial hatchery and vaccinated against Newcastle disease (7 and 18 D) and infection bronchitis (1 D). Chicks were weighted and randomly assigned into 36 experimental pens so that each pen had birds with similar initial weight and pen weight distribution (4 replicates for each treatment and 5 birds per replicate; 0.2 m^2/bird). Each pen was equipped with a through feeder and a through nipple waterer. Experimental pens were located in a solid-sided house with temperature control. Temperature, Moisture, ventilation and lighting was in accordance with the management handbook of the Aviagen Ross 308. Chicks were allowed *ad libitum* access to a corn-soybean meal starter diet until 10 days of old and then a current grower diet were fed from days 11 to 23 (Table 1).

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- Lysin	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	19.8
Metabolizable Energy (Kcal/Kg)	2850	2900
Met (%)	0.55	0.50
Met + Cys (%)	0.84	0.79
Lys (%)	1.15	1
Thre (%)	0.75	0.68
Calcium (%)	0.9	0.8
Available P (%)	0.45	0.39

aVitamin/ Mineral premix provided the per Kg of complete diet: vitamin A, 10,000 IU; vitamin D3, 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.

Table 1: composition of starter and grower diets were fed to chicks

On day 24, after an overnight fast, chicks were given *ad libitum* access to experimental diets (Table 2). There were nine dietary treatments: 8 semi-purified diets containing one of the CM samples as the only source of diet protein and one nitrogen free diet for determination of basal endogenous AA losses. The diets consisted of corn starch, dextrose and one of CM samples (CMs were included 560 to 620 mg/kg of diets according to its protein content). In the nitrogenfree diet, corn starch and dextrose were used as the energy source without any source of protein. All diets were balanced for calcium and phosphorus and supplemented with equal amounts of vitamin and minerals (NRC, 1994). Celite, (Celite 281) was included at 1% in all of the experimental diets and nitrogen free diet as an indigestible ash marker for determination of the ileal AA digestibility. All diets were fed in mash form. 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 [8]. Collected ileal digesta from 4 birds within a cage were pooled and stored at -20 ^{0}C for further analyses of AIA (acid insoluble ash) and AA. Frozen digesta samples were thawed, lyophilized, and ground using an electric coffee grinder (Moulimex, PRC) to obtain a finely ground sample while avoiding significant losses.

	Diets [a]								
ingredients	CM-1	CM-2	CM-3	CM-4	CM-5	CM-6	CM-7	CM-8	N-Free
Corn starch	160	160	160	160	160	160	160	160	358
Dextrose	100	100	100	100	100	100	100	100	430
Oil	60	60	60	60	60	60	60	60	50
Salt	2	2	2	2	2	2	2	2	3
Dicalcium phosphate	15	15	15	15	15	15	15	15	25
Limestone	12	12	12	12	12	12	12	12	11
Vit and Min premix [b]	9	9	9	9	9	9	9	9	9
Sodium bicarbonate	3	3	3	3	3	3	3	3	4
celite	10	10	10	10	10	10	10	10	10
Solka floc [c]	49	39	69	69	69	69	9	39	100
CM	580	590	560	560	560	560	620	590	0
SUM	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated nutrients content									
Dry matter (%)	89	89	89	89	89	89	89	89	94
Crude protein (%)	20	20	20	20	20	20	20	20	-
AMEn (kcal/kg)	2930	2940	2920	2920	2920	2920	2968	2940	3190
Calcium (%)	1.15	1.16	1.14	1.14	1.14	1.14	1.18	1.16	0.94
Available Pho (%)	0.49	0.49	0.48	0.48	0.48	0.48	0.51	0.49	0.44

Abbreviation: SID, standardized ileal digestibility; CM, canola meal [a]The canola meals (CM) were obtained from different origins: CM1= Behpak, CM2=Negine Nahavand, CM3=Talaye Sephid Gonbad, CM4=Roghan Talayee Neyshabor, CM5=Olva, CM6=Danehaye Roghanye Khorasan, CM7=Shokofeh Baharane Tehran, and CM8=Yeganeh Khazar. N-free = nitrogen free. [b]Vitamin/ Mineral premix provided per Kg of the 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]Purified cellulose

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

Chemical Analysis

Dry matter (DM), Ash, CP, crude fiber (CF) and ether extract (EE) of all CM samples were analyzed according to [32] analytical methods (930.15, 920.39, 990.03, 978.10 and 942.05 respectively). Neutral detergent fiber (NDF) was analyzed as described by [9] and then acid detergent fiber (ADF) analysis was performed as described by [10]. The GE of samples was measured by an adiabatic calorimetric bomb (Ika- Kalorimeter; C400 adiabatisch, Germany). Nitrogen content of all diets was determined by combustion with an automatic nitrogen analyzer [11]. All the analyses were performed in 3 replicates. Nitrogen free extract was determined by mathematical calculation. For AA analysis, samples (canola meals), test diets, and ileal digesta were digested by 6 N HCl for 24 h at 110 ^{0}C, afterward neutralized with 15 ml of 9.8 N NaOH, and then cooled to room temperature. After that, sodium citrate buffer was added, and the mixture was equalized to 100-ml volume [12]. Methionine and cystine (sulfur containing AA) were analyzed by performic acid oxidation at 0 ^{0}C and then hydrolyzed by 6 N HCL [13]. 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 in duplicate. The contents of diets, ileal digesta, and acid insoluble ash were determined after combusting the samples and then boiling the ash into 4 N HCL in duplicate according to the method by [14]. All analyses were conducted in the chemical laboratory of the College of Agriculture and Natural Resources, Tehran University.

Apparent ileal AA digestibility (AIAAD) was calculated using the following equation [15]: AIAAD = [(AA/AIA) diet - (AA/AIA) digesta]/ (AA/AIA) diet. Ileal endogenous AA (IEAA) flow in broilers fed with the nitrogen free diet was calculated as milligrams of AA flow per kilogram of DM intake (DMI) using the following equation [16]: 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 diet, g/kg DM)] × 100.

Statistical Analysis

Data were analyzed base on the randomized complete design [17]. The general linear model procedure and least-squares means method were used to compare means of chemical compositions, TAA, SIDAA, and SIDAA coefficients.

To predict TAA and SIDAA content of CM samples, the simple and multiple linear regressions were used by SPSS software version 19 with the following model [18]. In the equations, the dependent variables were individual TAA and SIDAA, and the independent variables were CP, DM, GE, CF, NDF, ADF, NFE, EE and ASH:

$$y_i = \beta_0 + \beta_{1\times1} + \beta_{2\times2} + \ldots + \varepsilon_i$$

where yi is the predicted concentration of individual TAA and SIDAA, β0 is the intercept of the regression equation, βj is the regression coefficient, xj is the independent variable (contains: CP, DM, GE, CF, NDF, ADF, NFE, EE and ASH) and i is the random error of the regression model. In order to define the equation with the best fit of independent variable, the coefficient of determination (R2), adjusted R2, P-value regression, P-value coefficients, and standard error of prediction (SEP) were used. Statistical significance was considered at P ≤ 0.05. The SEP was calculated according to the following equation [19]:

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

Where y is the TAA content or concentration of SIDAA determined in the chick's bioassay, y' is the predicted TAA or SIDAA value based on the prediction equation, and N is the number of test samples.

Results and Discussion

The determined total AA and CP content of semi-purified diets and nitrogen free diet are shown in Table 3. The determined CP content of experimental diets varied from 19.8% for CM-1 to 20.3% for CM-6 and CM-8. The total AA content of semi-purified diets varied among the different diets because the AAs content of different CM samples varied. Different in CP and AA content among the semi-purified diets have been shown in other studies [20,1].

	Diets [b]								
Item	CM-1	CM-2	CM-3	CM-4	CM-5	CM-6	CM-7	CM-8	N-Free
CP	19.8	20.0	20.2	20.0	20.1	20.3	19.9	20.3	0.18
Lys	1.15	1.00	1.08	1.13	1.14	1.15	0.99	0.97	0.003
Met	0.38	0.40	0.39	0.38	0.38	0.39	0.40	0.39	0.001
Cys	0.48	0.49	0.50	0.51	0.47	0.53	0.46	0.48	0.000
Thre	0.77	0.83	0.82	0.84	0.81	0.93	0.88	0.83	0.001
Val	0.99	1.01	1.10	1.14	1.07	1.12	1.00	0.98	0.002
Arg	1.23	1.18	1.22	1.28	1.15	1.33	1.18	1.29	0.002
Ileu	0.85	0.85	0.86	0.87	0.96	1.00	0.84	0.87	0.005
Leu	1.49	1.40	1.37	1.44	1.31	1.55	1.41	1.40	0.007
Phe	0.99	0.77	0.87	0.86	0.93	0.86	0.68	0.80	0.004
His	0.42	0.51	0.58	0.55	0.65	0.47	0.45	0.61	0.001

Abbreviation: TAA, total amino acid; CP, crude protein. [a]All means was obtained from average of 3 replicate. [b]The canola meals (CM) were obtained from different origins: CM1= Behpak, CM2=Negine Nahavand, CM3=Talaye Sephid Gonbad, CM4=Roghan Talayee Neyshabor, CM5=Olva, CM6=Danehaye Roghanye Khorasan, CM7=Shokofeh Baharane Tehran, and CM8=Yeganeh Khazar. N-free = nitrogen free.

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

The chemical composition of canola meal samples is shown in Table 4. DM, CP, GE, EE, ASH, CF, NDF, ADF and NFE contents among the samples were significantly different (*P*<0.05). the CP content varied from 32.21 to 36.18% for CM-7 and CM-3 respectively. The range GE content was 3680 to 4162 kcal/ kg DM with means of 3829 kcal/kg DM. For the EE content, CM-8 sample had the maximum level (4.12%), whereas the lowest level was found in CM-7 sample. various chemical compositions of different feed ingredients have been reported in other studies. [21] reported that 15 wheat samples, obtained from different origins, had different chemical compositions. Moreover, [7] reported that the range of CF content among 48 sorghum grain samples was 2.12 to 9.80 %. The means of CP (34.8%), EE (1.99%), and CF(10.66%) determined in the current study was relatively lower than those reported by [22] (CP (38%), EE (3.8%) and CF (11.1%)).

Sample	Moisture	CP	GE (kcal/kg)	EE	ASH	CF	NDF	ADF	NFE [c]
CM-1	22.57^{cd}	34.45^{c}	3763^{cd}	1.77^{c}	6.7^{d}	11.96^{a}	26.07^{c}	22.57^{bc}	35.86^{c}
CM-2	24.41^{a}	33.94^{d}	3693^{cd}	1.45^{d}	7.81^{c}	11.22^{b}	31.97^{a}	24.41^{a}	35.13^{d}
CM-3	21.04^{d}	36.18^{a}	3787^{b}	1.93^{b}	6.81^{d}	10.43^{c}	27.62^{b}	21.04^{d}	37.01^{b}
CM-4	18.68^{f}	35.74^{b}	3680^{d}	1.77^{c}	6.66^{d}	9.31^{d}	24.42^{d}	18.68^{f}	37.85^{a}
CM-5	19.17^{fe}	35.71^{b}	3711^{cd}	1.76^{c}	6.32^{e}	9.63^{d}	24.12^{d}	19.17^{ef}	37.34^{ab}
CM-6	19.8^{e}	35.77^{b}	3724^{cbd}	1.69^{c}	6.55^{ed}	10.47^{c}	23.93^{d}	19.82^{e}	35.03^{d}
CM-7	23.28^{b}	32.21^{e}	4110^{a}	1.39^{d}	9.37^{a}	11.87^{a}	26.89^{bc}	23.28^{b}	37.52^{ab}
CM-8	22.16^{c}	34.43^{c}	4162^{a}	4.12^{a}	9.17^{b}	10.47^{c}	26.57^{c}	22.16^{c}	34.71^{d}
Mean	21.39	34.8	3829	1.99	7.3	10.66	26.45	21.39	36.3
SEM	0.26	0.06	21.56	0.047	0.09	0.15	0.29	0.26	0.19

P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Means within a row with no common superscript letters (a-d) differ significantly (P≤0.05). Abbreviation: DM, Dry matter; CP, Crud protein; GE, Gross energy; EE, Ether extract, CF, Crud fiber, NDF, neutral detergent fiber; ADF, Acid detergent fiber, NFE, Nitrogen free extractaAll means was obtained from average of 3 replicatebThe canola meals (CM) were obtained from different origins: CM1= Behpak, CM2=Negine Nahavand, CM3=Talaye Sephid Gonbad, CM4=Roghan Talayee Neyshabor, CM5=Olva, CM6=Danehaye Roghanye Khorasan, CM7=Shokofeh Baharane Tehran, and CM8=Yeganeh Khazar. cNitrogen free extract = DM – (CP + EE + ASH + CF).

Table 4: Means [a]of canola meal chemical composition[b] (%, as-fed)

The TAA and SIDAA contents of CM samples have been shown in Tables 5 and 6 respectively. The levels of TAA content significantly differed among the CM samples (*P*<0.05). The total content of Lys ranged from 1.61 to 2.09% for CM-7 and CM-4, respectively, with a mean of 1.92%. The total Met content ranged from 0.69% for CM-1 sample to 0.74% for CM-6 sample with a mean of 0.7%. The mean of total Thr was 1.49%, and the highest level was 1.39% for CM-1 Sample and the lowest level was 1.69% for CM-6 sample. The highest level of SEM was for Leu, Arg, and Lys with 0.052, 0.045 and 0.039 values, respectively. The amino acid values reported by [23] for total Lys, Met, Cys and Thr were even higher than the values determined in this study (2.21 vs. 1.92% for Lys; 0.84 vs. 0.70% for Met; 1.06 vs. 0.87% for Cys and 1.64 vs. 1.49% for Thre based on DM).

	Canola Meal [b]										
Item	**CM-1**	**CM-2**	**CM-3**	**CM-4**	**CM-5**	**CM-6**	**CM-7**	**CM-8**	***P*-value**	**SEM**	**Mean**
Lys	2.01 ^{a}	1.82 ^{b}	2.00 ^{a}	2.09 ^{a}	2.08 ^{a}	2.08 ^{a}	1.61 ^{c}	1.65 ^{c}	0.0001	0.039	1.92
Met	0.69 ^{bc}	0.70 ^{abc}	0.73 ^{ab}	0.72 ^{ab}	0.71 ^{ab}	0.74 ^{a}	0.67 ^{c}	0.70 ^{abc}	0.0500	0.011	0.70
Cys	0.85 ^{bc}	0.85 ^{bc}	0.91 ^{ab}	0.91 ^{ab}	0.86 ^{bc}	0.95 ^{a}	0.76 ^{d}	0.81 ^{cd}	0.0011	0.017	0.87
Met+Cys	1.55 ^{bc}	1.56 ^{bc}	1.64 ^{ab}	1.63 ^{ab}	1.58 ^{bc}	1.69 ^{a}	1.43 ^{d}	1.51 ^{cd}	0.0045	0.029	1.57
Thre	1.39 ^{c}	1.45 ^{bc}	1.51 ^{b}	1.53 ^{b}	1.49 ^{bc}	1.69 ^{a}	1.45 ^{bc}	1.45 ^{bc}	0.0033	0.030	1.49
Val	1.77 ^{b}	1.77 ^{b}	2.06 ^{a}	2.10 ^{a}	1.99 ^{a}	2.06 ^{a}	1.66 ^{b}	1.67 ^{b}	0.0001	0.038	1.88
Arg	2.18 ^{cd}	2.04 ^{de}	2.21 ^{bc}	2.36 ^{ab}	2.12 ^{cd}	2.44 ^{a}	1.95 ^{e}	2.26 ^{bc}	0.0009	0.045	2.20
Ileu	1.51 ^{cde}	1.48 ^{de}	1.57 ^{cd}	1.61 ^{c}	1.77 ^{b}	1.94 ^{a}	1.40 ^{e}	1.50 ^{cde}	0.0001	0.032	1.60
Leu	2.66 ^{b}	2.44 ^{c}	2.52 ^{bc}	2.64 ^{b}	2.41 ^{c}	2.87 ^{a}	2.35 ^{c}	2.46 ^{c}	0.0017	0.052	2.55
Phe	1.77 ^{a}	1.36 ^{c}	1.61 ^{b}	1.57 ^{b}	1.72 ^{a}	1.58 ^{b}	1.13 ^{d}	1.39 ^{c}	0.0001	0.031	1.52
His	0.76 ^{d}	0.90 ^{c}	1.08 ^{b}	1.01 ^{b}	1.19 ^{a}	0.85 ^{c}	0.76 ^{d}	1.08 ^{b}	0.0001	0.019	0.95

Means within a row with no common superscript letters (a-d) differ significantly (p≤0.05). Abbreviation: TAA, total amino acid; CM, canola meal: DM, dry matter [a]All means was obtained from average of 3 replicate. [b]The canola meals (CM) were obtained from different origins: CM1= Behpak, CM2=Negine Nahavand, CM3=Talaye Sephid Gonbad, CM4=Roghan Talayee Neyshabor, CM5=Olva, CM6=Danehaye Roghanye Khorasan, CM7=Shokofeh Baharane Tehran, and CM8=Yeganeh Khazar.

Table 5: The total amino acid content of canola meal samples [a] (%, DM)

	Canola Meal [b]									
Item	**CM-1**	**CM-2**	**CM-3**	**CM-4**	**CM-5**	**CM-6**	**CM-7**	**CM-8**	***P*-value**	**SEM**	**Mean**
Lys	1.62^{d}	1.53^{e}	1.66^{c}	1.83^{b}	2.05^{a}	1.81^{b}	1.30^{g}	1.37^{f}	0.0001	0.010	1.650
Met	0.60^{e}	0.62^{d}	0.63^{c}	0.65^{b}	0.65^{b}	0.67^{a}	0.58^{f}	0.63^{d}	0.0001	0.002	0.633
Cys	0.68^{d}	0.71^{cd}	0.74^{b}	0.72^{c}	0.72^{c}	0.80^{a}	0.60^{f}	0.65^{e}	0.0001	0.006	0.708
Met+Cys	1.29^{d}	1.34^{c}	1.38^{b}	1.37^{b}	1.37^{b}	1.47^{a}	1.18^{e}	1.28^{d}	0.0001	0.006	1.34
Thre	1.01^{f}	1.08^{e}	1.10^{cd}	1.14^{b}	1.12^{bc}	1.27^{a}	1.02^{ef}	1.04^{e}	0.0001	0.008	1.102
Val	1.28^{d}	1.29^{d}	1.5^{c}	1.58^{a}	1.50^{c}	1.54^{b}	1.18^{e}	1.20^{e}	0.0001	0.007	1.388
Arg	1.94^{d}	1.87^{e}	1.98^{d}	2.18^{b}	1.97^{d}	2.28^{a}	1.67^{f}	2.02^{c}	0.0001	0.012	1.993
Ileu	1.22^{e}	1.21^{e}	1.26^{d}	1.33^{c}	1.48^{b}	1.62^{a}	1.11^{f}	1.20^{e}	0.0001	0.008	1.308
Leu	2.25^{b}	2.12^{cd}	2.13^{c}	2.26^{b}	2.08^{d}	2.51^{a}	1.93^{e}	2.08^{d}	0.0001	0.015	2.174
Phe	1.52^{a}	1.18^{d}	1.37^{c}	1.36^{c}	1.50^{a}	1.39^{b}	0.94^{e}	1.18^{d}	0.0001	0.006	1.309
His	0.66^{f}	0.79^{d}	0.93^{b}	0.89^{c}	1.06^{a}	0.75^{e}	0.64^{g}	0.92^{b}	0.0001	0.004	0.835

Means within a row with no common superscript letters (a-d) differ significantly (p≤0.05). Abbreviation: SIDAA, Standardized ileal digestible amino acids; CM, canola meal: DM, dry matter aAll means was obtained from average of 3 replicate. bThe canola meals (CM) were obtained from different origins: CM1= Behpak, CM2=Negine Nahavand, CM3=Talaye Sephid Gonbad, CM4=Roghan Talayee Neyshabor, CM5=Olva, CM6=Danehaye Roghanye Khorasan, CM7=Shokofeh Baharane Tehran, and CM8=Yeganeh Khazar.

Table 6: The SIDAA content of CM samples [a] (%, DM)

The SIDAA content among the different samples significantly differed (*P*<0.05). The contents of Lys varied from 1.30% (CM-7) to 2.05% (CM-5) with a mean of 1.65%. The highest level of Met SID was 0.67% (CM-6), whereas the lowest value was 0.58% (CM-7). The highest value of CP was 36.18% (CM-3), and the lowest was 32.21% (CM-7). However, the highest SIDAA was observed in CM-6 sample which had 35.77% CP, whereas the least content was found in CM-7 sample with 32.21% CP. Thus, the SIDAAs contents had positive relationship with CP content of CM samples. [24]reported that 9 samples of wheat cutivated in different locations had significant differences in term of contents of Lys, Met, Cys, Thr, Arg, Val, Pro, Leu, and Ileu. [25] showed that different samples of canola meal which were refined with different processing had significant differences in SIDAA and amino acids digestibility.

The standardized ileal digestibility coefficient (SIDC) of amino acids in different canola meals is shown in Table 7. Amino acids digestibility of different samples of CM significantly differed except for Leu (*P*<0.05). For example, SIDC of Lys varied from 74.9% for CM-1 to 81.8% for CM-4 with a mean of 78.42%. the range of SIDC for Thr was from 66.8% (CM-7) to 70.0% (CM-4). These references about the amino acids SIDC among the samples might be attributed to potential reactions among sugars, fibers, and amino acids by thermal processing during the oil extraction; thus, CM-4 and CM-5 samples with the lowest values of fibers (9.31 and 9.63%, respectively) had highest levels of SIDC. [26] reported that CP and AA digestibilities of expeller-extracted canola meals subjected to different proccesing conditions were different, most likely because of formation of indigestible complexces of AA with fiber. [19]reported that AA digetstibilities of 20 triticales from different genotypes were differed in laying hens. [27] illustrated that SIDC for amino acids among nine different samples of rapeseed meal differed and the highest variation of amino acids digestibility among rapeseed meals was for Thr (0.68 to 0.79%) and Lys (0.68 to 0.78%).

	Canola Meal[b]										
Item	CM-1	CM-2	CM-3	CM-4	CM-5	CM-6	CM-7	CM-8	*P*-value	SEM	Mean
Lys	74.9^{f}	77.2^{ed}	78.4^{cd}	81.8^{a}	81.0^{ab}	79.7^{bc}	76.0^{ef}	78.0^{cd}	0.0002	0.522	78.42
Met	81.2^{d}	81.7^{cd}	81.7^{cd}	84.7^{a}	84.4^{a}	82.7^{b}	82.5^{bc}	84.0^{a}	0.0001	0.267	82.92
Cys	74.9^{bcd}	76.3^{abcd}	77.2^{ab}	74.0^{d}	78.0^{a}	77.0^{ab}	74.4^{bcd}	75.0^{bcd}	0.0328	0.722	75.90
Thre	67.4^{bc}	67.9^{bc}	68.9^{ab}	70.0^{a}	69.8^{a}	68.9^{ab}	66.8^{c}	67.6^{bc}	0.0167	0.505	68.47
Val	67.1^{e}	67.2^{e}	68.8^{bc}	70.2^{a}	69.8^{ab}	68.5^{cd}	67.4^{de}	67.8^{cde}	0.0016	0.360	68.39
Arg	82.9^{c}	83.8^{bc}	84.2^{abc}	86.1^{a}	86.0^{a}	85.6^{ab}	83.9^{bc}	83.9^{bc}	0.0225	0.540	84.59
Ileu	74.9^{c}	75.0^{c}	75.8^{bc}	77.3^{ab}	77.6^{a}	76.2^{abc}	74.8^{c}	75.3^{c}	0.0249	0.509	75.90
Leu	78.4	79.6	80.1	79.9	79.9	79.9	77.7	79.5	0.1159	0.555	79.42
Phe	79.7^{bc}	79.7^{bc}	80.1^{abc}	81.4^{a}	81.0^{ab}	80.5^{ab}	79.1^{c}	79.9^{bc}	0.0484	0.399	80.24
His	80.2^{c}	80.3^{c}	82.0^{ab}	82.3^{ab}	82.7^{a}	81.1^{bc}	80.3^{c}	80.5^{c}	0.0129	0.434	81.23

Means within a row with no common superscript letters (a-d) differ significantly (p≤0.05). [a] there were 4 pens of 5 birds per each treatment. [b] The canola meals (CM) were obtained from different origins: CM1= Behpak, CM2=Negine Nahavand, CM3=Talaye Sephid Gonbad, CM4=Roghan Talayee Neyshabor, CM5=Olva, CM6=Danehaye Roghanye Khorasan, CM7=Shokofeh Baharane Tehran, and CM8=Yeganeh Khazar

Table 7: Coefficient of standardized ileal amino acid digestibility of broilers in 28 D of age [a]

In order to predict TAA content of CM based on its protein content and other chemical composition, linear regression equations were developed in the present study (Table 8). The adjusted R^2 for equations base on the CP as only an independent variable ranged from 0.585 to 0.999 for equations: total Phe = -2.832 + 0.117 × CP and total Met + Cys = 0.045 × CP, respectively, and the standard errors of prediction (SEP) for these equations ranged from 0.011 to 0.171% for equations: total Met = 0.02 × CP and total Phe = 0.043 × CP, respectively. The adjusted R^2 and SEP of these values represent that equations based on the CP as only an independent variable can predict TAA content of CM accurately. In addition to CP, inclusion of other chemical composition to the regression equation decreased SEP. for example, inclusion NFE into the regression equation for predicting total Cys decreased the SEP from 0.041 to 0.027%.

			Statistical parameters[2]					
Amino acids	**Basis**	**Prediction equations**	**R^2**	**Adjusted R^2**	**P-Value Regression**	**P-Value Coefficients**		**SEP**
T Met	CP	Met = 0.02 × CP	0.999	0.998	0.001	CP	0.001	0.011
	CPASH	Met = -0.534 + 0.03 × CP + 0.02 × ASH	0.942	0.918	0.001	ConsCPASH	0.0480.0020.038	0.009
T Cys	CP	Cys = 0.025 × CP	0.998	0.998	0.001	CP	0.001	0.041
	CPNFE	Cys = 0.034 × CP − 0.012 × NFE	0.999	0.999	0.001	CPNFE	0.0010.035	0.027

	CP	Cys = -0.649 + 0.041 × CP	0.845	0.819	0.001	ConsCP	0.0500.001	0.032
T Met + Cys	CP	Met + Cys = 0.045 × CP	0.999	0.999	0.001	CP	0.001	0.044
	CPNFE	Met + Cys = 0.054 × CP – 0.012 × NFE	0.999	0.999	0.001	CPNFE	0.0010.049	0.032
T Lys	CP	Lys = -2.648 + 0.123 × CP	0.778	0.741	0.004	ConsCP	0.0420.004	0.1
	CP	Lys = 0.054 × CP	0.995	0.995	0.001	CP	0.001	0.143
	CPDM	Lys = 0.115 × CP – 0.025 × DM	0.998	0.997	0.001	CPDM	0.0020.036	0.108
	ASHEE	Lys = 4.398 – 0.22 × ASH – 0.296 × EE	0.98	0.971	0.001	ConsASHEE	0.0010.0010.005	0.03
T Thre	CP	Thre = 0.042 × CP	0.998	0.997	0.001	Cp	0.001	0.078
T Val	CP	Val = 0.053 × CP	0.996	0.996	0.001	Cp	0.000	0.128
	CP	Val = -2.276 + 0.113 × CP	0.773	0.735	0.004	ConsCP	0.0500.004	0.092
	CPASH	Val = 0.067 × CP – 0.065 × ASH	0.999	0.998	0.001	CPASH	0.0000.049	0.093
T Arg	CP	Arg = 0.062 × CP	0.998	0.997	0.001	CP	0.001	0.115
T His	CP	His = 0.027 × CP	0.981	0.979	0.001	CP	0.001	0.142
	DMCF	His = 0.028 × DM – 0.126 × CF	0.992	0.990	0.001	DMCF	0.0010.014	0.101
T Ileu	CP	Ileu = 0.045 × CP	0.993	0.992	0.000	CP	0.000	0.145
	CPDM	Ileu = 0.098 × CP – 0.022 × DM	0.996	0.994	0.001	CPDM	0.0090.049	0.112
T Leu	CP	Leu = 0.072 × CP	0.997	0.997	0.001	CP	0.001	0.146
T Phe	CP	Phe = 0.043 × CP	0.989	0.988	0.001	CP	0.001	0.171
	CP	Phe = -2.832 + 0.117 × CP	0.645	0.585	0.016	ConsCP	0.0500.016	0.130

	CPASH	Phe = 0.065 × CP – 0.106 × ASH	0.997	0.996	0.001	CPASH	0.0010.011	0.095

Abbreviation; CM, canola meal; DM, dry matter; CP, crude protein; 1 Analyzed using SPSS statistical software and stepwise procedure. 2 R2 is the coefficient of determination, Adjusted R2 adjusted for the number of predictors in the model, P-value<0.05 is statistically significant [1]

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

In order to predict the total content of Met, Met + Cys, Lys, Thr, Trp and Arg, NRC (1994) proposed linear regression equations base on the CP content but not proposed equations based on other chemical compositions. For example, the equation for Met = 0.177 + 0.0157 × CP was proposed (NRC, 1994).

The linear regression equations developed by SPSS software to predict the SIDAA content of CM base on the its protein content and other chemical components are shown in Table 9. Due to some difficulties, such as time and cost, in determination of concentration of SIDAA before feed formulation, mathematical equations have been developed for fast and accurate SIDAA determination [1]. Use of chemical composition in order to predict the TAA and SIDAA contents of ingredients via linear regression equations has been reported in other studies [28,1, 27] used the multiple linear regression and artificial neural network models to predict the AA content in feed ingredients based on chemical analysis and sugested that AA contents of feedstuffs are highly correlated with the samples chemical analysis. [29] used multiple linear regression in order to precdict the apparent ileal digestible amino acids contetn of wheat grain from its CP content. [7] used chemical composition (CP, CF, EE, Ash and total phenols) in order to predict of digestible AA contetn of sorghum grain by multiple regression equations and reported that chemical composition was a good parameter with reasonable accuracy (e.g., Met = 0.3885 – 0.2454 × total phenols – 0.0109 × CP – 0.0336 × EE – 0.0158 × CF + 0.0830 × Ash, R^2 = 72%). Traditionally, the protein content was used to estimate AA digestibility coefficents [30-32]. However, the current study demonstrated that the equations base on the CP as only an indipendent varible in order to predict the content of SIDAA contetn of CM samples were feasible. The SEP of the equations ranged from 0.019 to 0.128% for eqations SID Met = 0.017 × CP and SID Lys = -3.338 + 0.13 × CP, respectively. In addition to CP, inclusion of other chemical components decreased the SEP. For example, inclusion of ASH into the equation (SID Met = -0.518 + 0.026 × CP + 0.018 × ASH) decreased SEP to 0.014% compare to the equation with only CP (Met = 0.017 × CP: SEP = 0.019%). [25] suggested that the multiple regression analysis based on the concentrations of CP and ASH together was a better approach.

			Statistical parameters [2]					
Amino acids	**Basis**	**Prediction equations**	**R^2**	**Adjusted R^2**	**P-Value Regression**	**P-Value Coefficients**		**SEP**
SID Met	CP	Met = 0.017 × CP	0.998	0.996	0.001	CP	0.001	0.019
	CPASH	Met = -0.518 + 0.026 × CP + 0.018 × ASH	0.936	0.911	0.001	ConsCPASH	0.0460.0030.050	0.014
SID Cys	CP	Cys= -0.664 + 0.036 × CP	0.868	0.846	0.001	ConsCP	0.0230.001	0.021
	CPDM	Cys= 0.034 × CP – 0.007 × DM	0.999	0.999	0.001	CPDM	0.0010.013	0.05
SID Met & Cys	CP	Met & Cys = -0.612 + 0.051 × CP	0.908	0.893	0.001	ConsCP	0.0500.001	0.024

	CPNFE	Met & Cys = 0.044 × CP – 0.011 × NFE	0.999	0.999	0.001	CPNFE	0.0010.030	0.022
SID Lys	CP	Lys = -3.338 + 0.13 × CP	0.705	0.656	0.009	ConsCp	0.0440.009	0.128
	CPASH	Lys = 0.066 × CP – 0.109 × ASH	0.996	0.994	0.001	CPASH	0.0010.017	0.109
SID Thre	CP	Thre = 0.029 × CP	0.997	0.997	0.001	CP	0.001	0.055
SID Val	CP	Val = -1.915 + 0.086 × CP	0.743	0.7	0.006	ConsCP	0.0530.006	0.078
	CPASH	Val = 0.048 × CP – 0.055 × ASH	0.997	0.996	0.001	CPASH	0.0010.050	0.077
SID Arg	CP	Arg= 0.052 × CP	0.997	0.996	0.001	CP	0.001	0.117
	DMASH	Arg= 0.033 × DM – 0.122 × ASH	0.995	0.994	0.001	ASHDM	0.0470.001	0.140
SID His	CP	His = 0.022 × CP	0.979	0.976	0.001	CP	0.001	0.122
	CPCF	His = 0.042 × CP – 0.065 × CF	0.990	0.986	0.001	CPCF	0.0020.047	0.086
SID Ileu	CP	Ileu = 0.034 × CP	0.992	0.991	0.001	CP	0.001	0.12
	CPDM	Ileu = 0.08 × CP – 0.019 × DM	0.995	0.994	0.001	CPDM	0.0080.049	0.090
SID Leu	CP	Leu = 0.057 × CP	0.997	0.997	0.000	CP	0.000	0.113
SID Phe	CP	Phe = -2.444 + 0.098 × CP	0.666	0.610	0.014	ConsCP	0.0480.014	0.104
	CPASH	Phe = 0.053 × CP – 0.09 × ASH	0.997	0.996	0.001	CPASH	0.0010.007	0.074

Abbreviation; CM, canola meal; DM, dry matter; CP, crude protein; Cons, intercept; 1. Analyzed using SPSS statistical software and stepwise procedure. 2. R2 is the coefficient of determination, Adjusted R2 adjusted for the number of predictors in the model, P-value<0.05 is statistically significant [1].

Table 9: Linear Regression equations for predicting the SIDAA content of CM from its chemical characteristics (DM basis)[1]

In the present study, linear regression equations were also developed for the determination of SIDAA content of CM from its TAA concentration (Table 10). The adjusted R^2 and SEP values for these equations raged from 82.2% (Met) to 99.9% (Phe and His) and from 0.009% (Met) to 0.039% (Lys), respectively. The SIDAA concentration of Met was predicted using the following equation: SID = -0.013 + 0.846 × Total (adjusted R^2 = 82.2% and SEP = 0.009 %). The SEP values for these equations were lower than equations base on the CP and other chemical componenets as indipendent variables. Use of TAA content in order to predict the SIDAA concentration of soybean meal was suggested as an accurate approach by [1].

Amino Acids	Prediction Equations	Statistical Parameters2				
		R2	Adjusted R2	P-Value Regression	P-Value Coefficents	SEP
Met	SID = -0.013 + 0.846 × Total	0.848	0.822	0.001	0.001	0.009
Cys	SID = 0.76 × Total	0.999	0.998	0.001	0.001	0.011
Met + Cys	SID = 0.791 × Total	0.998	0.997	0.001	0.001	0.014
Lys	SID = -0.24 + 0.9 × Total	0.972	0.967	0.001	0.001	0.039
Thre	SID = -0.145 + 0.775 × Total	0.967	0.961	0.001	0.001	0.014
Val	SID = -0.185 + 0.776 × Total	0.991	0.989	0.001	0.001	0.014
Arg	SID = -0.162 + 0.915 × Total	0.983	0.980	0.001	0.001	0.022
His	SID = 0.815 × Total	0.999	0.999	0.001	0.000	0.010
Ileu	SID = -0.102 + 0.819 × Total	0.993	0.991	0.001	0.000	0.013
Leu	SID = -0.106 + 0.833 × Total	0.984	0.981	0.001	0.001	0.020
Phe	SID = 0.803 × Total	0.999	0.999	0.000	0.000	0.011

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 [18].

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

Based on the current results, we concluded that the TAA content and amino acid digestibility of CMs from different origins are variable . Therefore, it is not suitable to consider one fixed value for amino acid content and its digestibility in diet formulation. Because it is difficult to measure the TAA and SIDAA contents of different origin and batches of CM, the prediction equations developed from the current study can be useful to predict the TAA and SIDAA contents of CM.

Acknowledgement

We are grateful to the staff of the Department of animal science of the Agriculture and Natural Resources College, University of Tehran, Iran, for their assistance in the chemical analysis of samples and their help in the management of this study.

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