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Original Article

Effect of Sunflower Kernel Peptides Produced by Dual-Degradation on the Growth Performance, Nutrient Digestibility, and Health Status of Broilers

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ABSTRACT

The purpose of this study was to investigate the effect of sunflower kernel peptides produced by enzymatic digestion, fermentation, or both on the growth performance, nutrient digestibility, and health status of broilers. Four diets contained 20% of sunflower kernel meal as its raw form (CON) or degraded by protease (ESM), *Bacillus pumilus* (FSM), or both (DSM). A total of 480 yellow broilers at one day old were randomly distributed to 4 groups with 6 replicates of 20 chicks each. The feeding trial lasted for 63 d. Results showed that peptide content was increased ($p<0.001$) from 3.97% (CON) to 32.5% (ESM), 24.2% (FSM), and 39.1% (DSM). The three sunflower peptide groups improved ($p\leq0.001$) feed intake and body weight gain. The peptide groups increased ($p\leq0.015$) ileal apparent digestibility of dry matter, energy, crude protein, and amino acids (methionine, lysine, tryptophan, and threonine). Furthermore, the peptide groups improved ($p\leq0.029$) the health status by increasing serum immunoglobulins (IgA, IgG) and glutathione peroxidase. Additionally, among the peptide groups, DSM showed more pronounced effects ($p<0.05$) on these parameters than ESM or FSM. It is concluded that dual-degradation by enzymolysis and fermentation has a better improvement in the nutrition and application of sunflower kernel meal in broilers.

INTRODUCTION

Soybean meal is a major source of feed products, but soybean meal prices remain on an upward trend in recent years (USDA, 2022). The feeding cost in the farming industry is rising and finding alternatives to soybean meal is now a key concern for the feed industry. In 2022, global sunflower seed production will be about 51 million metric tons, with sunflower seeds being the third largest oilseed producer after soybeans and rapeseed (USDA, 2022). Sunflower kernel meal can replace soybean meal as a major protein source for animal feed (Pedernera *et al.,* 2021). The protein content of sunflower seeds has been increased by nearly 40% through efficient hulling (González-Pérez, 2015). Compared to soybeans, sunflower seeds contain fewer or no anti-nutritional factors and are low in lysine, but high in crude fiber and sulfur-containing amino acids (González-Pérez, 2015). However, consuming too much of one miscellaneous meal can have a negative impact on the total cost and final balance since different miscellaneous meals include different hazardous elements and anti-nutrients. It will also affect the product's quality and endanger the health of farm animals. An investigation of the feed industry reveals that mixed grain and meal-based feed quality improvement is in full swing.

It is commonly known that using superior fermentation strains and effective enzyme preparations to ferment raw materials can decrease the quantity of anti-nutritional components, improve nutrient

digestibility, and reduce the environmental pollution caused by manure (Liu *et al.*, 2018 a,b). It has been shown that pretreatment with exogenous enzymes in pig and poultry diets can improve the nutritional value of sunflower kernel meal (Fafiolu *et al.,* 2015). Addition of exogenous enzymes (phytase, β-glucanase, etc.) to broiler feeds containing sunflower kernel meal can aid fiber digestion and dissolve phytate phosphorus, thereby reducing their deleterious effects on broiler performance parameters (Krieg *et al.,* 2020). However, the synergistic effect of enzymatic fermentation on sunflower kernel meal and its application to broiler chickens is still unidentified.

In this study, a selected enzyme and a probiotic were used for the dual degradation of sunflower kernel meal. By animal feeding tests, the sunflower kernel peptide content and its effects on broiler growth performance, nutrient digestibility, and health status were examined. The hypothesis that dual degradation is better than single enzymatic digestion or fermentation was finally tested.

MATERIAL AND METHODS

Animal ethics

Research on animals was conducted according to the institutional committee on animal use (2021010).

Experimental diets

Sunflower kernel meal is a by-product of the sunflower seed after oil extraction. Commercial acid protease (PROMAX® Protease, pH 1.5-6.0) was used for the enzymatic hydrolysis of sunflower kernel meal. *Bacillus pumilus* strain CGMCC 16061 was obtained from the China General Microbiological Culture Collection Center for the fermentation of sunflower meal.

The sunflower meal was ground (16 mesh size) and sterilized at 121 °C for 20 min. The sterilized sunflower meal and deionized water (100/35, weight/weight) were mixed as a fermented substrate for solid-state fermentation. The protease was incubated with the fermented substrate at 3 000 U/kg, 30 °C for 48 h, for preparing enzymatic hydrolysis of sunflower meal. *Bacillus pumilus* was incubated with the fermented substrate at 1.0×10^9 CFU/kg, 32 °C for 48 h, to prepare a fermented sunflower kernel meal. The same protease activity and *Bacillus pumilus* amount were dual-incubated with fermented substrates at 32 °C for 48 h for preparing a double-treated sunflower kernel meal. The products obtained by enzymolysis, fermentation, or both were freeze-dried, and ground (16 mesh size) for the preparation of the four diets.

Four diets contained 20% of non-degraded (control, CON), enzymatic (ESM), fermented (FSM), or dual-degraded (DSM) sunflower kernel meal. The diet was established according to the Nutritional Requirement of Chinese Yellow-feathered Broilers (Standards in Agricultural Industries in China, NY/T 3645-2020; Table 1).

Animals and samples

A total of 480 female yellow-feathered broilers at 1 d of age with similar body weight were randomly distributed into four groups, with six replicates of 20 broilers each (pens). The feeding trial lasted from 1 to 63 d with three phases, 1-21, 22–42 and 43–63 d. The chicken house was set at 20°C with automatic mechanical ventilation. All broilers were free to access diets and water throughout the feeding trial. Broilers and feed in each pen (20 birds) were weighed at 21, 42 and 63 d; feed efficiency was adjusted for mortality on a pen basis. General health status of broilers was monitored twice daily.

At 21, 42 and 63 d, ten birds per replicate were randomly selected for collecting blood samples from the wing vein. Serum samples were prepared by centrifuging the blood at 3 000 g for 10 min at 4°C (Wang *et al.,* 2011) and stored at –20°C until the assay of IgG, IgM, IgA, and antioxidant enzyme activities. After blood sampling, the ten birds at 63 d of age were euthanized by injection of sodium pentobarbital (1 mL per kg of body weight) into the wing vein, and then dissected. Ileal digesta was collected and pooled by replicate for detecting the apparent digestibility of nutrients.

Chemical and biological analysis

The contents of nutrients in the feed and digesta were determined according to AOAC (1990) for total P (964.06), phytate P (927.02), Ca (935.13), crude protein (976.05), ether extract (920.39), and ash (942.05). Gross energy was measured in an Oxygen Bomb Calorimeter (Model 6300, PARR, Moline, IL, USA). Metabolizable energy (ME) was detected using adult roosters according to the description by Wu *et al.* (2020). Dry matter was determined by drying a 2 g sample at 105 °C to a constant mass. Amino acids were measured using an AA analyzer (Beckman 6300; Beckman Coulter, Inc., Fullerton, CA). Acid-soluble protein was detected according to Standards in Agricultural Industries in China (NY/T 3801-2020).

Table 1 – Ingredients and nutrient levels of diets (air-dry basis) for yellow broilers.

Item	1-21 d				22-42 d				43-63 d			
	CON	Degraded			CON	Degraded			CON	Degraded		
		ESM	FSM	DSM		ESM	FSM	DSM		ESM	FSM	DSM
Ingredients (%)												
Sunflower kernel meal	20.0	-	-	-	20.0	-	-	-	20.0	-	-	-
Sunflower kernel peptide	-	20.0	20.0	20.0	-	20.0	20.0	20.0	-	20.0	20.0	20.0
Corn gluten	13.5	14.0	13.0	12.0	10.5	10.5	10.0	9.0	8.2	8.2	7.0	0
Corn	59.2	58.8	59.6	61.0	61.7	62.0	61.8	63.5	63.5	63.7	64.5	64.7
Soybean oil	1.8	1.6	1.8	1.3	2.8	2.5	3.0	2.5	3.8	3.6	4.0	3.8
Lysine	0.7	0.7	0.7	0.7	0.6	0.5	0.7	0.6	0.5	0.5	0.5	0.5
Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0	0
Limestone	1.3	1.4	1.4	1.5	1.4	1.5	1.5	1.4	1.3	1.3	1.3	1.3
Dicalcium phosphate	2.0	2.0	2.0	2.0	1.5	1.5	1.5	1.5	1.3	1.3	1.3	1.3
Sodium chloride	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Titanium dioxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Premix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated ME (MJ/kg)[2]	12.40	12.40	12.36	12.38	12.81	12.81	12.83	12.78	13.18	13.22	13.24	13.20
Detected nutrients (%)[2]												
Crude protein	21.44	21.53	21.46	21.28	19.54	19.37	19.73	19.48	18.03	17.94	17.75	18.10
Calcium	1.00	1.03	0.99	1.07	0.92	0.96	0.96	0.92	0.84	0.80	0.84	0.84
Non-phytate phosphorus	0.49	0.49	0.48	0.48	0.40	0.40	0.42	0.40	0.36	0.37	0.36	0.36
Lysine	1.22	1.22	1.21	1.20	1.09	0.98	1.18	1.07	0.97	0.94	0.95	0.95
Methionine	0.66	0.57	0.56	0.54	0.52	0.54	0.51	0.50	0.39	0.37	0.37	0.38
Methionine + Cysteine	0.90	0.94	0.92	0.89	0.86	0.85	0.84	0.83	0.70	0.69	0.68	0.71

[1] Supplied per kilogram of diet for 1-21/22-42/43-63 d: vitamin A 12 000/9 000/6 000 IU, vitamin D_3 600/500/500 IU, vitamin E 45/35/25 mg, vitamin K 2.5/2.2/1.7 mg, thiamin 2.4/2.3/1.0 mg, riboflavin 5.0/5.0/4.0 mg, niacin 42/35/20 mg, pantothenic acid 12/10/8 mg, pyridoxine 2.8/2.4/0.6 mg, biotin 0.12/0.10/0.02 mg, folic acid 1.0/0.7/0.3 mg, Vitamin B_{12} 1.3/1.0/0.75 mg, K 5.0/4.6/4.0 g, Mg 0.6/0.0.6/0.6 g, Fe 80/80/80 mg, Cu 7/7/7 mg, Mn 80/60/55 mg, Zn 85/80/75 mg, I 0.7/0.6/0.5 mg, Se 0.15/0.15/0.15 mg.

[2] Calculated according to China Feed Data (2020).

Titanium dioxide was added to all diets as the inert marker (Liu *et al.,* 2018a).

Peptides (≤1 kDa) were quantified using electrophoresis in sodium dodecyl sulfate-polyacrylamide gels (Fling & Gregerson, 1986). For organic acids estimation, the sample slurry was centrifuged at 1 × 10^4 g for 20 min. The supernatant was analyzed using HPLC (Model 1100, Agilent Technologies, Inc, Santa Clara, CA, USA) using 10 mM perchloric acid as mobile phase at 40 °C. Protease activity was analyzed according to China National Standard GB/T 28715-2012. A protease activity unit was the enzyme amount that hydrolyzes casein to produce color equivalent to 1.0 µmole of tyrosine per minute at pH 7.5 at 37 °C.

Concentrations of IgA and IgG in the serum were measured using an automatic biochemistry analyzer (Model 7600, Hitachi High-Tech, Tokyo, Japan). Commercial kits from Nanjing Jiancheng Biological Institutes (Nanjing, China) were used to detect superoxide dismutase (A001-0302), glutathione peroxidase (A005-102), and catalase (A007-1-1). *Bacillus pumilus* was enumerated according to Methods for the Examination of Import Microbial Blends in the Environmental Protection SN/T 4624.2-2016 using a nutrient agar base at 38 °C, pH 7.0, for 24 h.

Statistics

All data were expressed as mean and SEM using the GLM procedure of ANOVA (IBM SPSS, Armonk, NY, USA). The statistical unit was from all birds in a replicate for growth performance, the mean of ten birds for a blood parameter, and pooled digesta of ten birds for nutrient digestibility. Differences in variables of ANOVA analysis were separated using Tukey's-b test (assumption of homoscedasticity) or Tamhane T2 (heteroskedasticity) at a significance level of $p<0.05$.

RESULTS

Nutritional compositions

Sunflower kernel meal degraded with protease, *Bacillus pumilus*, or both did not affect the contents of dry matter, gross energy, and ash (some unaffected data not shown), increased ($p<0.001$) acid-soluble protein, peptides, and organic acid, but decreased ($p<0.001$) nitrogen-free extract and phytic P (Table 2). In contrast to CON, crude protein was increased ($p<0.05$) in FSM and DSM. There were differences ($p<0.05$) in peptide contents reflecting on FSM<ESM<DSM. Lysine, methionine, and total essential amino acid were unaffected by the dietary treatments.

Table 2 – Compositional differences of sunflower kernel meal degraded by protease, probiotic or both.

	CON	Degraded			SEM	*p*-value
		ESM	FSM	DSM		
Dry matter (%)	87.4	87.5	87.0	87.6	0.231	0.375
Crude protein (%)	36.2[b]	35.7[b]	38.2[a]	39.9[a]	0.385	<0.001
Ether extract (%)	1.01	1.02	1.02	1.01	0.045	0.997
Nitrogen-free extract (%)	34.2[a]	33.8[a]	19.4[b]	17.7[b]	0.814	<0.001
Phytate P (%)	0.73[a]	0.72[a]	0.48[b]	0.55[b]	0.018	<0.001
Ash (%)	5.75	5.98	5.86	6.01	0.211	0.813
Acid-soluble protein (%)	3.17[c]	19.1[a]	11.4[b]	19.6[a]	0.618	<0.001
Peptides (≤1 kDa, %)	3.97[d]	32.5[b]	24.2[c]	39.1[a]	0.816	<0.001
Organic acid (%)	0.52[b]	0.63[b]	3.58[a]	3.78[a]	0.115	<0.001
Lysine (%)	1.25	1.13	1.09	1.21	0.057	0.358
Methionine (%)	0.71	0.73	0.76	0.78	0.015	0.062
Total essential amino acid (%)	16.0	15.9	16.0	16.0	0.139	0.954
Gross energy (MJ/kg)	16.6[a]	16.2[a]	15.2[b]	15.0[b]	0.218	0.002
Metabolizable energy (MJ/kg)	9.75	10.2	9.55	9.62	0.306	0.069
Protease (U/kg)	-	241[a]	58.7[b]	255[a]	35.13	0.004
Bacillus pumilus (Log_{10} cfu/kg)	-	-	2.13	1.97	0.314	0.501

[a-d] Means among treatments without the same superscript were significantly different (p<0.05).

CON, control containing non-degraded sunflower kernel meal; ESM, degraded with enzyme PROMAX® Protease (pH 1.5-6.0, Challenge International Trade Co., Beijing, China); FSM, fermented with *Bacillus pumilus* strain CGMCC 16061 (China General Microbiological Culture Collection Center, Beijing, China); DSM, dual-degraded with protease and *Bacillus pumilus*.

Growth performance

As shown in Table 3, dietary treatment influenced (p≤0.001) feed take and body weight gain in the three periods. During 1-21 d of age, DSM had a more significant effect (p<0.05) on feed intake and body weight gain, compared to ESM and FSM. During 1-42 d, DSM had a better effect (p<0.05) on body weight gain and feed efficiency than FSM. During 1-63 d, FSM and DSM showed better (p<0.05) body weight gain and feed efficiency than ESM.

Ileal digestibility

The dietary treatment affected (p≤0.001) the ileal digestibility of dry matter, energy, crude protein, and amino acids (lysine, methionine, threonine and tryptophan). Compare to CON, all degraded groups increased (p<0.001) the digestibility of dry matter,

Table 3 – Effect of sunflower kernel peptides on the growth performance of yellow broilers.

	CON	Degraded			SEM	*p*-value
		ESM	FSM	DSM		
1-21 d of age						
Feed intake (kg/bird)	647[c]	670[b]	669[b]	689[a]	4.868	<0.001
Body weight gain (g/bird)	380[c]	409[b]	405[b]	421[a]	3.893	<0.001
Feed/gain	1.71	1.64	1.65	1.64	0.022	0.150
Mortality (%)	1.67	2.50	2.50	1.67	1.086	0.898
1-42 d of age						
Feed intake (kg/bird)	2.63[b]	2.74[a]	2.70[a]	2.72[a]	0.017	0.001
Body weight gain (kg/bird)	1.23[c]	1.29[ab]	1.26[bc]	1.34[a]	0.013	<0.001
Feed/gain	2.13[a]	2.13[a]	2.14[a]	2.03[b]	0.022	0.008
Mortality (%)	3.33	3.33	2.50	3.33	1.070	0.927
1-63 d of age						
Feed intake (kg/bird)	4.89[b]	5.15[a]	5.14[a]	5.24[a]	0.029	<0.001
Body weight gain (kg/bird)	1.78[c]	1.93[b]	2.07[a]	2.14[a]	0.022	<0.001
Feed/gain	2.75[b]	2.67[b]	2.49[a]	2.45[a]	0.032	<0.001
Mortality (%)	4.17	3.33	2.50	3.33	1.015	0.724

[a-c]Means among treatments without the same superscript were significantly different (p<0.05).

CON, control containing non-degraded sunflower kernel meal; ESM, degraded with enzyme PROMAX® Protease (pH 1.5-6.0, Challenge International Trade Co., Beijing, China); FSM, fermented with *Bacillus pumilus* strain CGMCC 16061 (China General Microbiological Culture Collection Center, Beijing, China); DSM, dual-degraded with protease and *Bacillus pumilus*.

energy, methionine, and total essential amino acids. DSM showed better effects (p<0.05) on the digestibility of energy, crude protein, lysine, methionine, threonine, tryptophan, and total essential amino acids than ESM or FSM.

Immunoglobulins and antioxidant

At 21 d of age, the dietary treament influenced (p<0.001) the levels of IgG, IgA, superoxide dismutase, and glutathione peroxidase. In contrast to CON, all degraded groups increased (p<0.05) IgG and IgA; FSM and DSM increased (p<0.05) superoxide dismutase; ESM and DSM increased (p<0.05) glutathione peroxidase. Among the three degraded groups, DSM was better (p<0.05) on IgG and glutathione peroxidase than ESM and FSM, whereas FSM is better (p<0.05) on superoxide dismutase than ESM. Similarly, at 42 d, the degraded groups increased (p≤0.001) the serum levels of IgG, IgA, and glutathione peroxidase, compared to CON; and DSM had a greater effect (p<0.05) on IgG than ESM and FSM. At 63 d of age, all degraded groups influenced (p≤0.029) the levels of IgG, IgA, superoxide dismutase and glutathione peroxidase, compared to CON; and DSM showed a greater effect (p<0.05) on IgA than ESM.

Table 4 – Effect of sunflower kernel peptides on the digestibility of yellow broilers at 63 d of age.

Item	CON	Degraded			SEM	*p*-value
		ESM	FSM	DSM		
Dry matter (%)	71.0^{b}	75.3^{a}	75.4^{a}	77.6^{a}	0.946	0.001
Energy (%)	65.3^{c}	73.3^{a}	69.9^{b}	73.9^{a}	0.782	<0.001
Crude protein (%)	70.3^{c}	74.7^{b}	72.0^{bc}	78.4^{a}	1.004	<0.001
Lysine (%)	63.6^{b}	70.2^{a}	65.5^{b}	72.4^{a}	1.210	<0.001
Methionine (%)	63.7^{c}	69.3^{b}	68.2^{b}	73.2^{a}	0.935	<0.001
Threonine (%)	67.0^{b}	72.8^{a}	68.1^{b}	73.7^{a}	0.969	<0.001
Tryptophan (%)	69.7^{b}	71.2^{b}	71.3^{b}	77.6^{a}	1.593	0.015
TEAA (%)	67.2^{d}	71.7^{b}	70.2^{c}	74.8^{a}	0.228	<0.001

$^{a-c}$Means among treatments without the same superscript were significantly different (p<0.05).

CON, control containing non-degraded sunflower kernel meal; ESM, degraded with enzyme PROMAX® Protease (pH 1.5-6.0, Challenge International Trade Co., Beijing, China); FSM, fermented with *Bacillus pumilus* strain CGMCC 16061 (China General Microbiological Culture Collection Center, Beijing, China); DSM, dual-degraded with protease and *Bacillus pumilus*. TEAA, total essential amino acids.

DISCUSSION

It has been reported that sunflower seed meal concentrate can be used to replace 70% of the soy component in broiler diets (Gaviley *et al.,* 2020). The application of unhulled sunflower seed meal in poultry

Table 5 – Effect of sunflower kernel peptides on serum immunoglobulins and antioxidative enzymes of yellow broilers.

Item	CON	Degraded			SEM	*p*-value
		ESM	FSM	DSM		
21 d of age						
IgG (g/L)	0.26^{c}	0.39^{a}	0.35^{b}	0.40^{a}	0.008	<0.001
IgA (g/L)	0.20^{b}	0.30^{a}	0.29^{a}	0.32^{a}	0.009	<0.001
SOD (U/mL)	30.7^{c}	35.0^{bc}	33.1^{ab}	37.5^{a}	0.729	<0.001
GPx (U/mL)	15.6^{b}	21.8^{a}	18.4^{b}	22.1^{a}	0.837	<0.001
42 d of age						
IgG (g/L)	0.22^{c}	0.27^{b}	0.26^{b}	0.30^{a}	0.007	<0.001
IgA (g/L)	0.16^{b}	0.21^{a}	0.21^{a}	0.23^{a}	0.009	0.001
SOD (U/mL)	30.9	31.8	33.1	33.1	1.287	0.058
GPx (U/mL)	17.5^{b}	24.1^{a}	23.3^{a}	24.9^{a}	0.919	<0.001
63 d of age						
IgG (g/L)	0.18^{b}	0.21^{a}	0.21^{a}	0.22^{a}	0.008	<0.001
IgA (g/L)	24.4^{b}	24.5^{b}	25.8^{ab}	28.7^{a}	0.007	0.026
SOD (U/mL)	16.1^{b}	19.9^{a}	20.6^{ab}	21.6^{a}	1.007	0.029
GPx (U/mL)	0.21^{b}	0.26^{a}	0.25^{a}	0.28^{a}	0.686	<0.001

$^{a-c}$ Means among treatments without the same superscript were significantly different (p<0.05).

CON, control containing non-degraded sunflower kernel meal; ESM, degraded with enzyme PROMAX® Protease (pH 1.5-6.0, Challenge International Trade Co., Beijing, China); FSM, fermented with *Bacillus pumilus* strain CGMCC 16061 (China General Microbiological Culture Collection Center, Beijing, China); DSM, dual-degraded with protease and *Bacillus pumilus*. GPx, glutathione peroxidase. SOD, superoxide dismutase.

diets is limited because of its high content of cell wall material content (Senkoylu & Dale, 1999). Therefore, the use of exogenous enzymes to increase the accessibility of cell contents to digestive enzymes may have some justification. The addition of no enzyme or too high amount of enzyme in the feed will cause the bird to produce excessive endogenous enzymes and lead to a decrease in production performance (Ravindran, 2013). In this study, dehulled sunflower seed meal or sunflower kernel meal was used to avoid the cell wall problem; the degraded sunflower kernel meal increased the acid-soluble protein, peptides, and organic acid, but decreased nitrogen-free extract and phytate P; the most significant changes in these parameters were observed in DSM after double degradation of sunflower kernel meal, indicating that the dual-degradation by enzymolysis and fermentation can have a more significant effect than a single method.

Exogenous enzyme and fermentation treatments can improve the digestion and utilization of feed by animals (Liu *et al.,* 2017, 2018a,b). Yaqoob *et al.* (2022) found that sunflower kernel meal at 3%, 6%, and 9% levels after treatment with multiple enzymes (β-xylanase, α-amylase, and protease) had a similar effect on broiler growth performance compared to soybean meal. Studies have shown that the effect of fermentation treatment of sunflower meal on the growth performance of broiler chickens is controversial. Horvatovic *et al.* (2015) reported that at 10% sunflower kernel meal level, enzyme (cellulase, B-glucanase, and xylanase) supplementation significantly improved broiler body weight in the grower phase, but had no effect on feed intake. Mbukwane *et al.* (2022) observed that 13% or 13.5% levels of sunflower meal treated with enzymes (xylanase, amylase and protease mixture) increased body weight gain and feed intake of broilers. In this experiment, protease, *Bacillus pumilus*, or dual degradation of 20% level of sunflower kernel meal increased the feed intake and body weight gain of broiler chickens.

Amerah *et al.* (2015) found that enzyme (endoxylanase and β-glucanase) supplementation improved the feed conversion of sunflower meal (0-100 g/kg), but did not restore the reduction in body weight caused by high levels of sunflower kernel meal. Abdelrahman & Saleh (2010) reported that the application of 10% level of sunflower meal in broiler diets without enzymes (β-glucanase) was better than the enzyme treatment, and without any negative effects. In addition, Berwanger *et al.* (2017) did not observe the effect of treated sunflower kernel meal with or without the addition of enzyme complexes (pectinase, protease, phytase, β-glucanase, xylanase, cellulase and amylase) on broiler growth performance. These inconsistencies may be due to the different levels and types of enzymes that have different effects on the application of non-starch polysaccharides.

Only used one enzyme or one probiotic was studied for the effect of sunflower kernel meal processing, which has some limitations. In addition, there is no report on the application of complex enzyme preparations and complex microbial fermenters in sunflower kernel meal processing, nor is it relevant. On the other hand, it is particularly important to determine the proportion of sunflower kernel meal in the feed and the level of fermentation. In the future studies, it is possible to combine different enzymes or probiotics to determine the role of dual fermentation in the fermentation of sunflower kernel meal to ensure its maximum nutritional values.

In this study, dual-degraded significantly improved the ileal digestibility of dry matter, energy, crude protein, and amino acids (lysine, methionine, threonine). Bilal *et al.* (2017) found that crude fiber digestibility improved when 20% and 25% levels of sunflower meal were used in broiler diets, while no improvement in crude fiber digestibility was observed with non-starch polysaccharide enzyme supplementation. The addition of enzymes (cellulase, β-glucanase and β-xylanase) to 35% level of sunflower kernel meal improves non-starch polysaccharide digestion in the jejunum and protein digestion in the ileum (Kocher *et al.,* 2000). Waititu *et al.* (2018) reported no effect on the apparent ileal digestibility of broiler chickens after multi-enzyme (xylanase, protease, and amylase by strains of *Trichoderma* and *Bacillus*) treatment of high-protein sunflower meal. Alagawany *et al.* (2017) found that the addition of an exogenous enzyme resulted in a better digestibility coefficient than non-supplemented treatments. Malathi & Devegowda (2001) discovered that an enzyme (xylanase and cellulase from Trichoderma) significantly reduced the relative viscosity of sunflower kernel meal digest. In addition, the digestibility values of the diet of the experimental group with enzymes were consistently better than those of the diet without enzyme supplementation when unhulled sunflower meal was applied to the diet in laying hens (Fafiolu *et al.,* 2015).

In this study, the IgG and IgA of broiler chickens in the experimental group were increased, and dual-degradation is more effective. The elevation of IgG, IgA, and IgM represents an increase in the immune

capacity of the animal (Zhang *et al.,* 2021a). Enting *et al.* (2007) has shown that a low-density breeder diets (containing 24.2 g/kg of sunflower meal) can improve offspring growth rates, reduce mortality, and reduce or increase immune responses, depending on breeder age and egg weight. Amer *et al.* (2021) reported that protease fermentation treatment of broiler diets (containing 5% sunflower) increases IgM levels. Antioxidant enzyme activities such as glutathione peroxidase and superoxide dismutase respond to the level of antioxidant capacity of animals (Zhang *et al.,* 2021b). In this study, both glutathione peroxidase and superoxide dismutase were elevated after fermentation treatment of sunflower kernel meal. In summary, it shows that sunflower seed peptides can improve the immune performance and antioxidant ability of broiler chickens, probably because of the nutritional improvement of sunflower kernel meal by degradation. To understand completely the effect of degraded sunflower meal on the immune performance of livestock, additional studies are needed.

CONCLUSION

Sunflower kernel meal degraded by protease, probiotic, or both increased its nutritional values and application in yellow broilers reflecting on growth performance, nutrient digestibility, and health status. The effect of synergistic fermentation treatment with both enzyme and probiotic was more significant than a single one. It is concluded that *in vitro* dual-degradation with exogenous enzymes and probiotics can improve the quality and application of plant-based proteins.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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