

Effect of Organic Acids and Probiotic On broiler Performance, Some Blood Parameters and Control of E. coli

Allam HH^{*}, Eman S Abd El Hamid^{**}, Halla Salah^{**}, Riham M El-Rashidy^{**}
and Adel E M^{**}

(Poultry^{*}, Biochemistry^{**} and Clinical pathology^{***} department)
Animal Health Research Institute (Zagazig branch)

ABSTRACT

One hundred, one-day old broiler chicks were divided into four equal groups (25 each). The 1st group kept as control, non infected,, chicks in 2nd, 3rd and 4th groups were experimentally infected with E coli (0.3 ml via nasal route of cultural suspension of E. coli O78 contain 3X10⁷viable organism/ml) at 20 day of age. Chicks in 2nd, group infected with E coli only. Chicks in 3rd and 4th groups supplemented with 1ml formic acid, 1ml probiotic/ liter drinking water respectively from 1st to 35th day of age and infected with E coli. At 1st day post supplementation 5 chicks in all groups were weighted individually for calculation weight gain and feed conversion rate. Swabes from cloaca and trachea were collected from all chicks for reisolation of E coli. Study the effect of organic acids and probiotic in mortality rate. Blood samples were collected at 1st &7th day post supplementation for hemato-biochemical analysis

Broiler chicks infected with E coli showed typical clinical signs of colibacelosis and mortality rate 24%. A significant reduction in body weight, lymphocytes, monocyts, eosinophils, basophils, serum total protein, albumin, total, α & γ globulin and a significant rise in feed conversion rate, leukocytic count, heterophils β globulin, AST, ALT, ALP, uric acid and creatinine levels. Also, beside insignificant reduction in phagocytosis, killing%, IgG, IGA and IgM were recorded. E. coli was reisolated from all infected chicks

Chicks infected with E coli and supplemented with organic acid or probiotic show less clinical signs, mortality rate was 4% coupled with reduction in reisolation of E. coli associated with significant elevation in weight gain, phagocytosis, killing %, IgG, IGA, IgM, total protein, albumin, α , β and γ globulin beside significant reduction in feed conversion rate and insignificant increase in leukocytic count, heterophils, lymphocyte, monocyte, eosinophils, basophils, A/G ratio, AST, ALT, ALP, uric acid and creatinine.

It could be concluded that the use of organic acid and probiotic reduced E. coli in digestive tract of broilers and improve body performance, immune response and some biochemical parameters.

INTRODUCTION

Escherichia coli (*E coli*) are one of the main spp of bacteria that normal inhabitants lower intestines of birds and mammals and causing colibacillosis (1). Colibacillosis, the affects poultry industry causing serious economic losses achieved by high mortality and loss of body weight (2).

Organic acids inhibit growth of bacteria (3). It has antimicrobial action in gastrointestinal tract of animal (4) and lowering pathogenic bacteria in intestine (5).

Probiotic is live microbial feed additives that beneficially affect intestinal microbial balance, improved weight gain and feed

conversion rate and reduction of mortality rate in broiler (6), decrease intestinal pathogenic bacteria (7), increase natural defense mechanism of chickens (8), promoting host defense mechanisms and modulating systemic immune system (9). It stimulates immune system cells to produce cytokines, which play a role in the induction immune response (10). Probiotic enhance intestinal health, stimulation of immunity and inhibition of epithelial invasion and production of antimicrobial substance (11).

The present work was conducted to study the effect of organic acids and probiotic on body performance, blood parameters and intestinal *E. coli* colonization in broiler.

MATERIAL AND METHODS

Birds

One hundred, one-day old, broiler chicks (Hubbard local breed) weighting 40.21-40.50 g obtained from a local hatchery were used in this study. Coloacal swabs were taken from all chicks for bacteriological examination to prove that all chicks free from bacterial infection. Chicks were fed a balanced commercial poultry ration. They were kept under hygienic conditions during experimental period.

Experimental design

Chickens were divided into 4 equal groups (25 each). The 1st group (healthy chicks) was used as control group. At 20 day of age, chicks of the 2nd, 3rd and 4th groups were experimentally infected with *E. coli* (0.3 ml via nasal route of cultural suspension of *E. coli* O78 contain 3×10^7 viable organism/ml) (12). The infected chicks in the 3rd and 4th group were supplemented with 1ml organic acid (formic acids)/liter drinking water and 1ml probiotic (sambioegen)/liter drinking water respectively from the 1st to 35th day of age.

E. coli strain

E. coli strain used in this study was isolated from a field case, identified, classified and serotyped as O78 according (13)

Reisolation *E. coli*

Cloacal and tracheal swabs samples from all chicks at 1st day post supplementation for reisolation of *E. coli*. Collected samples were incubated on nutrient broth at 37°C for 24h., then subcultured into nutrient agar according to (14), isolated bacteria were identified (15).

Body weight

Chicken in all groups were weighted individually at start of the experiment and at 1st day post supplementation for calculation weight gain and feed conversion rate.

Sampling and analysis

At 1st and 7th day post supplementation, 5 chicks from each group were sacrificed and two blood samples were collected.

First blood sample was taken in tube contained EDTA as anticoagulant for.

Determination of total and differential leucocytic count (16).

Determination of phagocytic and killing percentage. Blood was used to obtain polymorphonuclear cells (17). Mixtures of *Staph. aureus* and polymorphonuclear cell were incubated at 37°C for 2 hours with regular stirring and then the mixtures were centrifugated for 5 min at 4°C. The supernatant were used to estimate bacteria phago-cytosed %. The mixture of bacteria and polymorphnuclear cell were treated with one cycle of freezing and thawing and the bacteria killed% was estimated (18).

Second blood sample was used for obtain clear serum, for determination of serum total protein (19) and protein fractions were performed using cellulose acetate electrophoresis test (20), IgG, IgM and IgA was performed using Sandwich Elisa (21). Also, serum transaminases (AST and ALT) (22) alkaline phosphatase (ALP) (23) uric acid (24) and creatinine (25) were evaluated.

Statistical analysis

The obtained results were statistically analyzed using (T test) (26).

RESULTS AND DISCUSSION

Our results tabulated in table (1) revealed that a clinical signs of colibacillosis were appeared on chicks experimentally infected with *E. coli* as loss of appetite, ruffled feathers, depression, dropping of the wings, sneezing, frothy exudates in their eyes with conjunctivitis, yellowish watery diarrhea and mortality rate was 24% beside re-isolation of *E. coli* from cloacal and tracheal swabs. Organic acid or probiotic supplemented to broilers induce reduction in clinical signs,

mortality and re-isolation of *E. coli* from cloacal and tracheal swabs. Same observation was recorded (27-28) in broiler chicks infected with *E. coli*. Formic acid treatment induces reduction in clinical signs and absence of mortality in broiler (29). In addition, probiotic reduce intense of clinical signs of colibacellosis and decrease mortality rate in broiler chicks (30). Broiler infected with *E. coli* and supplemented with probiotic and organic acid showed reduction in re-isolation of *E. coli* organisms (31, 32). Probiotic is effective in reducing *E. coli* colonization (33). Antimicrobial activities of organic acids and probiotic may be due to pH reduction and dissociation capacity of their carboxyl groups so gut environment is too acidic and prevent growth pathogenic bacteria (34) and /or due to probiotic increase short-chain fatty acids with lowering pH of intestine and inhibition growth of pathogenic bacteria (35).

Table 1. Effect of organic acid and probiotic on clinical signs, mortality rate and re-isolation of *E. coli*.

Parameters Groups	Total No	Clinical signs		Mortality rate		Re-isolation of <i>E. coli</i>			
		No	%	No	%	Rectal swabs		Tracheal swabs	
						No	%	No	%
Control	25	00	00	00	00	00	00	00	00
E coli	25	23	92	6	24	17	89.47	11	57.89
Organic acid	25	4	16	1	4	3	12	1	4
Probiotic	25	3	12	1	4	2	8	2	8

Broilers infected with *E. coli* show a significant reduction in body weight gain and rise in feed conversion rate, but infected broiler and supplemented with organic acid or probiotic showed a significant increase in body weight gain and reduction in feed conversion rate (table, 2). Our findings were reinforced with previous report (36) in broiler infected with *E. coli*. Reduction in weight gain and raise in feed conversion rate in broiler infected with *E. coli* may be due to lower absorption of nutrient from inflamed intestinal tract (37).

The same improvement in body weight gain were recorded previously (38) in broiler chickens fed organic acid or probiotic. Organic acids or probiotic induce improvement in weight gain and feed conversion due to reduction intestinal pathogenic bacteria (39) beside improvement in bioavailability of nutrients (40). Dietary acidification inhibits of intestinal bacteria and reduction of toxic bacterial metabolites, thus improving weight gain (41).

Table 2. Effect of *E coli* , organic acid and probiotic on broiler performance (N =5 chicks)

Group	Parameters	Gp(1)	Gp(2)	Gp(3)	Gp(4)
	IBW (20 day of age) gm/bird	.21±0.86	40.42±0.79	40.30±0.90	40.55±0.85
	ABW gm/chick	1432.32±3.89	1261.43±2.63***	1470.07±2.95***	1479.16±2.75***
	Absolute body weight gm/chick	1392.11±3.87	1221.01±4.31***	1429.77±4.94***	1438.61±5.45***
	F.C. (gm/bird)	1431.43	1386.38	1405.45	1418.83
	FCR	1.03	1.14	0.98	0.99

*** Significant at P < 0.001 IBW= Initial Body weight consumption FCR = feed conversion rate ABW= Absolute body weight gm/chick F.C. = feed

The present work revealed a significant reduction in lymphocytes, monocytes eosinophils, basophils beside significant leukocytosis and heterophilia in broilers infected with *E coli* but broilers infected with *E coli* and supplemented with organic acid and probiotic showed insignificant leukocytosis heterophilia, lymphocytosis,

monocytosis, eosinophilia and basophilia (table, 3). similar results in leukogram was recorded in broiler infected with *E. coli* (42). Leukocytosis in infected broiler may be due to inflammatory response in intestinal tract (43). Same results were reported in broilers fed organic acid (44). Probiotic and organic acid induce leukocytosis due to lymphocytosis (45).

Table 3. Effect of *E coli* , organic acid and probiotic on leukogram of broiler (N =5 chicks)

Group	Parameter	Total WBCs X103/μl	Differential count (X103/μl)				
			Heterophilis	Lymphocytes	Eosinophils	Basophilis	Monocytes
1 day	Gp(1)	13.08±0.15	4.11±0.16	6.07±0.07	1.40±0.15	1.15±0.08	1.26±0.14
	Gp(2)	13.63±0.16*	5.10±0.33*	5.80±0.09*	0.95±0.05*	0.90±0.06*	0.88±0.03*
	Gp(3)	14.29±0.99	4.15±0.22	6.20±0.31	1.45±0.29	1.18±0.42	1.31±0.32
	Gp(4)	14.22±0.82	4.20±0.18	6.15±0.19	1.41±0.31	1.17±0.19	1.30±0.26
7 day	Gp(1)	14.20±0.15	4.15±0.35	6.21±0.06	1.46±0.12	1.15±0.07	1.23±0.10
	Gp(2)	14.64±0.10*	5.56±0.32*	6.01±0.05*	1.15±0.06*	0.96±0.02*	0.96±0.04*
	Gp(3)	14.24±0.13	4.20±0.10	6.25±0.16	1.55±0.17	1.24±0.10	1.30±0.08
	Gp(4)	14.72±0.25	4.31±0.22	6.27±0.18	1.56±0.20	1.23±0.09	1.25±0.17

* Significant at P < 0.05

Broiler infected with *E coli* showed insignificant reduction in phagocytosis %, killing %, IgG, IGA and IgM. Broilers infected with *E coli* and supplemented with organic acid and probiotic showed significant elevation in phagocytosis %, killing % IgG, IGA and IgM (Table, 4 &5). Nearly similar results were recorded in broiler chickens infected with *E coli* (46) and in mice fed

organic acid (47). Organic acids induce rise immunity response due to its activation of immune cells (48). Probiotic induce significant rise in phagocytosis and killing%, IgG, IGA and IgM (49). Probiotic stimulate immune system due to rise of T cells and serum protein (50). These effects were mediated by cytokines secreted by immune system cells stimulated with probiotic (51).

Table 4. Effect of *E coli*, organic acid and probiotic on immunoglobulin of broiler (N =5 chicks)

Parameters	Group					
	1 day			17day		
	IgG (gm/100ml)	IgM (gm/100ml)	IgA (gm/100ml)	IgG (gm/100ml)	IgM (gm/100ml)	IgA (gm/100ml)
Gp(1)	969.10±4.95	248.05±3.28	76.41±3.18	971.07±5.12	250.21±3.89	78.04±3.55
Gp(2)	960.93±5.64	242.12±4.79	72.29±4.05	967.15±4.21	247.32±3.49	77.41±2.16
Gp(3)	983.55±3.16*	257.11±2.17*	85.25±2.24*	974.31±3.47	254.84±2.54	81.13±1.41
Gp(4)	984.07±3.42*	258.05±2.32*	85.97±2.19*	975.16±3.83	253.38±1.78	80.59±1.62

* Significant at P < 0.05

Table 5. Effect of *E coli*, organic acid and probiotic on phagocytosis and Killing % (N =5 chicks)

Parameters	Group				
		Gp(1)	Gp(2)	Gp(3)	Gp(4)
Phagocytosis	1 day	48.18±1.47	44.31±1.94	54.79±2.36*	55.05±2.41*
	7 day	49.31±1.39	47.42±2.17	52.06±1.72	51.42±1.84
Killing %	1 day	39.42±1.41	37.08±1.21	45.22±1.51*	45.82±1.34*
	7 day	39.65±1.52	38.84±1.72	42.05±1.28	43.15±1.93

* Significant at P < 0.05

Broiler chicks infected with *E coli* showed significant reduction in total protein, albumin, total, α and γ globulin, significant increase in β globulin but broilers infected with *E coli* and supplemented with organic acids and probiotic show significant increase in total protein, albumin, total, α , β and γ globulins (Table, 6). Our results were confirmed by previous findings in broilers infected with *E coli* (52). Reduction in protein profile may be due to liver damage by *E coli* and its toxin (53). Also same results were obtained in

broiler chicks fed organic acid or probiotic (54). Improvement in protein profile was recorded in broiler fed organic acid (55) and in laying hens fed probiotic (56). Increase in protein profile in broiler fed probiotic may be due to improvement in the intestinal environment which leads to an improvement in digestion and absorption of nutrients with increase amino acids and protein (57) and/or due to proteolytic activities of bacillus spp. in probiotic that increase protein digestibility (58).

Table 6. Effect of *E coli*, organic acid and probiotic on protein profile of broiler (N =5 chicks)

Parameter	Group	T.P	Alb	Globulin (Gm/dl)				A/G ratio
				α	β	γ	Total	
1 day	Gp(1)	4.66±0.39	2.78±0.21	0.76±0.02	0.41±0.09	0.71±0.11	1.88±0.15	1.48±0.15
	Gp(2)	3.09±0.38*	1.66±0.37*	0.39±0.15*	0.67±0.08*	0.37±0.06*	1.43±0.11*	1.16±0.20
	Gp(3)	6.44±0.55*	3.91±0.35*	0.83±0.02*	0.61±0.03*	1.06±0.10*	2.50±0.20*	1.56±0.23
	Gp(4)	5.84±0.20*	3.53±0.21*	0.84±0.04*	0.62±0.04*	0.95±0.03*	2.33±0.12*	1.52±0.18
7 day	Gp(1)	4.26±0.14	2.67±0.10	0.69±0.07	0.35±0.05	0.61±0.04	1.61±0.04	1.66±0.16
	Gp(2)	3.82±0.10*	2.36±0.08*	0.47±0.05*	0.51±0.04*	0.48±0.03*	1.48±0.04*	1.48±0.14
	Gp(3)	4.84±0.56	3.65±0.49	0.79±0.12	0.57±0.15	0.73±0.16	2.09±0.24	1.74±0.21
	Gp(4)	4.49±0.45	2.77±0.20	0.66±0.08	0.45±0.09	0.61±0.09	1.72±0.15	1.61±0.27

* Significant at P < 0.05

Broilers infected with *E coli* show a significant increase in serum liver enzymes (AST, ALT and ALP) uric acid and creatinine levels but broilers infected with *E coli* and supplemented with organic acid and probiotic revealed insignificant increase in these previous parameters (Table, 7). Similar results were reported in broilers infected with *E coli* (59). Elevation liver enzymes reflect liver damage induced by infected *E coli* and its toxin these damage

leads leakage of enzymes into the blood stream (60). Increase in uric acid and creatinine in broiler infected with *E coli* may be due to degenerative changes in kidney tubules preventing excretion of uric acid and creatinine (61.). Organic acid and prebiotic induce insignificant rise in serum AST, ALT, ALP, uric acid and creatinine in broilers fed organic acid(54) and in broilers fed periodic(62).

Table 7. Effect of *E coli* , organic acid and probiotic on liver and kidney functions of broiler (N =5 chicks)

Group	Parameter	AST (u/ml)	ALT (U/ml)	ALP (U/L)	Uric acid (mg/dL)	Creatinine (mg/dL)
1 day	Gp(1)	38.48±0.98	28.03±1.05	29.14±1.79	6.23±0.97	1.42±0.27
	Gp(2)	45.03±1.95*	32.68±1.48*	34.29±1.24*	9.03±0.59*	2.49±0.33*
	Gp(3)	40.30±0.95	30.19±1.13	31.05±1.42	6.89±0.89	1.64±0.26
	Gp(4)	42.08±1.18	31.21±1.69	33.14±1.97	6.95±0.61	1.77±0.19
7 day	Gp(1)	38.37±0.64	27.98±0.87	29.59±0.90	6.58±0.48	1.44±0.21
	Gp(2)	44.14±2.13*	32.17±1.30*	32.05±0.40*	7.94±0.21*	2.60±0.40*
	Gp(3)	39.72±1.84	28.38±1.94	30.39±1.87	6.68±0.78	1.58±0.25
	Gp(4)	40.13±1.49	29.23±1.73	31.25±1.23	6.87±0.40	1.69±0.26

* Significant at P < 0.05 UA= Uricacid

It could be concluded that the use of probiotic and organic acid resulted in reduction in *E. coli* microorganisms in digestive tract of broilers, which can help to improve body performance, immune response and some biochemical parameters

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المخلص العربي

تأثير الأحماض العضوية والبروبيوتك على وزن الجسم
بعض مكونات الدم والتحكم في الميكروب القولوني العصوي في كتاكيت التسمين

حسام حسن علام* ، إيمان سعودي عبد الحميد** ، هاله صلاح**

ريهام رضا الرشيدى*** ، عادل السيد مصطفى**

معهد بحوث صحة الحيوان- فرع الزقازيق (أقسام الدواج*، الكيمياء** والباثولوجيا الاكلينيكية***

استخدمت في هذه الدراسة مائة كتكوت تسمين هبرد عمر يوم واحد يتم تقسيم الكتاكيت إلى أربع مجموعات متساوية كلا منها يحتوى على 25 كتكوت، كتاكيت المجموعة الأولى غذيت على عليقه بدون اى اضافات (مجموعه ضابطه) والكتاكيت فى المجموعات الثانية والثالثة والرابعة فى اليوم العشرين من العمر تم اجراء عدوى اصطناعية بالميكروب القولونى O78 بجرعه 1/2 سم⁷10⁷ عن طريق الفم . كتاكيت المجموعه الثانيه مصابه بالميكروب القولونى فقط اما كتاكيت المجموعه الثالثه والرابعه كتاكيت تم إعطائهم حمض الفورميك بجرعة [سم/لتر والبروبيوتك بجرعة [سم/لتر في مياه الشرب على التوالى ابتدا من اليوم الأول وحتى اليوم الخامس والثلاثون من العمر وفى اليوم العشرين من العمر تم اجراء عدوى

اصطناعية بالميكروب القولوني. عند اليوم الاول من نهايه اضافه حمض الفورميك والبروبيوتك تم وزن الكتاكيت فى كل المجموعه لدراسه تاثيرهما على وزن الجسم ومعامل التحويل الغذائى وكذلك تم اخذ مسحات من فتحه المجمع والقصبه الهوائيه لفحصها بكتريولوجيا لمحاولة اعاده عزل الميكروب القولونى وتم أخذ عينات دم من كل المجموعات عند اليوم الأول والسابع من نهاية المعاملات وذلك لدراسة تأثير البروبيوتك والاحماض العضويه على صورته الدم وبعض مكونات الدم.

أوضحت النتائج أن بدارى التسمين المصابه بالميكروب القولونى أدت إلى ظهور اعراض المرض على عدد 23 كتكوت من اجمالى 25 كتكوت بالمجموعه ومعدل الوفيات 24% وتم اعاده عزل الميكروب القولونى من كل الكتاكيت وعلاوة على ذلك وجد نقص معنوى فى وزن الجسم المكتسب, الخلايا الليمفاويه, الخلايا الملتهمه الكبيره, الخلايا الحامضيه, الخلايا القاعديه, البروتين الكلى, الزلال, الجلوبيولين الكلى, الفا وجاما جلوبيولين وزيادة معنويه فى معدل التحويل الغذائى. العدد الكلى لكرات الدم البيضاء, الهيتيروفيل, البيتا جلوبيولين الأسبريتيت امينوترانس راز, الالنين امينوترانسفيراز, الفوسفاتيز القاعدى, حمض اليوريك والكرياتينين مصحوبه بنقص غير معنوى فى قوه اللاتهام والقتل ونسبه IgG, IGA IgM والنسبه بين الزلال والجلوبيولين.

واسفرت النتائج ان الكتاكيت المصابه بالميكروب القولونى وتم امدادها بالاحماض العضويه والبروبيوتك ادى الى تقليل ظهور اعراض المرض ونقص فى نسبة الوفيات بجانب تقليل تركيز الميكروب القولونى فى مسحات المجمع والقصبه الهوائيه ووجود زياده معنويه فى وزن الجسم المكتسب, قوه اللتهام والقتل IgG, IGA IgM, البروتين الكلى, الزلال, الجلوبيولين الكلى, الفا, بيتا جاما جلوبيولين, مصحوبا بنقص معنوى فى معدل التحويل الغذائى وزيادة غير معنويه فى العدد الكلى لكرات الدم البيضاء, الهيتيروفيل, الخلايا اللمفاويه, الخلايا المالتهمه الكبيره, الخلايا القاعديه وفى نسبه الأسبريتيت امينوترانس فيراز, الالنين امينوترانس فيراز, الفوسفاتيز القاعدى, حمض اليوريك والكرياتينين.

وخلاصة هذه الدراسة أن البروبيوتك والاحماض العضويه لهما القدره على تقليل نمو الميكروب القولونى فى الامعاء ولهما تأثير محفز مناعى بالإضافة إلى كونه محفزا لمعظم الوظائف الحيويه فى بدارى التسمين