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Investigation of interleukin-6 gene expression in liver and spleen tissues of broiler chickens fed mealworm, probiotics, and mealworm with probiotics

Mohammadreza Mohammadabadi
*Corresponding Author. Professor, Animal Science Department, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail: mrm@uk.ac.ir

Amin Khezri
Associate Professor, Animal Science Department, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail: akhezri@uk.ac.ir

Mohsen Afsharmanesh
Associate Professor, Animal Science Department, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail address: mafshar@uk.ac.ir

Hamid Kheyrodin
Assistant Professor, Semnan University, Semnan, Iran. E-mail address: hamid.kheyrodin@semnan.ac.ir

Mohammad Reza Bahreini Behzadi
Animal Science Department, Faculty of Agriculture, Yasouj University, Yasouj, Iran. E-mail: bahreini2001@gmail.com

Olena Babenko
Assistant Professor, Department of Animal Science, Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine. E-mail: lelya.babenko1978@gmail.com

Svitlana Usenko
Department of Animal Productivity Biology, Named after academician O.V. Kvasnytskyi, Poltava State Agrarian University, Poltava, Ukraine. E-mail: svetlana.usenko@pdau.edu.ua

Mehdi Momen
Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA. E-mail: mmomen@wisc.edu

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Abstract

Objective

Given the growing concerns about the use of antibiotics in the poultry industry and the search for natural alternatives to improve the performance and health of broiler chickens, and the fact that the interleukin-6 (*IL-6*) gene is a key indicator in the immune and inflammatory responses of birds, the present study was conducted to investigate the nutritional effects of mealworms

(Tenebrio molitor) and probiotics, individually and in combination, on *IL-6* gene expression in liver and spleen tissues of broiler chickens.

Materials and Methods

In this study, 160 one-day-old male chicks of the Ross 308 strain were used in a completely randomized design with four treatments and four replicates (each replication included 10 birds). The experimental treatments included the control group (basal diet), probiotic group (0.2 g/kg feed), mealworm powder group (0.5% of feed), and probiotic and mealworm combination group. The rearing period lasted 42 days. At the end of the period, tissue samples were taken from the liver and spleen for RNA extraction and cDNA synthesis. *IL-6* gene expression was measured by Real-Time PCR using the *GAPDH* reference gene. Data were analyzed by ANOVA and Tukey's multiple comparison test ($P<0.05$).

Results

The results showed that the addition of probiotics, mealworm powder, and their combination led to a significant increase in *IL-6* gene expression in liver and spleen tissues compared with the control group ($P<0.05$). The highest increase in expression was related to the combined probiotic and mealworm treatment, which was significantly different from other treatments. Single-peak melting curves and the correct size of PCR products confirmed the specificity of the reaction. Increased *IL-6* expression in both tissues indicated stimulation of innate immune and acute phase inflammatory pathways at the systemic level.

Conclusion

The findings suggest that the combined use of probiotics and mealworm powder can have a synergistic effect on the immune activation of broiler chickens by increasing *IL-6* gene expression. These results indicate that the combination of these two natural additives can be used as an effective and safe approach to promote immune health and sustainability of the poultry farming system.

Keywords: immunity, probiotics, broiler chickens, mealworm, qRT-PCR

Paper Type: Research Paper.

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Introduction

In various countries, especially low-income African and Asian countries, livestock production, especially poultry production, is of particular importance (Mohammadabadi et al. 2024). Chickens have spread throughout the world due to their short generation interval and low demand and are raised by various rural and nomadic groups (Shahdadnejad et al. 2016). These birds are both a source of food for poor and low-income rural people and a good source of income to cover their other living expenses (Mohammadifar and Mohammadabadi, 2017). Because these animals are resistant to diseases, adapt to different climatic conditions, and easily use low-quality feed (Mohamadinejad et al. 2024). The poultry industry, as one of the main pillars of animal protein supply worldwide, plays an important role in food security and agricultural economics. In order to increase productivity, improve growth performance and promote the health of broilers, proper nutrition and optimal diet management are of particular importance (Khabiri et al., 2025). In recent years, with increasing concerns about the use of antibiotics as growth promoters and their negative effects on human health and the emergence of microbial resistance, research has been directed towards the use of natural and safe alternatives such as probiotics, prebiotics and unconventional protein sources (Mohammadifar & Mohammadabadi, 2018; Khezri et al., 2025). On the other hand, the increasing demand for poultry protein products and the simultaneous environmental and health pressures resulting from the widespread use of traditional feeds have led researchers and the poultry industry to search for sustainable and alternative nutritional sources (Khabiri et al., 2023). One of the emerging directions in the last decade is the use of edible pests and insects as an alternative protein source, which has attracted attention due to its high feed conversion efficiency, lower land and water requirements, and suitable nutritional profile (Mohammadabadi et al., 2025b). Research shows that black fly larva meal (Hermetia illucens) and mealworm or yellow mealworm or mealworm (Tenebrio molitor) powder are among the most important options for replacing part of the dietary protein in poultry and other production animals (Dalmoro et al., 2023). Among alternative protein sources, mealworm (Tenebrio molitor), as one of the most important edible insect species, has attracted special attention from researchers and the livestock and poultry industry due to its favorable chemical composition including high protein (about 45-55%), biologically valuable fats, vitamins, minerals, and chitin fiber (Hong et al., 2020). Several studies have shown that the use of mealworm powder in the diet of broiler chickens can lead to improved feed efficiency, increased body growth, improved carcass quality, and modulation of immune responses (Hong et al., 2020; Mohammadabadi et al., 2025b). However, the exact effects of mealworm use on molecular immune parameters, especially the expression of genes related to inflammation and immunity, have not yet been fully elucidated. At the same time, probiotics have been widely used as nutritional additives to improve intestinal health, increase nutrient absorption, and modulate immune responses in poultry (Mohammadabadi et al., 2025a). Among the most widely used probiotics in broilers are various species of Lactobacillus, Bacillus, and Enterococcus. Proposed mechanisms of action of probiotics include competition with pathogens, strengthening the intestinal epithelial barrier, production of beneficial metabolites, and stimulation of innate and adaptive immune pathways (Rehman et al., 2020). Meta-analyses and systematic reviews have indicated that probiotics can improve immune indices and various functional components in different conditions, although the consistency of results in individual studies is not complete and the efficacy depends on the microorganism species, dose, and environmental conditions (Yosi et al., 2023). The *IL-6* gene is located on chromosome 2 in chickens and roosters (Gallus gallus). The *IL-6* gene in chickens has a relatively more compact structure than its mammalian counterparts. In mammals, five exons are usually reported, while in the chicken genome the first two exons are partially fused together and have shorter non-coding sequences. This structural difference reflects different pathways of transcriptional regulation and gene expression in birds. Studies have shown that the promoter region of the *IL-6* gene in chickens contains conserved regulatory elements such as TATA-box, NF-κB binding site, and AP-1 motif, which play key roles in regulating inflammatory and innate immune responses. The mRNA sequence of this gene encodes a 212 amino acid long protein that plays a role in immune signaling pathways, especially JAK/STAT3 activation and induction of inflammatory factors (Kaiser et al., 2004). Therefore, the *IL-6* gene in chicken, while maintaining functional homology to mammals, but with structural differences in genomic organization, is considered one of the most important molecular indicators of inflammatory responses, and examining its expression in immune tissues such as liver and spleen can provide a deeper understanding of the effect of nutrition and biological additives on the regulation of poultry immunity. The liver and spleen, as two key organs in the host immune response, provide different perspectives. In addition to metabolic functions, the liver is the center of synthesis of acute phase proteins and a regulator of inflammatory reactions. The spleen serves as a site of accumulation and differentiation of immune cells and is part of the humoral and cellular response (Haq et al., 2022). Studying these two tissues simultaneously could provide a more comprehensive understanding of how nutritional interventions modulate immune responses at the tissue and molecular levels. Initial reports on the effects of mealworm on poultry immune parameters suggest that reasonable levels of replacement usually have synergistic or neutral effects on immune indices and in some cases cause mild activation of immune responses. However, inappropriate use or very high levels may induce adverse inflammatory responses. At the same time, studies investigating the combination of mealworm with probiotics have suggested the possibility of synergistic effects; this combination could lead to improved immune status by improving digestion and absorption of nutrients (providing substrates for the synthesis of immune molecules) while simultaneously modulating the gut microbiota (Mohammadabadi et al., 2025b). Considering the above, it is of practical and theoretical importance to investigate the separate and combined nutritional effects of mealworms and probiotics on *IL-6* gene expression in liver and spleen tissues of broiler chickens. The research hypothesis was the adding of mealworms and probiotics, alone or together, would influence IL-6 gene expression in immune tissues, with the combined treatment producing the most pronounced effect. So, the aim of this study was to investigate changes in *IL-6* gene expression in the liver and spleen of broiler chickens fed mealworms, probiotics, and a combination of the mealworms and probiotics.

Materials and methods

Experimental design: This study was conducted at the broiler farm of Shahid Bahonar University, Kerman. The initial temperature was 33°C and gradually reduced to 22°C at the end of the experimental period. Lighting was 23 hours per day in the first week and then 20 hours until the end of the period. Relative humidity was maintained between 50% and 60%. Ventilation and bedding were monitored daily. Vaccination was performed according to the standard Ross 308 strain (against Newcastle and Gumboro). All steps were performed in accordance with the university's ethical guidelines and the National Laboratory Animal Care and Use Protocol (IACUC Protocol #IR2018011). A total of 160 one-day-old male Ross 308 strain chicks were used in this study. The statistical design used was a completely randomized design (CRD) with four treatments and four replications, and 10 chicks were kept in each replicate (a total of 16 experimental units). The rearing period lasted 42 days and consisted of three nutritional phases: starter (1 to 14 days of age), grower (15 to 28 days of age), and finisher (29 to 42 days of age). The nutritional treatments used are shown in Table 1. Feed and water were freely available to the chicks throughout the period. Used probiotic included *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus licheniformis*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* (www.parsilact.com).

Table 1. Nutritional treatments used to breed the broiler chicks used in this study

Treatment number	Treatment name	Diet description
1	Control	Basal diet without additives
2	Probiotic	Basal diet + probiotic (0.2 g/kg feed)
3	Mealworm	Basal diet + Tenebrio molitor larval powder (0.5% of feed)
4	Probiotic + Mealworm	Basal diet + probiotic (0.2 g/kg) + Mealworm (0.5% of feed)

Tissue sampling: On day 42, one bird from each replicate (four birds per treatment, total 16 birds) was selected. After CO_2 anesthesia, the birds were humanely euthanized (breaking the cervical vertebrae). Samples of liver (right lobe) and spleen were immediately removed and placed in sterile Eppendorf tubes, then frozen in liquid nitrogen and stored at -80°C until RNA extraction.

RNA homogenization, extraction and quality assessment: About 100 mg of tissue was placed into a microtube containing 1 mL of Denazist RNA Lysis Buffer solution (Denazist Co., Tehran, Iran) and mixed with a mechanical homogenizer (IKA T25 digital homogenizer, Germany) on ice to obtain a uniform suspension. The samples were then incubated at room temperature for 5 minutes and centrifuged. Total RNA was extracted according to the instructions of the Total RNA Extraction Kit (Denazist Co., Tehran, Iran). The extracted RNA was washed with 70% ethanol and dissolved in RNase-free water. RNA concentration and purity were measured with a NanoDrop One (Thermo Fisher) at A260/A280 (acceptable ratio greater than 1.8). To assess RNA quality, 1 µL of sample was electrophoresed on a 1% agarose gel (containing ethidium bromide). The presence of clear 28S and 18S bands indicated RNA integrity. Smeared samples were discarded.

cDNA synthesis: From each sample, 1 µg of RNA was converted to cDNA using the ParsTous cDNA Synthesis Kit (ParsTous Biotechnology, Mashhad, Iran) according to the following instructions:

Reaction composition: RNA (1 µg), oligo(dT) primer (1 µL), dNTP mix (1 µL), RT buffer (4 µL), RNase inhibitor (1 µL), M-MLV reverse transcriptase (1 µL), RNase-free water to a final volume of 20 µL. Reaction conditions: 42°C for 60 min and then 70°C for 5 min to inactivate the enzyme. The resulting cDNA was diluted 1:10 and stored at −20°C.

Primer design and qRT-PCR reaction: The primers used for the *IL-6* gene (target gene) and the *GAPDH* reference gene are listed in Table 2 (Yu et al., 2020).

Table 2. Summary of the characteristics of the primers applied to study gene expression for *IL-6* and *GAPDH* genes and the effect of adding mealworm, probiotics, and mealworm plus probiotics on *IL-6* gene expression in liver and spleen tissues of broiler chickens

Gene	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)	Product size (bp)	GenBank accession no.	T_a (°C)
IL-6	CAAGGTGACGGAGGAGGAC	TGGCGAGGAGGGATTTCT	254	AJ309540	60
GAPDH	GGTGGTGCTAAGCGTGTTAT	ACCTCTGTCATCTCTCCACA	264	K01458	60

The qPCR reaction was performed using a Rotor-Gene Q (Qiagen) and a SYBR Green Master Mix kit (Applied Biosystems). The reaction was performed in a final volume of 25 μL and consisted of 12.5 μL SYBR Green Mix, 1 μL (10 μM) forward primer, 1 μL (10 μM) reverse primer, 2 μL cDNA, and 8.5 μL RNase-free water. The thermal profile was as follows: initial denaturation at 94°C for 1 min; 40 cycles of 94°C for 15 s, 60°C for 60 s with a final melt curve analysis (95°C for 15 s, 65°C for 1 min, incremental increase to 95°C). The combined annealing/extension step at 60°C was used according to the manufacturer's instructions and optimized for the Rotor-Gene Q system. The cycle threshold (Ct) values for each sample were calculated as the average of three technical replicates. The relative expression of the *IL-6* gene was determined according to the $2^{-\Delta\Delta Ct}$ method, which corresponding to $\log_2$-transformed fold-change values. *GAPDH* was used as a reference gene, and the control group was used as the calibrator. Results were reported as mean ± SEM. Statistical analysis was performed using one-way ANOVA and Tukey's multiple comparison test in GraphPad Prism 8.0 software. The significance level was considered to be $p < 0.05$.

Results

The quality assessment of RNA extracted from liver and spleen tissues of broiler chickens showed that the 28S and 18S bands in the agarose gel were quite clear and intact, and the intensity ratio of the bands was approximately 2:1, indicating the purity and integrity of the RNA (Figure 1). The absence of smearing further indicated minimal RNA degradation. These results confirmed that the isolated RNA was of sufficient quality for downstream cDNA synthesis and quantitative gene expression analysis. In Real-Time PCR reactions, the amplification curves of the target genes (*IL-6*) and the reference gene (GAPDH) clearly entered the exponential phase from cycles 23 to 24, and the melting curve showed only one distinct peak at 82°C, confirming the specificity of the reactions. Also, in the electrophoresis of the PCR products, a single band with a size of 254 bp was observed for the *IL-6* gene and a single band with a size of 264 bp for the *GAPDH* gene (Figure 2), confirming the accuracy of the results. In addition, the absence of secondary peaks or melt-shoulders (primer–dimers), stable baselines in amplification plots, and consistent Ct values across technical replicates (with no amplification in no-template controls) collectively indicate high assay reliability and robustness for downstream expression comparisons. The results showed that the addition of mealworm, probiotic, and the combination of both to the diet significantly increased *IL-6* gene expression in both liver (Figure 3) and spleen (Figure 4) tissues compared to the control group ($P<0.05$). Among the treatments, the simultaneous combination of mealworm and probiotic caused the greatest increase in the expression of this gene, and its difference with other treatments was also statistically significant.

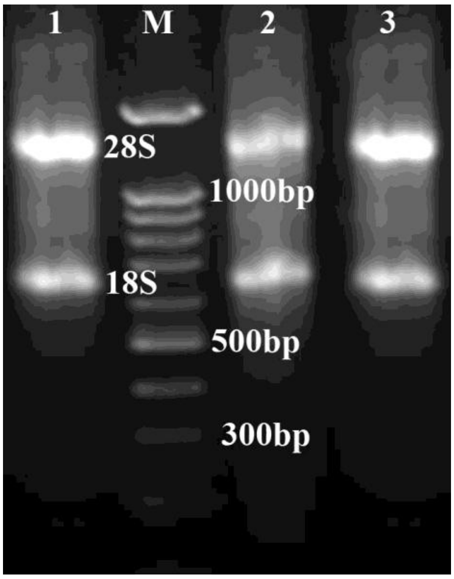

Figure 1. Some samples of extracted RNA on 2% agarose gel. For liver and spleen tissues of studied broiler chickens. Lanes 1, 2 and 3 are extracted RNA samples and lane M is size marker M100

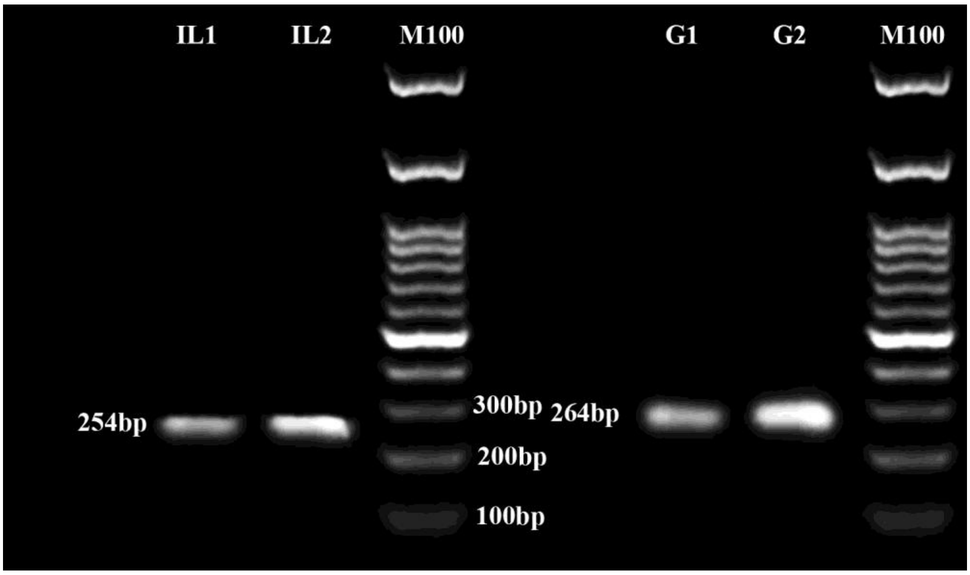

Figure 2. Some samples of PCR products of synthesized cDNA for *IL-6* and *GAPDH* genes on agaros gel electrophoresis. Lanes IL1 and IL2 show *IL-6* gene (254 bp) and lanes G1 and G2 display *GAPDH* gene (264 bp). Lanes M100 are the molecular size marker.

In general, the simultaneous use of mealworm and probiotic caused greater stimulation of immune pathways related to *IL-6* gene expression, which could be an indication of the synergistic effect of these two additives. The increase in *IL-6* expression in both main immune-related tissues (liver and spleen) indicates that this change is not only local but has a systemic effect on the immune response, reflecting a systemic enhancement of the host's immune readiness. These findings support the hypothesis that dietary inclusion of insect protein sources, such as mealworms, in combination with beneficial microorganisms, can modulate cytokine-mediated immune mechanisms. Such modulation may contribute to improved disease resistance and a more balanced inflammatory response, offering a potential natural alternative to antibiotic growth promoters in broiler production.

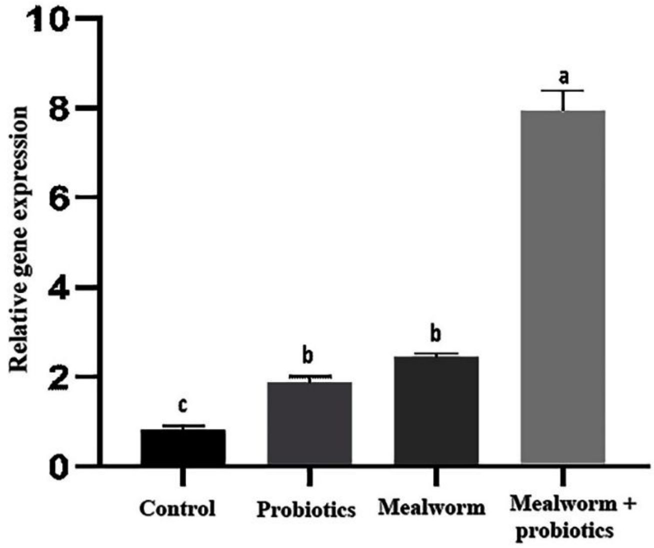

Figure 3. IL-6 gene expression levels in broiler liver tissue under the influence of mealworm, probiotics, and mealworm plus probiotic treatments. Different letters on the bars of the graph indicate a significant difference between them (P<0.05)

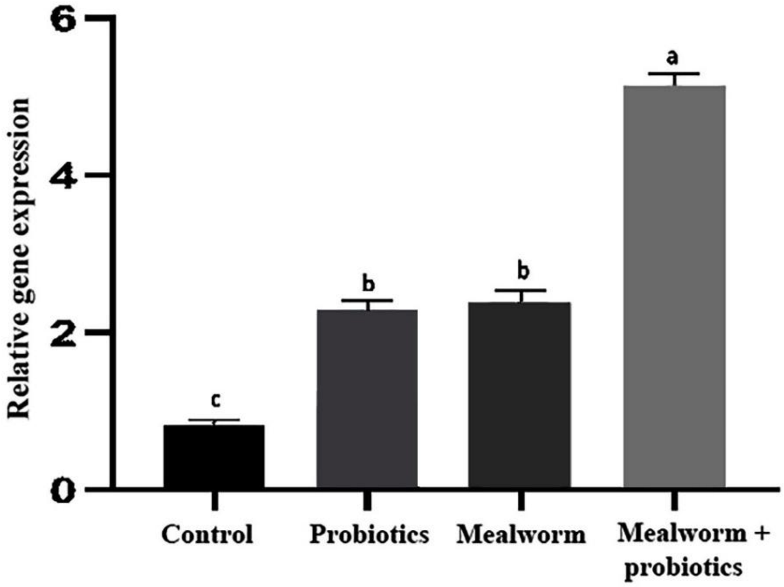

Figure 4. IL-6 gene expression levels in broiler spleen tissue under the influence of mealworm, probiotics, and mealworm plus probiotic treatments. Different letters on the bars of the graph indicate a significant difference between them (P<0.05)

Discussion

In the avian immune system, interleukin-6 (*IL-6*) is one of the most important inflammatory and immunogenic cytokines that plays a key role in regulating innate and adaptive immune responses. *IL-6* is produced by various immune cells, including macrophages, T and B lymphocytes, and Kupffer cells in the liver. In addition to its role in regulating inflammatory responses, this cytokine is also involved in B cell differentiation, T cell activation, and acute phase inflammatory stimulation in the liver. Increased *IL-6* gene expression is usually considered as an indicator of immune system stimulation and response to stressors, infections, or specific nutritional compounds (Bi et al., 2022). Therefore, examining changes in *IL-6* gene expression can be considered as a valuable molecular indicator for assessing the immune status of broiler chickens under different nutritional treatments. The findings of this study showed that the addition of mealworms, probiotics, and especially the simultaneous combination of the two to the diet significantly increased the expression of the *IL-6* gene in the liver and spleen tissues of broiler chickens. This increase in expression indicates the stimulation of innate immune pathways in the poultry body. Among the treatments, the simultaneous use of mealworms and probiotics showed the greatest effect, indicating a synergistic interaction between the biological components of these two substances. The *IL-6* gene is considered one of the most important pro-inflammatory cytokines and plays a key role in the recruitment and activation of immune cells. Therefore, a controlled increase in the expression of this gene in healthy birds can lead to improved immune system readiness against pathogens, without leading to harmful inflammation. The results of this study are consistent with previous reports on the immune system-stimulating effects of edible insects and probiotics. Kaiser et al. (2000) have shown *IL-6* is a secretory protein that plays an important role in the recruitment and control of cells in innate and adaptive immunity. Studies have shown that this cytokine is required for successful host immune responses to pathogens. They have confirmed the role of *IL-6* in the inflammatory response. They showed that *IL-6* contributes to short-term protection against infection or injury by alerting the immune system to the source of inflammation. Rodes et al. (2013) have displayed that if this molecule is not properly controlled and regulated, it can cause disease. They demonstrated that *IL-6* controls the immune response by stimulating the proliferation and differentiation of leukocytes that destroy pathogens. In a study by Elnagar et al. (2021), the prevalence of E. coli in diseased broiler chickens was investigated, and the pathological effects of these bacteria and interleukin gene expression in infected chickens were studied. They showed that there was a relationship between the levels of proinflammatory cytokines (*IL-6*, IL-8, and IL-10) and the challenge with different E. coli serotypes in broiler chickens. They observed an increase in ileal *IL-6* and IL-8 mRNA expression, but a decrease in ileal IL-10 expression after E. coli challenge. These changes occurred early after infection (day 2), and they concluded that *IL-6* and IL-8 are likely to participate in the host immune response to pathogens (Dalrymple et al., 1996; Eladl et al., 2019) and both cytokines are critical for initiating the acute phase immune response against invading pathogens and for stimulating a variety of immune cells such as T cells and macrophages (Wigley & Kaiser, 2003). They observed a significant increase in the expression of *IL-6* and IL-8 genes in the ileum of E. coli O78 (G2)-infected birds compared to other chickens and reported that the increased expression may be a result of the greater pathogenicity of E. coli O78 compared to the other serotypes tested. Therefore, E. coli O78 may overstimulate the immune system to a greater extent than the other serotypes tested and inoculation with E. coli induced the secretion of the proinflammatory cytokines IL-1b, *IL-6*, and IL-8, especially in spleen cells and lung tissue (Ariaans et al., 2008; Ateya et al., 2019; Huang et al., 2019). They concluded that cytokines have potential as therapeutic agents against infectious diseases, especially colibacillosis. Nishimichi et al. (2006) and Neurath et al. (2011) have shown that *IL-6* is a pleiotropic cytokine that plays an important role in inflammation, immune response, and hematopoiesis. Schett et al. (2013) have also reported that *IL-6* is mainly produced by macrophages, exerts protective functions in the repair of damaged tissues, and participates in acute-phase immune response and coagulation in chickens. Zhang and Zheng (2017) reported that *IL-6* stimulates systemic inflammatory signals to initiate host defense when the body is infected and damaged by pathogens. Rose-John et al. (2017) discovered that host defense against bacterial and fungal pathogen infection mainly relies on the classical *IL-6* signaling pathway. Sun et al. (2005) studied the effect of chicken *IL-6*-carrying DNA plasmids on infectious bursal disease virus (IBDV), and found that injection of *IL-6* plasmids resulted in a significant increase in the protective effect in chickens. Lin et al. (2015) used RNA-seq to sequence cecal tissue from an E. tenella-infected and an uninfected group at day 7 post-infection and showed that differentially expressed genes (DEGs) including *IL-6*, IL-12, and TGFB2 were significantly upregulated during coccidiosis infection. In another study, Zhang et al. (2014) showed that IL-1, *IL-6*, and TNF-α play critical roles in the inflammatory response of chicken embryo fibroblasts infected with avian reovirus. It seems that the presence of protein compounds and antimicrobial peptides in mealworms, together with the modulation of the gut microbial population by probiotics, led to the activation of immune signaling pathways and, consequently, increased *IL-6* gene expression. These simultaneous effects could explain the superiority of the combined treatment over each of the additives individually. The increased expression of *IL-6* in two key tissues, namely the liver and spleen, indicates a systemic rather than localized effect of these additives on the immune system. These two organs play a major role in regulating immune and metabolic responses; therefore, the stimulation of immune genes in them can help improve general health and resistance of poultry to diseases. Overall, the results of this study indicate that the use of mealworms and probiotics in the diet of broiler chickens can be probably considered as an effective biological strategy to improve immune health, without the need for antibiotics. However, further studies are needed to identify the bioactive compounds present in mealworms and investigate their effects in conditions of exposure to pathogens in order to determine the exact mechanism of action of these additives.

Conclusion: Based on the results obtained, the addition of mealworms and probiotics to the diet of broiler chickens significantly increased *IL-6* gene expression in liver and spleen tissues. This finding indicates that both additives can enhance the innate immune system of poultry. Among the experimental treatments, the simultaneous combination of mealworms and probiotics had the greatest effect, indicating a synergistic effect between these two biological compounds. Controlled increase in *IL-6* gene expression, as one of the key cytokines in immune responses, indicates positive and constructive stimulation of the immune system without causing harmful inflammation. Thus, it can be concluded that the combined use of mealworms and probiotics in poultry nutrition, while enhancing immune health, can be considered as a natural and efficient alternative to chemical growth promoters and antibiotics. Despite the promising results of this research, it is suggested that future studies focus on identifying the bioactive compounds of mealworms and examining their effects in conditions of infectious diseases in order to fully clarify the exact mechanism of their effect on immune genes.

Author contributions

Conceptualization, funding acquisition, investigation, methodology, resources, supervision, and experimental validation: M Mohammadabadi; data curation: AK, MA, and OB; formal analysis: M Mohammadabadi, M Momen, and HK; project administration: M Mohammadabadi, SU, and MRBB; software: M Mohammadabadi, and M Momen; manuscript drafting: M Mohammadabadi, AK, MA, and OB; manuscript review and editing: M Mohammadabadi, HK, SU, and M Momen. All authors reviewed and approved the final manuscript.

Data availability statement

All relevant data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Ethical considerations

This study was conducted in accordance with the standards of the Ethics Committee of Shahid Bahonar University of Kerman, and all experimental protocols and sampling techniques were approved by this committee. All procedures complied with institutional animal care and use committee (IACUC) guidelines (IACUC Protocol #IR2018011).

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Conflict of interest

There is no conflict of interest, financial or otherwise.

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محمدرضا محمدآبادی

*نویسنده مسئول: استاد بخش علوم دامی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، ایران. ایمیل: mrm@uk.ac.ir

امین خضری

دانشیار بخش علوم دامی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، ایران. ایمیل: akhezri@uk.ac.ir

محسن افشارمنش

دانشیار بخش علوم دامی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، ایران. ایمیل: mafshar@uk.ac.ir

حمید خیرالدین

استادیار، دانشکده کویرشناسی، دانشگاه سمنان، سمنان، ایران. ایمیل: hamid.kheyrodin@semnan.ac.ir

محمدرضا بحرینی بهزادی

گروه علوم دامی، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران. ایمیل: bahreini2001@gmail.com

اولنا بابنکو

استادیار، گروه علوم دامی، دانشگاه ملی کشاورزی بیلا تسرکوا، بیلا تسرکوا، اوکراین. ایمیل: lelya.babenko1978@gmail.com

سویتلانا اوسنکو

بخش زیست‌شناسی تولیدات دامی، به نام استاد دانشگاه او. وی. کواسنیتسکی، دانشگاه کشاورزی ایالتی پولتاوا، پولتاوا، اوکراین. ایمیل: svetlana.usenko@pdau.edu.ua

مهدی مومن

بخش علوم جراحی، دانشکده دامپزشکی، دانشگاه ویسکانسین-مدیسون، مدیسون، ویسکانسین، ایالات متحده آمریکا. ایمیل: mmomen@wisc.edu

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چکیده

هدف: با توجه به افزایش نگرانی‌ها در خصوص استفاده از آنتی‌بیوتیک‌ها در صنعت طیور و جستجوی جایگزین‌های طبیعی برای بهبود عملکرد و سلامت جوجه‌های گوشتی و این که ژن اینترلوکین-۶ (*IL-6*) به عنوان یکی از شاخص‌های کلیدی در پاسخ‌های

ایمنی و التهابی پرندگان مورد توجه قرار گرفته است، پژوهش حاضر با هدف بررسی اثرات تغذیه‌ای میلورم (Tenebrio molitor) و پروبیوتیک، به‌صورت جداگانه و ترکیبی، بر بیان ژن *IL-6* در بافت‌های کبد و طحال جوجه‌های گوشتی انجام شد.

مواد و روش‌ها: در این مطالعه، ۱۶۰ قطعه جوجه نر یک‌روزه سویه Ross 308 در قالب طرح کاملاً تصادفی با چهار تیمار و چهار تکرار (هر تکرار شامل ۱۰ پرنده) مورد بررسی قرار گرفتند. تیمارهای آزمایشی شامل گروه کنترل (جیره پایه)، گروه پروبیوتیک (۰/۲ گرم در کیلوگرم خوراک)، گروه پودر کرم آردی (۰/۵ درصد از خوراک) و گروه ترکیب پروبیوتیک و کرم آردی بودند. دوره پرورش ۴۲ روز به طول انجامید. در پایان دوره، نمونه‌های بافتی از کبد و طحال برای استخراج RNA و سنتز cDNA گرفته شدند. بیان ژن *IL-6* با روش Real-Time PCR و با استفاده از ژن مرجع *GAPDH* سنجش شد. داده‌ها با روش آماری ANOVA و آزمون چنددامنه‌ای توکی تحلیل شدند ($P<0.05$).

نتایج: نتایج نشان داد که افزودن پروبیوتیک، پودر کرم آردی و ترکیب آن‌ها منجر به افزایش معنی‌دار بیان ژن *IL-6* در بافت‌های کبد و طحال در مقایسه با گروه کنترل گردید ($P<0.05$). بیشترین میزان افزایش بیان مربوط به تیمار ترکیبی پروبیوتیک و کرم آردی بود که تفاوت آن با سایر تیمارها معنی‌دار بود. منحنی‌های ذوب تک‌پیک و اندازه صحیح محصولات PCR اختصاصیت واکنش را تأیید نمودند. افزایش بیان *IL-6* در هر دو بافت نشان‌دهنده تحریک مسیرهای ایمنی ذاتی و فاز حاد التهابی در سطح سیستمیک بود.

نتیجه‌گیری: یافته‌ها بیانگر آن است که استفاده توأمان از پروبیوتیک و پودر کرم آردی می‌تواند اثر هم‌افزایی بر فعال‌سازی ایمنی جوجه‌های گوشتی از طریق افزایش بیان ژن *IL-6* داشته باشد. این نتایج نشان می‌دهد که ترکیب این دو افزودنی طبیعی می‌تواند به‌عنوان رویکردی مؤثر و ایمن برای ارتقای سلامت ایمنی و پایداری سیستم پرورش طیور مورد استفاده قرار گیرد.

کلمات کلیدی: ایمنی، پروبیوتیک، جوجه گوشتی، میلورم، qRT-PCR

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