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CLINICOPATHOLOGICAL STUDIES ON THE EFFECT OF SOME FEED ADDITIVES IN BROILER CHICKS

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LIST OF ABBREVIATIONS

Abbr.	Description
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Alb.	Albumin
A/G	Albumin Globulin Ratio
ALP	Alkaline phosphatase
B.W	Body weight
BWG	Body weight gain
CAT	Catalase
CFU	Colony forming units
<i>E.coli</i>	Escherichia coli
FAO	Food and Agriculture organization
FCR	Feed conversion ratio
FI	Feed intake
Fig.	Figure
Gps.	Groups
Glob.	Globulin
HBSS	Hank's balanced salt solution
IgA	Immunoglobulin A
IL-6	interleukin-6
MDA	Malondialdehyde
P.B.S.	Phosphate Buffer Saline

List of Abbreviations

PM	Post Mortem
ROS	Reactive oxygen species
RPMI	Roswell park Memorial Institute
SOD	Superoxide dismutase
T.P.	Total proteins
WHO	world Health Organization

SUMMARY

In recent years, the feed additives are being studied extensively with great interest which have desirable effects by improving the animals performance and health, through enhancing digestibility of the feed materials, promoting growth rate, increasing feed conversion and preventing disease. Extensive using of antibiotics lead to imbalance of the intestinal microflora, appearance of resistant bacteria and also drug residues in the bird for that tend to restrict using of antibiotics and using natural feed additives. From this feed additives probiotics and prebiotics. Colibacillosis in Egypt and all over the world is considering one of the most chronic bacterial disease.

So this study aim to control the colibacillosis by using some feed additives as probiotics and prebiotics and studying their effects on biochemical & immunological parameters, as well as DNA damage and histopathological studies.

This study done on one hundred and twenty (120) chicks, one day old, commercial Hubbard chicks obtained from Dakahlia Poultry Company, divided equally into 6 groups of each 20 chicks.

Group 1: Chicks kept as normal control.

Group 2: Chicks administrated Curazym 1g/kg feed for 42 days

Group 3: Chicks administrated ZADO 0.6g/kg feed for 42 days.

Group 4: chicks infected orally by 1ml *E. coli* O78 (3×10^7 CFU) intra crop at 10th days old.

Group 5: Chicks administrated *Curazym* 1g/kg feed administrated from one day old then infected orally by 1 ml *E. coli* O78 (3×10^7 CFU) intra crop at 10th days old.

Group 6: Chicks administrated *ZADO* 0.6g/kg feed administrated from one day old then infected orally by 1 ml *E. coli* O78 (3×10^7 CFU) intra crop at 10th days old.

Two blood samples were collected from each birds groups via wing vein at 23rd and 37th days old. The first blood samples were taken in heparinized tube for phagocytic activity and phagocytic index test. The second blood samples without anticoagulant to take serum for biochemical, immunological and serum antioxidant.

Specimens from internal organs (liver, kidney and intestine) collected from all groups and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections prepared and stained with hematoxyline and eosin (H&E) and examined microscopically.

Specimens from internal organs (liver, intestine and lung) collected at day 23rd and 37th day from all groups placed in ice cold phosphate buffer saline (BPS) and were kept on -20°C until used for the comet assay.

The evaluation of effect of probiotic and prebiotic based on evaluating the indices of the body performance (BW, BWG, FI and FC) and blood chemistry as liver enzymes as (ALT, AST, ALP), kidney function tests (serum urea, creatinine and uric acid,), proteinogram (total proteins, albumin, globulin and A/G ratio), also immunological studies as serum Immunoglobulin A (IgA), interleukin-6 (IL-6) and phagocytic activity and phagocytic index, serum antioxidant activity (MDA, SOD and CAT) and comet assay test.

Clinical signs and mortality:

The result showed that birds infected with *E.coli* and non-treated exhibited ruffled feathers, inability to stand, dropping wings, sunken eyes, in-appetence, dullness, depression, decreased body weight, breathing difficulty and gasping, sneezing and coughing, beak fluid discharge, white to yellowish diarrhea and high mortality rate in a clinical course, where that clinical signs decreased in the infected treated groups.

Postmortem findings:

The result showed that birds infected with *E.coli* and non-treated were submitted to necropsy. They showed moderate to severe redness of airsac, intestine, heart and ballooning of intestine, liver has enlarged, congested and contained blood tinged materials, with presence of white pinpoint multifocal foci. While the severity of findings were decreased in the experimentally *E.coli* infected treated birds with probiotic and prebiotic as a moderate of aforementioned lesions were seen.

Body performance results:

Showed a high significant increase in the weekly live body weight, body weight gain, feed consumption with a significantly decreased of feed conversion rate were noticed in treated chicks with probiotic and prebiotic (gps.2&3) in comparison with normal control group (gp.1). On other hand the weekly live body weight, body weight gain and feed consumption of *E.coli* infected non-treated chicks (gp.4) was significantly decreased with a high significant increase in feed conversion rate when compared with control group. While the *E.coli* infected treated groups with probiotic and prebiotic (gps.5&6) showed a significant improvement in the body performance parameters in comparing with infected non treated group during the experimental period.

Biochemical findings:

A) Changes in some liver enzymes:

The liver enzymes ALT, AST and ALP showed a significant increase in *E.coli* infected non treated group and slight increase in *E.coli* infected treated groups. On other hand, a slight significant decrease in liver enzymes in treated groups with probiotic and prebiotic in all experimental periods.

B) Changes in proteinogram:

Gps. (2&3) showed a significant increase in the serum total protein, albumin and total globulin with non-significant difference recorded in albumin globulin ratio compared with

control group. While the 4th group revealed a significant decrease in total protein, albumin and albumin globulin ratio with a significant increase in total globulin at the beginning of experiment end with a significant decrease compared with normal group. On the other hands gps. (5&6) recorded an improvement in serum total protein, albumin and total globulin which showed a significant increase in a comparison with the *E.coli* infected non treated birds all over the experimental period.

C) Changes in some renal function tests

The serum biochemical markers for kidney functions evaluation (serum urea, creatinine and uric acid) showed a significant increase in *E.coli* infected non treated group and slight increase in *E.coli* infected treated groups. On other hand, a slight significant decrease in kidney function tests in treated groups with probiotic and prebiotic in all experimental periods.

Immunological results:

A)-Serum immunological parameters (IgA & IL6):

Serum IgA at 23rd & 37th days of experiment noticed a significant increase while, the serum IL6 showed a significant decrease in treated chickens with probiotic or prebiotic when compared with control group. However the *E.coli* infected non-treated birds showed a significant increase in serum IgA levels at 23rd days old followed by a significant decrease at 37th days with a significant increase in serum IL6 all over the experimental period compared with control. While the *E.coli* infected treated

groups with probiotic or prebiotic recorded a significant increase in the serum IgA and serum IL6 compared with the *E.coli* infected non treated group.

B)-Phagocytic activity and phagocytic index results:

Sera fed chickens groups with probiotic and exogenous enzymes showed a significant increase in phagocytic activity, and phagocytic index when compared with control group. Where the chickens infected with *E.coli* reported a significant decrease in phagocytic activity, and phagocytic index comparing with the control group all over the experimental period. While the chickens infected with *E.coli* and treated with probiotic or exogenous enzymes revealed a significant increase in phagocytic activity, and phagocytic index when compared with *E.coli* infected non-treated group.

Oxidative stress and Antioxidant activity:

The MDA levels were significantly decreased in gps. (2&3), but a significantly increased in gps. (4&5&6) respectively, on other hands a significant increase in serum (SOD) and (CAT) activities were observed in gps. (2&3) and a significant decrease in gps. (4&5&6) compared with other groups at 23rd and 37th days.

Evaluation of DNA damage:

Comet assay test in the liver, intestine and lung showed an improvement in the DNA degradation in the group treated with the probiotic. While the group treated with prebiotic showed

non-significant difference in DNA degradation compared to the control group during the course of experimental period. On the other hand the *E.coli* infected non treated group (gp.4) showed highly DNA degradation compared with control group during the course of experimental period. Where Gp.5 showed an improvement in DNA degradation, while there is a low DNA degradation improvement in the gp.6 compared to infected non treated group, at both 23rd & 37th days of experiment .

Histopathological results:

The histopathological results of *E.coli* infection:

- Liver of infected chickens with *E.coli* at 23rd days showing severe dilated and congestion of hepatic blood vessel with focal periportal infiltration with inflammatory cells, beside severe degenerative changes as vacuolar degeneration with atrophied hepatocytes were noticed. At 37th days showing disappear of basic architecture of liver, in addition to multifocal coagulative necrosis represented in karyolytic nuclei of hepatocytes, perivascular aggregation of leucocytic cells and fibroblast and hyperemia was seen.
 - Kidney of infected chickens with *E.coli* at 23rd days showing severe congestion of renal blood vessel with interstitial hemorrhage, beside degenerative changes in epithelial lining of renal tubule. At 37th days of infection showing massive aggregation of leucocytic cells of mainly lymphocytes in addition to focal necrosis of renal tubules and congestion of renal blood vessels
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- Intestine of infected chickens with *E.coli* at 23rd days showing vacuolation of submucosal glandular epithelium with edema among muscle fiber of muscularis mucosa. At 37th days showing sever inter glandular and lamina propria inflammatory cells infiltration of mainly lymphocytes in addition to atrophy of some intestinal glands.

The histopathological results of *E.coli* infection and treated with probiotic and prebiotic.

- Liver of chickens infected with *E.coli* treated with probiotic at 23rd days showing apparently normal hepatic parenchyma with mild degeneration in hepatocytes, dilated hepatic sinusoids and mild perivascular inflammatory cells infiltration with congestion of hepatic sinusoids. While at 37th days showing restore its normal histomorphologic picture of tissue architecture and cellular details with congestion of both hepatic blood vessels and hepatic sinusoids
- Kidney of chickens infected with *E.coli* treated with probiotic at 23rd days of experiment showing apparently normal renal cortex with normal renal medulla except mild focal intertubular hemorrhage. At 37th days showing reduction in renal lesions with few lesions still as degenerations and atrophy of some renal tubules.
- Intestine of chickens infected with *E.coli* treated with probiotic at 23rd showing normal mucosa and sub mucosa with congestion of serosa blood vessels, atrophy of some

submucosal glands. At 37th days showing normal intestinal layers with fusion of some intestinal villi.

- Liver of chickens infected with *E.coli* and treated with prebiotic at 23rd showing slight congestion of both hepatic blood vessels and sinusoids. At 37th day, liver sections showing apparently normal tissue architecture and cellular details with slight lymphocytic cells infiltration.
- Kidney of chickens infected with *E.coli* and treated with prebiotic at 23rd days showing normal renal cortex and medulla with cystic dilation of some renal tubules and moderate congestion of blood vessels. At 37th day, kidney sections of most chickens showed improvement of lesions with mild diffuse inter tubular hemorrhage and the glomeruli appeared apparently normal.
- Intestine of chickens infected with *E.coli* and treated with *prebiotic* at 23rd showing elongated villi with or without mild mucinous degeneration. At 37th day, intestinal sections showing apparently normal mucosal structure with inflammatory cells focally infiltrate lamina propria