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Fermented soybean meal increases nutrient digestibility via the improvement of intestinal function, anti-oxidative capacity and immune function of weaned pigs

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The nutritional components of fermented soybean meal (**FSBM**) vary because of the complex process of microbial fermentation. The objective of this study was to investigate the nutritional value of FSBM from two sources and explore the mode of actions of FSBM on the improvement of nutrient digestibility with the measurements of digestive enzymes and serum biomarkers. Eight weaned barrows (initial BW: 14.12 ± 0.24 kg) equipped with T-cannula in the distal ileum were allotted to a duplicated 4 × 4 Latin-square design with four experimental diets and four periods. Four experimental diets included a soybean meal control diet, two FSBM diets, and a nitrogen-free diet. The two sources of FSBM increased the contents of CP, amino acid and lactic acid, while decreased the levels of anti-nutritional factors, including glycinin, β-conglycinin and trypsin inhibitors. Compared to soybean meal control diet, both FSBM diets significantly increased the apparent and standardised ileal digestibility of CP and amino acids ($P < 0.05$), increased the activities of lipase, maltase and invertase in digesta ($P < 0.05$), increased total antioxidant capacity, activities of glutathione peroxidase and superoxide dismutase, the levels of interleukin-4, IgA, IgG and IgM in serum ($P < 0.05$), while decreased the levels of diamine oxidase, malondialdehyde, interleukin-6, and interleukin-2 in serum ($P < 0.05$). Additionally, the standardised ileal digestibility of amino acids were highly correlated with the aforementioned digestive enzymes and health-related serum biomarkers. In summary, FSBM diets showed an improved nutritional value evidenced by the higher nutrient digestibility, which may be partially derived from its beneficial effects on intestinal integrity, anti-oxidative capacity and immune function.

Implications

Microbial fermentation of soybean meal increased the contents of CP, amino acids and lactic acid, and decreased the levels of anti-nutritional factors. Weaned pigs fed with fermented soybean meal increased nutrient digestibility and improved health status in terms of intestinal integrity, anti-oxidative capacity and immune function. The notable correlations between nutrient digestibility and aforementioned health-related biomarkers suggested the better nutritional value of fermented soybean meal may be partially derived from its beneficial effects on animal health. The outcomes of this study would be a good reference for the application of fermented soybean meal with weaned pigs.

Introduction

Soybean meal (**SBM**) is the primary protein source for a swine diet with high protein content and well-balanced amino acid profiles (Stein et al., 2008; Mukherjee et al., 2016). However, the supply of SBM cannot meet its increasing demand due to the rapid development of the livestock industry in recent years. One option to alleviate this high demand is to improve the nutrient utilisation of SBM by animals. The anti-nutritional factors (**ANFs**) in SBM, including trypsin inhibitors, β-conglycinin and phytate, not only interrupt nutrient digestion and absorption (Sotak-peper et al., 2015) but also induce inflammation and oxidative stress associated

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with the impaired intestinal function (Kluess et al., 2007; Zhao et al., 2010). Specifically, weanling pigs are more sensitive to ANF, which causes intestinal malfunction and postweaning diarrhoea, due to the incomplete development of gastrointestinal tract and immature immune systems (Chi and Cho, 2016; Solomon et al., 2018).

Fermentation of SBM by microorganisms is a novel and efficient feed process that can effectively eliminate ANF, degrade large molecules of nutrients, produce bioactive compounds, and modulate the intestinal environment to eventually improve the digestibility (Yuan et al., 2017; Cheng et al., 2019). However, the nutritional composition of fermented soybean meal (**FSBM**) is variable due to the differences in microorganisms and procedures applied in fermentation (Song et al., 2008; Mukherjee et al., 2016). To our knowledge, limited data are reported on the nutritional value of FSBM, especially in weaned pigs. Therefore, the objective of this study was to investigate the nutritional value of FSBM and explore the mode of actions of FSBM on the improvement of nutrient digestibility with the measurements of digestive enzymes, intestinal integrity, anti-oxidative capacity, and immune function of weaned pigs.

Material and methods

Soybean meal and preparation of fermented soybean meals

The soybean meals used in animal trial or used for the preparation of FSBMs were all the by-products of solvent extracted soybeans, which were imported from the US and contained about 43% CP. Two FSBMs were prepared in two different companies and fermented by a similar mixture of *Bacillus subtilis*, *Lactobacillus*, and yeast but with different proportions and procedures. In FSBM A preparation, the fermenting microbes were prepared separately in a liquid medium and then mixed with SBM for an anaerobic solid fermentation at 30–40 °C for a 3-day period, followed by a high-temperature drying stage at 180 °C for about 20–30 minutes. FSBM B was produced in a 2-step fermentation, which included a liquid prefermentation with a mixture of SBM, microbes and water at 15–30 °C for less than 72 hours; and a solid fermentation with the prefermented product and SBM at 30–37 °C for a 3-day period, then followed by a low-temperature drying stage at 50–60 °C for about 48–72 hours. The analysed nutrient compositions of SBM and FSBMs were listed in Table 1.

Animals, diets and management

In this study, eight 6–7-week healthy barrows (Duroc × Landrace × Yorkshire) with initial BW equal to 14.12 ± 0.24 kg were subjected to a T-cannula surgery in the distal ileum as described previously (Stein et al. 1998). Followed by a 14-day convalescent period, all cannulated pigs were allotted to a duplicated 4 × 4 Latin-square design with four experimental diets and four periods (n = 8). Animals were housed individually in a metabolism cage (2.5 m × 1.8 m × 0.8 m) with free access to water and feed under a controlled light cycle, temperature, and humidity.

Four experiment diets, including an SBM diet, two FSBM diets (FSBM A and FSBM B), and a nitrogen-free diet, were prepared as shown in Table 2. SBM, FSBM A, and B diets were formulated with equal amounts of CP containing 37.2% SBM, 32.0% FSBM A, and 32.0% FSBM B as the sole nitrogen source, respectively. The nitrogen-free diet was used to estimate the basal endogenous loss of CP and amino acids (**AAs**). Chromic oxide (0.3%) was included in all diets as an indigestible marker. Vitamins and minerals were formulated to meet or exceed estimated nutrient requirements (NRC, 2012).

Table 1
Analysed chemical composition of ingredients for weaned pigs (as-fed basis).

Item	Ingredient		
	SBM	FSBM A	FSBM B
Gross energy, kcal/kg	4 072.28	3 984.13	4 364.14
DM, %	86.8	90.3	92.3
CP, %	42.80	50.21	50.47
Ether extract, %	2.1	0.9	1
Ash, %	5.5	6.7	6.2
Crude fibre, %	4.4	3.1	3.5
Ca, %	0.23	0.38	0.36
Total phosphorus, %	0.72	0.72	0.73
Amino acids, %			
Aspartic acid	4.82	5.78	5.82
Threonine	1.62	1.96	1.97
Serine	2.11	2.5	2.51
Glutamic acid	7.92	9.39	9.54
Glycine	1.76	2.14	2.14
Alanine	1.85	2.39	2.32
Cysteine	0.66	0.77	0.75
Valine	1.93	2.35	2.36
Methionine	0.72	0.79	0.75
Isoleucine	1.87	2.28	2.32
Leucine	3.19	3.9	3.94
Tyrosine	1.24	1.68	1.67
Phenylalanine	2.02	2.52	2.54
Lysine	2.59	2.77	2.82
Histidine	1.15	1.4	1.36
Arginine	3.10	3.27	3.35
Proline	2.06	2.53	2.47
Tryptophan	0.53	0.6	0.6

Abbreviations: SBM = soybean meal; FSBM A = fermented soybean meal A; FSBM B = fermented soybean meal B.

Feeding and sample collection

Each experimental period lasted 13 days, including a 5-day recovery stage with a commercial nursery diet followed by a 4-day adaption stage and a 4-day collection stage with experimental diets. During the collection stage, pigs were fed 4% BW of diet, which was equally divided into three meals and offered at 0800, 1400, and 1700 daily. Sample bags were used to collect ileal digesta from the T-cannulas for a continuous 12 h on each collection day. Bags were changed whenever they were full of digesta or at least every 25 min and stored at −20 °C. Ileal digesta collected from each period were mixed. Partial samples were lyophilised and ground for chemical analyses, and the rest were used to determine the activities of digestive enzymes.

Blood samples were collected from the anterior vena cava at the end of each period. Weaned pigs fasted for 12 hours before blood collection. Blood samples were centrifuged at 3 000g for 15 min at 4 °C, and serum was collected from the supernatant and stored at −20 °C until analyses.

Chemical analysis

The nutrient composition of ingredients, diets and ileal digesta were analysed according to the method of AOAC International (2007), including DM (method 930.15; AOAC Int., 2007), CP (method 930.15; AOAC Int., 2007), ether extract (method 920.39; AOAC Int., 2007), Ash (method 942.05; AOAC Int., 2007), crude fibre (method 978.10; AOAC Int., 2007), Ca (method 968.08; AOAC Int., 2007), total phosphorus (method 964.06; AOAC Int., 2007), AA (method 982.30E (a, b, c); AOAC Int., 2007) and Cr (method 930.15; AOAC Int., 2007). The gross energy of ingredients was measured by an automatic adiabatic oxygen bomb calorimeter (Parr Instruments Co., Morin, IL, USA).

Table 2
Ingredient composition and nutritional level of the experimental diets for weaned pigs (as-fed basis).

Item	Diet			
	SBM	FSBM A	FSBM B	N free
Ingredient, %				
SBM	37.2	0	0	0
FSBM A	0	32	32	0
FSBM B	0	0	0	0
Cornstarch	46.2	51.4	51.4	78.9
Sucrose	10	10	10	10
Dicalcium phosphate	2.2	2.2	2.2	2.2
Limestone	0.5	0.5	0.5	0.5
Chromic oxide	0.3	0.3	0.3	0.3
Salt	0.4	0.4	0.4	0.4
Solka floc	0	0	0	4
Soybean oil	3	3	3	3
Potassium carbonate	0	0	0	0.4
Magnesium oxide	0	0	0	0.1
Vitamin premix[1]	0.05	0.05	0.05	0.05
Mineral premix[2]	0.15	0.15	0.15	0.15
Total	100	100	100	100
Nutritional level[3]				
ME, MJ/kg	14.63	14.96	14.98	15.26
CP	16.01	16.02	16.02	0
Ca	0.82	0.80	0.80	0.69
Total phosphorus	0.64	0.62	0.62	0.42
Lysine	1.00	0.84	0.84	0
Methionine	0.22	0.21	0.21	0
Threonine	0.64	0.53	0.53	0
Tryptophan	0.21	0.22	0.22	0

Abbreviations: SBM = soybean meal; FSBM A = fermented soybean meal A; FSBM B = fermented soybean meal B; ME = metabolisable energy.
[1] The premix provides the following per kilogram of diet: vitamin A 15 000 IU, vitamin D_3 5 000 IU, vitamin E 40 mg, vitamin K_3 5 mg, vitamin B_2 12.5 mg, vitamin B_6 6 mg, vitamin B_{12} 0.06 mg, folic acid 2.5 mg, nicotinic acid 50 mg, biotin 2.5 mg, D-pantothenic acid 25 mg.
[2] The premix provides following per kilogram diet: Fe 100 mg, Cu 5 mg, Mn 3 mg, Zn 80 mg, I 0.14 mg, Se 0.25 mg.
[3] Calculated values.

Anti-nutritional factor analysis

The contents of β-conglycinin, glycinin, and trypsin inhibitors in SBM, FSBMs, and the corresponding diets were determined with a commercially available ELISA kit (Beijing Longke Ark Bioengineering Technology Co., Ltd, Beijing, China). A total of 300 mg samples were incubated in the extraction solution at room temperature for 16 hours, followed by a centrifuge at 4 000 rpm for 5 min to collect the supernatant. The supernatant was subjected to the anti-nutritional factor measurements according to the manufacturer's instructions.

Digestive enzyme analysis

Ileal digesta was homogenised in ice-cold saline at the ratio of 1:9 (g/ml). The homogenate was centrifuged at 3 000*g* for 10 min at 4 °C to collect the supernatant. The activities of maltose, lactose, sucrose, α-amylase, lipase and trypsin in the supernatant were measured by using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

Serum analysis for intestinal integrity, anti-oxidative capacity and immune function

The serum level of diamine oxidase (**DAO**) is an indicator of intestinal integrity. DAO was determined by using the commercially available porcine-specific ELISA kits (Jiangsu Meimian Industrial Co., Ltd, Yangcheng, China) according to the manufacturer's instructions. The anti-oxidative capacity of weaned pigs was determined by measuring total antioxidant capacity (**T-AOC**), activities of glutathione peroxidase (**GSH-Px**), catalase (**CAT**) and superoxide dismutase (**SOD**), and malondialdehyde (**MDA**) level in the serum samples with commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The systemic immune function of weaned pigs was determined by measuring the innate immunity and inflammation-related molecules (interleukin-6, IL-6; interleukin-2, IL-2; interleukin-4, IL-4; interferon-γ, IFN-γ) and adaptive immunity-related immunoglobulins (immunoglobulin M, IgM; immunoglobulin A, IgA; immunoglobulin G, IgG) with commercial porcine-specific ELISA kits (Jiangsu Meimian Industrial Co., Ltd, Yangcheng, China) according to the manufacturer's instructions.

Calculations and statistical analysis

The apparent ileal digestibility and the standardised ileal digestibility of CP and AAs were calculated for three semi-purified diets containing SBM, FSBM A, and FSBM B (Stein et al., 2007). Data were sorted and organised using Excel (Microsoft) or GraphPad Prism (GraphPad Software Inc.). All data were analysed using the Generalised Linear Model of SAS (version 9.4) as a duplicated 4×4 Latin-square design. The statistical model for this design was $Y_{ij} = \mu + \alpha_i + \gamma_j + \varepsilon_{ij}$ where μ is the overall mean, α_i is the treatment effect, γ_j is the block effect, and ε_{ij} is the error term. Data were expressed as least square mean with pooled SEM. $P < 0.05$ was considered statistically significant. The correlations between standardised ileal digestibility of AA and intestinal integrity, anti-oxidative capacity, and immunity function were determined by Pearson correlation analysis in SAS.

Results

Fermentation of soybean meal improved nutrient compositions, reduced anti-nutrient factors and increased lactic acid content

Compared to SBM, FSBM A and B exhibited an increase of CP by 17.3 and 17.9% with the increase of all the measured AAs, a decrease of crude fibre by 29.5 and 20.5%, and a decrease of ether extract by 57.1 and 52.4%, respectively (Table 1). FSBMs also showed a higher calcium level than SBM (Table 1).

Anti-nutritional factors in SBM, including glycinin, β-conglycinin and trypsin inhibitors, impede nutrient utilisation and gut development. The glycinin, β-conglycinin, and trypsin inhibitors of FSBM A were 60.5, 58.3, and 95.9% lower than those of SBM, respectively. Similarly, FSBM B had 63.3, 61.1 and 97.5% lower of glycinin, β-conglycinin and trypsin inhibitors compared to SBM (Table 3). Furthermore, the lactic acid in FSBM A and B was 9.8 and 9.4-fold higher, and the pH values in FSBM A and B were 21.8 and 24.8% lower compared to SBM, respectively (Table 3). The experimental diets exhibited the changes in ANFs, lactic acid, and pH values correspondingly.

Fermented soybean meals improved nutrient digestibility and activity of digestive enzymes in weaned pigs

The apparent ileal digestibility and standardised ileal digestibility of CP and AAs for three semi-purified diets containing SBM, FSBM A, and FSBM B were reported in Tables 4 and 5. Compared to SBM, FSMB A and FSMB B significantly increased ($P < 0.05$) apparent ileal digestibility of CP by 34.7 and 24.1%, and increased standardised ileal digestibility of CP by 15.3 and 8.2%, respectively. Also, FSBM A and FSBM B significantly increased ($P < 0.05$) apparent ileal digestibility and standardised ileal digestibility of six indispensable AAs (isoleucine, leucine, phenylalanine, threonine, tryptophan and valine) and four dispensable AAs (alanine, glutamic acid, glycine and serine). Additionally, FSBM A showed higher apparent ileal digestibility of threonine, and higher standardised ileal digestibility of threonine, glycine and glutamic acid than FSBM B. Cautions were needed for the proline data as its apparent ileal digestibility value was negative and standardised ileal digestibility value was greater than 100%.

The activity of digestive enzymes in ileal digesta was reported in Table 6. The results showed that the two FSMB groups significantly increased the activities of lipase and maltase ($P < 0.05$), and FSMB A significantly increased the invertase activity ($P < 0.05$). The activities of trypsin and α-amylase were numerically increased in FSBM groups compared to SBM group.

In general, the activities of digestive enzyme were positively correlated with the standardised ileal digestibility of AAs (Supplementary Fig. S1). Specifically, invertase activity was positively ($P < 0.05$) correlated with the standardised ileal digestibility of CP, aspartic acid, threonine, serine, glycine, alanine, valine, isoleucine, leucine and phenylalanine; lipase activity was positively ($P < 0.05$) correlated with standardised ileal digestibility of aspartic acid, glycine, alanine, valine, isoleucine and phenylalanine; maltase activity was positively ($P < 0.05$) correlated with standardised ileal digestibility of threonine, serine, isoleucine and histidine.

Table 3
Lactic acid, pH and anti-nutritional factors in ingredients and diets for weaned pigs.

Item	Ingredients			Diets		
	SBM	FSBM A	FSBM B	SBM	FSBM A	FSBM B
Lactic acid, mg/g	10.25	110.30	106.94	4.13	62.64	66.96
pH	6.33	4.95	4.76	6.83	5.6	5.47
Glycinin, mg/g	160.81	63.45	58.95	61.77	37.62	25.52
β-conglycinin, mg/g	144.87	60.38	56.34	58.88	36.95	25.68
Trypsin inhibitor, mg/g	8.39	0.34	0.21	0.69	0.16	0.05

Abbreviations: SBM = soybean meal; FSBM A = fermented soybean meal A; FSBM B = fermented soybean meal B.

Table 4
Apparent ileal digestibility of CP and AAs in semi-purified diets with SBM, FSBM A and B by weaned pigs.

	Ingredient			SEM[1]	*P*-value
	SBM	FSBM A	FSBM B		
CP	40.61^{b}	54.7^{a}	50.36^{a}	2.17	<0.01
Indispensable AA, %					
Arginine	74.38	78.52	78.88	1.83	0.189
Histidine	56.82	68.86	61.55	4.56	0.204
Isoleucine	58.84^{b}	69.69^{a}	71.21^{a}	1.69	<0.01
Leucine	63.91^{b}	71.85^{a}	72.12^{a}	1.56	<0.01
Lysine	60.65	63.64	59.58	1.50	0.174
Methionine	68.52	68.23	71.77	2.16	0.456
Phenylalanine	64.41^{b}	74.28^{a}	71.43^{a}	1.49	<0.01
Threonine	33.43^{c}	55.6^{a}	47.33^{b}	1.89	<0.01
Tryptophan	44.45^{b}	60.24^{a}	55.45^{a}	2.87	<0.01
Valine	50.71^{b}	64.68^{a}	62.48^{a}	2.13	<0.01
Dispensable AA, %					
Alanine	35.42^{b}	53.19^{a}	59.3^{a}	3.76	<0.01
Aspartic acid	57.22	63.79	60.19	1.77	0.058
Cysteine	46.96	51.27	48.38	1.87	0.280
Glutamic acid	63.37^{b}	68.98^{a}	65.41^{ab}	1.35	0.031
Glycine	14.63^{b}	33.74^{a}	27.29^{a}	3.41	<0.01
Proline	−64.37	−7.86	−24.27	26.99	0.340
Serine	48.89^{b}	63.5^{a}	59.82^{a}	1.88	<0.01
Tyrosine	59.94	65.15	63.93	3.32	0.525

Abbreviations: AA = amino acid; SBM = soybean meal; FSBM A = fermented soybean meal A; FSBM B = fermented soybean meal B.
[1] Data expressed as least square mean values (n = 8) with pooled SEM. Different superscript letters indicate significant difference ($P < 0.05$).

Table 5
Standardised ileal digestibility of CP and AAs in SBM, FSBM A and B by weaned pigs.

Item	Ingredient			SEM[1]	*P*-value
	SBM	FSBM A	FSBM B		
CP	78.40[b]	90.43[a]	84.84[ab]	2.17	<0.01
Indispensable AA, %					
Arginine	94.20	99.67	98.03	1.86	0.137
Histidine	74.68	85.25	79.01	4.58	0.289
Isoleucine	71.40[b]	81.81[a]	81.47[a]	1.69	<0.01
Leucine	74.31[b]	82.89[a]	81.33[a]	1.59	<0.01
Lysine	73.20	76.81	71.72	1.51	0.080
Methionine	83.03	88.03	87.27	2.19	0.251
Phenylalanine	73.69[b]	83.33[a]	79.79[a]	1.49	<0.01
Threonine	59.47[c]	77.30[a]	70.32[b]	2.03	<0.01
Tryptophan	61.82[b]	76.02[a]	71.25[a]	2.87	0.010
Valine	66.77[b]	79.81[a]	77.07[a]	2.15	<0.01
Dispensable AA, %					
Alanine	66.24[b]	84.00[a]	82.77[a]	3.73	<0.01
Aspartic acid	69.26	75.18	71.14	1.78	0.087
Cysteine	68.07	73.94	70.30	1.71	0.080
Glutamic acid	74.72[b]	79.53[a]	75.43[b]	1.35	0.048
Glycine	84.32[b]	102.43[a]	92.56[b]	3.26	<0.01
Proline	232.40	252.63	230.18	27.38	0.818
Serine	69.87[b]	82.66[a]	78.35[a]	1.88	<0.01
Tyrosine	78.67	84.35	81.50	3.32	0.498

Abbreviations: AA = amino acid; SBM = soybean meal; FSBM A = fermented soybean meal A; FSBM B = fermented soybean meal B.
[1] Data expressed as least square mean values (n = 8) with pooled SEM. Different superscript letters indicate significant difference ($P < 0.05$). Values for standardised ileal digestibility were calculated by correcting the values for apparent ileal digestibility with basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of DM) as CP, 17.95; arginine, 0.27; histidine, 0.09; Isoleucine, 0.10; leucine, 0.16; lysine, 0.14; methionine, 0.06; phenylalanine, 0.09; threonine, 0.18; tryptophan, 0.04; valine, 0.13 alanine, 0.25; aspartic acid, 0.24; cysteine, 0.07; glutamic acid, 0.37; glycine, 0.57; proline, 2.31; serine, 0.18; and tyrosine, 0.11.

Table 6
The effect of dietary SBM and FSBMs on activities of digestive enzymes in ileal digesta of weaned pigs.

Item	SBM	FSBM A	FSBM B	SEM[1]	*P*-value
Trypsin (U/mg prot)	40 238.10	44 046.47	46 115.95	9 051.53	0.91
Lipase (U/mg prot)	334.56[b]	587.75[a]	615.23[a]	45.90	<0.01
α-Amylase (U/mg prot)	1.84	2.38	2.23	0.32	0.48
Invertase (U/mg prot)	246.42[b]	455.00[a]	393.55[ab]	39.20	<0.01
Maltase (U/mg prot)	359.22[b]	546.41[a]	579.56[a]	36.03	<0.01
Lactase (U/mg prot)	90.65	103.93	78.90	14.31	0.48

Abbreviations: SBM = soybean meal; FSBM A = fermented soybean meal A; FSBM B = fermented soybean meal B.
Different superscript letters indicate significant difference ($P < 0.05$).
[1] Data expressed as least square mean values with pooled SEM (n = 8).

Fermented soybean meals improved intestinal integrity, anti-oxidative capacity and immune function in weaned pigs

As the surgery of cannulation induced a stress to pigs in this study, the serum biomarkers were evaluated to explore the possible benefits of FSBMs. Serum DAO is well correlated with the intestinal mucosal lesions; a higher value indicates a severer damage. FSBM A and FSBM B significantly reduced ($P < 0.05$) serum DAO levels compared to the SBM group (Table 7), suggesting a better intestinal integrity. Oxidative stress occurs with the accumulation of free radicals, which is associated with cell death and tissue damage. Generally, FSBM A and B significantly increased ($P < 0.05$) serum T-AOC of weaned pigs compared to SBM. GSH-Px, SOD, and CAT are key anti-oxidative enzymes to maintain oxidative homeostasis. Compared to SBM, FSBM A and B significantly increased ($P < 0.05$) activity of GSH-Px; FSBM B significantly increased activity of SOD; and FSBM A had a tendency to increase the activity of CAT (Table 7). MDA is a by-product of lipid peroxidation, and the accumulation of MDA reflects oxidative stress in animals. Compared to SBM, FSBM A and FSBM B significantly reduced ($P < 0.05$) the level of MDA in serum (Table 7). Additionally, FSBM A and FSBM B significantly reduced pro-inflammatory cytokines of IL-6 and IL-2 ($P < 0.05$) and increased anti-inflammatory cytokine of IL-4 ($P < 0.05$) compared to SBM group (Table 7), suggesting the alleviation of inflammation in FSBM groups. Immunoglobulins are indispensable immune molecules protecting the body from pathogen infection, especially after surgery. The results showed that pigs fed with FSBM A and FSBM B exhibited ($P < 0.05$) higher levels of IgA, IgM, and IgG in serum compared to pigs fed with SBM (Table 7), indicating an enhanced immunity of cannulated pigs with FSBM supplementations. FSBM A group even showed a significantly higher level of IgA than FSBM B group ($P < 0.05$). Briefly, FSBM diets enhanced intestinal integrity, anti-oxidative capacity and immune function compared to SBM diet, which indicated that FSBMs were able to maintain a better health status of cannulated pigs than SBM.

Serum biomarkers were highly correlated with nutritional value of fermented soybean meals in weaned pigs

The correlations between standardised ileal digestibility of AAs and health-related serum indicators of weaned pigs were presented in Fig. 1. The results revealed that serum DAO had comprehensive correlations with multiple measurements. Besides the negative correlation with standardised ileal digestibility of glycine and phenylalanine (Fig. 1, $P < 0.05$), serum DAO was negatively cor-

Table 7
The effect of dietary SBM and FSBMs on intestinal integrity, anti-oxidative capacity and immune function of weaned pigs.

Item	SBM	FSBM A	FSBM B	SEM[1]	P-value
Intestinal integrity					
DAO (IU/L)	26.69[a]	21.78[b]	21.98[b]	1.30	0.04
Anti-oxidative capacity					
T-AOC (U/mL)	0.96[b]	2.72[a]	2.24[a]	0.21	<0.01
CAT (U/mL)	26.83	38.71	28.52	3.92	0.10
SOD (U/mL)	34.78[b]	47.28[ab]	55.43[a]	5.11	0.04
GSH-Px (U/mL)	367.71[b]	488.09[a]	459.81[a]	23.28	<0.01
MDA (nmol/mL)	3.61[a]	2.64[b]	2.47[b]	0.22	<0.01
Immune function					
IL-6 (ng/L)	860.00[a]	752.96[b]	704.54[b]	21.62	<0.01
IL-2 (pg/mL)	489.79[a]	421.90[b]	422.15[b]	8.07	<0.01
IL-4 (ng/L)	91.58[b]	120.53[a]	118.29[a]	6.01	0.02
IFN-γ (pg/mL)	1 476.11	1 444.17	1 616.67	131.41	0.62
IgA (μg/mL)	35.18[c]	86.25[a]	64.68[b]	4.97	<0.01
IgM (μg/mL)	26.98[b]	33.73[a]	32.54[a]	1.62	0.02
IgG (μg/mL)	330.16[b]	411.54[a]	395.19[a]	18.37	0.02

Abbreviations: SBM = soybean meal; FSBM A = fermented soybean meal A; FSBM B = fermented soybean meal B; DAO = diamine oxidase; T-AOC = total anti-oxidative capacity; MDA = malondialdehyde; CAT = catalase, SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; IL-2 = interleukin-2; IL-6 = interleukin-6; IL-4 = interleukin-4; IFN-γ = interferon gamma; IgA = immunoglobulin A; IgM = immunoglobulin M; IgG = immunoglobulin G.
Different superscript letters indicate significant difference ($P < 0.05$).
[1] Data expressed as least square mean values with pooled SEM (n = 8).

related with the activities of invertase and lipase in digesta (Supplementary Fig. S1, $P < 0.05$), negatively correlated with the levels of serum IgA and IgG (Fig. 1, $P < 0.05$), negatively correlated with serum SOD activity (Fig. 1, $P < 0.05$), and positively correlated with serum MDA (Fig. 1, $P < 0.05$). These comprehensive correlations suggested the intestinal integrity played a central role in promoting animal health and nutrient utilisation.

Additionally, the results showed the standardised ileal digestibility of seven indispensable AAs and four dispensable AAs were positively correlated with anti-oxidative capacity of weaned pigs (Fig. 1). Specifically, the standardised ileal digestibility of threonine, serine, glycine, alanine, valine, isoleucine, leucine, phenylalanine, arginine and tryptophan were positively correlated with T-AOC activity ($P < 0.05$), the standardised ileal digestibility of threonine, serine, cysteine, valine, isoleucine, leucine, phenylalanine and tryptophan were positively correlated with GSH-Px activity ($P < 0.05$), the standardised ileal digestibility of valine, isoleucine, phenylalanine and tryptophan were positively correlated with SOD activity ($P < 0.05$), and the standardised ileal digestibility of glycine and tryptophan were negatively correlated with serum MDA ($P < 0.05$).

The standardised ileal digestibility of eight indispensable AAs and three dispensable AAs were negatively correlated with the inflammatory status of weaned pigs (Fig. 1). Specifically, the standardised ileal digestibility of threonine, serine, alanine, valine, isoleucine, leucine, phenylalanine and tryptophan were negatively correlated with IL-2 ($P < 0.05$), the standardised ileal digestibility of isoleucine was negatively correlated with IL-6 ($P < 0.05$), and the standardised ileal digestibility of threonine, serine, glycine, valine, phenylalanine, histidine, arginine and tryptophan were positively correlated with IL-4 ($P < 0.05$).

The standardised ileal digestibility of seven indispensable AAs and five dispensable AAs were positively correlated with serum immunoglobulin of weaned pigs (Fig. 1). The standardised ileal digestibility of threonine, serine, valine, isoleucine, phenylalanine, and tryptophan were positively correlated with serum IgA, IgM and IgG ($P < 0.05$). Additionally, the standardised ileal digestibility of aspartic acid, glycine, alanine, cysteine and leucine were positively correlated with IgA ($P < 0.05$), the standardised ileal digestibility of cysteine and methionine were positively correlated with IgG ($P < 0.05$), and the standardised ileal digestibility of methionine and leucine were positively correlated with IgM ($P < 0.05$).

Discussion

Weanling pigs have a limited capacity to digest plant proteins and are sensitive to anti-nutritional factors of SBM. The *in vitro* microbial fermentation has been commonly used to increase the nutrient utilisation of SBM (Upadhaya and Kim, 2014). In this study, the nutritional components, digestibility of CP and AAs from two fermentation processes were investigated, which provided the nutritional value of FSBMs to apply in swine industry. Additionally, the significant correlations of AA digestibility with serum biomarkers (in terms of intestinal integrity, anti-oxidative capacity and immune function) were identified, which demonstrated that the better health status of pigs fed with FSBMs may partially account for the improvement of nutrient utilisation of FSBMs.

Currently, the nutritional value of FSBM mainly refers to the data reported by NRC (2012), which does not take into account the effect of different growth stages on nutrient digestibility. In this study, the nutritional value of FSBM was investigated with pigs at young age, when the FSBMs were commonly supplemented in practice. Interestedly, the apparent ileal digestibility of CP and AAs for pigs fed with SBM diet or FSBM diets reached the lower limits of previously published data, while the standardised ileal digestibility values were around the average (NRC, 2012; Cervantes-Pahm and Stein, 2010; Pedersen et al., 2016), which was probably due to higher endogenous losses of AAs from weaned pigs than that from growing pigs (Leterme and Thewis, 2004; Presto et al., 2011). The reason is that the gastrointestinal tract of weaned pigs is vulnerable to various stimuli resulting in the higher endogenous AA losses than that of growing pigs (Adeola et al., 2016). Therefore, enhancing intestinal health is critical to minimise the endogenous losses and improve nutrient utilisation in weaned pigs. Notably, the apparent ileal digestibility of proline was negative and its standardised ileal digestibility was over 100% because of the extremely high endogenous loss of proline. Similar results for proline, especially for young pigs, were reported in previous studies (Park and Adeola, 2022; Rojas and Stein, 2013).

The increased digestibility of CP and AAs from FSBMs were related to the changes in chemical compositions. One obvious change is the elimination of ANF naturally existed in SBM. The ANFs are generally unfavourable to animal growth, as they interrupt nutrient digestion, decrease food intake, impair gastrointestinal function and induce metabolic disorders and stress (Woyengo

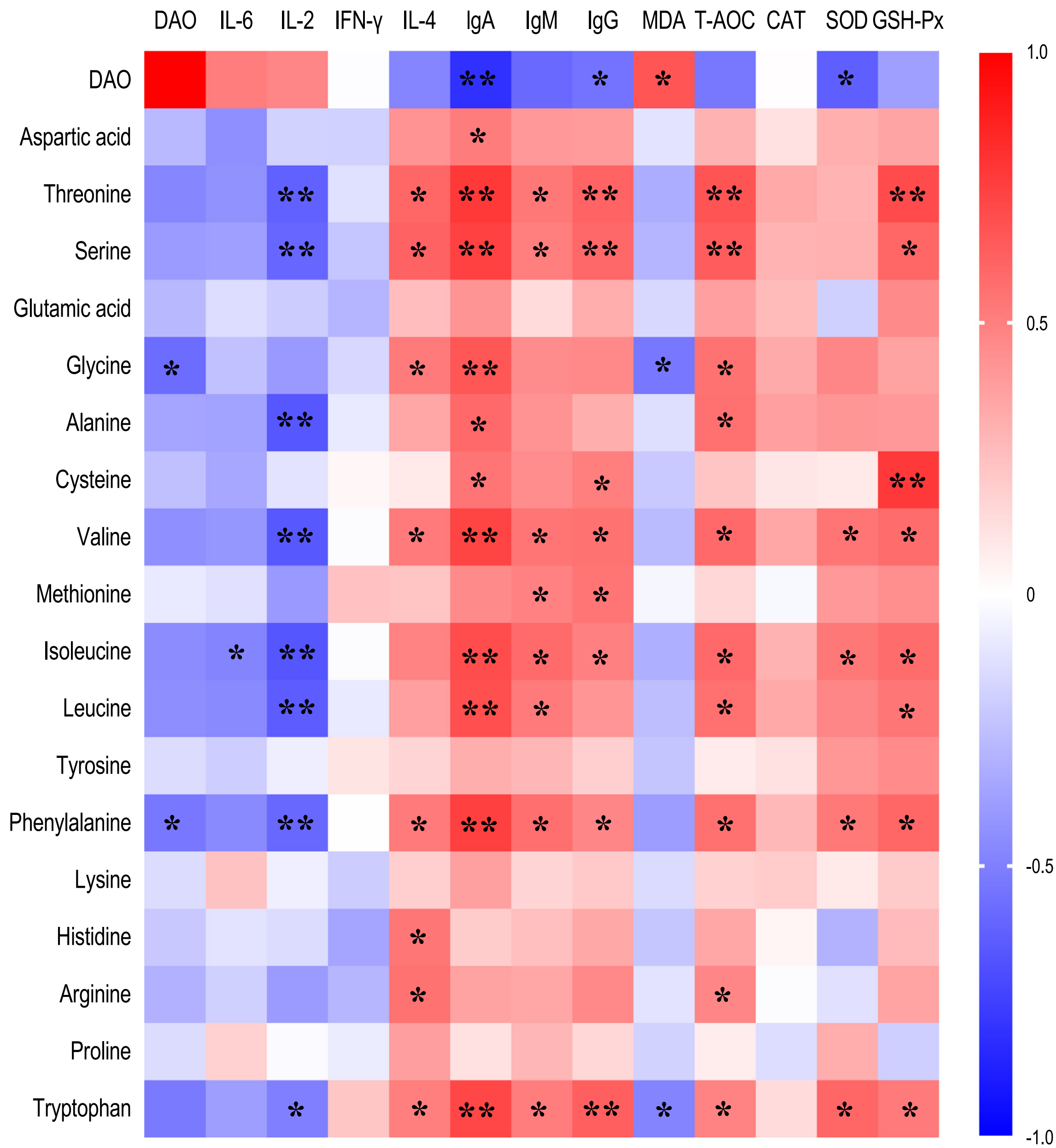

Fig. 1. The Pearson correlation analysis between the standardised ileal digestibility of AAs and health-related serum markers in weaned pigs. *Abbreviations:* standardised ileal digestibility = standardised ileal digestibility; AA = amino acid; DAO = diamine oxidase; T-AOC = total anti-oxidative capacity; MDA = malondialdehyde; CAT = catalase, SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; IL-2 = interleukin-2; IL-6 = interleukin-6; IL-4 = interleukin-4; IFN-γ = interferon gamma; IgA = immunoglobulin A; IgM = immunoglobulin M; IgG = immunoglobulin G. Positive correlations are shown in red and negative correlations in blue. Colour intensity represents R-values of correlation ranging from −1 to 1. *, ** indicate significant correlations between two groups with $P < 0.05$ and $P < 0.01$, respectively.

et al., 2017; Pan et al., 2018; Wang et al., 2021). In this study, FSBMs reduced the content of glycinin, β-conglycinin, and trypsin inhibitors by about 60–96%, respectively. Another change is the enrichment of digestible nutrients. The fermenting microorganisms are able to decompose macromolecules to facilitate the digestion and absorption (Mukherjee et al., 2016). In this study, FSBM increased CP by ~17% and decreased crude fibre by about 20–29% compared to SBM. Additionally, the microbial metabolites from fermentation, specifically lactic acid by *Lactobacillus*, are beneficial for animal intestinal health. In this study, FSBMs exhibited a ~9-fold increase in lactic acid content and a 20–24% reduction in pH value. Studies indicated that lactic acid compensates the insufficient gastric acid secretion in weanling pigs (Aumaitre et al., 1995; Heo et al., 2013), and the reduced pH value of lactic acid in stomach could delay the propagation of pathogens (Thompson and Lawrence, 1981; Wang et al., 2015). Lactic acid also acts as an energy source to promote the growth and recovery of intestinal epithelial cells (Suiryanrayna and Ramana, 2015).

In addition to the changes in chemical compositions, the increased activities of invertase, maltase and lipase in ileal digesta were observed with FSBM supplementations in this study. The improved enzyme activity resulted from the acidic environment of gastrointestinal tract and reduced ANF content by microbial fermentation (Yue et al., 2016; Zhang et al., 2018; Zaworska-Zakrzewska et al., 2020). Interestingly, standardised ileal digestibility of CP and AAs were positively correlated with the activities of invertase, maltase and lipase, although these enzymes do not directly digest protein and peptides. Because the limited number of enzymes was evaluated, these enzymes may reflect overall enzyme activities on some degree.

Animal health is essential to maintain digestive enzyme activity and nutrient digestion (Metges, 2010; Campbell et al., 2013). The health issue of weaned pigs caused by various stress and infection is the major concern in swine industry. The surgery is a strong stimulus to induce inflammation and oxidative stress, and it requires immunity to prevent infection (Faure et al., 2017; Hari and Summers, 2019). Thus, the cannulated pig may serve as a stress model to study the interaction between animal health and nutrient digestibility. Moreover, the anti-oxidative and immune systems are highly active and sensitive; they respond to stimuli, nutrient, even diet patterns fast from minutes to hours (Murphy and Weaver, 2016). Therefore, the 8-days of experimental diet in each period of Latin-square design was feasible to investigate the effect of nutrient on animal health.

Among the multiple serum measurements, DAO is a well-established indicator for intestinal integrity, and serum DAO increases when intestinal integrity is impaired (Li et al., 2019). In this study, FSBMs decreased serum DAO level, which was negatively correlated with AA digestibility, enzyme activity, anti-oxidative capacity and immune function and was positively correlated with inflammation. These results suggested the intestinal integrity played a critical role in mediating the benefits of FSBMs on nutrient utilisation and animal health.

Impaired intestinal integrity is highly associated with oxidative stress and inflammation (Vancamelbeke and Vermeire, 2017). Oxidative stress occurs when there is an imbalance between the production of potentially destructive reactive oxygen species and the antioxidant defences in the body, which would result in cellular and tissue damage. T-AOC reflects the capacity of antioxidants to alleviate oxidative stress. GSH-Px helps reduce toxic peroxides. MDA is a common indicator for oxidative stress, and the higher level of MDA reflects the more severe oxidative stress (Chen et al., 2022; Yu et al., 2018; Guo et al., 2021; Liu et al., 2021). In this study, FSBMs increased the anti-oxidative capacity of weaned pigs as indicated by an increase in T-AOC and GSH-Px activity, and a decrease of MDA in serum. Moreover, the positive correlations between standardised ileal digestibility of AAs and anti-oxidative capacity in weaned pigs were detected, which suggested the elevated anti-oxidative capacity may account for the improved nutrient digestibility of FSBMs.

Inflammation belongs to innate immunity that rapidly responds to danger signals, but uncontrolled activation of inflammation with over-production of pro-inflammatory cytokines leads to cell death, organ dysfunction, and reduced animal performance (Guo et al., 2016; Wang et al., 2020). In some severe infectious diseases, such as African swine fever and Covid-19, the hyper-activation of inflammation-induced cytokine storm syndrome is the leading cause of death (Post et al., 2017; Chen et al., 2021). In this study, standardised ileal digestibility of AAs and intestinal integrity were negatively correlated with pro-inflammatory cytokines and were positively correlated with anti-inflammatory cytokines. Therefore, alleviation of inflammation would be plausible to improve intestinal health and nutrient utilisation. Given that FSBMs reduced the IL-2 and IL-6 levels and increased IL-4 level in weaned pigs, FSBMs may improve nutrient digestibility partially through the alleviation of inflammation in weaned pigs.

Immunoglobulins such as IgM, IgG and IgA belong to adaptive immunity and play an important role in preventing pathogen infection (Schroeder and Cavacine, 2010; Chen et al., 2017). IgM is the most rapidly released antibody when infection occurs. IgG and IgA are the most abundant and the second most abundant immunoglobulin in serum, respectively; they recognise pathogens, coordinate cytotoxicity and neutralise toxins (Snoeck et al., 2006; Mallery et al., 2010). In this study, FSBM increased levels of immunoglobulins in serum indicating an improvement in immunity of weaned pigs. The positive correlations between standardised ileal digestibility of AAs and immunoglobulin levels were observed, which indicated that the enhanced immune function may contribute to the increased nutrient digestibility of FSBMs.

Conclusion

In summary, FSBM altered the nutritional composition, with an increase of CP, amino acids and lactic acid, while a decrease in anti-nutritional factors, including glycinin, β-conglycinin and trypsin inhibitors. Weaned pigs fed with FSBM diets showed better digestibility of CP and AAs, which were highly correlated with the enhanced activities of digestive enzymes, intestinal integrity, anti-oxidative capacity and immune function. Therefore, the increased nutrient utilisation with FSBM was not only derived from the changes in nutritional composition but also benefited from the improved animal health.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100557.

Ethics approval

In this study, the use of animals and all procedures of animal experimentation complied with the ethical standards and were approved by the Animal Care Committee of Sichuan Agricultural University with approval number 20200058.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study can be requested from the author.

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Declaration of interest

We declare that no conflict of interest in this study.

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