# Evaluation of the standardized ileal digestibility of amino acids of rapeseed meals varying in protein solubility for Pekin ducks

K. X. Zhang,<sup>\*</sup> K. Y. Zhang,<sup>\*</sup> T. J. Applegate,<sup>†</sup> S. P. Bai,<sup>\*</sup> X. M. Ding,<sup>\*</sup> J. P. Wang,<sup>\*</sup> H. W. Peng,<sup>\*</sup> Y. Xuan,<sup>\*</sup> Z. W. Su,<sup>\*</sup> and Q. F. Zeng<sup>\*,1</sup>

\*Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan, China; Key Laboratory for Animal Disease-Resistance Nutrition, Ministry of Education, Ministry of Agriculture and Rural Affaires, Sichuan Province, China, 611130; and <sup>†</sup>Department of Poultry Science, University of Georgia, 110 Cedar St. Athens, GA, 30602, USA

ABSTRACT This study was conducted to determine whether protein solubility (PS) of rapeseed meals (RSM) can affect standardized ileal amino acid digestibility (SIDAA) in meat ducks. A total of 1,168, 14-days-old ducks were randomly allotted to 23 treatments (6 cages per diet, 8 ducks per cage) and 1 nitrogen-free diet treatment (8 cages, 8 ducks per cage) based on body weight. The 23 experimental diets consisted of a corn–soybean meal basal diet, and 22 diets containing 15% RSM: 85% basal diet. Titanium dioxide (0.5%) was included in all diets as an indigestible marker. On day 18, all ducks were euthanized by carbon dioxide asphyxiation and digesta samples from the ileum. The contents of PS, ether extract (**EE**), glucosinolate, isothiocyanate, and oxazolidine were significantly different (P < 0.05) in the 22 RSM, with the CV being 52.62, 49.23, 86.84, 90.19, and 81.98%, respectively. The content of lysine (Lys) and methionine in the 22 RSM samples ranged from 1.03 to 2.71% (CV

24.19%) and from 0.33 to 0.65% (CV 15.17%), respectively. The SIDAA, except for leucine (Leu) and tyrosine, of the 22 RSM samples varied significantly (P < 0.05). A positive correlation was observed (P < 0.05) between PS and standardized ileal digestibility (SID) of Lys, isoleucine, valine, phenylalanine, histidine, serine, cysteine, and tyrosine. The  $R^2$ value of multiple linear regression equations for predicting the SID of amino acids (AA) was best for Lys  $(R^2 = 0.958 \text{ using dry matter, crude protein, EE, crude})$ fiber, acid detergent fiber, and PS) and least significant for Leu ( $R^2 = 0.348$  using crude fiber and ash) with intermediate values for other AA ( $R^2 = 0.359-0.837$ , P < 0.05). These results suggest that PS varying from 15.06 to 98.08%, also varied considerably in the proximate nutrient content, AA composition, and antinutritional factor content, which was reflected in considerable differences in the duck's SID of AA in RSM. Therefore, PS value can partly reflect the quality of RSM.

Key words: rapeseed meal, ducks, standard ileal amino acid digestibility, protein solubility

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#### INTRODUCTION

Rapeseed meal (**RSM**) is a potential source of vegetable protein and is an important source of essential amino acids (**AA**), especially methionine (**Met**) (Woyengo et al., 2010) for poultry production that can replace soybean meal at a certain level. In addition, because of high and unpredictable prices of soybean meal, alternative feedstuffs such as RSM could be valuable alternative protein for feed formulation (Li, 1995; Cowieson, 2009). Some reports have been observed that there was large variation in chemical composition and ileal AA digestibility between RSM batches sourced from different processing plants (Xi et al., 2002; Messerschmidt et al., 2014; Adewole et al., 2016) as well as the content of toxic components such as glucosinolate (**GS**), isothiocyanate (**ITC**), and oxazolidine (**OZT**), which are the major limiting factors that cause change in the thyroid and liver tissues. inhibitfeed intake and growth, and subsequently decrease growth performance of poultry (Tripathi and Mishra, 2007; Wickramasuriya et al., 2015). In our previous study, Qin et al. (2017) found that the growth performance and standardized ileal digestibility (SID) of amino acids (SIDAA) of meat ducks

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<sup>&</sup>lt;sup>1</sup>Corresponding author: zqf@sicau.edu.cn

(except for Met, threenine **[Thr]**, and valine **[Val]**) linearly decreased with increasing dietary RSM concentrations from 6.66 to 24.64%. Kluth and Rodehutscord (2006) reported that digestibilities were lower in ducks than in broiler chickens and turkeys, and significant differences between soybean meal (SBM) and RSM were detected for some AA in ducks, which suggested that AA digestibilities determined with broilers should not be used in formulating feed for ducks. Studies with pigs (Fan et al., 1996) and broiler chickens (Gallardo et al., 2017) have indicated that there were differences in apparent ileal digestibility (AID) and SID (Adewole et al., 2017; Gallardo et al., 2017) of AA when pigs and broilers were fed RSM from different sources. However, to our best knowledge, there are few studies on the evaluation of SIDAA of RSM varying in protein solubility (**PS**) in meat ducks.

Protein solubility value in a 0.2% potassium hydroxide solution of soybean meal could reflect the intensity of the heat treatment on soybean meal quality, and meals with PS values below 70% probably were of impaired nutritive value for the chick, and values of less than 65% almost certainly indicate overprocessing (Araba and Dale, 1990). Thermal treatment of RSM may cause Maillard reactions, forming AA complexes which have been significantly associated with the SIDAA in poultry (Muir, 2010). Similarly, the production process of RSM involves toasting to remove the organic solvent remaining after solvent extraction of the oil and to inactive antinutritional factors present such as GS (Classen et al., 2004). The toasting process time usually ranges from 60 to 90 min at 100°C to 110°C, which can increase the variation in the lysine (Lys) content and ileal digestibility of most AA in RSM (Newkirk et al., 2003a). Subsequently, Salazar-Villanea et al. (2016) observed that the decrease in PS with increasing toasting time were indications of protein aggregation in RSM, and the rate of protein hydrolysis linearly decreased with increasing toasting time, which was largely correlated to the decrease in PS; however, in contrast, the authors did not find the decrease of the coefficient of in vitro CP digestibility. No study has been conducted to determine the effect of PS on the SIDAA of RSM in meat ducks. Rapeseed meal endusers desire more information on the nutritive value and heat damage of AA in RSM (Spragg and Mailer, 2007). In many countries, PS is an important index to determine the quality of RSM when feed companies buy RSM. Therefore, in the current study, 22 samples of RSM were collected according to their PS and subsequently evaluated their SIDAA in meat ducks. Prediction equations for SIDAA of RSM based on chemical compositions, such as PS and proximate composition, were then established.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Sichuan Agricultural University approved all procedures used in the study.

#### Rapeseed Meal Samples

The RSM samples were collected from all over China according to their PS (PS < 20%, 20% < PS < 40%, 40% < PS < 60%, 60% < PS < 80%, PS > 80%, with each group including 4 or 5 RSM samples; 22 RSM samples in total). It is of interest to note that there is a wide range for RSM varieties and processes in China. According to the appearance of RSM, the forms of RSM include mash, pellet, and flaky, and the color changed from yellow to dark. The RSM studied herein had a PS was from 15.06 to 98.08%.

## Standardized Ileal Amino Acid Digestibility Assay

A total of 1,168 1-day-old ducks were obtained from a commercial hatchery and received a standard starter diet from day 0 to 14 posthatch. On day 14 posthatch, the birds were randomly allotted by body weight to 23 dietary groups or nitrogen-free diet (Han et al.,2017) (**NFD**; corn starch 71.00%, glucose 21.24%, carboxy-methyl cellulose 3.00%, CaCO<sub>3</sub> 0.51%, and CaHPO<sub>4</sub> 2.33%) in a randomized complete block design. The 23 experimental diets included a basal diet (Table 1) and 22 RSM diets (15% RSM:85% basal diet). All diets contained 0.5% titanium dioxide (**TiO**<sub>2</sub>) as an indigestible

**Table 1.** Composition and nutrient levels of the basal diet(air dry basis) %.

Item	15  to  18  d of age
Ingredients, %	
Corn	65.25
Soybean meal, 43% SBM	27.68
Soybean oil	1.62
Calcium carbonate	1.10
Dicalcium phosphate	1.56
L -Lysine.HCl	0.05
DL- Methionine	0.17
Threonine	0.03
Tryptophan	0.02
Sodium chloride	0.35
Mineral premix <sup>1</sup>	0.50
Vitamin premix <sup>2</sup>	0.03
Choline chloride $(50\%)$	0.15
Rice bran	1.99
Titanium dioxide	0.50
Total	100.00
Formulated energy and nutrient	
composition	
${ m ME}~{ m (MJ/kg)}$	12.12
Crude protein %	17.50
Calcium %	0.85
Total phosphorus $\%$	0.60
Available phosphorus %	0.40
Lysine %	0.85
Methionine %	0.40
Threenine $\%$	0.68
Tryptophan $\%$	0.22

<sup>1</sup>Mineral premix provides the following per kg of final diet: Fe (FeS-O4·H2O) 80 mg; Cu (CuSO4·5H2O) 8 mg; Mn (MnSO4·H2O) 70 mg; Zn (ZnSO4·H2O) 90 mg; I (KI) 0.4 mg; Se (Na2SeO3) 0.3 mg.

<sup>&</sup>lt;sup>2</sup>Vitamin premix provides the following per kg of final diet: vitamin A 12,000 IU; vitamin D3 3,000 IU; vitamin E 7.5 mg; vitamin K2 1.5 mg; vitamin B1 0.6 mg; vitamin B2 4.8 mg; vitamin B6 1.8 mg; vitamin B12 0.009 mg; niacin 10.5 mg; DL-calcium pantothenate 7.5 mg; folic acid 0.15 mg.

marker. There were 6 cages of 6 birds/cage for all dietary treatment except for NFD, which had 8 replicates of 8 birds/cage to ensure a sufficient amount of digesta sample for analyses.

All ducks were reared in cages  $(1.2 \times 1.2 \times 0.9 \text{ m})$ and ad libitum provided fresh water and feed throughout the experiment. Room temperature was maintained at 28°C to 32°C during the first week, with a weekly reduction of 2°C until reaching 22°C. Birds had ad libitum access to feed and water throughout the study. On day 18, ducks were euthanized by carbon dioxide asphysiation and the digesta from the terminal two-thirds of ileum were collected by gently squeezing the contents of the ileum into sample bags. The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction. Digesta from ducks within a cage were pooled and frozen immediately after collection and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at -4°C until needed for chemical analysis.

### **Chemical Analysis**

The RSM samples were finely ground and were analyzed for DM, CP, ether extract (**EE**), crude fiber (**CF**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), ash, PS, GS, ITC, OZT, and AA. Test diets and digesta samples were finely ground and were all analyzed for AA and TiO<sub>2</sub>. For AA (Lys; Met; **Arg**: arginine; isoleucine; **Leu**: leucine; Thr; Val; **Phe**: phenylalanine; **His**: histidine; **Asp**: aspartic acid; **Ser**: serine; **Glu**: glutamic acid; **Gly**, glycine; **Ala**, alanine; **Cys**, cysteine; **Tyr**, tyrosine; **Pro**, proline) analyses, first, diets and ileal digesta were hydrolyzed with 6 N HCl for 24 h at 110°C (method 982.30 E; AOAC, 2005), filtered, and subsequently content of AA were analyzed by an automatic amino acids analyzer (HITACHI L-8900). Tryptophan was not determined.

The TiO<sub>2</sub> of test diets and ileal digesta samples were measured by dissolving in hot concentrated sulfuric acid to form titanium sulfate acyl which can form stable orange  $[\text{TiO}(\text{H}_2\text{O}_2)]^{2+}$  with  $\text{H}_2\text{O}_2$ . The solution was colorimetric to determine absorbance values at 410 nm wavelength, then the content of TiO<sub>2</sub> is calculated according to the standard curve of TiO<sub>2</sub> (Brandt and Allam, 1987).

The GS, ITC, and OZT of RSM samples were analyzed by high-performance liquid chromatography, thiourea colorimetric method, and spectrometric method, respectively, by the Laboratory of the Oil Crops Research Institute, Chinese Academy of Agricultural Science (Wuhan city, China) according to the study of Salazar-Villanea et al. (2016). The DM content was determined according to ISO 6496 (1999). Nitrogen content was analyzed by combustion according to AOAC 968.06 (1969). A conversion factor of 6.25 was used for the calculation of CP content from nitrogen. Crude fat content was determined according to ISO 734-2 (2008). Analyses for CF, NDF, and ADF content of experimental diets was analyzed using an ANKOM A2000i Fiber Analyzer (Eklund et al., 2015).

As for PS, protein dispensability index in water was measured using a modification of the method of AOCS (1997a). Soluble protein in 0.2% (w/v) KOH, equivalent to nitrogen solubility index, was measured according to ISO 14244 (2014).

## Standardized Ileal Amino Acid Digestibility Calculations

The AID of amino acids was calculated using the formula reported by Nyachoti et al. (1997). The basal ileal endogenous loss of AA (IAA, g/kg DM intake [**DMI**]) was calculated according to Qin et al. (2017). The SIDAA for diets or for RSM were calculated according to the following equations:

SIDAA% of diets =  $AID + (IAA / Ad) \times 100$ 

SIDAA% of RSM = B - (B - A)/F

where Ad is the concentration of AA in the diet (g/kg DM); A is the SID of a specific AA in assay diet; B is the SID of a specific AA in basal diet; and F is the proportion of a specific AA from the RSM to it from the assay diet.

#### Statistical Analysis

Data were analyzed using the Mixed procedure of SAS (SAS Institute Inc., Cary, NC). Means were compared using the Tukey's studentized range test when there was a significant difference at  $P \leq 0.05$  between means. Cage was the experimental unit. The regression procedure of SAS was used to develop prediction equations for determining SIDAA from the chemical composition (Adewole et al., 2017). Statistical significance was considered at  $P \leq 0.05$ .

## RESULTS

## Chemical Composition of Rapeseed Meal Samples

The analyzed composition of RSM samples is presented in Tables 2 and 3. The chemical compositions of the 22 RSM samples significantly varied (P < 0.05). The content of DM, CP, EE, Ash, CF, NDF, ADF, GS, ITC, OZT, and PS are from 88.34 to 94.23%, 32.04 to 38.75%, 1.92 to 14.70%, 6.94 to 13.27%, 9.44 to 17.32%, 24.22 to 53.74%, 7.24 to 18.57%, 3.21 to 82.04 µmol/g, 0.18 to 6.80 mg/g, 0.15 to 2.82 mg/g, and 15.06 to 98.08%, respectively. A higher CV was noted for EE, GS, ITC, OZT, and PS which were 49.23, 86.84, 90.19, 81.98, and 52.62%, respectively.

For essential AA values, the concentration of Lys, Met, Arg, Ile, Leu, Thr, Val, Phe, and His were from 1.03 to 2.71%, 0.33 to 0.65%, 1.81 to 2.89%, 1.24 to 1.97%, 2.34 to 3.19%, 1.46 to 2.90%, 1.58 to 2.17%, 1.27 to 1.75%, and 0.85 to 1.10%, respectively. For

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Table 2. The chemical properties of the 22 rapeseed meal samples ranging in protein solubility (DM basis).

Sample number	DC 07	DM oz	CP	EE vz	CF oz	NDF 07		Ach 07	CS umol/a	ITC mg/g	O7T mg/g
Sample number	10/01	/0	/0	/0	/0	NDF /0	ADF /0	ASII /0	G5 µmoi/g	IIC mg/g	OZ1 mg/g
1	15.06	93.99	38.00	5.52	16.34	50.06	14.05	7.78	20.18	-	-
2	15.93	90.70	37.11	9.62	17.32	53.74	16.04	8.07	9.01	0.62	-
3	17.69	89.80	37.82	6.58	13.49	49.92	18.57	7.21	22.48	1.11	-
4	19.83	91.83	37.05	10.72	16.58	49.80	16.19	7.05	19.11	2.21	1.26
5	23.67	93.73	37.00	10.24	13.14	40.22	15.19	7.45	50.78	-	0.52
6	25.79	94.23	36.36	13.84	12.83	38.81	14.91	6.94	8.63	1.33	-
7	27.72	93.91	35.34	14.70	12.19	43.14	17.53	6.96	8.34	1.46	0.52
8	34.95	93.84	37.17	8.78	13.44	43.66	18.23	7.24	10.40	0.42	-
9	45.45	89.91	36.94	2.63	14.10	40.24	16.42	7.30	3.21	-	-
10	49.50	91.25	32.04	10.30	10.43	37.20	12.18	13.27	39.08	4.83	0.40
11	53.87	88.34	36.70	1.92	12.38	29.25	10.94	7.65	11.60	0.21	1.86
12	55.77	89.54	38.75	2.89	12.04	30.07	13.62	7.27	7.18	0.18	-
13	59.26	89.54	36.50	2.89	12.69	33.88	14.05	7.72	5.62	-	-
14	60.42	89.35	36.12	3.86	13.30	36.38	15.81	7.15	9.52	0.77	0.32
15	65.81	89.84	36.80	3.48	13.86	30.76	12.19	7.23	9.53	0.22	0.15
16	68.87	89.32	36.26	3.47	13.95	30.34	11.57	7.18	8.92	-	-
17	70.73	93.06	33.42	9.19	13.96	33.12	11.10	7.83	19.61	-	-
18	85.86	93.73	36.20	9.02	10.57	26.47	7.24	7.04	29.84	6.80	1.68
19	89.72	93.29	37.14	10.69	9.44	26.04	9.68	7.28	39.88	3.61	2.82
20	92.60	90.04	34.19	8.52	11.60	24.22	8.32	7.49	50.53	3.05	2.35
21	96.24	92.00	35.97	8.69	10.19	26.21	9.46	7.07	34.74	2.12	-
22	98.08	92.87	38.52	8.86	9.68	28.29	11.15	8.23	82.04	5.08	0.49
Mean	53.31	91.55	36.43	7.56	12.89	36.45	13.40	7.66	22.74	2.18	1.12
SD	28.05	1.96	1.57	3.72	2.13	8.96	3.24	1.30	19.75	1.97	0.92
$\mathrm{CV}\%$	52.62	2.14	4.31	49.23	16.50	24.57	24.15	17.03	86.84	90.19	81.98
Max	98.08	94.23	38.75	14.70	17.32	53.74	18.57	13.27	82.04	6.80	2.82
Min	15.06	88.34	32.04	1.92	9.44	24.22	7.24	6.94	3.21	0.18	0.15

Abbreviations: ADF, acid detergent fiber; CF, crude fiber; EE, ether extract; GS, glucosinolate; ITC, isothiocyanate; Max, maximum; Min, minimum; NDF, neutral detergent fiber; OZT, oxazolidine thione; PS, protein solubility.

nonessential AA values, the content of Asp, Ser, Glu, Gly, Ala, Cys, Tyr, and Pro were from 2.29 to 3.19%, 1.42 to 1.91%, 5.97 to 8.08%, 1.70 to 2.31%, 1.48 to 1.95%, 0.24 to 0.60%, 0.95 to 1.33%, and 1.99 to 2.59%, respectively. The highest CV for Lys and Cys were 24.19 and 30.83\%, respectively.

## Standardized Ileal Digestibility of Amino Acid

Table 4 shows the basal endogenous AA losses (**BEL**) of ducks fed NFD. The estimated BEL of indispensable AA for ducks ranged from 0.184 (Met) to 2.153 (Leu)

Table 3. The amino acid composition of 22 rapeseed meal samples ranging in protein solubility (DM basis) %.

Sample number	Lys	Met	Arg	Ile	Leu	Thr	Val	Phe	His	Asp	Ser	Glu	Gly	Ala	Cys	Tyr	Pro
1	1.28	0.49	1.92	1.50	2.73	1.68	1.85	1.56	0.96	2.57	1.64	7.17	2.01	1.66	0.28	1.11	2.37
2	1.03	0.52	1.81	1.56	2.87	1.66	1.92	1.66	0.88	2.65	1.60	7.30	2.03	1.73	0.25	1.16	2.35
3	1.43	0.49	2.23	1.52	2.91	1.71	1.95	1.62	1.01	2.68	1.72	7.76	2.07	1.77	0.26	1.14	2.46
4	1.36	0.49	1.89	1.37	2.63	1.56	1.75	1.47	0.92	2.38	1.54	7.00	1.87	1.59	0.29	1.05	2.28
5	1.81	0.48	2.10	1.34	2.59	1.62	1.73	1.41	0.93	2.45	1.57	6.61	1.85	1.61	0.27	1.07	2.16
6	1.66	0.48	2.23	1.52	2.73	1.69	1.79	1.50	0.95	2.65	1.65	6.85	2.00	1.66	0.28	1.16	2.21
7	1.56	0.48	2.15	1.47	2.66	1.61	1.81	1.47	0.93	2.54	1.54	6.62	1.91	1.61	0.24	1.13	2.16
8	1.99	0.57	2.43	1.75	3.04	2.90	2.11	1.68	1.03	2.90	1.75	7.51	2.18	1.83	0.39	1.25	2.47
9	2.18	0.36	2.30	1.70	2.93	1.82	2.05	1.62	0.99	2.87	1.72	7.21	2.16	1.80	0.45	1.24	2.39
10	1.83	0.33	1.95	1.24	2.34	1.46	1.58	1.27	0.85	2.29	1.42	5.97	1.70	1.48	0.25	0.95	1.99
11	2.71	0.65	2.73	1.97	3.19	1.96	2.16	1.70	1.10	3.13	1.91	7.65	2.30	1.95	0.60	1.33	2.56
12	2.65	0.59	2.69	1.82	3.14	1.93	2.17	1.75	1.10	3.19	1.87	8.08	2.31	1.93	0.58	1.26	2.59
13	2.40	0.44	2.51	1.56	2.89	1.82	1.96	1.55	0.99	2.94	1.76	7.15	2.10	1.78	0.53	1.21	2.38
14	2.43	0.43	2.40	1.54	2.80	1.75	1.91	1.52	0.98	2.76	1.69	6.80	2.05	1.72	0.50	1.17	2.27
15	2.66	0.49	2.64	1.66	3.10	1.95	2.09	1.69	1.09	3.11	1.88	7.69	2.30	1.90	0.56	1.27	2.54
16	2.58	0.38	2.48	1.56	2.88	1.83	1.95	1.55	1.03	2.90	1.77	7.18	2.13	1.76	0.53	1.19	2.39
17	2.15	0.46	2.23	1.34	2.54	1.58	1.73	1.38	0.91	2.50	1.55	6.32	1.90	1.61	0.40	1.07	2.10
18	2.36	0.50	2.33	1.44	2.67	1.64	1.78	1.44	1.00	2.55	1.63	7.18	2.00	1.65	0.41	1.07	2.31
19	2.44	0.52	2.60	1.57	2.92	1.74	1.92	1.58	1.02	3.04	1.71	7.15	2.09	1.74	0.43	1.20	2.30
20	2.35	0.51	2.21	1.53	2.57	1.58	1.81	1.42	0.94	2.52	1.52	6.65	1.90	1.60	0.34	1.07	2.13
21	2.41	0.49	2.60	1.48	2.71	1.65	1.85	1.47	0.98	2.84	1.66	7.00	2.04	1.68	0.46	1.15	2.19
22	2.33	0.56	2.89	1.61	2.81	1.61	1.94	1.69	1.01	3.08	1.68	7.55	2.12	1.70	0.32	1.18	2.22
Mean	2.07	0.49	2.33	1.55	2.80	1.76	1.90	1.55	0.98	2.75	1.67	7.11	2.05	1.72	0.39	1.16	2.31
SD	0.50	0.07	0.29	0.17	0.21	0.29	0.15	0.12	0.07	0.26	0.12	0.50	0.15	0.12	0.12	0.09	0.16
CV%	24.19	15.17	12.62	10.67	7.54	16.30	7.98	7.95	6.72	9.57	7.45	7.01	7.56	6.90	30.83	7.65	6.82
Max	2.71	0.65	2.89	1.97	3.19	2.90	2.17	1.75	1.10	3.19	1.91	8.08	2.31	1.95	0.60	1.33	2.59
Min	1.03	0.33	1.81	1.24	2.34	1.46	1.58	1.27	0.85	2.29	1.42	5.97	1.70	1.48	0.24	0.95	1.99

Abbreviations: Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Max, maximum; Met, methionine; Min, minimum; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

Amino acid	$\frac{\rm Endogenous}{\rm loss~(g/kg~of~DMI)}$	Amino acid	Endogenous loss (g/kg of DMI)
Lys	0.968	Asp	2.706
Met	0.184	Ser	1.639
Arg	1.500	Glu	4.467
Ile	1.277	Gly	1.865
Leu	2.153	Ala	1.992
Thr	1.805	Cys	0.214
Val	1.603	Tyr	1.339
Phe	1.426	Pro	1.728
His	0.677	Total amino acid	27.543

Abbreviations: Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; DMI, DM intake; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

<sup>1</sup>Data are means of 8 replicate cages with 8 birds per cage.

g/kg of DMI, corresponding lowest and highest value of dispensable AA were 0.214 (Cys) and 4.467 (Glu) g/kg of DMI. The total of BEL of AA is 27.543 g/kg of DMI.

Table 5 and 6 show the SIDAA of the 22 RSM samples in meat ducks. The SID of indispensable and dispensable AA in 22 RSM samples were significantly different (P < 0.05), except for Leu and Tyr. For SID of essential AA, the SID of Lys, Met, Arg, Ile, Thr, Val, Phe, and His were from 54.62 to 74.43%, 81.44 to 95.93%, 76.42 to 84.93%, 72.18 to 79.72%, 67.67 to 79.65%, 72.30 to 79.91%, 78.75 to 85.95%, and 77.59 to 85.79%, respectively. The highest SID of Met and lowest SID of Lys were 90.74% (from 81.44–95.93%) and 66.21% (from 54.62-74.43%), respectively.

For SID of nonessential AA, the SID of Asp, Ser, Glu, Gly, Ala, Cys, Pro were from 66.87 to 79.86%, 72.91 to 81.58%, 67.78 to 81.48%, 73.59 to 85.85%, 73.22 to 84.80%, and 75.53 to 85.32%, respectively. The highest SID of Glu and lowest SID of Asp were 82.91 (from 77.82-88.72%) and 73.97% (from 66.87-79.86%), respectively.

## Correlation Coefficient and the Regression Equations

Correlation analysis (Table 7) found that there was a negative correlation (P < 0.05) between PS and CF and NDF and ADF, and a positive correlation (P < 0.05) between PS and GS or ITC. A positive correlation was observed (P < 0.05) between PS and SID of Lys, Ile, Val, Phe, His, Ser, Cys, and Tyr.

Linear regression equations for predicting SID of Lys, Ile, Val, Phe, His, Ser, Cys, and Tyr from a simple measure of PS are presented in Table 8. Regression equations predicting SID of Lys and Cys based on PS had a higher  $R^2$  value, 0.7129 and 0.8160 (P < 0.05), respectively.

Multiple linear regression equations for predicting the SID of Lys, Arg, Ile, Leu, Thr, Val, Phe, His, Asp, Ser, Glu, Gly, Ala, Cys, Tyr, and Pro based on the chemical components of RSM are presented in Table 9. The  $R^2$  value of multiple linear regression equations for predicting the SID was the best for Lys ( $R^2 = 0.96$  using DM,

CP, EE, CF, ADF, and PS), then followed by Cys ( $R^2 = 0.837$  using NDF and PS), and least significant for Leu ( $R^2 = 0.348$  using CF and ash) with intermediate values for SID of Arg, Ile, Thr, Val, Phe, His, Asp, Ser, Glu, Gly, Ala, Tyr, and Pro ( $R^2 = 0.359$ –0.767, P < 0.05).

#### DISCUSSION

The PS in 22 RSM samples was from 15.06 to 98.08%, and PS < 20%, 20% < PS < 40%, 40% < PS < 60%, 60% < PS < 80%, and PS > 80% had 4, 4, 5, 4, and 5 RSM samples, respectively. The PS of RSM depends on extraction and processing techniques (Andersonhafermann et al., 1993), as well as the content of dietary fiber fractions, EE, AA, and GS (Newkirk et al., 2003b). The GS concentration can also be regarded as an index of RSM heating degree because GS would be partially destroyed by heat during process (Jensen et al., 1995; SchoNe et al., 1997). Overheating can lead to the Maillard reaction that during the heating drying process can bind CP (Van Soest et al., 1994) and the free amino group of AA to the reducing sugars, which may increase neutral detergent insoluble substance (Fastinger et al., 2006). For example, Lys, Arg, His, and Thr are susceptible to heat treating, resulting in a decrease in their contents. Among them, Lys is the primary amino acid involved in the early stage of Maillard reactions because of the presence of the highly reactive  $\varepsilon$ -amino group (Mauron, 1981), and its contents will inevitably affect the quality of RSM (Derycke et al., 1999). In the present study, we found there is a high variation in the EE (1.92-14.70%), NDF (24.22-53.74%), GS  $(3.21-82.04 \ \mu mol/g)$ , Lys (1.03-2.71%), and PS (15.06-98.08%) contents of the 22 RSM samples, which is wider variation than the report by Adewole et al. (2016), who found that the variations in the contents of NDF (26.3–33.5% DM), Lys (2.00–2.29% DM), GS  $(1.90-9.70 \ \mu mol/g DM)$  for canola meals produced in Canada. These results indicate that varieties and processes of RSM in China are more complex than them in Canada.

In our study, we also found that PS and CF, including NDF and ADF, showed a negative correlation, whereas PS and GS or ITC showed a positive correlation. This result agreed with Salazar-Villanea et al. (2016), who found that the contents of NDF and acid detergent insoluble nitrogen (**ADIN**) increased, and the content of Lvs and Arg decreased with increasing toasting time of canola meals. First-order reactions calculated from the measured parameters showed that GS was degraded faster than Lys and Arg and that physical changes to proteins seem to occur before chemical changes during toasting. The increasing NDF, ADF, and ADIN contents with increasing toasting time was previously described after hydrothermal treatments of canola and RSM (Pastuszewska et al., 2003; Eklund et al., 2015). It is possible that the increase in the content of NDF, ADF, and ADIN resulted from the inability of the solvents used to solubilize the aggregated and chemically

Table 5. Standardized ileal digestibility of essential amino acid of 22 rapeseed meal samples varying in protein solubility in Pekin ducks $^{1}\%$ .

Sample number	$\mathbf{PS}$	Lys	Met	Arg	Ile	Leu	Thr	Val	Phe	His	TEAA
1	15.06	61.15	89.65	78.15	72.25	77.62	68.87	72.30	80.63	80.36	78.32
2	15.93	54.68	88.23	76.42	72.18	75.92	67.83	72.51	78.75	77.59	76.88
3	17.69	60.91	94.53	84.93	77.72	84.49	79.65	78.81	85.55	83.65	82.57
4	19.83	54.62	93.47	81.49	74.15	80.58	74.36	74.78	81.75	79.27	78.44
5	23.67	61.92	92.59	82.95	75.76	82.36	77.94	77.10	83.69	81.80	80.81
6	25.79	61.98	93.88	78.78	74.11	78.28	69.61	73.93	81.79	80.37	79.37
7	27.72	58.06	95.71	83.49	76.73	82.64	77.64	77.06	83.73	82.35	80.93
8	34.95	66.45	83.18	79.46	75.09	78.59	72.25	74.83	81.31	81.53	80.13
9	45.45	60.38	81.44	81.20	73.37	78.99	73.95	73.82	79.24	79.57	77.67
10	49.50	68.40	92.86	80.16	74.35	78.69	67.67	74.45	82.84	83.42	81.70
11	53.87	69.15	91.04	79.51	74.43	78.37	70.21	74.39	81.15	82.37	80.43
12	55.77	70.32	93.35	81.73	76.84	80.68	73.40	76.99	83.55	83.57	82.66
13	59.26	74.43	87.50	83.71	79.72	83.30	75.57	79.74	85.92	85.65	85.01
14	60.42	71.22	95.67	82.26	76.30	81.10	73.11	76.66	83.67	83.43	82.73
15	65.81	70.77	93.74	80.61	75.41	80.16	71.82	75.62	82.69	83.72	81.92
16	68.87	69.64	95.78	82.45	75.96	80.55	73.44	76.22	83.25	83.12	82.51
17	70.73	70.02	87.55	81.14	75.61	79.97	72.29	76.17	82.84	83.34	81.91
18	85.86	73.70	91.82	83.93	79.45	82.70	75.60	79.91	85.15	85.41	84.71
19	89.72	72.20	87.25	82.60	78.25	82.35	75.29	78.91	85.60	85.79	84.14
20	92.60	69.77	86.51	80.53	76.43	80.17	72.38	75.95	84.01	83.86	82.00
21	96.24	64.88	84.68	82.37	74.71	80.45	76.30	76.10	81.70	81.61	79.79
22	98.08	71.98	95.93	83.33	78.67	82.48	74.53	78.19	85.95	84.19	84.21
SEM	-	2.25	2.15	1.41	1.64	1.49	1.74	1.55	1.48	1.14	1.52
<i>P</i> -value	-	< 0.0001	< 0.0001	0.005	0.055	0.012	< 0.0001	0.016	0.019	< 0.0001	0.004
Mean	53.31	66.21	90.74	81.42	75.80	80.47	73.35	76.11	82.94	82.54	81.18
SD	28.05	6.05	4.35	2.09	2.10	2.10	3.21	2.15	2.03	2.13	2.18
$\mathrm{CV}\%$	62.62	9.13	4.79	2.57	2.77	2.61	4.38	2.82	2.45	2.58	2.69
Max	98.08	74.43	95.93	84.93	79.72	84.49	79.65	79.91	85.95	85.79	85.01
Min	15.06	54.62	81.44	76.42	72.18	75.92	67.67	72.30	78.75	77.59	76.88

Abbreviations: Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Max, maximum; Met, methionine; Min, minimum; Phe, phenylalanine; PS, protein solubility; TEAA, total essential amino acid; Thr, threonine; Val, valine. <sup>1</sup>Means represent 6 cages of ducks, 6 ducks per cage, fed diets containing 15% RSM:85% corn-SBM basal diet from 14 to 18 d of age. A

nitrogen-free diet was used for standardization of apparent digestibility.

Sample number	$_{\rm PS}$	Asp	Ser	Glu	Gly	Ala	Cys	Tyr	Pro	TNEAA
1	15.06	69.02	75.33	79.23	70.00	73.59	74.38	76.14	75.59	78.89
2	15.93	66.87	72.91	77.82	67.78	73.59	73.22	76.20	75.53	77.53
3	17.69	79.86	81.09	88.72	81.48	85.85	81.89	81.88	85.32	84.53
4	19.83	73.77	74.77	85.41	77.11	80.61	76.14	77.52	80.83	80.05
5	23.67	77.55	77.87	87.13	80.28	83.27	76.56	79.63	83.78	82.50
6	25.79	70.61	74.75	79.94	71.25	74.71	74.40	79.39	76.99	79.98
7	27.72	78.24	78.08	87.12	79.98	83.44	77.67	80.88	83.64	82.65
8	34.95	70.35	75.88	80.37	71.66	75.77	78.01	77.26	78.68	80.39
9	45.45	74.56	74.80	83.80	77.31	79.44	76.77	75.91	81.67	79.37
10	49.50	71.45	73.61	78.54	71.99	75.85	81.14	77.20	79.91	82.45
11	53.87	69.57	76.54	80.22	71.71	75.23	81.17	77.37	78.53	80.40
12	55.77	73.87	79.11	82.46	74.16	78.62	80.13	81.50	81.08	83.15
13	59.26	76.37	81.58	83.91	76.46	80.84	82.32	83.29	82.23	84.94
14	60.42	74.12	77.84	82.18	74.64	77.99	80.05	80.63	79.53	82.58
15	65.81	72.99	78.51	81.56	73.78	77.06	82.36	79.45	79.30	82.18
16	68.87	74.12	78.36	82.11	74.31	77.89	81.99	80.85	80.10	82.76
17	70.73	72.68	77.38	81.82	73.64	77.65	81.17	79.70	80.44	82.47
18	85.86	77.23	80.89	84.83	76.45	81.86	84.80	82.90	83.06	85.51
19	89.72	76.54	80.79	83.79	76.61	80.71	83.37	82.40	82.56	84.97
20	92.60	73.07	78.14	82.21	74.27	78.00	81.10	79.11	80.54	82.79
21	96.24	76.71	77.45	86.63	79.52	82.92	82.97	77.94	84.45	82.11
22	98.08	77.81	79.64	84.19	75.85	79.49	83.50	83.79	81.87	84.87
SEM	-	1.80	1.71	1.25	1.40	1.79	1.60	1.91	1.10	1.37
<i>P</i> -value	-	< 0.0001	0.007	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.059	< 0.0001	0.001
Mean	53.31	73.97	77.51	82.91	75.01	78.84	79.78	79.59	80.71	82.02
SD	28.05	3.38	2.47	2.95	3.52	3.36	3.34	2.43	2.65	2.07
$\mathrm{CV\%}$	62.62	4.57	3.19	3.56	4.69	4.27	4.18	3.05	3.28	2.52
Max	98.08	79.86	81.58	88.72	81.48	85.85	84.80	83.79	85.32	85.51
Min	15.06	66.87	72.91	77.82	67.78	73.59	73.22	75.91	75.53	77.53

Table 6. Standardized ileal digestibility of nonessential amino acid in 22 rapeseed meal samples varying in protein solubility in Pekin ducks  $^{1}\%$ .

Abbreviations: Ala, alanine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; Max, maximum; Min, minimum; Pro, proline; PS, protein solubility; Ser, serine; TNEAA, total nonessential amino acid; Tyr, tyrosine. <sup>1</sup>Means represent 6 cages of ducks, 6 ducks per cage, fed diets containing 15% RSM:85% corn-SBM basal diet from 15 to 18 d of age.

A nitrogen-free diet was used for standardization of apparent digestibility.

Table 7. Correlation coefficient among protein solubility and chemical composition and standardized ileal digestibility of amino acid of rapeseed meal in 18-day-old Pekin ducks (n = 22).

Component	$_{\rm PS}$	SIDAA	$\mathbf{PS}$	SIDAA	$\mathbf{PS}$
PS DM CP EE CF NDF ADF GS ITC OZT	$\begin{array}{c} 1.000\\ -0.126\\ -0.201\\ -0.723^*\\ -0.920^*\\ -0.830^*\\ 0.467^*\\ 0.541^*\\ 0.430 \end{array}$	PS SID Lys SID Met SID Arg SID Ile SID Leu SID Thr SID Val SID Phe SID His	$\begin{array}{c} 1.000\\ 0.776^{*}\\ -0.140\\ 0.356\\ 0.532^{*}\\ 0.292\\ 0.144\\ 0.489^{*}\\ 0.464^{*}\\ 0.659^{*} \end{array}$	PS SID Ser SID Glu SID Gly SID Ala SID Cys SID Tyr SID Pro SID Asp	$\begin{array}{c} 1.000\\ 0.506*\\ 0.103\\ 0.143\\ 0.151\\ 0.817*\\ 0.461*\\ 0.326\\ 0.319\end{array}$

Abbreviations: ADF, acid detergent fiber, Ala, Alanine; Arg, arginine; Asp, aspartic acid; CF, crude fiber; Cys, cysteine; EE, ether extract; Glu, glutamic acid; Gly, glycine; GS, thioglycoside; His, histidine; Ile, isoleucine; ITC, isothiocyanate; Leu, leucine; Lys, lysine; Met, methionine; NDF, neutral detergent fiber; OZT, oxazolidine thione; Phe, phenylalanine; Pro, proline; PS, protein solubility; Ser, serine; SID, standardized ileal digestibility; Thr, threonine; Tyr, tyrosine; Val, valine.

\*P < 0.05.

modified proteins (e.g., melanoidins) (Alimeida et al., 2014).

The total of BEL of AA in ducks fed NFD in the present study is 27.543 g/kg of DMI. This result was consistent with a report of Kong and Adeola (2013) which noted a 27.395 g/kg BEL of DMI in white Pekin ducks. Both were higher than the BEL of AA in broilers fed a NFD. The reason may be because of the mass and turnover of intestinal mucosa, which is known to be a major component of ileal endogenous AA flows (Adedokun et al., 2007). Meanwhile, BEL of Leu and Glu were the largest proportion at 2.153 g/kg and 4.467 g/kg of DMI among indispensable and dispensable AA, respectively, in the current study. These results are similar to the results of previously reported studies (Adedokun et al., 2008; Golian et al., 2008).

To the best of our knowledge, there has not been any study on the evaluation the SIDAA of RSM with varying PS in meat ducks. In the present study, we found that there were significant differences in SIDAA of RSM varying in PS, except Leu and Tyr. These results agreed with studies with pigs and broiler chicks (Fan et al., 1996; Xi et al., 2002; Gallardo et al.,2017), which have indicated that there were differences in AID and SID

Table 8. Prediction equations of standardized ileal digestibility of amino acid based on protein solubility of rapeseed meal in 18-dayold Pekin ducks (n = 22).

Prediction equation	$\mathbb{R}^2$	P-value
SID Lys = 0.193 PS + 56.359	0.7129	< 0.0001
SID ILe = 0.038 PS + 73.574	0.3186	0.0077
SID Val = 0.047 PS + 73.380	0.3810	0.0029
SID Phe = $0.043$ PS + $80.454$	0.3525	0.0045
SID His = $0.051 \text{ PS} + 80.113$	0.5158	0.0004
SID Ser = 0.057 PS + 74.194	0.4327	0.0012
SID Cys = 0.111 PS + 73.578	0.8160	< 0.0001
SID Tyr = $0.040 \text{ PS} + 77.460$	0.2128	0.0307

Abbreviations: Cys, cysteine; Gly, glycine; His, histidine; Ile, isoleucine; Lys, lysine; Phe, phenylalanine; PS, protein solubility; Ser, serine; SID, standardized ileal digestibility; Tyr, tyrosine; Val, valine. of AA when pigs and birds were fed RSM from different sources. In general, processing methods and cultivars of rapeseed were responsible for the variability of the SID of AA values (Xi et al., 2002). Similarly, in our study, we also found there was a positive relationship between PS and SID of Lys, Ile, Val, Phe, His, Ser, Cys, and Tyr in RSM, indicating that processing factors have an important effect on the SIDAA of RSM in ducks. A decrease in PS in heat-treated materials is an indication of the aggregation of proteins after denaturation (Amin et al., 2014). In addition, it was reported that greater AID of AA for nontoasted than toasted canola meal fed to broiler chickens and attributed it to Maillard reaction because the color of the meal was changed from light yellow to brown by this process (Newkirk et al., 2003a, 2003b). These Maillard products have been associated with lower SID of AA, with Lys being the most sensitive AA (González-Vega et al., 2011). Woyengo et al. (2010) also found that lower AID and SID of AA in solvent extracted canola meal than in expeller extracted meal.

Moreover, we found that the SID of Lys had a wide range (54.62-74.43%, CV 9.13%, mean 66.21%), and SID of Met had the highest value (mean 90.74%) when compared with the SID of other AA in RSM in the present study. These results agree with a report of Newkirk et al. (2003a), which reported that both Lys content and its digestibility were reduced by the desolventization or toasting process. Similarly, Adewole et al. (2016) found that Lys damage due to heat treatment in the desolventizer/toaster was well supported by a positive relationship between Lys and heat sensitive GS contents, as well as a negative relationship between Lys and NDF and total dietary fiber contents. These results indicated that Lys damage by heat treatment maybe a major cause for a lower SID of Lys in RSM. Meanwhile, Adewole et al. (2017) also found that the SID of Met in canola meal was higher (85.4-91.4%) than the SID of other AA in broiler chickens. Similarly, Kluth and Rodehutscord (2006) observed that the SID of Glu and Met in RSM was higher than the SID of other AA in meat ducks, but in soybean meal, SID of Met was lower than the SID of Phe, Glu, and Ser. These results suggest that the SID of each AA in different oilseed meal is different for meat ducks.

At the same time, we found that the R<sup>2</sup> value of linear regression equations for predicting SID of Lys, Ile, Val, Phe, His, Ser, Cys, and Tyr based on PS ranged from 0.21 to 0.82 and also found that the R<sup>2</sup> value of multiple regression for predicting the SID of Lys, Arg, Ile, Leu, Thr, Val, Phe, His, Asp, Ser, Glu, Gly, Ala, Cys, Tyr, and Pro based on the chemical components of RSM ranged from 0.35 to 0.96. The regression equations indicate that PS, NDF, ADF, CF, CP, EE, DM, and Ash could be a suitable predictor of SIDAA content in RSM. These results were in line with previous findings. Anderson-hafermann et al. (1993) found that PS and the SIDAA in cecectomized broilers were reduced with increasing autoclaving time of a commercial RSM; Almeida (2013) reported there was a negative

Prediction equations	$\mathbb{R}^2$	<i>P</i> -value
SID Lys = 0.264 + 1.231DM - 0.914CP - 1.342EE - 1.081CF + 0.334ADF + 0.131PS	0.958	< 0.0001
SID $Arg = 92.741-0.564CF-0.529Ash$	0.359	0.0146
SID $IIe = 90.568-0.790$ CF-0.602Ash	0.717	< 0.0001
SID Leu = $91.928-0.543$ CF- $0.583$ Ash	0.348	0.0171
SID Thr = $97.514-1.441$ CF + $0.227$ NDF- $1.800$ Ash	0.684	0.0003
SID Val = 89.243-0.681 CF-0.569 Ash	0.476	0.0022
SID Phe = $90.650-0.598$ CF	0.390	0.0019
SID His = $95.146-0.220$ EE- $0.826$ CF	0.767	< 0.0001
SID Asp = 92.557-1.390CF + 0.548ADF-1.001Ash	0.584	0.0016
SID Ser = 95.511-0.174 EE-0.765 CF-0.891 Ash	0.563	0.0016
SID $Glu = 101.576-1.188CF + 0.191NDF-1.368Ash$	0.479	0.0099
SID Gly = 192.358-0.800 DM - 0.785 CP - 2.024 CF + 0.612 NDF - 2.169 Ash + 0.085 PS	0.631	0.0154
SID Ala = 186.586 - 0.754 DM - 0.703 CP - 2.002 CF + 0.637 NDF - 2.065 Ash + 0.093 PS	0.652	0.0107
SID Cys = 80.803-0.140NDF + 0.071PS	0.837	< 0.0001
SID Tyr = $93.710-0.708$ CF- $0.653$ Ash	0.416	0.0060
SID $Pro = 94.529-0.756CF-0.551Ash$	0.400	0.0101

Table 9. Prediction equations of standardized ileal digestibility of amino acid based on the chemical components of rapeseed meal in 18day-old Pekin ducks (n = 22).

Abbreviations: ADF, acid detergent fiber, Ala, Alanine; Arg, arginine; Asp, aspartic acid; CF, crude fiber; Cys, cysteine; EE, ether extract; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; NDF, neutral detergent fiber; Phe, phenylalanine; Pro, proline; PS, protein solubility; Ser, serine; SID, standardized ileal digestibility; Thr, threonine; Tyr, tyrosine; Val, valine.

correlation between the concentration of SIDAA for pigs and ADF and concluded that the concentration of ADIN may be a suitable predictor of SIDAA, and ADF could be a suitable predictor of SIDAA content in canola meal (Adewole et al., 2017); Eklund et al. (2015) also reported that SIDAA linearly decreased with increasing NDF, ADF, ADL and NDIN content of RSM in pigs; Almeida et al. (2013) found, for some AA, close linear relationships between SIDAA and content of lignin and ADF in differently heat treated (autoclaved) canola meal; Wovengo et al. (2010) observed that broilers fed RSM with the greater fat content had the greater AID and SID of AA. These results suggest that PS value can partly reflect the quality of RSM and can act a suitable predictor of SIDAA coefficients in RSM for meat duck.

#### CONCLUSIONS

In conclusion, results from the current study indicate that the proximate nutrients content, AA composition, antinutritional factor content, and duck's SID of AA in RSM showed a huge difference with varying PS from 15.06 to 98.08%. Protein solubility is associated with the content of CF, NDF, ADF, GS, and ITC in RSM and also positively correlated with the SID of Lys, Ile, Val, Phe, His, Ser, Cys, and Tyr in RSM. Protein solubility when combined with proximate analyses could be a suitable predictor of SIDAA coefficients in RSM for ducks based on linear regression equations.

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