

EFFICACY OF PROBIOTICS AND ESSENTIAL OILS AS ALTERNATIVES TO ANTIBIOTIC GROWTH PROMOTERS IN BROILER CHICKENS

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ABSTRACT

The current study was carried out on broiler chickens to assess whether probiotics or essential oils could be utilized instead of antibiotics growth promoters (AGP) in typical feed formulations, to determine their effectiveness. A total of 2250 broiler chicks at one-day old age were allocated into 5 dietary treatments in a completely randomized design with 9 replicates per treatment and 50 chicks/replicate. The Probiotic included three strains of *Bacillus amyloliquefaciens*, the essential oil (EO) was a blend of Cinnamaldehyde-Thymol, and the AGP was Lincomycin™. These additives were added to the basal diets (BD) to formulate the following dietary treatments (T): (T1) control-fed BD that is regularly used by broiler farmers (T2) control-fed BD that is regularly used by broiler farmers and supplemented with Lincomycin™ (100 g/ton) as AGP; (T3) fed BD + Probiotics (Enviva® PRO 60 g/ton); (T4) fed BD + EO (Enviva® 100g/ton); and (T5) fed BD + combination of both probiotics and EO with the same doses used in T3 and T4. The study findings demonstrated that the use of probiotics or essential oils did not have any significant impact on the average body weight gain, final body weight, feed conversion ratio, or footpad lesion scores when compared to the AGP group. It was concluded that replacing the AGP with probiotics or EO, as natural feed additives, had no negative effects on broiler performance. The probiotic strains or the EO could be safe alternatives to AGP.

Keywords: cinnamaldehyde, thymol, feed conversion ratio, footpad lesion, additives

اليونس وآخرون

مجلة العلوم الزراعية العراقية- 643-633:(2)55:2024

فاعلية المعزز الحيوي والزيوت الاساسية كبديل للمضادات الحيوية في دجاج اللحم

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المستخلص

هذه الدراسة اجريت لاختبار كفاءة اضافة المعزز الحيوي وخليط من الزيوت الأساسية كبديل لمحفزات النمو من المضادات الحيوية على أداء الدجاج اللحم. تمت تربية اجمالي 2250 كتكوتاً ذات عمر يوم واحد، من سلالة روس 308، مقدمة من مفرخ تجاري في بيئة خالية من التحديات حتى عمر 42 يوماً. وزعت الطيور بشكل عشوائي في 5 معاملات غذائية في تصميم عشوائي كامل بحيث تحتوي كل معاملة على 450 طائر مقسمة الى 9 مكررات في كل مكرر 50 طائر. تم استخدام 3 سلالات من المعزز الحيوي وخليط الزيوت الاساسية والمضاد الحيوي لينكوميسين كإضافات تمت اضافتها على خلطة علفية اساسية للحصول على 5 معاملات: المعاملة الأولى خلطة العلف الأساسية، المعاملة الثانية خلطة العلف الأساسية مع اضافة المضاد الحيوي لينكوميسين كمحفز نمو (100غ/طن) و المعاملة الثالثة خلطة العلف الأساسية والمعزز الحيوي (60غ/طن) والمعاملة الرابعة خلطة العلف الأساسية والزيوت الأساسية (100غ/طن) والمعاملة الخامسة خلطة العلف الأساسية وخليط من المعزز الحيوي والزيوت الأساسية باستخدام نفس الجرعات. تم تسجيل تأثير المعاملات الغذائية على متغيرات أداء النمو لدى الطيور بشكل أسبوعي وحسابها بشكل اجمالي في نهاية التجربة وكانت النتيجة انه لم يكن هناك اي فروق معنوية بين المعاملات المختلفة على وزن الطيور النهائي أو مقدار زيادة وزن الطيور او معدلات التحويل الغذائي أو اصابات التهاب جلد القدم لدى الطيور. يمكن الاستنتاج انه يمكن استخدام البدائل الطبيعية المتوفرة كبديل امن للمضادات الحيوية.

الكلمات المفتاحية: القرقة، الزعتر، معامل التحويل الغذائي، تقرحات باطن القدم، إضافات

Received: 14/1/2022, Accepted:29/5/2022

INTRODUCTION

The growing awareness of the interconnection between health, and nutrition in the broiler industry has sparked a surge of interest in seeking suitable alternative products to antibiotic growth promoters (AGP) which used to improve performance (5, 11). One of the main concerns is developing antibiotic-resistant bacteria. European Union banned the use of most AGP in broiler diets due to multiple and/or cross-resistance (37). Cross-resistance against pathogens and antibiotic residues in tissues was previously confirmed by (14). (40) proposed that the intensive use of antibiotics in broiler diets led to increased resistance lines of microorganisms. Numerous types of alternatives to AGP are developed such as probiotics, prebiotics, symbiotic, organic acids, and essential oils (EO). Alternative efficacy can differ depending on several factors such as nutrition, management practices, and environmental conditions (22). Probiotics are live microorganisms (bacteria or yeast) that are naturally found in the gut of animals. Probiotics work by keeping balance of useful bacteria in the gut, in order to help improve nutrient digestion, absorption, boost immunity, and reduce the disease threats (39, 40). Additionally, they support inhibition harmful bacteria growth in the gut by competing for nutrients and adhesion sites (16, 41, 34). Essential oils are organic plant extracts known as phytochemicals (35). They have several benefits as improving growth performance (4,53), gut health (24, 50, 20), enhancing immunity (1,19), and improving` meat quality (44). Beneficial effects of probiotics and EO reported widely such as improving weight gain, promoting feed intake, enhancing digestion, and increasing feed conversion efficiency (6, 13, 33), Another advantage is that it increases enzyme activity by stimulating the animal's digestive system, increases metabolism and use of digestive products, and improves liver functioning (23, 25). Probiotics and EO play an essential role as antimicrobial active substances that reduce colonization of pathogens and invasion in the host digestive system to eliminate various enteric infections (17,48,52). Thymol was identified by (21) as one of the strongest bioactive antimicrobials

among all phytochemicals. It is the major component of thyme EO, and the substance is found in plants such as *Thymus vulgaris* it is recognized for its ability to target bacterial membranes and generate pores that permit the release of substances from the cell and the entry of chemicals from outside the cell (36). Cinnamaldehyde is naturally occurring plant-derived chemical with antibacterial action that has the potential to be used instead of conventional antibiotics (18). Cinnamaldehyde attacks proteins involved in generating energy for bacteria (36). This mode of action is considered to be crucial in giving broad coverage against both types of bacteria the gram-negative and gram-positive (36). The efficacy of each thymol and Cinnamaldehyde blend to have antimicrobial activities against *C. perfringens* is stated in several poultry researchs (32). *Bacillus* strains, particularly those of the *amyloliquefaciens* type, have been discovered as probiotic bacteria that help to create and maintain a balanced microbial population in the host animal's gut (9). Increased beneficial bacteria colonization makes the gut environment less favorable to coliform colonization and aids in maintaining optimal villi height and crypt depth, assuring the gut's capacity to absorb nutrients for animal maintenance and growth (28). On the other hand, probiotics have been demonstrated to elicit a beneficial impact on the immune system of broiler by stimulating the development of gut-associated lymphoid tissue and enhancing the activity of immune cells (15, 30). Probiotics interact with the goblet cells, M cells from the Peyer's patches, and enterocytes, increasing the number of IgA-producing cells along with producing IgM and IgA, which is important for the immunity of the mucosa because it forms a barrier against pathogenic microorganisms (29). This study aims to find safe alternative for AGP. The study hypothesized that using either probiotics, EO, or both will improve the overall performance of broiler chickens and will be a substitute for AGPs.

MATERIALS AND METHODS

Study Location, Feed, and Test Products

The University of Jordan's Scientific Research Council authorized the experimental methodology. This experiment was carried out

at AL-Estesharia company research facility, Amman, Jordan. AL-Estesharia Research facility is a state-of-art research facility for field experiments on poultry. The laboratory works and analyses were carried out at the University of Jordan, school of Agriculture and AL-Estesharia lab. Feed was produced at AL-Estesharia feed mill. Three phases basal diets (starter, grower, and finisher) were produced and used for all treatments. The basal diets were formulated to be Antibiotic- and Coccidiostat-free. The composition and nutrients content of the basal diets were formulated in a specialized computer program (Brill Software V1.36.017) to meet the broiler nutrients requirement according to the strain commercial guideline (Ross 308 Strain Guideline). All diet types were iso-caloric and iso-nitrogenous. Table 1 shows the nutritional composition of the basal diets. Five samples of 200 g each, from each diet type were collected and thoroughly mixed (total=1000g) and sent to perform chemical analysis of feed composition (Gross energy, crude protein, amino acids profile, moisture, ash, crude fat, starch, sugar, crude fiber, phosphorus, calcium, and sodium). Feed analyses was conducted at AL-Estesharia Lab. A sub-sample of 200g sent to Danisco labs-Germany for a recovery test of *Bacillus amyloliquefaciens* and thymol-cinnamaldehyde blend to ensure the homogeneity of mixing before launching up the trial. The experiment started upon receiving homogeneity approval from Danisco. At the research center, one metric ton from each diet produced firstly to flush out the production lines then excluded to make sure that production lines are clean of any previous residues from other rations. Test products weighed separately and added to feeds in automated highly sensitive injectors. Three commercial products were used in this

experiment. The recommended dosage of these products was according to the manufacturer. The description of the three commercial products were as the following: *Bacillus amyloliquefaciens* Strains (Probiotics) provided by Enviva® PRO (Danisco Animal Nutrition, USA). It is a probiotic contains three strains of *Bacillus amyloliquefaciens* isolated from intestinal tracts of healthy chickens: BS15AP4, BS8 and BS2084. It is provided in powder form with a concentration of 2.5×10^9 CFU/g, and it is a thermo-stable product. The recommended dose for broiler chickens is 60 g of the product/MT feed.

Essential Oils (EO) provided by Enviva® EO (Danisco Animal Nutrition, UK). It is a combination of Cinnamaldehyde and Thymol aromatic molecules that have been formed by the carrier phase using maltodextrin gum mixed with oil phase of cinnamaldehyde and oil then homogenized carefully, encapsulated with glass like surface, dried as early stage drying then dried again as later stage drying and finally agglomerated in the final form of product from specific plants or plant parts. The concentration of cinnamaldehyde (2E)-3-Phenylprop-2-enal is 4.5 %, while the Thymol (2-isopropyl-5-methylphenol, IPMP) concentration is 13.5%. The product is provided in encapsulated powder form, it is a thermo-stable product. The recommended dose for broiler chickens is 100 g/MT feed.

The Antibiotic Growth Promoter (AGP) provided by LINCOMIX 44 (Zoetis). It is a broad-spectrum antibiotic used for poultry and consists of Lincomycin hydrochloride. The Lincomycin concentration is 4.4% as Lincomycin hydrochloride. The recommended dose as a growth promoter in broiler chickens is 50-100 g/ MT feed. This AGP was chosen because it is widely used in Jordan broiler farms as prophylactic and as AGP (Personal communication).

Table 1. Basal diets composition and nutrient.

Ingredients	unit	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42)
Corn ¹	kg	579.5	616.7	666.5
Soybean Meal ²	kg	370	335	285
Soy oil	kg	14	15	20
CaCO ₃	kg	14	14	13
Lysine Sulphate	kg	3.1	2.3	2.3
MCP	kg	7	5.5	5
Salt	kg	2	2	2
Sodium Bicarbonate	kg	1.5	1.5	1.5
Threonine	kg	1.3	1	0.7
Methionine	kg	3.5	3	0
L- Valine	kg	0.1	0	0
Minerals & Vitamins	kg	2	2	2
Choline Chloride 70%	kg	0.4	0.4	0.4
Betaine HCl	kg	0.3	0.3	0.3
Mycotoxin binder	kg	1	1	1
Enzymes ³	kg	0.3	0.3	0.3
Total kg		1000	1000	1000
Calculated Analysis				
Metabolizable Energy ⁴	Kcal/Kg	3,100.0	3,150.0	3,240.0
Crude Protein	%	23	21.5	19.5
Crude Fat	%	4.0	4.3	4.9
Crude Fiber	%	2.65	2.64	2.6
Calcium	%	1	0.95	0.9
Avi.Phosphorus	%	0.48	0.435	0.395
Dig.Lysine	%	1.28	1.15	1.03
Dig.Methionin +Cysteine	%	0.95	0.87	0.8
Dig.Threonin	%	0.86	0.77	0.69
Dig. Valine	%	0.96	0.87	0.78
Sodium	%	0.16	0.16	0.16
Chloride	%	0.19	0.19	0.19
1 Crude protein 7.6%, crude fat 3.5%, metabolizable energy 3377 kcal/kg				
2 Crude protein 47.5%, crude fat 1.93%, metabolizable energy 2287 kcal/kg				
3 Multiblend enzyme consist of amylase, xylanase, protease and phytase enzymes, 145 kcal/kg				
4 Metabolizable energy calculated based on equations consider digestibility coefficient factors for each raw material				

Five samples of 200 g each, from each diet type were collected and thoroughly mixed (total=1000g) and sent to perform chemical analysis of feed composition (Gross energy, crude protein, amino acids profile, moisture, ash, crude fat, starch, sugar, crude fiber, phosphorus, calcium, and sodium). Feed analyses was conducted at AL-Estesharia Lab. A sub-sample of 200g sent to Danisco labs-Germany for a recovery test of *Bacillus amyloliquefaciens* and thymol-cinnamaldehyde blend to ensure the homogeneity of mixing before launching up the trial. The experiment started upon receiving homogeneity approval from Danisco. At the research center, one metric ton from each diet produced firstly to flush out the production lines then excluded to make sure that production lines are clean of any previous

residues from other rations. Test products weighed separately and added to feeds in automated highly sensitive injectors. Three commercial products were used in this experiment. The recommended dosage of these products was according to the manufacturer. The description of the three commercial products were as the following: *Bacillus amyloliquefaciens* Strains (Probiotics) provided by Enviva® PRO (Danisco Animal Nutrition, USA). It is a probiotic contains three strains of *Bacillus amyloliquefaciens* isolated from intestinal tracts of healthy chickens: BS15AP4, BS8 and BS2084. It is provided in powder form with a concentration of 2.5×10^9 CFU/g, and it is a thermo-stable product. The recommended dose for broiler chickens is 60 g of the product/MT feed. Essential Oils (EO) provided by Enviva® EO

(Danisco Animal Nutrition, UK). It is a combination of Cinnamaldehyde and Thymol aromatic molecules that have been formed by the carrier phase using maltodextrin gum mixed with oil phase of cinnamaldehyde and oil then homogenized carefully, encapsulated with glass like surface, dried as early stage drying then dried again as later stage drying and finally agglomerated in the final form of product from specific plants or plant parts. The concentration of cinnamaldehyde (2E)-3-Phenylprop-2-enal is 4.5 %, while the Thymol (2-isopropyl-5-methylphenol, IPMP) concentration is 13.5%. The product is provided in encapsulated powder form, it is a thermo-stable product. The recommended dose for broiler chickens is 100 g/MT feed. The Antibiotic Growth Promoter (AGP) provided by LINCOMIX 44 (Zoetis). It is a broad-spectrum antibiotic used for poultry and consists of Lincomycin hydrochloride. The Lincomycin concentration is 4.4% as Lincomycin hydrochloride. The recommended dose as a growth promoter in broiler chickens is 50-100 g/ MT feed. This AGP was chosen because it is widely used in Jordan broiler farms as prophylactic and as AGP (Personal communication).

Birds and Experimental Design

A commercial hatchery provided 2250 1-day-old broiler chicks from Ross 308 strain. Chicks are reared till 42 days of age in accordance with standard management and practices provided by the strain management guidelines. Birds were reared on floor pens at a fully closed poultry house equipped with full automated systems installed specifically for research purposes. The dimensions of the house are 48 X 10 m. It is composed of 54 floor pens (1.7m X 1.64m), where each pen can accommodate up to 50 broiler birds. Each two adjacent pens are separated by a robust heavy-duty plastic mesh with an ideal opening size of 181 cm height to prevent birds from escaping or mingling, especially at young ages. This house heating is provided by an autonomous infrared heating system that maintains the proper temperature as needed. In this housing, an autonomous negative pressure system with the necessary sensors is built to provide adequate ventilation that is consistent with the age and weight of the birds. This

house's precise environmental parameters (temperature, ventilation, and air velocity) are ensured by an automatic computerized control panel. Feeding is controlled through manual hanging circular feeders with adjustable height to align age of birds. Similarly, automated hanging nipple drinkers supply birds with clean water in each pen. The rearing cycle was divided into 3 stages: started with starter phase at 0 days of age up to 14 days followed with grower phase from 15 days up to 28 days and ended up with finisher phase from 29 days to 42 days. According to Ross 308 strain specifications, feed and water were supplied ad libitum, with a lighting schedule includes 20 hours of light followed by 4 hours of darkness. In a fully randomized design (CRD), broiler chicks were randomly assigned to one of four nutritional treatments, with nine repetitions (pens) per treatment and 50 birds each replication. There were 450 birds in each treatment. The Probiotic, essential oils, and AGP were added to the standard commercial basal diets (BD) for broiler chickens to formulate 5 dietary treatments (T) as the following:

T1: Control diet; the regular commercial broiler diets (Table 1).

T2: Control diet+ Lincomycin (100 g/ton; recommended dosage), used as AGP.

T3: Control diet + Probiotics “Enviva® PRO” (60 g/ton; recommended dosage).

T4: Control diet + Essential oils “Enviva® EO” (100g/ton; recommended dosage).

T5: Control diet + Mixture of probiotics and EO (same doses as in T3&T4).

Growth Performance Parameters

Average Body Weight

Birds were weighed at weekly basis and overall period results recorded at 42 days of age, all birds in each pen used for weighing (the whole pen were taken), and then treatment average body weight (BW) determined.

Feed Intake: Feed intake (FI) for each floor pen was calculated in weekly basis and the total consumed feed measured at 42 days of age, then calculated as total amount of provided feed for each pen each day divided by total numbers of chicks.

Feed Conversion Ratio

Feed conversion ratio (FCR) was calculated at weekly basis and total FCR at 42 of age

recorded. It was calculated as the amount of FI divided by average body weight gain (differences between the current weight and previous weight). FCR was corrected for mortality in each pen by weighing the dead bird and use below formula to calculate estimated feed intake:

$$\text{Estimated feed intake} = \text{FCR} * \text{Weight}$$

To calculate BW gain the initial BW subtracted from final BW. Feed intake was calculated by subtracting estimated feed intake for mortality and remined feed from the whole quantity of feed offered to the birds. The FCR was calculated weekly and as a total at the end of the trial using data from FI and BW gain.

Mortality

On a pen-by-pen basis, all dead birds have been recorded and weighed. In order to find the reason, dead birds were necropsied.

Foot Pad Lesion Score (FBD). Two birds from each floor were collected for foot and pad lesion examination at day 28 of age. The methodology for foot pad lesion scoring was conducted according to (10) where: 0: No no visible symptoms of FPD, and the skin of the foot pad feels soft to the touch with no swelling or necrosis; 1: shows slight redness to the foot pad, and the pad textures look tougher and denser than a non-affected foot, the central part of the pad is higher, reticulate scales are detached, besides presence of small black necrotic areas; 2: shows visible lesions appear on foot pad surface, obvious swelling appearance of the foot pad, reticulate scales are black in addition to forming scale shaped necrotic areas.

Statistical Analysis

All statistical analysis was carried out using the Statistical Analysis System (SAS Institute, 2010, Version 9.1.3) (45). The replicate was

considered as the experimental unit (pen) and was analyzed using one-way ANOVA implemented in the proc GLM method of SAS. When $P \leq 0.05$, differences were accepted as statistically significant differences. Tukey's test used to split means for significant interactions.

RESULTS AND DISCUSSION

Growth Performance Parameters

Tables 2 and 3 show the effect of various treatments on growth performance parameters. At D0, the initial body weight of chicks was similar, no significant variation between treatments (Table 2). The growth rate for the birds in the different treatments followed the normal pattern and was the same. The results of final weight were the same with no significant differences found in the treatments compared to the control treatment (Table 2). In addition, the results for average body weight gain showed that there is no statistical differences in the treatments relative to the control treatment (Table 2). Recorded Feed intake data did not show any significant variation between groups as demonstrated below in (Table 3). Likewise, the outcomes of the calculated FCR demonstrated no significant variances in the treatments when compared to the control treatment (Table 3). Recorded mortality % were within the normal accepted values under large scall field trial in Jordan, no disease challenges observed during the trial and no significant differences recorded among groups (Table 3).

Footpad Lesions

Table 4 illustrates the impact of various treatments on footpad lesion scores. The results revealed that none of the treatments had a significant impact on the foot pad lesions scores in comparison with control diet.

Table 2. Effect of Different Treatments (T) on initial body weight, final body weight and average body weight gain (Mean \pm standard deviation)

Treatment	T1	T2	T3	T4	T5	P-Value
Initial by weight (g)	39.3 \pm 0.60	39.3 \pm 0.67	39.7 \pm 0.5	40 \pm 0.57	39.6 \pm 0.52	0.3235
Final body weight (g)	2898 \pm 173	2964 \pm 210	2807 \pm 143	2926 \pm 103	2904 \pm 227	0.388
Average body weight gain (g)	2858 \pm 173	2925 \pm 210	2767 \pm 143	2887 \pm 103	2786 \pm 227	0.323

T1: Control diet (CD); the regular commercial broiler diets.
T2: CD + Lincomycin (100 g/ton; recommended dosage), used as AGP.
T3: CD + Probiotics "Enviva® PRO" (60 g/ton; recommended dosage).
T4: CD + Essential oils "Enviva® EO" (100g/ton; recommended dosage).
T5: CD + Mixture of probiotics and EO (60 g/ton and 100g/ton).

Table 3. Effect of Different Treatments (T) on total feed intake, FCR and mortality % (Mean ± standard deviation)

Treatment	T1	T2	T3	T4	T5	P-Value
Total feed intake (g)	4288±157	4463±470	4168±160	4242±100	4250±223	0.21
FCR	1.5±0.04	1.53±0.14	1.51±0.04	1.47±0.04	1.53±0.04	0.406
Mortality %	10.8±4.66	12.6±7.77	11.9±6.36	12±3.85	10.8±3.59	0.406

T1: Control diet (CD); the regular commercial broiler diets.
T2: CD + Lincomycin (100 g/ton; recommended dosage), used as AGP.
T3: CD + Probiotics “Enviva® PRO” (60 g/ton; recommended dosage).
T4: CD + Essential oils “Enviva® EO” (100g/ton; recommended dosage).
T5: CD + Mixture of probiotics and EO (60 g/ton and 100g/ton).

Table 4. Effect of Different Treatments (T) on foot pad lesion score (Mean ± standard deviation)

Treatment	T1	T2	T3	T4	T5	P-Value
Foot pad lesion score	0.56±0.51	0.44±0.51	0.39±0.50	0.44±0.51	0.57±0.51	0.791

T1: Control diet (CD); the regular commercial broiler diets.
T2: CD + Lincomycin (100 g/ton; recommended dosage), used as AGP.
T3: CD + Probiotics “Enviva® PRO” (60 g/ton; recommended dosage).
T4: CD + Essential oils “Enviva® EO” (100g/ton; recommended dosage).
T5: CD + Mixture of probiotics and EO (60 g/ton and 100g/ton).

The purpose of this study was to investigate if three *Bacillus amyloliquefaciens* probiotic strains and a thymol-cinnamaldehyde mix (EO) might improve broiler chicken performance and if the supplements could possibly be used as an alternative to AGP. During the trial period, no significant effect of the probiotic or EO on broiler chicken performance parameters as body weight and/or body weight gain or FCR was seen. relative to the control treatment that had supplemented with AGP. This results in consistence with results obtained by (8) in trial conducted to evaluate adding probiotics, prebiotics and synbiotics. The lack of the differences between broilers supplemented with probiotics and the control treatment (supplemented with AGP) indicating that broilers in T2 were able to get the beneficial effects out of the probiotic and to successfully substitute the AGP. Under normal conditions (non-challenging circumstances), the utilization of probiotic strains and EO resulted in performance results comparable to broiler chickens in the control treatments, with no significant differences found. Probiotic supplementation has been suggested as an alternative to AGP in poultry diet due to their potential to improve performance and health (2,7). The potential

benefits of providing probiotics in broiler diets have been extensively investigated. Probiotics are known to combat harmful microorganisms in the digestive system of chickens (47) improve growth rate (3), enhance immune system (43) improve gut health by promote beneficial microorganism and suppress the pathogenic once (52). Probiotics have been known to improve gut health by promote beneficial bacteria and suppressing pathogenic bacteria with create aggressive environment for harmful bacterial through reducing pH; competing for nutrients, inhibit bacterial adherence and translocation and produce antibacterial substances as bacteriocins (38, 46). In addition, probiotics have been reported to have a positive effect on footpad lesions in broiler chickens. Footpad lesions are a common welfare issue in broiler chickens, it might be caused by different reasons such as poor litter quality, high ammonia levels, and bacterial infections. The beneficial probiotics effects such as improving gut health, increasing feed absorption and utilization in the gut might help in reducing harmful bacterial and thus prevent or reduce the incidence of footpad lesions. Our findings indicated that probiotics supplementation in broiler diets improved broilers growth

parameters. Likewise, the results obtained by EO supplementation were similar to what was obtained by probiotics. (26) and (49) found a significant positive effect of EO on FCR and body weight gain of broiler chickens in comparison with an unsupplemented control group. Essential oils are aromatic compound found in plants that are hydrophobic in nature. They can either be naturally derived from plants or created synthetically (31). It was reported that EO have both preventative and healing effects on necrotic enteritis in broiler chickens (27), exerts a beneficial influence on broilers growth, meat and carcass quality and overall health (31), and improved FCR (42). Similarly, it was found that using both probiotics and EO in the broiler diets improved the performance parameters during the experimental period.

Conclusion

The results indicated that using probiotics, EO, or their combination in broiler diets might be one of the promising strategies that might be applied in the field to stop using AGP without any negative consequences. However, further research is needed to examine the potential advantages of these natural additives under several environmental and management circumstances.

REFERENCES

- 1.,Abd El-Hack M. E, El-Saadony M. T, Saad AM, et al. (2022) Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. *Poult Sci* 101(2):101584
<https://doi.org/10.1016/j.psj.2021.101584>
- 2.Abd El-Hack M. E, El-Saadony M. T, Shafi ME, et al. (2020) Probiotics in poultry feed: A comprehensive review. *J Anim Physiol Anim Nutr (Berl)* 104(6):1835-1850
<https://doi.org/10.1016/j.psj.2021.101584>
- 3.Al-Khalaifa H, Al-Nasser A, Al-Surayee T, et al. (2019) Effect of dietary probiotics and prebiotics on the performance of broiler chickens. *Poultry Science* 98(10):4465-4479
doi:<https://doi.org/10.3382/ps/pez282>

- 4.Alali WQ, Hofacre CL, Mathis GF, and Faltys G. (2013) Effect of essential oil compound on shedding and colonization of *Salmonella enterica* serovar Heidelberg in broilers. *Poultry Science* 92(3):836-841
doi:<https://doi.org/10.3382/ps.2012-02783>
- 5.Ameen, N. A., N. A.Rahman, , and A. H. Hassan, 2021. Preventative effects of probiotic (miaclost) on experimentally induced hypocalcemic rickets in broiler chicks. *Iraqi Journal of Agricultural Sciences*, 52(6), 1461-1474. <https://doi.org/10.36103/ijas.v52i6.1487>
- 6.Amerah AM, Jansen van Rensburg C, Plumstead PW, Kromm C, and Dunham S. (2012) Effect of feeding diets containing a probiotic or antibiotic on broiler performance, intestinal mucosa-associated avian pathogenic *E. coli* and litter water-soluble phosphorus. *Journal of Applied Animal Nutrition* 1:e7
<https://doi.org/10.1017/jan.2013.4>
- 7.Ayalew H, Zhang H, Wang J, et al. (2022) Potential feed additives as antibiotic alternatives in broiler production. *Front Vet Sci* 9:916473
<https://doi.org/10.3389/fvets.2022.916473>
- 8.Aziz, H. I., and Al-Hawezy, D. J. 2021. Effect of probiotics, prebiotics and synbiotic on growth performance of broiler under different stock density. *Iraqi Journal of Agricultural Sciences*, 52(5), 1126-1138.
<https://doi.org/10.36103/ijas.v52i5.1451>
- 9.Bahaddad SA, Almalki MHK OA, Alghamdi et al. (2023) *Bacillus* species as direct-fed microbial antibiotic alternatives for monogastric production. *Probiotics Antimicrob Proteins* 15(1):1-16
doi: [10.1007/s12602-022-09909-5](https://doi.org/10.1007/s12602-022-09909-5)
- 10.Bilgili SF, Hess JB, Blake JP, Macklin KS, Saenmahayak B, and Sibley JL. (2009) Influence of bedding material on footpad dermatitis in broiler chickens. *Journal of Applied Poultry Research* 18(3):583-589
doi:<https://doi.org/10.3382/japr.2009-00023>
- 11.Broom L. J. (2017) The sub-inhibitory theory for antibiotic growth promoters. *Poult Sci* 96(9):3104-3108
<https://doi.org/10.3382/ps/pex114>
- 12.Brown M. (2011) Modes of action of probiotics: Recent Developments. *J Anim Vet Adv* 10(14):1895-1900

13. Calik A, Omara, II, White MB, Li W, and Dalloul RA. (2019) Effects of dietary direct fed microbial supplementation on performance, intestinal morphology and immune response of broiler chickens challenged with coccidiosis. *Front Vet Sci* 6:463
14. Chattopadhyay MK. (2014) Use of antibiotics as feed additives: a burning question. *Front Microbiol* 5:334
[doi: 10.3389/fmicb.2014.00334](https://doi.org/10.3389/fmicb.2014.00334)
15. Dalloul RA, Lillehoj HS, Shellem TA, and Doerr JA. (2003) Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. *Poult Sci* 82(1):62-6 <https://doi.org/10.1093/ps/82.1.62>
16. Dibner JJ, and Richards JD. (2005) Antibiotic growth promoters in agriculture: history and mode of action. *Poult Sci* 84(4):634-43 [https://doi: 10.1093/ps/84.4.634](https://doi.org/10.1093/ps/84.4.634)
17. Dorman HJ and Deans SG. (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 88(2):308-16 <https://doi.org/10.1046/j.1365-2672.2000.00969.x>
18. Doyle AA, and Stephens JC. (2019) A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia* 139:104405 <https://doi.org/10.1016/j.fitote.2019.104405>
19. El-Shall NA, Shewita RS, ME and Abd El-Hack, et al. (2020) Effect of essential oils on the immune response to some viral vaccines in broiler chickens, with special reference to Newcastle disease virus. *Poult Sci* 99(6):2944-2954 [doi: 10.1016/j.psj.2020.03.008](https://doi.org/10.1016/j.psj.2020.03.008)
20. Ezzat, H. N. 2023. Effect of supplementation fenugreek oil to the diet on the physiological anatomical and histological traits of broilers. *Iraqi Journal of Agricultural Sciences*, 54(1), 106-113.
<https://doi.org/10.36103/ijas.v54i1.1681>
21. Falcone P, Speranza B, Del Nobile MA, Corbo MR, and Sinigaglia M. (2005) A study on the antimicrobial activity of thymol intended as a natural preservative. *J Food Prot* 68(8):1664-70
[doi: 10.4315/0362-028x-68.8.1664](https://doi.org/10.4315/0362-028x-68.8.1664)
22. Gadde U, Kim WH, Oh ST, and Lillehoj HS. (2017) Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Anim Health Res Rev* 18(1):26-45
[doi: 10.1017/S1466252316000207](https://doi.org/10.1017/S1466252316000207)
23. Hernández F, Madrid J, García V, Orengo J, and Megías MD. (2004) Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poult Sci* 83(2):169-74 [doi: 10.1093/ps/83.2.169](https://doi.org/10.1093/ps/83.2.169)
24. Hu Z, Liu L, Guo F, et al. (2023) Dietary supplemental coated essential oils and organic acids mixture improves growth performance and gut health along with reduces *Salmonella* load of broiler chickens infected with *Salmonella Enteritidis*. *J Anim Sci Biotechnol* 14(1):95 [doi: 10.1186/s40104-023-00889-2](https://doi.org/10.1186/s40104-023-00889-2)
25. Jamroz D, Wertelecki T, Houszka M, and Kamel C. (2006) Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *J Anim Physiol Anim Nutr (Berl)* 90(5-6):255-68
<https://doi.org/10.1111/j.1439-0396.2005.00603.x>
26. Jamroz D, Wiliczekiewicz A, Wertelecki T, Orda J, and Skorupińska J. (2005) Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. *Br Poult Sci* 46(4):485-93
[doi: 10.1080/00071660500191056](https://doi.org/10.1080/00071660500191056)
27. Jerzsele A, Szeker K, Csizinszky R, et al. (2012) Efficacy of protected sodium butyrate, a protected blend of essential oils, their combination, and *Bacillus amyloliquefaciens* spore suspension against artificially induced necrotic enteritis in broilers. *Poult Sci* 91(4):837-43 [doi: 10.3382/ps.2011-01853](https://doi.org/10.3382/ps.2011-01853)
28. Lee KW, Li G, Lillehoj HS, et al. (2011) *Bacillus subtilis*-based direct-fed microbials augment macrophage function in broiler chickens. *Res Vet Sci* 91(3):e87-91
[doi: 10.1016/j.rvsc.2011.01.018](https://doi.org/10.1016/j.rvsc.2011.01.018)
29. Luciana Kazue O, Marcelo Biondaro Gi, Elis Regina de Moraes G, and Maria Marta L. (2012) Variations on the Efficacy of Probiotics in Poultry. In: Everlon Cid R (ed) *Probiotic in Animals*. IntechOpen, Rijeka, p Ch. 9
30. Lutful Kabir SM. (2009) The role of probiotics in the poultry industry. *Int J Mol Sci* 10(8):3531-46 [doi: 10.3390/ijms10083531](https://doi.org/10.3390/ijms10083531)
31. Mehdi Y, Létourneau-Montminy M-P, Gaucher M-L, et al. (2018) Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition* 4(2):170-178

doi:

<https://doi.org/10.1016/j.aninu.2018.03.002>

32.Mitsch P, Zitterl-Eglseer K, Köhler B, Gabler C, Losa R, and Zimpernik I. (2004) The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. *Poult Sci* 83(4):669-75

[doi: 10.1093/ps/83.4.669](https://doi.org/10.1093/ps/83.4.669)

33.Mookiah S, Sieo CC, Ramasamy K, Abdullah N, and Ho YW. (2014) Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *J Sci Food Agric* 94(2):341-8

[doi: 10.1002/jsfa.6365](https://doi.org/10.1002/jsfa.6365)

34.M'sadeq, S. A. 2019. Effect of dietary supplementation of miaclost on performance and gut morphology in broiler chickens challenged with *Escherichia coli*. *Iraqi Journal of Agricultural Sciences*, 2(50).

<https://doi.org/10.36103/ijas.v2i50.650>

35.Mustafa, M. M. 2022. Effect of different levels of oleobiotec on production performance and egg quality traits in Japanese quail. *Iraqi Journal of Agricultural Sciences*, 53(3),578-583.

<https://doi.org/10.36103/ijas.v53i3.1566>

36.Nazzaro F, Fratianni F, De Martino L, Coppola R, and De Feo V. (2013) Effect of essential oils on pathogenic bacteria. *Pharmaceuticals (Basel)* 6(12):1451-74

[doi: 10.3390/ph6121451](https://doi.org/10.3390/ph6121451)

37.Neu HC. (1992) The crisis in antibiotic resistance. *Science* 257(5073):1064-73

[doi: 10.1126/science.257.5073.1064](https://doi.org/10.1126/science.257.5073.1064)

38.Ng SC, Hart AL, Kamm MA, Stagg AJ, and Knight SC. (2008) Mechanisms of Action of Probiotics: Recent Advances. *Inflammatory Bowel Diseases* 15(2):300-310

39.Panda AK, Rao SVR, Raju MVLN, and Sharma SR. (2006) Dietary Supplementation of *Lactobacillus Sporogenes* on Performance and Serum Biochemico - Lipid Profile of Broiler Chickens. *The Journal of Poultry Science* 43(3):235-240

<https://doi.org/10.2141/jpsa.43.235>

40.Park YH, Hamidon F, Rajangan C, et al. (2016) Application of Probiotics for the Production of Safe and High-quality Poultry Meat. *Korean J Food Sci Anim Resour* 36(5):567-576

[doi: 10.5851/kosfa.2016.36.5.567](https://doi.org/10.5851/kosfa.2016.36.5.567)

41.Patterson JA, and Burkholder KM. (2003) Application of prebiotics and probiotics in poultry production. *Poult Sci* 82(4):627-31

[doi: 10.1093/ps/82.4.627](https://doi.org/10.1093/ps/82.4.627)

42.Peng QY, Li JD, Li Z, Duan ZY, and Wu YP. (2016) Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. *Animal Feed Science and Technology* 214:148-153

[doi: https://doi.org/10.1016/j.anifeedsci.2016.02.010](https://doi.org/10.1016/j.anifeedsci.2016.02.010)

43.Qamar A, Waheed J, Hamza A, et al. (2021) The role of intestinal microbiota in chicken health, intestinal physiology and immunity. *J Anim Plant Sci-Pak* 31(2):342-351

[doi:10.36899/Japs.2021.2.0221](https://doi.org/10.36899/Japs.2021.2.0221)

44.Rimini S, Petracci M, and Smith DP. (2014) The use of thyme and orange essential oils blend to improve quality traits of marinated chicken meat. *Poult Sci* 93(8):2096-102

<https://doi.org/10.3382/ps.2013-03601>

45.SAS Institute, Inc., 2010. SAS OnlineDoc Version 9.1.3, Cary, NC, USA).

46.Schneitz C. (2005) Competitive exclusion in poultry 30 years of research. *Food Control* 16(8):657-667

[doi:https://doi.org/10.1016/j.foodcont.2004.06.002](https://doi.org/10.1016/j.foodcont.2004.06.002)

47.Shini S, Zhang D, Aland RC, et al. (2020) Probiotic *Bacillus amyloliquefaciens* H57 ameliorates subclinical necrotic enteritis in broiler chicks by maintaining intestinal mucosal integrity and improving feed efficiency. *Poult Sci* 99(9):4278-4293

[doi:10.1016/j.psj.2020.05.034](https://doi.org/10.1016/j.psj.2020.05.034)

48.Singh G, Kapoor IP, Pandey SK, Singh UK, and Singh RK. (2002) Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. *Phytother Res* 16(7):680-2

[doi:10.1002/ptr.951](https://doi.org/10.1002/ptr.951)

49.Spernakova D, Mate D, Rozanska H, and Kovac G. (2007) Effects of dietary rosemary extract and alpha-tocopherol on the performance of chickens, meat quality, and lipid oxidation in meat stored under chilling conditions. *B Vet I Pulawy* 51(4):585-589

50.Stappen I, Wanner J, Tabanca N, et al. (2014) Chemical composition and biological effects of *Artemisia maritima* and *Artemisia nilagirica* essential oils from wild plants of

western Himalaya. *Planta Med* 80(13):1079-87
[doi:10.1055/s-0034-1382957](https://doi.org/10.1055/s-0034-1382957)

51.Valero M, and Salmerón MC. (2003) Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *Int J Food Microbiol* 85(1-2):73-81
[https://doi.org/10.1016/S0168-1605\(02\)00484-1](https://doi.org/10.1016/S0168-1605(02)00484-1)

52.Wu Y, Wang B, Zeng Z, et al. (2019) Effects of probiotics *Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10 on

intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. *Poult Sci* 98(10):5028-5039
[doi:10.3382/ps/pez226](https://doi.org/10.3382/ps/pez226)

53.Zeng Z, Zhang S, Wang H, and Piao X. (2015) Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. *Journal of Animal Science and Biotechnology* 6(1):7

[doi:10.1186/s40104-015-0004-5](https://doi.org/10.1186/s40104-015-0004-5)