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Using Bacteriophage in Poultry Diet as Therapeutics Against *Escherichia coli* in Broiler Chickens

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Abstract | Bacteriophages (BP) are viruses that invade bacteria and propagate inside them, leading to the lysis of the bacterial cells. The aim of this study was to investigate the effect of adding BP to the broiler's diet and its effect on body performance, bacterial population of the gut and immune responses against infectious bronchitis. For this purpose, a total of 250 one-day-old Ross308 broilers were randomly allotted five treatments with two replicate pens of 25 birds per pen. Different treatments regimens were practiced where G1 chicken were orally inoculated with *E. coli* at a dose of 0.5 ml containing 7.5x10^6 CFU/ml and were treated with bacteriophage (3.2×10^{8}PFU/g) through feeding. In the G2 group, chicken were orally inoculated with *E. coli* at a dose of 0.5 ml containing 7.5x10^6 CFU/ml and were treated with enrofloxacin 100mg/kg and neomycin 200mg/kg orally through drinking water. In the group G3, chickens were orally inoculated with *E. coli* at a dose of 0.5 ml containing 7.5x10^6 CFU/ml and were treated with both combination bacteriophage 3.2×10^{8}PFU/g with feeding and enrofloxacin 100mg/kg and neomycin 200mg/kg orally through drinking water. The G4 acted as control group, where chickens were orally inoculated with *E. coli* at a dose of 0.5 ml containing 7.5x10^{6}CFU/ml whereas G5 control group remained untreated and un-infected. Parameters of body weight, weight gain, and feed conversion were observed. Data analysis indicated a statistically significant ($P < 0.05$) increased value of body weight, weight gain, feed consumption and feed conversion in G1, G3 then G2 and G5 respectively, as compared to G4 (control positive). Meanwhile, bacteriophage fed birds had higher Lactobacillus and lowered coliform bacteria counts in G1, G3 than G2 and G5 respectively, compared to G4 (control positive). The bacteriophage additives groups showed significant ($P<0.05$) increased in Ab titer against IBV. In conclusion, bacteriophage 3.2×10^{8}PFU/g additives to broiler chicks feed enhanced growth performance, decreased number of *E. coli* in the gut and showed significant ($P < 0.05$) increased in antibodies titer against IBV.

Keywords | Broiler chicken, *E. coli*, Bacteriophage, Enrofloxacin, Neomycin, Infectious bronchitis, Body performance, H120 vaccination

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INTRODUCTION

Avian colibacillosis is one of the most important avian bacterial diseases producing a wide spectrum of diseases in baby chicks, broilers, and layers leading to financial losses as well as a high rate of mortality and morbidity (Paixao et al., 2016). In broiler chickens, mortality varied from 20% to 40% depending on the

disease severity and it most typically affects 3 to 12 week-old broiler chicks. *E. coli* is a typical component of the intestinal microbiota in chicken. The pathogenic *E. coli* strains are linked to extra intestinal illness resulting in high mortality and morbidity in poultry as a result of septicemia (Guabiraba et al., 2015), which is characterized by high mortality rates with acute septicemia as well as airsacculitis, pericarditis, perihepatitis and peritonitis (Younis et al., 2017). Antimicrobial drugs are unfortunately utilized not just for treatment, but also to improve animal productivity, growth rate and feed conversion rate in food-producing animals in many developing countries (Snary et al., 2004). Many studies have shown that using antibiotics inappropriately boost productivity but increases the selection pressure for antimicrobial resistance bacteria (Levy, 2014). Antibiotic resistance showed no borders and bacteria that have gained resistance to specific antibiotics can quickly travel from nations with effective surveillance programs to those that do not. As a result, a coordinated strategy between developing countries is required (WHO, 2017). Antimicrobial resistance has the potential to wreak havoc on the treatment and management of infectious diseases in humans and animals alike (Mamza et al., 2010).

The application of an alternate rational approach is crucial in APEC control with non-antibiotic treatments. bacteriophages have been tested (Wang et al., 2017). The significant appearance of multidrug-resistant *E. coli* infections in chicken farms is obvious making treatment with standard antibiotics unfeasible and necessitating the use of alternative medicines. Phage therapy is one of the most effective ways to treat drug-resistant bacterial infections in animals without disrupting their normal gut flora. Bacteriophages have a number of qualities that make them potentially appealing therapeutic agents for bacterial illnesses. The excellent specificity and efficacy in lysing targeted harmful bacteria is one of them (Nabil et al., 2018). In addition, other studies have also reported successful reduction of *E. coli* spp. It is found in the internal organs and droppings of chickens (Tawakol et al., 2019), as well as in poultry products Whichard et al. (2003) and Higgins et al. (2005) with the application of phages. Rydman and Bamford (2002) have explained the strategy adopted during the phage bacterium interaction which based on the degradation of extracellular polymeric substance present in the biofilm by polysaccharide de-polymerases production allowing the access of phage which encased bacterial cells and cause their lysis.

Therefore, the goal of this study was to assess the positive effective when used bacteriophage on body performance and the gut microbiome under *E. coli* challenge.

MATERIALS AND METHODS

Antibiotic *in-vivo* study

Enrofloxacin 100 mg was procured from Cenavisa – Spain and Neomycin 200 mg was from Univet-United Arab Emirates.

Bacteriophage

E. coli Bacteriophage 3.2×10^8 PFU/g was procured from EASY BIO- Korea and used in the study as such.

Experimental design

Experimental animals

The chicks in this study were weighed at one day old (average chick weight 43 g±2) and then transported to the poultry hall. The chicks are in the houses divided by wooden barriers, the area of each barrier is 1 x 1.5 m. The bedding in the poultry house was sawdust and was 7 cm thick. Access to drinking water and food is done freely and using a continuous lighting system. No clinical signs of disease were noted before beginning the experiment. In this current study, a total of 250 one-day-old Ross308 broilers were randomly allotted five treatments with two replicate pens of 25birds per pen. The treatments where first group was inoculated orally with *E. coli* at a dose of 0.5 ml containing $7.5x10^6$ CFU/ml of *E. coli* and given bacteriophage 3.2×10^8PFU/g with feeding Tottori et al. (1997) and Rabee et al. (2023) second group was inoculated orally with *E. coli* at a dose of 0.5 ml containing $7.5x10^6$ CFU/ml of *E. coli* and given enrofloxacin 100mg/kg ad neomycin 200mg/kg orally with drinking water. Third group was inoculated orally with *E. coli* at a dose of 0.5 ml containing $7.5x10^6$ CFU/ml of *E. coli* and treated by bacteriophage 3.2×10^8PFU/g with feeding and enrofloxacin 100mg/kg and neomycin 200mg/kg orally with drinking water. Fourth group (control positive) was inoculated orally with *E. coli* at a dose of 0.5 ml containing $7.5x10^6$CFU/ml of *E. coli*. Five group represented the control negative untreated and no infected. Chicks were given starter diet (1-15) days and a finisher diet (15-28) days Table 1.

Vaccination of chicks

The birds were vaccinated against infectious bronchitis at one day old IB QX strain was given by eye drop and at 10 days old IB H120 was given by drinking water, ND at one day old LaSota attenuated strain was given by eye drop and at 10 days old Clone attenuated strain was given by drinking water and 20 days old LaSota attenuated strain was given by drinking water, avian influenza day 1 and infectious bursal disease day 14.

Statistical analysis

Analysis was done by ready-made statistical program

SAS. The data were expressed as mean±standard deviation (STD) unless otherwise stated SAS (2018) and the RCBD (Randomized Completely Block Design) was used in analyzing the data. The differences between the coefficients of the first experiment were tested using Dunkin's multi-level test, at a significant level of 0.05 Duncan (1955).

Table 1: The nutritional composition of dietary treatments.

Ingredients (%)	Starter (1-15) days	Finisher (15-28) days
Yellow corn	35	40
Wheat	25	25
Soybean meal (44 %)	25	25
Protein concentration	10	5.0
Dicalcium phosphate	2.0	2.0
Limestone	1.0	1.0
Vitamin/mineral premix	1.5	1.5
Salt	0.5	0.5
Total	100	100
Calculation composition		
Crude protein (%)	22.5	20.4
Kcal ME/Kg diet	3155	3213
Calorie: protein ratio	140	157.5
Calcium (%)	0.9	0.8
Phosphorus (%)	0.8	0.5

RESULTS AND DISCUSSION

The measurement of live body weight

The results of the current study reflected the presence of significant (P<0.05) differences among all groups with respect to live body weight at 7, 14, 21 and 28-days old chicks. However, at 7 days old chicks, the highest mean of the live body weight in G1 and G3 was 208.6 gm and 207.4gm, respectively followed by G2, G4 and G5with weight of 206.3, 204.1 and 204.2 gm, respectively (Table 2).

While the results recorded a significantly increased level in the live body weight at 14 days old chicks between groups the highest mean was observed in G1 and G3 which were 516.8 and 516.6 gm, followed by G2, G4 and G5 which were 516.1, 512.2 and 512.2, respectively (Table 2).

Similar to body weight on 14 days, the results also showed a significant increase (P<0.05) in the live body weight at 21 days old chicks. The highest mean was observed in G1 and G3 which was 950.6 and 948.9, followed by G2, G4 and G5, which were 945.8, 937.3 and 937.7, respectively (Table 2).

Whereas the live body weight increased in all groups at day 28 as compared with the live body weight at days 7, 14 and 21. The highest the live body weight was given by G1 and G3 which was 1628 and 1625.2, followed by G2, G4 and G5 which were 1600.2, 1429 and 1518, respectively (Table 2).

The measurement of body weight gain (g/bird)

The results of the current study reflected the presence of significant differences (P<0.05) among all groups in body weight gain at 7-, 14-, 21- and 28-days old chicks. However, first week old chicks recorded superior progress towards body weight gain among all groups was given by the G1 and G3, which was 164.65 and 164.2 followed by G2, G4 and G5 which were 162.95, 163.9 and 163.7, respectively (Table 3).

Table 2: Live body weight in experimental study.

AGE/ Groups	G1	G2	G3	G4	G5
7 Day	208.6±0.565D a	206.3±1.55D b	207.4±0.28D a	204.1±0.42D d	204.2±0D c
14 Day	516.8±1.6C a	516.1±1.55C b	516.6±0C a	512.2±1.131C d	512.2±1.4C c
21 Day	950.6±3.95B a	945.8±1.13B b	948.9±0.707B a	937.3±4.10B d	937.7±4.38B c
28 Day	1628±0.28A a	1600.2±5.091A b	1625.2±2.26A a	1429±4.525A d	1518.1±3.25A c

* Means ± Std Error. *Means with the same capital letters in the same column are not significantly different. *Means with the same small letters in the same row are not significantly different.

Table 3: Body weight gain (gm/ bird) in experimental study.

AGE/ Groups	G1	G2	G3	G4	G5
7 Day	164.65±1.34D a	162.95±0.777D b	164.2±1.979D a	163.9±0.42D d	163.7±2.687D c
14 Day	309.2±2.26C a	308.8±0C b	309.2±0.282C a	305.1±0.7C d	305±1.41C c
21 Day	433.8±5.65B a	429.7±2.687B b	432.3±0.707B a	425.1±5.23B d	425.5±2.96B c
28 Day	677.4±4.242A a	654.4±3.95A b	676.3±1.55A a	491.7±0.42A d	580.4±1.13A c

* Means ± Std Error. *Means with the same capital letters in the same column are not significantly different. *Means with the same small letters in the same row are not significantly different.

Table 4: Feed conversion intake ratio between experiential study.

AGE/ Group	G1	G2	G3	G4	G5
7 Day	1.035±0.007D d	1.045±0.007D b	1.035±0.007D c	1.04±0 D a	1.04±0.014D c
14 Day	1.75±0.014C d	1.75±0C b	1.75±0 C c	1.77±0C a	1.77±0.014C c
21 Day	2.765±0.035B d	2.795±0.0212B b	2.775±0.007B c	2.825±0.035B a	2.82±0.014B c
28 Day	3.185±0.021A d	4.48±0.028A b	3.545±0.007A c	10.065±0.007A a	3.415±0.007A c

* Means ± Std Error. *Means with the same capital letters in the same column are not significantly different. *Means with the same small letters in the same row are not significantly different.

While the results recorded a significant increase (P<0.05) in the body weight, gain at second weeks old chicks, where the progress senior body weight gain mean was given by the G1 and G3 respectively which were 309.2 and 309.2, followed by G2, G4 and G5 which were 308.8, 305.1 and 305, respectively (Table 3)

Similarly, a significant increase (P<0.05) in the body weight gain at third weeks old chicks was observed where the highest mean was recorded in G1 and G3 respectively, which were 433.8 and 432.3, followed by G2, G4 and G5 which were 429.7, 425.1 and 425.5, respectively (Table 3).

Whereby the body weight gain increased in all groups at fourth weeks as compared with the body weight gain at weeks first, second and third. The largest the body weight gain was given by G1 and G3 respectively which were 677.4 and 676.3, followed by G2, G4 and G5, which were 654.4, 491.7 and 580.4, respectively (Table 3).

The measurement of Feed conversion ratio (gm feed / gm weight)

The results of the current study reflected the presence of significant differences (P<0.05) among all groups in feed conversion ratio at 7, 14, 21 and 28-days old chicks. However, at 7 days old chicks, the lowest mean FCR among all groups was given by the G1 and G3, respectively, which were 1.035 and 1.035 followed by G2, G4 and G5 which were 1.045, 1.04 and 1.04, respectively (Table 4).

While the results recorded the significant descending at level (P<0.05) in the FCR at 14 days old chicks, the lowest mean was given by the G1 and G3 which were 1.75 and 1.75 followed by G2, G4 and G5 which were 1.75,1.77 and 1.77, respectively (Table 4).

Furthermore, the results continue recorded the significant decline at level (P<0.05) in the FCR at 21 days old chicks, the minimum mean was given by the G1 and G3 which were 2.765 and 2.775, followed G2, G4 and G5 which were 2.795, 2.825 and 2.82, respectively (Table 4).

Nevertheless, the result observed the significant decline at level (P<0.05) in the FCR at 28 days old chicks, the dropped off mean was given by the G1 and G3 which were 3.185 and 3.545 followed by G2, G4 and G5 which were 4.48, 10.065 and 3.415, respectively (Table 4).

The significant increase in the live body weight, weight gain and decrease FCR in G1 and G3 was due to the bacteriophage supplementation in feeding broiler chickens. It is plausible that the BF improve the intestinal morphology, such as increased villus height, enhancing the digestion and absorption of nutrients as reported before (Sarrami et al., 2022). They have showed bacteriophage supplementation in broiler chickens increased body weight gain, improved live body weight, and reduced feed conversion ratio compared to colistin treatment, indicating positive growth performance effects. Tawakol et al. (2019) have showed phage treatment in broiler chickens reduced clinical signs, mortality, and shedding of *E. coli*, potentially improving live body weight, weight gain, and FCR. Li et al. (2012) have documented that BF improve weight gain and feed conversion rate in broiler chickens infected with pathogenic *E. coli*, indicating a positive effect on live body weight. However, Shaufi et al. (2023) have suggested that the combination of phage cocktail and probiotics significantly improved live body weight, weight gain, and feed conversion ratio (FCR) in broiler chickens, suggesting a promising alternative to antibiotic growth promoters. On the other hands, Al-Bawi and Rabee (2020) have showed that Zingiber officinale additives to broiler chicks feed, enhanced growth performance. As for the G2 where antibiotic acted and inhibited harmful microorganisms that produce toxins which it effected on micro flora, absorption and digestion are thus affected on body performance. These results are in agreement with Lev et al. (1957) and Abdel-Azeem (2002) who showed antibiotics can eliminate undesirable microorganisms that produce toxins or metabolic products that irritate and increase thickness of intestinal wall and subsequently decrease the absorption of nutrients. Uzair et al. (2022) have showed neomycin increase weight gain and inhibition of *E. coli* in broiler chicken. Some studied found *E. coli* resistant to neomycin causes defective gut flora ,absorption and effect on dietary digestion, so weight gain was medium.As for the G4 the body performance affected due to *E.coli* causes damage in flora gut led to decrease absorption nutrient by intestinal as shown before by Dziva and Stevens (2008) and Kuldeep et al. (2013) who have explained pathogenic *E. coli*

Table 5: *E. coli* colony from 1 day to 28 days in different groups.

Age/ Groups	7 days	14 days	21 days	28 days
G1	6.11±0.10C c	8.45±1.6C b	10.23±1.13Bb	12.95± 1.6Ca
G2	7.95±0.21C c	10.32±1.12Bb	12.93±1.6Ba	14.12± 1.3Ba
G3	7.61±0.13C c	9.84±1.21Bb	11.38±1.10Ba	13.52± 1.4Ba
G4	11.72 ±0.12Cc	14.65±1.7Ab	17.13±1.5Ab	21.64± 1.8Aa
G5	8.59 ± 0.23Cc	10.65±1.13Bb	12.86±1.11Ba	13.86± 1.05Ba

* Means ± Std Error. *Means with the same capital letters in the same column are not significantly different. *Means with the same small letters in the same row are not significantly different.

invades the columnar epithelium of intestine, produces toxin, also known as enterotoxin leads to the activation of adenylate cyclase and guanylate cyclase which results into the production of increased cAMP and cGMP, respectively. This possibly affect the absorption of sodium, chloride and water balance, ultimately produces watery diarrhea and death occurs due to dehydration and hypo-volumic shock. On the other hands, Salim et al. (2013) have showed infected untreated group had a low significant level weekly body weight, feed consumption. Pallavali et al. (2019) have suggested that decrease in weight gain due to oxidative stress caused by colibacillosis.

Coliform bacteria

The study results revealed the percentage of *E. coli* colonies was decreased significantly ($p<0.05$) in the first group followed by the third and the second groups when compared with the control groups (fourth group which represent the positive control, and fifth group represented the control negative group), respectively. The results in *E. coli* colony gradually increased to a significant level ($p<0.05$) from the seven days to the last day of the experiment (28 days) (Table 5). The first group that consumed the bacteriophage (3.2×10^8 PFU/g) from one day old until the end of the experiment (28 day) show significantly reduced ($p<0.05$) count of coliform bacteria compared to the control positive, indicating highly effective than antibiotic on decreasing intestinal count of coliform bacteria. These results were in agreement with Sarrami et al. (2022) who have found that BP with diet led to reduced population of coliform bacteria in the gut.

Use of antibiotic instead of bacteriophage caused disturbances in the balance of normal microbiota leading to dysbiosis, immunosuppression, and the development of secondary infections (Abbas et al., 2022). On the other hands, Noor et al. (2020) have showed the bacteriophage had improved positive effects on growth performance and reduced E. *coli*. Bacteriophage treatment significantly reduced *E. coli* and Bacteriophages isolated from poultry effectively combat Shiga-toxic *E. coli* in calves, indicating potential for controlling E. coli infections in broiler chickens (Maram et al., 2019; Mohammed et al., 2016).

Bacteriophages have shown significant potential in combating *E. coli* infections in broiler chickens. Studies have isolated lytic phages such as CE1, which effectively lysed high pathogenic strains of avian pathogenic *E. coli* Zhaohui et al. (2023). Additionally, phage cocktails have demonstrated the ability to alleviate intestinal lesions caused by *C. perfringens* in broilers, showcasing the therapeutic potential of bacteriophages (Keerqin et al., 2022).

Moreover, phages targeting non-pathogenic *E. coli* have been identified, with unique characteristics suitable for gut modulation studies in chickens (Li et al., 2012). Honorio et al. (2021) have showed that Bacteriophages effectively combat *E. coli* in broiler chickens, reducing pathogenic bacteria while promoting beneficial ones, surpassing antibiotics as growth promoters in poultry production. Zahra et al. (2023) have showed the Bacteriophage supplementation in broiler chickens feed inhibited coliform bacteria counts, indicating potential usefulness in controlling *E. coli* growth, but this result is in contrast with result from previous studies (Sophie et al., 2020) where Bacteriophages did not reduce *E. coli* strain E28 in broiler chicken gut but showed potential for replication could be demonstrated.

Immunity against the infectious bronchitis disease

Results of maternal immunity against IBV

The result of 10 serum samples from 250 one-day old chicks (before division into groups) for estimation of the maternal immunity against IBV which revealed high level with a mean value of 5300 through ELISA. Local anti-IBV antibodies, particularly IgA and cytotoxic T cells, have been shown as crucial for restricting or eliminating IBV from the host (Awad et al., 2016). IBV had the second highest transfer rate (maternal Ab titer) (Kadhem and Mahdi, 2015).

These results may explain that breeders infected by IBV previously or might be vaccinated by IBV vaccines for many times which eventually gave a high titre of IgG in the sera of breeders. These antibodies passed from breeders to chicks through the passive immunity in high levels

of IgG in which approximately 30% of IgG of breeders. Consequently, the chicks were hatched with that level of circulating IgG from their mother and would show high levels of maternal antibodies against IBV at the first day of age as shown previously (Murphy et al., 1999; De Herdt et al., 2001; Abozeid, 2023; Cavanagh and Naqi, 2003; Sharma, 2003; Hamal et al., 2006; Alazawy, 2013). These studies have also confirmed that several serotypes of IBV might reached to the reproductive system of chickens when the reproductive tract of the hen has developed, some of stimulated lymphocytes localized in the lamina propria of the oviduct and in the stroma of the ovary that led to antibodies produced locally in these organs usually represented a significant increase of the transferred antibody to the eggs.

Results of antibody response by ELISA against IBV

The results of the current study reflected the presence of significant differences (P<0.05) among all groups in Ab titre against IBV at 7, 14, 21 and 28-days old chicks. However, on 7 days old chicks, the highest mean maternal antibody level titre among the vaccinated groups was given by the first group which was 3211 followed by medium mean antibody level titre in third and second groups, which were 2877 and 2372, respectively as compared to the control positive group and control negative group, respectively which was 1967 and 1595 (Table 6).

Table 6: Results of antibody titer against infectious bronchitis disease (M± Std) in different days by ELISA test of the experiment.

Age/ Groups	Day 7	Day 14	Day 21	Day 28
G 1	3211±9.23A	3954±7.67A	2953±3.34A	6767±7.89A
G 2	2372±4.22 B	3380±8.79B	1778±2.56C	3428±4.55B
G 3	2877±5.13A	3653±7.56A	2089±4.22B	5348±8.91A
G 4	1967±4.57C	2013±3.77C	1219±3.59C	1350±3.34C
G 5	1595± 5.33C	1997±3.54C	729±1.5D	790±1.7D

* Means ± Std Error. *Means with the same capital letters in the same column are not significantly different. *Means with the same small letters in the same row are not significantly different.

While the results recorded the significant increase (P<0.05) in antibody titre against IBV at 14 days old chicks, the highest mean was given by the first group which was 3954 followed by medium mean antibody level titre in the third and second groups, which were 3653 and 3380 respectively as compared to the control positive group and control negative group respectively which was 2013 and 1997 (Table 6).

On the other hand, Ab titers decreased in all immunized groups at day 21 as compared with antibody level titre at days 14, the lowest antibody level titre was given by forth group which was 1219 followed by the second, third and first groups, which were 1778 and 2089, respectively, as compared with the control negative group was 729 (Table 6).

Whereas Ab titers increased in all immunized groups at day 28 as compared with antibody level titre at days 7, 14 and 21. The highest antibody level titre was observed in first groups which was 6767 followed by the third and second groups, which were 5348 and 3428 respectively as compared to the control positive group and control negative group, respectively, which were 1350 and 790 (Table 6).

It is plausible that due to vaccination with IB vaccine QX strain at 1 day and H120 at 10 days of age then infected with *E. coli* at 21 days of age, or due to maturation of immune system, as observed by Hegazy et al. (2010) and Li et al. (2017). These studies have found that infection with *E. coli* post IB vaccination showed no differences in antibody titre and in both infected and non-infected groups. In addition, H120 induced a local humoral response, as demonstrated by an increase in IgA levels in tracheal washes of vaccinated birds (Kadhem and Mahdi, 2015).

Bacteriophage leads to prevention of infectious bronchitis virus (IBV) in broiler chickens, reducing mortality and decreasing shedding with *E. coli* (Maram et al., 2019). On the other hand, Andrzej *et al.* (2017) have showed Bacteriophages can combat bacterial infections in poultry effectively, including infectious bronchitis. Their specificity and safety make them a promising alternative to antibiotics in preventing infections in broiler chickens. While Aizhen et al. (2006) have showed Bacteriophage containing a specific short peptide can be used in diagnosing and treating avian infectious bronchitis virus in chickens, aiding in vaccination strategies for broiler chickens. However, Sarrami et al. (2022) have shown that phage supplement in broiler chickens enhances immunity by up regulating IL-10 gene expression and improving gut morphology, potentially aiding in combating infectious bronchitis.

In the control negative group, the low antibody titer in these groups represent the represent the termination of maternal antibody (mab). These results are in accordance with Mondal and Naqi (2001) and Al-Khafaji (2013) who have studied the role of IBV Mab on the development of active immunity to vaccine and they found that the mab declined gradually, remaining on detectable levels up to 17 days as reported by Darbyshire and Peters (1984) and Al-Khafaji (2013).

CONCLUSIONS AND RECOMMENDATIONS

Our results concluded that bacteriophages are useful alternative to antibiotics specially for preventing and treating the multidrug resistance infections. It is also concluded that combination between the antibiotic and bacteriophage therapy reduce and rationalize the levels of antibiotics used in treating bacterial diseases. Finally, future investigations recommended to focus mechanisms of phage-bacterium interactions and application of phage - based products intended for treatment colibacillosis in poultry farms.

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NOVELTY STATEMENT

Using Bacteriophage in Poultry Diet as Therapeutics Against *Escherichia coli* is a novel approache specially as detary inclustion method and can be more considered in compare with inhections of vaccine in the future.

AUTHOR'S CONTRIBUTION

Aljuhaishi and Albawi were participated in proposing, designing and all experimental process of the study. In the writing steps, Aljuhaishi participate in drafting and proofreading of article.

Ethics approval and consent to participate

Ethical and animal walfare status during study were checked and monitoring by universiy research comitee. The proposal and plan of experiment are approved byedical comittee of Al-Qasim Green University.

Conflict of interests

The authors have declared no conflict of interest.

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