Clinicopathological Studies on the Effect of Acidifier and Probiotics in Broilers

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Abstract

This study was conducted on 250 Cobb broilers, healthy chicks one-day- old, to evaluate and compare the impact of using water natural additives (such as acidifier and probiotics) on broiler. Complete blood pictures and immune response, as well as histopathological in treated and experimentally infected chickens with *E.coli* compared to control.The birds were randomly allotted to ten treatment groups (A - J). Control, probiotic (HerriC 20%) as 0.5 gm, 1gm / 4 liters of drinking water respectively, acidifier (AniGut) as at dose of 1 and 2 ml/ litre of drinking water respectively and another same treatment groups and infected with *E coli* O78 containing 4 x 10^6 colony-forming units CFU /ml in phosphate buffered saline (PBS) at 21 days of age. The result indicated that, probiotic treated groups 'showed significant increase ($P<0.05$) in RBCs count and PCV value at 4 and 6 weeks, showed non-significant changes in Hb conc., MCV, MCH and MCHC in compare with the control, while acidifier treated groups showed non-significant changes in erythrogram. The leukogram studies revealed that, there was increase in TLC with characteristic heterophilia, lymphocytosis, and monocytosis in infected non treated group and in probiotic treated groups, while acidifier treated groups showed non-significant changes in TLC and D.L.C all over the experiment. The result of immunological parameters showed an elevation in the serum of IgG and IgM in infected group and in probiotic treated groups. While acidifier treated groups showed non-significant changes. The results of IL-6 and IL-12 showed that there were a significant increase in all infected groups and in probiotic treated groups, while acidifier treated groups showed non-significant changes. Histopathological results showed that addition of probiotic improved all the examined organs. The study concluded that Probiotic has marked growth promoter as well as immunomodulatory effects in broilers, the low dose of probiotic (0.5 gm) proved to be more beneficial than the higher dose.

Introduction

The poultry industry has been considered one of the most dynamic and ever expanding sectors in the world; it helps to fill the gap between requirement and availability of high quality protein for human consumption ***(Pervez and Abdul Sajid, 2011)***. Poultry are exposed to pathogenic microorganisms such as *Escherichia coli* and *Salmonella species* in the small intestine of the host which compete with the host for nutrients and reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids, this could result in reduction of growth performance and increases the incidence of disease ***(Engberg et al, 2000).*** Since the proposed total ban on sub-therapeutic feed antibiotics. Therefore, the importance of using alternative growth promoters products such as probiotics, organic acids and prebiotics is receiving considerable attention in animal nutrition because of their non-residual and non-resistant properties ***(Kocher, 2005; Plail, 2006)***.The acidifiers are naturally occurring substances, many of which play an important role in the metabolism ***(Freitag, 2007).*** Acidifier suppress the growth of certain species of bacteria, particularly acid-intolerant species such as *E.coli, Salmonella spp.* and *Campylobacter ssp.* ***(Ricke, 2003; Dibner, 2004 and Lückstadt, 2005).*** Acidified diets containing 1.5 and 3% citric acid had better immune response in chicks indicated by higher serum globulin and relative lymphoid organs than the control ***(Abdel-Fattah et al, 2008)***. Probiotics are new products which are live microbes grow in the gastrointestinal tract and create beneficial conditions for nutrients utilization and inhibit the growth of pathogenic bacteria in the host**.** **(*Amer and Khan, 2012).*** Different strains of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* could be considered as the main microbial species that have been use as probiotics**.** ***(Shahin, 2007 and Ranadheera et al, 2010).*** Probiotic effects on intestinal microflora and pathogen inhibition, intestinal histological changes, immunomodulation, some hemato-biochemical parameters and subsequently improve growth performance of broilers. ***(Kabir, 2009).***This study aimed to investigate the effect of acidifier and probiotic on broiler chicken experimentally infected with *E.coli* from many aspects including Hematological parameters, Some selective cellular and humeral immunological parameters to evaluate the immunomodulatory effect of probiotic and acidifier and hitopathological examination of heart, liver, intestine, spleen, kidney and bursa in both treated and infected chickens.

Materials and Methods

1-Experimental Chicks:

Two hundred and fifty, one day old, Cobb chicks with an average body weight 45-50 gm. were obtained from Ismailia/Misr poultry Company. Chicks were randomly assigned to ten treatment groups each of 25 birds and reared for 6 weeks, the end of experimental period. Feed and water were provided *adlibitum***.** All chickens were vaccinated at 5^{th} days of age with Hitchner, at 14^{th} and 28^{th} days of age with Gumboro. Whereas, at 21^{st} and 31^{st} day of age with Lasota vaccine.

2- Bacterial strain : *Escherichia coli* strain (O78) was kindly obtained from National laboratory for Quality control of Poultry production (NLQP) - Dokki - Giza.

3-Probiotic: Commercial name (HerriC 20%): Lactic acid bacteria $3x10^{12}$C.F.U; Vitamin C 1000 % (200,000mg); Carrier up to1 Kg and include lactobacillus acidophilus DDS-1 .It was used in water in rate of 0.5 and 1 gm / 4 Liter.

4-Acidifier: Commercial name (AniGut): Contained citric acid (35gm), formic acid (70gm), lactic acid (60gm), propionic acid (65gm), phosphoric acid (72gm),benzoic acid (12gm),malic acid (14gm),ascorbic acid (5gm),copper penta sulphate (10gm),sorbitol (60gm) and distilled water up to 1 liter. It was used in water in rate of 1ml and 2ml/liter.

5-Experimental design: Two hundred and fifty, one day old, apparent healthy chicks were classified into ten equal groups; each group was fed for 6 weeks. Group (A): Control group. Group (B) and (C) were fed probiotics with dose 0.5 and 1 gm / 4 liters of drinking water respectively. Group (D) and (E) fed acidifier with dose 1 and 2 ml/liters of drinking water respectively. Groups (F), (G), (H) and (I) were received probiotic and acidifier at dose which mentioned above and infected with *E.coli* (O78) at 21 days of age. Group (J): Only infected with *E.coli* at 21 days of age.

6- Sampling: Five birds from each group were randomly selected, two blood samples were collected from wing vein from each bird, whole blood and serum sample from all experimental groups at 2nd, 4th and 6th weeks. Blood sample used for: complete blood picture (RBCs, Hb, PCV, TLC and differential leukocytic count), serum sample used for estimation of immunological parameters: IgG, IgM, IL6 and IL12. Specimen from liver, intestine, kidney, spleen, pancreas, bursa and heart were used for histopathological studies.

6- Pathogenicity test: Colonies of *E.coli* strain were grown in nutrient broth for 24 hours at 37 ^{o}C and viable number adjusted to 4 x 10^{6} colony-forming units CFU viable organism /ml by phosphate buffered saline (PBS) according to ***Macfaddin (1980).*** Chicken were inoculated with 0.5 ml by intranasal route at 21 days of age according to method described by ***Peighambari et al (2000)***.

7-Haematological Studies: The haematological studies were performed on the whole blood sample within two hours of blood collection. Total erythrocyte count (TEC) was determined by Neubauer Haemocytometer with Natt and Herrick's solution as diluting fluid according to the method described by ***Natt and Herrick (1952)***.Packed cell volume (PCV) was measured by microhaematocrit centrifuge according to ***Coles (1986).*** Hemoglobin(Hb) estimation was performed using the cyanomet-hemoglobin colorimetric method after centrifugation according to ***Zijlstra (1960).***

8-Leukogram studies: Leukocytic counts were performed using an improved Neubaur Haemocytometer and Natt & Herick solution. Total white blood cells and differential leukocyte count were calculated according to standard technique described by ***Jain (1986) and Terry (1988).*** For differential leukocytic count, blood films were made on clean slides, dried on air, fixed with absolute methyl alcohol and stained with Giemsa stain, the percentage and absolute value for each type of white cells were calculated according to ***Feldman et al (2000).***

9-Immunological studies:

A) Antibodies estimation: Detection of immunoglobulin's (IgG and IgM) in serum of chickens was performed by Enzyme Linked Immunosorbent Assay (ELISA) using commercial kits (Biochek B. V., Holland).

B) Cytokines estimation:

- **Interleukin 6 assay**: using GSI Chicken IL6 ELISA Kit**.**
- **Interleukin 12 assay**: using GSI Chicken IL-12 ELISA Kit.

10-Pathological Studies: Birds were slaughtered and necropsied. Representative tissue samples from liver, spleen, bursa of Fabricious, pancreas, heart, intestine and kidney were collected in 10% buffered formal saline for histopathological examination, until further processing. Specimen were cut into 5-mm thickness sections and put into tissue cassettes, they were dehydrated by transferring through a series of alcohols with increasing concentrations, cleared in xylol and embedded in paraffin. A 6µm sections were obtained by using rotator microtome, the obtained sections were stained with hematoxline and eosin (H&E) ***Bancroft et al (1996)***.

11-Statistical analysis: Data collected from hematological and serum biochemical analysis of treated groups of chicks were statically analyzed in compare to control group for the mean and standard error using **SPSS 16** ***(Coakes et al, 2009).*** Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests according to ***Snedecor and Cochran (1989)***.

Results

1- Hematological parameters: (Table 1, 2, 3, 4, 5 and 6)

Normocytic normochromic anemia in the infected non treated group. The group treated with probiotic showed significant increase in RBCs count and PCV value at 4 and 6 weeks, showed non- significant changes in Hb conc., MCV, MCH and MCHC in compare with the control, while groups treated with acidifier showed non- significant changes in erythrogram.

There was increase in TLC with characteristic heterophilia, lymphocytosis, and monocytosis in infected non treated group and in groups treated with probiotic, while groups treated with acidifier showed non -significant changes in TLC and D.L.C all over the experiment.

2- Immunological study: (Table 7)

An elevation in the serum of IgG and IgM in infected group and in probiotic treated groups. While acidifier treated groups showed non -significant changes.

The results of IL-6 and IL-12 showed that there was significant increase in all infected groups and probiotic treated groups, while acidifier treated groups showed non- significant changes.

Discussion

In modern poultry production, different types of growth promoters are used. The public concern about resistant pathogenic bacteria in humans leads to increasing pressure by the consumer to a reduction or a ban on the use of nutritive antibiotics ***(Awaad and Zouelfeker, 2001).*** Since the proposed total ban on sub-therapeutic feed antibiotics, products such as probiotics, organic acids and probiotics are receiving considerable attention in animal nutrition because of their non-residual and non-resistant properties ***(Plail, 2006).***

The erythrogram results, the infected non treated group (J) showed normocytic normochromic anemia. This result agreed with ***Marcel (1994)*** who reported that experimental infection of chickens with *E.coli* (O78) induced normocytic normochromic anemia, this may be attributed to suppression of the bone marrow's ability to manufacture more blood cells due to bacterial endotoxins ***(Feldman et al, 2000).*** On the other hand, probiotic treated groups (B and C) showed a significant increase ($P< 0.05$) in RBCs count and PCV value at 4 and 6 weeks in comparing with the control, this improvement in erythrogram could be attributed to the hepatostimulatory and hepatoprotective effect of probiotic leading to production of more RBCs by the bone marrow under control of erythropoietic factors released by hepatic cells ***(Sarma et al, 2003).*** The most likely explanations are improved of bioavailability of essential nutrients and enhancing vitamin B absorption resulted from increased small intestinal absorption

(Jenkin et al, 1999). The hemoglobin concentration (Hb g/dl) showed non-significant changes in groups treated with probiotic. The same finding was obtained by ***Alkhalfa et al (2010a)*** who explained that, administration of probiotic (Bactocell® , lactic acid bacteria strain Pedicoccus acidilactici) at different concentration (1.6 g ,0.8 g and 1.0 g per kg of feed) resulted in no significant changes in the Hb concentrations in broiler chickens at 7, 28, 42 days of age. Concerning, data related to Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC), showed non-significant changes in probiotic treated groups in comparing with the control. The same results obtained by ***Abd El-Rahman et al (2012)*** who showed no significant changes in the value of MCV and MCHC after Bactocell or Revitilyte-plusTM (probiotic of a mixture of Lactobacillus acidophilus, Lactobacillus casei and Enterococcus faecium) supplementation. Also, acidifier treated groups showed non-significant changes in their erythrogram in comparison with the control. Similar results have been reported by ***Baruah et al (2009)***.

Avian leukocytes act as the first line of defense against invading microorganism ***(Powell, 1987),*** the results of leukogram study showed that, the infected non treated group (J) was significantly increased ($P< 0.05$) T.L.C at 4^{th} and 6^{th} weeks of age with characteristic heterophilia, lymphocytosis, and monocytosis. This result completely agreed with ***Barry (1998)*** who reported that **,**leukocytosis with a primary heterophilia is consistent with the hematologic response to *Escherichia coli* airsacculitis in chickens , acute staphylococcal infection ,and coccidiosis in chickens. Also the result goes with accordance with ***Manimaran et al (2003)***; ***Hanan (2002)*** and ***Fatma (2005)***. Where, ingested microbes are taken up in the Peyer's patches which serve as inductive sites for mucosal immune responses. The lymphoid nodules in the wall of the small intestine which containing macrophages and other antigen presenting cells, B cells and T cells are usually, invasive, so, pathogenic microbes are the best inducers of immunity, which is probably due to their superior capacity to multiply in the intestine and penetrate across the mucosal barrier, a prerequisite in order to stimulate the immune system ***(Agnes, 2001).*** On the other hand probiotic treated groups, showed a significant ($P< 0.05$) increase in T.L.C (Leukocytosis) at 4 and 6 weeks of age compared with the control group. This Leukocytosis could be attributed to the stimulatory effect of probiotic for bone marrow to produce more leukocytes ***(Gheith et al, 2011).*** On contrary, although, ***Capcarova et al***

(2008 and 2010) reported non-significant decrease in TLC (leukopenia) in the probiotic - treated group as compared with the control. This difference of obtained results may refer to; the authors apply their experiment on different bird species (turkey), age (layers) with another challenged bacterial stain *Enterococcus faecium.* Regarding, probiotic treated groups showed a significant increase in heterrophil % at 6 weeks and slight increase at 4^{th} weeks of age compared with control one. Nearly, same finding was obtained by ***Talebi et al (2008).*** While, ***Riad et al (2010)*** reported non-significant increase in heterophilic counts after probiotic supplementation. Lymphocytes are the bulky leukocyte in the peripheral blood of most normal chicken that play a major role in the humeral and cell mediated immunity of bird, therefore, lymphocytosis is suggestive of immunogenic stimulation ***(Thrall, 2004).*** Lymphocytosis was observed in chickens treated with probiotic. This result coincided with the finding obtained by ***Khaksar et al (2012).*** Chickens received probiotic showed monocytosis at 4 weeks of age. Our results were in agreement with ***Khajepour et al (2011).*** Worthwhile, ***Abd El-Rahman et al (2012)*** mentioned that, monocytes count nearly not affected when diets supplemented with probiotic. Also the result explained that, acidifier treated groups showed non-significant changes in TLC and D.L.C all over the experiment period. These results were similar to ***Tollba (2010)*** who stated that citric acid as acidifier, did not affect the leukocytic count and their differential counts in broilers till 40 days. Moreover, ***Mahdavi and Torki (2009)*** noted that dietary, inclusion of butyric acid didn't affect the counts of lymphocytes, heterophils, monocytes, basophils and eosinophils at days 21, 42 and 49 of broilers life.

Concerning the obtained data for the immunological analysis from our study, an elevation in the serum of IgG and IgM in infected group and in probiotic treated groups when compared to the control. Similary, administration of probiotic bacteria in chickens was shown to enhance specific, systemic antibody response and to stimulate the production of natural antibodies such as serum IgG and IgM ***(Haghighi et al, 2006).*** Previous studies have indicated that the modulation of innate and adaptive immunity by probiotics is a dose and strain- dependent phenomenon ***(Alberda et al, 2007).*** The same findings obtained by ***Khaksar et al (2012).*** On the other hand, ***Mountzouris et al (2010)*** found that concentration of IgM and IgG didn't differ between probiotic supplemented chicken and non-supplemented groups. While in groups treated with acidifier showed non-significant changes in

comparing with control group, this result is the same obtained by ***Rosyidah et al (2011)*** who reported that the addition of either *L.Plantarum* or acidifier to the diet did not influence the immunoglobulin status of the chicken.

Interleukins or cytokines are small proteins which allow the cells of the immune system to communicate with one another via receptors expressed at the cell surface, come from hematopiotic and non hematopiotic cells. Interleukin 6 is B-cell stimulatory factor-2 and interferon beta-2, a cytokine involved in a wide variety of biological functions. It plays an important role in the final differentiation of B cells into immunoglobulin's-secreting cells, T-cells, as well as inducing myeloma plasmacytoma growth, nerve cell differentiation, and in hepatocytes, acute- phase reactants ***(Frans and Michael, 2005).*** Since T-cells are the major source of cytokines, the ability of these cells to proliferate in response to their mitogens has been used to determine the development of the immune responses of chickens ***(Lowenthal et al, 1994).*** The present results showed that there were significant increased ($P \leq 0.05$) in IL6 levels in infected groups and in probiotic treated groups compared with control group, this result agreed with that of ***Rajput et al (2013)*** who found that Intestinal cytokines interleukin-6 was improved in the probiotic receiving groups. The results of ***Huang et al (2012)*** are in agreement that inflammatory cytokine concentration increased and this might vary from probiotic species to species. Another study reported that, invasion of *Salmonella typhi* into human or murine epithelial cells resulted in the production of high levels of IL-6 ***(Weinstein et al, 1997)***. Initially, probiotic interacts with commensal bacterial and mucosal epithelial cells of the small intestine and the modulation in intestinal epithelial cells secreting IL-6 might be predicted ***(Deplancke and Gaskins, 2001)***. In contrast, findings of ***Hong et al (2006)*** supported the data, demonstrating that *Salmonella enteritidis* (an infection) has little effect on IL-6 cytokine production and downregulates IL-6 mRNA expression. In contrast ***Haghighi et al (2008)*** reported that, there was no significant difference in Il-6 gene expression in cecal tonsils of chicken belonging to various treatment groups with probiotic. Acidifiers treated groups showed non-significant changes in IL-6. This result agrees with ***Ao et al (2012)*** who stated that no effect on IL-6 production in birds given acidifier diets. Meanwhile, ***Abdalla et al (2013)*** mentioned that, supplementation of 0.2% benzoic acid recorded a significant decrease in interleukin 6 (IL 6) at 6 weeks when compared with the control group. Also ***Haque et al (2010)***

stated that, the lymphocyte cells associated with immunity in the lymphoid organs (caecal tonsil, bursa Fabricius and ileum) of broilers were more densely populated, suggesting an increased level of innate immunity in the o.5% citric acid group. Interleukin-12 (IL-12) is a heterodimeric cytokine produced mostly by phagocytic cells in response to bacteria, bacterial products, and intracellular parasites, and to some degree by B lymphocytes. IL-12 induces cytokine production, primarily of IFN-gamma, from NK and T cells, acts as a growth factor for activated NK and T cells, enhances the cytotoxic activity of NK cells, and favors cytotoxic T lymphocyte generation ***(Trinchieri, 1995).*** Also ***Hamza et al (2010)*** reported that**,** Interleukin-12 holds considerable promise as an immunotherapeutic agent because it plays a central role in regulating innate and adaptive immune responses, and synergizes with several other cytokines for increased immunoregulatory activities. Animal and human studies have shown improved outcomes in treating or preventing infections based on the mechanisms of IL-12-dependent therapies. Concerning, the results, showed a significant increase (P≤ 0.05) of Interleukin-12 (IL-12) production in *E.coli* infected groups. This result confirmed by ***Hamza et al (2010)*** who reviewed that, the Interleukin-12 is a Key Immunoregulatory Cytokine in Infection; IL-12 has potential clinical uses in treating and preventing bacterial infections. Produced mainly by antigen-presenting cells during infection and regulates innate responses and determines the type of adaptive immune responses. Also the result indicated that , probiotic treated groups showed showed a significant increase (P≤ 0.05) in IL-12 production, this result agree with ***Jennifer et al (2010)*** who demonstrated that live lactobacilli commonly used as probiotic and isolated from gastrointestinal tract induced expression of IL-12 in chicken mononuclear cells cultured in vitro. Meanwhile, acidifier treated groups showed non-significant changes.

Regarding histopathology, probiotic treated groups only showed normal organ architecture of liver and heart.Normal healthy long intestinal villi and normal kidney architecture mild hyperplasia of lymphoid follicles in bursa and spleen. This result came in agreement with ***Metwali et al (2006)*** who found that chicks fed commercial feed and supplemented with drinking water containing 0.5gm/L of lactobacilli preparation (AVI-BAC) till the end of experiment showed no defined lesions in the heart, liver and lung. Infected non treated group showed severe perihepatitis, Pericarditis which extended to the parts of the myocardium resulting in

myocarditis. Destruction and shortening of intestinal villi. Moderate to severe changes in kidney include congestion and focal non suppurative interestial nephritis. Spleen and bursa showed sever congestion and lymphoid deplestion. Changes recorded in the liver, intestine and heart in the present study are consistant with that of ***Manimaran et al (2003)***. Also there was lymphoid depletion in bursa and spleen, this result came in agreement with ***Nakamura et al (1990)*** who mentioned that *E. coli* infection induce damage in the immune systems of the chickens including lymphocyte depletion in both bursa and thymus. Groups that infected and treated showed mild to moderate perihepatitis and mild to moderate pericarditis. Destruction and shortening of intestinal villi. Mild to moderate changes in kidney include focal non suppurative interestial nephritis. Spleen and bursa showed mild to moderate congestion with depletion of lymphoid follicles. This result came in agreement with ***Metwali et al (2006)*** who found that when lactobacilli was used as a treatment after the *E.Coli* infection, lesions were more pronounced showed more severe lesions either in the heart or in the liver and lung which appeared as pericarditis and coagulative necrosis in the liver.

Table (1): *Effect of Acidifier and Probiotics on Hematological parameters at 2weeks age.*

Group	Hematological Parameters					
	RBCs 10^6/µl	Hb g/dl	PCV %	MCV fl	MCH Pg	MCHC %
A (control)	2.74 ± 0.42[a]	8.19 ±0.49[a]	27.33 ±1.45[a]	102.96 ±10.09[a]	31.02 ±3.77[a]	30.03 ±1.71[a]
B (probiotic 0.5g)	2.53 ±0.21[a]	8.59 ±0.30[a]	27.33 ±1.86[a]	108.78 ±5.83[a]	34.30 ±1.95[a]	31.55 ±1.06[a]
C (probiotic 1g)	2.35 ±0.23[a]	7.85 ±0.17[a]	28.00 ±2.00[a]	121.48 ±14.78[a]	31.23 ±3.91[a]	28.25 ±1.66[a]
D (acidifier 1ml)	2.60 ±0.12[a]	8.57 ±0.23[a]	28.33 ±0.67[a]	109.45 ±5.74[a]	36.77 ±3.01[a]	30.24 ±0.51[a]
E (acidifier 2ml)	2.49 ±0.21[a]	8.29 ±0.34 [a]	28.67 ±0.33[a]	117.01 ±11.14[a]	34.00 ±4.26[a]	28.89 ±0.88[a]

Values are expressed as mean (Mean± SE).
Means with the same letter in the same column are non-significant at P<0.05

Table (2): *Effect of Acidifier and Probiotics on Hematological parameters at 4weeks age of chickens experimentally infected with E. coli.*

Groups	Hematological parameters					
	RBCs 10^6/µl	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %
A (control)	2.12 ±0.01^{c}	7.16 ±0.10^{ab}	28.00 ±0.58^{b}	131.89 ±3.21^{a}	33.74 ±0.49^{a}	25.62 ±0.90^{a}
B (probiotic 0.5g)	2.40 ±0.06 ^{a}	7.81 ±0.12^{a}	31.00 ±0.58^{a}	129.38 ±4.88^{a}	32.56 ±0.30^{a}	25.22 ±0.74^{a}
C (probiotic 1g)	2.34 ±0.02^{ab}	7.73 ±0.44^{a}	30.67 ±0.33^{a}	130.50 ±0.79 ^{a}	32.93 ±2.19^{a}	25.22 ±1.56^{a}
D (acidifier 1ml)	2.20 ±0.06^{bc}	7.44 ±0.24^{a}	29.00 ±0.58 ^{b}	131.93 ±3.05^{a}	33.87 ±1.67^{a}	25.69 ±1.32^{a}
E (acidifier 2ml)	2.19 ±0.10^{bc}	7.80 ±0.15^{a}	28.33 ±0.88^{b}	129.80 ±2.60^{a}	35.85 ±2.08^{a}	27.58 ±1.03^{a}
F (probiotic0.5g+*E.coli*)	1.78 ±0.04^{d}	6.60 ±0.21^{b}	25.00 ±0.58^{c}	140.20 ±0.68^{a}	37.02 ±1.02^{a}	26.40 ±0.60^{a}
G (probiotic 1g+*E.coli*)	1.83 ±0.01^{d}	6.74 ±0.12^{b}	25.00 ±0.58^{c}	139.42 ±3.05^{a}	36.91 ±0.46^{a}	26.98 ±0.23^{a}
H (acidifier1ml+*E.coli*)	1.80 ±0.06^{d}	6.63 ±0.18^{b}	25.00 ±0.58^{c}	138.84 ±4.19^{a}	36.84 ±1.54^{a}	26.58 ±1.32^{a}
I (acidifier2ml+*E.coli*)	1.80 ±0.03^{d}	6.52 ±0.14^{b}	24.67 ±0.33^{c}	137.16 ±3.89^{a}	36.64 ±1.06^{a}	26.72 ±0.36 ^{a}
J (*E.coli*)	1.78 ±0.03^{d}	6.54 ±0.03^{b}	24.67 ±0.33^{c}	138.70 ±3.89^{a}	36.19 ±0.92^{a}	26.10 ±0.08^{a}

Values are expressed as mean (Mean± SE).
Means with the same letter in the same column are non-significant at P<0.05.

Table (3): Effect of Acidifier and Probiotics on Hematological parameters at 6weeks age of chickens experimentally infected with *E.coli*.

Group	RBCs 10^6/µl	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %
A (control)	2.23 ±0.03^{bc}	8.54 ±0.23^{a}	26.67 ±0.33^{bc}	119.50 ±3.23^{a}	38.21 ±0.48^{a}	32.04 ±1.29^{a}
B (probiotic 0.5g)	2.65 ±0.13^{a}	8.62 ±0.24^{a}	30.33 ±1.86^{a}	114.38 ±2.71^{a}	32.63 ±1.22^{a}	28.55 ±1.06 ^{a}
C (probiotic 1g)	2.64 ±0.08^{a}	8.59 ±0.15^{a}	30.00 ±1.15^{b}	114.12 ±6.97^{a}	32.62 ±0.69^{a}	28.72 ±1.08^{a}
D (acidifier 1ml)	2.39 ±0.20^{ab}	8.24 ±0.52^{a}	29.33 ±0.88^{ab}	124.07 ±9.18^{a}	34.77 ±2.64^{a}	28.04 ±1.60 ^{a}
E (acidifier 2ml)	2.45 ±0.16^{ab}	8.66 ±0.50^{a}	29.33 ±0.33^{ab}	120.87 ±7.45^{a}	35.91 ±3.99^{a}	29.58 ±2.01 ^{a}
F (probiotic 0.5g+*E.coli*)	2.10 ±0.19^{bc}	7.10 ±0.50^{a}	26.33 ±0.88^{bc}	127.19 ±10.81^{a}	34.50 ±4.63^{a}	27.15 ±2.78 ^{a}
G (probiotic 1g+*E.coli*)	2.19 ±0.09^{bc}	7.20 ±0.15^{a}	26.33 ±0.88^{bc}	120.68 ±6.29^{a}	33.05 ±2.23^{a}	27.39 ±1.06 ^{a}
H (acidifier 1ml+ *E.coli*)	2.13 ±0.09^{bc}	7.16 ±0.17^{a}	26.33 ±0.33^{bc}	123.95 ±6.34^{a}	33.68 ±1.64^{a}	27.21 ±0.88 ^{a}
I (acidifier 2ml +*E.coli*)	2.14 ±0.07^{bc}	7.66 ±0.67^{a}	26.67 ±0.88^{bc}	124.80 ±0.39^{a}	35.75 ±2.21^{a}	28.65 ±1.83 ^{a}
J (*E.coli*)	1.98 ±0.02^{c}	7.22 ±0.85^{a}	25.00 ±0.58^{c}	126.45 ±1.86^{a}	36.59 ±4.66^{a}	29.02 ±4.02 ^{a}

Values are expressed as mean (Mean± SE).
Means with the same letter in the same column are non-significant at P<0.05

Table (4): *Effect of Acidifier and Probiotics on leukogram parameters at 2weeks age.*

Groups	TLC $10^3/\mu l$	Heterophils $10^3/\mu l$	Lymphocyte $10^3/\mu l$	Monocyte $10^3/\mu l$	Eosinophils $10^3/\mu l$	Basophils $10^3/\mu l$
A (control)	58.64 ±1.32 ^{a}	18.46 ±2.16 ^{a}	31.09 ±2.74^{ab}	6.53 ±1.36^{ab}	2.36 ±1.53^{a}	0.20 ±0.20^{a}
B (probiotic0.5g)	51.33 ±2.40 ^{a}	15.39 ±0.98 ^{a}	28.24 ±1.37^{b}	2.69 ±1.82 ^{b}	4.84 ±1.51^{a}	0.17 ±0.17^{a}
C (probiotic 1g)	53.33 ±5.70 ^{a}	16.27 ±3.03 ^{a}	28.25 ±2.49^{b}	4.21 ±1.71^{ab}	4.61 ±1.93^{a}	0.00 ±0.00^{a}
D (acidifier 1ml)	60.00 ±2.89 ^{a}	19.30 ±2.01 ^{a}	27.80 ±2.00^{b}	8.70 ±1.60 ^{a}	4.20 ±0.62^{a}	0.00 ±0.00^{a}
E (acidifier 2ml)	63.67 ±4.10 ^{a}	13.35 ±1.89 ^{a}	37.83 ±3.51^{a}	9.36 ±1.28^{ab}	3.12 ±1.61^{a}	0.00 ±0.00^{a}

Values are expressed as mean (Mean± SE).
Means with the same letter in the same column are non-significant at P<0.05

Table (5): *Effect of Acidifier and Probiotics on leukogram parameters at 4weeks age of chickens experimentally infected with E.coli.*

time / Group	TLC $10^3/\mu l$	Heterophs $10^3/\mu l$	Lymphocyte $10^3/\mu l$	Monocyte $10^3/\mu l$	Eosinophils $10^3/\mu l$	Basophils $10^3/\mu l$
A (control)	55.33 ±0.88^{d}	22.12 ±0.69^{b}	28.66 ±0.59 ^{d}	2.66 ±0.32^{d}	1.89 ±0.35 ^{a}	0.00 ±0.00^{a}
B (probiotic0.5g)	71.00 ±3.79^{bc}	23.52 ±0.28^{ab}	39.31 ±2.32^{bc}	5.97 ±1.04^{abc}	2.20 ±0.85 ^{a}	0.00 ±0.00^{a}
C (probiotic 1g)	70.00 ±2.89^{bc}	23.17 ±1.86^{ab}	39.07 ±0.07^{bc}	5.65 ±0.93^{abc}	2.12 ±0.47 ^{a}	0.00 ±0.00^{a}
D (acidifier 1ml)	66.17 ±3.94^{cd}	26.00 ±1.15^{ab}	33.67 ±2.52^{cd}	4.52 ±0.17^{bcd}	1.98 ±0.46 ^{a}	0.00 ±0.00^{a}
E (acidifier 2ml)	64.00 ±2.76^{cd}	25.07 ±1.33^{ab}	33.40 ±2.43^{cd}	3.76 ±1.03^{cd}	1.77 ±0.51 ^{a}	0.00 ±0.00^{a}
F (probiotic0.5g+*E.coli*)	84.33 ±6.17^{a}	27.98 ±5.24^{ab}	47.90 ±0.78 ^{a}	7.29 ±0.66^{a}	1.16 ±0.38 ^{a}	0.00 ±0.00^{a}
G (probiotic 1g+*E.coli*)	80.33 ±4.91^{ab}	27.23 ±2.71^{ab}	46.80 ±3.67 ^{a}	5.52 ±0.97^{abc}	0.78 ±0.06 ^{a}	0.00 ±0.00^{a}
H (acidifier 1ml+*E.coli*)	80.49 ±2.83^{ab}	27.09 ±1.63^{ab}	45.43 ±2.08^{ab}	7.17 ±0.52^{a}	0.80 ±0.02 ^{a}	0.00 ±0.00^{a}
I (acidifier 2ml+*E.coli*)	84.00 ±4.16^{a}	28.62 ±4.75^{ab}	47.23 ±1.27 ^{a}	6.72 ±0.33^{ab}	1.43 ±0.33 ^{a}	0.00 ±0.00^{a}
J (*E.coli*)	84.67 ±2.91^{a}	31.38 ±2.51 ^{a}	45.97 ±2.18 ^{a}	6.47 ±0.13^{ab}	0.85 ±0.03 ^{a}	0.00 ±0.00^{a}

Values are expressed as mean (Mean± SE).
Means with the same letter in the same column are non-significant at P<0.05

Table (6): *Effect of Acidifier and Probiotics on leukogram parameters at 6weeks age of chickens experimentally infected with E.coli.*

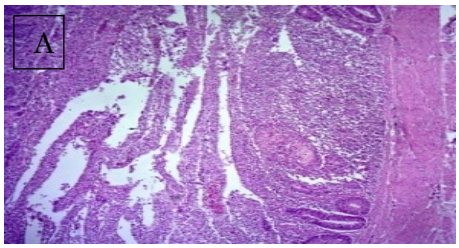
	TLC $10^3/\mu l$	Heterophils $10^3/\mu l$	Lymphocye $10^3/\mu l$	Monocyte $10^3/\mu l$	Eosinophils $10^3/\mu l$	Basophils $10^3/\mu l$
A (control)	55.33 ±4.37^{b}	14.87 ±0.94^{b}	26.97 ±2.53^{b}	8.79 ±0.76^{ab}	4.70 ±1.15^{a}	0.00 ±0.00^{a}
B (probiotic0.5g)	76.33 ±1.86^{a}	25.48 ±1.72^{a}	34.35 ±0.83^{a}	11.45 ±0.28^{a}	5.05 ±1.18^{a}	0.00 ±0.00^{a}
C (probiotic 1g)	72.12 ±4.86^{a}	22.93 ±1.68^{a}	33.35 ±0.96^{a}	10.34 ±2.31^{ab}	5.22 ±1.57^{a}	0.27 ±0.27^{a}
D (acidifier 1ml)	49.83 ±3.44^{b}	14.94 ±1.38^{b}	26.78 ±3.07^{b}	5.59 ±1.05^{b}	2.52 ±0.52^{a}	0.00 ±0.00^{a}
E (acidifier 2ml)	50.13 ±2.65^{b}	13.75 ±0.14^{b}	24.92 ±1.16^{b}	8.00 ±1.89^{ab}	3.47 ±1.35^{a}	0.00 ±0.00^{a}
F (probiotic0.5g+*E.coli*)	80.00 ±4.62^{a}	26.80 ±2.72^{a}	37.84 ±0.44^{a}	9.76 ±2.17^{ab}	5.60 ±0.32^{a}	0.00 ±0.00^{a}
G (probiotic 1g+*E.coli*)	78.58 ±1.80^{a}	28.17 ±3.32^{a}	34.58 ±0.71^{a}	10.05 ±0.81^{ab}	5.78 ±0.94^{a}	0.00 ±0.00^{a}
H (acidifier 1ml+ *E.coli*)	81.00 ±6.66^{a}	28.24 ±3.24^{a}	39.00 ±2.57^{a}	8.42 ±1.22^{ab}	5.35 ±1.10^{a}	0.00 ±0.00^{a}
I (acidifier 2ml +*E.coli*)	84.07 ±3.72^{a}	31.03 ±2.97^{a}	37.38 ±1.61^{a}	11.15 ±1.12^{a}	4.51 ±1.15^{a}	0.00 ±0.00^{a}
J (*E.coli*)	84.67 ±8.51^{a}	31.23 ±4.22^{a}	39.96 ±3.74^{a}	8.79 ±1.09^{ab}	4.69 ±0.09^{a}	0.00 ±0.00^{a}

Values are expressed as mean (Mean± SE).
Means with the same letter in the same column are non-significant at P<0.05

Table (7): *Effect of Acidifier and Probiotics on Interleukins and antibodies at 6weeks age of chickens experimentally infected with E.coli.*

Group	IgG mg/ml	IgM mg/ml	IL6 pg/ml	IL12 pg/ml
A (control)	3.37±0.19^{c}	1.10±0.06^{c}	240.00±5.77^{d}	38.93±3.33^{d}
B (probiotic 0.5g)	4.15±0.01^{ab}	1.87±0.09^{b}	292.67±4.67^{c}	54.87±3.55^{c}
C (probiotic 1g)	4.18±0.16^{ab}	1.94±0.03^{b}	294.67±3.93^{c}	52.80±3.65^{c}
D (acidifier 1ml)	3.68±0.06^{bc}	1.07±0.09^{c}	249.33±5.21^{d}	35.40±0.58^{d}
E (acidifier 2ml)	3.69±0.06^{bc}	1.13±0.09^{c}	256.00±1.15^{d}	34.67±1.76^{d}
F (probiotic 0.5g+*E.coli*)	4.30±0.25^{a}	2.20±0.11^{ab}	323.67±8.57^{b}	55.00±0.00^{c}
G (probiotic 1g+*E.coli*)	4.46±0.23^{a}	2.47±0.26^{ab}	326.00±3.05^{b}	77.20±3.55^{b}
H(acidifier 1ml+ *E.coli*)	4.39±0.31^{a}	2.77±0.50^{a}	348.67±6.96^{a}	90.10±0.76^{a}
I (acidifier 2ml +*E.coli*)	4.29±0.16^{a}	1.97±0.26^{b}	340.00±5.77^{ab}	78.30±2.54^{b}
J (*E.coli*)	4.35±0.20^{a}	2.87±0.09^{a}	350.00±5.77^{a}	94.00±2.46^{a}

Values are expressed as mean (Mean± SE).
Means with the same letter in the same column are non-significant at P<0.05



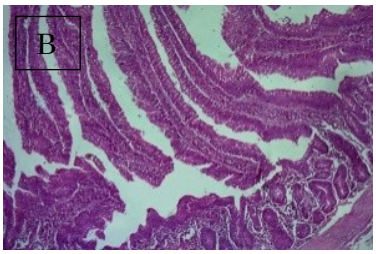
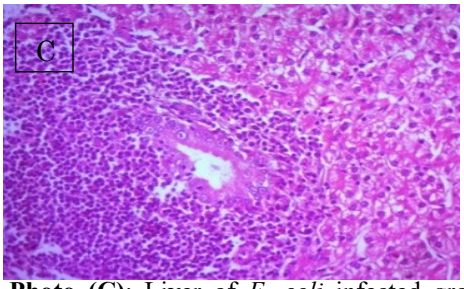


Photo (A): Duodenum of *E. coli* infected group, showing destruction of villi, loss and necrosis of duodenal glands along with severe leukocytic infiltration. H&E. X 200.
Photo (B): Duodenum of probiotic treated group (VII) showing long healthy villi, normal glandular and intestinal epithelium have numerous goblet cells. H&E. X 200.



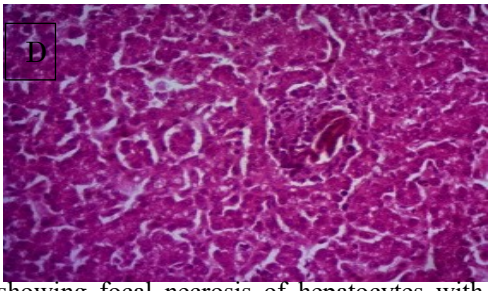
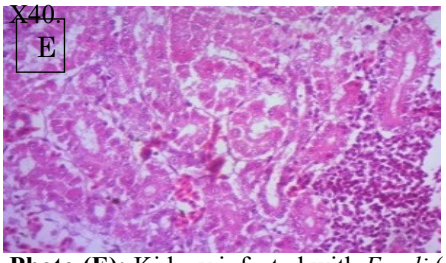


Photo (C): Liver of *E. coli* infected group, showing focal necrosis of hepatocytes with replacement of hepatic areas with massive aggregation of lymphocytes. H&E. X400.
Photo (D): Liver of probiotic treated group (VIII) showing normal polyhedral hepatocytes, normal arrangement of hepatic cords and mild small focal lymphocytic aggregation. H&E. X40.



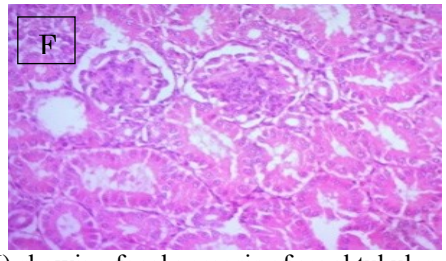
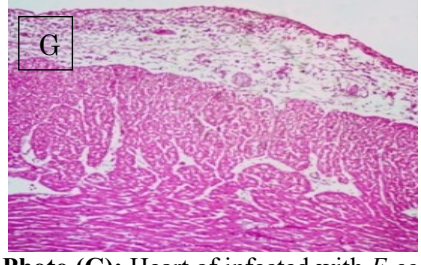


Photo (E): Kidney infected with *E.coli* (group VI) showing focal necrosis of renal tubules with massive aggregation of lymphocytes. H&E. X400.
Photo (F): Kidney of probiotic treated group (VIII) showing mild degeneration of tubular epithelium. H&E. X400



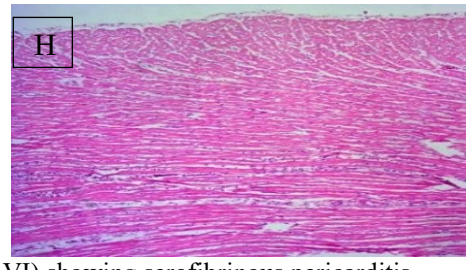
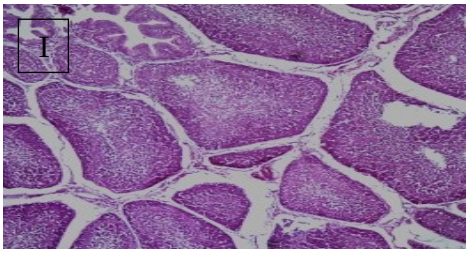


Photo (G): Heart of infected with *E.coli* (group VI) showing serofibrinous pericarditis. H&E. X400
Photo (H): Heart of probiotic treated group (VIII) showing fairly normal myocardium with mild vacuolation. H&E. X400



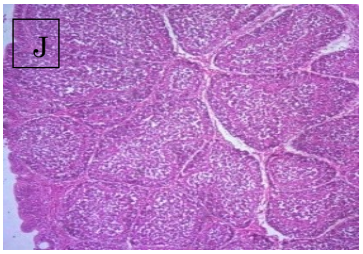
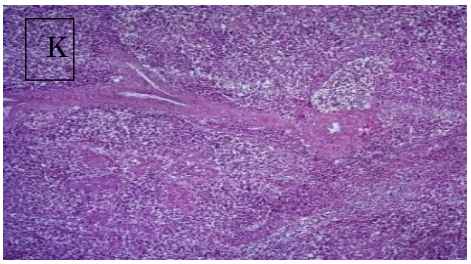


Photo (I): Bursa of *E .coli* infected group interfollicular edema, depletion and necrosis of lymphocytes. H&E. X200.
Photo (J): Bursa of probiotic group (VIII) showing hyperplasia of lymphoid follicles. H&E. X200.



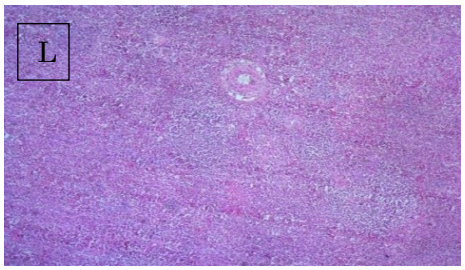


Photo (K): Spleen of *E. coli* infected group, showing mild to moderate depletion of lymphoid follicles with necrotic changes of lymphocytes. H&E. X200.
Photo (L): Spleen of probiotic group (X), showing lymphoid follicles hyperplasia of white pulp and normal red pulp. H&E. X200.

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الملخص العربي

دراسات باثولوجية إكلينيكية على تأثير المحمضات والمحفزات الحيوية في بداري التسمين

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أجريت هذه الدراسة علي عدد ٢٥٠ كتكوت تسمين كب عمر وقد تم تقسيم الكتاكيت عشوائيا فى عمر يوم الى عشر مجموعات المجموعة الأولى :المجموعة الضابطة، المجموعة الثانية والثالثة: وفيها تم إضافة البروبيوتك (هارى سى ٢٠%) في ماء الشرب بمعدل ٠,٥، ١ جم لكل ٤ لتر ماء بالترتيب. المجموعة الرابعة والخامسة : تم إضافة المحمضات (انى جت) بمعدل ١، ٢ ملليتر/ لتر فى ماء الشرب بالترتيب. بينما المجموعات السادسة والسابعة والثامنة والتاسعة:عولجت باضافة البروبيوتك والمحمضات بالجرعات المذكورة سابقا مع اجراء العدوى الاصطناعية بالميكروب القولونى O78 عند عمر ٢١ يوم.المجموعة العاشرة: تم اجراء العدوى الاصطناعية بالميكروب القولونى O78 عند عمر ٢١ يوم فقط. وقد تم إجراء القياسات الدموية وبعض القياسات المناعية والهستوباثولوجية.وقد أوضحت نتائج القياسات الدموية ان الطيور المصابة اصطناعيا بالميكروب القولونى تعانى من أنيميا من النوع التى تتميزبحجم خلية وكمية هيموجلوبين طبيعيين. وجود زيادة معنوية فى عدد كرات الدم الحمراء وحجم الخلايا المضغوطة وذلك عند ٤ و٦ اسابيع من العمر فى المجموعات المعالجة بالبروبايوتيك مع عدم وجود تأثير معنوى فى نسبة الهيموجلوبين.عدم وجود تغيير معنوى المجموعات المعالجة بالاحماض العضوية.أظهرت الدراسة وجود زيادة معنوية فى العدد الكلى والنوعى لخلايا الدم البيضاء فى المجموعات المصابة بميكروب الايشريشيا القولوني ولم تعالج والمجموعات المعالجة بالبروبايوتك بينما لم تظهر اى تغير معنوى فى المجموعات المعالجة بالاحماض العضوية.وقد أظهرت نتائج القياسات المناعية وجود زيادة معنوية ($P<0.05$) فى الاجسام المناعية (IgG) و (IgM) فى المجموعات المصابة والمجموعات المعالجة بالبروبايوتك بينما المجموعات المعالجة بالاحماض العضوية لم تظهر اى تغير معنوى. اظهرت النتائج زيادة معنوية ($P<0.05$) فى مستوى الانترلوكين ٦والانترلوكين ١٢ فى المجموعات المصابة والمجموعات المعالجة بالبروبايوتك بينما المجموعات المعالجة بالاحماض العضوية والغير مصابة لم تظهر اى تغير معنوى.وقد أظهرت التغيرات النسيجية للاعضاء ان المجموعات المعالجة بالبدائل الحيويه ادت الى تحسين ملحوظ فى القياسات النسيجيه . وبذلك أكدت نتائج الفحص النسيجى للأعضاء الداخلية ما تم التوصل اليه من النتائج السابقة.مما سبق نستنتج أن يمكن استخدام البروبايوتك كمحفز حيوى للنمو ومنشط للمناعة فى بدارى التسمين وبديل طبيعى امن.أثبتت الجرعة القليلة (٠,٥جم) من البروبايوتك أنها أكثر فاعلية من الجرعة العالية.البروبايوتك اكثر فاعلية كمحفز حيوى للنمو ومنشط للمناعة من الاحماض العضوية .