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EFFECT OF PROBIOTIC ON GROWTH PERFORMANCE AND CERTAIN PARAMETERS IN BROILER INFECTED WITH ESCHERICHA COLI (E. coli)

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***ABSTRACT* : In this study was suspected Escherichia coli (E .Coli) were detected nine pooled samples out of (120) examined pool samples with an incidence (7.5%); while nine(9) farms were positive for E. coli with an incidence of the farm infection of 47.3%.The nine suspected E. coli isolates were subjected to morphological and microbiological characterization of the colony (size, colour and shape), motility and gram reaction. All suspected colonies have pink colonies on Maconkey agar media, round, moist and raised.The serogroup analysis nine (9) different E. coli showed three different group were identified (2) *O78*, (3) *O111* and (4) untyped group.**

Nine isolates of E. coli were subjected to PCR. All isolates of E. coli were proved positive used this method of characterization and showed the specific expected PCR products at (720 pb).Performance parameter (body weight & body weight gain) at 28 days post infection in group 2 broilers showed lowest means body weight (1120 gm) in comparison of control group (1640 gm), while in groups 3, 4 and 5 body weight gain were showing significant increase (2060 gm), (1995 gm) and (1680 gm) respectively . feed consumption in control group (2673 gm) mean while in group 3, 4, 5 were (3069 gm), (3072 gm) (2973 gm) respectively.Total protein showed high significant increase when compared with control and antibiotic groups.It coluded that the Probiotic and prebiotic are resolve of major problems in broilers and increase encome in poultry industry. Probiotic and prebiotic cause significant increase in body weight gain, feed consumption body weight and FCR.

KEYWORDS: Escherichia coli (E .Coli), , broiler chicken, probiotic, prebiotic, antibiotic.

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I. INTRODUCTION

Studies on the beneficial impact on poultry performance have indicated that probiotic supplementation can have positive effects. It is clearly evident from the result of Kabir et al., (**Kabir 2004**) that the live weight gains were significantly (*P<0.01*) higher in experimental birds as compared to control ones at all levels during the period of 2nd, 4th, 5th and 6th weeks of age, both in vaccinated .and nonvaceinated birds. This result is in agreement with many investigators (**Kalavthy *et al.*, 2003**), (**Islam *et al.*, 2004**), (**Khakse Fidi and Ghoorchi 2006**), who demonstrated increased live weight gain in probiotic fed birds. On the other hand, Lan *et al.*, (**Lan *et al.*, 2003**), found higher (*P<0.01*) weight gains in broilers subjected to two probiotic species. (**Huang *et al.*, 2004**) and **(Wang J, *et al.*, 2021)** Demonstrated that inactivated probiotics, disrupted by a high-pressure homogenizer, have positive effects on the production performance of broiler chickens when used at certain concentrations. In addition, (**Torres-Rodriguez *et al.*, 2007**) reported that administration of the selected probiotic (FM-B11) to turkeys increased the average ckuly gain and market BW, representing an economic alternative to improve turkey production. However, Karaoglu and Durdag (**Karaoglu and Durdag 2005**) used *Saccharomyces cerevisiae* as a dietary probiotic to assess performance and found no overall weight gain difference.

Kalbande *et al.*, (1992) and **EnanG *et al.*, (2022)**have observed probiotic consistent improvements in body weight gain of chicken fed lactobacillus sporogenes culture.and also**Mohan *et al.*, (1995)** reported a quadratic increase in egg production in chickens supplemented with 0.100, and 150 mg probiotic.

Direct or indirect contact with other animals or feces can introduce new strains into poultry flock. Free-living birds are especially important as they colonized with strains that are already adapted to avian species (**Morishita *et al.*, 1999**). E. coli can transmit only horizontally in ducklings (**Islam *et al.*, 2004**).

Diarrheal Disease: primary enteritis in poultry caused by E. coli has been considered rare, enterotoxogenic E. coli (ETEC) that elaborate toxins capable of causing fluid accumulation in intestinal loops of chicken have been recovered from chickens with diarrhea (**Saif 2010**).

The currant study aimed to

Isolation of E .coli from chickens;Effect of E. coli on performance of boiler and Effect of E. coli on liver and kidney function.

II. MATERIALS AND METHODS

2.1. Collected chicks:

Two hundred and thirty chicks either freshly dead or moribund, 1 - 40 days – old of different breeds (Balady broilers and Saso) were collected from different localities of Sharkia Governo-rate and subjected to either clinical and/or postmortem examination. Specimens form liver, lung, kidney, heart and yolk sac were aseptically collected and subjected for bacterial isolation and identification.

One hundred, one day old avian 48 chicks obtained from El Salhy Poultry Company used for experimental injection with isolate E.coli.

2.2. Commercial probiotic and prebiotics:

Lypholac: Produced by Microbiotech USA containing Bacillius subtilis (Lactobacillus acidophilus) 1 x 108 CFU.

Levoxyl: Produced by New Feed Team (NFT), Italy containing manoligo sacharid and betaglocan.

2.3. Bacteriological media: Nutrient agar medium (Oxoid, CMS), Buffered peptone water (Difco), Rappaport–Vassiliadis Soy Peptone (RVS) Broth (MERCK), MacConkey's agar (Oxoid, CM7), Christensen's urea agar bases medium (Difco),Muller- Hinton broth (Oxoid), Muller- Hinton agar (Oxoid code: CM0337).

2.4. Reagents of API 20 E: API NaCl 0.85 % medium, 5 ml (Ref. 20 230) or APIsuspension medium, 5 ml (Ref. 20 150).

2.5. Antisera : The antisera were kindly supplied by Prof. Dr. Samy Adaiel, Animal HealthResearch Institute, Zagazig Branch. Polyvalent O, H and monovalent E. coli antisera.

2.6. METHODS:

Cultivation and isolation of E. coli was carried after (Siam l998).

Biotyping using API 20E (Bio-Merieux, 1992).

Isolation and identification of bacteria from commercial probiotic prepara-tions (Collins et al., 1995).

Serological identification of E. coli was carried out according to Kok et al., 1996).

Extraction of DNA according to QIAamp DNA mini kit.

Preparation E .coli O111and resistant strain performed after (Siam l998).

2.7. Experimental design:

Experiment to study the Pathogencity of most prevalent isolated E. coli spp. In experimentally 7 days old broiler chicks.

One hundred, one day old avian 48 broiler chicks were grouped into five equal groups (1, 2, 3, 4 and 5) each containing 20 broiler chicks. Chicks in groups 2, 3, 4 and 5 were inoculated orally with a dose 1 ×108 cfu of naldixic acid resistant E. coli serotype O111.

Chicks of each group were reared separatly part and fed on starter ration .Group 2 not treated, group 3 treated with lypholac 1m/L FOR 5 succesive days but group 4 treated with leveoxil 1mL/L for 5 succesive days.

Broiler were reared separately on the floor during the experimental period (6 week). Clinical observation of the infected chicks were carried out for recording morbidity, mortalities, clinical and gross lesion reisolation trails of inoculated pathogens were preformed using colocal swabs from each group at post infection.

2.8. Blood analysis: The sample were collected without addition of anticoagulant for serum sepration.

Determination of Liver and Kidney function, serum samples at 7, 14, 21 and 28 post treatment:

Determination of serum aspirates amino transferees (AST) and alanine amin transferees (ALT) according to (Reitman and frankel 1957).

Determination of serum total proteins according to (Gronal et al., 1949)

Determination of serum albumin according to (Bauer, 1982)

Determination of serum uric acid according to (Tajarman et al., 1988)

Determination of serum creatinine according to (Folin, 1934).

2.9. Statistical analysis: The obtained data was statistically analyzed according to Tamhane and Dunlop, (2000).

III. RESULTS

From the table (1) its clear that suspected E. coli were detected nine (9) pooled samples out of (120) examined pool samples with an incidence (7.5%); while (9) farms were positive for E. coli with an incidence of the farm infection of 47.3%.

Table (1): Incidence of infection in each farms and samples

Examined farm	+ve sample	No. total samples	%	+ve farms	Total farms	%
Zagazig	1	16	6.2%	1	4	25%
Abo Hamad	1	13	7.6%	1	3	33.3%
Kanayat	2	13	15.2%	2	4	50%
Abo Kabier	1	15	6.6%	1	3	33.3%
Fakous	-	16	0%	-	-	-
Menea Al Kamh	-	14	0%	-	-	-
Dearb Negm	3	17	17.6%	3	5	60%
El-Salhia	1	16	6.2%	1	-	25%
Total	**9**	120	7.5	9	19	47.3

Identification of E. coli isolates from chickens:

The nine suspected E. coli isolates were subjected to morphological and microbiological characterization of the colony (size, colour, and shape), motility and gram reaction. All suspected colonies have variable sizes.

The size varies from 2 mm till 4 mm in media pink colonies on Maconkey's agar media, round moist and raised on media, gram negative and motile. So, they are all having the same microbiological and morphological pattern.

Gram staining of E. coli isolates showed gram-negative, medium size bacilli non spore forming and arranged singly, in pairs and in groups.

Biochemical characters: All E. coli isolates were indole positive (red ring), methyl red positive (red colour), voges Proskauer vegative (copper like colour) and citrate negative (green colour). E. coli isolates gave yellow slant and bottom with gas formation and no H_2S production on TSI agar medium and urease negative (yellow colour)

API20 E kits: The profile 7144572 was the most prevalent one as it was referred to 6 isolates (Figure 1). API 20 E results were in parallel to the conventional biochemical identification results for these 9 E. coli isolates as both identified the isolates as E. coli.

Figure (1): Biochemical identification of E. coli isolate using API 20 E kits showing very good E. coli identification (seven – digit code number 7144572, id: 99.8 T index : 0.63)

Nine isolates of E. coli were subjected to PCR. All isolates of E. coli were proved positive used this method of characterization and showed the specific expected PCR products at (720bp) **(Figure 2).**

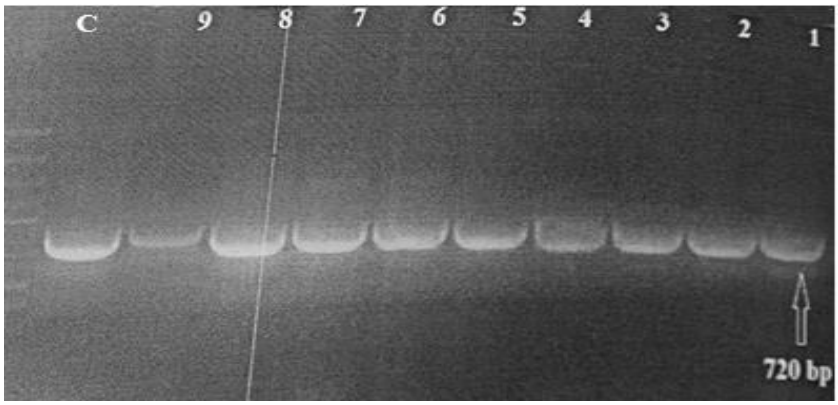

Figure (2): Agrose gel electrophoresis for PCR results of serotyping of all isolates for detecting E. coli by molecular method using PCR techniques

Serotyping of E. coli from examined chickens: Nine E. coli isolates were serogrouped as the following O78, O111, O78, O111 and O111 were recorded 22.2% and 33.3% receptively, while 4 isolate 44.4 were untyped **Table (2): Frequency of occurrence of different E. coli serogroup isolated from infected chickens**

No	O78	O111	Untyped
1	+ve	-	-
4	-	+ve	-
5	-	-	+ve
12	+ve	-	-
22	-	+ve	-
27	-	-	+ve
35	-	-	+ve
40	-	+ve	-
42	-	-	+ve

Pathogenesity of E. coli species: The clinical signs were in both general symptoms such as depression, weakness, ruffed feathers, and loss of appetite and specific symptom in the form of closed eyes, gasping profuse greenish diarrhea 3 days post infection and lameness 14 days post infection. Mortality experimental infected broilers of group 2 were (6) 30%, mortality rate in groups 3, 4 & 5 were (0) 0%, (1) 5% and (1) (5%) respectively **(Table 3).**

Postmortem examination of the early freshly dead and scarified experimentally infected broilers with E. coli spp. isolates revealed gross lesion in the form of congestion of all paranchyma organ lung congestion kidney, enlargement and distension of ureter with urates.

Table (3): Pathogenesity test of E. coli isolates:

No	No. of broiler	Dose of broilers	Route of injection	Mortality	Re-isolation
Control group	20	0	0	0	0
Infected group with *E. coli*	20	1×10^7	Orally	6/20	6/20
Infected and treated by probiotic group	20	1×10^7	Orally	0/20	0/20
Infected and treated with prebiotic group	20	1×10^7	Orally	1/20	1/20
Infected and treated with antibiotic group (Apramycin)	20	1×10^7	Orally	1/20	1/20

Effect of probiotics and prebiotics on body weight gain: Performance parameter (body weight & body weight gain) at 28 days post infection in group 2 broilers showed lowest means body weight (1120 gm) in comparison of control group (1640 gm), while in groups 3, 4 and 5 body weight gain were showing significant increase

(2060 gm), (1995 gm) and (1680 gm) respectively. feed consumption in control group (2673 gm) mean while in group 3, 4, 5 were (3069 gm) ,(3072 gm) (2973 gm) respectively in **(Table 4).**

Table (4): Effect of probiotic and prebiotic given in drinking water for 5 successive days on body weight and body weight gain

Parameter / Group No.	B. weight gain		Feed consumption		FCR	
	14 day PI	28 day PI	14 day PI	28 day PI	14 day PI	28 day PI
1	1001 ± 22^{b}	1640 ± 24^{c}	1641±16 ^{b}	2673±22 ^{c}	1.64 ^{c}	1.63 ^{c}
2	580 ± 57^{d}	1120 ± 12^{d}	1218 ± 42^{d}	2464 ± 55 ^{c}	2.1 ^{a}	2.2 ^{a}
3	1250 ± 62^{a}	2060 ±24 ^{a}	1900 ± 54^{a}	3069 ± 81^{a}	1.49 ^{d}	1.52 ^{d}
4	1202 ±32^{a}	1995 ± 34^{b}	1827 ± 38^{a}	3072±72 ^{a}	1.52^{d}	1.54 ^{d}
5	920 ± 21^{c}	1680 ± 52^{c}	1582 ± 14 ^{c}	2973 ± 62^{b}	1.72^{b}	1.77 ^{b}

Table (5): Effect of probiotics and prebiotics giving in drinking water for 5 successive days on total protein, globulin and albumin on healthy and experimental infected broilers with E.coli organism.

Parameter / Groups	Total protein		Albumin		Globulin	
	7 day PI	14 day PI	4 day PI	14 day PI	7 day PI	14 day PI
Control group	6.4 ±^{a} 0.02	6.5 ±^{a} 0.02	3. 8 ±^{a} 0.01	3.82±^{a} 0.01	2.83 ±^{b} 0.02	2.79 ±^{b} 0.03
Infected group	5.14 ±^{b} 0.2	5.3 ± ^{c} 0.12	1.8 ±^{d} 0.16	1.94 ±^{d} 0.02	3.34 ±^{a} 0.24	3.36 ±^{a} 0.16
Infected group and treated with probiotic	5.74±^{b} 0.01	5.8 ±^{b} 0.05	2.12 ±^{c} 0.01	3.04 ±^{b} 0.01	3.42 ±^{a} 0.02	2.66 ±^{b} 0.06
Infected group and treated with prebiotic	6.71 ±^{c} 0.01	6.54±^{a} 0.01	2.9 ±^{b} 0.02	3.2 ±^{b} 0.08	2.79 ±^{b} 0.03	2.9 ±^{b} 0.06
Infected group and treated with antibiotic	5.6 ±^{b} 0.1	6.2 ±^{b} 0.01	2.8 ±^{b} 0.02	3.1 ±^{b} 0.04	2.6 ±^{b} 0.02	2.75 ±^{b} 0.05

V. DISCUSSION

Collibacillosis considred one of the most serious problem affecting the poultry industry either by direct infectious processes or indire city following infection of other pathogeus **(Saif *et al.*, 2008).**

In The present study were isolated E. coli positive with incidence (7.5%) in positive farms, The positive of E- coli proved positive using method of characterization expeed PCR products at (720 bp) these results full agreed with **Hu. *et al.*, (2011)**.

In the current study biochemical serological and PCR were used to detect two (O78) three (O111) and four untyped *E.coli* , these result similar to **Johnson, *et al.*, (2002)**

Experimental infection of broiler chickens with E. coli (O111) . Orally showed clinical signs after incubation period 72 hours. Similar results were reported by **Khodary and Elsayed (1997)**.

The result of clinical signs, lesion and mortality rate of chickens of group 2,3,4 and 5 were (30%), (0%), (5%) and (5%) respectively. These results disagreed with the result obtained from **Stavric *et al.*, (1992). While Kempf *et al.*, (1994)** observed depression and (8%) mortality in 6 old day infected chickens.

The percentage of reisolation of inoculated nalidexic acid resistant E.coli from different group (0%), (30%), (0%), (5%) and (5%) respectively. These result agreed with the result obtained by **Khaled (2015)**

Performance parameter (body weight & body weight gain) at 28 days post infection in group 2 broilers showed lowest means body weight (1120 gm) in comparison of control group (1640 gm), while in groups 3, 4 and 5 body weight gain were (2060 gm) , (1995 gm) and (1680 gm) respectively . feed consumption in control group (2673 gm) mean while in group 3, 4, 5 were (3069 gm) ,(3072 gm)(2973 gm) and also These results is similar to that obtained by **Rajeswari *et al.*, (2002)**.

Their for it could be coaculeded that the probiotic and prebiotic improve feed consumption and consencuntly the positive impact of the life weight **Andreeva and Dimitrov (2002)**. .

Also there is no difference liver enzymes between the probiotic, prebiotic group and control both at stand and the end of expirement, and the same conculsion suggested by **Isolauri *et al.*, (2001)** who ascartainted the addative with probiotic and prebiotic to broiler rations induced statically significant difference between the groups only in terms of the life weight and the end of experiment ($P \leq 0.05$) and difference between the expiremental group and control both at the stant and the end of the experimental.

Serum had been shown that after one week post treatment probiotic and prebiotic treated broilers refelod a significant increase in serum total protein that were coutained till the end of the experiment in comparsion with control group showed that the administration of probiotic and orebiotic avaked non significant changes in serum Albumin level in broiler chickens this could be credited to the fact the probiotic and prebiotic in directly stimulate the activity of Beta cell. These result are cleary reinforced by **Mohan et al., (1995)** who suggested that increase of total protein and albumin were eviduet to propbiotic and prebiotic to stimulate the lymphocyte.

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