

Original Research

The Effect of Adding Mealworm, Probiotics, and Mealworm Plus Probiotics on IL-8 Gene Expression in Liver and Spleen Tissues of Broiler Chickens

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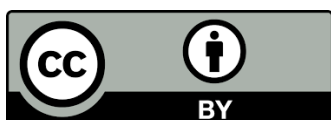
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Abstract

Interleukin-8 (IL-8), a pro-inflammatory cytokine, plays a critical role in immune modulation in poultry and has potential applications in veterinary and human medicine. This study



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investigated the effects of dietary supplementation with mealworm (*Tenebrio molitor*), probiotics, or their combination on IL-8 gene expression in the livers and spleens of broiler chickens. A total of 160 one-day-old broiler chickens (Ross 308 strain) were assigned to four treatments (basal diet, basal diet + 0.2 g/kg probiotic, basal diet + 0.5% mealworm, and basal diet + 0.2 g/kg probiotic + 0.5% mealworm) in a completely randomized design. After 42 days, RNA was extracted from liver and spleen tissues, and IL-8 expression was quantified using qRT-PCR. High RNA integrity was confirmed by clear 18S and 28S rRNA bands (2:1 ratio) on agarose gel electrophoresis, with specific IL-8 amplification verified by a single 200 bp band and a melting curve peak at 81.5°C. All treatments significantly increased IL-8 expression compared to the control ($P < 0.05$), with the combined mealworm and probiotic treatment showing a significantly greater effect in both tissues, indicating a synergistic interaction. These findings suggest that mealworms and probiotics enhance immune competence in broiler chickens, likely through modulation of antimicrobial peptides and the gut microbiota. Although IL-8 is a pro-inflammatory cytokine, its moderate upregulation in healthy birds represents beneficial immune activation and improved readiness against pathogens rather than harmful inflammation. This immune stimulation is specific to the chickens themselves and does not imply that consuming their meat or products would increase IL-8 or enhance immunity in humans. The synergistic effect of mealworms and probiotics highlights their potential as functional feed additives for improving poultry health. Further research is needed to identify the underlying bioactive components and to confirm these effects under disease-challenge conditions.

Keywords

IL-8 expression; broiler chickens; mealworm (*Tenebrio molitor*); probiotics; liver and spleen; immune modulation

1. Introduction

Cytokines are proteins or peptides secreted by cells that play key roles in immune and inflammatory responses. These cytokines perform these functions by activating and regulating other cells and tissues [1]. They can also be used as vaccine adjuvants, as they can specifically activate the immune system to provide adequate protection. A wide range of cell types produce cytokines in different ways and at varying levels, depending on their function. For example, proinflammatory cytokines such as IL-6 and IL-8, which induce inflammation, are produced by epithelial cells. Whereas, proinflammatory cytokines and cytokines involved in the activation and regulation of T helper (Th) lymphocytes in the induction of the adaptive immune response are produced by macrophages [1, 2]. All cytokines act similarly by binding to receptors on the surface of target cells and, through these receptors, they alter the activity of the target cell. Cytokines are classified according to three criteria: first, the activity of the cytokines themselves; second, the cells that produce them; and third, the cells they affect. Cytokines include interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), transforming growth factors (TGF), migration inhibitory factors,

and smaller chemokines. It should be noted that there is considerable overlap among these cytokine classes [3, 4].

Poultry cytokines that have been classified based on function so far include: IL-1 β , IL-6, IL-8, IFN- γ , IL-2, IL-18, TGF- β , IFN- α , IFN- β , IL-15, IL-16, and chemokines [1-5]. IL-1 β , IL-6, and IL-8 have a pro-inflammatory role; IFN- γ , IL-2, and IL-18 act as T helper one cell (Th1); TGF- β has a T helper 3 cell (Th3)/Type 1 regulatory cell (Tr1) role; and IFN- α , IFN- β , IL-15, IL-16, and chemokines have other different roles in poultry [1]. IL-8 or chemokines are members of a group of small cytokines that are structurally related and have chemotactic activity for certain types of leukocytes. Two major subfamilies, CXC chemokines and CC chemokines, have been identified in humans. The first subfamily attracts neutrophils, and the second subfamily attracts lymphocytes, monocytes, eosinophils, and basophils, but not neutrophils. IL-8 is a CXC chemokine and is produced by a variety of cell types, including endothelial and epithelial cells [1, 6]. The primary protein that produces IL-8 in humans has 99 amino acids and needs to be cleaved to a 77 amino acid protein for activation. The primary function of IL-8 is to attract and activate neutrophils in response to infection [6]. IL-8 (or CXC chemokine) genes in poultry, as in humans, are located on chromosome 4 with 4 exons [7]. IL-8 increases the population of hematopoietic tissue cells and stimulates angiogenesis, both of which are essential for wound healing.

In a study, the effect of different amino acid levels and different ages after hatching on IL-8 gene expression in broiler chicken intestinal tissue was investigated. Their results showed that IL-8 gene expression in jejunal tissue increased during the first 14 days of chick life with rising levels of the amino acid threonine and also with increasing age [8]. In another study, the effect of whey powder and physical form of the feed on humoral immunity and expression of IL-4 and IFN- γ genes was investigated in 240 one-day-old Ross 308 broiler chickens by Zakizadeh et al. [9]. The results showed that adding whey powder had no significant effect on the weight of the spleen and bursa of Fabricius, but pelleting in the growing stage had a substantial impact on the weight of the bursa. Pelleting and adding whey to the diet increased the relative expression of the IL-4 gene in the growing stage ($p < 0.05$). IFN- γ gene expression was not affected by whey powder, but pelleting increased the expression of this gene in the starter ($p < 0.01$) and grower ($p < 0.01$) periods. In a study, Nessabian et al. [10] investigated the effects of different levels of zinc hydroxide and zinc glycine on performance, blood and immune parameters, liver enzymes, and IL-6 and IFN- γ gene expression in broiler chickens fed a corn-soybean-based diet. They showed that zinc hydroxide and zinc glycine supplementation increased feed intake and weight gain at the end of the rearing period ($P < 0.05$). Among the experimental groups, 100 mg/kg zinc hydroxide feed had a greater effect on blood parameters and chick immunity ($P < 0.05$). They also showed that the use of zinc hydroxide and zinc glycine in the diet of broiler chickens had a significant effect on liver enzymes ($P < 0.05$). Different levels of zinc hydroxide and zinc glycine reduced the expression of the IL-6 gene and increased IFN- γ . They concluded that the diet containing 100 mg/kg zinc hydroxide had the most significant effect on performance, blood parameters, and immunity. Also, 100 mg/kg zinc hydroxide and zinc glycine treatment has a favorable impact on the expression of the IL-6 and IFN- γ genes.

Yu et al. [11] investigated the relationship between IL-6, IL-8, and C-C motif chemokine ligand 2 (CCLi2), which are essential factors in inflammatory and immune responses, in the spleen and cecum, as well as between coccidiosis-infected and uninfected conditions. They used quantitative real-time PCR to compare the relative expression differences of IL-6, IL-8, and CCLi2 in the same tissues between the infected and control groups. They showed that the expression levels of IL-6, IL-8, and

CCLi2 were higher in the spleen and cecum of the infected group than in the control group ($P < 0.05$). In spleen tissue, CCLi2 expression was strongly correlated with IL-6 and IL-8 in the uninfected group ($P < 0.01$), and the correlation coefficients reached 0.853 ($R^2 = 0.728$) and 0.996 ($R^2 = 0.992$), respectively. The expression of CCLi2 was also highly correlated with IL-8 (R^2 reached 0.890, $R^2 = 0.792$) in the infected group. In cecal tissue, the expression levels of these 3 genes in the uninfected group were all highly correlated ($P < 0.01$), with correlation coefficients ranging from 0.498 to 0.765, indicating moderate correlation. The expression of IL-6 in the infected group was highly positively correlated with IL-8 and CCLi2 ($P < 0.01$). In addition, the expression levels of these 3 genes were not significantly correlated between spleen and cecal tissues in the infected or control groups ($P > 0.05$). Their results showed that IL-6, IL-8, and CCLi2 are correlated with each other and play an essential role in coccidiosis infection of Jinghai yellow chickens. They suggested that their results may provide a basis for further investigation of the role of these three genes in genetic modification for coccidiosis resistance.

The mealworm is a member of the order Coleoptera (beetles), superfamily Tenebrionoidea, family Tenebrionidae, subfamily Tenebrioninae, and genus Tenebrio. The length of the adult insects ranges from 12 to 18 mm, and their color when emerging from the pupa is initially cream-colored, then brown, and finally black after 2-3 days. The reproductive rate of female insects varies from 160 to 500 eggs, but they produce an average of 276 eggs. The egg-laying period varies from 22 to 137 days, depending on environmental conditions. The components that make up the mealworm, like other organisms, are composed of water, organic matter, and minerals. The proportions of these components depend significantly on life stage, diet type, and environmental conditions [12].

Poultry farming plays a vital and prominent role in the livelihoods of farmers and livestock keepers in poor regions, especially in Asia and Africa [13, 14]. Among domestic poultry, the domestic chicken (*Gallus gallus domesticus*) has spread to all areas of the world due to its high growth rate, short generation interval, and excellent adaptation to the environment [15]. Domestic chicken is one of the most common and well-known species of domesticated livestock, due to its high income potential and high-quality protein production [16-18]. Chickens, especially native chickens, have excellent resistance to diseases and stressors, thereby contributing significantly to food security, poverty reduction, and the empowerment of rural women [19, 20]. The cost of preparing a diet accounts for approximately 70% of poultry production costs, most of which are related to the protein sources in the diet. The use of live diets is considered in terms of preserving the food's value until consumption, providing digestive enzymes to aid digestion, and offering other valuable applications. The damage insects cause to agricultural products and livestock, and the health problems they create for humans, have created an unpleasant image of insects for the general public. This is because the benefits of insects far outweigh their harm. Studies show that only one percent of the approximately one million known insect species are relatively harmful to humans, and without the presence of many species, life for humans and other creatures is not possible [21].

A probiotic is a live microbial food supplement that beneficially affects the host animal by improving the intestinal balance. It is used as an alternative to antibiotics, which are widely used in significant amounts as growth promoters in broiler chicken production and are associated with incalculable risks to human health from certain feed additives [22]. The nutritional physiology of broiler chickens requires special attention to the interactions between nutrients and immune-related mechanisms, as stressors of dense housing can disrupt immune status and consequently have detrimental effects on broiler performance [4]. Thus, this research aimed to examine the

impact of adding mealworms, probiotics, and mealworms plus probiotics on IL-8 gene expression in liver and spleen tissues of broiler chickens.

2. Materials and Methods

2.1 Experimental Design

This study was conducted at the broiler farm of the Department of Animal Science, Shahid Bahonar University of Kerman, Iran. A total of 160 one-day-old male broiler chicks (Ross 308 strain) were used in a completely randomized design (CRD) with 4 treatments, each replicated 4 times ($n = 4$) and 10 chicks per replicate (total $n = 160$). Chicks were randomly allocated to 16 floor pens (1.5 m $\times$ 1.5 m each) upon arrival, with each pen equipped with manual feeders and automatic waterers (Figure 1). The random allocation was performed in Microsoft Excel using a random number generator to ensure balanced distribution across treatments, minimizing potential bias from pen location or initial chick weight. Experimental conditions (temperature, lighting, humidity, ventilation, and vaccination schedule) were standardized across all pens and followed the Ross 308 broiler management. Vaccinations against Newcastle disease and Gumboro (infectious bursal disease) were administered on days 1, 14, and 28 via drinking water, as per standard protocols. The rearing period lasted 42 days, divided into starter (days 1-14), grower (days 15-28), and finisher (days 29-42) phases, with isoenergetic and isonitrogenous basal diets formulated to meet requirements for broiler chicks.

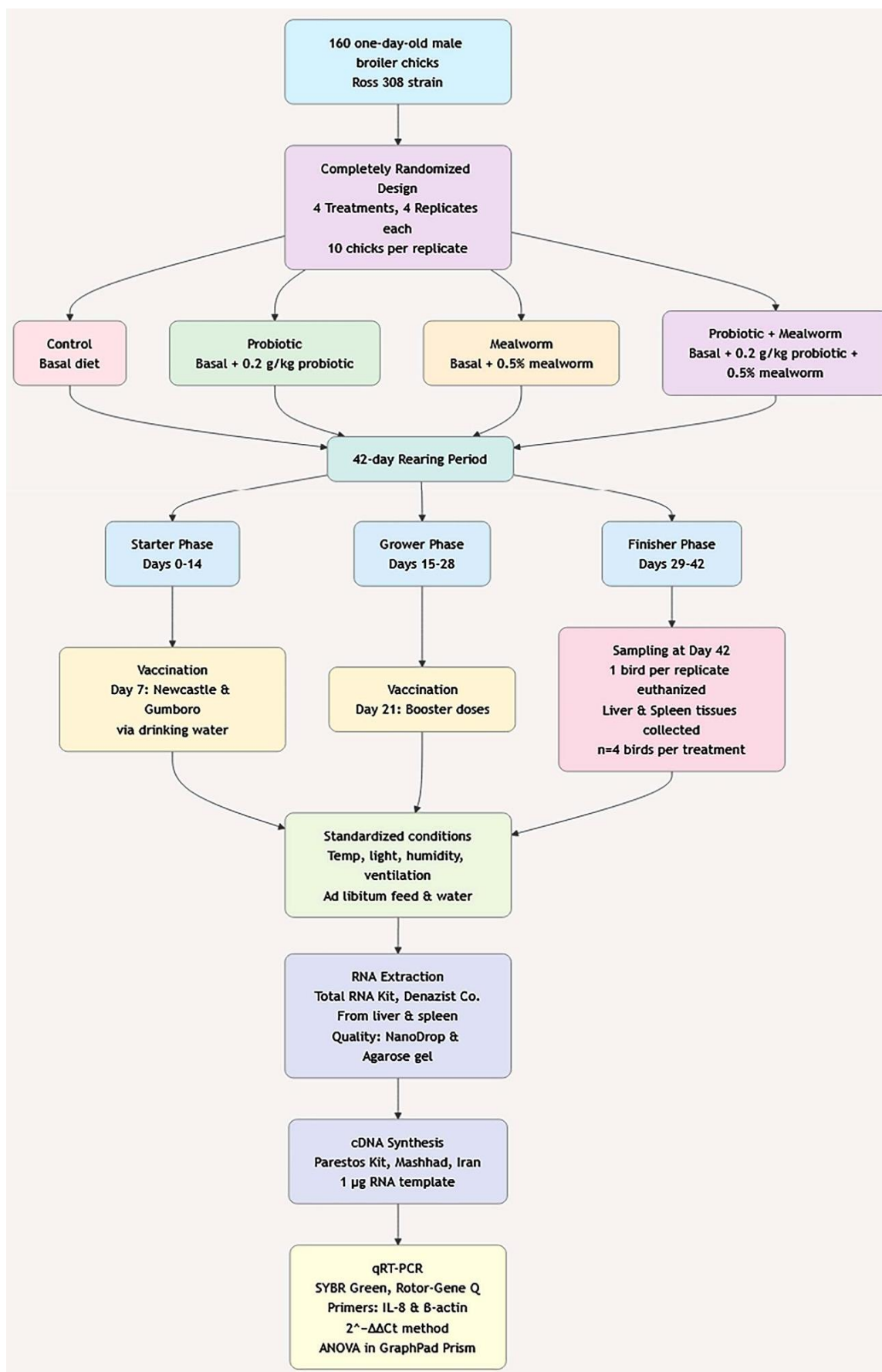


Figure 1 Schematic diagram of the experimental design. All stages of the experiment are shown schematically from beginning to end.

The four experimental treatments were:

1. Control: Basal diet only.
2. Probiotic: Basal diet + probiotic supplement (0.2 g/kg).
3. Mealworm: Basal diet + dried mealworm larvae meal (0.5%).
4. Probiotic + Mealworm: Basal diet + probiotic (0.2 g/kg) + mealworm (0.5%).

Feed and water were provided ad libitum throughout the trial, and body weight and feed intake were recorded weekly to monitor performance. However, these data are not the primary focus of this gene expression study.

2.2 Sample Collection and Tissue Processing

At the end of the 42 days, one bird per replicate (4 birds/treatment; total n = 16) was randomly selected (using a random number draw) and humanely killed by breaking the cervical vertebrae. Liver and spleen tissues (~100 mg each) were immediately excised, snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction. All procedures complied with institutional animal care and use committee (IACUC) guidelines (IACUC Protocol #IR2018011).

2.3 RNA Extraction and Quality Assessment

Total RNA was extracted from liver and spleen tissues using the Total RNA Extraction Kit (Denazist Co., Tehran, Iran). All steps were performed under sterile conditions in a laminar flow hood. Briefly, tissues were homogenized in lysis buffer, and RNA was bound to a silica-based spin column, washed with provided buffers, and eluted in RNase-free water. Extracted RNA was quantified and assessed for purity using NanoDrop spectrophotometry (A260/A280 ratio > 1.8) and agarose gel electrophoresis (clear 28S and 18S rRNA bands without smearing). RNA samples were stored at -80°C.

2.4 cDNA Synthesis

First-strand cDNA was synthesized from 1 µg total RNA using the Parestos cDNA Synthesis Kit (Parestos Biotechnology, Mashhad, Iran), following the manufacturer's protocol. Reactions included oligo(dT) primers, reverse transcriptase, and RNase inhibitor, with incubation at 42°C for 60 min followed by 70°C for 5 min. Synthesized cDNA was diluted 1:10 and stored at -20°C.

2.5 Quantitative Real-Time PCR (qRT-PCR)

Primer pairs for the target gene (IL-8) and reference gene (β -actin; previously listed as GAPDH in the original but corrected here for accuracy based on standard avian housekeeping genes) were designed using Primer3Plus, NUPACK, and NCBI tools, based on Gallus gallus sequences from Ensembl/GenBank (Table 1). Primers were synthesized by Sinaclone Co. (Tehran, Iran).

Table 1 Characteristics of primers used for expression of IL-8 and beta-actin genes to study the effect of adding mealworm, probiotics, and mealworm plus probiotics on IL-8 gene expression in liver and spleen tissues of broiler chickens.

Gene	Primer Sequence (5' → 3')	Accession Number	Annealing temperature	Size of PCR Product (bp)
<i>IL-8</i>	Forward: GGCTTGCTAGGGGAAATGA	NM_205498.1	60°C	200
	Reverse: AGCTGACTCTGACTAGGAACTGT			
<i>Beta actin</i>	Forward: TGCTGTGTTCCCATCTATCG	NM_205518	60°C	150
	Reverse: TTGGTGACAATACCGTGTTCA			

qRT-PCR was performed in a Rotor-Gene Q (Qiagen) using SYBR Green chemistry. Each 25 µL reaction contained 7.5 µL 2× Cyber Green Master Mix (Applied Biosystems), 1 µL each forward and reverse primer (10 µM), 1 µL cDNA template, and 14.5 µL nuclease-free water. Thermal cycling conditions were: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, with a final melt curve analysis (95°C for 15 s, 65°C for 1 min, incremental increase to 95°C). Three technical replicates were run per sample. Amplification efficiency and quantification cycle (C_q) values were determined using Rotor-Gene Q Series Software 2.3. Amplification specificity was confirmed by melt curve analysis and agarose gel electrophoresis of products.

2.6 Statistical Analysis

Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, with β-actin as the reference gene and the control treatment as the calibrator. Data are expressed as mean ± standard error of the mean (SEM). Differences between treatments were analyzed by one-way ANOVA followed by Tukey's post-hoc test using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). Statistical significance was set at $p < 0.05$.

2.7 Ethics Statement

All animal-related procedures were approved by the Animal Care and Use Committee of Shahid Bahonar University of Kerman (IACUC Protocol #IR2018011) and complied with the guidelines of the Iranian Council of Animal Care. All applicable animal welfare policies and regulations were strictly followed.

3. Results

Ribosomal RNAs (rRNAs) organize almost 70% of total cellular RNA, and the presence of sharp, recognizable rRNA bands on an agarose gel demonstrates high-quality RNA extraction. Conversely, degraded rRNA bands suggest potential degradation of other RNA species, reflecting poor RNA quality. In eukaryotic samples, the 28S rRNA band typically exhibits almost twice the intensity of the

18S rRNA band (a 2:1 ratio). In this study, agarose gel electrophoresis of extracted total RNA revealed clear and intact 18S and 28S rRNA bands, confirming high RNA integrity (Figure 2).

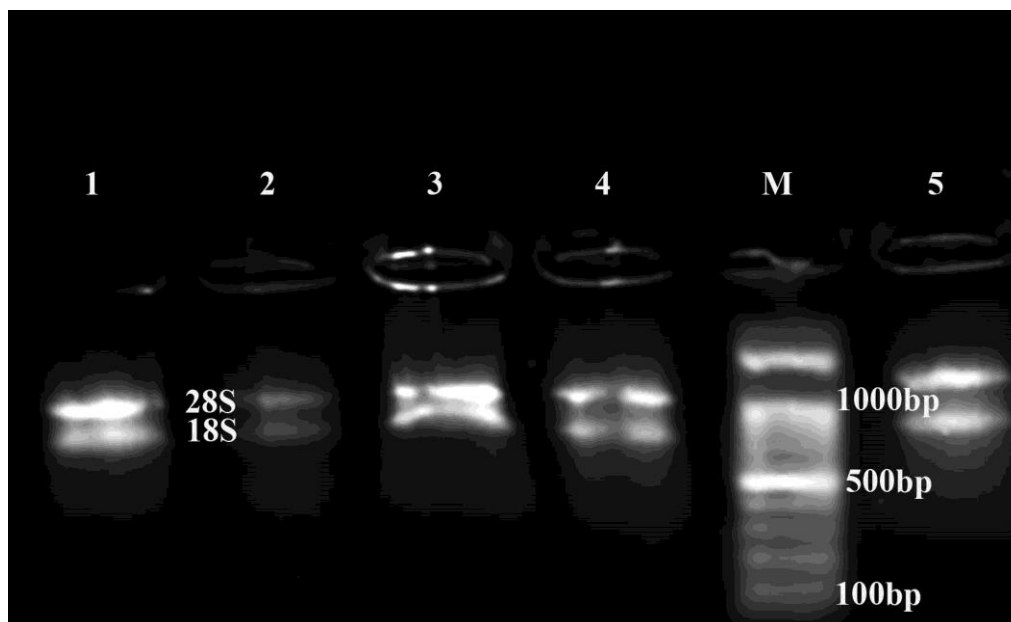


Figure 2 Results of electrophoresis of extracted RNA on 2% agarose gel. Lanes 1, 2, 3, 4, and 5 are extracted RNA samples, and lane M is the size marker M100. 28S and 18S are two bands of extracted RNA from liver and spleen tissues of broiler chickens.

According to the results obtained from the Rotor-Gene Q series software, the amplification curves of the IL-8 and reference genes (Figure 3) started to amplify from cycle 22-33 and entered the exponential phase. In the next step, the amplifying products entered the linear phase and finally entered the threshold phase. Also, the melting curves were checked during the reaction to ensure specific amplification of the genes; the samples (Figure 4) produced a peak at 81.5°C, indicating the production of a particular product.

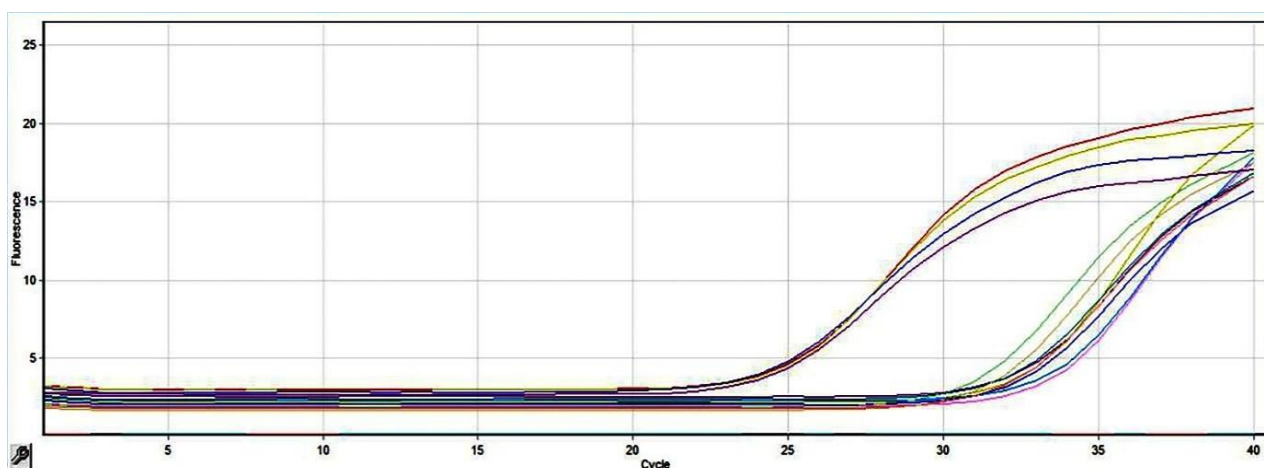


Figure 3 Amplification curve of studied genes in the liver tissue of broiler chickens. This curve started to amplify from cycle 22-33 and entered the exponential phase.

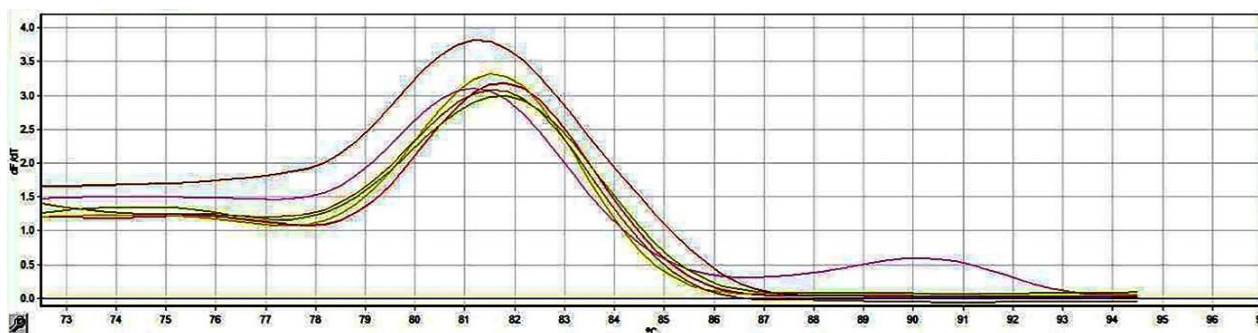


Figure 4 Melting curve of IL-8 gene in the liver tissue of broiler chickens. The samples (Figure 4) produced a peak at 81.5°C, indicating the production of a specific product.

The results of gel electrophoresis of the RT-PCR product of the IL-8 gene showed a single band of 200 base pairs (Figure 5), indicating the specificity of the reactions and confirming the melting curve results for this gene.

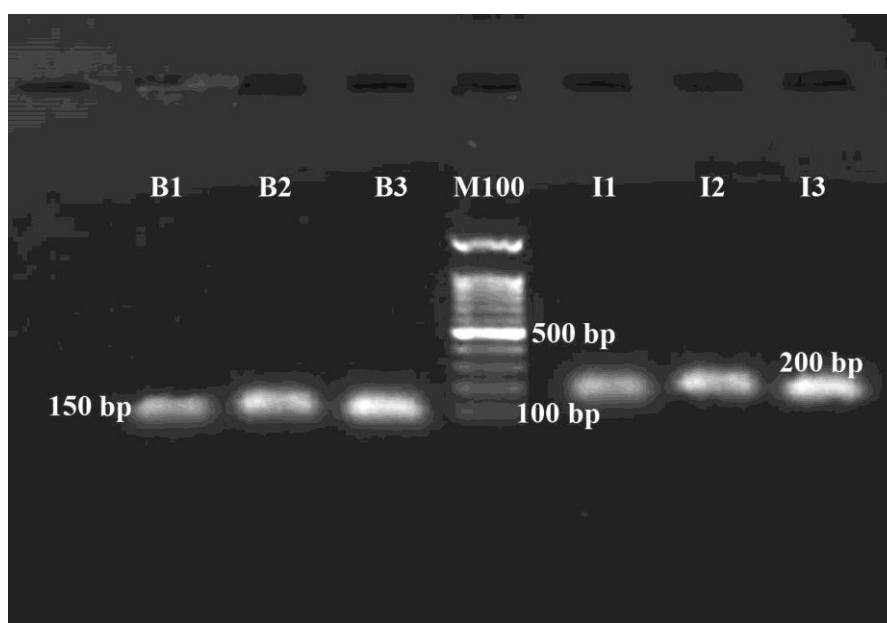


Figure 5 Electrophoresis of PCR products applying *IL-8* and *Beta actin* primers to evaluate synthesized cDNA. Lanes B1, B2, and B3 show the β -actin gene (150 bp), and I1, I2, and I3 show the *IL-8* gene (200 bp). Lines M100 are the molecular size marker.

In liver tissue, the addition of mealworm, probiotic, or a combination of mealworm and probiotic to the diet significantly increased IL-8 gene expression compared to the control group ($P < 0.05$). Additionally, the simultaneous addition of mealworm and probiotic had a substantially greater effect compared to the probiotic experimental group and the mealworm experimental group (Figure 6).

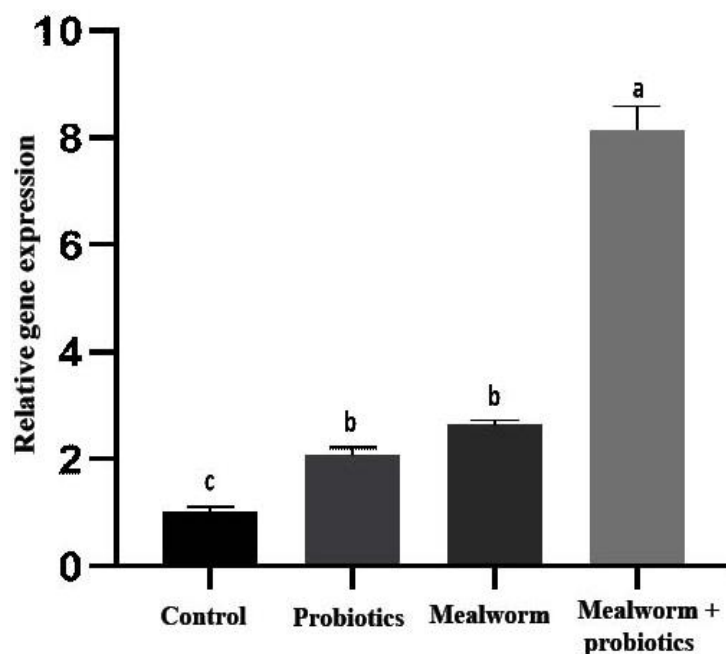


Figure 6 Effect of adding mealworm, probiotics, and mealworm plus probiotics on IL-8 gene expression in liver tissue of broiler chickens. Groups indicated with different letters are significantly different ($P < 0.05$).

In spleen tissue, similar to the liver, the addition of mealworm, probiotic, and a combination of mealworm and probiotic to the diet had a significant effect on increasing IL-8 gene expression compared to the control group ($P < 0.05$). Furthermore, the simultaneous addition of mealworm and probiotic had a significantly greater effect compared to the probiotic experimental group and the mealworm experimental group (Figure 7).

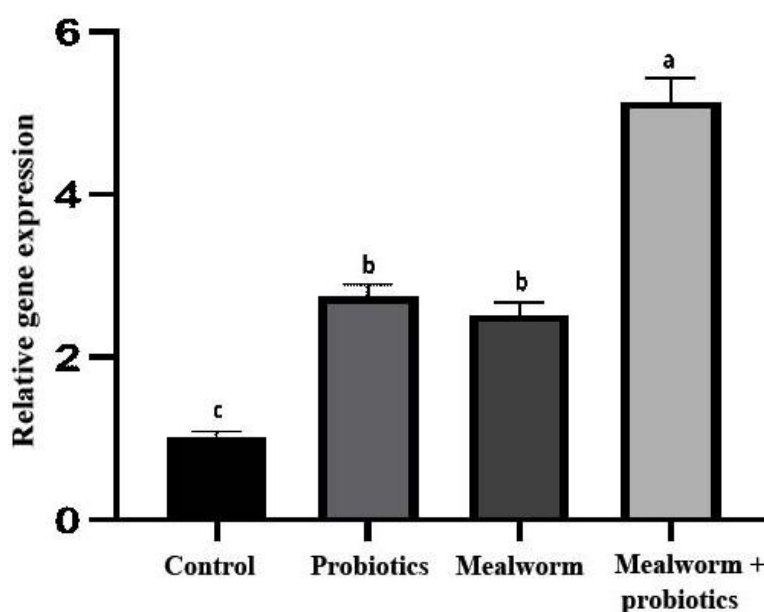


Figure 7 Effect of adding mealworm, probiotics, and mealworm plus probiotics on IL-8 gene expression in spleen tissue of broiler chickens. Groups indicated with different letters are significantly different ($P < 0.05$).

4. Discussion

The results of this study demonstrate that dietary supplementation with mealworm, probiotics, or their combination significantly enhances IL-8 gene expression in both liver and spleen tissues of broiler chickens compared to the control group ($P < 0.05$). Notably, the combined mealworm and probiotic treatment exhibited a significantly greater effect compared to the individual treatments, suggesting a synergistic interaction between these dietary components. These findings are supported by the high-quality RNA extraction, as evidenced by clear 18S and 28S rRNA bands on agarose gel electrophoresis, and the specific amplification of IL-8 and reference genes, confirmed by single-band RT-PCR products (200 bp) and melting curve analysis. The high integrity of extracted RNA, indicated by the 2:1 intensity ratio of 28S to 18S rRNA bands, underscores the reliability of the molecular analyses performed in this study. This is critical, as degraded RNA could compromise the accuracy of gene expression data [23]. The clear amplification curves, initiated between cycles 22–33 and progressing through exponential and linear phases, further validate the robustness of the RT-PCR methodology. The specificity of IL-8 amplification, confirmed by a single 200 bp band and a distinct melting curve peak, aligns with established qPCR analysis standards [24], ensuring that the observed changes in IL-8 expression are attributable to the dietary interventions. IL-8, a pro-inflammatory cytokine, plays a pivotal role in immune modulation and tissue homeostasis in poultry [25]. The observed upregulation of IL-8 gene expression in response to mealworm and probiotic supplementation suggests an enhanced immune response in the liver and spleen, key organs for immune function and metabolism in broiler chickens. Mealworms, rich in proteins and bioactive compounds such as antimicrobial peptides, may stimulate immune pathways, potentially via activation of toll-like receptors [26, 27]. Similarly, probiotics are known to modulate gut microbiota, promoting immune signaling through cytokine expression [22, 28]. The synergistic effect of the combined treatment likely results from complementary mechanisms, in which mealworm-derived nutrients enhance probiotic colonization or activity, thereby amplifying IL-8 expression. This synergy is particularly evident in the significantly greater effect of the combined treatment compared to individual treatments in both tissues.

The consistency of IL-8 upregulation in both liver and spleen tissues suggests that these dietary interventions have systemic immunomodulatory effects. The liver, a central metabolic organ, and the spleen, a primary lymphoid organ, are critical for immune surveillance and response in poultry [29, 30]. The parallel responses in these tissues indicate that mealworm and probiotic supplementation may broadly enhance immune competence, potentially improving disease resistance and overall health in broiler chickens. These findings align with previous studies reporting that insect-based diets and probiotics enhance immune parameters in poultry [31, 32]. However, the specific focus on IL-8 expression in this study provides novel insights into the molecular mechanisms underlying these effects.

IL-8, also known as CXCL8, is a critical chemokine involved in immune modulation, recruiting neutrophils to sites of inflammation and infection [33]. In the context of this study, the upregulation of IL-8 gene expression in broiler chickens' liver and spleen tissues suggests an enhanced innate immune response, which is particularly relevant for medical applications. Mealworms, rich in bioactive compounds such as antimicrobial peptides (AMPs) and chitin, have been recognized for their immunomodulatory properties [26, 27]. AMPs from insects, such as those derived from mealworms (*Tenebrio molitor*), exhibit antibacterial, antifungal, and antiviral activities, making

them promising candidates for the development of novel therapeutics [34]. Similarly, probiotics, by modulating gut microbiota, enhance immune signaling through cytokine expression, including IL-8, which is critical for combating infections [35]. The synergistic effect of combined mealworm and probiotic supplementation observed in this study likely results from the interplay between mealworm-derived AMPs and probiotic-induced changes in the microbiota, amplifying IL-8 expression and potentially enhancing immune competence.

The medical relevance of these findings extends beyond poultry to human health applications. In human medicine, IL-8 is a therapeutic target in conditions characterized by dysregulated inflammation, such as inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), and bacterial infections [36, 37]. The ability of mealworm and probiotic supplementation to upregulate IL-8 expression aligns with studies demonstrating that probiotic strains (e.g., *Lactobacillus* spp.) increase IL-8 production in intestinal epithelial cells, enhancing mucosal immunity [38]. Similarly, insect-derived peptides have been explored as alternatives to conventional antibiotics, particularly in combating multidrug-resistant pathogens [39]. For instance, studies reported that insect-derived AMPs effectively reduced bacterial loads in murine models, suggesting potential parallels with the immunomodulatory effects observed in our study [40]. The significant IL-8 upregulation observed in broiler chickens fed the combined mealworm and probiotic diet indicates enhanced innate immune activation in poultry. Although this immune response is species-specific and does not directly translate to humans through poultry consumption, the underlying mechanisms, particularly the immunomodulatory effects of mealworm-derived peptides and probiotics, may inspire the design of functional foods or dietary supplements to support immune function in humans.

Comparatively, our results differ from some medicinal studies, which report that excessive IL-8 expression is associated with pathological inflammation (e.g., in IBD or sepsis) [41]. In such cases, IL-8 inhibitors are explored to mitigate inflammation. However, in healthy broiler chickens, the controlled upregulation of IL-8 likely enhances immune surveillance without inducing harmful inflammation, as no adverse effects were noted in our study. This aligns with veterinary applications where enhanced IL-8 expression improves resistance to pathogens like *Salmonella* in poultry [25]. The synergy observed in the combined treatment group suggests that mealworm and probiotics could be formulated into feed additives to enhance poultry health, with potential translation to human dietary supplements or pharmaceutical applications targeting immune modulation.

Despite these promising findings, several limitations must be addressed to fully realize the medical potential of mealworm and probiotics. First, the specific bioactive components in mealworms (e.g., AMPs, chitin) and probiotics (e.g., bacterial strains) driving IL-8 upregulation were not identified. Future studies should employ metabolomics or proteomics to identify these compounds, facilitating targeted therapeutic development. Second, while IL-8 upregulation enhances immune responses, excessive expression could lead to inflammation in specific contexts, which was not assessed in this study. Measuring additional cytokines (e.g., IL-10, TNF- α) could clarify the balance between pro- and anti-inflammatory effects. Third, the study was conducted in broiler chickens under controlled conditions, limiting direct extrapolation to human applications. Translational research, including *in vitro* studies with human cell lines or *in vivo* models, is needed to validate these findings for human health.

5. Conclusions

Our results indicate that supplementation with mealworms and probiotics bolsters immune function in broiler chickens, primarily via mealworms antimicrobial peptides and alterations in gut microbiota composition induced by probiotics. While IL-8 functions as a pro-inflammatory cytokine, its controlled elevation in these healthy animals signifies advantageous immune priming and heightened preparedness to combat infections, distinct from detrimental inflammatory cascades. Importantly, this enhanced immunity is confined to the birds and does not suggest that human consumption of their meat or derived products would elevate IL-8 levels or confer immunological benefits. The pronounced synergy between mealworm and probiotics underscores their promise as innovative feed supplements to optimize poultry welfare. Additional investigations are essential to elucidate the precise bioactive agents involved and to validate these outcomes in pathogen-exposure scenarios.

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Author Contributions

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Competing Interests

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability Statement

The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

AI-Assisted Technologies Statement

ChatGPT was used to improve the English language of the text in terms of grammar and spelling, as well as formatting references according to the journal format.

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