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USING OF PROBIOTICS AND PROPIONIC ACID AGAINST *E. COLI* INFECTION IN BROILER CHICKENS

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ABSTRACT

A total of 400 swabs (cloacal, oral) were collected from 200 one-day-old chicks for bacteriological examination. One hundred one-day-old chicks were divided into 5 equal groups, Gp (1) healthy chicks (control), Gp (2) infected (positive control), Gp (3 and 4) chicks received ½ kg probiotic/ton ration and 0.1 ml propionic acid/liter drinking water for 30 days from respectively, and Gp (5) treated by 10 mg doxycycline/kg Bwt in drinking water for 5 consecutive days (from 15-20 days old). Gp (2, 3, 4, and 5) broilers infected with *E coli* at 15th day old. On the 20th and 30th days old. Blood and tissue samples were collected, and re-isolation of *E. coli* was carried out. Broilers of group (2) showed signs of colibacillosis, reduction in body performance, and antioxidant enzymes, in addition to an increase in WBCs and heterophiles, and impairment in liver and kidney functions at 20 days old. Broilers infected with *E. coli* and receiving probiotic, propionic acid, or doxycycline treatment showed improvement in clinical signs, body performance, leukogram, liver and kidney function tests, antioxidant enzyme activity, and immunity. However, doxycycline residues were found in the kidney, liver, and breast muscle at the 20th day old and completely disappeared from all examined tissues by the 30th day old. The high residue was detected in the kidney, followed by the liver then the breast muscle. It could be concluded that probiotics, propionic acid and doxycycline control *E. coli* infection, *and* improve growth performance, and hematological and biochemical parameters in broilers, so it is good to use probiotic and propionic acid throughout the entire fattening period of broilers as growth promoters and to control *E. coli* infections.

Keyword: Performance - *E coli* - doxycycline - residue - hematobiochemical

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INTRODUCTION

Chicken meat is considered a good source of protein of high biological value (Karine 2002). Chicken meat contains many essential amino acids (Ohimain and Ofongo 2012). Poultry industry needs to prevent and control chicken diseases to increase meat and egg production (Nava *et al.,* 2005). Control broiler diseases leading to increased poultry production (Hassan *et al.,* 2010). *Escherichia coli,* a member of Entero-bacteriaceae, is short Gr-ve, rod-shaped, non-spore forming inhabitants in the lower part of the intestine causing enteritis and high mortalities (Abd El-Tawab *et al.,* 2015). Pathogenic *E coli* induces many diseases in poultry as septicemia, omphalitis, pan-ophthalmitis, arthritis, peritonitis, salpingitis, and perihepatitis (Mohamed *et al.,* 2022).

Antibiotics are used in the treatment of bacterial infection and growth promoters, but many antibiotics can depress the immune system (Shalaby 1989). Doxycycline is a broad-spectrum antibiotic, one member of the tetracycline class, used in the treatment of bacterial infections, it slows or kills bacteria by inhibiting protein biosynthesis (Nelson and Levy 2011). Antibiotic growth promoters induce antibiotic-resistant strains of bacteria and have compelled researchers to use other alternatives like probiotics and organic acids (Gunal *et al.,* 2006). Growth promoters are important for improved performance and productivity (Shahid *et al.,* 2015).

Probiotics is a live microbial feed additives (Tannock 2002). Probiotics improve intestinal microbial balance and body performance (Sethiya 2016). Many types of micro-organisms are used as probiotics (various spp of lactobacilli or bifidobacteria) (Bengmar 1998). Probiotics lower gut pH and inhibit pathogenic bacteria or kill pathogenic bacteria and maintain intestinal flora (Qiao *et al.,* 2019). Organic acids play an important role in controlling and preventing foodborne diseases (Oakley *et al.,* 2014). Organic acids are either simple monocarboxylic acids (formic and propionic acid) or carboxylic acids with a hydroxyl group (lactic and citric acid) that penetrate the semipermeable bacterial cell wall, enter the cytoplasm and decrease the internal pH affecting the enzyme system (Artur *et al.,* 2020). Propionic acid is a member of organic acids used in poultry ration (Broom 2015). Using organic acids and probiotics together improved weight gain (Jadhao *et al.,* 20019).

The present study was carried out to compare the effect of dietary probiotics, propionic acid and sensitive antibiotic (doxycycline) on *E. coli* infection in broiler chickens by studying body performance, and hematobiochemical parameters, in addition to detecting the antibiotic residue.

MATERIALS AND METHODS

Ethical approval

This animal protocol was approved by the Agriculture Research Center ARC-IACUC committee by IACUC protocol number: ARC-AHRI-26-24. Egypt.

Bacteriological examination

A total of 400 swabs (200 oral - 200 cloacal) were taken aseptically from 200, one-day old healthy broilers then inoculated into the nutrient broth at 37^0C for 12 hrs then, re-inoculated on Mac Conkey agar, blood agar and nutrient agar media plates for 24 hr at 37^0C. The colonies were identified *(*Quinn *et al.,* 2002). Suspected colonies were selected for further morphological and biochemical identification (Cruickshank *et al.,* 1975).

Antibiotic sensitivity: The susceptibility of isolated *E. coli* to antibiotics was tested by disc diffusion methods (Quinn *et al.,* 2002).

Serological identification of isolated E coli: Antisera of *E. coli* was used for serological identification of somatic antigen "O"using a slide agglutination test (Bopp *et al.,* 1999). (Antisera of *E. coli* were obtained

from Denka Sciken Co. Ltd Tokyo. Japan).

Drug

Probiotic bacteria (Probax) [R] is an American product directed for poultry feed produced by Pro Byn International, Inc. USA in powder form, composed of: Lactic acid bacteria, 1.6×10^9 CFU/gm (*L.acidophilus, L.Planterum, L.bervis*), Amylase, 224 AU/ gm and β-glucanas 144 BGU/gm.

Propionic acid (CH3CH2COOH) is used in a concentration of about 99% from Sigma ALDRICH®

Doxyrall 80% each gm contains doxycycline hydrate 923.32 mg equiv doxycyclin base 800 mg manufactured by Lely Pharma BV (Netherlands) for Emdokabvba- Belgium.

***E coli* titration:** At 15th day old, broilers in groups 2,3, 4, and 5 were experimentally infected with *E. coli* (0.3 ml via nasal route of cultural suspension of *E. coli* O78 contain $3X10^7$viable organism/ml) (Nakamura *et al.,* 1992).

Chicks and Experimental Design:

One hundred, one day old Hubbard chicks, 43-47gm proved free from any bacterial infection. Chicks were fed a balanced ration (Table 1, 2 & 3) and clean drinking water ad libtium during the experimental period. Chicks were divided randomly into 5 equal groups (٢0/each), Gp (1) healthy chicks (negative control), Gp (2) infected with *E coli* at 15th day old (positive control), Gp (3 &4) healthy chicks received ½ kg probiotic/ton ration (Shawky, et al. 2011) and 0.1ml propionic acid/liter drinking water (Thompson and Hinton 1997) from 1st to 30 days old respectively and at 15th days old, infected with *E. coli.* Chicks in the fifth group, were infected with *E coli* at 15th days old and treated with 10 mg doxycycline/kg Bwt in drinking water for 5 consecutive days (15-20 days old) (Croublels *et al.,* 1997)

Body weight: Chicks in all groups were weighted individually at the start and the end of the experiment (30 days old) for calculation of weight gain and FCR

Samples: - At 20 & 30 days of age 5 chicks from each group were sacrificed for

a) Re-isolation of *E coli*: Swabs from intestine, liver and heart blood were taken for re-isolation of *E. coli.* Collected samples were incubated on nutrient broth at 37 ºC for 24h., then subcultured into Mac Conkey agar and nutrient agar plates at 37 ºC for 24hr., and isolated bacteria were identified (Quinn *et al.,* 2002).

b) Doxycycline residues: Samples from liver, kidney and Breast muscle were taken for estimation of doxycycline residues (Roudaut and Moretain 1990)

c) Blood samples three blood samples were collected

1st sample was taken in EDTA tubes for estimation of leukogram (Feldman *et al.,* 2000**)**

2nd sample was taken in heparinized tubes for estimation of phagocytic % and killing % according to **Wilkinson (1977)** and **Lee and Bacon (1983).**

3rd sample was taken for separation of serum and estimation of T. protein (Doumas *et al.,* 1981), albumin Bauer (1982), globulins fractions which were performed using cellulose acetate electrophoresis (Henry *et al.,* 1974), AST, ALT Reitman and Frankel (1957), ALP, John (1982), uric acid Artiss (1981), creatinine Husdan and Roporpot (1968), total lipid (Knight *et al.,* 1972), cholesterol White *et al.* (1970), triglyceride (Wahlefeld and Bergmeyer (1974), SOD (Nishikimi et al., 1972), CAT (Sinha 1972) MDA (Nielsen *et al.,* 1997).

Statistical analysis was performed using analysis of variance (ANOVA). Computerized SPSS program version 16, Duncan's Multiple Range Using (Tamhane and Dunlop 2000).

Table 1: Physical composition of experimental diets.

Ingredient/ kg	starter stage	Grower stage	Finisher stage
GroundYellow corn	59.5	60.5	64.5
Soya bean meal 44% CP	26.1	28.1	22.36
Corn gluten 60% CP	10.7	6.7	5.7
Oil	00.0	1.00	3.74
Lysine Hcl 78%	0.1	0.1	0.1
DL- methionine 98%	0.2	0.2	0.2
Calcium dibasic phosphate	1.7	1.7	1.7
Calcium carbonate	1.3	1.3	1.3
Vit. Premix	0.1	0.1	0.1
Common salt	0.3	0.3	0.3
Total	100	100	100

Calculated according to the feed composition by **NRC (1994)**
Vit. Premix: each 2.5 kg contains vit. A(12000000 IU), Vit. D3(2000000 IU)
Vit. E(10000 mg), Vit. K(1000 mg), vit B1(1000 mg), vit. B2(5000 mg), Vit. B6(1500 mg)
Pantothenic acid(1000 mg), Vit. B12(10 mg), niacin (3000 mg), folic acid(1000 mg), Biotin(50 mg)
Fe(30000 mg), Mn(60000 mg), Cu(4000 mg), I(300 mg), Co(100 mg), Se(100 mg)&Zn(5000 mg)

Table 2: Chemical composition of experimental diets.

Calculated chemical analysis	starter stage	Grower stage	Finisher stage
Metabolic energy Kcal/Kg	2973.3	2990.6	3200.5
Crude protein %	23	21	18.52
Ether extract %	2.65	2.61	2.66
Crud fiber %	3.35	3.46	3.12
Ca %	0.94	0.98	0.98
Available Phosphorus %	0.459	0.506	0.494
Lysine Hcl 78%	1.085	1.057	1.03
DL DL-methionine 98%	0.57	0.53	0.52

Crude protein% and Ether extract % were chemically analyzed according to the method described by AOAC (1990)
Calcium & Available Phosphorus calculated according to the feed composition by NRC (1994)

Table 3: Chemical analysis of feedstuffs used in formulation of experimental diets (analyzed).

Ingredient	crude protein %	Ether extract %	crude fiber %	Ca %	Available Ph %	Metabolic energy Kcal /Kg	Moisture
Ground Yellow corn	7.9	3.5	2.2	0.05	0.1	3350	10.7
Soya bean meal	43.5	1.2	7.3	0.35	0.27	2230	10.8
Fish meal	65	5	1	3.73	2.43	2580	7
Corn gluten 60%	60	2.4	1.3	0.07	0.14	3720	9.5
Soy bean oil	0.0	00	00	0.0	0.0	8800	0
Calcium dibasic phosphate	00	00	00	21.3	18.5	00	0
Calcium carbonate	00	00	00	38	00	00	0

Crude protein%, Ether extract % and moisture were chemically analyzed according to **AOAC (1990)**
Calcium & Available Phosphorus calculated according to the feed composition given by **NRC (1994)**

RESULTS

Out of examined oral swabs 15 (7.50%) were +ve (9 single isolate and 6 mixed) beside 25 (12.50%) cloacal swabs were +ve (9 single isolate and 16 mixed). High single insolent +ve swabs were *E coli* (10 isolates). Serologically isolated E coli, O78 (3), O157 (5) and O11 (2). Isolated *E coli* were sensitive to doxycycline (Tables 4, 5, 6, and 7)

Broilers infected with *E coli* (Gp 2) at 20 days old, showed depression, diarrhea, dropping wings, listlessness, frothy exudate in eyes, and respiratory signs, with a significant decrease ($P< 0.05$) in body weight, weight gain, albumin, A/G ratio, CAT, and SOD, compared to the normal control group (Gp 1). Significant increase ($P< 0.05$) in WBCs, heterophils, phagocytic %, killing %, AST, ALT, ALP, α, β,γ globulin, T. globulin, MDA, uric acid, and creatinine. Nonsignificant ($P< 0.05$) changes in other parameters, compared to the normal control group (Tables 8, 9, 10, 11 and 12). Most of these parameters returned to normal at 30 days old.

Broilers received probiotic or propionic acid from 1st to 30th days old and infected with *E coli* at days 15th old (Gp 3 and 4), showed no signs of colibacillosis, reduced ($P< 0.05$) mortality and *E coli* reisolation associated with significant elevation ($P<0.05$) in weight gain, phagocytic %, killing %, T. protein, albumin coupled with insignificant increase in total and differential leukocytic counts, AST, ALT, ALP, A/G ratio, uric acid, creatinine, total lipids, cholesterol, triglyceride, beside insignificant changes in α, β, γ, total globulin, CAT, SOD according to normal control group at 20 and 30 days old. But according to a positive control group (Gp 2), these groups showed improvement in body performance, liver and kidney function tests, MDA and antioxidant enzymes (Tables 8, 9, 10, 11 and 12).

Infected broilers with *E coli* and doxycycline-treated (Gp 5) showed no clinical signs, zero mortality, reduced *E coli* reisolation and insignificant elevation ($P<0.05$) in weight gain, WBCs, heterophils, lymphocyte, monocyte, eosinophils, basophils, phagocytic%, killing %, AST, ALT, ALP, A/G ratio, uric acid, creatinine, T lipid, cholesterol, triglyceride beside significant elevation in uric acid and MDA, coupled with insignificant reduction α, β,γ globulin, T. globulin CAT and improved in FCR as compared to control broilers according to normal control group (Gp 1) (Tables 8, 9, 10, 11 and 12). This group also showed improvement in all examined parameters, compared to the positive control infected group.

Doxycycline residues were found in liver, kidney and breast muscle at the 20th day of age and completely disappeared from all examined tissues at the 30th day of age. The high residue was detected in the kidney followed by the liver then the breast muscle.

Table 4: Incidence of different bacterial pathogens isolated from examined oral and cloacal swabs

Swabs type	Oral swabs (200)				Coloacal swabs (200)			
+ ve swab	15 isolate (7.5%)				25 isolate (12.5%)			
Isolate type	Single 9(60%)		Mixed 6(40%)		Single 9(36%)		Mixed 16(64%)	
isolate	*E. coli*	5	*E coli +Staph spp*	2	*E. coli*	5	*Strept sp + E. coli*	6
	Staph. spp	2	*E. coli+ Sal. Spp*	1	*Proteus sp*	2	*Sal. Sp+ Strept sp*	1
	Strep spp	2	*Proteus +E. coli*	2	*Sal sp*	1	*sal .sp + Staph sp*	2
			Sal Sp+ Staph sp	1	*Klebsiella sp*	1	*E. coli + Proteus*	7

Table 5: Serological identification of isolated *E. coli* strains type

Isolated bacteria	*E. coli* serotype	Oral (5)	Coloacal (5)	Total
E. coli	O157	3	2	5
	O78	1	2	3
	O11	1	1	2

Table 6: Result of sensitivity test for *E coli* isolated from chickens to antibiotics

Antibiotic disc	Mark of sensitivity disc	Disc-potency (ug)	Inhibitory Zone(mm)	Sensitive
Spectinomycin	SP	10ug	17	++
Doxycycline	DX	30 ug	20	++++
Florfenicol	FF	30 ug	19.5	++++
Gentamycin	Gm	10 ug	16	+++
Amoxycylline	AM	25ug	13	++
Neomycine	NM	30 ug	10	+

Table 7: Effect of *E coli* on mortality rate and reisolated *E coli* of chicks

Parameters		Gp (1)	Gp (2)	Gp (3)	Gp (4)	Gp (5)
total chicks number		20	20	20	20	20
Mortality rate	No	00	6	1	1	0
	%	00	30	5	5	0
Reisolated E *coli*	20^{th} day	00	8/20	2/20	2/20	1/20
	30^{th} day	00	8/20	2/20	2/20	1/20

Table 8: Effect of *E coli*, probiotic and propionic acid on body performance of broiler (n= 5)

Groups	Initial weight (1^{th} day old) (gm)	final body weight (30^{th}day old) (gm)	Weight gain (gm)	feed consumption	feed conversion ratio
Gp1	45.96± 0.73	1726.81 ± 5.25^{b}	1680.95 ± 5.63^{b}	2521.42 ± 4.96^{b}	1.5 ± 0.16^{a}
Gp2	46.58±0.44	1531.55 ± 5.61^{c}	1484.97 ± 5.52^{c}	2178.95 ± 4.76^{c}	1.42 ± 0.19^{b}
Gp3	47.49± 0.32	1783.59 ± 6.52^{a}	1736.10 ± 4.74^{a}	2656.23 ± 4.88^{a}	1.53 ± 0.23^{a}
Gp4	45.89±0.85	1773.62 ± 5.52^{a}	1727.53 ± 5.57^{a}	2608.87 ± 4.97^{a}	1.51 ± 0.19^{a}
Gp5	47.38± 0.77	1753.50 ± 6.34^{ab}	1706.12 ± 5.7^{ab}	2559.18 ± 5.55^{b}	1.50 ± 0.27^{a}

Means with different superscripts of same column indicate significant difference at P< 0.05

Table 9: Effect of *E coli*, probiotic and propionic acid on leukogram, Phagocytic% and Killing% of broilers (n=5)

Groups		WBCs X10³/µl	Deferential X10³/µl					Phagocytic %	Killing %
			heterophil	lymphocyt	esinophil	basophil	monocyte		
20th day	Gp1	11.93±0.82[b]	3.40±0.32[b]	4.32±0.51	1.31±0.36	1.50±0.21	1.42±0.55	58.05±0.71[b]	36.21±0.39[b]
	Gp2	15.19±0.89[a]	5.69±0.61[a]	4.52±0.89	1.85±0.40	1.17±0.21	1.56±0.27	62.54±0.78[a]	39.02±0.81[a]
	Gp3	12.25±0.87[b]	3.46±0.76[b]	4.40±0.25	1.38±0.32	1.55±0.13	1.46±0.21	63.06±0.39[a]	39.21±0.32[a]
	Gp4	12.20±0.78[b]	3.49±0.29[b]	4.35±0.43	1.36±0.21	1.58±0.32	1.45±0.30	63.32±0.28[a]	39.55±0.42[a]
	Gp5	14.12±0.69[b]	5.45±0.75[a]	4.42±0.49	1.31±0.32	1.52±0.18	1.43±0.23	58.59±0.95[b]	37.95±0.73[b]
30th day	Gp1	11.95±0.99[b]	3.36±0.27[b]	4.37±0.49	1.35±0.32	1.47±0.18	1.40±0.27	58.38±0.58[b]	36.69±0.84[b]
	Gp2	14.85±0.96[a]	5.08±0.53[a]	4.63±0.71	1.80±0.33	1.90±0.19	1.54±0.32	65.13±0.89[a]	37.98±0.69[b]
	Gp3	12.42±0.91[b]	3.50±0.46[b]	4.44±0.48	1.41±0.28	1.58±0.19	1.49±0.33	63.21±0.49[a]	39.72±0.94[a]
	Gp4	12.47±0.97[b]	3.53±0.48[b]	4.43±0.61	1.39±0.31	1.63±0.40	1.49±0.27	63.82±0.53[a]	39.84±0.63[a]
	Gp5	12.48±0.84[b]	3.49±0.59[b]	4.48±0.64	1.41±0.29	1.58±0.21	1.52±0.23	58.38±0.58[b]	37.77±0.85[b]

Means with different superscripts of the same column indicate significant difference at $P < 0.05$

Table 10: Effect of effect of probiotic, propionic acid and *E coli* on liver function in broiler chickens (mean ± SE) (n= 5)

Groups		liver enzymes (U/L)			Protein profile (gm/dl)						
		AST	ALT	ALP	Total protein	albumin	Globulin				A/G ratio
							α	β	γ	total	
20th day	Gp1	35.99± 0.74[b]	48.87± 0.89[b]	67.45± 0.28[b]	5.83± 0.68[b]	2.93± 0.49[b]	1.01± 0.05[b]	0.90± 0.07[b]	0.99± 0.07[b]	2.90± 0.17[b]	1.01± 0.19[a]
	Gp2	45.60± 0.58[a]	55.49± 0.74[a]	74.98± 0.63[a]	5.98± 0.33[b]	2.07± 0.49[c]	1.22± 0.12[a]	1.11± 0.11[a]	1.12± 0.15[a]	3.55± 0.17[a]	0.58± 0.11[b]
	Gp3	36.12± 0.89[b]	49.12± 0.83[b]	67.67± 0.79[b]	6.95± 0.35[a]	3.37± 0.32[a]	0.95± 0.14[b]	0.75± 0.15[b]	0.98± 0.14[b]	2.68± 0.39[b]	1.24± 0.18[a]
	Gp4	36.01± 0.79[b]	49.21± 0.94[b]	67.68± 0.74[b]	6.12± 0.65[a]	3.29± 0.51[a]	0.95± 0.15[b]	0.88± 0.19[b]	0.96± 0.19[b]	2.83± 0.34[b]	1.14± 0.21[a]
	Gp5	36.03± 0.36[b]	49.17± 0.37[b]	67.60± 0.83[b]	5.74± 0.71[b]	3.00± 0.42[ab]	0.99± 0.20[b]	0.89± 0.16[b]	0.95± 0.18[b]	2.74± 0.80[b]	1.09± 0.26[a]
30th day	Gp1	35.53± 0.55	48.79± 0.53	67.78± 0.59	5.78± 0.93[b]	2.90± 0.37[b]	0.99± 0.09[b]	0.90± 0.12[b]	0.99± 0.11[b]	2.88± 0.27[b]	1.01± 0.23[a]
	Gp2	34.92± 0.59	48.21± 0.88	67.59± 0.59	5.48± 0.93[b]	2.11± 0.68[c]	1.17± 0.19[a]	1.04± 0.16[a]	1.16± 0.19[a]	3.37± 0.32[a]	0.63± 0.11[b]
	Gp3	35.61± 0.68	49.58± 0.58	67.98± 0.64	6.43± 0.46[a]	3.67± 0.29[a]	0.93± 0.11[b]	0.82± 0.13[b]	1.01± 0.20[b]	2.76± 0.47[b]	1.33± 0.28[a]
	Gp4	35.81± 0.83	49.15± 0.74	67.89± 0.35	6.49± 0.81[a]	3.67± 0.63[a]	0.95± 0.12[b]	0.86± 0.18[b]	1.01± 0.16[b]	2.82± 0.38[b]	1.25± 0.19[a]
	Gp5	35.87± 0.63	48.99± 0.85	67.96± 0.71	5.76± 0.58[b]	2.71± 0.59[b]	1.00± 0.23[b]	0.91± 0.25[b]	1.01± 0.32[b]	2.92± 0.72[b]	0.93± 0.21[a]

Means with different superscripts of the same column indicate significant difference at $P < 0.05$

Table 11: Effect of probiotic, propionic acid and *E coli* on kidney function, Lipid profile, antioxidant enzymes in broilers (mean ± SE)(n= 5)

	Groups	Kidney functions (mg/dL)		Lipid profile(mg/dl)			MDA (mmol/ml)	Antioxidant enzymes ((U/mL)	
		Uric acid	Creatinine	Triglyceride	choles terol	Total lipid		SOD	CAT
20th day	Gp1	5.69± 0.60^{c}	1.08± 0.16^{b}	98.05± 1.21^{a}	69.32± 1.62^{a}	189.3± 1.07^{a}	15.21± 0.98^{b}	94.31± 0.88^{a}	56.17± 0.62^{b}
	Gp2	9.38± 0.42^{a}	1.96± 0.11^{a}	90.12± 1.32^{b}	62.92± 1.31^{b}	168.23± 1.52^{b}	19.89± 0.69^{a}	83.36± 0.83^{c}	51.52± 0.84^{c}
	Gp3	5.53± 0.78^{c}	1.21± 0.23^{b}	99.13± 1.78^{a}	70.12± 1.33^{a}	190.04± 1.64^{a}	15.98± 0.79^{b}	96.86± 0.96^{a}	59.89± 0.69^{a}
	Gp4	5.99± 0.63^{c}	1.12± 0.19^{b}	98.57± 1.93^{a}	70.32± 1.21^{a}	189.73± 1.57^{a}	15.93± 0.59^{b}	96.56± 0.74^{a}	59.98± 0.22^{a}
	Gp5	7.98± 0.58^{b}	1.24± 0.17^{b}	98.98± 1.32^{a}	70.15± 1.73^{a}	190.02± 1.84^{a}	18.74± 0.88^{a}	88.95± 0.94^{b}	55.49± 0.99^{b}
30th day	Gp1	5.73± 0.29	1.12± 0.19	98.33± 1.69^{a}	69.54± 1.37^{a}	188.45± 1.28^{a}	15.67± 0.83	94.86± 0.73^{b}	56.39± 0.87^{b}
	Gp2	5.96± 0.72	1.08± 0.13	95.05± 1.32^{b}	65.54± 1.42^{b}	176.20± 1.94^{b}	16.60± 0.84	93.09± 0.85^{b}	55.97± 0.79^{b}
	Gp3	5.86± 0.63	1.25± 0.15	99.19± 1.58^{a}	70.43± 1.51^{a}	190.21± 1.87^{a}	16.06± 0.85	96.97± 0.83^{a}	59.55± 0.83^{a}
	Gp4	5.89± 0.83	1.18± 0.21	99.51± 1.71^{a}	70.16± 1.44^{a}	190.52± 1.48^{a}	16.09± 0.83	96.86± 0.88^{a}	59.06± 0.42^{a}
	Gp5	5.89± 0.43	1.19± 0.22	97.89± 1.90^{a}	68.58± 1.21^{a}	190.72± 1.71^{a}	15.93± 0.76	93.98± 0.99^{b}	55.88± 0.85^{b}

Means with different superscripts of the same column indicate significant differences at $P < 0.05$

Table 12: Doxycycline residues (µg/gm) in liver, kidney and breast muscle of chicks of group(5) (n=5).

Antibiotic disc	20th day			30th day		
Organ	Liver	Kidney	Breast muscle	Liver	Kidney	Breast muscle
Residues	0.46±0.11	0.69±0.15	0.28±0.04	00	00	00

DISCUSSION

Bacteriological examination of collected swabs revealed the presence of bacteria in 15 oral swabs and 25 cloacal swabs, single and mixed infection. The main prominent isolated bacteria was *E. coli* with 10 isolates. An antibiogram for isolated *E coli* revealed doxycycline was an effective antibiotic. Similar results were recorded by Mohamed (2005) and Haji; *et al.* (2009) who found that *E coli* is sensitive to doxycycline. It is a broad spectrum bacteriostatic antibiotic that inhibits the synthesis of bacterial proteins by binding to the 30S ribosomal subunit, which is only found in bacteria, this prevents the binding of tRNA to mRNA at the ribosomal subunit, so the amino acids cannot be added to polypeptide chains and new proteins cannot be made (Hitchings *et al.,* 2015). This stops bacterial growth, giving the immune system time to kill and remove the bacteria (Maaland *et al.,* 2013).

Birds of group (2), infected with *E coli*, showed signs of colibacillosis with a high mortality rate of 30%, these signs may be due to the *E coli* endotoxins. These signs were previously observed by Hashem, *et al.*

(2019) and Reham, *et al.* (2021) who reported diarrhea, loss of appetite, mouth breathing, sneezing, ruffled feathers, weight loss and mortality reached 30% in chicken infected with *E.coli*. The decrease in body weight and weight gain and increase in FCR occurred due to inappetence, intestinal damage, poor digestion and diarrhea induced by the *E coli.* This is supported by Fadl, *et al.* (2020) and El-Tahawy, *et al.,* (2022).

Broilers in Gp 3 and Gp 4, received probiotic or propionic acid respectively from 1st to 35th days old and infected with *E coli* at the 15th day old revealed significant elevation in body weight gain and reduced FCR. This may be due to the dietary organic acids which inhibit the growth of pathogenic bacteria and improve body performance in broilers (Skinner *et al.,* 1991). Marin, *et al.* (2014) reported that probiotic or organic acids increased weight gain and reduced FCR in broilers infected with *E coli*. These findings agreed with Cao *et al.* (2013) and Mohamed, et al., (2022), who stated that probiotics induce improvement in body performance in broilers infected with *E coli*. The elevation in body weight, weight gain and reduced FCR may be due to the change in PH of the intestine which protects the birds from the pathological bacteria. Probiotics lower the gut PH and so inhibit or kill the pathogenic bacteria and maintain intestinal flora (Qiao *et al.,* 2019).

Broilers of Gp 5 that were infected with *E coli* and treated with doxycycline showed improvements of clinical signs and reduced mortality rate with reduced *E coli* reisolation. This may be due to the antimicrobial effect of doxycycline (Abd El-Aziz 2000). The improvement in the body's performance may be due to the antimicrobial effect of doxycycline (Abd El-Aziz 2000 & Milles *et al.,* 2006). Similar results were obtained by Abd Allah (1992) in broilers infected with *E. coli* doxycycline-treated. In keeping with this line, Koji *et al.* (1989) stated that chickens infected with *E. coli* medicated with doxycycline revealed no clinical signs, zero mortality, and reduced *E. coli* reisolation besides better weight gain and FCR. This improvement in body performance and protein profile may be due to the antimicrobial effect of doxycycline (Abd El-Aziz, 2002). Antibiotics play an important role in reducing intestinal pathogenic bacteria, leading to reduced competition for microbial nutrients in the host, increasing availability of nutrients, improved body performance and improved biochemical parameters (Miles *et al.*, 2006). Broilers suffering from colibacillosis doxycycline treated showed improvement in body performance (Amer *et al.,* 2009).

The leucocytosis and hetarophilia in addition to the increase of phagocytic% and killing% in broiler of Gp 2, infected with *E coli* may be due to stimulation of the immune system and defence mechanism occurred by *E coli* and their endotoxins. This was in accordance with Mithin, *et al.* (2022), who reported that broilers infected with *E. coli* showed an increase in WBCs and heterophils.

The insignificant elevation of total and differential leucocytic counts in addition to the increase of the phagocytic% and killing% in broiler of Gp 3 and Gp 4 compared with the normal control group may be due to the improvement of the immunity and blood picture occurred by probiotic or propionic acid. Similar results in leukogram were recorded previously by Sabry *et al.* (2016) in broilers infected with *E coli* and received probiotics or organic acid. Broilers infected with *E coli,* which received probiotics or organic acid showed an insignificant increase in WBCs, heterophil, lymphocyte, eosinophil, and basophil coupled with an increase in phagocytic % and killing % (Allam *et al.,* 2014). Organic acids reduced bacterial pathogens and improved blood picture (Dana *et al.,* 2018).

Birds in Gp 5 that were infected with *E coli* and treated with doxycycline showed a decrease in total leucocytic count and heterophils in addition to a decrease in the phagocytic% and killing%, compared to the infected group (Basak *et al.,* 2004; Amer *et al.,* 2009).

Broilers in Gp 2 showed a significant increase in liver and kidney function tests in addition to a decrease in the antioxidant enzymes may be due to the *E coli* endotoxin which induces oxidative stress, severe inflammation and damage of the internal organs, especially the liver and kidneys (Nana *et al.,* 2022; Mohamed *et al.*, 2022)

Broilers received probiotic or propionic acid (Gp 3 & Gp 4) each alone and infected with *E coli* showed significant improvement in liver, kidney function tests and antioxidant enzymes, compared to Gp 2 infected with *E coli.* This improvement in liver function may be due to the antimicrobial and antioxidant activity of probiotics besides decreased pathogenic bacterial population (Abdelhady and El-Abasy 2015). Probiotics enhance the activity of SOD and CAT in chickens infected with *E. coli* (Dong *et al.,* 2019). Broilers infected with *E. coli* received probiotics and showed a decrease in serum MDA due to protection against lipid peroxidation by the anti-oxidant effect of probiotics and improved CAT and SOD (Abd-El-Rhman *et al.,* 2012). Probiotic has antibacterial and antioxidant activity, so increase CAT, SOD and decrease MDA (Abdelhady and El-Abasy 2018). Organic acids and probiotics improved the antioxidant status of broilers and increased CAT and SOD (Alaeldein *et al.,* 2017). Our results were consistent with Allam *et al.* (2014), who stated that broilers infected with *E. coli* and received organic acids and probiotics revealed insignificant increases in uric acid, creatinine total lipid, triglycerides and cholesterol. In addition, Abd-El-Rhman *et al.* (2012) stated that broilers infected with *E coli* and supplemented with probiotics showed no significant increase in uric acid, creatinine total lipid, triglycerides and cholesterol.

Infected broilers with *E coli* doxycycline-treated (Gp 5) showed insignificant changes in AST, ALT, ALP, A/G ratio, uric acid, creatinine, T lipid, cholesterol, triglyceride, MDA besides significant elevation in T protein, albumin coupled with insignificant reduction α, β,γ, total globulin, CAT, SOD and improved in FCR as compared to control broilers. Our obtained results are similar to those recorded by Mohamed (2005) who found that infected broilers with *E. coli,* doxycycline-treated revealed improve in body performance and insignificant increase in liver enzymes, A/G ratio, uric acid, creatinine, total lipid, cholesterol and triglyceride. Our results also agreed with Basak *et al.* (2004), who reported that broilers infected with *E. coli* treated with doxycycline revealed an insignificant decrease in CAT, SOD.

It is clear from the present study that doxycycline residues were found in kidneys, liver, and breast muscle on the 20^{th} day of age and completely disappeared from all examined tissues on the 30^{th} day of age and the residue was high in the kidney followed by the liver then breast muscle. Our finding agreed with that reported in chickens by Reham and Eladl (2014), who stated that the highest doxycycline residue was found in the kidney followed by the liver and the lowest residues in breast muscle. Doxycycline residue was detected in muscle up to 5 days of administration (Wijayanti and Rosetyadewi 2011). Doxycycline residue was present in the liver, kidney and muscle up to 5 days post-dosing (Laczay *et al.,* 2001). Doxycycline was eliminated from the kidney and liver after 4 days and present in muscles up to 5 days post-dosing (Donoghue 2003)

It could be concluded that probiotics, propionic acid and doxycycline control *E.*

coli infection, improved body performance, and hematological and biochemical parameters in broilers. So it is recommended to use any of them to control *E coli* infection in broilers and it is good to use probiotic or propionic acid over the fatting period of broilers as growth promoters.

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إستخدام المحفزات الحيوية وحمض البروبيونك للحد من الاصابة بالميكروب القولوني فى بداري التسمين

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تم تجميع عدد ٤٠٠ عينة، ٢٠٠ من فتحة المجمع و ٢٠٠ من المنقار للفحص البكتيريولوجى. واتضح ان مسحات المنقار كان ١٥ (٧,٥٪) موجب للبكتريا وموزعة ٩ (٦٠٪) معزولات منفردة و6 (٤٠٪) معزولات مشتركة اما مسحات فتحة المجمع كانت ٢٥ (١٢,٥٪) موجب للبكتريا وكانت موزعة ٩ (٣٦٪) معزولات منفردة و١٦ (٦٤٪) معزولات مشتركة. وكان اعلى المعزولات هى الميكروب القولونى العصوى (١٠ معزولات) وبالفحص السيريولوجى كانت O157 , O78 و O11 .بعمل أختبار الحساسية لهذه المعزولات وجد أن الميكروب القولونى العصوى حساس للدوكسي سكلين. بعد الفحص البكتيريولوجى تم استخدام عدد ١٠٠ كتكوت عمر يوم واحد ووزنهم ٤٣-٤٧ خالية من اى عدوى بكتيرية تم تقسيمهم الى٥ مجموعات (20 بكل مجموعة). المجموعة الاولى تركت بدون اى اضافات (مجموعة ضابطة), الثانية تم اصابتها بميكروب العصوي القولوني (مجموعة ضابطة موجبة)، اما المجموعة الثالثة والرابعة تم اعطائها نصف كجم بروبيوتك لكل طن علف و ٠,١ ملى من حمض البربيونك/ لتر من مياة الشرب من اليوم الاول من العمر حتى اليوم ٣٠ من العمر وفى اليوم ١٥ من العمر تم عمل عدوى اصطناعية بالميكروب القولونى العصوى اما المجموعة الخامسة تم عمل عدوى اصطناعية بالميكروب القولونى العصوى للكتاكيت عند اليوم ١٥ من العمر وتم علاجها باستخدام ١٠ مجم دوكسى سيكلين/ كجم من ون الجسم. عند اليوم الاول من نهاية الامداد تم وزن الكتاكيت فى كل المجموعات وحساب كمية العلف المستخدمة لتعيين تأثير البروبيوتك وحمض البربيونك والميكروب القولونى العصوى على وزن الجسم ومعدل التحويل الغذائى. عند اليوم ٢٠ و٣٠ من العمر تم ذبح ٥ كتاكيت من كل مجموعة وتم اخذ عينات من الكبد والكلى وعضلة الصدر لتعيين بقايا الدوكسى سيكلين وتم أخذ مسحات من الامعاء لمحاولة اعادة عزل الميكروب القولونى العصوى وتم اخذ ٣ عينات دم لتعيين بعض الوظائف الدموية والبيوكيميائية.

وقد اتضح من النتائج ان الكتاكيت التى تم اصابتها بالمكروب العصوي القولوني عانت من اعراض الاسهال، انتفاش الريش، نقص الاوزان وقلة استهلاك العليقة وزيادة في معدل التحويل الغذائي وزيادة في الوفيات عند مقارنتها بالمجموعة الضابطة السلبية. كما اظهر فحص الدم زيادة في عدد كرات الدم البيضاء وعدد الهيتروفيل، وكذلك اظهر الفحص الكيميائي للسيروم خلل في وظائف الكبد والكلي ونشاط للجهاز المناعي. وتم اعادة عزل الميكروب العصوي القولوني من الطيور المصابة.

أدى اعطاء البروبيوتك وحمض البربيونك الى عدم ظهور اعراض مرضية ونقص فى الوفيات ونقص في اعادة عزل الميكروب القولونى العصوى ووجود زيادة معنوية فى وزن الجسم المكتسب، قوه اللتهام والقتل، البروتين الكلى الزلال وتحسن فى معدل التحويل الغذائى بجانب وجود زيادة غير معنوية فى عدد كرات الدم البيضاء, الخلايا المتعادلة، الخلايا الليمفاوية, الخلايا الملتهمة الكبيرة, الخلايا الحامضية, الخلايا القاعدية, A/G ratio AST –ALT- ALP, حمض اليوريك والكرياتينين الدهون الكلية ,الكليستيرول, الدهون الثلاثية MDA ,ووجود نقص غير معنوى فى , α, β, γ جلوبيولين والجلوبيولين الكلى, الكتاليز, السوبر اكسيد دسميوتيز عند مقارنتها بالكتاكيت بالمجموعة الضابطة.

أظهرت النتائج ان الكتاكيت المصابة بالمكيروب القولونى العصوى وتم علاجها بالدوكسى سيكلين ادى الى عدم ظهور اى اعراض مرضية ولم يحدث اى وفيات ووجود زيادة غير معنوية فى وزن الجسم المكتسب, كرات الدم البيضاء, الخلايا المتعادلة، الخلايا الليمفاوية, الخلايا الملتهمة الكبيرة, الخلايا الحامضية, الخلايا القاعدية, قوه اللتهام والقتل, A/G ratio AST –ALT- ALP حمض اليوريك والكرياتينين الدهون الكلية ,الكليستيرول, الدهون الثلاثية, MDA مصحوب بزيادة معنوية فى البروتين الكلى الزلال ونقص غير معنوى فى α,β,γ جلوبيولين والجلوبيولين الكلى, الكتاليز, السوبر اكسيد دسميوتيز وتحسن فى معدل التحويل الغذائى عند مقارنتها بالكتاكيت بالمجموعة الموجبة. وقد وجد ان الدوكسي سيكلين له بقايا في الأنسجة عند اليوم ٢٠ من العمر واختفى تماما عند اليوم ٣٠ من العمر وكان أعلى تركيز للبقايا في الكلى يليها الكبد واقلها كان فى عضلات الصدر.

من النتائج السابقة يتضح ان البروبيوتك وحمض البربيونك والدوكسي سيكلين لهم دور فى مقاومة الميكروب القولونى العصوى، تحسين معدل النمو وتحسين الوظائف الدموية والبيوكيميائية. لذلك من المستحسن استخدام البروبيوتك وحمض البربيونك طوال مدة التسمين فى كتاكيت التسمين نظرا لقله اثارهم الضارة وتاثيرهم المهم على وزن الجسم ومعامل التحويل الغذائى ومقاومة البكيريا الضارة.