Original Article

Improving the Antioxidant Status, Meat Quality, and Immune Response of Broilers by Fermented Feather Meal

Hassan Safari*, Ardeshir Mohit, Maziar Mohiti-Asli

Department of Animal Science, Faculty of Agriculture Science, University of Guilan, Rasht, Iran.

Use your device to scan and read the article online

How to Cite This Article Safari, H., Mohit, A., & Mohiti-Asli, M. (2025). Improving the Antioxidant Status, Meat Quality, and Immune Response of Broilers by Fermented Feather Meal. *Iranian Journal of Veterinary Medicine, 19*(2), 279-298. https://crossmark.crossref.org/dialog/?doi=10.32598/ijvm.19.2.1005483

doi: **http://dx.doi.org/10.32598/ijvm.19.2.1005483**

ABSTRACT

Background: Feather biodegradation is an effective alternative to other processing methods.

Objectives: This study aims to investigate the effects of hydrolyzed feather meal (HFM) on broilers' antioxidant status, meat quality, and immune response.

Methods: In October 2022, 480-day-old Ross 308 male broilers were used for 42 days in a completely randomized design with eight treatments and five replicates (12 chicks/replicate) in the research farm of Agriculture Faculty, Guilan University (Rasht City, Iran). The experimental diets were as follows: (1) control diet (without feather meal [FM]), (2), (3), (4), (5), (6), (7), and (8) all containing 4% raw FM (RFM), HFM by autoclave (Au-HFM), fermented FM (FFM) by *Bacillus licheniformis* (Bl-FFM), FFM by *Bacillus subtilis* (Bs-FFM), FFM by *Aspergillus niger* (An-FFM), FFM by *B. licheniformis+B. subtilis+A. niger* (Co-FFM), and HFM by an enzyme (En-HFM), respectively.

Results: The results of the in vitro experiment showed that 2,2-diphenyl-1-picrylhydrazl (DPPH) and 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical-scavenging activities, as well as microorganism count of FFM, increased with increasing fermentation time ($P \le 0.05$). Also, the pH of FFM significantly decreased by increasing the fermentation time ($P \le 0.05$). The DPPH and ABTS radical-scavenging activities of FFM were higher than those of RFM, Au-HFM, and En-HFM ($P \le 0.05$). The in vivo experiment results showed that the total antioxidant capacity (TAC) serum levels, glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) in the FFM were higher than those in the control and other treatments ($P \le 0.05$). Compared to control and other treatments, broilers fed FFM had lower meat malondialdehyde (MDA) levels, higher meat pH, and higher water-holding capacity at 0, 7, and 14 d of the breast meat storage, as well as lower serum MDA levels ($P \le 0.05$). Broilers fed FFM had higher antibody titers against Newcastle disease virus, lower heterophil count, and lower heterophil-to-lymphocyte ratio, as well as higher total anti-sheep red blood cell (anti-SRBC) titer, immunoglobulin G (IgG), and immunoglobulin M (IgM), compared to the control and other treatments ($P \le 0.05$).

Conclusion: Fermentation of FM increased its antioxidant properties, improved the body's antioxidative status, and contributing to improved the meat quality and immune response of broilers.

Keywords: Biodegradation, Broiler chicken, Feather meal (FM), Fermentation, Radical scavenging

Article info:
Received: 05 Mar 2024
Accepted: 02 Apr 2024
Publish: 01 Apr 2025

*** Corresponding Author:**
Hassan Safari, PhD.
Address: *Department of Animal Science, Faculty of Agriculture Science, University of Guilan, Rasht, Iran.*
Phone: *+98 (13) 33690274*
E-mail: *h.safari6813@gmail.com*

Copyright © 2025 The Author(s);
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-By-NC: https://creativecommons.org/licenses/by-nc/4.0/legalcode.en), which permits use, distribution, and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Introduction

Reducing feed costs, reducing the cost of treatment, and increasing the quality of products in livestock production are the main challenges confronted by many researchers (Safari et al., 2016; Al-Muhammadawi & Jassim Hammoudi, 2022). Up to 70% of the total cost of commercial poultry production is spent on feed costs (Safari et al., 2016). As a result of the rising global price of soybean meal, the most commonly used source of protein in the diet formulation of poultry, competition between humans and animals for soybean has led researchers to seek unconventional, alternative, or cheap sources of feed (Safari et al., 2016). Feathers make up approximately 5%-10% of the live weight of poultry. Feathers, a major by-product of the poultry industry, contain 90% crude protein, mainly keratin. The disulfide bonds, hydrogen bonds, and hydrophobic properties of keratin result in its low solubilities and digestibility, hard to hydrolyze by enzymes, and limited bioavailability (Huang et al., 2022). Feathers are extremely resistant to common proteases and stable against different processes, including thermo-chemical, autoclave, chemical, and mechanical (Prajapati et al., 2021). These intense processes denature and lose some amino acids, resulting in poor digestible feather meal (FM) (Huang et al., 2022). For example, autoclave conditions launch large amounts of waste gases, including ammonia and sulfur dioxide, making them contaminating and unsustainable. Additionally, these methods produce non-nutritive amino acids, such as lanthionine and lysinoalanine, from Cys and Lys (Abdelmoteleb et al., 2023).

Among the different procedures, the biodegradation of feather keratin by keratinolytic microorganisms is feasible as an effective alternative to other methods due to their quick growth and development, accessibility, large yield, cost-effective, sustainability, improved digestibility, and bioavailability (Abdelmoteleb et al., 2023). Additionally, fermentation increases the palatability of feed, improves growth performance, enhances beneficial gut microbiota, and improves immunity in broilers (Ibrahim et al., 2021). Microorganisms, including fungi, yeasts, actinomycetes, and *Bacillus* species, can biodegrade feathers, utilizing feathers as the only source of nitrogen and carbon (Pei et al., 2023). The main mechanisms of feather keratin degradation in the fermentation process are as follows: 1) Breaking the structural bonds and formation of the secondary structure of feather keratin: A decrease in the disulfide link in feather keratin from cystine (-S-S-) to cysteine (-SH) causes the strongly bound structure of keratin to alter its biodegraded state (Abdelmoteleb et al., 2023). 2) Hydrolysis: Macromolecules hydrolysis into soluble peptides and easily digestible small-size molecules (Huang et al., 2022). 3) The keratin is completely degraded into bioavailable amino acids (Abdelmoteleb et al., 2023). Feather keratin, an ideal source of bioactive peptides and oligopeptides, frequently has various biological properties, including potent antibacterial, immunomodulatory, and strong antioxidant properties (Kshetri et al., 2022). Feathers are rich in functional groups, such as -SH, -COOH, -OH, and $-NH_2$, which are present on keratin polypeptide chains and lead to biological activities, such as strong antioxidant activity (Fakhfakh et al., 2011). In this study, we extracted an antioxidative octapeptide from broiler feather hydrolysate. Furthermore, it is well known that the intestinal mucosa's peptide transport system facilitates the direct, energy-free absorption of small peptides (Huang et al., 2019). It is well known that the quality of poultry meat is strongly related to its diet; therefore, modulation of animal feed can improve the quality and nutritional value of poultry products. Meat oxidation, water-holding capacity indicators, pH, and intramuscular fat directly impact meat quality. Moreover, meat quality parameters, such as flavor, color, and nutrient content, are all impacted by oxidation (Safari et al., 2016). Diseases are the most significant economic challenge in the poultry industry after feed cost. Immunity and antioxidant levels are key metrics for assessing health, and nutrition and health are inextricably linked (Huang et al., 2022; Morovati et al., 2022; Mohammed Awad, 2023). Fermented feed improves humoral responses (immunoglobulin content, lysozyme activity, and cytokine production) and, cell-mediated immunity and gastrointestinal health (Huang et al., 2022). Consequently, the current study was conducted to investigate the impacts of various hydrolyzed FMs (HFM) on antioxidant status, meat quality, and immune response in broilers.

Materials and Methods

Preparation of various HFM

Preparation of FM: White broiler chicken feathers were collected from a slaughterhouse in Rasht City, Guilan Province, Iran. The feathers were removed mechanically, washed (by hand in tap water), dirt-free, and then sun-dried. The material was milled (MAKI-M-1282-1) and powdered (TS-2700) to an average particle size of less than 0.5 mm. Powdered feathers were sterilized by autoclave (15 min, 121 °C, 1.8 bar), then dried for 48 h at 45 °C in a sterile oven. Finally, one part of the dried FM was stored in sterile bags for raw FM (RFM). The remainder was hydrolyzed using an autoclave, fermentation, or enzyme.

Fermentation of FM: A solid-state fermentation process was developed according to methods described in previous studies (Belewu et al., 2008; Jazi et al., 2017; Oluseun et al., 2016). The steps for fermentation of FM were as follows: 1) *Bacillus licheniformis* (Persian Type Culture Collection [PTCC]: 1595), *Bacillus subtilis* (PTCC: 1720), and *Aspergillus niger* (PTCC: 5154) were prepared from the Persian Type Culture Collection of the Iranian Research Organization for Science and Technology. 2) *B. licheniformis* and *B. subtilis* were cultured on nutrient agar (Merck, Germany) and incubated for 48 h at 37 ˚C and 30 ˚C, respectively. *A. niger* was cultured on potato dextrose agar (PDA; Merck, Germany) and incubated for 72 h at 26 ˚C. 3) One liter of distilled water was mixed with each kilogram of FM and 15 g of corn starch as a source of easily digestible carbohydrates and inoculated with either *B. licheniformis* (10^9 colony-forming units [CFU]/mL), *B. subtilis* (10^9 CFU/mL), *A. niger* (10^9 spores/mL), or co-fermented FM (Co-FFM) (10^3 CFU or spores/mL of each strain was used for Co-FFM) as FFM treatments in a fermenter (10 L), with a one-way valve to the outflow of produced gases and prevent air entry (B. Braun Biostat B Fermenter/Bioreactor Controller, type 8840334) for 12 days at 30 ˚C. 4) FFM was dried at 50 ˚C for two days.

Autoclave hydrolysis: For treatment, FM was hydrolyzed in an autoclave at 140 ˚C and 2.5 bar steam pressure for 60 minutes (Wiradimadja et al., 2014).

Enzymatic hydrolysis: The enzyme used in this experiment was Cibenza IND900 (Novus International Inc.). Cibenza IND900 is a heat-resistant protease designed solely to increase the nutritional value of FM (Novus International, Inc). According to the manufacturer, Cibenza IND900 has an enzyme activity of 65,000 U/g. The steps for hydrolysis of FM by enzymes were as follows in compliance with the manufacturer's instructions: 1) 50 kg of raw feathers (50-60% moisture) were loaded into a batch. 2) 150 g Cibenza IND900 was dissolved in two liters of water (Cibenza IND900 solution). 3) Cibenza IND900 solution was added to the feathers and mixed well. 4) 50 kg of additional raw feathers (50%-60% moisture) were added to the batch, mixed well again, and thoroughly mixed before being placed in the oven. 5) It was incubated at 55 ˚C for 45 minutes. 6) The temperature was gradually increased to 120 ˚C and a pressure of 1.8 bar with agitation for 20 minutes in an autoclave. 7) The HFM was dried at 45 ˚C for 48 h (Ajayi & Akoma, 2017).

In vitro experiment

Measurement of the physiochemical characteristics of FFMs

To measure the pH value and microorganism count on 0, 4, 8, and 12 d of fermentation, ten samples from each FFM were taken. After homogenizing five grams of each sample in 45 mL of distilled water for one minute, the pH of the homogenate was measured using a digital pH meter (Inolab, Germany) (Safari et al., 2016). To determine the microorganism count, 1 g of feed was added to 9 mL of sterile water and homogenized. The supernatants were diluted 10 times with buffered peptone water. Then 100 µL of supernatant were cultured on nutrient agar (Merck, Germany) and incubated for 48 h at 37 ˚C and 30 ˚C for *B. licheniformis* and *B. subtilis*, respectively. Also, *A. niger* was cultured on potato dextrose agar (PDA; Merck, Germany) and incubated for 72 h at 26 ˚C (Belewu et al., 2008; Jazi et al., 2017; Oluseun et al., 2016).

Measurement of antioxidative activity of various HFM

Preparation of various HFM and RFM for antioxidative assay: Various HFM and RFM were prepared using the method described by Jeampakdee et al. (2020). Ten samples from each replicate of HFM (on 0, 4, 8, and 12 d of fermentation for FFM) and RFM were milled and powdered to a particle size of 150-µm. Then, HFM and RFM (0.5 g) were mixed with 10 mL of 20-mmol phosphate buffer (pH=7.2), homogenized (High-Speed Homogenizer, D-500 Pro), and stirred (Magnetic Stirrer C-MAG HS) with 10 mL of 150-mmol NaCl overnight at 4 ˚C. Next, the samples were centrifuged for ten minutes at 10,000× g at 4 ˚C, and clear supernatants were collected. Supernatants were clarified by filtering through 0.45-µm filters (Whatman filter paper, GE, Buckinghamshire, UK) to remove the insoluble substrate. The filtered supernatants were subsequently frozen at −20 ˚C to evaluate antioxidant potency using 2,2-diphenyl-1-picrylhydrazl (DPPH) and 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging activity assays. Samples were measured in triplicate in the laboratory for DPPH and ABTS radical-scavenging activities.

DPPH radical-scavenging activity assay: Methods described by Saisavoey et al. (2019) and Jeampakdee et al. (2020) were used to assay the DPPH radical scavenging activity. Briefly, 100 µmol of a DPPH radical solution in methanol was added to each sample at a ratio of 1:4 (v/v), amounting to 80 µL of the sample with

320 μL of the DPPH radical solution. The mixture was incubated at 25 ˚C for 15 minutes in the dark. After 5 minutes of centrifugation at 12,500× g, the solution was measured for absorbance at 517 nm (A517) using a microplate reader. Ascorbic acid (100 μg/mL) was used as the positive control.

2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical-scavenging activity assay: Methods described by Saisavoey et al. (2019) and Jeampakdee et al. (2020) were employed in the assay for ABTS radical-scavenging activity. Briefly, a 1:1 (v/v) mixture of potassium persulphate (2.45 mmol) and ABTS solution (7 mmol) was placed in the dark and allowed to sit at 25 ˚C for 12 h to produce ABTS cation radicals. Next, the cation radical solution ABTS was diluted to reach an absorbance of 0.7±0.02 at 734 nm (A734). This solution was mixed with the test hydrolysate at a 1:30 (v/v) ratio, requiring 25 μL of the sample and 750 μL of the ABTS cation radical solution. A734 was measured using a microplate reader after a 10-minute incubation period in the dark. Ascorbic acid (100 μg/mL) was used as the positive control.

Calculations of percentage inhibition: The radical scavenging percentage of samples was calculated (Saisavoey et al., 2019; Jeampakdee et al., 2020), as follows (Equation 1):

1. *[(Abs control–Abs blank)–(Abs sample–Abs background)/(Abs control–Abs blank)]×100.*

Abs control is the control's absorbance (no sample), Abs blank is the absorbance of deionized water, Abs sample denotes the various HFM or RFM absorbances, and Abs background shows the level of color absorbance in the samples.

In vivo experiment

Experimental birds and diets

This study was performed at the Poultry Research Station and Nutrition Laboratory of the University of Guilan Province, Rasht City. A total of 480-one-day-old male broiler chickens (Ross 308) were obtained from a commercial hatchery, individually weighed, and allocated to a completely randomized design with eight dietary treatments and five replicates with 12 birds per replicate. The main housing conditions of chickens (chicken density, light regime, microclimate parameters, feeding, and drinking space) were identical for all groups. They corresponded to the Ross 308 broilers raising guide for 2019. The chemical compositions of RFM, and various HFM were analyzed and used for diet formulation. All diets were formulated according to the Ross 308 broiler chicken nutrient requirement guidelines. The following were the experimental treatments: 1) Control diet (based on corn and soybean meal, without FM), 2) diet containing 4% RFM, 3) diet containing 4% HFM by autoclave (Au-HFM), 4) diet containing 4% FFM by *B. licheniformis* (Bl-FFM), 5) diet containing 4% FFM by *B. subtilis* (Bs-FFM), 6) diet containing 4% FFM by *A. niger* (An-FFM), 7) diet containing 4% FFM by *B. licheniformis* + *B. subtilis* + *A. niger* (Co-FFM)", and 8) diet containing 4% HFM by an enzyme (En-HFM). Tables 1, 2, and 3 present the ingredients and chemical compositions of the starter (1–10 days), grower (11–24 days), and finisher (25–42 days) diets. All diets were fed in the mash form, and the birds were provided ad libitum access to feed and water during the study. The experiment lasted for 42 days.

Serum antioxidant capacity

On day 42 of the experiment, three birds from each replicate were selected randomly, and blood samples were collected 4 h after feed withdrawal from the brachial vein. Then blood samples were centrifuged at 3000 × g for 15 minutes, and collected serum samples were kept at -20˚C for further analysis (Hosseinian et al., 2021; Xu et al., 2022). Finally, the serum levels of malondialdehyde (MDA), as well as the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and total antioxidant capacity (TAC), were assessed using commercial kits (Kushanzist Azma- Parseh, Tehran, Iran), according to the manufacturer's instructions. Samples were analyzed in triplicate in the laboratory to determine serum antioxidant capacity.

Meat quality measurements

At the end of the study (day 42), three birds from each pen were slaughtered and breast meat samples were individually sliced, washed in a solution of 0.9% sodium chloride (NaCl), vacuum packaged in plastic bags, and kept frozen at -20 ˚C for 0 (immediately after sampling and before freezing), 7, and 14 days to assess meat quality characteristics. The samples were tested in triplicate in the laboratory to measure the meat quality.

Lipid oxidation: After dilution of 1 g of breast meat sample with 9 mL of ice-cold phosphate-buffered saline (pH=7.2), homogenization and centrifugation at 9000× g for 15 minutes at 4 ˚C were used to obtain muscle homogenates (Alahyaribeik et al., 2022). Clear supernatants of breast meat homogenates were poured into vials to measure MDA. MDA levels were determined using the corresponding diagnostic kits (Kushanzist Azma- Parseh, Tehran, Iran) in compliance with the manufacturer's instructions.

Table 1. Ingredients and nutrient composition of the starter (1–10 days) diets

	Ingredients (%)	Treatments							
		Control	RFM	Au-HFM	Bl-FFM	Bs-FFM	An-FFM	Co-FFM	En-HFM
	Corn	52.97	59.98	59.83	58.93	58.99	58.94	58.76	59.93
	Soybean meal (CP: 44%)	40.12	30.58	30.71	31.58	31.56	31.6	31.8	30.65
	Soybean oil	2.44	0.82	0.86	0.99	0.95	0.97	1.01	0.83
	RFM	0	4	0	0	0	0	0	0
	Au-HFM	0	0	4	0	0	0	0	0
	Bl-FFM	0	0	0	4	0	0	0	0
	Bs-FFM	0	0	0	0	4	0	0	0
	An-FFM	0	0	0	0	0	4	0	0
	Co-FFM	0	0	0	0	0	0	4	0
	En-HFM	0	0	0	0	0	0	0	4
	Dicalcium phosphate	1.9	1.89	1.88	1.86	1.86	1.86	1.86	1.88
	Calcium carbonate	0.95	1	1	1	1	1	1	1
	Sodium bicarbonate	0.21	0.23	0.23	0.21	0.2	0.2	0.19	0.23
	Sodium chloride	0.23	0.15	0.15	0.16	0.16	0.16	0.16	0.15
	Mineral and vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	DL-Met	0.34	0.32	0.31	0.3	0.3	0.3	0.3	0.31
	L-Lys	0.24	0.44	0.43	0.4	0.39	0.39	0.37	0.43
	L-Thr	0.11	0.1	0.1	0.08	0.08	0.08	0.05	0.1
Nutrient composition	ME (kcal/kg)	2900	2900	2900	2900	2900	2900	2900	2900
	Crude protein (%)	22.23	22.23	22.23	22.23	22.23	22.23	22.23	22.23
	Lys (%)	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39
	Met + cys (%)	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04
	Thr (%)	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
	Calcium (%)	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
	Available phosphorus (%)	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
	Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Abbreviations: RFM: Raw feather meal; Au-HFM: Hydrolyzed feather meal by autoclave; Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: Fermented feather meal by *B. subtilis*; An-FFM: Fermented feather meal by *A. niger*; Co-FFM: Fermented feather meal by *B. licheniformis* + *B. subtilis* + *A. niger*; En-HFM: Hydrolyzed feather meal by an enzyme; ME: Metabolizable energy.

Note: Control: A diet based on corn and soybean meal, without FM; Vitamin A: 11000 U; Vitamin D3: 5000 U; Vitamin E: 36.75 U; Vitamin K3: 3.4 mg; Vitamin B1: 1.98 mg; Vitamin B2: 5.25 mg; Pantothenic acid: 10.5 mg; Niacin: 31.5 mg; Vitamin B6: 2.87 mg; Folic acid: 1.2 mg; Vitamin B12: 0.024 mg; Biotin: 0.105 mg; Choline: 800 mg; Manganese: 120 mg; Zinc: 100 mg; Iron: 50 mg; Copper: 12 mg; I: 1.3 mg; Selenium: 0.3 mg; Antioxidant: 100 mg.

Table 2. Ingredients and nutrient composition of the grower (11–24 days) diets

Ingredients (%)	Treatments							
	Control	RFM	Au-HFM	Bl-FFM	Bs-FFM	An-FFM	Co-FFM	En-HFM
Corn	56.51	62.96	63.05	62.43	62.52	62.48	62.15	63.22
Soybean meal (CP: 44%)	35.25	26.74	26.42	26.74	26.69	26.73	27.22	26.22
Corn gluten meal	1	0.31	0.62	1	1	1	0.8	0.71
Soybean oil	3.18	1.82	1.74	1.74	1.7	1.72	1.82	1.68
RFM	0	4	0	0	0	0	0	0
Au-HFM	0	0	4	0	0	0	0	0
Bl-FFM	0	0	0	4	0	0	0	0
Bs-FFM	0	0	0	0	4	0	0	0
An-FFM	0	0	0	0	0	4	0	0
Co-FFM	0	0	0	0	0	0	4	0
En-HFM	0	0	0	0	0	0	0	4
Dicalcium phosphate	1.7	1.69	1.68	1.67	1.66	1.65	1.65	1.69
Calcium carbonate	0.88	0.91	0.92	0.92	0.93	0.93	0.93	0.92
Sodium bicarbonate	0.19	0.2	0.21	0.19	0.19	0.18	0.17	0.21
Sodium chloride	0.24	0.17	0.16	0.17	0.17	0.17	0.17	0.16
Mineral and vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-Met	0.28	0.26	0.25	0.24	0.24	0.24	0.24	0.25
L-Lys	0.2	0.37	0.38	0.35	0.36	0.36	0.33	0.38
L-Thr	0.07	0.07	0.06	0.04	0.04	0.04	0.02	0.06
Nutrient composition								
ME (kcal/kg)	3000	3000	3000	3000	3000	3000	3000	3000
Crude protein (%)	20.81	20.81	20.81	20.81	20.81	20.81	20.81	20.81
Lys (%)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Met + cys (%)	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Thr (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Calcium (%)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Available phosphorus (%)	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Abbreviations: RFM: Raw feather meal; Au-HFM: Hydrolyzed feather meal by autoclave; Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: Fermented feather meal by *B. subtilis*; An-FFM: Fermented feather meal by *A. Niger*; Co-FFM: Fermented feather meal by *B. licheniformis* + *B. subtilis* + *A. niger*; En-HFM: Hydrolyzed feather meal by an enzyme; ME: Metabolizable energy.

Note: Control: A diet based on corn and soybean meal, without FM; Vitamin A: 11 000 U; Vitamin D3: 5000 U; Vitamin E: 36.75 U; Vitamin K3: 3.4 mg; Vitamin B1:1.98 mg; Vitamin B2: 5.25 mg; Pantothenic acid: 10.5 mg; Niacin: 31.5 mg; Vitamin B6: 2.87 mg; folic acid: 1.2 mg; Vitamin B12: 0.024 mg; Biotin: 0.105 mg; Choline: 800 mg; Manganese: 120 mg; Zinc: 100 mg; Iron: 50 mg; Copper: 12 mg; I: 1.3 mg; Selenium: 0.3 mg; Antioxidant: 100 mg.

Table 3. Ingredients and nutrient composition of the finisher (25–42 days) diets

Ingredients (%)	Treatments							
	Control	RFM	Au-HFM	Bl-FFM	Bs-FFM	An-FFM	Co-FFM	En-HFM
Corn	60.24	67.24	67.08	66.18	66.26	66.19	66.02	67.14
Soybean meal (CP: 44%)	31.68	22.15	22.29	23.16	23.12	23.17	23.36	22.23
Soybean oil	4.34	2.72	2.76	2.89	2.85	2.88	2.91	2.75
RFM	0	4	0	0	0	0	0	0
Au-HFM	0	0	4	0	0	0	0	0
Bl-FFM	0	0	0	4	0	0	0	0
Bs-FFM	0	0	0	0	4	0	0	0
An-FFM	0	0	0	0	0	4	0	0
Co-FFM	0	0	0	0	0	0	4	0
En-HFM	0	0	0	0	0	0	0	4
Dicalcium phosphate	1.52	1.5	1.5	1.48	1.48	1.48	1.48	1.5
Calcium carbonate	0.82	0.86	0.86	0.87	0.87	0.86	0.86	0.86
Sodium bicarbonate	0.3	0.33	0.32	0.29	0.29	0.3	0.28	0.34
Sodium chloride	0.13	0.05	0.06	0.07	0.07	0.06	0.07	0.06
Mineral and vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-Met	0.26	0.23	0.23	0.22	0.22	0.22	0.21	0.23
L-Lys	0.17	0.37	0.36	0.32	0.32	0.32	0.3	0.36
L-Thr	0.05	0.04	0.04	0.02	0.02	0.02	0	0.04
Nutrient composition								
ME (kcal/kg)	3100	3100	3100	3100	3100	3100	3100	3100
Crude protein (%)	18.89	18.89	18.89	18.89	18.89	18.89	18.89	18.89
Lys (%)	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
Met + Cys (%)	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Thr (%)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Calcium (%)	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77
Available phosphorus (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16

Abbreviations: RFM: Raw feather meal; Au-HFM: Hydrolyzed feather meal by autoclave; Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: fermented feather meal by *B. subtilis*; An-FFM: Fermented feather meal by *A. niger*; Co-FFM: Fermented feather meal by *B. licheniformis* + *B. subtilis* + *A. niger*; En-HFM: Hydrolyzed feather meal by an enzyme; ME: Metabolizable energy.

Note: Control: A diet based on corn and soybean meal, without FM; Vitamin A: 11 000 U; Vitamin D3: 5000 U; Vitamin E: 36.75 U; Vitamin K3: 3.4 mg; Vitamin B1:1.98 mg; vitamin B2: 5.25 mg; Pantothenic acid: 10.5 mg; Niacin: 31.5 mg; Vitamin B6: 2.87 mg; folic acid: 1.2 mg; Vitamin B12: 0.024 mg; Biotin: 0.105 mg; Choline: 800 mg; Manganese: 120 mg; Zinc: 100 mg; Iron: 50 mg; copper: 12 mg; I: 1.3 mg; Selenium: 0.3 mg; Antioxidant: 100 mg.

pH: After homogenizing the five grams of breast meat for one minute in 45 mL of distilled water, the pH of the homogenate was measured using a digital pH meter (Inolab, Germany) calibrated at pH 4.0 and pH 7.0 (Safari et al., 2016; Partovi et al., 2021).

Water-holding capacity (WHC): Aristides et al.'s method was followed (with small modifications) to determine the WHC (Aristides et al., 2018). Samples of meat were taken from the same location in the breast muscle and cut into 15-g (wet weight) cubes. First, the samples were gently sandwiched between two sheets of filter paper (Whatman Filter Paper, No. 2, 150 mm circle, 1002-150). Following a 5-minute exposure to a 35 kg force using a pressure instrument (Guangzhou Runhu Instrument Co., China), the samples were weighed again. Finally, the WHC percentage was determined using the Equation 2:

2. *WHC (%)=100–{([Initial weight–Final weight]/Initial weight)×100}*.

Immune responses

Anti-sheep red blood cell (SRBC) antibody assay: In the current study, as methods described by Rafat Khafar et al. (2019) and Hosseini-Vashan and Piray. (2021) non-pathogenic antigens of SRBC were used to measure the broiler chickens' humoral immune response. SRBC was collected and diluted in phosphate-buffered saline (PBS; pH 7.5) to obtain a 10% (v/v) suspension. At 28 and 35 d of age, 1 mL of 10% suspension SRBC was intravenously administered to the right wings of three birds per replicate. Seven days after the first and second injections (at 35 and 42 days of age), the same broilers were bled through brachial venipuncture, and 3 mL samples were collected for primary and secondary antibody responses, respectively. Blood samples were centrifuged for 15 minutes (at 2500× g) at 4 ˚C, and the obtained serum was stored at -20 ˚C for further analysis. In the current study, samples were assayed for total anti-SRBC antibody and serum levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) by the hemagglutination assay (HA) test, according to the method previously explained by Rafat Khafar et al. (2019) Finally, antibody titers were reported as log2 of the reciprocal of the highest dilution, giving visible agglutination. The samples were tested in triplicate for the anti-SRBC antibody assay.

Anti-Newcastle disease virus (NDV) antibody assay: In our study, to assay the humoral immune responses of broilers antigens NDV, all the birds were subcutaneously vaccinated in the back of the neck against Newcastle-influenza (H9N2 subtype) with 0.3 mL per chick on 8 days of age. Birds were orally vaccinated against Newcastle disease (Lasota) at 18 days of age. To measure antibody titers against NDV on days 24 and 42, blood samples were taken from the brachial vein of three broilers from each replicate, and the antibody titer production response against NDV was assayed using the hemagglutination inhibition (HI) method (Hosseini-Vashan & Piray, 2021). The samples were measured in triplicate in the laboratory. Finally, the HI antibody titers were expressed as log2 of the reciprocal of the highest dilution, giving visible agglutination.

Heterophile (H) and lymphocyte (L) counts: In the present study, to measure the immune responses of broilers on day 42, blood samples were taken from the brachial vein from three selected birds per replicate in heparinized tubes (containing heparin to avoid blood clot formation). One drop of each blood sample was smeared onto each of the three glass slides to acquire heterophile (H) and lymphocyte (L) counts and H/L ratios. Then May-Grunwald-Giemsa staining was used to prepare blood smears. Then, by counting 100 leukocytes on each slide, the heterophile (H) and lymphocyte (L) levels, as well as the H/L ratio, were determined for each broiler (Jazi et al., 2019).

Statistical analysis

Levene's and Shapiro-Wilk's tests were used for variance homogeneity and normality of distributions, respectively. Data were analyzed in a completely randomized design using the GLM procedures of SAS software (SAS, 2009). Significant differences among the treatment means were determined using Tukey's multiple-range test. Significance was based on P≤0.05.

Results

In vitro experiment

DPPH and ABTS radical scavenging activity assay

As shown in Table 4, the DPPH and ABTS radical scavenging activities, as well as the microorganism count of FFM, significantly increased with increasing fermentation time (P≤0.05). Also, the pH value of FFM significantly decreased by increasing the fermentation time (P≤0.05). As illustrated in Table 5, the DPPH and ABTS radical scavenging activity of FFM was significantly higher than RFM, Au-HFM, and En-HFM (P≤0.05).

Table 4. Antioxidant potential and physiochemical characteristics of various fermented feather meals at different times of fermentation.

Treatments			Fermentation Times (d)				SEM	P
			0	4	8	12		
Antioxidant potential	Bl-FFM	DPPH scavenging (%)	3.17^{d}	13.20^{c}	24.70^{b}	39.44^{a}	2.194	0.001
		ABTS scavenging (%)	4.28^{d}	15.24^{c}	29.24^{b}	47.35^{a}	2.604	0.001
	Bs-FFM	DPPH scavenging (%)	3.17^{d}	13.52^{c}	24.81^{b}	38.58^{a}	2.137	0.001
		ABTS scavenging (%)	4.28^{d}	14.72^{c}	27.73^{b}	46.14^{a}	2.526	0.001
	An-FFM	DPPH scavenging (%)	3.17^{d}	12.87^{c}	23.87^{b}	37.92^{a}	2.104	0.001
		ABTS scavenging (%)	4.28^{d}	14.92^{c}	28.38^{b}	45.96^{a}	2.515	0.001
	Co-FFM	DPPH scavenging (%)	3.17^{d}	14.13^{c}	25.11^{b}	40.11^{a}	2.214	0.001
		ABTS scavenging (%)	4.28^{d}	15.63^{c}	30.36^{b}	49.04^{a}	2.704	0.001
pH value		Bl-FFM	7.00^{a}	6.68^{a}	5.63^{b}	4.45^{c}	0.168	0.001
		Bs-FFM	6.98^{a}	6.73^{a}	5.71^{b}	4.36^{c}	0.174	0.001
		An-FFM	7.02^{a}	6.80^{a}	5.61^{b}	4.38^{c}	0.177	0.001
		Co-FFM	6.98^{a}	6.51^{b}	5.55^{c}	4.25^{d}	0.175	0.001
Microorganisms count, log CFU/g feed		Bl-FFM	6.73^{d}	7.94^{c}	8.97^{b}	9.83^{a}	0.185	0.0001
		Bs-FFM	6.71^{d}	7.96^{c}	8.95^{b}	9.84^{a}	0.187	0.0001
		An-FFM	6.72^{d}	7.95^{c}	8.99^{b}	9.87^{a}	0.188	0.0001
		Co-FFM	6.70^{d}	7.94^{c}	8.98^{b}	9.85^{a}	0.188	0.0001

Abbreviations: Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: Fermented feather meal by *B. subtilis*; An-FFM: fermented feather meal by *A. niger*; Co-FFM: Fermented feather meal by *B. licheniformis*+ *B. subtilis*+ *A. niger*; DPPH: 2,2-diphenyl-1-picrylhydrazl; ABTS: 2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid; 0-day: Initial condition.

[a, b, c, d]Significant difference within groups (P≤0.05).

In vivo experiment

Antioxidant enzymes

As presented in Table 6, the serum levels of TAC, GPX, SOD, and CAT in broilers fed the FFM diet were significantly higher than those in the control, RFM, Au-HFM, and En-HFM diets (P≤0.05). Birds on the En-HFM diet had higher GPX and SOD levels compared to the control and RFM (P≤0.05). Broilers fed Au-HFM diet had higher GPX and SOD levels compared to the control (P≤0.05).

Meat quality and serum MDA

Table 7 presents the effects of various HFM on pH, WHC, and MDA in breast meat and serum MDA concentrations in broilers. The results showed that at 0 d of breast meat storage, broilers fed En-HFM had lower breast meat MDA compared to the control (P≤0.05), while those fed the diet containing FFM had the lowest level of breast meat MDA compared to the control, En-HFM, and other treatments (P≤0.05). Compared with the control and other treatments, broilers fed FFM had lower breast meat MDA levels at 7 and 14 days of storage and lower serum MDA levels (P≤0.05). Broilers fed FFM had higher breast meat pH and WHC at 0, 7, and 14 days of storage compared with the control and other treatments (P≤0.05).

Immune response

Table 8 presents the effects of various HFM on antibody responses against SRBC, NDV, heterophils, and lymphocytes in broilers. At 35 days, broilers fed FFM had higher total anti-SRBC, IgG, and IgM titers than

Table 5. Antioxidant potential of various hydrolyzed feather meals (the 12th day of fermentation in fermented feather meals)

Treatments	%	
	Parameters	
	DPPH Free Radical Scavenging Activity	ABTS Free Radical Scavenging Activity
RFM	3.17[b]	4.28[b]
Au-HFM	4.23[b]	5.15[b]
Bl-FFM	39.44[a]	47.35[a]
Bs-FFM	38.58[a]	46.14[a]
An-FFM	37.92[a]	45.96[a]
Co-FFM	40.11[a]	49.04[a]
En-HFM	5.07[b]	6.11[b]
SEM	2.1	2.514
P	0.001	0.001

Abbreviations: RFM: Raw feather meal; Au-HFM: Hydrolyzed feather meal by autoclave; Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: Fermented feather meal by *B. subtilis*; An-FFM: Fermented feather meal by *A. niger*; Co-FFM: Fermented feather meal by *B. licheniformis*+ *B. subtilis*+ *A. niger*; En-HFM: Hydrolyzed feather meal by an enzyme; DPPH: 2,2-diphenyl-1-picrylhydrazl; ABTS: 2, 2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid.

[a, b] Significant difference among groups (P≤0.05).

Table 6. Effects of various hydrolyzed feather meals on antioxidant enzyme activities in serum of broilers at 42 d of age

Treatments	Antioxidant Enzyme Activities (U/mL)			
	TAC	GPX	SOD	CAT
Control	9.93[b]	210.42[d]	124.93[d]	4.12[b]
RFM	10.23[b]	215.22[cd]	128.06[cd]	4.22[b]
Au-HFM	10.41[b]	223.54[bc]	133.41[bc]	4.25[b]
Bl-FFM	13.82[a]	285.66[a]	173.48[a]	6.88[a]
Bs-FFM	13.70[a]	288.30[a]	173.64[a]	6.92[a]
An-FFM	13.61[a]	279.40[a]	170.08[a]	6.71[a]
Co-FFM	14.01[a]	290.26[a]	176.24[a]	7.02[a]
En-HFM	10.72[b]	230.65[b]	139.08[b]	4.30[b]
SEM	0.226	3.274	2.100	0.126
P	0.001	0.001	0.001	0.001

Abbreviations: RFM: Raw feather meal; Au-HFM: Hydrolyzed feather meal by autoclave; Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: Fermented feather meal by *B. subtilis*; An-FFM: Fermented feather meal by *A. niger*; Co-FFM: Fermented feather meal by *B. licheniformis* + *B. subtilis* + *A. niger*; En-HFM: hydrolyzed feather meal by an enzyme; TAC: Total antioxidant capacity; GPX: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase; Control: Diet based on corn and soybean meal, without feather meal.

[a, b, c, d] Significant difference among groups (P≤0.05).

Table 7. Effects of various hydrolyzed feather meals on MDA concentrations in serum at 42 d of age and breast meat quality of broilers during 14 d of storage

Treat-ments	Breast Meat									Serum
	MDA (nmol/mg of protein)			pH			WHC (%)			
	0 d	7 d	14 d	0 d	7 d	14 d	0 d	7 d	14 d	MDA (nmol/mL)
Control	0.456[a]	0.764[a]	1.211[a]	6.11[b]	5.86[b]	5.53[b]	82.15[b]	75.46[b]	65.46[b]	6.12[a]
RFM	0.451[ab]	0.762[a]	1.196[a]	6.12[b]	5.88[b]	5.56[b]	82.18[b]	76.12[b]	66.49[b]	6.10[a]
Au-HFM	0.445[ab]	0.753[a]	1.178[a]	6.15[b]	5.89[b]	5.56[b]	83.17[b]	76.42[b]	68.27[b]	5.98[a]
Bl-FFM	0.280[c]	0.442[b]	0.668[b]	6.84[a]	6.60[a]	6.32[a]	94.12[a]	89.17[a]	86.19[a]	2.88[b]
Bs-FFM	0.278[c]	0.446[b]	0.664[b]	6.85[a]	6.61[a]	6.35[a]	94.02[a]	90.41[a]	85.66[a]	2.83[b]
An-FFM	0.283[c]	0.452[b]	0.678[b]	6.81[a]	6.58[a]	6.29[a]	93.04[a]	89.04[a]	85.73[a]	2.94[b]
Co-FFM	0.275[c]	0.439[b]	0.653[b]	6.87[a]	6.64[a]	6.35[a]	95.10[a]	91.11[a]	88.55[a]	2.80[b]
En-HFM	0.441[b]	0.745[a]	1.171[a]	6.17[b]	5.92[b]	5.60[b]	85.58[b]	78.20[b]	70.73[b]	5.98[a]
SEM	0.007	0.014	0.024	0.033	0.034	0.036	0.697	0.790	1.073	0.146
P	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Abbreviations: RFM: Raw feather meal; Au-HFM: Hydrolyzed feather meal by autoclave; Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: Fermented feather meal by *B. subtilis*; An-FFM: Fermented feather meal by *A. niger*; Co-FFM: Fermented feather meal by *B. licheniformis* + *B. subtilis* + *A. niger*; En-HFM: Hydrolyzed feather meal by an enzyme; MDA: Malondialdehyde; WHC: Water-holding capacity; Control: Diet based on corn and soybean meal, without feather meal.

[a, b, c] Significant difference among groups (P≤0.05).

the control and other treatments (P≤0.05). At 42 days, birds fed the En-HFM diet had a higher total anti-SRBC titer compared to the control (P≤0.05), while those fed FFM had the highest total anti-SRBC titer than the control, En-HFM, and other groups (P≤0.05). At 42 days, broilers fed FFM had higher IgG and IgM titers than the control and other treatments (P≤0.05). Broilers fed FFM had higher antibody titers against NDV at 24 and 42 days than the control and other treatments (P≤0.05). Birds on the FFM diet had lower heterophil count and heterophil-to-lymphocyte ratio compared to the control and other treatments (P≤0.05). No significant changes were observed in lymphocyte counts among the different treatment groups (P>0.05).

Discussion

In vitro experiment

The physiochemical characteristics of FFMs: The fermentation process results in the growth of many fermenter microorganisms, which produce a high amount of organic acids, reducing the pH of fermented feed (Engberg et al., 2009). This is consistent with the observation from the current study, which exhibited a decrease in pH and an increase in microorganism count with increasing fermentation time.

DPPH and ABTS radical scavenging activity assay: In the current study, the in vitro antioxidant capacities of various HFM were assessed using DPPH and ABTS assays. The results of the current study are consistent with the results of the previous studies, which demonstrated that fermented feathers (Callegaro et al., 2018; Bezus et al., 2021; Prajapati et al., 2021; Kshetri et al., 2022; Abdelmoteleb et al., 2023; Pei et al., 2023) as well as oligopeptides and peptides produced from fermented feathers (Huang et al., 2022) had a significantly higher antioxidant activity with DPPH and ABTS radical scavenging activity than the control and RFM. The DPPH is a strong free radical that is scavenged when it comes into contact with a proton-donating material, such as an antioxidant, and its purple color changes to yellow, resulting in a decrease in absorbance (Ben Hamad Bouhamed et al., 2020). Various mechanisms, such as radical scavenging, chelation, reduction of metal ions, or a combination of these processes, can provide protein hydrolysates with antioxidant properties. In the DPPH assay, the lat-

Table 8. Effects of various hydrolyzed feather meals on antibody response against SRBC, (log2), NDV, (log2), heterophil, and lymphocyte in broilers

Treatments	SRBC Titer at 35 d of Age			SRBC Titer at 42 d of Age			Antibody Titer Against NDV		Leucocytes Count 42 d of Age		
	Total Anti-SRBC	IgG	IgM	Total Anti-SRBC	IgG	IgM	24 d	42 d	H (%)	L (%)	H:L Ratio
Control	3.13^{b}	1.53^{b}	1.60^{b}	4.33^{c}	2.20^{b}	2.13^{b}	5.00^{b}	3.13^{b}	33.20^{a}	51.33	0.654^{a}
RFM	3.33^{b}	1.66^{b}	1.66^{b}	4.66^{bc}	2.33^{b}	2.33^{b}	5.13^{b}	3.26^{b}	36.13^{a}	52.26	0.696^{a}
Au-HFM	3.4^{b}	1.66^{b}	1.73^{b}	4.66^{bc}	2.46^{b}	2.20^{b}	5.13^{b}	3.33^{b}	34.33^{a}	52.40	0.664^{a}
Bl-FFM	7.33^{a}	4.13^{a}	3.20^{a}	9.33^{a}	5.33^{a}	4.00^{a}	8.33^{a}	5.53^{a}	19.13^{b}	55.13	0.353^{b}
Bs-FFM	7.46^{a}	4.13^{a}	3.33^{a}	9.33^{a}	5.13^{a}	4.20^{a}	8.26^{a}	5.46^{a}	19.20^{b}	54.33	0.357^{b}
An-FFM	7.06^{a}	4.26^{a}	2.80^{a}	9.00^{a}	5.06^{a}	3.93^{a}	8.13^{a}	5.33^{a}	20.06^{b}	54.06	0.374^{b}
Co-FFM	7.60^{a}	4.40^{a}	3.20^{a}	9.60^{a}	5.33^{a}	4.26^{a}	8.46^{a}	5.60^{a}	18.20^{b}	55.13	0.336^{b}
En-HFM	3.4^{b}	1.66^{b}	1.73^{b}	5.00^{b}	2.66^{b}	2.33^{b}	5.20^{b}	3.33^{b}	34.26^{a}	52.33	0.662^{a}
SEM	0.193	0.126	0.084	0.220	0.135	0.105	0.157	0.115	0.736	0.556	0.015
P	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.307	0.001

Abbreviations: RFM: Raw feather meal; Au-HFM: Hydrolyzed feather meal by autoclave; Bl-FFM: Fermented feather meal by *B. licheniformis*; Bs-FFM: Fermented feather meal by *B. subtilis*; An-FFM: Fermented feather meal by *A. niger*; Co-FFM: Fermented feather meal by *B. licheniformis* + *B. subtilis* + *A. niger*; En-HFM: Hydrolyzed feather meal by an enzyme; SRBC: Sheep red blood cell; IgG: Immunoglobulin G; IgM: Immunoglobulin M; H: Heterophile; L: Lymphocyte; H/L ratio: Heterophile to lymphocyte ratio; Control: Diet based on corn and soybean meal, without feather meal.

[a, b, c] Significant difference among groups ($P \le 0.05$).

ter becomes stable when an antioxidant transfers a hydrogen atom to a DPPH radical (Callegaro et al., 2018). An electron-donating (antioxidant) molecule decreases the pre-formed cation (ABTS*+) to ABTS in the ABTS radical-scavenging assay, a decolorization technique that results in reduced absorbance (Callegaro et al., 2018). According to reports, electron and hydrogen atom transfer are antioxidant mechanisms that can be reflected by the ABTS method (Callegaro et al., 2018). Therefore, loss of absorbance reflects the antioxidant activity of the compound.

Studies have shown that the peptide chain length, molecular weight, and amino acid sequence are connected to the antioxidant potential and scavenging ability of DPPH and ABTS free radicals (Pei et al., 2023). Feather keratin hydrolyzed by microbes contains low-molecular-weight free amino acids and small peptides that donate electrons to free radicals to create more stable products (Ben Hamad Bouhamed et al., 2020). The low molecular weight of peptides can increase their ability to interact more readily with radicals, resulting in increased antioxidant activity and hindering oxidization (Jeampakdee et al., 2020). As part of the precursor protein, these peptides are inactive; they only become active when they are released via hydrolytic cleavage and converted to bioactive peptides (Callegaro et al., 2018). Since aromatic and hydrophobic amino acids promote interactions with DPPH and ABTS, there is a tendency for protein hydrolysis to increase ionizable groups and produce hydrophobic or aromatic groups, which have a high degree of antioxidant potential (Callegaro et al., 2018; Pei et al., 2023). This is due to the peptide sequences' hydrophobic amino acid residues can improve peptide solubility at the water-lipid interface, enabling more interaction with any radical species that may be present (Callegaro et al., 2018; Pei et al., 2023). About 50% of the amino acid residues in feather keratins' sequences are hydrophobic or aromatic (Pei et al., 2023). This explains why unfermented FM exhibits DPPH and ABTS radical-scavenging activities. The arrangement of the amino acids within the peptide sequence is one element that influences the antioxidant activity of peptides (Pei et al., 2023). For example, His located at the C-terminal functions as an efficient various radicals scavenger, whereas His located at the N-terminal functions as an efficient chelator of metal ions (Jeampakdee et al., 2020). In the meantime, tripeptides with Tyr or Trp at the C-terminal function as potent radical scavengers (Jeampakdee et

al., 2020). Diverse proteases, which are highly active across a wide pH range and primarily classified as serine proteases that cleave peptide bonds near aromatic and/or hydrophobic residues, are produced by bacteria and fungi during their growth on feather media (Pei et al., 2023). In addition, due to microbial fermentation breaking down the feather keratin's disulfide bonds, cysteine, which has strong antioxidant properties, is created. Since feather peptide's cysteine-SH is a potent hydrogen donor to free radicals, cysteine has antioxidative activity (Prajapati et al., 2021). Sulfenic acid (–SOH) and sulfhydryl (–SH) are yet another prime antioxidant in feather keratin hydrolysate, which are produced when the feather is under microbial fermentation process (Prajapati et al., 2021). Reductones in fermented feathers have antioxidant activity due to radical chain breaking by donating a hydrogen atom. Free radicals can react with hydrogen or electron-donating amino acids and peptides to be stabilized (Bezus et al., 2021).

In vivo experiment

Antioxidant enzymes

The quick induction of protective antioxidant enzymes is necessary for cells to maintain functional homeostasis. GPX, SOD, and CAT enzymes, which constitute the first barrier in the antioxidant defense system, are crucial in protecting cells and tissues from the damaging effects of free radicals by scavenging oxygen free radicals via a chain reaction mechanism (Xu et al., 2022; Obaid Saleh et al., 2022). The dismutation of superoxide anions to hydrogen peroxide, the breakdown of H_2O_2 and hydroperoxides derived from unsaturated fatty acids at the expense of reduced glutathione, and the conversion of H_2O_2 into water are all catalyzed by the SOD, GPX, and CAT (Liu et al., 2020). Our results are similar to those of the previous studies, which reported that FFM and other fermented feeds increased the content of TAC, GPX, SOD, and CAT in the serum, muscle, and liver of broilers (Liu et al., 2020; Ibrahim et al., 2021; Xu et al., 2022), piglets (Gu et al., 2021), and laying hen chicks (Zhu et al., 2020). There are several potential methods by which FFM enhances the broiler's antioxidant status. (1) The increased antioxidant capacity of the fermented feed can be attributed to the production of lactic acid, small and bioactive peptides, and antioxidant vitamins during fermentation (Sugiharto et al., 2019). (2) Microbial enzymes hydrolyze phenolic compounds, such as phenolic glycosides, produced by microorganisms during fermentation and release free aglycones, which have the potential for high antioxidant activity (Ibrahim et al., 2021). (3) Keratinase produced by microorganisms can enhance the digestibility of minerals (Xu et al., 2022), which might improve the utilization of selenium. The activity of GPX as a selenium-dependent enzyme can be improved by increasing selenium utilization (Xu et al., 2022). (4) As well as keratinase of microorganisms can improve the hydrolysis of the proteins containing cysteine to release cysteine, which is a precursor of glutathione, thus increasing the body's synthesis of glutathione (Huang et al., 2022).

Meat quality

pH

Our results are consistent with those of previous studies, which reported that fermented feeds, including FFM, significantly increased the pH value of the breast and thigh meat of broilers (Liu et al., 2020). The pH value of breast meat in broilers fed FFM was higher than that of the broilers from the control and unfermented FM groups, which indicates a positive correlation between strong antioxidant capacity and higher meat pH in broilers. The postmortem conversion of muscle glycogen to lactic acid primarily determines the pH value, one of the most significant indices of meat quality. In other words, the pH value directly reflects the meat acidity (Xu et al., 2022). Normally, the muscle cells in a broiler chicken that has been exsanguinated and subsequently suffer hypoxia eventually have to turn to anaerobic glycolysis to maintain metabolic activities. This is the only energy source for the postmortem muscles (Aristides et al., 2018). Under these conditions, body glycogen stores are depleted as they are converted to energy and lactic acid, reducing the pH (Aristides et al., 2018). However, an antioxidant can neutralize the H^+ cation by direct reduction via electron donation and prevent pH reduction and acidification of the meat (Callegaro et al., 2018). The pH decrease leads to meat myofibrillar protein denaturation, which impairs the proteins' ability to hold onto water inside the cell, tenderness, and color, thereby creating economic losses by decreasing carcass quality and reducing production efficiency in the poultry industry (Aristides et al., 2018; Majidi et al., 2023).

Water-holding capacity

Consistent with the results of the current study, other studies have also shown that fermented feed increases the WHC in muscles, including the breast and thigh of broilers (Liu et al., 2020; Xie et al., 2021). The results of the present study showed that WHC was consistent with the pH values. The improved pH and WHC of muscle can be related to the enhanced antioxidative status of

broilers fed FFM. Loss of soluble nutrients, poor flavor, and drier and tougher meat can result from increased liquid outflow in muscles with low WHC (Xie et al., 2021). The inverse correlation between meat moisture and intramuscular fat, directly related to meat juiciness, could cause elevated WHC in muscle meat (Liu et al., 2020; Xie et al., 2021). Since unsaturated fatty acids comprise the cell membrane, oxidation status and meat WHC are closely associated. Membrane fluidity and structure are specifically affected by the oxidation of these fatty acids (Xie et al., 2021). Muscle WHC is reduced due to altered cell permeability due to the oxidation of the membrane phospholipids. High WHC in meat indicates low water loss, which can benefit meat processing, value, and appearance for consumers (Liu et al., 2020). The higher levels of antioxidants in broiler meat, which demonstrated potent free-radical scavenging activity and decreased oxidatively induced conformational changes and myofibrillar protein fragmentation, may be related to the higher WHC in meat (Xie et al., 2021). Furthermore, the denaturation of sarcoplasmic proteins to myofibrils may affect the WHC in meat (Liu et al., 2020).

MDA of muscle and serum

Compared to other meats, chicken meat has a comparatively high concentration of polyunsaturated fatty acids, such as the crucial n-3 fatty acids, and is more susceptible to free radical damage (Safari et al., 2016). In the current study, FFM feeding improved broiler meat quality, as reflected by reduced meat postmortem MDA content. These results are consistent with other research, which found that FFM or other fermented feeds decreased MDA amount in the serum (Drażbo et al., 2018; Gu et al., 2021; Xu et al., 2021; Elbaz et al., 2023), and muscle including thigh and breast of broilers, egg yolk homogenate, turkeys, and piglets (Aristides et al., 2018; Ibrahim et al., 2021; Alahyaribeik et al., 2022; Xu et al., 2022). MDA, a crucial product of lipid peroxidation, indicates the degree of lipid peroxidation and the generation of free radicals by reactive oxygen species (Safari et al., 2016; Gholipour-Shoshod et al., 2023; Al-Abdaly et al., 2023). Also, lipid peroxidation damages the integrity of the muscle cell membrane, which can cause meat to lose nutrients and exudate (Safari et al., 2016). Moreover, reactive oxygen species harm biological macromolecules, such as proteins and nucleic acids. Lipid peroxidation produces MDA, which can be incorporated into proteins through interactions with biomolecules. This can lead to the generation of carbonyl derivativeswith cytotoxic and genotoxic impacts (Zhu et al., 2020). The possible reasons for decreasing MDA through FFM in broilers are as follows: 1) The activity of microbial proteases during the fermentation process changes substrate proteins in a manner that exposes the more active R groups of amino acids. Thus, bioactive peptides present in fermented products display greater antioxidant activity (Alahyaribeik et al., 2022). Also, bioactive peptides could prevent the accumulation of cholesterol in meat (Alahyaribeik et al., 2022), which is sensitive to oxidation. 2) Additionally, bioactive polysaccharides that have antioxidant properties may be produced by fermentor microorganisms, particularly fungi and bacteria (Sugiharto et al., 2019). 3) Live microorganisms in the fermented feed, including lactic acid bacteria, fungi, and *Bacillus* spp., help maintain the balance of antioxidants and pro-oxidants in chickens (Sugiharto et al., 2019). 4) The antioxidant enzymes produced during fermentation inhibit lipoxygenase enzymes, improve oxidative stability, and increase meat quality and shelf life (Aristides et al., 2018; Ibrahim et al., 2021; Alahyaribeik et al., 2022; Xu et al., 2022).

Immune SRBC

Previous studies have demonstrated that FFM or other fermented feeds significantly increase total anti-SRBC, IgG, and IgM levels in broilers (Liu et al., 2020; Xie et al., 2021; Xu et al., 2021), laying hen chicks (Zhu et al., 2020), and pigs (Huang et al., 2022). The three main immunoglobulin classes in poultry are IgG, IgM, and IgA (Zhu et al., 2020), produced by B cells (Xu et al., 2021). It has been established that animal immunity and disease resistance are positively correlated with antibody contents (IgA, IgM, and IgG) (Xie et al., 2021). In other words, during acquired immunity, blood cells derived from B cells produce IgA, IgM, and IgG immunoglobulins in response to pathogen attack (Drażbo et al., 2018). IgM is the primary antibody isotype in the primary antibody response (Drażbo et al., 2018). There are several possible mechanisms by which FFM increases serum concentrations of total anti-SRBC, IgG, and IgM. The formation of small-sized peptides and bioactive compounds during feed fermentation can improve the humoral immune response of animals by stimulating B cell proliferation and immunoglobulin production (Zhu et al., 2020; Xie et al., 2021). Additionally, microbial fermentation can produce a variety of advantageous compounds, including organic acids, vitamins, and exoenzymes, all of which can strengthen an animal's immune function (Gu et al., 2021). In addition to increasing lactic acid bacteria in the gut, the use of fermented protein feeds can stimulate immune cells to produce Th2 cytokines, such as interleukin-4 (IL-4) and interleukin-10 (IL-10), which in turn promote B cell development and antibody production (Chang et al., 2022). Moreover, the immune status of animals is strongly related to their antioxidant

capacity. The FFM has strong antioxidant activity; this property helps improve immune functions and reduce oxidative stress by eliminating harmful free radicals produced by environmental stressors or normal cellular activity (Huang et al., 2022).

Antibody titer against Newcastle disease virus (NDV)

Similar outcomes to the present study's results have been reported in previous studies, which have indicated that fermented feed significantly increases antibody titers against NDV in broilers (Salehi et al., 2021; Elbaz et al., 2023). The generally suggested mechanisms of action for improving antibody titers against NDV by FFM may be due to the following reasons. Since FFM has an antioxidant capacity, antioxidase can enhance immunity by encouraging bacterial clearance and controlling phagocyte numbers (Gu et al., 2021). Lactic and acetic acids produced by bacteria in fermented feed result in an acidic environment. Acidic molecules can permeate pathogen bacteria's cell membranes and increase their acidity, disrupting enzymatic processes, killing pathogenic bacteria, and modulating the immune response of poultry (Jazi et al., 2019). Live microbes in fermented feeds can function as probiotics and stabilize intestinal inflammation by balancing the intestinal microflora, preserving the mucosal barrier, and regulating and improving the intestinal mucosal immune system response (Chang et al., 2022; Shihab & Nafea, 2023).

Heterophil and lymphocyte

Consistent with the current study, it has been reported that fermented feed significantly decreases the heterophil-to-lymphocyte ratio and increases the antibody titer against phytohaemagglutinin injection (Ashayerizadeh et al., 2017; Jazi et al., 2019). The heterophil-to-lymphocyte ratio is a reliable indicator of nutritional or environmental stress, as well as the efficiency of the body's immune response in poultry, and this ratio increases during stress and/or illnesses (Ashayerizadeh et al., 2017; Jazi et al., 2019). Heterophils are polymorphonuclear poultry cells essential to the innate immune system (Jazi et al., 2019). Increasing the number of heterophils indicates the induction of innate immune responses to combat pathogenic bacteria via phagocytosis and antimicrobial activities, which include the production of proteolytic enzymes, reactive oxygen and nitrogen species, and antimicrobial peptides (Jazi et al., 2019). Under normal circumstances, most white blood cells are lymphocytes produced in lymphoid tissues, including the spleen, thymus, and lymph glands, and are involved in both humoral immunity and cells (Ashayerizadeh et al., 2017). The improvement in the heterophil count and heterophil-to-lymphocyte ratio by FFM may be due to the following reasons. Fermented feed has the potential to boost intestinal mucosal immunity and induce recirculating antibodies in broilers, which can lower the heterophile-to-lymphocyte ratio and reduce oxidative stress (Chang et al., 2022). Increasing lactic acid bacteria in the gut due to using fermented feeds stimulates mucin production and activates the immune responses to create a barrier of defense against pathogenic bacteria (Jazi et al., 2019). Furthermore, *B. subtilis* and *B. licheniformis* can, with bactericidal and bacteriostatic properties, compete with pathogens, balance intestinal microbiota, and enhance immunity in broilers (Xu et al., 2021). Feeding fermented feeds to broilers has been shown to increase antibody-mediated and cell-mediated immune responses (Chang et al., 2022).

Conclusion

In vitro experiments of the current study demonstrated that the fermentation of FM increased its antioxidant activity, with a high performance recorded in DPPH and ABTS assays for free radical scavenging activity. In vivo experiments also showed that it improved the broiler's body antioxidative status and contributed to the improved antioxidant enzymes and immune response of broilers, which resulted in increased antibody titers against NDV and SRBC, as well as decreased heterophil count and heterophil-to-lymphocyte ratio. Additionally, this study illustrated that including FFM in broiler diets led to an increase in meat quality by increasing WHC and pH and reducing the lipid oxidation of meat, which was reflected in the decreased MDA and increased shelf life of the product. The current study also suggests that the fermentation process through the bioreactor on an industrial scale is a sustainable method to remove the vast feather waste biomass and upgrade its feeding value to produce valuable feed ingredients from unconventional and cheaper components.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were performed following the standards outlined in the University of Guilan's Animal Welfare, Ethics, Sample Collection, and Experimentation Committee guidelines, Rasht, Iran (Code: 94131/p97).

Funding

This study was conducted as part of the PhD dissertation of Hassan Safari, approved by the Department of Animal Science, Faculty of Agriculture Science, University of Guilan, Rasht, Iran. This research did not receive specific grants from public, commercial, or not-for-profit funding agencies.

Authors' contributions

Project administration, and writing the original draft: Hassan Safari; Conceptualization, investigation, methodology, formal analysis, data curation, software, supervision, review and editing: Hassan Safari, Ardeshir Mohit, and Maziar Mohiti-Asli.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors thank the University of Guilan, Rasht, Iran for its cooperation.

References

Abdelmoteleb, A., Gonzalez-Mendoza, D., Tzintzun-Camacho, O., Grimaldo-Juárez, O., Mendez-Trujillo, V., & Moreno-Cruz, C., et al. (2023). Keratinases from Streptomyces netropsis and Bacillus subtilis and their potential use in the chicken feather degrading. *Fermentation, 9*(2), 96. [DOI:10.3390/fermentation9020096]

Ajayi, H., & Akoma, O. (2017). Performance and carcass characteristics of broiler chickens fed low protein diets containing hydrolysed feather meal, with or without protease supplementation. *BIU Journal of Basic and Applied Sciences, 3*(1), 20-30. [Link]

Al-Abdaly, Y. Z., Alfathi, M. Y., & Al-mahmood, S. S. (2023). Comparison of azithromy cin toxicity in chickens and quails. *Iranian Journal of Veterinary Medicine, 17*(4), 321-332. [DOI:10.32598/IJVM.17.4.1005354]

Alahyaribeik, S., Nazarpour, M., Tabandeh, F., Honarbakhsh, S., & Sharifi, S. D. (2022). Effects of bioactive peptides derived from feather keratin on plasma cholesterol level, lipid oxidation of meat, and performance of broiler chicks. *Tropical Animal Health and Production, 54*(5), 271. [DOI:10.1007/s11250-022-03244-1] [PMID]

Al-Muhammadawi, N. A., & Jassim Hammoudi, S. (2022). Effect of adding different levels of therapeutic curcuma on productive traits in broiler chickens. *Archives of Razi Institute, 77*(6), 2059-2064. [PMID]

Aristides, L. G. A., Venancio, E. J., Alfieri, A. A., Otonel, R. A. A., Frank, W. J., & Oba, A. (2018). Carcass characteristics and meat quality of broilers fed with different levels of Saccharomyces cerevisiae fermentation product. *Poultry Science, 97*(9), 3337–3342. [DOI:10.3382/ps/pey174] [PMID]

Ashayerizadeh, A., Dastar, B., Shams Shargh, M., Sadeghi Mahoonak, A., & Zerehdaran, S. (2017). Fermented rapeseed meal is effective in controlling Salmonella enterica serovar Typhimurium infection and improving growth performance in broiler chicks. *Veterinary Microbiology, 201,* 93-102. [DOI:10.1016/j.vetmic.2017.01.007] [PMID]

Belewu, M., Asafa, A., & Ogunleke, F. (2008). Processing of feather meal by solid state fermentation. *Biotechnology, 7*(3), 589-591. [DOI:10.3923/biotech.2008.589.591]

Ben Hamad Bouhamed, S., Krichen, F., & Kechaou, N. (2020). Feather protein hydrolysates: A study of physicochemical, functional properties and antioxidant activity. *Waste and Biomass Valorization, 11,* 51-62. [DOI:10.1007/s12649-018-0451-2]

Bezus, B., Ruscasso, F., Garmendia, G., Vero, S., Cavello, I., & Cavalitto, S. (2021). Revalorization of chicken feather waste into a high antioxidant activity feather protein hydrolysate using a novel psychrotolerant bacterium. *Biocatalysis and Agricultural Biotechnology, 32,* 101925. [DOI:10.1016/j.bcab.2021.101925]

Callegaro, K., Welter, N., & Daroit, D. J. (2018). Feathers as bioresource: Microbial conversion into bioactive protein hydrolysates. *Process Biochemistry, 75,* 1-9. [DOI:10.1016/j.procbio.2018.09.002]

Chang, Y., Omer, S. H. S., Li, G., Lian, H., & Liu, Y. (2022). Research advance on application of microbial fermented fodder in broilers production: A short review. *Open Journal of Animal Sciences, 12*(2), 200-209. [DOI:10.4236/ojas.2022.122015]

Drazbo, A., Ognik, K., Zaworska, A., Ferenc, K., & Jankowski, J. (2018). The effect of raw and fermented rapeseed cake on the metabolic parameters, immune status, and intestinal morphology of turkeys. *Poultry Science, 97*(11), 3910-3920. [DOI:10.3382/ps/pey250] [PMID]

Elbaz, A. M., El-Sheikh, S. E., & AbdelMaksoud, A. (2023). Growth performance, nutrient digestibility, antioxidant state, ileal histomorphometry, and cecal ecology of broilers fed on fermented canola meal with and without exogenous enzymes. *Tropical Animal Health and Production, 55*(1), 46. [DOI:10.1007/s11250-023-03476-9] [PMID]

Engberg, R. M., Hammershøj, M., Johansen, N. F., Abousekken, M. S., Steenfeldt, S., & Jensen, B. B. (2009). Fermented feed for laying hens: Effects on egg production, egg quality, plumage condition and composition and activity of the intestinal microflora. *British Poultry Science, 50*(2), 228-239. [DOI:10.1080/00071660902736722] [PMID]

Fakhfakh, N., Ktari, N., Haddar, A., Mnif, I. H., Dahmen, I., & Nasri, M. (2011). Total solubilisation of the chicken feathers by fermentation with a keratinolytic bacterium, Bacillus pumilus A1, and the production of protein hydrolysate with high antioxidative activity. *Process Biochemistry, 46*(9), 1731-1737. [DOI:10.1016/j.procbio.2011.05.023]

Gholipour-Shoshod, A., Rahimi, S., Zahraei Salehi, T., Karimi Torshizi, M. A., Behnamifar, A., & Ebrahimi, T., et al. (2023). Evaluating the competitiveness of medicinal plants with antibiotics to control salmonella enterica serovar typhimurium in broiler chickens. *Iranian Journal of Veterinary Medicine, 17*(2), 155-166. [DOI:10.32598/IJVM.17.2.1005233]

Gu, X., Li, Z., Wang, J., Chen, J., Jiang, Q., & Liu, N., et al. (2021). Fermented cottonseed meal as a partial replacement for soybean meal could improve the growth performance, immunity and antioxidant properties, and nutrient digestibility by altering the gut microbiota profile of weaned piglets. *Frontiers in Microbiology, 12,* 734389. [DOI:10.3389/fmicb.2021.734389] [PMID]

Hosseinian, A., Abdi Hachesoo, B., Nazifi, S., Hashemi Hezaveh, A., Hashemi Tabar, H., & Rezapoor, R., A. (2021). Cardioprotective and hepatoprotective activity of silymarin in broiler chickens fed on mash and pellet diets. *Iranian Journal of Veterinary Medicine, 15*(1), 104-121. [DOI:10.22059/ijvm.2020.302033.1005084]

Hosseini-Vashan, S. J., & Piray, A. H. (2021). Effect of dietary saffron (Crocus sativus) petal extract on growth performance, blood biochemical indices, antioxidant balance, and immune responses of broiler chickens reared under heat stress conditions. *Italian Journal of Animal Science, 20*(1), 1338-1347. [DOI:10.1080/1828051X.2021.1921628]

Huang, H. J., Weng, B. C., Lee, Y. S., Lin, C. Y., Hsuuw, Y. D., & Chen, K. L. (2022). The effects of two-stage fermented feather meal-soybean meal product on growth performance, blood biochemistry, and immunity of nursery pigs. *Fermentation, 8*(11), 634. [DOI:10.3390/fermentation8110634]

Huang, Y., Liu, X., Ran, Y., Cao, Q., Zhang, A., & Li, D. (2019). Production of feather oligopeptides by a newly isolated bacterium Pseudomonas otitis H11. *Poultry Science,* 10.3382/ps/pez030. Advance online. [PMID]

Ibrahim, D., Moustafa, A., Shahin, S. E., Sherief, W. R. I. A., Abdallah, K., & Farag, M. F. M., etal. (2021). Impact of fermented or enzymatically fermented dried olive pomace on growth, expression of digestive enzyme and glucose transporter genes, oxidative stability of frozen meat, and economic efficiency of broiler chickens. *Frontiers in Veterinary Science, 8,* 644325. [DOI:10.3389/fvets.2021.644325] [PMID]

Jazi, V., Boldaji, F., Dastar, B., Hashemi, S. R., & Ashayerizadeh, A. (2017). Effects of fermented cottonseed meal on the growth performance, gastrointestinal microflora population and small intestinal morphology in broiler chickens. *British Poultry Science, 58*(4), 402–408. [DOI:10.1080/00071668.2017.1315051] [PMID]

Jazi, V., Mohebodini, H., Ashayerizadeh, A., Shabani, A., & Barekatain, R. (2019). Fermented soybean meal ameliorates Salmonella Typhimurium infection in young broiler chickens. *Poultry Science, 98*(11), 5648-5660. [DOI:10.3382/ps/pez338] [PMID]

Jeampakdee, P., Puthong, S., Srimongkol, P., Sangtanoo, P., Saisavoey, T., & Karnchanatat, A. (2020). The apoptotic and free radical-scavenging abilities of the protein hydrolysate obtained from chicken feather meal. *Poultry Science, 99*(3), 1693-1704. [DOI:10.1016/j.psj.2019.10.050] [PMID]

Kshetri, P., Singh, P. L., Chanu, S. B., Singh, T. S., Rajiv, C., & Tamreihao, K., et al. (2022). Biological activity of peptides isolated from feather keratin waste through microbial and enzymatic hydrolysis. *Electronic Journal of Biotechnology, 60,* 11-18. [DOI:10.1016/j.ejbt.2022.08.001]

Liu, S., Yan, W., Ma, C., Liu, Y., Gong, L., & Levesque, C., et al. (2020). Effects of supplemented culture media from solid-state fermented Isaria cicadae on performance, serum biochemical parameters, serum immune indexes, antioxidant capacity and meat quality of broiler chickens. *Asian-Australasian Journal of Animal Sciences, 33*(4), 568-578. [DOI:10.5713/ajas.19.0179] [PMID]

Majdi Abdelfaraj, K., Mohamed Idris, A., & Mohamed M, I. (2023). Effect of diet supplemented with different levels of moringa powder on growth performance, carcass characteristics, meat quality, hematological parameters, serum lipids, and economic efficiency of broiler chickens. *Archives of Razi Institute, 78*(5), 1647–1656. [DOI:10.32592/ARI.2023.78.5.1647] [PMID]

Mohammed Awad, A. (2023). Effect of supplementation of diets with L-Carnitine and Methionin on broiler chicken productive performance. *Archives of Razi Institute, 78*(1), 345-352. [PMID]

Morovati, S., Bassami, M. R., Kalidari, G. A., Tavassoli, A., Razmyar, J., & Seno, M. M. G. (2022). Characterization of the Full length P and M genes in a Newcastle Disease Virus Isolated from Chicken Farms in Northeast of Iran Sarcoid. *Iranian Journal of Veterinary Medicine, 16*(2), 126-143. [DOI:10.22059/ijvm.2021.315021.1005144]

Obaid Saleh, R., Jawdat Abdul-Abbas, S., Naji Aziz, S., Ghazi Al-Shawi, S., Yacoob Yousif, A., & Ahmed Hamza, T., et al. (2022). Evaluation of the effect of peppermint extract and probiotics on biochemical factors in the blood of ascites-induced chickens. *Archives of Razi Institute, 77*(6), 2243-2250. [PMID]

Oluseun, A. I., Oluwaseun, A. C., Ogundipe, K., & Ndudi, O. S. (2016). Chemical composition and amino acid profile of differently processed feather meal. *Journal of Agricultural Sciences (Belgrade), 61*(3), 237-246. [DOI:10.2298/JAS1603237A]

Partovi, R., Seifi, S., Samakkhah, S. A., & Nikpay, A. (2021). Appraisal of dietary prebiotic supplementation on meat properties and carcass characteristics of broiler chickens after experimental infection with eimeria species. *Iranian Journal of Veterinary Medicine, 15*(3), 346-357. [Link]

Pei, X. D., Li, F., Gao, T. T., Su, L. Y., Yu, F. T., & Shi, P., et al. (2023). Utilization of feather keratin waste to antioxidant and migration-enhancer peptides by Bacillus licheniformis 8-4. *Journal of Applied Microbiology, 134*(2), lxad005. [DOI:10.1093/jambio/lxad005] [PMID]

Prajapati, S., Koirala, S., & Anal, A. K. (2021). Bioutilization of chicken feather waste by newly isolated keratinolytic bacteria and conversion into protein hydrolysates with improved functionalities. *Applied Biochemistry and Biotechnology, 193*(8), 2497–2515. DOI:10.1007/s12010-021-03554-4] [PMID]

Rafat Khafar, K., Mojtahedin, A., Rastegar, N., Kalvani Neytali, M., & Olfati, A. (2019). Dietary inclusion of thyme essential oil alleviative effects of heat stress on growth performance and immune system of broiler chicks. *Iranian Journal of Applied Animal Science, 9*(3), 509-517. [Link]

SAS Institute. (2009). *SAS®/STAT Software, Release 9.2*. Cary, NC: SAS Institute, Inc. [Link]

Safari, H., Mohit, A., & Mohiti-Asli, M. (2016). Influence of dietary supplementation of dried purslane (Portulaca oleracea L.) powder on growth performance and susceptibility of chicken thigh muscle to lipid oxidation during frozen storage conditions. *Indian Journal of Animal Nutrition, 33*(2), 169-175. [DOI:10.5958/2231-6744.2016.00029.3]

Saisavoey, T., Sangtanoo, P., Reamtong, O., & Karnchanatat, A. (2019). Free radical scavenging and anti-inflammatory potential of a protein hydrolysate derived from salmon bones on RAW 264.7 macrophage cells. *Journal of the Science of Food and Agriculture, 99*(11), 5112-5121. [DOI:10.1002/jsfa.9755] [PMID]

Salehi, S., Sadeghi, A., & Karimi, A. (2021). Growth performance, nutrients digestibility, caecum microbiota, antioxidant status and immunity of broilers as influenced by kombucha fermented on white sugar or sugar beet molasses. *Italian Journal of Animal Science, 20*(1), 1770-1780. [DOI:10.1080/1828051X.2021.1941335]

Shihab, S. K., & Nafea, H. H. (2023). Effect of adding cordyceps sinensis extract and probiotic to the diet on productive performance of broiler. *Archives of Razi Institute, 78*(2), 659-666. [DOI:10.22092/ari.2022.359478.2430]

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H. I., & Sartono, T. A. (2019). Fermented feed as a potential source of natural antioxidants for broiler chickens-a mini review. *Agriculturae Conspectus Scientificus, 84*(4), 313-318. [Link]

Wiradimadja, R., Rusmana, D., Widjastuti, T., & Mushawwir, A. (2014). Chicken slaughterhouse waste utilization (chicken feather meal treated) as a source of protein animal feed ingredients in broiler chickens. *Lucrări Ştiinţifice - Seria Zootehnie, 62,* 120-124. [Link]

Xie, M., Wang, R., Wang, Y., Liu, N., & Qi, J. (2021). Effects of dietary supplementation with fermented Chenopodium album L. on growth, nutrient digestibility, immunity, carcase characteristics and meat quality of broilers. *Italian Journal of Animal Science, 20*(1), 2063-2074. [DOI:10.1080/1828051X.2021.1996289]

Xu, K. L., Gong, G. X., Liu, M., Yang, L., Xu, Z. J., & Gao, S., et al. (2022). Keratinase improves the growth performance, meat quality and redox status of broiler chickens fed a diet containing feather meal. *Poultry Science, 101*(6), 101913. [DOI:10.1016/j.psj.2022.101913] [PMID]

Xu, Y., Yu, Y., Shen, Y., Li, Q., Lan, J., & Wu, Y., et al. (2021). Effects of Bacillus subtilis and Bacillus licheniformis on growth performance, immunity, short chain fatty acid production, antioxidant capacity, and cecal microflora in broilers. *Poultry Science, 100*(9), 101358. [DOI:10.1016/j.psj.2021.101358] [PMID]

Zhu, F., Zhang, B., Li, J., & Zhu, L. (2020). Effects of fermented feed on growth performance, immune response, and antioxidant capacity in laying hen chicks and the underlying molecular mechanism involving nuclear factor-κB. *Poultry Science, 99*(5), 2573-2580. [DOI:10.1016/j.psj.2019.12.044] [PMID]

مقاله پژوهشی

پودر پَر تخمیر شده وضعیت، آنتی اکسیدانی، کیفیت گوشت و پاسخ ایمنی جوجه های گوشتی را بهبودمی‌بخشد

*حسن صفری ، اردشیر محیط ، مازیار محیطی اصلی

گروه علوم دامی، دانشکده کشاورزی، دانشگاه گیلان، رشت، ایران.

How to Cite This Article Safari, H., Mohit, A., & Mohiti-Asli, M. (2025). Improving the Antioxidant Status, Meat Quality, and Immune Response of Broilers by Fermented Feather Meal. *Iranian Journal of Veterinary Medicine*, *19*(2), 279-298. https://crossmark.crossref.org/dialog/?doi=10.32598/ijvm.19.2.1005483

http://dx.doi.org/10.32598/ijvm.19.2.1005483

چکیده

زمینه مطالعه: تجزیه بیولوژیکی پودر پَر یک جایگزین موثر بر سایر روش‌های فرآوری است.

هدف: این آزمایش برای بررسی اثرات پودر پَر هیدرولیز شده به روش‌های مختلف بر وضعیت آنتیاکسیدانی، کیفیت گوشت و پاسخ ایمنی جوجه‌های گوشتی انجام شد.

روش کار: تعداد ۴۸۰ قطعه جوجه گوشتی نر یکروزه به مدت ۴۲ روز در قالب طرح کاملاً تصادفی با هشت تیمار، پنج تکرار و ۱۲ جوجه در هر تکرار استفاده شد. تیمارهای آزمایشی شامل: ۱) گروه کنترل (جیره شاهد بدون پودر پَر)، تیمار ۲، ۳، ۴، ۵، ۶، ۷ و ۸ به ترتیب حاوی ۴ درصد پودر پَر خام، پودر پَر اتوکلاو شده، پودر پَر تخمیری با باسیلوس لیچنیفرمیس، پودر پُر تخمیری با باسیلوس سوبتیلیس، پودر پُر تخمیری با آسپرژیلوس نایجر، پودر پُر تخمیری با مخلوطی از باسیلوس لیچنیفرمیس+باسیلوس سوبتیلیس+آسپرژیلوس نایجر و پودر پَر هیدرولیز شده با آنزیم تجاری بودند.

نتایج: مطالعه آزمایشگاهی نشان داد که فعالیت پاکسازی رادیکال‌های آزاد ۲،۲-دیفنیل-۱-پیکریل هیدرازیل و ۲،۲-آزینو-بیس-۳-اتیل بنزوتیازولین-۶-سولفونیک اسید و تعداد میکروارگانیسم‌ها در پودر پَر تخمیری با افزایش زمان تخمیر افزایش یافت (P≤۰/۰۵). پاکسازی رادیکال‌های آزاد ۲،۲-دیفنیل-۱-پیکریل‌هیدرازیل و ۲،۲-آزینو-بیس-۳-اتیل بنزوتیازولین-۶-سولفونیک اسید در پودر پَر تخمیری در مقایسه با پودر پَر خام، پودر پَر اتوکلاو شده و هیدرولیز شده توسط آنزیم تجاری بیشتر بود (P≤۰/۰۵). میزان pH در پودر پَر تخمیری با افزایش زمان تخمیر کاهش یافت (P≤۰/۰۵). آزمایش مزرعه‌ای نشان داد که فعالیت آنزیم‌های آنتی‌اکسیدانی در جوجه‌های تغذیه شده با پودر پَر تخمیری در مقایسه با گروه کنترل و سایر تیمارهای آزمایشی بیشتر بود (P≤۰/۰۵). در مقایسه با گروه کنترل و سایر تیمارهای آزمایشی، جوجه‌هایی که با پودر پَر تخمیری تغذیه شده بودند دارای سطوح مالوندی‌آلدهید پایین‌تری در گوشت و سرم، همچنین میزان pH و ظرفیت نگهداری آب بالاتری در روزهای ۰، ۷ و ۱۴ ذخیره‌سازی گوشت نشان دادند (۰/۰۵ P≤). جوجه‌های تغذیه شده با پودر پَر تخمیری تیتر آنتیبادی بالاتری علیه ویروس نیوکاسل، تعداد کمتری هتروفیل، نسبت هتروفیل به لنفوسیت پایین‌تر، تیتر آنتی‌بادی بالاتری علیه گلبول قرمز گوسفندی (SRBC)، و همچنین ایمونوگلوبولین G و M بالاتری را در مقایسه با گروه کنترل و سایر تیمارهای آزمایشی نشان دادند (P≤۰/۰۵).

نتیجه‌گیری نهایی: پودر پَر تخمیر شده وضعیت آنتیاکسیدانی بدن، کیفیت گوشت و پاسخ ایمنی جوجه‌های گوشتی را بهبود بخشید.

کلیدواژه‌ها: پودر پر، تجزیه زیستی، تخمیر، جوجه گوشتی

تاریخ دریافت: ۱۵ اسفند ۱۴۰۲

تاریخ پذیرش: ۱۴ اردیبهشت ۱۴۰۳

تاریخ انتشار: ۱۲ فروردین ۱۴۰۴

* نویسنده مسئول:

دکتر حسن صفری

نشانی: رشت، دانشگاه گیلان، دانشکده کشاورزی، گروه علوم دامی.

تلفن: ۳۳۶۹۰۲۷۴ (۱۳) ۹۸+

رایانامه: *h.safari6813@gmail.com*

Copyright © 2025 The Author(s);
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-By-NC: https://creativecommons.org/licenses/by-nc/4.0/legalcode.en), which permits use, distribution, and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

This Page Intentionally Left Blank