



Effects of Probiotics Mixture and Blend of Mannan and Beta Glucan on Gene Expression of Cytokines in Broiler



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Abstract

A TOTAL of 350 broiler chicks (Ross) were used to examine the effects of pro-, prebiotic and symbiotic use on the immune response. The chicks were assigned into 14 groups and different mixtures of probiotics, prebiotics and symbiotics were added to the drinking water / feed. The 1st group (T1) was fed diet with no additives, the second (T2) was fed probiotic mixture at a count of 10⁸ CFU/ml and the rest of groups were fed prebiotics (blend of Mannan and Beta Glucan) at different concentrations (50, 150, 250, 350, 450 and 550 ppm) with probiotic mixture (T3, T5, T7, T9, T11 and T13 and without probiotic mixture (T4, T6, T8, T10, T12 and T14), respectively. During the experiment (42 days), body weights, body weight gain and feed intake were calculated weekly and feed conversion ratio was calculated at the end of the experiment. At the end of the experiment, representative number from each group was slaughtered and its intestines were collected for estimation of the expression of IL4, IL6, TLR2 and TLR4 genes. The findings revealed that providing probiotics in drinking water and prebiotics at a concentration of 150 ppm in feed enhanced the immune response of broilers and improved their feed conversion ratio (FCR). This suggests that probiotics and prebiotics may serve as reliable, safe, and natural alternatives to antibiotic growth promoters. Although probiotics did not significantly impact performance parameters, they exhibited a positive effect as an immune stimulant, as demonstrated by the increased mRNA levels of immune-related genes.

Keywords: Broilers, probiotics, prebiotics, symbiotic, mannan, beta glucan, growth performance and immunity.

Introduction

Antimicrobial growth promoters (AGPs) have been an essential part of animal feed for decades. Strengthening the immune system of farm animals and enhancing their growth can assist in the prevention of gastrointestinal illnesses [1]. However, concerns about the rise of antibiotic-resistant bacteria led the European Union to ban their use as growth promoters in 2006. This decision aimed to curb the spread of these "superbugs," which can cause untreatable and deadly infections in both animals and humans due to the ineffectiveness of most existing antibiotics [2]. Following the ban on AGPs,

researchers have been on a quest for safe, effective and affordable alternatives for animal nutrition. The goal is to find substances that boost immunity, prevent disease, and improve growth or production. Probiotics stand out as a promising contender, demonstrating their ability to enhance growth performance and strengthen immune response in broiler poultry without harming consumers [3,4,5,6]. Essentially, probiotics work to restore and maintain a healthy gut microbiome, leading to improved immunity. Various bacterial and fungal strains like *Lactobacillus* spp., *Bifidobacteria* spp., *Saccharomyces cerevisiae*, *Bacillus subtilis*, and *Enterococci* are approved as feed additives

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worldwide, with many more under consideration. Scientists have extensively tested these bacteria as feed additives, proving their effectiveness in improving gut health, feed efficiency, and immune response in broilers [7]. Prebiotics, on the other hand, are non-digestible food components that selectively nourish beneficial gut bacteria, ultimately enhancing overall health. Studies have shown that prebiotics both alone and combined with probiotics (symbiotics) can act as growth promoters in broilers. Mannan, B-Glucan, Inulin and many other polysaccharides are approved prebiotics in various regions, including the EU, FDA and Egypt [8, 9]. Molecular messengers called cytokines, produced by various cells, are the key players in communication within the body. They come in many forms, including interleukins, chemokines and lymphokines, each with specific roles. Some cytokines act locally, influencing nearby cells, while others travel further, like hormones, to reach distant targets. Interleukins, in particular, are crucial for immune cell activation, differentiation and function. They can also trigger inflammation or dampen it down, depending on their type. These versatile molecules bind to receptors on cell surfaces and unleash a cascade of reactions, influencing everything from antibody production to cell migration. TLR2 and TLR4 act like alert systems in our bodies, detecting foreign invaders and triggering immune responses. TLR2, in particular, activates IL-6, a key player in both healthy and unhealthy processes. While normally undetectable, IL-6 levels surge during inflammation [10]. Similarly, TLR4 recognizes bacterial threats but also senses internal damage, making it central to both infectious and non-infectious inflammation [11]. This study aims to see if probiotics, prebiotics, or their combination (symbiotics) can influence the expression of IL4, IL6, TLR2 and TLR4 genes in broilers, potentially enhancing their immune function.

Ethical approval

The experimental design and procedures were in compliance with the ethical standards of relevant national and institutional committee on animal experimentation approved No. CU-I-F-6b-23 (CU-IACUC) by Institutional Animal Care and Use Committee (CU-IACUC), Cairo University, Egypt.

Material and Methods

This experiment, conducted at the experimental poultry farm (faculty of agriculture, Benha university), examined the effects of different dietary treatments on broiler immunity. 350 chicks (one-day-old Ross) were divided into 14 groups of 25 birds each as shown in Table 1. The chicks were housed on the floor with wire borders under continuous fluorescent lighting (10 watts/m²). The chicks were fed specially formulated diets (Table 2) designed to meet their nutritional needs throughout various

growth stages (starter, grower and finisher). Probiotic mixture added to drinking water at a count of 10⁸ CFU/ml and blend of mannan and beta glucan were added in feed during experimental period in different concentrations (50, 150, 250, 350, 450 and 550 ppm). After 42 days, 5 birds from each group were conventional (partial) neck cutting both jugular veins, both carotid arteries, trachea, and the oesophagus, and intestinal samples (ileum) were collected and frozen for gene expression analysis. Importantly, the probiotic strains used (*E. faecium*, *L. acidophilus*, *B. subtilis* and *S. cerevisiae*) were generously provided by the Food Safety Lab of the Regional Center for Food and Feed (RCFF) within the Agriculture Research Center (ARC) in Egypt. Each probiotic strain was prepared to reach a final concentration of 10⁸ colony-forming units (CFU) per milliliter of drinking water and stored at 4-8°C for the duration of the experiment.

RNA extraction

Total RNA was extracted from caecal tissue using Trizol Reagent (Invitrogen Canada Inc., Burlington, Ontario, Canada) according to the manufacturer's protocol.

The RNA assessment

With the NanoDrop Spectrophotometer (Nano Drop 1000, USA), the amount and quality of extracted RNA were measured. By measuring the absorbance at 260 nm as well as at 280 nm, the concentration of nucleic acids was precisely determined.

Reverse transcription

As soon as RNA has been extracted and quality checked, reverse transcription begins to create cDNA using RNA as a template. Making use of Maxima's First Strand cDNA Synthesis software Based on the manufacturer's instructions, the reverse transcriptase enzyme synthesizes first strand cDNA from the RNA template and short-sequence primers using the kit for RT-qPCR. The first strand cDNA is then used as a template for qPCR. 80°C was used to store the cDNA.

Quantitative real-time PCR

RT-qPCR reactions were conducted with a total volume of 25 µL, 0.3 µM of each primer each primer (Table 3), and 2 µL of diluted cDNA (70 ng/l), There were 12.5 µL of Maxima SYBR Green qPCR Master Mix (Thermo Scientific/Fermentas, Vilnius, Lithuania) in the reaction mixture, 10nM ROX solution and nuclease free water up to 25 µl. The Applied Biosystems 7500 Real-Time PCR System (Foster City, CA, Applied Biosystems) using the following thermal program, denaturation for 15 min at 95 °C, annealing for 15 s at 58 °C and extension for 30 s at 72 °C for 40 cycles (Table 4). During each extension step, the fluorescence was measured. The

melting curve was generated following the completion of the thermal program, which indicated the specificity of the amplification. For the melting curve, the temperature was gradually raised to 98°C and the melting amplicon's fluorescence was measured

Measurement of Gene Expression

To normalize target gene expression levels (Ct—cycle threshold), the geometric mean of two reference genes was calculated. In order to calculate the Ct value for each gene, the reference gene's Ct was subtracted from the target gene's Ct (Ct target - Ct reference). The Ct values were used in all statistical analyses. A correlation coefficient was calculated using the $\Delta\Delta\text{Ct}$ algorithm. In the $\Delta\Delta\text{Ct}$ algorithm, a controlled calibration coefficient (control Ct) is subtracted from the experimental Ct. A fold change is calculated based on the difference between the experimental and control groups for the target gene. Using this approach, the relative expression levels of mRNA were calculated and normalized using the housekeeping gene, β -actin. As a result of the treatment's means and standard errors, mean \times SE is calculated. We calculated relative transcript levels and fold changes in transcript abundance using Pfaffl's efficiency-adjusted methodology [12].

Statistical analysis

In order to analyze the data, SAS 9.4 software (SAS Institute, Cary, NC, USA) was used. The differences between means was examined using Duncan's multiple range test [13]. This study examined how the treatments affected the expression of specific genes using the GLM method. $P < 0.05$ was considered significant for differences.

Results

From the data represented in Table 5, it was clear that, feed intake was increased in all groups significantly if compared to the control group except in T8 and T14 groups which showed lower FI than the control group. The highest feed intake level was recorded in T9 group and the lowest one belonged to T14 group. Also, it was clear from the same table that, weight gain and body weight were significantly positively affected by all treatments (except in T2 and T3 groups) if compared to the control group. The highest positive affect was obtained in T11 and T12 groups while the lowest values were recorded in T2 and T3 groups which were significantly lower than the control group in both BW and BWG. Feed conversion ratio was significantly positively affected by all treatments except for T2 and T3 groups in which the highest values were recorded which were also higher than that of the control group.

TLR2 expression was significantly increased in all treatments if compared to the control group except T14 group which showed the same trend as

the control group and T13 which showed lower value than the control group as shown in Figure 1 and Table 6. In Figure 2 and Table 6 it was clear that, the expression of TLR4 responsible gene was significantly affected as all obtained values were higher than the value obtained in the control group. T8 group scored the highest gene expression value while T11 group showed the lowest significantly increased value. From the same table 6 and figure 3, the obtained values concerning the expression of IL4 responsible gene showed that, all obtained values were significantly higher than the control group except in T2 and T13 which showed significant lower values. T8 group scored the highest gene expression value while T14 group had the lowest significant increased value. Data illustrated in the same Table 6 and figure 4 concluded that, there was no significant change in the expression of IL6 responsible gene.

Discussion

The diverse commensal microbiome in the gastrointestinal system is essential for its integrity and effective performance. The gut microbiota has a well-established symbiotic relationship with its hosts [14]. It has also been shown to enhance nutritional absorption, storage, and digestion [15] and support immunological responses [16]. According to [17], probiotics are live, non-pathogenic bacteria that, when administered in sufficient doses, could enhance health of the host. By using antimicrobial processes such as competitive exclusion and the synthesis of various biological products such as bacteriocins, organic acids, hydrogen peroxide, and carbon dioxide, they are able to inhibit the growth of intestinal pathogens [18]. A healthy gut requires prebiotics in order for probiotics to survive. It is essential for probiotics to survive in the gut if they are given prebiotics. By making probiotics able to endure anaerobic conditions, such as low oxygen, low pH, and low temperature, prebiotics help them grow and survive in the digestive tract. For probiotics to survive and multiply in a symbiotic lower intestinal region, prebiotics provide substrates [19]. The use of prebiotics has been demonstrated to decrease infections such as *Salmonella* and *Escherichia coli* as well as promote *Bifidobacteria* and *Lactobacilli* growth. An example of a prebiotic (MOS) is mannan oligosaccharides. *Saccharomyces cerevisiae* produces mannan oligosaccharides in its outer cell wall. These are the outer layers of yeast cells, which contain glucans (30%), mannans (30%), and proteins (12%). Methionine, serine, aspartic acid, and glutamic acid are all abundant in the protein [20].

Several studies have been conducted on the impact of beta glucan and MOS on the gut flora. Most MOS additions can significantly improve the composition of the microbial community. On the other hand, few studies have been performed on how

MOS affects immune response mechanisms in broilers [21].

In the present study, the beneficial effects of prebiotic and/or symbiotic on broiler performance parameters including BWG, FI and consequently FCR was clear by using 150 ppm of mannan and beta glucan mixture with or without probiotics (T5 and T6) which showed significant improvement of FCR. Although FCR values in T11, T12 and T14 were better (lower) than those recorded in T5 and T6, but from economical point of view it is recommended to rely on amounts and types used in T5 and/or T6 for growth promoting purposes. This data agreed with previous studies [22,23,24] which reported the positive effect of prebiotic and/or symbiotics as it positively affected FCR. The supplementation of probiotics decreased gastric emptying time, which leads to higher FI [25], and this was clear from the data obtained in this study as FI was significantly increased by the addition of pro, pre and symbiotics. Results of FI agreed with [26] who found that feed intake was improved by the supplementation of prebiotics, probiotics and symbiotics who reported significant increase of FI by the effect of pro and symbiotics.

In this study while the expression of TLR2 responsible gene in the treated groups increased, no significant changes were recognized in the expression of IL6 values which indicated that, there were no inflammatory response in the body of the experimental animals during the time of the experiment which needed no IL6 to be produced as this type of interleukin increases in the serum only due to inflammatory response as stated by [10]. The increased IL4 gene expression values indicated that, all the treatments had positive stimulatory effect for antibodies production which enhances the defense mechanism of the body. moreover, the increase of TLR4 gene expression values indicates the elevated immune response against gram negative bacteria which may be introduced into the body through feed, water or the surrounding environment or those which are present normally in the gut of the broilers throughout the period of the experiment. The absence of deleterious effect of grow negative bacteria on the body of the broilers indicates a positive immune stimulants effect of the dietary supplements used in this study. This data was approved by that obtained from [27,28,29], who reported the positive effect of pro, pre and symbiotic on the expression of immunoglobulins and interleukins in broilers and other form animals.

From this study it was concluded that, using pro, pre and symbiotic in drinking water at a level of 150 ppm could enhance the immune response of broilers together with improving the FCR which is considered as a great, reliable, safe and natural source that can be used as alternative(s) to antimicrobial growth promoters. Also, while

probiotics alone showed no significant improvement in the obtained values concerning performance parameters. It had a significant positive effect as an immune stimulant which was clear in the obtained increased values of mRNA related to immune responsible gene expression.

Data obtained in Table 4 reported that there were significant differences in the growth performance observed during the experiment because of the main effects of prebiotic and symbiotics. As shown in Table 4, BW in broiler chicks fed prebiotics and symbiotics were significantly higher than that in broiler chicks in the control group $P > 0.001$ except group 13 which was offered 550 ppm prebiotic only. Also, BW in broiler chicks in (T6, T8 and T12 groups) were significantly higher than in broiler chicks in the other groups ($P > 0.01$). FI was improved because of the main effect of probiotics ($p > 0.01$) Table 4. The probiotics strains and prebiotics significantly increased BW and improved Feed intake and FCR of birds in comparison with the control group ($P > 0.001$).

Conclusion

From this study it was concluded that, using pro, pre and symbiotic in drinking water or feed at a level of 150 ppm could enhance the immune response of broilers together with improving the feed conversion ratio which is considered as a great, reliable, safe and natural source that can be used as alternative(s) to antimicrobial growth promoters. Also, while probiotics alone showed no significant improvement in the obtained values concerning performance parameters. It had a significant positive effect as an immune stimulant which was clear in the obtained increased values of mRNA related to immune responsible gene expression.

The declarations

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Conflict of interest

No conflicts of interest exist.

Data Availability

This article includes all data collected or analyzed during this study.

Authorship contribution statement

The manuscript was read and approved by all authors who consented to participate.

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TABLE 1. Experimental design and treatments

Treatments	Groups
T1	Control
T2	Probiotics ^a (10 ⁸ cfu / ml)
T3	50 ppm prebiotics ^b / ton
T4	Probiotics (10 ⁸ cfu / ml) + 50 ppm prebiotics/ ton
T5	150 ppm prebiotics/ ton
T6	Probiotics + 150 ppm prebiotics
T7	250 ppm prebiotics/ ton
T8	Probiotics (10 ⁸ cfu / ml) + 250 ppm prebiotics
T9	350 ppm prebiotics/ ton
T10	Probiotics (10 ⁸ cfu / ml) + 350 ppm prebiotics
T11	450 ppm prebiotics/ ton
T12	Probiotics (10 ⁸ cfu / ml) + 450 ppm prebiotics
T13	550 ppm prebiotics/ ton
T14	Probiotics (10 ⁸ cfu / ml) + 550 ppm prebiotics

^aprobiotics strains of (*E. faecium*, *L. acidophilus*, *B. subtilis* and *S. cerevisiae*).

^b Prebiotics beta glucan and MOS added for feed.

TABLE 2. Ingredients and nutrient composition of diets:

Item	Starter (1– 15d)	Grower (16– 27d)	Finisher (28– 35d)
Ingredient (%):			
Corn	50.74	54.96	58.82
Soybean meal	41.96	37.83	33.73
Corn oil	3.09	3.40	3.96
Dicalcium Phosphate	1.72	1.53	1.35
Calcium carbonate	1.07	0.98	0.90
Salt	0.25	0.25	0.24
Sodium bicarbonate	0.15	0.15	0.16
Premix ¹	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25
DL-methionine	0.24	0.20	0.18
L-lysine HCl	0.16	0.11	0.10
L-Threonine	0.09	0.05	0.03
Chemical Analysis:			
Kcal/Kg	2900	3000	3100
Crude protein %	22.71	20.91	18.93
Dry matter (DM%)	89.4	89.4	89.3
Crud fat %	5.01	5.12	5.65
Crude fiber %	4.21	3.99	3.84

1 Vitamin premix supplied the followings per kg of diet: vitamin A, 9000 IU; vitamin D3, 2000 IU; vitamin E, 36 mg; vitamin K3, 2 mg; vitamin B1, 1.75 mg; vitamin B2, 6.6 mg; vitamin B6, 2.94 mg; vitamin B12, 0.015 mg; nicotinic acid, 29.7 mg; folic acid, 1 mg.

2 Mineral premixes supplied the followings per kg of diet: calcium pantothenate, 9.8 mg; choline chloride, 250 mg; Mn, 99.2 mg; Zn, 84.7 mg; Cu, 10 mg; Fe, 50 mg; Se, 0.2 mg; I, 0.99 mg.

TABLE 3. Primer sequences of the used genes for amplification:

Gene	Primer sequence forward	Primer sequence revers
IL4	TGTGCCACGCTGTGCTTACA	CTTGTGGCAGTGCTGGCTCTCC
IL6	AGAGAGGACTAACCCACAGAG	CCAGCTTCTCCAGTCTTGTC
TLR2	CGCTTAGGAGAGACAATCTGTGAA	GCCTGTTTTAGGGATTTCAGAGAATTT
TLR4	AGTCTGAAATTGCTGAGCTCAAAT	GCGACGTTAAGCCATGGAAG
β-Actin	CAACACAGTGCTGTCTGGTGGTA	ATCGTACTCCTGCTTGCTGATCC

TABLE 4. Thermal profile used for DNA augmentation:

Stage	Duration	Temperature	Cycles
Initial denaturation	15 min	95 °C	
Denaturation	10 s	95 °C	40
Annealing	15 s	58 °C	
Extension	30 s	72 °C	

TABLE 5. Effect of prebiotic and symbiotics concentrations on performance parameters of the experiment:

Treatment*	FI 0-35	WG 0-35	BW 0-35	FCR 0-35day
T1	3070.66 ^h	1870.80 ^{cd}	1916.62 ^{de}	1.64 ^{abc}
T2	3139.66 ^b	1852.23 ^e	1898.64 ^e	1.69 ^a
T3	3133 ^{cd}	1860.18 ^e	1906.35 ^e	1.68 ^a
T4	3137 ^{cb}	1883.69 ^{ced}	1929.85 ^{ede}	1.66 ^{ab}
T5	3110 ^f	1991.31 ^{ab}	2037.30 ^{ab}	1.57 ^{def}
T6	3073 ^h	1937.50 ^{bcd}	1984.22 ^{bcd}	1.58 ^{cdef}
T7	3135 ^{cb}	1943.98 ^{abcd}	1990.70 ^{abcd}	1.60 ^{cde}
T8	3064 ⁱ	2004.30 ^{ab}	2051.74 ^{ab}	1.53 ^f
T9	3154 ^a	1954.11 ^{abc}	2000.09 ^{abc}	1.61 ^{bcd}
T10	3136 ^{cb}	1961.75 ^{ab}	2008.94 ^{ab}	1.60 ^{cde}
T11	3129 ^d	2019.99 ^a	2064.84 ^a	1.55 ^{ef}
T12	3092 ^g	2021.77 ^a	2067.32 ^a	1.53 ^f
T13	3115 ^e	1874.33 ^{ed}	1921.41 ^{de}	1.66 ^b
T14	3047 ^j	1972.16 ^{ab}	2018.12 ^{ab}	1.54 ^{ef}
SE	3.41	23.88	23.89	0.019

* Treatments as defined in Table 1

^{a-j} Means followed by different superscript in the same column are significant at $P \leq 0.05$ **TABLE 6. Effects of prebiotic and symbiotic concentrations on TLR2, TLR4, IL4 and IL6 mRNA expression of broiler chickens:**

Treatments*	TLR2	TLR4	IL4	IL6
T 1	3.25 ^l	1.03 ⁿ	1.51 ^l	1.01
T 2	3.66 ⁱ	5.53 ⁱ	1.22 ^m	1.52
T 3	4.33 ^g	5.26 ^j	1.09 ⁱ	2.12
T 4	5.8 ^b	8.88 ^b	2.55 ^h	1.54
T 5	5.01 ^e	6.08 ^g	1.9 ^f	3.32
T 6	6.01 ^a	8.06 ^c	4.41 ^d	1.91
T 7	5.54 ^c	7.94 ^d	1.88 ^b	5.38
T 8	6.09 ^a	9.89 ^a	5.76 ^a	2.35
T 9	4.05 ^h	2.47 ^l	0.59 ^e	4.23
T 10	4.76 ^f	6.56 ^f	5.06 ^c	1.56
T 11	2.83 ^l	1.68 ^m	1.24 ^j	2.04
T 12	5.14 ^d	5.74 ^h	3.14 ^g	1.36
T 13	3.02 ^k	5.01 ^k	1.19 ⁿ	1.20
T14	3.18 ^j	6.84 ^e	1.86 ^k	1.62

* Treatments as defined in Table 1

^{a-l} Means followed by different superscript in the same column are significant at $P \leq 0.05$

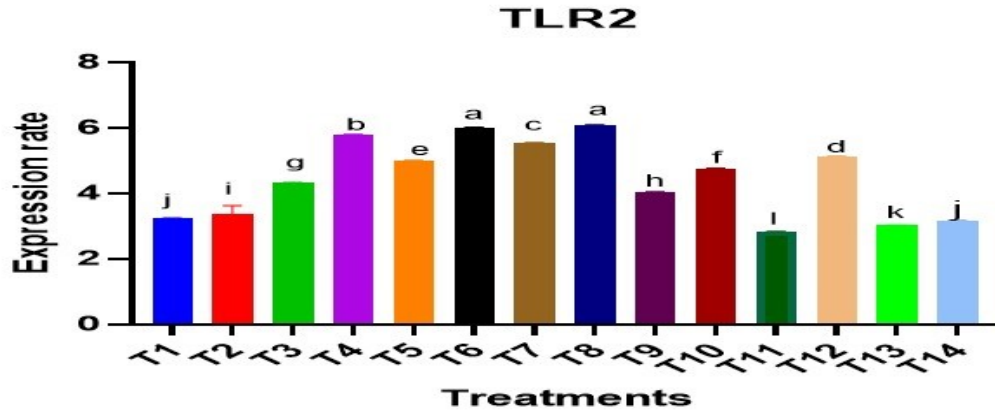


Figure (1): Effects of probiotics and prebiotic concentrations on mRNA expression of TLR2 of broiler chickens.

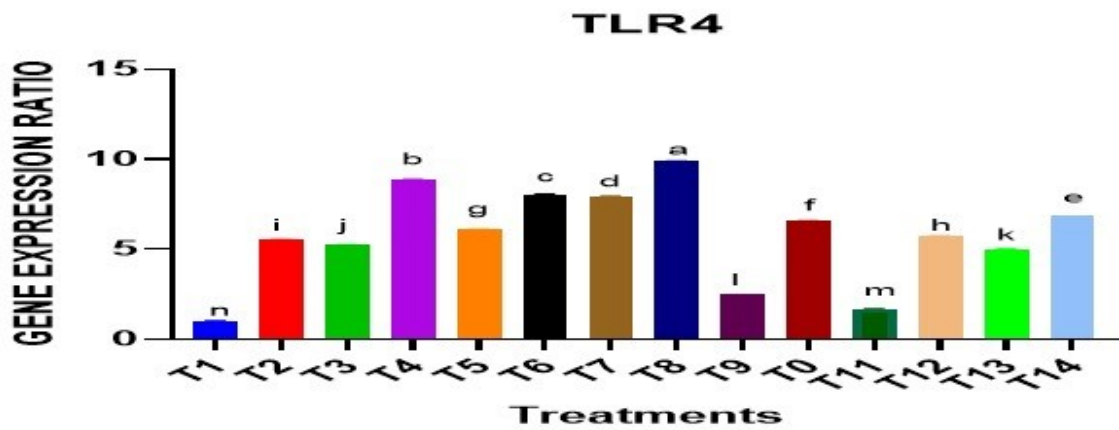


Figure (2): Effects of probiotics and prebiotic concentrations on mRNA expression of TLR4 of broiler chickens.

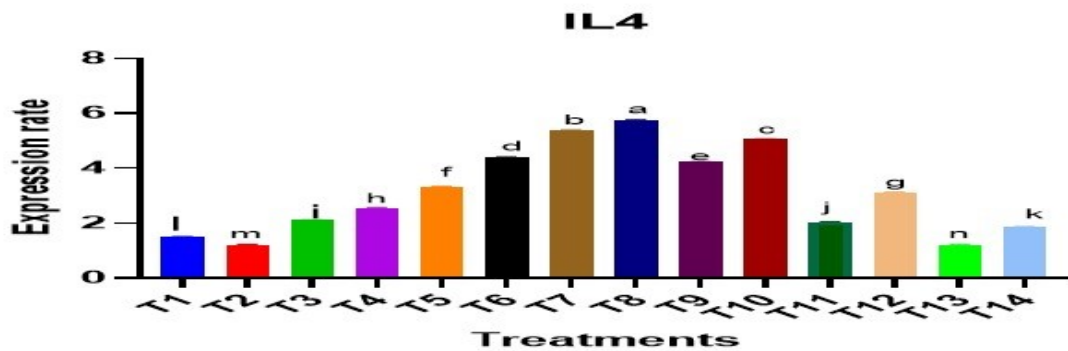


Figure (3): Effects of probiotics and prebiotic concentrations on mRNA expression of IL4 of broiler chickens.

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تأثير خليط البروبيوتيك ومزيج المنان والبيتا جلوكان على التعبير الجيني للسيتوكينات في دجاج اللحم

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الملخص

تم استخدام 350 كتكوت لاجم من سلالة (روص) لفحص تأثيرات استخدام البريبايوتيك والبروبيوتيك والسيمبيوتيك على الاستجابة المناعية. تم تقسيم الكتاكيت إلى 14 مجموعة وتمت إضافة خليط مختلف من البروبيوتيك والبريبايوتك والسيمبيوتيك إلى مياه الشرب/العلف. غذيت المجموعة الأولى (T1) على علف بدون إضافات، وغذيت المجموعة الثانية (T2) بخليط بروبيوتيك بمعدل 10⁸ مستعمرة بكتيرية / مللي وغذيت بقية المجموعات على البريبايوتك (خليط مانان وبيتا جلوكان) بتركيزات مختلفة (50، 150، 250، 350، 450 و 550 جزء في المليون) مع خليط بروبيوتيك T3، T5، T7، T9، T11 و T13 وبدون خليط بروبيوتيك (T4، T6، T8، T10، T12 و T14)، على التوالي. خلال التجربة (42 يوماً)، تم حساب أوزان الجسم، زيادة وزن الجسم وتناول العلف أسبوعياً وتم حساب نسبة التحويل الغذائي في نهاية التجربة. في نهاية التجربة تم ذبح العدد التمثيلي من كل مجموعة وجمعت أمعائها لتقدير التعبير الجيني لكل من IL4 و IL6 و TLR2 و TLR4 وأظهرت النتائج التي تم الحصول عليها أن استخدام البروبيوتيك في مياه الشرب والبريبايوتيك بمستوى 150 جزء في المليون في العلف يمكن أن يعزز الاستجابة المناعية لدجاج التسمين مع تحسين نسبة التحويل الغذائي الذي يعتبر مصدراً رائعاً وموثوقاً وأمناً وطبيعياً وقد يعمل كبديل واحد أو أكثر. أيضاً، في حين أن البروبيوتيك وحده لم يظهر أي تحسن كبير في القيم التي تم الحصول عليها فيما يتعلق بمعايير الأداء، إلا أنه كان له تأثير إيجابي كبير كمنشط مناعي والذي كان واضحاً في القيم المتزايدة التي تم الحصول عليها من mRNA والمتعلقة بالتعبير الجيني المسؤول عن المناعة.

الكلمات الدالة: دواجن التسمين، البروبيوتيك، البريبايوتكس، التكافلية، المنان، بيتا جلوكان، أداء النمو والمناعة.