

SHORT COMMUNICATION

Energy values and protein digestibility of soybean milk by-product in pigs based on *in vitro* assays

Valores energéticos y digestibilidad proteica del subproducto de la leche de soja en cerdos con base en ensayos in vitro

Valores energéticos e digestibilidade protéica do subproduto do leite de soja em suínos com base em ensaios in vitro

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Abstract

Background: Soybean milk by-product (SMBP) is a potential alternative feed ingredient in swine diets due to its high protein content. However, information on energy and nutritional values of SMBP used as swine feed ingredient is limited.

Objective: To estimate energy values and protein digestibility of SMBP in pigs based on *in vitro* assays.

Methods: Four SMBP samples were obtained from 3 soybean milk-producing facilities. *In vitro* total tract disappearance (IVTTD) and *in vitro* ileal disappearance (IVID) of dry matter (DM) in the SMBP samples were determined. *In vitro* ileal disappearance of crude protein was determined by analyzing crude protein content in undigested residues after determining IVID of DM. Digestible and metabolizable energy of SMBP were estimated using gross energy, IVTTD of DM, and prediction equations.

Results: Sample 4 had greater IVTTD of DM than that of sample 3 (97.7 vs. 94.4%, $p < 0.05$), whereas IVID of DM in sample 4 was lower compared with sample 1 (53.5 vs. 65.0%, $p < 0.05$). *In vitro* ileal disappearance of crude protein in sample 2 was greater than that in sample 1 and 3 (92.6 vs. 90.6 and 90.1%; $p < 0.05$). The estimated metabolizable energy of SMBP ranged from 4,311 to 4,619 kcal/kg as-is basis and the value of sample 3 was the least ($p < 0.05$) among SMBP samples.

Conclusion: Energy values and protein digestibility should be determined before using SMBP in swine diets.

Keywords: *alternative feedstuffs; digestibility; energy; feed byproduct; in vitro assay; pig; protein; soybean milk by-product; soy byproduct; swine; trypsin inhibitor.*

Resumen

Antecedentes: El subproducto de la leche de soja (SMBP) es un ingrediente alimenticio alternativo con uso potencial en dietas porcinas dado su alto contenido de proteína. Sin embargo, la información sobre sus valores energéticos y nutricionales para alimentación de cerdos es muy limitada.

Objetivo: Estimar los valores de energía y la digestibilidad de la proteína del SMBP en cerdos con base en ensayos *in vitro*.

Métodos: Se obtuvieron cuatro muestras de SMBP de tres empresas productoras de leche de soja. Se determinaron la desaparición de tracto total *in vitro* (IVTTD) y la desaparición ileal *in vitro* (IVID) de la materia seca (DM) en las muestras de SMBP. La desaparición ileal *in vitro* de proteína cruda se determinó analizando el contenido de proteína cruda en residuos no digeridos después de determinar la IVID de la DM. La energía digestible y metabolizable de SMBP se estimó utilizando la energía bruta, IVTTD de la DM y ecuaciones de predicción.

Resultados: La muestra 4 tuvo una mayor IVTTD de la DM que la muestra 3 (97,7 vs. 94,4%, $p<0,05$), mientras que la IVID de la DM en la muestra 4 fue menor en comparación con la muestra 1 (53,5 vs. 65,0%, $p<0,05$). La desaparición ileal *in vitro* de la proteína cruda en la muestra 2 fue mayor que la de las muestras 1 y 3 (92,6 vs. 90,6 y 90,1%; $p<0,05$). La energía metabolizable estimada de SMBP varió de 4.311 a 4.619 kcal/kg (en base húmeda) y el valor de la muestra 3 fue el menor ($p<0,05$) entre las muestras de SMBP.

Conclusión: Los valores de energía y la digestibilidad de la proteína deben determinarse antes de usar el SMBP en dietas porcinas.

Palabras clave: cerdo; digestibilidad; energía; ensayo *in vitro*; inhibidor de tripsina; piensos alternativos; proteína; subproducto de alimentación; subproducto de leche de soja; subproducto de soja; suino.

Resumo

Antecedentes: O subproduto do leite de soja (SMBP) é um potencial ingrediente alternativo na dieta de suínos, considerando seu alto teor de proteínas. No entanto, as informações sobre os valores energéticos e nutricionais do SMBP usado como ingrediente alimentar para suínos são limitadas.

Objetivo: Estimar valores energéticos e digestibilidade protéica do SMBP em suínos com base em ensaios *in vitro*.

Métodos: Foram obtidas quatro amostras de SMBP de três instalações produtores de leite de soja. Foram determinados o desaparecimento total do trato *in vitro* (IVTTD) e o desaparecimento ileal *in vitro* (IVID) da matéria seca (DM) nas amostras de SMBP. O desaparecimento ileal *in vitro* da proteína bruta foi determinado pela análise do conteúdo de proteína bruta em resíduos não digeridos após a determinação da IVID do DM. A energia digerível e metabolizável do SMBP foi estimada usando energia bruta, IVTTD do DM e equações de predição.

Resultados: a amostra 4 apresentou maior IVTTD de DM do que a amostra 3 (97,7 vs. 94,4%, $p<0,05$) enquanto a IVID do DM na amostra 4 foi menor em comparação com a amostra 1 (53,5 vs. 65,0%, $p<0,05$). O desaparecimento ileal *in vitro* da proteína bruta na amostra 2 foi superior ao da amostra 1 e 3 (92,6 vs. 90,6 e 90,1%; $p<0,05$). A energia metabolizável estimada do SMBP variou de 4.311 a 4.619 kcal/kg no estado em que se encontra e o valor da amostra 3 foi o menor ($p<0,05$) entre as amostras do SMBP.

Conclusão: os valores energéticos e a digestibilidade das proteínas devem ser determinados antes do uso do SMBP nas dietas suínas.

Palavras-chave: alimentos alternativos; digestibilidade; energia; ensaio *in vitro*; inibidor de tripsina; porco; proteína; subproduto da ração; subproduto do leite de soja; subproduto da soja; suínos.

Received: 29/08/2019

Accepted: 18/06/2020

Introduction

Soybean milk has become popular as a protein supplement for humans. After squeezing the soybean slurry, raw soybean milk is produced. The residue is called soybean milk by-product (SMBP; Toda *et al.*, 2007; Kim *et al.*, 2016). Soybean milk by-products are used in human and animal diets, but the majority is disposed (Woo *et al.*, 2001). As SMBP often contains more than 30% of crude protein (CP), this ingredient can be used as an alternative protein source for animals (Woo *et al.*, 2001; Li *et al.*, 2012).

An accurate nutritional evaluation of feed ingredients is required for a precise feed formulation (Son *et al.*, 2017). However, information on available energy and nutrients in SMBP as a swine feed ingredient is very limited (Kortelainen *et al.*, 2014). *In vitro* methods have been widely used to estimate nutritional values because these assays are highly correlated with *in vivo* data (Boisen and Fernández, 1997; Son *et al.*, 2017). Therefore, the objective of this study was to determine energy concentrations and protein utilization of SMBP using *in vitro* assays simulating digestion and absorption by pigs.

Materials and methods

Sample preparation

Four soybean milk by-product samples were obtained from three soybean milk-producing facilities in the Republic of Korea. Samples 2 and 3 were from the same facility, and sample 3 was dried at the soybean milk-producing facility. Samples 1, 2, and 4 were dried to a constant weight at 55 °C using a forced-air oven (FC- PO-150, Dongseo Science LTD., Seongnam, Republic of Korea). Dried test ingredients were finely ground to pass a 1-mm screen (Cyclotech 1093; Foss Tecator AB, Höganäs, Sweden).

In vitro assays

In vitro total tract disappearance (IVTTD) of dry matter (DM) in ground SMBP was determined using a three-step procedure (Boisen and Fernández, 1997). In the first step, 0.5 g of sample was weighed into a 125 mL conical flask, and 25 mL of phosphate buffer solution (0.1 M and pH 6.0), and 10 mL of 0.2 M HCl were added to the flask. The pH of the solution was adjusted to 2.0 using 1 M HCl and NaOH solution, and 1 mL of freshly prepared pepsin (25 mg/mL; ≥ 250 U/mg solid, P7000, Pepsin from porcine gastric mucosa, Sigma-Aldrich, St. Louis, MO, USA) was added. Thereafter, 0.5 mL of chloramphenicol (C0378, Chloramphenicol, Sigma-Aldrich, St. Louis, MO, USA; 0.5 g/100 mL of ethanol) was added to prevent bacterial fermentation. The flasks were incubated in a shaking incubator for 2 h at 39 °C.

In the second step, the flasks were added with 10 mL of phosphate buffer (0.2 M and pH 6.8), and 5 mL of 0.6 M NaOH solution. Then the pH was adjusted to 6.8 using 1 M HCl and NaOH solution, and 1 mL of freshly prepared pancreatin solution (100 mg/mL; 4 × USP, P1750, pancreatin from porcine pancreas, Sigma-Aldrich, St. Louis, MO, USA) was added. The flasks were incubated in a shaking

incubator for 4 h at 39 °C.

After the second incubation, 10 mL of 0.2 M EDTA solution was added, and the pH was adjusted to 4.8 using 30% of acetic acid and 1 M NaOH solution. The flasks were added with 0.5 mL of Viscozyme (V2010, Viscozyme® L, Sigma-Aldrich, St. Louis, MO, USA), and incubated for 18 h at 39 °C.

After the incubation, undigested residues were filtered through pre-dried and weighed glass filter crucibles containing 0.4 g of celite using the Fibertec System (Fibertec System 1021 Cold Extractor, Tecator, Höganäs, Sweden). The test flasks were rinsed twice by distilled water, followed by rinsing twice with 10 mL of 95% ethanol and 99.5% acetone. Then, glass filter crucibles with undigested residues were dried at 130 °C for 6 h. Glass filter crucibles were weighed after cooling for 1 h.

In vitro ileal disappearance (IVID) of DM in ground SMBP was determined using a two-step procedure (Boisen and Fernández, 1995). The first and second steps were similar to the procedures of IVTTD. For IVID, 1.0 g of sample was used, and the concentrations of pepsin and pancreatic solutions were reduced to 10 and 50 mg/mL, respectively, while the incubation times were increased to 6 and 18 h, respectively. After the incubation, the test flasks were added 5 mL of 20% sulfosalicylic acid solution and left at room temperature for 30 min to precipitate indigested protein. After 30 min of precipitation, undigested residues were filtered through pre-dried and weighed glass filter crucibles containing 0.5 g of celite using the Fibertec System (Fibertec System 1021 Cold Extractor, Tecator, Höganäs, Sweden). The test flasks were rinsed twice by distilled water followed by rinsing twice with 10 mL of 95% ethanol and 99.5% acetone.

Then, glass filter crucibles with undigested residues were dried at 80 °C for 24 h. Glass filter crucibles were weighed after cooling for 1 h. After conducting the two-step procedure, undigested residues on filter crucibles were collected for analyzing CP concentration to calculate IVID of CP. The IVTTD and IVID for each ingredient were measured in triplicate.

Chemical analysis

Test ingredients were analyzed for DM (method 930.15; AOAC, 2005), CP (method 990.03; AOAC, 2005), ether extract (method 920.39; AOAC, 2005), calcium (method 978.02; AOAC, 2005), phosphorus (method 946.6; AOAC, 2005), and ash (method 942.05; AOAC, 2005). Test ingredients were also analyzed for neutral detergent fiber (NDF) and acid detergent fiber using an Ankom A2000 Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Gross energy (GE) was analyzed in each ingredient using a bomb calorimeter (Parr 1261 bomb calorimeter; Parr Instruments Co., Moline, IL, USA). Amino acids in SMBP were analyzed using ion-exchange chromatography with postcolumn derivatization with ninhydrin (AOAC, 2005; method 994.12). Methionine and cysteine in test ingredients were determined by method 985.28 (AOAC, 2005).

Calculations and statistical analysis

The IVTTD or IVID of DM and IVID of CP were calculated with the following equations, respectively:

$$\text{IVTTD or IVID of DM (\%)} = [(\text{DM}_{\text{TI}} - \text{DM}_{\text{UR}}) \div \text{DM}_{\text{TI}}] \times 100,$$

where: DM_{TI} and DM_{UR} are the weight of DM concentration in the test ingredient and undigested residues, respectively.

$$\text{IVID of CP (\%)} = [(\text{DM}_{\text{TI}} \times \text{CP}_{\text{TI}}) - (\text{DM}_{\text{UR}} \times \text{CP}_{\text{UR}})] \div (\text{DM}_{\text{TI}} \times \text{CP}_{\text{TI}}) \times 100,$$

Where: CP_{TI} and CP_{UR} are CP concentration expressed as DM basis in the test ingredient and undigested residues, respectively. Based on determined IVTTD, digestible energy (DE):GE ratio and

DE in SMBP were estimated with the following equations (Park *et al.*, 2012):

$$\text{DE:GE ratio} = 0.5986 + 0.0030 \times \text{IVTTD of DM (\%)} \\ \text{DE (kcal/kg)} = \text{GE (kcal/kg)} \times \text{DE:GE ratio}$$

Metabolizable energy (ME) was calculated using an equation as follows (Noblet and Perez, 1993):

$$\text{ME (kcal/kg DM)} = \text{DE (kcal/kg DM)} - 0.68 \times \text{CP (g/kg DM)}$$

Tryptophan concentration in SMBP was estimated using an average value of tryptophan to lysine ratio in soybean meal and full-fat soybean according to Goebel and Stein (2011) and NRC (2012). The average value of tryptophan to lysine ratio was 0.22.

The GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA) was used, and least squares means for response criteria were calculated for each ingredient. Each flask was considered as an experimental unit. Differences among least squares means were tested using the PDIFF option with Tukey's adjustment (Seo *et al.*, 2018).

Results

Crude protein and GE concentrations in SMBP ranged from 32.2 to 37.0% and 5,155 to 5,477 kcal/kg on an as-is basis (Table 1), respectively. The average ratio of methionine and threonine to lysine in SMBP was 0.22 and 0.73, respectively. The IVTTD of DM in sample 4 was greater than that of sample 3, whereas sample 4 had less IVID of DM compared with sample 1 ($p < 0.05$; Table 2). Sample 2 showed greater IVID of CP than samples 1 or 3 ($p < 0.05$). Metabolizable energy was not different between samples 1 and 2, and sample 3 had the least value ($p < 0.05$) among SMBP samples (Table 3).

Table 1. Analyzed composition of soybean milk by-product, as-is basis.

Item	Sample number				Mean
	1	2	3	4	
Dry matter, %	97.0	95.2	91.4	94.9	94.6
Gross energy, kcal/kg	5,477	5,462	5,155	5,201	5,324
Crude protein, %	36.9	37.0	34.5	32.2	35.1
Ether extract, %	18.5	17.8	14.1	14.6	16.3
Neutral detergent fiber, %	12.8	11.0	15.2	18.8	14.5
Acid detergent fiber, %	6.95	5.07	6.18	8.06	6.57
Ash, %	3.87	4.11	3.99	4.55	4.13
Essential amino acid, %					
Arginine	2.28	2.32	2.21	1.89	2.17
Histidine	0.95	0.94	0.91	0.84	0.91
Isoleucine	1.14	1.15	1.11	0.96	1.09
Leucine	2.88	2.88	2.77	2.41	2.74
Lysine	2.17	2.14	1.99	1.81	2.03
Methionine	0.48	0.47	0.43	0.43	0.45
Phenylalanine	1.69	1.71	1.64	1.43	1.62
Threonine	1.56	1.52	1.47	1.35	1.48
Tryptophan ¹	0.49	0.48	0.45	0.41	0.45
Valine	1.32	1.30	1.27	1.13	1.26
Non-essential amino acid, %					
Alanine	1.72	1.69	1.61	1.43	1.61
Aspartic acid	3.84	3.91	3.68	3.31	3.69
Cysteine	0.49	0.48	0.46	0.46	0.47
Glutamic acid	5.66	5.82	5.48	4.98	5.48
Glycine	1.51	1.49	1.42	1.29	1.43
Proline	1.93	1.93	1.83	1.65	1.83
Serine	1.90	1.90	1.82	1.63	1.81
Tyrosine	1.16	1.17	1.10	0.92	1.09
Calcium, %	0.37	0.35	0.41	0.41	0.39
Phosphorus, %	0.52	0.49	0.47	0.46	0.49

¹Tryptophan was estimated using the average value of tryptophan to lysine ratio in soybean meal and full-fat soybean (Goebel and Stein, 2011; NRC, 2012).

Table 2. *In vitro* disappearance of soybean milk by-product¹.

Item ² , %	Sample number				SEM ³	p-value
	1	2	3	4		
IVTTD of dry matter	96.8 ^{ab}	97.3 ^{ab}	94.4 ^b	97.7 ^a	0.7	0.030
IVID of dry matter	65.0 ^a	59.8 ^{ab}	58.1 ^{ab}	53.5 ^b	1.8	0.012
IVID of crude protein	90.6 ^b	92.6 ^a	90.1 ^b	91.2 ^{ab}	0.4	0.011

^{a-b}Least squares means within a row without a common superscript differ at $p < 0.05$.

¹Each least squares mean represents 3 observations except for IVID of crude protein in sample 4 (2 observations); ²IVTTD, *in vitro* total tract disappearance; IVID, *in vitro* ileal disappearance; ³SEM, standard error of the means.

Table 3. Estimated energy concentrations in soybean milk by-product (as-is basis).

Item ¹ , %	Sample number				SEM ²	p-value
	1	2	3	4		
GE, kcal/kg	5,477	5,462	5,155	5,201		
DE, kcal/kg	4,870 ^a	4,864 ^a	4,546 ^c	4,637 ^b	10	<0.001
ME, kcal/kg	4,619 ^a	4,613 ^a	4,311 ^c	4,418 ^b	10	<0.001
DE to GE ratio	0.889 ^{ab}	0.891 ^{ab}	0.882 ^b	0.892 ^a	0.002	0.030

^{a-c}Least squares means within a row without a common superscript differ at $p < 0.05$.

¹GE, gross energy; DE, digestible energy; ME, metabolizable energy; ²SEM, standard error of the means.

Discussion

Soybean milk by-products produced from soybean milk-producing facilities have been regarded as potential substitutes for other protein sources in swine diets because of their high protein concentration (Li *et al.*, 2012). However, very limited information is available on CP and essential amino acid digestibility of SMBP by pigs (Kortelainen *et al.*, 2014), and energy values in SMBP for pigs, to our knowledge, have not been documented.

The analyzed composition of SMBP tested in the present work was within the range of reported values (Ma *et al.*, 1997; Li *et al.*, 2012; Kortelainen *et al.*, 2014). The ratios of methionine and threonine to lysine of SMBP in the current study were very similar to the values in previous studies (Hermann and Honeyman, 2004; Kortelainen *et al.*, 2014; Jiang *et al.*, 2018).

In vitro total tract disappearance of DM represents the three-step digestion of the stomach, small intestine, and large intestine of pigs (Boisen and Fernández, 1997). In contrast to IVTTD of DM, IVID of DM and IVID of CP represent two-step digestion of the stomach and small intestine (Boisen and Fernández, 1995). The IVID of CP in SMBP in the present study was greater than the apparent ileal digestibility of CP (90.1 to 92.6 % vs. 81.5%) in SMBP by pigs determined by Kortelainen *et al.* (2014). This difference is likely due to endogenous losses of protein. Apparent ileal digestibility does not reflect endogenous losses of protein from the gastrointestinal tract (Park *et al.*, 2013). In contrast to apparent values, the secretion of endogenous losses does not occur in the *in vitro* system, which is very similar to true digestibility (Boisen and Fernández, 1997). Another possible reason is the difference in trypsin inhibitor activity of SMBP. Trypsin inhibitor is a principal anti-nutritional factor in soybean and has been known to lower protein and DM digestibility due to a reduction in the activity of proteolytic enzymes (Goebel and Stein, 2011). In previous studies, *in vitro* disappearance of CP in raw legume grains ranged from 58 to 80%, and these values increased up to 88% when grains were adequately heat-treated (Khokhar and Chauhan, 1986; Nergiz and Gökgöz, 2007; Shimelis and Rakshit, 2007). Standardized ileal digestibility of essential amino acids reported by Kortelainen *et al.* (2014) and both IVTTD of DM and IVID of CP of SMBP in the present study exceeded 90%. Although trypsin inhibitor activity was not analyzed in both studies, negative influence of trypsin inhibitor in SMBP was not observed considering the high digestibility values.

Unlike IVTTD of DM or IVID of CP, the value of IVID of DM was relatively low (53.5 to 65.0%). The low values of IVID of DM in SMBP compared with IVTTD of DM are supported by previous studies. In the studies by Park *et al.* (2016) and Navarro *et al.* (2018), IVID of DM in soybean meal was less than IVTTD of DM by 20 and 15% unit, respectively. This difference may be due to the absence of simulation of fiber-degrading enzymes that are secreted by microbes in the large intestine in the IVID procedure. In contrast to the IVID procedure, Viscozyme (V2010, Viscozyme® L, Sigma-Aldrich, St. Louis, MO, USA) is added in the third step when determining IVTTD. As Viscozyme contains various carbohydrases, such as arabanase, cellulase, beta-glucanase, hemicellulose, and xylanase, most dietary fiber in SMBP is expected to be digested in the third step.

The reason for less IVID of DM in sample 4 compared with sample 1 may be due to greater NDF concentration in sample 4 (18.8 vs. 12.8%). However, no difference in IVID of CP between sample 1 and 4 was observed. Generally, a greater amount of undigested fiber in the small intestine could interfere with protein utilization, but dietary fiber may not always be a promising predictor of IVID of CP (Huanget *al.*, 2003; Fang *et al.*, 2007). In the study of Huang *et al.* (2003), NDF concentration (11.0 to 15.1%) was negatively correlated with IVID of DM in various barley cultivars ($r = -0.97$). In contrast, the correlation between NDF and IVID of CP was not significantly strong. Another possible reason is that variability in NDF concentrations (11.0 to 18.8%) of SMBP in the current study might not be sufficient to verify a consistent negative impact of fiber on protein utilization.

The equation employed for estimating DE:GE ratio of SMBP in the present study was developed by Park *et al.* (2012). In the study by Park *et al.* (2012), the prediction equation was developed by a regression analysis based on a high correlation ($r^2 = 0.91$) between IVTTD of DM and DE:GE ratio of by-products in pigs. The reason for the least ME of sample 3 is attributed to low GE concentration and DE:GE ratio. The average value of estimated ME in SMBP (4,490 kcal/kg as-is basis) is greater than ME in soybean meal (approximately CP 47% and 3,283 kcal/kg as-is basis) reported by Sauvant *et al.* (2004) and NRC (2012). This difference could be attributed to a higher ether extract concentration in SMBP (14.1 to 18.5% as-is basis) compared with values in soybean meal (1.5%; Sauvant *et al.*, 2004; NRC, 2012). As oil extraction does not occur during the production of soybean milk, SMBP contains high ether extract concentration.

Hermann and Honeyman (2004) fed a diet containing 25% SMBP replacing corn and soybean meal to nursery pigs with 13 kg initial body weight. This inclusion did not show compromised growth performance compared with pigs fed a corn-soybean meal-based diet. Considering the inclusion rate and energy and protein utilization of SMBP determined in the present study, SMBP could be used as a high-quality feed ingredient for swine. However, as potential difference in energy values and protein digestibility among SMBP samples were found in the present study, energy and nutrient utilization should be determined before using SMBP in swine diets. Furthermore, available energy and protein values in SMBP should be carefully applied to the industry. As stated earlier, trypsin inhibitor of SMBP was regarded inactive considering the high *in vitro* disappearance value of DM and CP. However, active trypsin inhibitor may exist in SMBP, resulting in the necessity of down adjusting the nutritional values of SMBP found in the present study.

In conclusion, estimated ME ranged from 4,311 to 4,619 kcal/kg as-is basis, and *in vitro* protein utilization of soybean milk by-product exceeded 90%. Considering these values, soybean milk by-product can be a good protein source for pigs. However, as energy values and protein utilization of soybean milk by-products are variable, different energy and protein values should be used for each soybean milk by-product considering the trypsin inhibitor effects.

Declarations

Acknowledgments

This study was supported by the KU Research Professor Program of Konkuk University.

Funding

The authors received no financial support for the research.

Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Jung Yeol Sung: Guided the experimental procedures, interpreted data and wrote the article; Youngeun Song: Obtained the samples, collected the data and analyzed data; Bokyoung Hong: Conducted the experiment and revised the article; Beob Gyun Kim: Constructed the research concept and experimental design, and also revised and approved the manuscript.

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