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Effects of fermented crickets (*Gryllus bimaculatus*) with pineapple for supplementation in feeds on growth performance, immune function, antioxidant activities and microflora in broiler chickens

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ABSTRACT

The poultry industry is vulnerable to pathogenic bacteria, leading to antibiotic use for disease prevention and growth enhancement. However, overuse causes resistance and disrupts gut microbiota, affecting consumers. Therefore, discovering alternatives to antibiotics is crucial. Crickets are highly nutritious insects, and fermentation enhances their nutritional value and biological activities. This study evaluated the effects of fermented crickets supplementation, where the crickets were fermented together with pineapple using natural fermentation relying on endogenous microbial activity in raw materials (submerged fermentation), on growth performance, inflammatory responses, immune function, antioxidative activity, and gut microbiota in broiler chickens. This study used 315 one-day-old unsexed Arbor Acres broiler chicks, which were divided into three groups with seven replicates per group: a control (CT) and two treatments supplemented with 1% (FC1) or 3% (FC3) fermented crickets. Results showed that no significant growth differences among groups ($P > 0.05$). At 21 days, the FC3 group had lower serum IgY (1,008.50 µg/ml) and large intestine IgA levels (834.05 µg/g) than CT group (serum IgY: 1,652.03 µg/ml; large intestine IgA: 1,345.72 µg/g, $P < 0.01$). The FC3 group also had lower serum nitric oxide at 21 days (195 nmole/ml, $P < 0.05$) than FC1 group and reduced IL-6 at 42 days (853.91 pg/ml, $P < 0.05$) compared to the CT group. At 21 days, *E. coli* and *Salmonella* spp. were absent in the FC3 group, and lactic acid bacteria (LAB) levels increased significantly by day 42 ($P < 0.05$). Antioxidant activity in the FC3 group was higher than in the CT group at 21 days and the FC1 group at 42 days ($P < 0.05$). In summary, the FC3 group reduced pathogenic bacteria, lowered IgY and IgA, suppressed inflammatory markers, and enhanced antioxidant activity, suggesting potential to mitigate gut oxidative stress and inflammation in broilers.

Keywords: Broiler chickens, Fermented crickets, Immunity, Intestinal microorganisms, Antioxidants

INTRODUCTION

The poultry production industry prioritizes environmental and food safety because poultry is vulnerable to bacterial infections such as *Escherichia coli*, *Salmonella* sp., and *Clostridium perfringens*. These diseases significantly impact poultry farming, leading to substantial economic losses and prompting the use of antibiotics for disease treatment, prevention, and growth promotion [1]. However, the reliance on antimicrobials in modern feed production has contributed to the spread of antibiotic-resistant bacteria, posing a serious threat to both human and animal health [2]. In response, the World Health Assembly introduced a global action plan to mitigate antimicrobial resistance in 2015 (WHA68.7) [3]. Growing concerns about the impact of antibiotics have spurred research into natural alternatives such as probiotics, organic acids and various bioactive compounds (e.g., phytogenics, bacteriocins, peptides, and essential oils). These bioactive compounds [4-6] promote growth, reduce disease incidence, and improve gut health by modulating gut microflora, enhancing immune responses, inhibiting pathogens, and maintaining gut integrity [7, 8].

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The use of insects as an alternative protein source in animal feed is gaining increasing attention as a substitute for conventional protein sources (CPS) such as fishmeal and soybean meal. The rising costs of these traditional ingredients, along with their environmental impacts have driven interest in sustainable alternatives. Insects like crickets, mealworms (*Tenebrio molitor*), and black soldier fly larvae (*Hermetia illucens*) contain high protein levels and can be used in smaller quantities compared to CPS when included in feeds for animals such as pigs, poultry and fish [9].

Economically, incorporating insect protein into animal diets offers advantages since insects can be mass-produced using low-cost materials like organic waste, making them a cost-effective and sustainable protein source [10]. Additionally, insects typically contain 30-70% protein, which is considered high quality compared to many plant and animal sources [11]. Given these benefits, edible insects are increasingly seen as a promising, sustainable protein source with broad potential applications in both animal feed and human food industries in the future. They also contribute to reducing environmental impact and addressing global challenges in animal feed production. Most insects have high nutritional value and serve as a natural food source for many animals [12]. Consequently, insects are now used as an alternative protein source containing bioactive compounds such as polyphenols, antimicrobial peptides, amino acids, fatty acids, vitamins, minerals and antioxidants. These compounds exhibit various biological activities including anti-inflammatory, antidiabetic, antihypertensive, hypolipidemic, immune-modulating, and growth-promoting effects, making them suitable for inclusion in poultry feed [13-15]. In Thailand, crickets farming is widespread, with over 20,000 farmers [16]. Crickets (*Gryllus bimaculatus*) are rich in crude protein (54.10%), fiber (6.90%), and fat (26.90%) and have a total digestible nutrient content of 78.90%. They also contain essential amino acids such as methionine, lysine, histidine, valine, and leucine. Many studies have examined insects as a protein source in poultry, swine, and fish feed [17], with crickets showing significant potential as a protein source and bioactive peptide provider [18].

The fermentation process enhances nutrient absorption as health-promoting components and improves sensory properties. Fermentation stimulates the production of bioactive compounds, which may be insufficient or present in small quantities in the unprocessed substrate [19]. The fermentation process enhances the functional properties of insects, inhibiting the growth of pathogenic microorganisms, aiding in protein digestion, and generating bioactive peptides with antibacterial and immune-boosting properties [20-22]. Several studies have demonstrated that the production of small peptides during fermentation is associated with increased immunoglobulin (Ig) levels in chickens [23, 24] while fermented feed with low pH increases gastrointestinal acidity and enhances the stomach's defense against harmful pathogens. Fermented feed creates an unfavorable environment for the spread of pathogens in broiler chickens [25] and also improves the antioxidant properties of feed. Polysaccharides and peptides generated by hydrolysis are products of microbial activity or biotransformation [26]. Protein-rich insects such as larvae of *Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, and Orthoptera insects like crickets and grasshoppers have been widely studied as alternative protein sources for animal feed [27]. Research by Ardra et al. [28] demonstrated that organic acid-fermented black soldier fly larvae (*Hermetia illucens*) can completely replace fishmeal without negatively affecting the growth performance or nutrient utilization of Pangasius catfish (*Pangasius hypophthalmus*). Similarly, a study by Xiang et al. [29] evaluated the partial replacement of marine fishmeal with fermented black soldier fly larvae (BSFL) in the diet of Asian swamp eel (*Monopterus albus*). The results showed that BSFL could substitute part of the fishmeal and also improve the health and survival rate of the eel. In another study, Vasilopoulos et al. [30] investigated the effects of including whole dried yellow mealworm larvae (*Tenebrio molitor*) at 5% and 10% levels in broiler chicken diets. The findings indicated beneficial modulation of gut morphology and microbiota diversity without compromising intestinal integrity. Benzertiha et al. [31] research examined the effects of supplementing broiler chicken diets with full-fat larvae of *Tenebrio molitor* and *Zophobas morio*. This supplementation enhanced growth performance and positively influenced certain aspects of the immune system. Additionally, Vasilica et al. [32] studied the fermentation of house crickets (*Acheta domesticus*) powder using the lactic acid bacterium *Lactobacillus plantarum*. This fermented product proved to be a sustainable, nutrient-rich feed ingredient, containing high levels of protein, fatty acids, amino acids, minerals, and vitamins, while having a low environmental footprint.

Although numerous studies have explored the use of fermented insects or other insect-based products, research specifically focusing on fermented insects in poultry nutrition-especially using natural microbial communities during fermentation-remains limited. Therefore, our study focuses on supplementing broiler chicken diets with fermented crickets produced through natural microbial fermentation. We aim to evaluate the effects on growth performance, immune function, antioxidant activity, and intestinal microbiota composition in broilers.

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MATERIALS AND METHODS

Crickets fermentation

This study utilized two-spotted crickets using a specific formula comprising whole crickets, brown sugar, and pineapple in a 1:1:1 ratio with 1.5 liters of distilled water. This fermentation formula utilizes submerged fermentation, which relies on the natural microbial activity of the raw materials. Pineapples, including both the peel, flesh and core are used because of the bromelain enzymes, which function by initially hydrolyzing insect proteins, providing a nitrogen source for microorganism growth and adjusting to an acidic pH (4.5-4.6) to suppress the growth of undesirable microorganisms. The crickets and pineapples were finely ground using a blender, with water gradually added during blending until the total volume reached 1.5 L. The mixture was then poured into a fermentation container, sugar was added, and the mixture was stirred until the sugar completely dissolved. The container was then closed tightly. During the first week of fermentation, the lid was opened daily to release gas and then closed. From weeks 2 to 4 the lid was opened once a week for gas release. Fermentation continued for two months, and the mixture was stored at -20°C for analysis.

Chickens, design, and experimental diets

This research was conducted at the Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi, Thailand, from August 2023 to February 2024. A total of 315 1-day-old, mixed-sex Arbor Acres broiler chicks were used. From days 1 to 10, chicks were housed at a density of 50 per m², with a plastic slate floor covered in a plastic net topped with 5 cm of finely ground sawdust and provided an 80-watt lamp for hatched chickens. At 10 days of age, chicks were separated for dietary supplementation experiments with fermented crickets. The chicks were divided into three groups, each with seven replicates of 15 chicks per replicate. Group 1: A broiler diet without fermented crickets (Control: CT), Group 2: A broiler diet supplemented with 1% fermented crickets (FC1), and Group 3: A broiler diet supplemented with 3% fermented crickets (FC3). All groups were fed a commercial Betagro diet, with Betagro pellets (223) for newborns to 3 weeks (starter phase; days 1-21), and Betagro pellets (224) for 3 to 6 weeks (grower phase; days 21-42). In the FC1 and FC3 groups, the diets were supplemented with 1% and 3% fermented crickets, respectively. The nutrient composition of the diets is shown in Table 1. Diets were prepared each morning and fed three times daily with clean water ad libitum. The experiment was conducted in an environmentally controlled evaporative cooling system (EVAP), which maintained temperature between 28-30°C, with 72% relative humidity and an airspeed of approximately 2.04 m/s to ensure 100% air exchange. Light intensity was kept at the minimum of 10 lux. Chickens were housed in cages constructed from plastic netting on steel frames (1 x 1.5 x 1 m) with plastic slate floors elevated 50 cm off the ground. The experimental design was Completely Randomized Design (CRD) and vaccination was performed according to veterinary recommendations. At 21 and 42 days, blood, small intestine, and large intestine samples were collected from the control and two treatment groups, and the experiment was completed at 42 days. The researchers obtained animal use approval under license number U1-06537-2560, and the study entitled "Testing the use of fermented crickets-based bioactivities as a feed supplement to enhance growth and immune response in broiler chickens" was ethically approved by the Institutional Animal Care and Use Committee under Certificate Number P-67003.

Table 1 Nutrient compositions of experimental diets fed to newborn to 6 weeks broiler chickens.

Item	Treatment		
	CT	FC1	FC3
1–21 days			
Crude protein (%)	21	21.034	21.118
Fat (%)	4	4.015	4.045
Fiber (%)	5	5.004	5.012
21–42 days			
Crude protein (%)	19	19.034	19.118
Fat (%)	4	4.015	4.045
Fiber (%)	5	5.004	5.012

Note: CT = control group, FC1 = supplemented with 1% fermented crickets, FC3 = supplemented with 3% fermented crickets.

Growth performance

The remaining feed was recorded daily. On the first day of the experiment (day 10), as well as on days 21 and 42, the body weight of all broiler chickens was measured to calculate the average body weight (ABW), average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR). These parameters were assessed for the starter phase (days 1-21), the grower phase (days 21-42), and the overall period (days 1-42).

Formula of ABW, ADG, FI and FCR

- Average Body Weight (ABW)

$$ABW = \frac{\text{Initial Body Weight} + \text{Final Body Weight}}{2} \quad (1)$$

- Average Daily Gain (ADG)

$$ADG = \frac{\text{Final Body Weight} - \text{Initial Body Weight}}{\text{Number of Days}} \quad (2)$$

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- Average Feed Intake (FI)

$$FI = \text{Total Feed Intake} \quad (3)$$

- Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Total Feed Intake}}{\text{Total Weight Gain}} \quad (4)$$

Sample collection

On days 21 and 42, chickens from each experimental group (7 chickens per replicate) were weighed and euthanized. Blood samples (3 ml) were collected from the brachial vein, along with samples from the small and large intestines. The blood samples were allowed to clot at room temperature for 1-2 hours, followed by centrifugation at 8,000 rpm for 15 minutes. The serum was then extracted and stored at -80°C for further analysis. Intestinal samples (1 g) were obtained by scraping the small and large intestines with a scalpel to remove fecal matter and contents and diluted with 4 ml of PBS (pH 7). The mixtures were homogenized using a vortex mixer for 5-10 minutes, then centrifuged at 8,000 rpm for 15 minutes. The supernatants were collected and stored at -80°C for subsequent analysis of immune function and microbial populations in the large intestine.

Effects of fermented crickets on gut microflora in broiler chickens (in Vivo)

Large intestine samples (1 g) from broiler chickens at 21 and 42 days of age were weighed and diluted in 9 ml of 0.85% saline solution. Serial dilutions were performed, and each dilution was spread plated onto Nutrient Agar (NA), De Man, Rogosa, and Sharpe Agar (MRS), Eosin Methylene Blue Agar (EMB), and *Salmonella*-Shigella Agar (SS). The plates were incubated in an anaerobic chamber at 37°C for 24-48 hours. All experiments were conducted in duplicate for each agar medium, and colony counts were recorded to quantify the microbial population.

Biochemical analysis

IgA levels were measured using a commercial ELISA Kit for Chicken IgA (ab157691, Abcam, Cambridge, UK) according to the manufacturer's instructions. Briefly, this assay involves the reaction of IgA in the samples with anti-IgA antibodies adsorbed to the surface of polystyrene microtiter wells. Unbound proteins were removed through washing, and anti-IgA antibodies conjugated with horseradish peroxidase (HRP) were added. These enzyme-labeled antibodies formed complexes with the bound IgA. After an additional washing step, the amount of enzyme bound in the complex was quantified by adding the chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme correlated proportionally with the concentration of IgA in the sample; thus, absorbance at 450 nm indicated the IgA concentration. The concentration of IgA in the test samples was determined by interpolation from a standard curve constructed using known standards.

IgY levels were quantified using a commercial ELISA Kit for Chicken IgY (ab189577, Abcam, Cambridge, UK) according to the manufacturer's instructions. In summary, this assay employs an affinity tag-labeled capture antibody and a reporter-conjugated detector antibody to immunocapture the analyte in the sample solution. This complex (capture antibody/analyte/detector antibody) is then immobilized via immunoaffinity with an anti-tag antibody coating the well. To conduct the assay, samples or standards were first added to the wells, followed by the antibody mixture. After incubation, the wells were washed to remove unbound materials. A TMB substrate was added and catalyzed by HRP during incubation, resulting in a blue coloration. This reaction was halted by the addition of stop solution, which changed the color from blue to yellow. The signal, which is proportional to the amount of bound analyte, was measured at 450 nm. TMB development can also be recorded kinetically at 600 nm instead ofby an endpoint reading by comparing the IgY concentration in the test sample to a standard curve derived from known standards.

Nitric oxide levels were assessed using the Griess Reagent System (Promega, USA) according to the manufacturer's instructions. This system relies on a chemical reaction involving sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED) under acidic conditions (phosphoric acid). The Griess Reagent System detects nitrite (NO_2^-) in various biological and experimental liquids such as plasma, serum, urine, and tissue culture media. Sensitivity to nitrite varies with the matrix, with a detection limit of 2.5 µM (125 pmol). The nitrite concentration in the test sample was determined using a standard curve derived from known standards.

The cytokine IL-6 levels were measured using a Chicken IL-6 ELISA Kit (ab273258, Abcam, Cambridge, UK) according to the manufacturer's instructions. This assay uses a specific antibody for Chicken IL-6 coated on a 96-well plate. Standards and samples were added to the wells, where IL-6 bound to the immobilized antibody. After washing to remove unbound substances, biotinylated anti-Chicken IL-6 antibody was added. Following another wash to remove unbound biotinylated antibody, HRP-conjugated streptavidin was added. The wells were then rewashed and a TMB substrate solution was added, producing color proportional to the amount of bound IL-6. The color change from blue to yellow was measured at 450 nm. The concentration of IL-6 in the test sample was determined from a standard curve constructed from known standards.

The total antioxidant capacity was measured using a Total Antioxidant Capacity Assay Kit (MAK187,

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Sigma-Aldrich, USA) following the manufacturer's instructions. This assay quantifies the combined concentration of small molecule and protein antioxidants or only the concentration of small molecule antioxidants by reducing Cu^{2+} ions to Cu^{+}. The reduced Cu^{+} ions then complex with a colorimetric probe, producing a broad absorbance peak at 570 nm, which is proportional to the total antioxidant capacity. The results were expressed in Trolox equivalents, with values ranging from 4-20 nmole/well. Trolox, a water-soluble vitamin E analog, was used as the antioxidant standard.

Statistical analysis

Data on microbial population in the large intestine, inflammatory responses, immunoglobulin levels and total Antioxidant Capacity (TAC) were analyzed by one-way ANOVA to compare the mean values between CT, FC1, and FC3 experimental groups using SPSS software, version 26, and the Tukey test determined statistical significance at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Growth performance

The effects of fermented crickets supplementation on the growth performance of broiler chickens are presented in Table 2. Throughout the initial phase (days 0-10), the growth phase (days 10-21), and the overall period (days 0-42) the average body weight (ABW), average daily gain (ADG), average feed intake (ADFI), and feed conversion ratio (FCR) were similar across all the experimental groups. These values increased during the growth phase compared to the initial phase, but no statistically significant differences were observed ($P > 0.05$).

Table 2 Effect of fermented crickets supplementation on growth performance during 0-42 days of broiler chickens.

Days	Treatment			P-value
	CT	FC1	FC3	
Body Weight, BW				
BW 10	286.79 ±2.30	283.39 ±3.53	281.79 ±2.20	0.437
BW 21	1,244.79 ±13.16	1,245.50 ±10.97	1,233.02 ±5.91	0.646
BW 42	2,707.52 ±29.07	2,665.58 ±31.33	2,650.65 ±14.17	0.300
Average Daily Gain, ADG				
ADG 0-10	23.64 ±0.23	23.30 ±0.93	23.14 ±0.22	0.434
ADG 0-21	56.88 ±0.63	56.91 ±0.52	56.32 ±0.28	0.647
ADG 0-42	63.27 ±0.69	62.27 ±0.74	61.91 ±0.34	0.300
Feed Intake, FI				
FI 0-10	231.9 ±0.00	231.9 ±0.00	231.9 ±0.00	0.000
FI 0-21	1,078.9 ±1.09	1,068.0 ±0.00	1,068.0 ±0.00	0.387
FI 0-42	2,859.3 ±3.50	2,806.2 ±2.64	2,787.8 ±1.42	0.171
Feed Conversion Ratio, FCR				
FCR 0-10	0.81 ±0.02	0.82 ±0.03	0.82 ±0.02	0.368
FCR 0-21	0.87 ±0.02	0.86 ±0.02	0.87 ±0.01	0.749
FCR 0-42	1.06 ±0.03	1.05 ±0.03	1.05 ±0.01	0.948

Note: CT = control group, FC1 = supplemented with 1% fermented crickets, FC3 = supplemented with 3% fermented crickets.

Limited research has studied the addition of fermented *Gryllus bimaculatus* crickets as a feed supplement for broiler chickens. Crickets are highly nutritious and widely available insects, with their protein, energy, amino acids, saturated and unsaturated fatty acids, and vitamin and mineral contents making them a sustainable feed source [14]. Supplementation with fermented feed enhanced growth performance, antioxidant activity, immune function, digestive enzyme activity, and gut microflora in poultry [33]. However, our study found no significant effects of fermented crickets supplementation on average weight gain (AWG), average daily gain (ADG), average daily feed intake (ADFI), or feed conversion ratio (FCR) compared to the control group across the three experimental periods (0-10, 0-21, and 0-42 days). Chitin is a major component of insect exoskeletons and impedes protein digestion in mammals and poultry. One possible explanation is the presence of chitin in insects, which serves as an external structural component. Chitin acts as an anti-nutritional factor that hinders protein digestion in mammals and poultry, as these animals lack the enzymes required to break it down. However, the fermentation process can degrade chitin into chito-oligosaccharides and glucosamine compounds that are more readily utilizable by animals [34-38]. However, the low supplementation levels of fermented crickets (1% and

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3%) used in this study may have been insufficient to elicit a significant growth response. Li et al. [39] found that higher levels of fermented feed (10% dry and wet enzyme-bacteria fermented feed) significantly improved ADFI and ADG from days 22-42. Liu et al. [40] observed that a 6% solid-state fermented cicada supplement increased AWB and ADFI from days 1-21, while Fisher et al. [41] reported that 15% and 20% crickets meal additions improved growth performance metrics, especially during days 0-21, supplementation at 5% resulted in reduced growth performance. Our findings are consistent with previous studies that found low levels of fermented feed or insects supplementation (<5%) often do not enhance growth performance when compared to higher inclusion levels (6-20%). This suggests that the amount of supplementation has a clear impact on growth parameters relative to the control group. Therefore, supplementing with low levels (1-3%) of fermented crickets may not be sufficient to significantly improve the growth performance of broiler chickens. However, it does not have any adverse effects on animal health or feed intake.

Microbial population in the large intestine

The effects of fermented crickets supplementation on the microbial balance in the large intestine of broiler chickens (in *vivo*) were assessed at 21 and 42 days. At 21 days, the total plate count, lactic acid bacteria (LAB), and *Salmonella* spp. levels in the CT and FC1 groups were similar, but *E. coli* levels were high in the FC1 group. The FC3 group showed a lower total plate count than the other groups and an absence of *Salmonella* spp. and *E. coli*, with a higher lactic acid bacteria count than the CT and FC1 groups (10.80, 10.0, and 9.8 logCFU/g feces, respectively) (Fig. 1). By 42 days, the total microbial count in the FC3 group increased significantly (6.2-7.8 logCFU/g) compared to the CT and FC1 groups ($P < 0.05$), with lactic acid bacteria higher in the CT group ($P < 0.05$). However, *Salmonella* spp. and *E. coli* increased in the FC3 group compared to day 21 (Fig. 2).

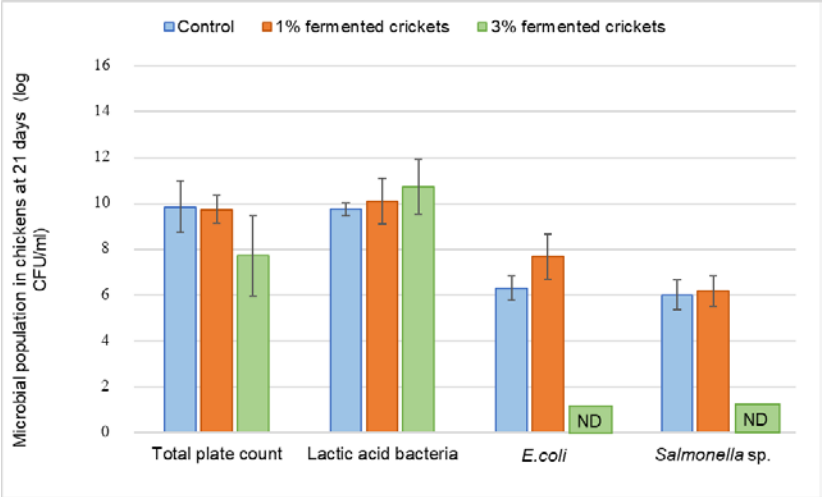

Figure 1 Effects of fermented crickets on microbial populations in 21 days old broiler chickens.

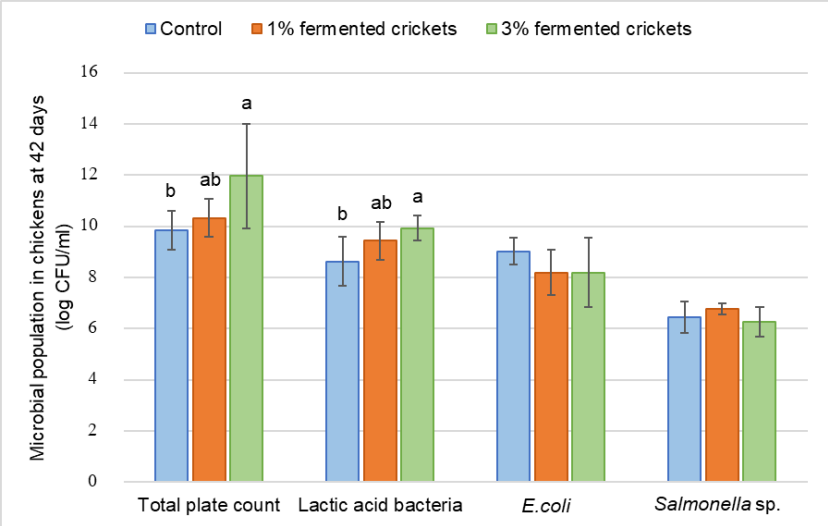

Figure 2 Effects of fermented crickets on microbial populations in 42 days old broiler chickens. [a, b] within a row indicates statistically significant differences at $P < 0.05$.

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At 21 days, broiler chickens in the FC3 group exhibited a higher quantity of lactic acid bacteria (LAB) compared to the CT group, with no detectable levels of *E. coli* or *Salmonella* spp. (Fig. 1) This finding concurred with several studies. Sun et al. [42] reported that supplementing fermented cottonseed meal in chicken feed reduced *E. coli* in the small intestine and cecum at 21 days ($P < 0.05$) while increasing *Lactobacillus* levels ($P < 0.05$). Similarly, Jazi et al. [43] found that fermented cottonseed meal resulted in higher LAB counts and lower pH in the intestines of chickens compared to non-fermented meal. Peng et al. [44] investigated the effects of supplementing feed with fermented materials at both high and low levels, as well as with antibiotics. Their findings revealed that the group receiving high levels of fermented feed experienced a significant reduction in pathogenic *E. coli*. Collectively, these studies indicated that fermented feed supplementation resulted in a decrease in pH, promoted the growth of LAB, and inhibited the growth of *E. coli* and *Salmonella* spp. [45, 46] The fermentation process also stimulates the growth of lactic acid bacteria (LAB), which can lower the intestinal pH by producing organic acids and inhibit the colonization of pathogenic bacteria in the gut through the production of antibacterial substances [47]. Generally, the optimal pH range for the growth of *Lactobacillus* spp. It's between 5.5 and 6.2, and they can grow within a pH range of 4.5 to 6.5, or even lower in some strains [48, 49]. The pH in the intestines of broiler chickens is approximately 5.7 to 6.5 [50]. This information is consistent with our study, which found that the intestinal pH of groups supplemented with fermented crickets (FC3 and FC1) was around 6.0, within the optimal range for LAB growth, whereas the control group (CT) had a pH of 6.8, which may be less favorable for the colonization of beneficial microbiota compared to the fermented feed groups. Moreover, fermented feed benefits poultry health by producing bioactive compounds such as peptides, surfactants, bacteriocins, gamma-aminobutyric acid (GABA), exopolysaccharides, organic acids, short-chain fatty acids (SCFAs), vitamins, minerals, and polyphenols through the activity of naturally occurring microorganisms. These compounds help regulate the microbiota, reduce stress, and maintain the physiological balance and homeostasis of the gut environment. Fermented feed contains living organisms that can modulate gut microbiota, physiology and maintain intestinal environmental balance [51, 52].

At 42 days of age, the broiler chickens showed an increase in pathogenic microorganisms; however, the quantity of lactic acid bacteria (LAB) remained higher than in the control (CT) group. (Fig. 2) This observation aligned with Alshelmani et al. [53], who noted that feeding broiler chickens with fermented palm kernel cake resulted in a decrease in Enterobacteriaceae (ENT) levels during the first 3 weeks of age, followed by an increase at 6 weeks. This indicates that environmental factors and the duration of rearing influence the gut microbial population. At the beginning of the rearing period, the poultry house environment was relatively clean and free from contamination, allowing fermented cricket to effectively inhibit pathogenic bacteria. However, as the rearing period progressed, waste accumulation inside the poultry house increased, raising the animals' exposure to environmental pathogens-particularly at 42 days of age. At this stage, the birds' body size had increased while the cage space remained unchanged, leading to overcrowding and increased contact with the floor. These unfavorable environmental factors, such as the accumulation of animal feces and the presence of carrier animals like cockroaches, rats, or other pets, may increase the risk of contamination by *Salmonella* spp. Moreover, data from the Department of Livestock Development reports that *Salmonella* can be found in poultry farms throughout the production cycle. The periods with the highest risk of infection are during the rearing of breeder chickens in the pullet phase and broiler chickens after 35-36 days of age, which corresponds to the timing of increased bacterial levels observed in this study [54-56].

Inflammatory cytokine IL-6 and nitric oxide levels

As shown in Table 3, on day 21, IL-6 levels in serum, small intestine, and large intestine did not significantly differ across the groups ($P > 0.05$). However, by day 42, serum IL-6 levels in the CT group were significantly higher (1,062.24 pg/ml) compared to the FC1 and FC3 groups (887.34 and 853.91 pg/ml, respectively; $P < 0.05$), while intestinal IL-6 levels remained statistically similar ($P > 0.05$). Fermented crickets supplementation significantly affected nitric oxide levels on day 21, with the FC1 group showing the highest levels in serum (512.14 nmole/ml; $P < 0.05$). By day 42, serum nitric oxide levels were highest in the CT group (1,318.57 nmole/ml), while the FC3 group had the lowest levels in the small intestine (3,478.57 nmole/g feces). In the large intestine, the FC1 group had the highest nitric oxide levels on day 21 (3928.57 nmole/g feces) but the lowest on day 42 (2,857.14 nmole/g feces) in Table 4.

Results showed that the levels of nitric oxide (NO) and IL-6 in the serum were lower compared to those in the small and large intestines, which are the sites directly exposed to pathogens. Intraepithelial lymphocytes (IELs), the immune cells within the intestinal lining, are a major source of IL-6 and NO secretion. When the gastrointestinal tissue is damaged, the body responds with an acute inflammatory process. This begins with changes in the blood vessels of the intestinal lining, where mast cells and monocytes release mediators such as histamine and serotonin.

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These substances cause blood vessels to dilate and increase the permeability of the vessel walls, which disrupts the integrity of tight junctions between epithelial cells. As a result, the intestinal barrier weakens, allowing fluids, molecules, immune cells, and even pathogens to pass more easily into the tissue. This increased permeability activates macrophages to release cytokines, including IL-6, IL-1, TNF-α, and chemokines, as well as reactive oxygen species like superoxide, hydroxyl radicals, hydrogen peroxide, and nitric oxide. These molecules work together to eliminate pathogens and regulate inflammation [57-59]. In this study, at 42 days, the FC3 group showed significantly lower serum IL-6 levels compared to the CT group, due to higher LAB counts in the FC3 group (1-2 log CFU/g feces) compared to the CT group ($P<0.05$) (Fig 2). Chen et al. [60] also determined that LAB reduced inflammatory cytokine production like IL-6 associated with *Salmonella* infection. The FC3 group had a significantly higher total bacterial count at day 42 (Fig 2), which included beneficial bacteria from fermentation such as probiotics [61]. Probiotics can reduce inflammation by regulating intracellular signaling pathways, such as the MAPK (mitogen-activated protein kinase) pathway, which plays a key role in controlling the production of inflammatory cytokines like IL-6 and IL-8. This regulation occurs particularly through the inhibition of phosphorylation of ERK1/2 and p38, which are involved in cellular inflammatory responses across various animal species [62, 63]. Bacillus licheniformis and Bacillus subtilis are probiotics known for their potential to enhance gut health, especially when used together in multispecies probiotic formulations. Previous studies have shown that these mixtures can significantly reduce IL-6 and IL-8 levels in IPEC-J2 cells stimulated with *E. coli* and *Salmonella* Typhimurium [64]. Additionally, a study by Aghamohammad et al. [65] reported that probiotics such as *Lactobacillus* spp. and *Bifidobacterium* spp. can downregulate genes involved in inflammatory signaling pathways that drive strong immune responses. These probiotics also help decrease the production of inflammatory cytokines, including IL-6 and IL-1β. Although the number of pathogenic microbes increased in the FC3 group at day 42 (Figure 2), the presence of beneficial microbes from fermented crickets may have contributed to reducing inflammation and pathogen invasion. This highlights the potential of probiotics to modulate immune balance and support gut health even under conditions of microbial contamination.

Table 3 Effect of fermented crickets supplementation on cytokine (IL-6) levels in serum, small intestine and large intestine of broiler chickens at 21 and 42 days of age.

Items Treatment	IL-6 levels			P-value
	CT	FC1	FC3	
Serum				
21 days	959.36 ± 31.23	869.34 ± 69.32	838.48 ± 22.07	0.186
42 days	1,062.24 ± 20.09[a]	887.34 ± 27.24[b]	853.91 ± 80.15[b]	0.022
Small intestine				
21 days	4,320.99 ± 160.62	4,488.17 ± 317.78	3,806.58 ± 83.74	0.091
42 days	4,153.80 ± 288.99	4,076.65 ± 148.31	3,780.86 ± 82.14	0.379
Large intestine				
21 days	11,792.70 ± 986.81	7,973.25 ± 1630.58	11,818.42 ± 628.57	0.051
42 days	6,262.86 ± 426.09	5,761.32 ± 411.04	5,182.61 ± 433.48	0.229

Note: CT = control group, FC1 = supplemented with 1% fermented crickets, FC3 = supplemented with 3% fermented crickets. [a, b] Means within a row indicate statistically significant differences. Number of experimental units per treatment = 6. Concentrations of serum (pg/ml), small and large intestines (pg/g feces).

Table 4 Effect of fermented crickets supplementation on nitric oxide levels in serum, small intestine and large intestine of broiler chickens at 21 and 42 days of age.

Items Treatment	Nitric oxide levels			P-value
	CT	FC1	FC3	
Serum				
21 days	227.86 ±16.73[b]	512.14 ±137.38[a]	195.00 ±13.39[b]	0.031
42 days	1,318.57 ±272.48	658.57 ±111.54	1,072.86 ±302.37	0.196
Small intestine				
21 days	3,296.43 ±321.66	3,035.71 ±213.76	3,067.86 ±1965.74	0.823

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Items Treatment	Nitric oxide levels			P-value
	CT	FC1	FC3	
42 days	3,635.71 ±287.19	3,778.57 ±408.40	3,478.57 ±481.79	0.870
Large intestine				
21 days	3,717.86 ±127.45	3,928.57 ±610.36	3,421.42 ±206.68	0.647
42 days	4,482.14 ±841.49	2,857.14 ±500.03	4,232.14 ±555.52	0.205

Note: CT = control group, FC1 = supplemented with 1% fermented crickets, FC3 = supplemented with 3% fermented crickets. [a, b] Means within a row indicate statistically significant differences. Number of experimental units per treatment = 5. Concentrations of serum (nmol/ml), small and large intestines (nmol/g feces).

Table 5 Effect of fermented crickets supplementation on Immunoglobulin (IgY) levels in serum, small intestine, and large intestine of broiler chickens at 21 and 42 days of age.

Items	IgY levels			P-value
	CT	FC1	FC3	
Serum				
21 days	1,652.03 ± 184.48[a]	1,647.64 ± 96.36[a]	1,008.50 ± 127.68[b]	0.008
42 days	3,636.29 ± 158.02	3,178.70 ± 321.53	4,035.20 ± 198.62	0.066
Small intestine				
21 days	9.52 ± 2.65	5.41 ± 1.37	3.39 ± 0.62	0.072
42 days	42.80 ± 23.18	13.12 ± 3.56	30.72 ± 18.76	0.493
Large intestine				
21 days	140.99 ± 31.96	103.16 ± 14.64	65.49 ± 13.96	0.081
42 days	264.64 ± 99.04	74.19 ± 18.41	290.21 ± 174.94	0.382

Note: CT = control group, FC1 = supplemented with 1% fermented crickets, FC3 = supplemented with 3% fermented crickets. [a, b] Means within a row indicate statistically significant differences. Number of experimental units per treatment = 6. Concentrations of serum (µg/ml), small and large intestines (µg/g feces).

Table 6 Effect of fermented crickets supplementation on Immunoglobulin (IgA) levels in serum, small intestine, and large intestine of broiler chickens at 21 and 42 days of age

Items	IgA levels			P-value
	CT	FC1	FC3	
Serum				
21 days	240.95 ± 29.24	222.06 ± 21.30	166.52 ± 18.81	0.100
42 days	355.99 ± 20.23	317.52 ± 34.68	413.80 ± 103.70	0.577
Small intestine				
21 days	463.00 ± 37.45[b]	695.69 ± 71.26[a]	448.28 ± 23.57[b]	0.004
42 days	1,504.85 ± 124.97	1,563.15 ± 210.77	1,418.75 ±163.48	0.835
Large intestine				
21 days	1345.72 ± 35.78[a]	1141.16 ± 113.46[a]	834.05 ± 39.99[b]	0.001
42 days	3,874.82 ± 1054.57	1,769.93 ± 277.71	2,449.17 ± 386.72	0.108

Note: CT = control group, FC1 = supplemented with 1% fermented crickets, FC3 = supplemented with 3% fermented crickets. [a, b] Means within a row indicate statistically significant differences. Number of experimental units per treatment = 6. Concentrations of serum (µg/ml), small and large intestines (µg/g feces).

In our study, nitric oxide (NO) levels in the serum at 21 days were significantly higher in the FC1 group, correlating with elevated *E. coli* levels compared to other groups (Fig 1). Lipopolysaccharides (LPS) from the cell wall of *E. coli* can stimulate macrophages to produce nitric oxide (NO) by inducing the expression of the enzyme inducible nitric oxide synthase (iNOS). The NO produced plays a role in promoting inflammation and stimulating the migration of immune cells to the site of infection [66,67]. On day 42, increased levels of *E. coli* were also observed in the CT and FC3 groups (Figure 2), which may contribute to the long-term stimulation of NO production. The sustained increase in NO resulting

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from continuous infection can negatively impact gut health and cause inflammatory conditions in animals. Furthermore, a report by Poljakovic et al. [68] found that *E. coli* can rapidly induce iNOS expression in epithelial and immune cells of the urinary tract in mice, with nitrite levels in the urine rising significantly within 6-12 hours post-infection. This confirms the acute role of *E. coli* in stimulating NO production. Therefore, supplementation with probiotics, prebiotics, or antioxidants, combined with proper environmental and dietary management, may be an effective strategy to reduce the impact of pathogens such as *E. coli* and to control excessive NO production [69].

Immunoglobulin levels

The supplementation of fermented crickets in broiler chickens showed higher levels of IgY in serum compared to the small and large intestines. At day 21, the FC3 group had the lowest serum IgY level at 1,008.50 µg/ml, significantly lower ($P < 0.01$) than the CT and FC1 groups, but no significant differences were observed in the intestines ($P > 0.05$). At 42 days, no significant differences were recorded in IgY levels in the serum and small and large intestines across all the groups. At day 21, the FC3 group had significantly lower IgA levels in the small ($P < 0.001$) and large intestines ($P = 0.001$) compared to other groups, with no significant differences observed at day 42. Results are shown in Table 5-6.

At 21 days, the FC3 group exhibited lower levels of serum IgY and intestinal IgA compared to the CT group. This difference was attributed to the roles of IgA and IgY in the adaptive immune response [70]. This is triggered by the innate immune system, which releases inflammatory mediators such as cytokines, chemokines, and nitric oxide that stimulate the humoral immune response in B cells, leading to the production of antibodies, which are proteins known as immunoglobulins. These antibodies circulate in the bloodstream and permeate other body fluids, binding to specific antigens that trigger their production [71, 72]. In chickens, the humoral immune system consists of three types of immunoglobulins: IgM, IgA, and Ig. IgY is transferred from the mother to the egg yolk through passive natural immunity, providing short-term protection (approximately two weeks). The chick begins to produce its own IgY (active immunity) once its immune system is fully developed, which usually occurs after about three weeks of age. Therefore, at 21 days old, the IgY levels in the FC3 group may be low because passive immunity has declined, while active immunity is not yet fully developed. Additionally, no pathogens were detected at day 21, which may explain the IgY levels observed [73-75]. The production of IgA, specifically Secretory IgA (SIgA), is generated by plasma cells in mucosal tissues. IgA is primarily produced within the mucosa lining the intestines, where SIgA plays a crucial role in preventing pathogen adhesion and invasion of the intestinal barrier [76]. Our findings aligned with microbial analysis in the intestines of 21 -day old chickens, where no pathogenic *E. coli* or *Salmonella* spp. were detected (Fig. 1). Pathogenic microorganisms act as antigens, triggering the immune system upon entry into the body. When antigen levels were low, the immune response in the FC3 group was lower compared to the CT and FC1 groups, where pathogens were detected. This result concurred with Jazi et al. [77]. They conducted a study with five experimental groups: one supplemented with Xylo-oligosaccharides, one with Lactic acid bacteria, one with fermented soybean meal, a positive control (normal feed mixed with *Salmonella* Typhimurium), and a negative control (normal feed without *Salmonella* Typhimurium). They measured heterophil levels, which play a key role in pathogen phagocytosis. The group supplemented with fermented soybean meal had lower levels of both *Salmonella* Typhimurium and heterophils compared to the positive control group, demonstrating that lower pathogen levels correlated with reduced immune response.

Total antioxidant capacity (TAC)

The effects of fermented crickets supplementation on antioxidant capacity in the serum, small intestine, and large intestine are summarized in Table 7. At 21 and 42 days, the FC3 group exhibited the highest antioxidant capacity in serum, with values of 21.61 and 24.68 nmole/ml, respectively, although these differences were not statistically significant ($P > 0.05$). In the small intestine, all groups showed similar antioxidant levels. However, in the large intestine at 21 and 42 days, the FC3 group exhibited significantly the highest antioxidant capacities, measuring 71.65 and 45.54 µmole/g feces, respectively ($P < 0.05$)

Oxygen toxicity in most organisms arises from ROS, which are byproducts of normal cellular metabolism and can lead to oxidative stress, causing damage to cells and biomolecules. Antioxidants mitigate the harmful effects of oxidative stress [33]. Fermented feed can help modulate the production of reactive oxygen species (ROS) and antioxidants within cells, and the live microorganisms present in fermented feed contribute to balancing ROS and antioxidants in chickens. The antioxidant properties are mainly produced by lactic acid bacteria (LAB), which are capable of producing antioxidant enzymes, glutathione, and exopolysaccharides, which directly stimulate ROS and enhance the host's antioxidant defenses [78, 79]. Fu et al. [80] demonstrated that a 5.0% fermented feed significantly increased total antioxidant capacity (T-AOC) in the upper small intestine ($P < 0.05$). Similarly, Niu et al. [81] found that supplementation with fermented Ginkgo biloba leaves (FGBL) significantly enhanced T-AOC in the

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small intestine. Our study aligned with these findings, showing that at 21 and 42 days, the FC3 group had significantly higher antioxidant levels in the large intestine compared to the CT and FC1 groups. Results indicated that fermented crickets supplementation enhanced antioxidants, benefiting intestinal health by reducing inflammation caused by bacterial or viral invasion and protecting cells and tissues through immune system mechanisms [40]. The large intestine serves as a habitat for a diverse range of microorganisms that play a vital role in the host's health. Gut microbes produce beneficial bioactive compounds such as glutathione (GSH), butyrate, and folate, which possess antioxidant properties and help regulate the microbial balance within the gastrointestinal tract [82, 83].

Insect fermentation generates various bioactive substances, including polyphenols, amino acids, short-chain fatty acids, vitamins, minerals, peptides, and antioxidants. These compounds may support gut health by promoting the production of metabolites in the large intestine and help maintain amino acid balance in the body [21, 34, 84-86]. For example, the amino acid N-acetyl-cysteine is a potent antioxidant that plays a role in maintaining intracellular glutathione levels [87]. Meanwhile, vitamin E stabilizes cell membranes by reducing lipid oxidation [88], and polyphenols effectively scavenge free radicals, stimulate antioxidant enzymes, and inhibit oxidase enzymes [89]. Therefore, fermented crickets supplementation can provide antioxidant benefits.

CONCLUSIONS

Supplementation with the FC3 group did not improve the growth performance of broiler chickens but increased the population of lactic acid bacteria and reduced the number of pathogenic *E. coli* and *Salmonella* spp. in chickens at 21 days of age, a period when their immune system declines. Fermented crickets supplementation effectively decreased pathogen levels during this early stage. However, levels of IgY and IgA were relatively low in the supplemented chickens. A significant reduction was recorded in inflammatory mediators such as IL-6 and nitric oxide in the serum at 21 and 42 days. This effect did not extend to the intestinal tract. Fermented crickets demonstrated the ability to enhance antioxidant levels in the chickens' intestines, thereby mitigating oxidative stress-related damage.

Table 7 Effect of fermented crickets supplementation on antioxidant capacity in serum, small intestine, and large intestine of broiler chickens at 21 and 42 days of age.

Items	Antioxidant capacity			P-value
	CT	FC1	FC3	
Serum				
21 days	20.09 ± 0.90	19.72 ± 0.45	21.61 ± 0.46	0.127
42 days	23.93 ± 2.14	20.03 ± 2.49	24.68 ± 5.28	0.628
Small intestine				
21 days	51.44 ± 0.44	49.08 ± 2.08	46.88 ± 0.66	0.079
42 days	50.08 ± 2.32	53.49 ± 3.05	52.02 ± 1.48	0.607
Large intestine				
21 days	58.45 ± 1.62^{b}	59.97 ± 2.28^{b}	71.65 ± 1.33^{a}	0.000
42 days	40.60 ± 1.47^{ab}	38.14 ± 0.94^{b}	45.54 ± 2.16^{a}	0.020

Note: CT = control group, FC1 = supplemented with 1% fermented crickets, FC3 = supplemented with 3% fermented crickets. $^{a, b}$ Means within a row indicate statistically significant differences. Number of replicates/experimental units: 5. Concentrations of serum (nmol/ml), small and large intestines (µmol/g feces).

REFERENCES

1. Roth N, Käsbohrer A, Mayrhofer S, Zitz U, Hofacre C, Domig KJ. The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview. Poult Sci. 2019;98(4):1791-804.
2. Aarestrup FM. Veterinary Drug Usage and Antimicrobial Resistance in Bacteria of Animal Origin. Basic Clin Pharmacol Toxicol. 2005;96 (4):271-81.
3. World Health Organization. Global action plan on antimicrobial resistance (WHA68.7) [Internet]. World Health Assembly, 68th session; 2015. Available form: https://www.who.int/publications/i/item/9789241509763.
4. Gupta R, Sharma S. Role of alternatives to antibiotics in mitigating the antimicrobial resistance crisis. Indian J Med Res. 2022;156(3):464-77.
5. Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, et al. Use of antibiotics in broiler production: Global impacts

DOI: 10.60101/jarst.2025.261344

and alternatives. Animal Nutrition. 2018;4(2): 170-8.

6. Thong HT, Thu LNA, Duc HV. Chapter potential substitutes of antibiotics for swine and poultry production. Rijeka: IntechOpen; 2022.
7. Arsene MMJ, Jorelle ABJ, Sarra S, Viktorovna PI, Davares AKL, Ingrid NKC, et al. Short review on the potential alternatives to antibiotics in the era of antibiotic resistance. J Appl Pharm Sci. 2022;12(1):29-40.
8. Ayalew H, Zhang H, Wang J, Wu S, Qiu K, Qi G, et al. Potential Feed Additives as Antibiotic Alternatives in Broiler Production. Front Vet Sci.2022;9:916473.
9. Kolobe SD, Manyelo TG, Sebola NA, Malematja E, Mabelebele M. Prospects of rearing selected southern African swarming insects for animal feed: a review on insect farming and the economic value of edible insects. Agric Food Secur. 2024; 13:6.
10. Tilami SK, Turek J, červený D, Lepič P, Kozák P, Burkina V, et al. Insect meal as a partial replacement for fish meal in a formulated diet for perch Perca fluviatilis. Turk J Fish Aquat Sci. 2020;20(12): 867-78.
11. Veldkamp T, van Duinkerken G, van Huis A, Lakemond CMM, Ottevanger E, Bosch G, et al. Insects as a sustainable feed ingredient in pig and poultry diets: A feasibility study [Internet]. Wageningen UR Livestock Research; 2012. Available from: https://edepot.wur.nl/234247
12. Abd El-Hack ME, El-Saadony MT, Salem HM, El-Tahan AM, Soliman MM, Youssef GBA, Taha AE, et al. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poult Sci.2022; 101(4):101696.
13. Malematja E, Manyelo TG, Sebola NA, Mabelebele M. The role of insects in promoting the health and gut status of poultry. Comp Clin Pathol. 2023; 32:501-13.
14. Khan SH. Recent advances in role of insects as alternative protein source in poultry nutrition. J Appl Anim Res. 2018;46(1):1144-57.
15. Sánchez-Estrada M de la L, Aguirre-Becerra H, Feregrino-Pérez AA. Bioactive compounds and biological activity in edible insects: A review. Heliyon. 2024;10(2):e24045.
16. Magara HJO, Niassy S, Ayieko MA, Mukundamago M, Egonyu JP, Tanga CM, et al. Edible crickets (orthoptera) around the world: distribution, nutritional value, and other benefits-a review. Front Nutr. 2020;7:537915.
17. Halloran A, Hanboonsong Y, Roos N, Bruun S. Life cycle assessment of cricket farming in north-eastern Thailand. J Clean Prod. 2017;156: 83-94.
18. Phesatcha B, Phesatcha K, Viennaxay B, Matra M, Totakul P, Wanapat M. Cricket meal (*Gryllus bimaculatus*) as a protein supplement on in vitro fermentation characteristics and methane mitigation. Insects. 2022;13(2):129.
19. Liceaga AM. Processing insects for use in the food and feed industry. Curr Opin Insect Sci. 2021;48:32-6.
20. Fashakin OO, Tangjaidee P, Unban K, Klangpetch W, Khumsap T, Sringarm K, et al. Isolation and identification of antioxidant peptides derived from cricket (*Gryllus bimaculatus*) protein fractions. Insects. 2023;14(8):674.
21. Kewuyemi YO, Kesa H, Chinmaa CE, Adebo OA. Fermented Edible Insects for Promoting Food Security in Africa. Insects. 2020;11(5):283.
22. Martinez-Villaluenga C, Peñas E, Frias J. Chapter 2 - Bioactive peptides in fermented foods: production and evidence for health effects. In: Frias J, Martinez-Villaluenga C, Peñas E, editor. Fermented Foods in Health and Disease Prevention. Boston: Academic Press; 2017.
23. Bangar SP, Suri S, Trif M, Ozogul F. Organic acids production from lactic acid bacteria: A preservation approach. Food Biosci. 2022;46:101615.
24. Gao J, Zhang HJ, Wu SG, Yu SH, Yoon I, Moore D, et al. Effect of *Saccharomyces cerevisiae* fermentation product on immune functions of broilers challenged with *Eimeria tenella*. Poult Sci. 2009;88(10):2141-51.
25. Sugiharto S, Yudiarti T, Isroli I, Widiastuti E, Kusumanti E. Dietary supplementation of probiotics in poultry exposed to heat stress-a review. Annals of Animal Science. 2017;17: 591-604.
26. Sugiharto S, Ranjitkar S. Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review. Anim Nutr. 2019;5(1):1-10.
27. Solecka K, Drażbo A. The use of insects in poultry nutrition - a review. Anim Sci Genet. 2024;20(3): 23-36.
28. Ardra M, Pradhan C, Das S, Pillai D. The effect of fishmeal replacement with organic acid fermented black soldier fly (Hermetia illucens) larvae meal on growth, nutrient utilization, metabolic enzyme activity, antioxidant status and immunity in

DOI: 10.60101/jarst.2025.261344

Pangasius (Pangasianodon hypophthalmus). Aquaculture. 2024;591:741114.

29. Xiang Y, Gao S, Luo Y, Tang G, Zou X, Xie K, et al. Fermented black soldier fly larvae as a sustainable replacement for marine fish in Asian swamp eel diets. Vet World. 2025;18(4):1002-13.
30. Vasilopoulos S, Giannenas I, Mellidou I, Papadopoulos E, Tzora A, Bonos E, et al. Diet replacement with whole insect larvae affects intestinal morphology and microbiota of broiler chickens. Sci Rep.2024; 14:6836.
31. Benzertiha A, Kierończyk B, Kołodziejski P, Pruszyńska-Oszmałek E, Rawski M, Józefiak D, et al. Tenebrio molitor and Zophobas morio full-fat meals as functional feed additives affect broiler chickens' growth performance and immune system traits. Poult Sci. 2020;99(1):196-206.
32. Vasilica BTB, Chiș MS, Alexa E, Pop C, Păucean A, Man S, et al. The impact of insect flour on sourdough fermentation-fatty acids, amino acids, minerals and volatile profile. Insects. 2022;13(7):576.
33. Xu F, Wu H, Xie J, Zeng T, Hao L, Xu W, et al. The Effects of fermented feed on the growth performance, antioxidant activity, immune function, intestinal digestive enzyme activity, morphology, and microflora of yellow-feather chickens. Animals. 2023;13(22):35-45.
34. Rivas-Navia DM, Duenas-Rivadeneira AA, Duenas-Rivadeneira JP, Aransiola SA, Maddela NR, Prasad R. Bioactive compounds of insects for food use: potentialities and risks. J Agric Food Res. 2023;14:100807.
35. Abassi S, Emtiazi G, Hosseini-Abari A, Kim BG. Chitooligosaccharides and thermostable chitinase against vulvovaginal candidiasis and saprophyte fungi: lc mass studies of shrimp shell fermentation by *bacillus altitudinis*. Curr Microbiol. 2020;77(1):40-8.
36. Jung WJ, Park RD. Bioproduction of Chitooligosaccharides: Present and Perspectives. Marine Drugs. 2014;12(11):5328-56.
37. Cheng G, Hu T, Zeng Y, Yan L, Liu Y, Wang Y, et al. Enhancing immune response, antioxidant capacity, and gut health in growing beagles through a chitooligosaccharide diet. Front Vet Sci. 2024;10:1283248.
38. Gu YF, Chen YP, Jin R, Wang C, Wen C, Zhou YM. Dietary chitooligosaccharide supplementation alleviates intestinal barrier damage, and oxidative and immunological stress in lipopolysaccharide-challenged laying hens. Poult Sci. 2022;101(4): 101701.
39. Li J, Tao L, Zhang R, Yang G. Effects of fermented feed on growth performance, nutrient metabolism and cecal microflora of broilers. Anim Biosci. 2022;53(4):596-604.
40. Liu S, Yan W, Ma C, Liu Y, Gong L, Levesque C, et al. 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-Australas J Anim Sci. 2020;33(4):568-78.
41. Fisher HJ, Empey M, MacIsaac JL, Rathgeber BR, Mason B, Colombo SM, et al. The effects of cricket meal (*Gryllus sigillatus*) on the meat quality, growth, and internal morphology of broiler chickens. Can J Anim Sci. 2023;104:1-38.
42. Sun H, Tang JW, Fang CL, Yao XH, Wu YF, Wang X, et al. Molecular analysis of intestinal bacterial microbiota of broiler chickens fed diets containing fermented cottonseed meal. Poult Sci. 2013;92 (2):392-401.
43. Jazi V, Boldaji F, Dastar B, Hashemi SR, Ashayerizadeh A. Effects of fermented cottonseed meal on the growth performance, gastrointestinal microflora population and small intestinal morphology in broiler chickens. Br Poult Sci. 2017;58(4):402-8.
44. Peng W, Talpur MZ, Zeng Y, Xie P, Li J, Wang S, et al. Influence of fermented feed additive on gut morphology, immune status, and microbiota in broilers. BMC Veterinary Research. 2022;18: 218.
45. Hong TTT, Thuy TT, Passoth V, Lindberg JE. Gut ecology, feed digestion and performance in weaned piglets fed liquid diets. Livestock Science. 2009;125:232-7.
46. Murry AC Jr, Hinton A Jr, Morrison H. Inhibition of Growth of *Escherichia coli*, *Salmonella* Typhimurium, and *Clostridia perfringens* on Chicken Feed Media by *Lactobacillus salivarius* and *Lactobacillus plantarum*. Int J Poult Sci. 2004;3(9):603-7.
47. Lv J, Guo L, Chen B, Hao K, Ma H, Liu Y, et al. Effects of different probiotic fermented feeds on production performance and intestinal health of laying hens. Poult Sci. 2022;101(2): 101570.
48. Śliżewska K, Chlebicz-Wójcik A. Growth kinetics of probiotic *Lactobacillus* strains in the alternative, cost-efficient semi-solid fermentation medium. Biology (Basel). 2020;9(12):423.
49. Loo JS, Oslan SNH, Mokshin NAS, Othman R, Amin Z, Dejtisakdi W, et al. Comprehensive review of strategies for lactic acid bacteria production and

DOI: 10.60101/jarst.2025.261344

metabolite enhancement in probiotic cultures: multifunctional applications in functional foods. Fermentation. 2025;11(5):241.

50. Morgan NK, Walk CL, Bedford MR, Burton EJ. The effect of dietary calcium inclusion on broiler gastrointestinal pH: quantification and method optimization. Poult Sci. 2014;93(2): 354-63. doi:10.3382/ps.2013-03305.

51. Fitsum S, Gebreyohannes G, Sbhatu DB. Bioactive compounds in fermented foods: health benefits, safety, and future perspectives. Appl Food Res. 2025;5(2):101097.

52. Verardo V, Gómez-Caravaca AM, Tabanelli G. Bioactive components in fermented foods and food by-products. Foods. 2020;9(2):153.

53. Alshelmani MI, Loh TC, Foo HL, Sazili AQ, Lau WH. Effect of feeding different levels of palm kernel cake fermented by Paenibacillus polymyxa ATCC 842 on nutrient digestibility, intestinal morphology, and gut microflora in broiler chickens. Anim Feed Sci Technol. 2016;216:216-24.

54. Thanadna B, Niyamosot C. Epidemiological characteristics and risk factors for *Salmonella* spp. in GAP certified broiler and breeder farms in Lopburi in 2020. Research Report No. 64(2)-0116(1)-102. Lopburi Provincial Livestock Office and Saraburi Provincial Livestock Office, Thailand; 2020.

55. Rugpudsa P, Kasemsuwan S, Saengthongpinit C. Prevalence of *Salmonella* spp. in broiler industrial production chain. In: Proceedings of 51st Kasetsart University Annual Conference. Bangkok; Veterinary Medicine, Fisheries; 2013. p. 1-8.

56. Yegani M and Korver DR. Factors affecting intestinal health in poultry. Poult Sci. 2008;87 (10):2052-2063.

57. Cruvinel WM, DM Jr, Araújo JAP, Catelan TTT, de Souza AWS, da Silva NP, et al. Immune system-Part I fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. Bras J Rheumatol. 2010;55(4):434-61.

58. Shahini A, Shahini A. Role of interleukin-6-mediated inflammation in the pathogenesis of inflammatory bowel disease: focus on the available therapeutic approaches and gut microbiome. J Cell Commun Signal. 2023;17(1):55-74.

59. Soufli I, Toumi R, Rafa H, Touil-Boukoffa C. Overview of cytokines and nitric oxide involvement in immuno-pathogenesis of inflammatory bowel diseases. World J Gastrointest Pharmacol Ther. 2016 6;7(3):353-60.

60. Chen CY, Tsen HY, Lin CL, Yu B, Chen CS. Oral administration of a combination of select lactic acid bacteria strains to reduce the *Salmonella* invasion and inflammation of broiler chicks. Poult Sci. 2012;91(9):2139-47.

61. Voidarou C, Antoniadou M, Rozos G, Tzora A, Skoufos I, Varzakas T, et al. Fermentative foods: microbiology, biochemistry, potential human health benefits and public health issues. Foods. 2020;10(1):69.

62. Pechrkong T, Kaewkong W, Hwanhlem N, Subsoontorn P, Tartrakoon W, Incharoen T, et al. The interleukin 6 (IL-6) gene expression is decreased by probiotic supplementation in young Barbary ducks. Khon Kaen Agric J. 2021;49(Suppl 1):89-94.

63. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. Front Immunol. 2021;26(12):578386.

64. Palkovicsné Pézsa N, Kovács D, Rácz B, Farkas O. Effects of *Bacillus licheniformis* and *Bacillus subtilis* on Gut Barrier Function, Proinflammatory Response, ROS Production and Pathogen Inhibition Properties in IPEC-J2-*Escherichia coli*|*Salmonella* Typhimurium Co Culture. Microorganisms. 2022;10:936.

65. Aghamohammad S, Sepehr A, Miri ST, Najafi S, Pourshafie MR, Rohani M. The role of combining probiotics in preventing and controlling inflammation: a focus on the anti-inflammatory and immunomodulatory effects of probiotics in an in vitro model of IBD. Can J Gastroenterol Hepatol. 2022;2022(1):2045572.

66. Choi EY, Lee SS, Hyeon JY, Choe SH, Keum BR, Lim JM, et al. Effects of β-glucan on the release of nitric oxide by macrophages stimulated with lipopolysaccharide. Asian-Australas J Anim Sci. 2016;29(11):1664-74.

67. Lieb K, Engels S, Fiebich BL. Inhibition of LPS-induced iNOS and NO synthesis in primary rat microglial cells. Neurochemistry International. 2003;42(2):131-7.

68. Poljakovic M, Svensson ML, Svanborg C, Johansson K, Larsson B, Persson K. *Escherichia coli*-induced inducible nitric oxide synthase and cyclooxygenase expression in the mouse bladder and kidney. Kidney Int. 2001;59(3):893-904.

69. Shehata AA, Yalçın S, Latorre JD, Basiouni S, Attia YA, Abd El-Wahab A, et al. Probiotics, prebiotics, and phytogenic substances for optimizing gut health in poultry. Microorganisms. 2022;10(2):395.

DOI: 10.60101/jarst.2025.261344

70. Sun Y, Liu Z, Ren L, Wei Z, Wang P, Li N, et al. Immunoglobulin genes and diversity: what we have learned from domestic animals. J Animal Sci Biotechnol. 2012;3:18.

71. Alberts B, Johnson A, Lewis J. Molecular Biology of the Cell. 4th edition. Chapter 24, The Adaptive Immune System. New York: Garland Science; 2002.

72. Janeway CAJ, Travers P, Walport M. Immunobiology: The Immune System in Health and Disease. 5th edition. Principles of innate and adaptive immunity. New York: Garland Science; 2001.

73. Ulmer-Franco AM. Transfer of Chicken Immunoglobulin Y (IgY) from the Hen to the Chick. Avian Biology Research. 2012;5(2):81-7.

74. Abbas AT, El-Kafrawy SA, Sohrab SS, Azhar EIA. IgY antibodies for the immunoprophylaxis and therapy of respiratory infections. Human Vaccines & Immunotherapeutics. 2019;15(1):264-75.

75. Dias da Silva W, Tambourgi DV, Pietrzak B, Tomela K, Olejnik-Schmidt A, Mackiewicz A, et al. Secretory IgA in intestinal mucosal secretions as an adaptive barrier against microbial cells. Int J Mol Sci. 2020;21(23):9254.

76. Jazi V, Mohebodini H, Ashayerizadeh A, Shabani A, Barekatain R. Fermented soybean meal ameliorates *Salmonella* Typhimurium infection in young broiler chickens. Poult Sci. 2019;98 (11):5648-60.

77. Sugiharto S, Yudiarti T, Isroli I, Widiastuti E, Wahyuni HI, Sartono TA. Fermented feed as a potential source of natural antioxidants for broiler chickens -A mini review. Agric Conspec Sci. 2019;84(4):313-8.

78. Gao Y, Liang Q, Sun J, Wu X, Song Y, Xu Y, et al. Probiotic potential of lactic acid bacteria with antioxidant properties in modulating health: mechanisms, applications, and future directions. Food Biosci. 2025;66:106181.

79. Fu Z, Ao N, Liang X, Chen J, Wang Y, Wang Q, et al. Effects of fermented feed on growth performance, serum biochemical indexes, antioxidant capacity, and intestinal health of lion-head goslings. Front Vet Sci. 2023;10:1284523.

80. Niu Y, Zhang JF, Wan XL, Huang Q, He JT, Zhang XH, et al. Effect of fermented Ginkgo biloba leaves on nutrient utilisation, intestinal digestive function and antioxidant capacity in broilers. Br Poult Sci. 2019;60(1):47-55.

81. Kennedy MS, Chang EB. The microbiome: Composition and locations. Prog Mol Biol Transl Sci. 2020;176:1-42.

82. Rajoka MSR, Thirumdas R, Mehwish HM, Umair M, Khurshid M, Hayat HF, et al. Role of food antioxidants in modulating gut microbial communities: novel understandings in intestinal oxidative stress damage and their impact on host health. Antioxidants (Basel). 2021;10(10): 1563.

83. Lucas AJS, Oliveira LM, Rocha M, Prentice C. Edible insects: An alternative of nutritional, functional and bioactive compounds. Food Chemistry. 2020;311:126022.

84. Kittibunchakul S, Whanmek K, Santivarangkna C. Physicochemical, microbiological and nutritional quality of fermented cricket (*Acheta domesticus*) paste. LWT. 2023;189:115444.

85. Diether NE, Willing BP. Microbial fermentation of dietary protein: an important factor in diet-microbe-host interaction. Microorganisms. 2019;7(1):19.

86. Kerksick C, Willoughby D. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr. 2005;2(2):38-44.

87. Kilicarslan YD, Fuwad A, Lee KH, Kim HK, Kang L, Kim SM, et al. Evaluation of the protective role of vitamin E against ROS-driven lipid oxidation in model cell membranes. Antioxidants (Basel). 2024;13(9):1135.

88. Guedes LM, Torres S, Sáez-Carillo K, Becerra J, Pérez CI, Aguilera N. High antioxidant activity of phenolic compounds dampens oxidative stress in Espinosa nothofagi galls induced on Nothofagus obliqua buds. Plant Sci. 2022;314:111114.

DOI: 10.60101/jarst.2025.261344