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Efficacy of Probiotic Enterococcus Faecium (M74) against Necrotic Enteritis in Experimentally Infected Broilers

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Abstract: Enterococcus faecium (M74), a lactic acid bacterium and normal inhabitant in the gut, is a probiotic that may be useful in animal health; likewise, including E. faecium (M74) in the feed reduced the faecal concentrations of Clostridium perfringens and increased the levels of the potentially beneficial Bifidobacteria and Lactobacilli. Aim of the study: This study explores the possible use of Enterococcus Faecium (M74) probiotics as an alternative to antibiotics for treating necrotic enteritis (NE) in broilers, with the emerging public health concern about antibiotic resistance. Investigate the possible mechanism involved in the beneficial effect of suggested Enterococcus faecium (M74) on growth performance and carcass characteristics of broiler chickens via evaluating the growth performance profile, haematological profile, Liver and kidney functions, immunological response, histopathological examination of the GIT Tissue, Re-isolation of Cl. perfringens from the infected chickens I am running a few minutes late; my previous meeting is running over. post-treatment. Results: Both C. perfringens and total coliform counts were significantly the lowest in the prophylactic and treated groups. Furthermore, the prophylactic and treated groups showed significant improvement in the count compared to the positive control group. The relative length of the jejunum and ileum in control group on day 35 was significantly shorter than that in other groups. Adding E. faecium significantly reduced the relative length of the cecum on day 21. Conclusion: E.Faecium (M74) was able to significantly lesser the C. perfringens–induced Necrotic enteritis and its consequences on the growth performance, haematological picture, liver and kidney functions, immunological profile in addition to its positive impacts on the bacterial burden and histopathological picture of the gut intestine of the infected broilers. Considering the antibiotic resistance problem, E.Faecium (M74) could be used as one of the best alternatives to antibiotics in dealing with C. perfringens (NE), with special references to the preference for a prophylactic regimen over the therapeutic one

Keywords: *Broilers , C. perfringens , Enterococcus faecium , Necrotic enteritis*

1. Introduction

Enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality, reduced welfare of birds and the associated contamination of poultry products for human consumption. Necrotic enteritis (NE) is one of the most important enteric diseases in poultry and it is of a high cost to the industry worldwide. It is caused by avian-specific, Necrotic Enteritis Beta toxin (NetB)-producing, strains of Clostridium perfringens, a Gram-positive spore-forming anaerobe **[1].** For the past four decades,

antibiotics have been supplemented to animal and poultry feed to improve growth performance and efficiency and protect animals from adverse effects of pathogenic and non-pathogenic enteric microorganisms**.** Field observations have indicated that low levels of lincomycin in the feed would reduce and/or eliminate the incidence of Necrotic enteritis **[2].** Antibiotics have come under increasing scrutiny by some scientists, consumers and government regulators because of the potential development of antibiotic-resistant human pathogenic bacteria after prolonged use **[3].** Antimicrobial resistance represents a global health problem that contributes to tens of thousands of deaths per year. Furthermore, the global demand for meat and poultry consumption is increasing at a rapid and unprecedented rate **[4].** However, the utility of antimicrobial agents as a preventive measure has been questioned, given extensive documentation of the evolution of antimicrobial resistance among pathogenic bacteria. So, the possibility of antibiotics ceasing to be used as growth stimulants for poultry and the concern about the side-effects of their use as therapeutic agents has produced a climate in which both consumer and manufacturer are looking for alternatives. Probiotics are being considered to fill this gap **[5].** Probiotics are live bacteria, fungi, or yeasts that supplement the gastrointestinal flora and help to maintain a healthy digestive system. The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) working group have defined probiotics as "live microorganisms that, when administered in adequate amounts, confers a health benefit on the host" **[6].** A good probiotic must fulfil some selection criteria such as membership among the normal intestinal microbiota, acid and bile tolerance, gut colonization, production of antimicrobial substances or bacteriocin. Then, it must easily to survive growth on a large scale, retain its viability under storage and field conditions, and be cost-effective to use for farm animals**.** Enterococcus faecium (M74), a lactic acid bacterium and normal inhabitant in the gut, is a probiotic that may be useful in animal health, Likewise, including E. faecium (M74) in the feed reduced the fecal concentrations of Clostridium perfringens and increased the levels of the potentially beneficial Bifidobacteria and Lactobacilli **[7].** The purpose of this study is to explore a possible use of Enterococcus Faecium (M74) probiotic as an alternative to antibiotics for treatment of necrotic enteritis (NE) in broilers, with the emerging public health concern about antibiotic resistance. Investigate the possible mechanism involved in the beneficial effect of suggested Enterococcus faecium (M74) on growth performance and carcass characteristics of broiler chickens via evaluating: Growth performance profile, hematological profile, Liver and kidney functions, immunological response, histopathological examination of the GIT Tissue, Re-isolation of Cl. perfringens from the infected chickens I am running a few minutes late; my previous meeting is running over .post-treatment. The study was conducted on healthy one hundred and eighty, one-day old, commercial Hubbard chickens from Al-Kahira Poultry Company, 10th of Ramadan city, Egypt was used for this investigation. They divided into one control group and 5 tested groups. Each group contain 30 broiler chickens. The chickens were allocated into 4 groups as follows: 1st **group: (Negative control group):** non infected fed on a basal diet only. 2nd **group :(Positive control group) :** fed on a basal diet and was infected with 2 ml broth culture Cl. perfringens type (1.9×10^9 organism/mL) orally on 19th day old **[9]. 3rd group**: **(Prophylaxis E.Faecium M74):** given dehydrated E.Faecium M74(1 gm /l) in drinking water at concentration (15×10^9 CFU kg^{-1}) from day one till the end of the experiment and orally infected with 2 ml broth culture of Cl. perfringens (1.9×10^9 organism/mL) on 19th day old **[8]. 4th Group (Therapeutic E.Faecium M74) :** Infected with 2 ml broth culture (1.9×10^9 organism/ml) orally on 19th day old then treated with E.Faecium M74 (1 gm /l) in drinking water at concentration (15×10^9 CFU kg^{-1}) for 5 days after appearance of clinical signs.

2. Materials and Methods

2.1. Materials

2.1.1. Drugs and Bacteria

2.1.1.1. Enterococcus Faecium M74

The product "Enterococcus faecium M74" is already provisionally authorised in Council Directive 70/524/EEC as a feed additive under the category "Micro-organism", for use in calves and piglets until 6 January 2004 (first authorisation: Commission Regulation (EC) Nº 2690/1999). The Scientific Committee on Animal Nutrition (SCAN) issued a favourable opinion on the safety of "Lactiferm" for the mentioned animal categories. Company Medipharm AB is seeking provisional Community authorization of its micro-organism product "Enterococcus faecium M74" as a feed additive intended for use in chickens for fattening under category "Micro-organism".

2.1.1.2. Description of the Probiotic

Product category: Micro-organism, **Description:** Enterococcus faecium M74 (NCIMB 11181), **Target animal category:** Chickens for fattening, **Applicant :**Medipharm AB and **Import:** Sweden. **Dose of the Enterococcus**

faecium M74: Dehydrated E.Faecium M74 (15 x 10^9 CFU kg^{-1}) in drinking water [8**]. Inoculum preparation and chick challenge:** On the 19th day of age, all infected groups were individually orally inoculated with one dose of 1ml / chicken field strain Cl. perfringens type "A" (1.9×10^9 organism/ml), which was kindly obtained from the Anaerobes Unit, Animal Health Research Institute, Dokki, Giza, Egypt **[9].** The Cl. perfringens type "A" reference strain (ATCC™ 13124™) was cultured in meat broth and incupated for 24-48 h at 37° c under anaerobic conditions using Gas Pak. The bacterial cells attained were then resuspended in phosphate –buffered saline (PBS).

2.1.2. Experimental Birds

One hundred and twenty, one-day old, 38-42 gm, commercial Hubbard chicks from Al-Kahira Poultry Company, 10th of Ramadan city, Egypt were used for this investigation. The chicks will be reared under standard environmental and hygienic conditions and food added ad libitum. All chicks will be vaccinated against Newcastle disease on 7th and 18th days old using Hitchner B1 and LaSota live virus vaccines (IntervetBoxmeer Company, Boxmeer, Netherlands); the vial contains 106 EID50 Newcastle disease virus, dissolved in physiological saline (30 mL per 1000 doses) as eye drops. Vaccination of all chicks against Gumboro disease was done on 15th day old using Holland. Gumboro vaccine (Rhone- Merieau Company, France); the Vial was dissolved in 50 mL physiological saline / 1000 bird as eye drops. The experiment was performed at lab Animal house, Faculty of Vet. Medicine Zagazig University. The poultry house was maintained at temperature (25±20C). Feed and water will be provided ad lib and normal managemental practices will be followed to stay the birds free from stress. The experiment was performed in accordance with the rules set by the moral Committee of zagazig university, Egypt.

2.1.3. Experimental Design

The study was conducted on healthy one hundred and twenty, one-day old, commercial Hubbard chickens from Al-Kahira Poultry Company, 10th of Ramadan city, Egypt was used for this investigation. They divided into one control group and 3 tested groups. Each group contain 30 broiler chickens. The chickens were allocated into 4 groups as follows: 1st **group: (Negative control group):** non infected fed on a basal diet only. 2nd **group :(Positive control group) :** fed on a basal diet and was infected with 2 ml broth culture Cl. perfringens type (1.9×10^9 organism/mL) orally on 19th day old **[9]. 3rd group**: **(Prophylaxis E.Faecium M74):** given dehydrated E.Faecium M74(1 gm /l) in drinking water at concentration (15 x 10^9 CFU kg^{-1}) from day one till the end of the experiment and orally infected with 2 ml broth culture of Cl. perfringens (1.9×10^9 organism/mL) on 19th day old (**8). 4th Group (Therapeutic E.Faecium M74) :** Infected with 2 ml broth culture (1.9×10^9 organism/ml) orally on 19th day old then treated with E.Faecium M74 (1 gm /l) in drinking water at concentration (15 x 10^9 CFU kg^{-1}) for 5 days after appearance of clinical signs.

2.1.4. Sampling

2.1.4.1. Blood Samples collected for hematological analysis and blood serum (for biochemical analysis and immunological parameters)

Five birds, from each group, were used for collecting blood samples 2nd, 9th, 16th days post infection in prophylactic groups and 2nd, 9th, 16th days after treatment in therapeutic groups. Each blood sample was subdivided into two parts. The 1st part was taken on dipotassium salt of EDTA (1mg/1mL blood) for hematological examination. The 2nd part was collected into clean centrifuge tube for obtaining serum by centrifugation for biochemical studies **[10].**

2.1.4.2. Tissue Samples collected for Histopathological Examination

Small intestine tissue was collected after animal sacrifice for histopathological examination purposes.

2.1.5. Methods

2.1.5.1. Methods used for hematological analysis: For hematological examination (CBC)

Detection of (Total Erythrocytic count, Total Leucocytic count, Hemoglobin (Hb)) by Rayto 7200 (Auto Hematolgy Analyzer -MEDINICS R)**.**

2.1.5.2. Methods used for biochemical analysis: For determination of liver function

Detection of blood serum (AST-ALT-ALP-Total protein -Albumin-globulin) Chem 7 (Semi Automated Clinical Chemistry Analyzer - Erba R) according to [**11]**.

2.1.5.3. For determination of Kidney function

Detection of blood serum (urea- creatinine –uric acid –potassium) by Chem7 (Semi Automated Clinical Chemistry Analyzer - Erba R) according to [**12].**

2.1.5.4. For determination of Immunological Parameters

Detection of blood serum (IgM, IgA, CD4, CD8, IL-2, IL4, TNF-α,) by Absorbance microplate reader ChroMate 4300 (ELISA) according to the following principle: The IgM assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to IgM is added to the sample. Sample containing IgM is incubated with a buffer (R1) and a sample blank determination is performed prior to the addition of IgM antibody (R2). In the presence of an appropriate antibody in excess, the IgM concentration is measured as a function of turbidity. The IgA assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to IgA is added to the sample. Sample containing IgA is incubated with a buffer (R1) and a sample blank determination is performed prior to the addition of IgA antibody (R2). In the presence of an appropriate antibody in excess, the IgA concentration is measured as a function of turbidity. The assay was done according to manufacturer's instructions. The A450 was measured using a multiscan plate reader, Power wave XS2 (Biotek, India). Antibody levels were calculated by plotting the absorbencies on the graph obtained by plotting absorbency of calibrator serum against their concentration. CD4 T cells are human helper T cells that express cluster determinant 4 (CD4) molecules. The CD4 molecule is a member of the immunoglobulin family and primarily mediates adhesion to major histocompatibility complex molecules. CD4 T cells are selectively targeted and infected by HIV. HIV proliferates rapidly during acute infection leading to high levels of viremia and rapid impairment and death of CD4 T cells (**13**). The antibody MEM-31 recognizes a conformationally-dependent epitope of CD8, a cell surface glycoprotein found on most cytotoxic T lymphocytes that mediates efficient cell-cell interactions within the immune system. CD8 is a disulfide-linked dimer and exists as a CD8 alpha/alpha homodimer or CD8 alpha/beta heterodimer (each monomer approx. 32-34 kDa). IL-4 (Interleukin-4, Ia inducing factor (IaIF), B-cell stimulating factor-1, BSF-1, Hodgkin's cell growth factor, HCGF, Mast cell growth factor-2, MCGF-2, Macrophage fusion factor, MFF, T cell growth factor-2, TCGF-2) is a pleiotropic cytokine that is produced by activated T cells, mast cells and basophils. IL-4 elicits many different biological responses but has two dominant functions. IL-4 regulates differentiation of naïve CD4+ T cell to Th2 cells. Th2 cells produce IL-4, IL-5, IL-10 and IL-13, which tend to favor a humoral immune response while suppressing a cell-mediated immune response controlled by Th1 cells. In addition, IL-4 regulates IgE and IgG1 production by B cells. The tumor necrosis factor (TNF)-α kit is a solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA). A monoclonal antibody specific for human TNF-α has been coated onto the wells of the provided microtiter strips provided. Samples and standards of known human TNF-α content were pipetted into these wells. During the first incubation, the human TNF-α antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for human TNF-α was added. During the second incubation, this antibody binds to the immobilized human TNF-α which was captured during the first incubation. After removal of excess second antibody, streptavidin-peroxidase (enzyme) was added to bind to the biotinylated antibody and then the four-member sandwich was completed. After a third incubation and washing to remove all the unbound enzyme, a substrate solution was added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of human TNF-α present in the original specimen. This assay was performed using TNF alpha ELISA kit.

2.1.6. Growth performance (Body weight, Average daily gain, Average feed intake, Feed conversion ratio)

Performance characteristics monitored included initial body weight, final body weight, body weight gain, total feed intake and feed conversion. Body weight (BW, g) and feed intake (FI, g/bird) were weekly recorded to determine body weight gain (BWG, g) as following equation: **BWG** = final weight (g) – initial weight (g).

Feed conversion ratio (FCR) was calculated as the amount of feed required (g) for producing a unit of gain (g) according to the following equation.

FCR = feed intake (g)/weight gain (g)

2.1.7. Histopathological Examination

Small intestine tissue was collected after animal sacrifice for histopathological examination purposes. All tissue samples were fixed in 10 % neutral buffered formalin solution for 12 hours, then dehydrated through increasing ethanol series after dehydration until they reached the absolute alcohol (1hour). The specimens

were cleared in xylol (1:1) in oven (60 °C) for 30 min and embedded in paraffin wax (melting point 56 °C for 3 hours. Then cut into 5 µm-thick sections using a microtome (Leica RM2255, Germany) and stained with Della field's hematoxylin (a blue, basic or nuclear stain) and counterstained by eosin (a red, acidic or cytoplasm stain) hematoxylin and eosin (H & E) dye for microscopic investigation. Slides were dipped in haemtoxylin for 10 min and then washed with distilled water to remove the excess stain. Then slides were transferred to acidify 70% alcohol till the proper density of color is attained. The slides were rinsed in alkaline water to neutralize any acid present and then counterstained with eosin (1 % saturated aqueous solution) for 5 min. The excess stain was removed with distilled water until the correct staining effect was stained. Sections were again dehydrated by passing in series of 70, 80, 90 and 96 % alcohol for 2 min in each, then twice in 100% alcohol. Sections were cleared by passing xylol twice for 2 min each to ensure getting rid of the alcohol. Finally, sections were embedded in Canada balsam, covered with a thin cover glass, and dried in an oven (40 °C) to harden the balsam [**14**]. The slides were examined and photographed with an Olympus UTU1X-2 camera connected to an Olympus CX41 microscope (Tokyo, Japan). Reagents used for Histopathological Examination: 10% neutral buffered formalin solution, ethanol, xylol (1:1), paraffin wax, Hematoxylin and Eosin (H &E) dye, distilled water, 70 % alcohol and alkaline water according to [**14**].

2.1.8. Reisolation of C. perfringens

Intestinal samples from each group (n = 10) were collected once at 35-day-old and immediately plated. Ten-fold dilutions per each sample were prepared, and 0.1 mL was spread (triplicate) on a blood agar base containing 5% sheep blood supplemented with 100 mg/L neomycin sulfate for C. perfringens enumerations. The plates were anaerobically incubated at 37°C for 16 to 24 h. The α- and β-hemolytic colonies were counted as C. perfringens, and the presumptive colonies were randomly picked, Gram-stained, and microscopically examined to confirm them as C. perfringens. Counts were expressed as $\log_{10}$ CFU/g of the intestinal contents **[15]**.

2.1.9. Statistical Analysis

The present data is subjected to statistical analysis. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) computer program, version 25.00 produced by IBM Software, Inc. Chicago, USA, and Graph Pad prism software version 9.4. The data were analyzed by one-way analysis of variance (ANOVA).All data were presented as means ± standard error (SE). Differences were considered significant at $P \leq 0.05$.

3. Results

3.1. Histopathological changes

A) Intestine of chicken in negative control group revealing normal intestinal coats structures (villus height and width) and intestinal crypts with few goblet cells in villus enterocytes.

(B) Intestine of chicken in positive control group showing broad ulcerated villus surface (arrow), thickened lamina propriainfiltrated by heterophils (arrow) and intense desuamation of villus epithelial cells.

(C) Intestine of chicken in Group 3 showing broad and thickened villus tips in most intestinal villi (arrow) and an increase in absorptive surfaces without exudate in the lumen.

(D) Intestine of chicken in Group 5 revealing normal villi tall and thin lined by hyperplastic villus epithelium containing numerous goblet cells beside a few necrotic tissue.

4. Discussion

Poultry is the fastest-growing source of meat and eggs worldwide. This development resulted from proper genetic selection, improved feeding, health management practices, and antibiotics. The poultry industry is an important sector in the economies of many countries worldwide. Therefore, any contribution towards better understanding of poultry production could help to improve this sector. Gaps in research and knowledge show that there is a need to develop alternative substances that will reduce the burden of over reliance on antibiotics **[16].** Digestive tract infections are a major concern in the poultry industry and have led to severe economic losses. Necrotic enteritis (NE) in either clinical or subclinical form is a major enteric poultry disease that has a detrimental effect on profitability in the broiler industry. A survey by **[17]** estimated that the cost of subclinical NE can be as high as 5 cents per bird, and NE outbreaks have the potential to cost the global broiler industry approximately $2 billion per year [**18**]. The C. perfringens is responsible for the enteric disorder in animals and humans. It can be found anywhere, commonly in wastewater, dust, air, and healthy human and animal intestinal tracts. NE mostly occurs in broiler chicks between 2 and 6 week of age and is caused by C. perfringens, anaerobic, Gram-positive, endospore-forming, nonmotile bacteria, which could form resistant endospores, allowing it to survive and remain persistent in extreme environmental conditions, such as decaying organic matter and soil. Since C. perfringens cannot synthesize several essential amino acids, it releases enzymes that degrade host tissue to meet its demand **[19]**. Necrotic enteritis is a multifactorial disease. The ubiquitous

nature of C. perfringens makes it difficult to attribute a single cause to the development of NE. C. perfringens is a serious pathogen which attacks the intestinal cells and disturbs the ecosystem within the intestine resulting in dysbiosis. Previous studies estimated that C. perfringens colonize over three-quarters of birds in any flock at any given time, but only small percentages develop necrotic enteritis **[20]**. The overgrowth of C. perfringens in the intestines has been suggested to occur because of a combination of events, including damage to the intestinal mucosa, low pH level in the intestine, and co-infection with coccidia, breed, sex, and age [**21]**.The clinical signs of clostridiosis include dehydration, depression, orange colored frothy diarrhea and, in some cases, tinged with blood, and ruffled feathers. Large sections of the intestine are necrotic and coated in a yellow brown pseudo-membrane filled with necrotic cells, bacterial colonies, and tissue fragments postmortem [**22**]. In 1951, the United States Food and Drug Administration approved the use of antibiotics as animal additives to prevent disease in general and, in some cases, to improve efficiency without veterinary prescription. In the 1950s and 1960s, each European state approved its own national regulations about the use of antibiotics in animal feeds. However, using antibiotics may develop bacteria resistant to these drugs. Accordingly, the use of antibiotics has been minimized and replaced by effective dietary supplements such as probiotics and/or prebiotics that are claimed to enhance growth and positively modulate the immune response [**23**]. Antibiotic growth promoters (AGPs) have been widely used in food animal production for decades, the Antibacterial drugs, such as avoparcin, lincomycin, amoxicillin, tylosin, virginiamycin, and bacitracin, were commonly used for the treatment and prevention of NE **[24]**. Using the veterinary medications particularly antibiotics while not the priority of adequate Withdrawal Time (WDT) of the medicine have a great risk to human health as hypersensitivity or alteration on the conventional microflora.it is important to place the most residual limits (MRLs) in thought**.** Understanding the effects of feeding practices on animal health, growth performance, and gut microbiota using alternatives (such as probiotics) is important for strategies to reduce antibiotic use in poultry production and for reducing environmental pollution. Environmental microorganisms had huge diversity, and the development and utilization of these microorganisms had great potential for sustainable agricultural development and green-friendly production [**25**]. Probiotics are live bacteria, fungi, or yeasts that supplement the gastrointestinal flora and help to maintain a healthy digestive system. The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) working group have defined probiotics as "live microorganisms that, when administered in adequate amounts, confers a health benefit on the host" **[6].** A good probiotic must fulfill some selection criteria such as membership among the normal intestinal microbiota, acid and bile tolerance, gut colonization, production of antimicrobial substances or bacteriocin. Then, it must easily survive growth on a large scale, retain its viability under storage and field conditions, and be cost-effective to use for farm animals**.** Enterococcus faecium (M74), a lactic acid bacterium and normal inhabitant in the gut, is a probiotic that may be useful in animal health, Likewise, including E. faecium (M74) in the feed reduced the fecal concentrations of Clostridium perfringens and increased the levels of the potentially beneficial Bifidobacteria and Lactobacilli **[7].** Enterococcus faecium was the first to be used as a probiotic feed additive and permitted by the European Union and FDA. Enterococcus faecium has been shown to improve the intestinal immunity and the jejunal mucus secretion in the broiler chickens. Enterococcus faecium improved the resistance to pathogenic bacteria in animals. The poultry gut was rich in lactic acid bacteria (LAB), which has been used as an important biological resource for the isolation of probiotics [**12**]. The current study aimed to investigate the possible best alternative to antibiotic drugs in treatment of necrotic enteritis with the emerging public health concern about antibiotic resistance and Investigating the possible mechanism involved in the beneficial effect of suggested Enterococcus faecium (M74) on performance and carcass characteristics of broiler chickens. One hundred and eighty, one-day old, commercial Hubbard chicks from Al-Kahira Poultry Company, 10th of Ramadan city, Egypt were used for this investigation. The chickens were allocated into 4 groups as follows: 1st group: (Negative control group) non infected fed on a basal diet only., 2nd group: (Positive control group) fed on a basal diet and was infected with 2 ml broth culture (1.9×10^{9} organism/ml) orally on 19th day old **[9]**, 3rd group: (Prophylaxis E.Faecium M74): Fed on a diet containing dehydrated E.Faecium M74(1 gm /l) in drinking water at concentration (15 x 10^{9} CFU /kg) from day one till the end of the experiment and orally infected with 2 ml broth culture (1.9×10^{9} organism/ml) on 19th day old **[8],** 4th Group (Therapeutic E.Faecium M74): Infected with 2 ml broth culture (1.9×10^{9} organism/ml) orally on 19th day old then treated with E.Faecium M74 (1 gm /l) in drinking water at concentration (15 x 109 CFU /kg) for 5 days after appearance of clinical signs [**26]**. Several investigations were performed; Hematological studies : Erythrocytic count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and total leukocytic count. Biochemical studies were conducted; serum total proteins,

serum albumin, serum globulins, serum alkaline phosphatase (ALP), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum levels of potassium, serum uric acid and serum creatinine.Immunological studies were performed ;Serum IgM, IgA, CD+4, CD+8**,** Inflammatory mediators (Tumor necrosis factor; TNF) and Interleukin IL-4. Growth performances were evaluated as total body weight, Average daily gain, Average feed intake and Feed conversion ratio. Intestinal morphology was performed;Histopathology of the gut with detection of lesion score %. Histopathology for intestine with detection of villus height and crypt depth and re-isolation of Cl. Perfringens after treatment. Our results showed that, WBCs showed significant increase in positive control, (leukocytosis).There was no significant difference between the studied groups and negative control. Positive control showed a significant decrease in RBCs (10^{12}/L), while there was no significant difference between prophylaxis examined group (group 3)and negative control followed by group 4. According to HGB (g/L), as showed in Table 1 and Figure 1; there was a significant difference between the studied group and negative control while there is no significant difference between negative control and group 3 followed by a significance difference on group 4, positive control showed the lowest HGB level. According to MCV (fL), as showed in Table 1 and Figure 1; there was a no significant difference between the studied group and positive control while there is significant difference between negative control and group 3. MCH (pg) results showed no significant difference between the studied group and positive control while there is significant difference with negative control (figure 2).

Complete blood pictures (hemoglobin concentration, packed cell volume, red blood cell count, and white cell count, as well as differential leukocytic count) were examined according to **[27]**. All infected chickens had immobility, weakness, decreased appetite, tiredness, and brown diarrhea throughout the post infection period (Table 2). Broiler chicks treated with lincomycin among the infected groups showed fewer severe clinical symptoms, particularly in the preventive dosage groups .Small intestinal lesions of varied severity appeared in the chicks (Figure 3). The mortality rate in the group under positive control (group 2) was 20%. Anemia was caused by severe intestinal lesions in the dead chickens from this group, which also had bloody diarrhea and a buildup of blood. Negative control (group 1) group did not see any incidents of fatality,Our results were agreed to [**28].** There was a substantial impact on C. perfringens bacterial counts,which reduced considerably in all treated groups when compared to the positive control (Table 3). While leukocytosis, heterophilia, and monocytosis were seen in G2 infected with C. perfringens compared to the control, showing a substantial reduction in RBCS, HB, and PCV according to **[28].** A significant decrease in RBCs, HB, and PCV was observed, which may be due to the destruction of RBCs by C. perfringens toxins inducing hemolytic anemia, whereas leukocytosis, heterophilia, and monocytosis were detected in G2 compared to the control (Figure 4). Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, total protein, serum albumin and globulin. Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (AP) act as markers of biliary function and cholestasis. Total protein,Albumin and globulin reflect liver synthetic function. The aminotransferases AST and ALT are normally < 30-40 units/l (Table 4). Elevations of aminotransferases greater than eight times the upper limit of normal reflect either acute viral hepatitis, ischemic hepatitis, or drug- or toxin-induced liver injury (Figure 5). Much more common than patients with acute hepatitis, however, are patients with chronic mild elevation of aminotransferases. Higher-than-normal levels of liver function levels may indicate liver damage or disease, such as a blocked bile duct, or certain bone diseases. An alkaline phosphatase (ALP) test measures the amount of ALP in the blood. It is commonly used to diagnose liver damage or bone disorders [**29**]. **[9]** study showed that treatment with probiotic extract, and amoxicillin reduced a considerable rise in serum total protein (Figure 6). Since lysozyme and related components make up a small portion of plasma protein, an increase in overall plasma protein denotes improved immunity. Albumin is one of the major proteins synthesized in the liver (Table 5). Energy supply is a very important determinant for the normal physiology of albumin production. Indeed, reduced serum albumin levels are observed in medical conditions associated with malnutrition, whereas high serum albumin levels have been reported to be associated with metabolic syndrome, an indicator of obesity and over nutrition (Figure 7). In addition, recently, serum albumin has been suggested to be associated with insulin resistance [**30**]. In our study infected non treated group showed a significant elevation in AST, ALT, ALP and total protein, albumin, followed by improvements with non-significant variations in the examined groups in each liver function parameters representing liver injury due to bacterial infection while in case of globulin level there was no significant difference between positive control, negative control and examined groups (Figure 8).

The prophylaxis group showed marked improvement in liver function test that became better in group 4 which treated with probiotic. Kidney function is important in the toxicity assessment because it is essential for the organism's survival (Table 6). Renal function indices are typically used to determine the normal functions of the different parts of the nephrons. Electrolyte, urea, uric acid, and creatine serum concentrations could provide insight into the effect of a compound or drug on the kidney's tubular or glomerular portions **[16].** kidney function parameters were evaluated for each group versus positive and negative control group. Our results showed that there was a significant increase in urea, creatinine, uric acid, and potassium in positive control group that demonstrated the presence of kidney injuries followed by non-significant increase in urea, creatinine, uric acid, and potassium in groups (Figure 9). The urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys. Measurement of serum urea and creatinine levels are commonly used to study the effect on renal function **[31].** Our results are supported by [**32]** who noted that, there were mild elevation in serum urea and creatinine which the non-effects on the renal function indexes may indicate that nephron normal function at the tubular and glomerular level was not affected. Our results showed that immunological parameters were estimated in studied chicken groups. Our study showed that IgM and IgA were significantly decreased in positive control and evaluated in prophylaxis group (group 1) while it is slightly evaluated. Our study showed that TNF, IL4, CD+4and CD+8 was significantly increased in positive control and non-significantly decreased in prophylaxis group, therapeutic and negative control (Figure 10). Many microbial pathogens make initial contacts with their hosts at mucosal surfaces, especially in the alimentary tract, and S-IgA acts as the first line of defense against invading pathogens **[33].** Our results showed that the challenged birds had higher concentrations of IL-4, TNF-α, and S-IgA in the jejunal mucosa compared with unchallenged birds at the starting challenge phase, [**34]** reported that the intake of probiotics enhanced production of pro inflammatory cytokines such as IL-1, TNF-α,and IL-6. [**35]** reported that the E. faecium elicited protective immune responses against various infections at mucosal Enterococcus faecium induced immune stimulation in broilers is still under investigation, and many factors, including dosage and administration, may affect potential immune modulation. **[36]** findings are agreed with our results which noted that serum lysozyme activity at day 21 increased linearly with dietary E. faecium concentration the highest activity was observed in the 1×10^8 and the 2×10^8 CFU E. faecium groups Serum levels of pro inflammatory cytokines IL-1β, IL-2, IL-6, IFN-γ, and anti-inflammatory IL-4, IL-10 changed linearly or quadratically both at the initial and final phases (Table 7).

As the first line of defence in the small intestine and other luminal surfaces, the pro inflammatory cytokine-involved genes are immunoregulatory peptides that can change tight junction gene expression (Figure 11). Toxins produced by C. perfringens and other virulence factors might affect cellular receivers enzymatically to activate immune cells and cause a genetic inflammatory response [**37**]. This finding fitted closely with those of **[38**] who noted that in vitro experiment showed that C. perfringens challenge markedly increased cellular cytotoxicity and the mRNA expression of interleukiens. Our study agreed with those of **[39]** who discovered the relative weights of intestine, spleen and Bursa Fabricius were heavier in chickens fed E. faecium probiotic, they concluded that dietary supplementation of E. faecium could improve immune organ development. They also found that the E. faecium probiotic decreased the inflammation conditions in the intestinal mucosa of broilers. This means less energy costs and probably explains the improved feed conversion ratio. Probiotic promote endogenous host defense mechanisms (table 8). Therefore, probiotics enhance humoral immune response in the intestine. Interleukiens has been shown to reduce the virulence of pathogens by activation T cell proliferation **[40].** CD4+/CD8+ ratio is a direct index for evaluating the condition of body immunity [**41].** Recent studies demonstrated that probiotics can improve T cell subsets through regulation of gut microbiota **[42]**. **[43]** observed that combined probiotics can induce T cell subsets in the intestine of broiler chicks. These probiotics mainly cause an influx of CD8+ T cells into the intestinal mucosa; this influx may enhance intestinal immunity. The obtained results also point to the possibility that the C. perfringens strain's virulence affects the severity of NE infection, most likely by changing the expression of intestinal genes involved in immunity, intestinal integrity, mucus production, apoptosis, and nutrition transporters. The healthy digestive system is essential for nutrition absorption as well as acting as an essential barrier against bacterial disease (Figure 12). According to the experimental findings, dietary supplementation with enterococcus faecium or its unique commercial product improves performance by reducing the negative effects of NE on intestinal cell integrity and immunological responses. In challenged broilers, both demonstrated their substantial effectiveness by altering genes involved in the mucosal barrier, tight junctions, and immunology, particularly as preventative measures. Body weight was recorded at the beginning (day 0), during the experiment (day 5, 10), and post-administration (day 23) to evaluate the effects on body weight gain, average weight gain, and feed conversion

ratio of each group (Figure 13). The FCR value was calculated as grams of feed consumed per gram of body weight gain. Our results showed that; according to body weight and average daily gain there were a significant elevation of negative control, group 3 and group 4, According to feed conversion ratio; there were significant decrease in groups 3. **[44]** reported that dietary supplementation of E. faecium probiotic improved broiler chickens weight gain and feed efficiency (Figure 14). The same findings have been reported by **[45].** Our results agreed with **[36]** that administered different dosages of E. faecium (0, 5 × 107, 1 × 10^8, and 2 × 10^8 CFU E. faecium/kg diet). The results revealed that average daily gain (ADG) changed quadratically, while feed conversion rate (FCR) increased linearly from day 22 to 35 and day 1 to 35 ($P < 0.05$). Supplementation of E. faecium at 5 × 10^7CFU/kg diet resulted in increased ADG ($P < 0.05$) compared with the other groups. In another study using a multi species containing Enterococcus strains in feed and water, **[45]** found a growth rate comparable to feeding 2.5 mg/kg antibiotic. Surprisingly, probiotic included in drinking water was more effective (Figure 15). **[46]** studied the supplementation of E. faecium, The improved feed conversion ratio in birds fed both the supplements was attributed to the increased phagocytes. **[47**] discovered At day1-21, compared with the control group, dietary supplement with antibiotics or E. faecium at low (1 ×10^8 CFU/kg feed) and medium (1×10^9 CFU/kg feed) levels significantly reduced FCR in broiler chickens and that agree with our study. **[48**] found that Enterococcus faecium-fed chickens showed an improved body weight and a lower mortality rate (17–34%). Body weight gain and increased feed intake because of dietary probiotics supplementation were reported. For example, **[45]**, supplemented broiler chickens with a probiotics mixture at 1 g/kg of feed from 1 to 42 d of age (Figure 16). The probiotic mixture included microbes that were isolated from the gut of healthy chickens. These included Lactobacillus reuteri, Enterococcus faecium, Bifidobacterium animalis, Pediococcus acidilactici and Lactobacillus salivarius. The probiotic product had a total bacterial count, expressed as colony-forming units of 2 × 10^{12} CFU/kg of product. **[49]** showed that dietary probiotics in the feed of broiler chickens significantly promoted growth performance. Similarly, supplementing broiler chickens with a probiotic mixture spores at 0.05% of feed significantly improved the feed conversion ratio, compared to the unsupplemented control group. E. faecium replaced antibiotics to reduce the feed conversion rate. Furthermore, E. faecium improved intestinal morphology and altered the composition of microbiota in the cecum to reduce feed conversion rate. Thus, it can be used as an alternative for antibiotics in broiler production to avoid the adverse impact of antibiotics by altering the gut microbiota. The clostridium perfringens-infected group's histopathological examination of the intestine revealed significant changes in the tunica mucosa and lamina propria. These changes included degenerative changes in the apical part of intestinal villi in the ileum to the entire length of intestinal villi and thickening of some villi in addition to complete loss of villi in some areas in the duodenum and jejunum in positive control group (group 2). The effects of antibiotics and E. faecium on intestinal morphology were evaluated. On day 21, the villus height in the antibiotics and E. faecium treated groups was non-significantly higher (Figure 17). On day 21, the villus height in E. faecium treated groups was higher than that in the other groups. The crypt depth in E. faecium treated group was higher than that in the antibiotics group. on day 35, the crypt depth in the antibiotics group was lower than that in E. faecium treated group, and E. faecium treated group and antibiotic group were non-significantly higher than the control group. Other intestinal morphologies with insignificant differences.

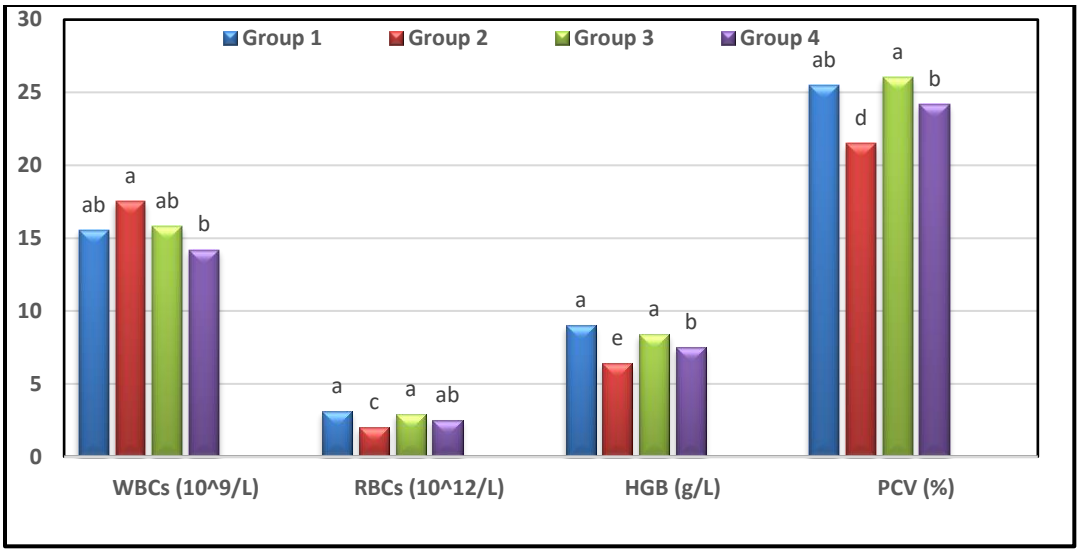

Figure 1: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg) on hematological parameters of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day old of broilers.

Table 1: The effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg) hematological parameters of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day old of broilers.

Hematological parameters				
	Group (1) Control (-Ve)	**Group (2) Control (+Ve)**	**Group (3)**	**Group (4)**
WBCs (10^9/L)	**15.5±0.3^{ab}**	**17.5±0.3^{a}**	**15.8±0.2 ^{ab}**	**14.2±0.5 ^{b}**
RBCs (10^{12}/L)	**3.1±0.33^{a}**	**2±0.02 ^{c}**	**2.9±0.03 ^{a}**	**2.5±0.04^{bc}**
HGB (g/L)	**9.0±0.13 ^{a}**	**6.4±0.01 ^{e}**	**8.4±0.01 ^{a}**	**7.5±0.1 ^{d}**
PCV (%)	**25.5±3 ^{ab}**	**21.5±2^{d}**	**26±3^{a}**	**24.2±4 ^{b}**
MCV (fL)	**95±3 ^{c}**	**145±4 ^{b}**	**145±1^{b}**	**150±4 ^{a}**
MCH (pg)	**33±2 ^{d}**	***50*±2 ^{a}**	**45±3 ^{c}**	**47±3 ^{b}**
MCHC (g/L)	***27*±1 ^{c}**	***45*±1.9 ^{a}**	***28*±2.1 ^{c}**	***40*±1.5 ^{a}**

Table 2: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU/kg) on Liver function Parameters of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers

(mean ± SE) (n = 5)

Liver function Parameters						
Groups	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Albumin g/Dl**	**Total protein g/dL**	**Globulin g/Dl**
Group(1) Control (-Ve)	**33.8 ± 1.5^{c}**	**23.2 ±2.5^{c}**	**48.6 ±1.2 ^{c}**	**3.20 ±0.21 ^{b}**	**3.36 ±0.2^{c}**	**0.88 ±0.16 ^{b}**
Group (2) Control (+Ve)	**82.4 ± 7.2 ^{a}**	**58.4 ± 3.6 ^{a}**	**137.4 ±6.9 ^{a}**	**4.9 ±0.1 ^{a}**	**6.8 ±0.01^{a}**	**0.8 ±0.01 ^{b}**
Group (3)	**51.1 ± 1.3 ^{b}**	**35.6 ±1.4 ^{b}**	**82.4 ±2.4 ^{b}**	**3.2 ±0.05 ^{b}**	**3.7 ±0.05 ^{b}**	**0.92 ±0.6 ^{a}**
Group (4)	**55.8 ± 2.4 ^{b}**	**38.3 ±1.8 ^{b}**	**88.6 ±1.9 ^{b}**	**2.9 ±0.6 ^{b}**	**3.5 ±0.4^{b}**	**0.91 ±0.06 ^{a}**

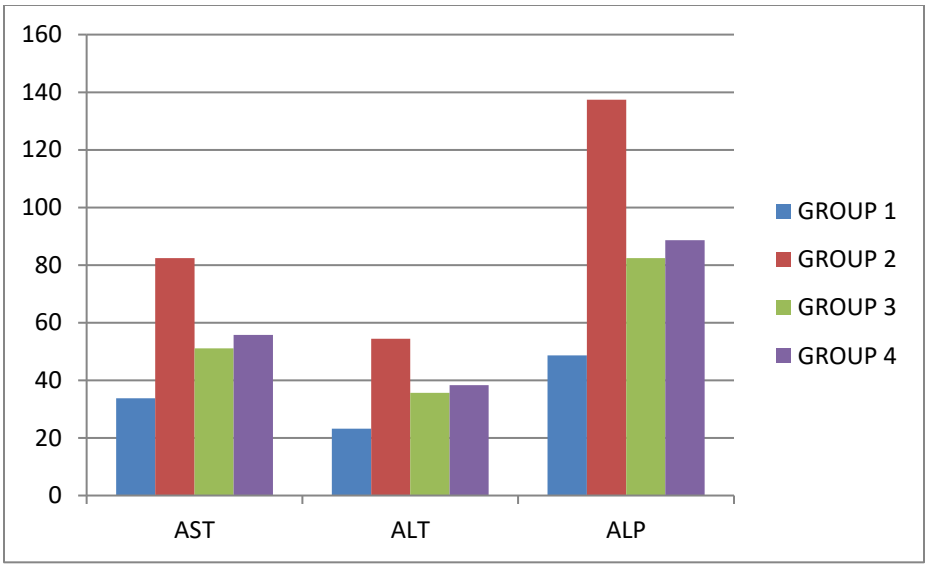

Figure 2: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU/kg) on Liver function Parameters of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

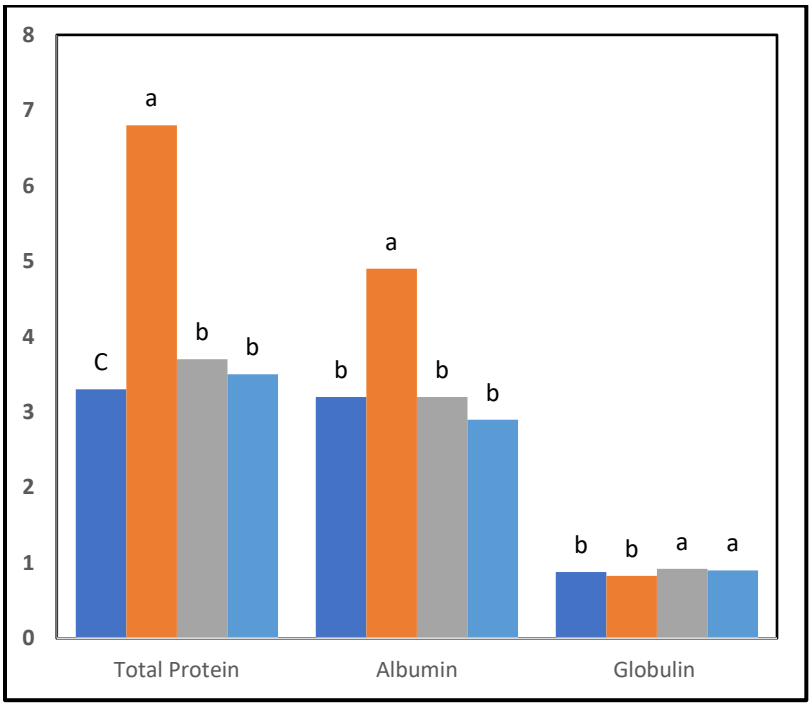

Figure 3: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU/kg) on serum protein of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

Table 3: Effect of Enterococcus Faecium (15x 10^9 CFU/kg) on kidney functions parameters of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

	Kidney's function parameters			
Groups	**Urea (mmol/L)**	**Creatinine (mg/dL)**	**Uric acid (mg/dL)**	**Potassium (mg/dL)**
1st Control (-Ve)	**28.8± 1.20 b**	**0.77±0.12 c**	**2.52±0.04 b**	**135.5±1.0 b**
2nd Control (+Ve)	**46.7±3.41 a**	**1.34±0.21 a**	**5.61±0.06 a**	**150.3±1.8 a**
3rd Group	**29.6±2.32 b**	**0.85±0.13 b**	**2.85±0.60 b**	**138±1.8 b**
4th Group	**29.8±1.28 b**	**0.91±0.16 b**	**2.97±0.07 b**	**138.9±1.7 b**

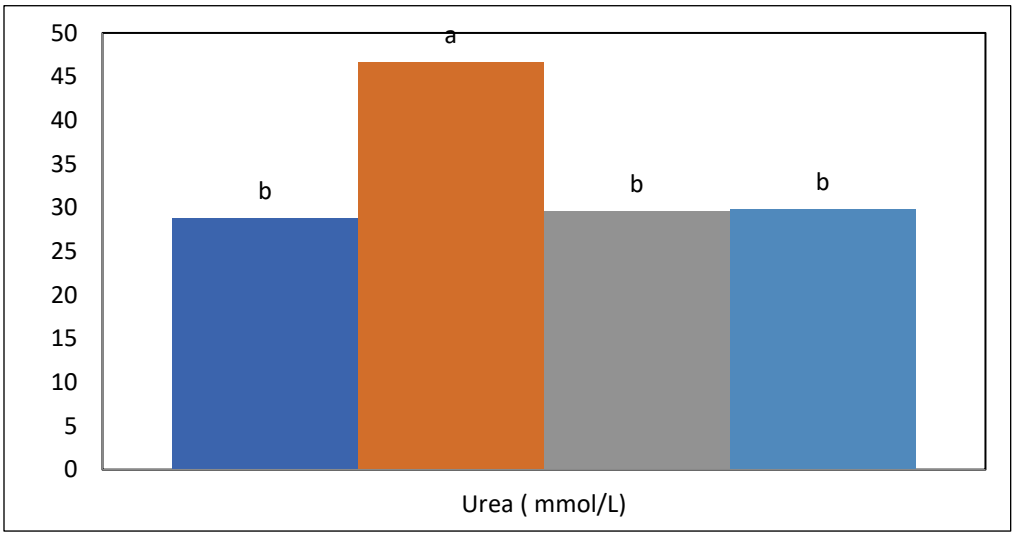

Figure 4: Effect of Enterococcus Faecium (15 x 10^9 CFU /kg) on urea (mmol/l) of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

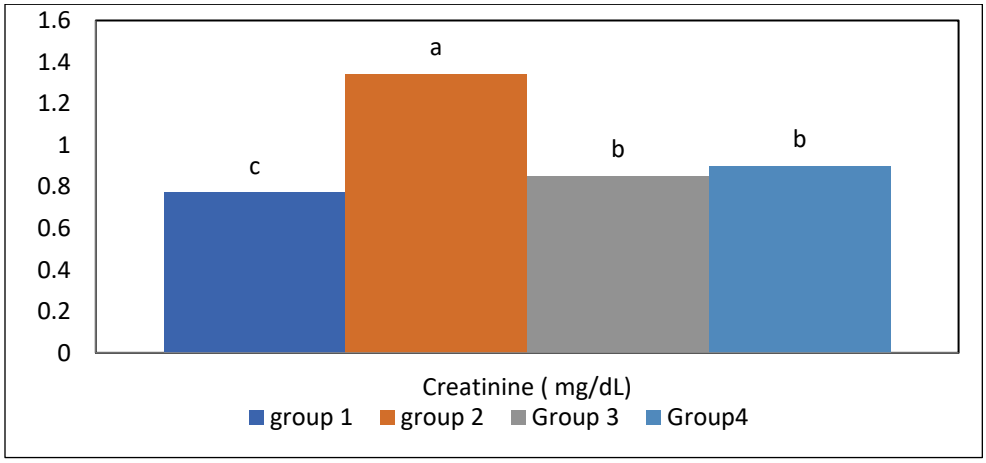

Figure 5: Effect of Enterococcus Faecium (15 x 10^9 CFU /kg) on creatinine (mg/dl) of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

Table 4: Effect of Enterococcus Faecium M74 (15x 10^9 CFU /kg) on immunological Parameters of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers
(mean ± SE) (n = 5).

Groups	IgM mg/ml	IgA mg/ml	TNF (mg/dl)	IL4 (pg/ml)	CD+4	CD+8
1st Control (-Ve)	1± 0.20 c	1.02±0.12 d	2.52±0.3c	220.5±1.0 b	600.5±1.0c	180.5±1.0 c
2nd Control (+Ve)	2.8±0.01 c	3.6±0.02 c	5.61±0.4 a	230.3±1.8 a	1195.3±1 a	900.3±1.8 a
3rd Group	10±2.32 a	13±0.13 a	4.88±0.1 ab	230.7±1.8 a	980.7±1.8ab	450.7±1.8b
4th Group	9.1±1.28 a	11.1±0.16ab	4.38±0.16 ab	228.4±1.72 ab	960.4±1.7ab	420.4±1.7b

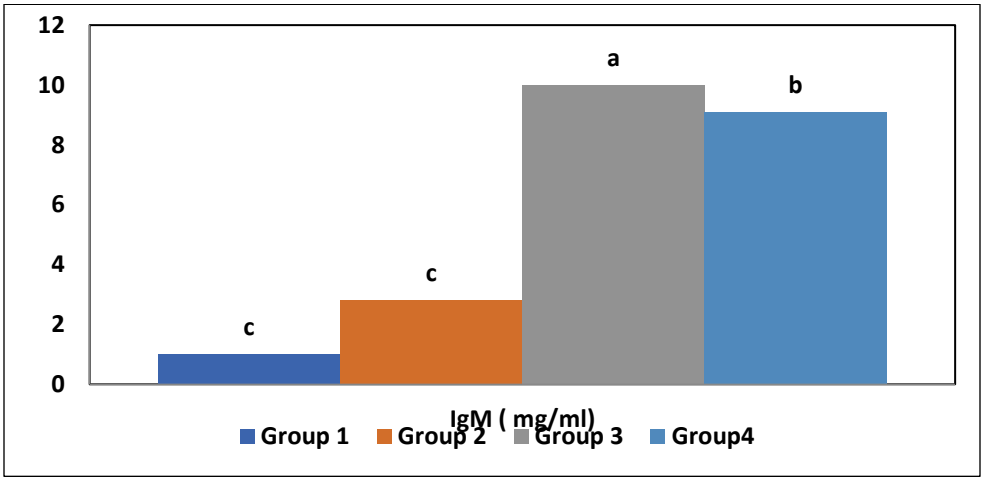

Figure 6: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg) on IgM (mg/ml) of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

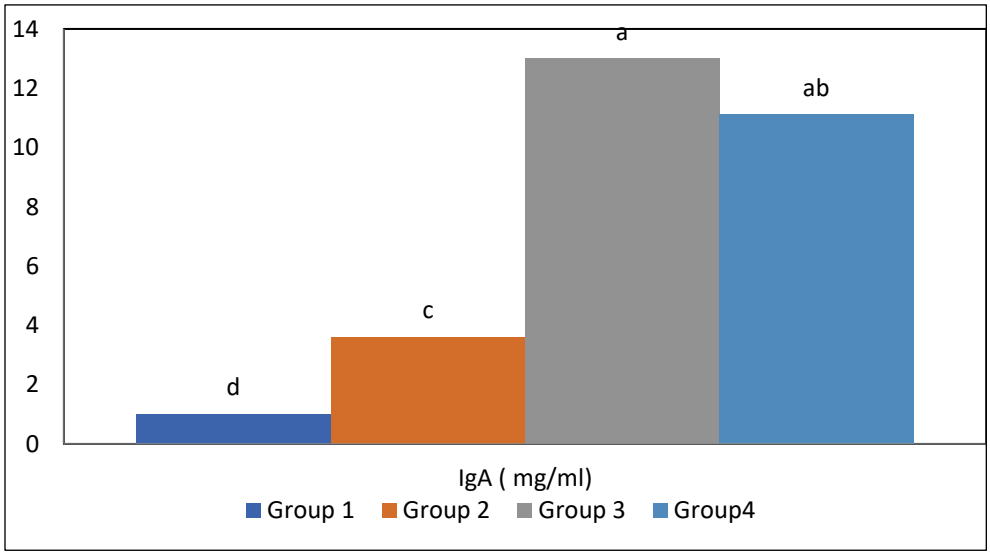

Figure 7: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg)on IgA (mg/ml) of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

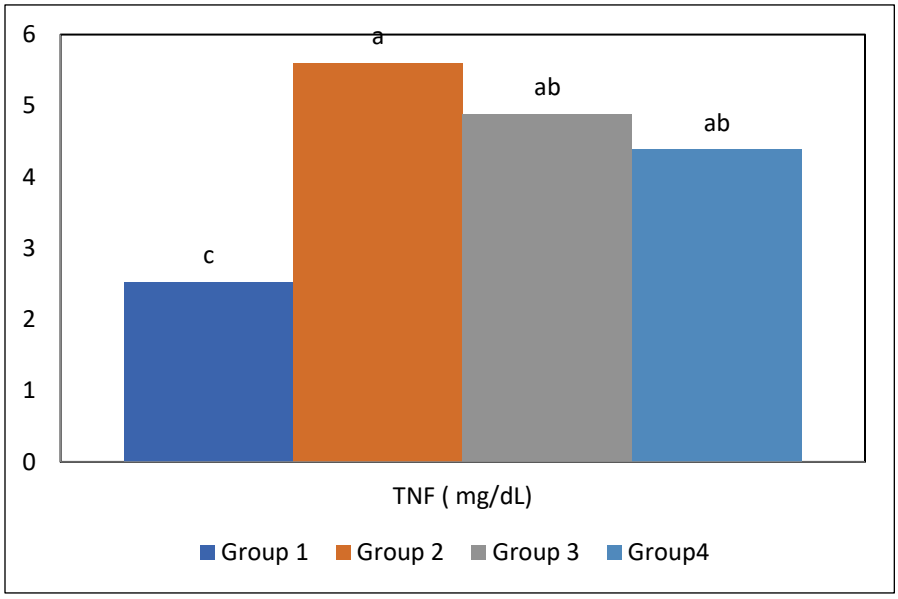

Figure 8: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg on Tumor necrotic factor (mg/ml) of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

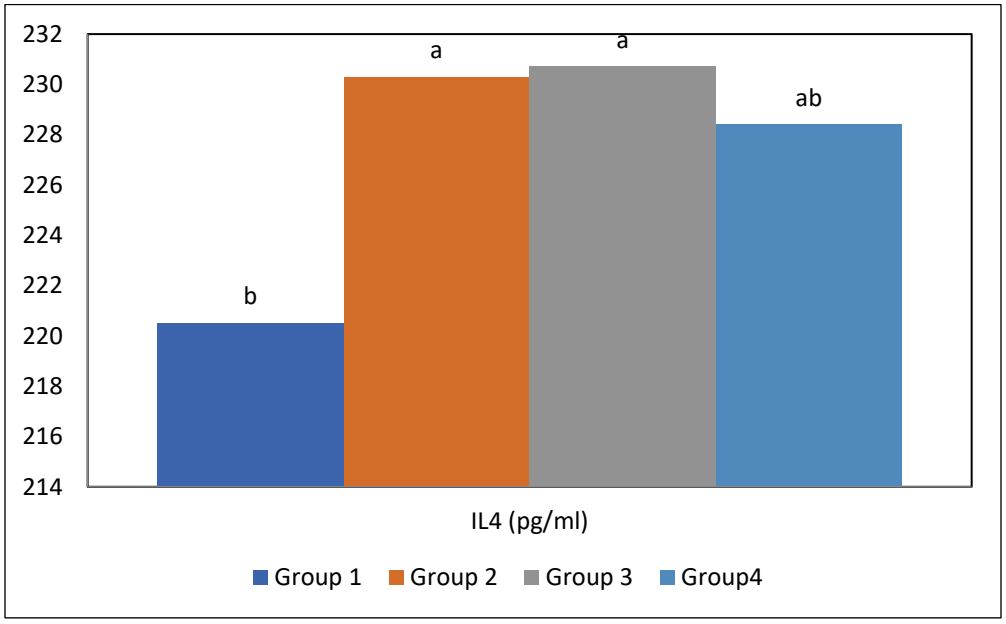

Figure 9: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg) on IL4 (pg/ml) of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

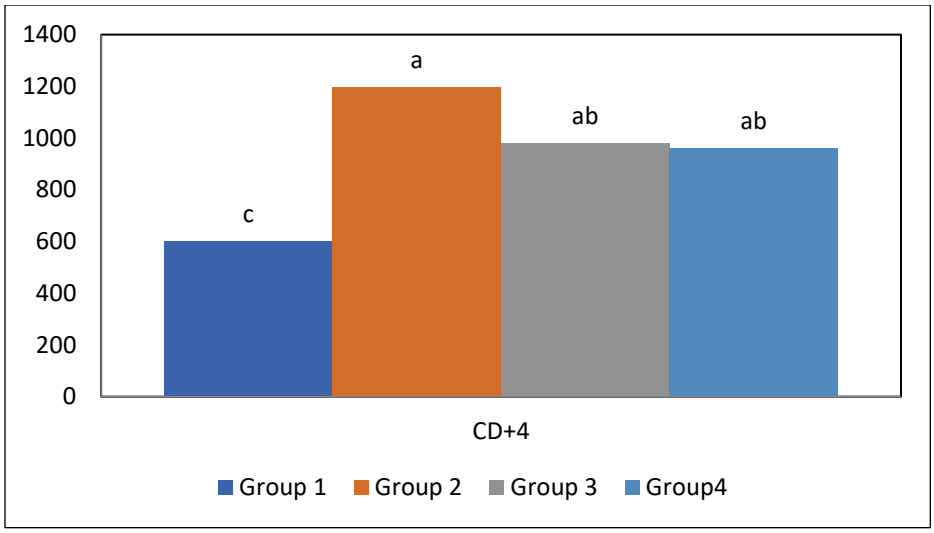

Figure 10: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg on CD+4 of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

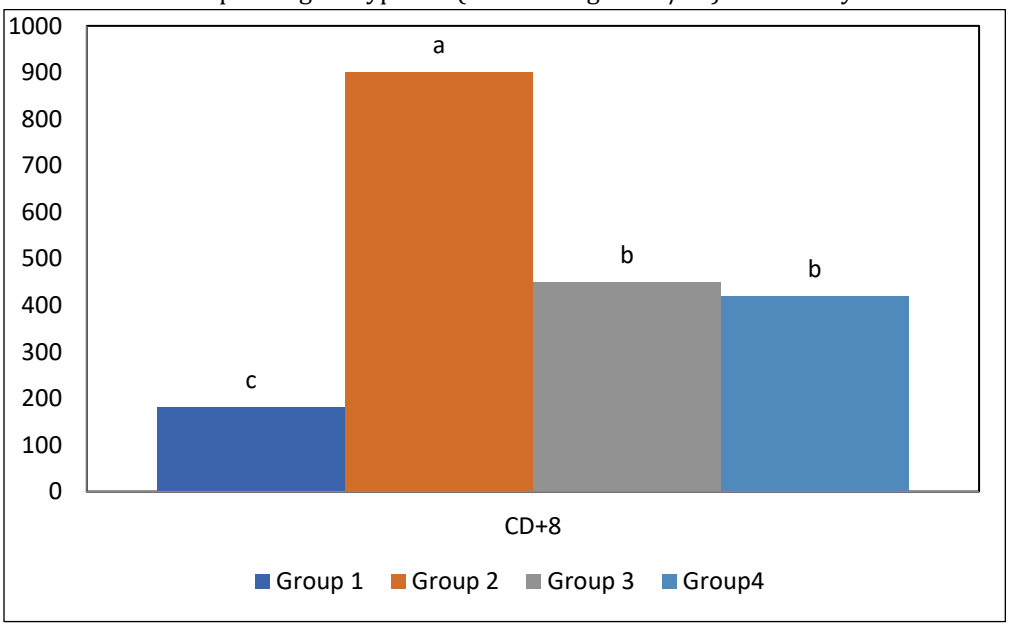

Figure 11: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg) on CD+8 of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

Table 5: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg) on growth Performance parameters (initial BWT, final BWT [FBWT], body weight gain [BWG], average feed intake [AFI], feed conversion ratio [FCR] of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

Groups	**Final BWT [FBWT] (gm)**	**Body weight gain[BWG]** (gm)	**Average feed intake [AFI]** (gm)	**Feed conversion ratio [FCR]**
1st Control (-Ve)	**1,676± 47.66^{b}**	**1,631 ±47.66^{b}**	**2,475 ±5.01^{ab}**	**1.51^{b}**
2nd Control (+Ve)	**1,465 ± 45.67^{b}**	**1,430 ±45.67^{c}**	**2,495 ±6.43^{a}**	**1.74^{a}**
3rd Group	**1,904 ± 39.79^{a}**	**1,880 ±39.79^{a}**	**2,310 ±5.24^{b}**	**1.3^{c}**
4th Group	**1,764±34.34^{a}**	**1,720 ±39.79^{a}**	**2,260 ±5.24^{b}**	**1.31^{c}**

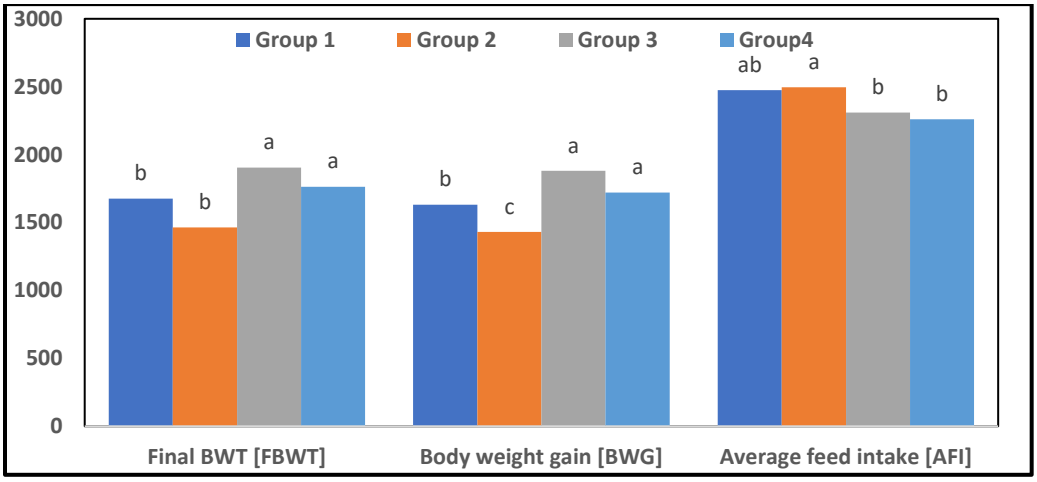

Figure 12: Effect of Enterococcus Faecium M74 (15 x 10^9 CFU /kg) on growth Performance parameters (initial BWT, final BWT [FBWT], body weight gain [BWG], average feed intake [AFI] of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

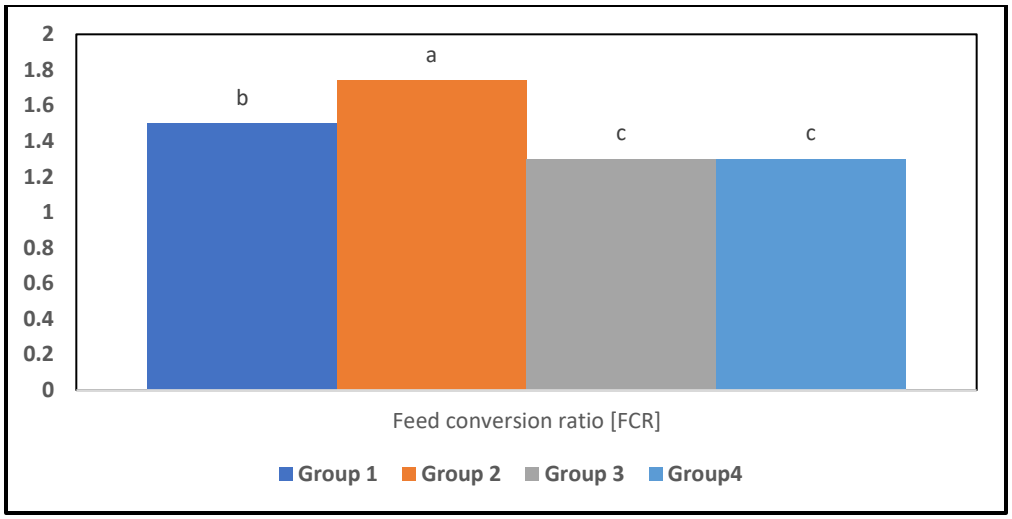

Figure 13: Effect of Enterococcus Faecium M74 (15x 10^9 CFU/kg) on feed conversion ratio [FCR] of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

Table 6: Effect of Enterococcus FaeciumM74 (15 x 10^9 CFU /kg) on Villus Height & Crypt Depth of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

(mean ± SE) (n = 5).

Groups	**Villus Height µm**	**Crypt Depth µm**	**Villus Height / Crypt Depth Ratio**
1st Control (-Ve)	651.67±56.51^b	97.93±16.47^c	6.65^b
2nd Control (+Ve)	580.67±64.46^b	89.65±7.94^a	5.83^c
3rd Group	828.33±56.37^a	107.97±24.34^b	7.73^a
4th Group	676.18±68.73^b	92.81±5.87^c	7.28^a

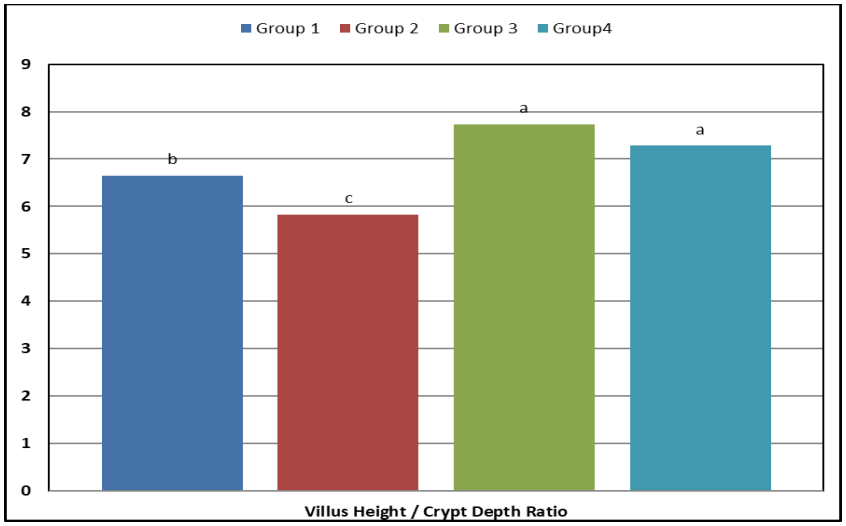

Figure 14: Effect of Enterococcus FaeciumM74 (15 x 10^9 CFU/kg) and Lincomycin (10 mg/liter) on Villus Height & Crypt Depth of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

Table 7: Effect of Enterococcus.Faecium M74 (15 x 10^9 CFU /kg) on Histopathological lesion scoring of intestines of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers. (mean ± SE) (n = 5).

Groups	**Histopathological lesion scoring of intestines**	
	Degeneration	**Congestion**
1st Control (-Ve)	0.2 ± 0.13^d	0 ± 0^d
2nd Control (+Ve)	3.4 ± 0.22^a	3.2 ± 0.2^a
3rd Group	0.6 ± 0.22^c	0.8 ± 0.13^c
4th Group	0.7 ± 0.18^c	0.9 ± 0.26^c

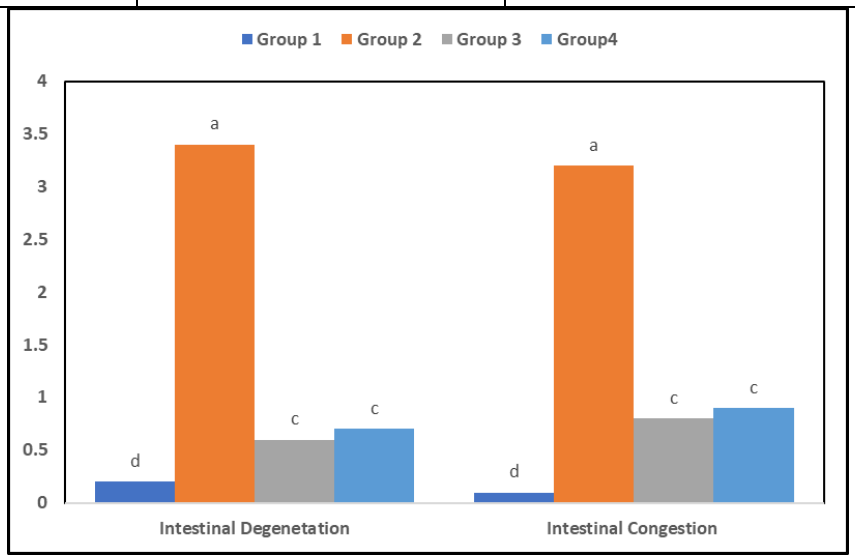

Figure 15: Effect of Enterococcus.Faecium M74 (15 x 10^9 CFU/kg) on Histopathological lesion scoring of intestines of prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

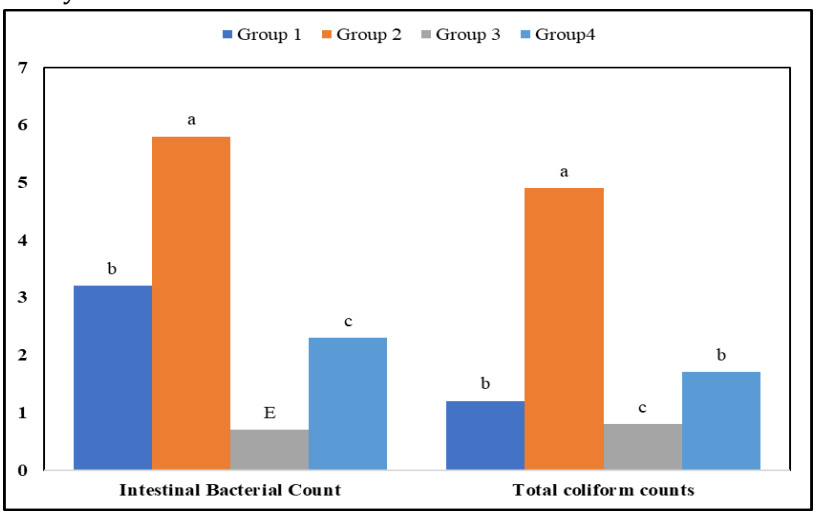

Figure 16: Effect of Enterococcus.Faecium (15x10^9 CFU/kg) on Re-isolation of C. perfringens and Intestinal Bacterial Count of normal prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers.

Table 8: Effect of Enterococcus.Faecium (15x10^9 CFU/kgon Re-isolation of C. perfringens and Intestinal Bacterial Count of normal prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers (mean ± SE) (n = 5).

Groups	Intestinal Bacterial Count CFU/mL	Total coliform counts CFU/Ml
1st Control (-Ve)	3.2 ± 0.1^b	1.2 ± 0.1^b
2nd Control (+Ve)	5.8 ± 0.1^a	4.9 ± 0.1^a
3rd Group	0.7 ± 0.21^e	0.8± 0.19^c
4th Group	2.3± 0.19^c	1.7± 0.23^b

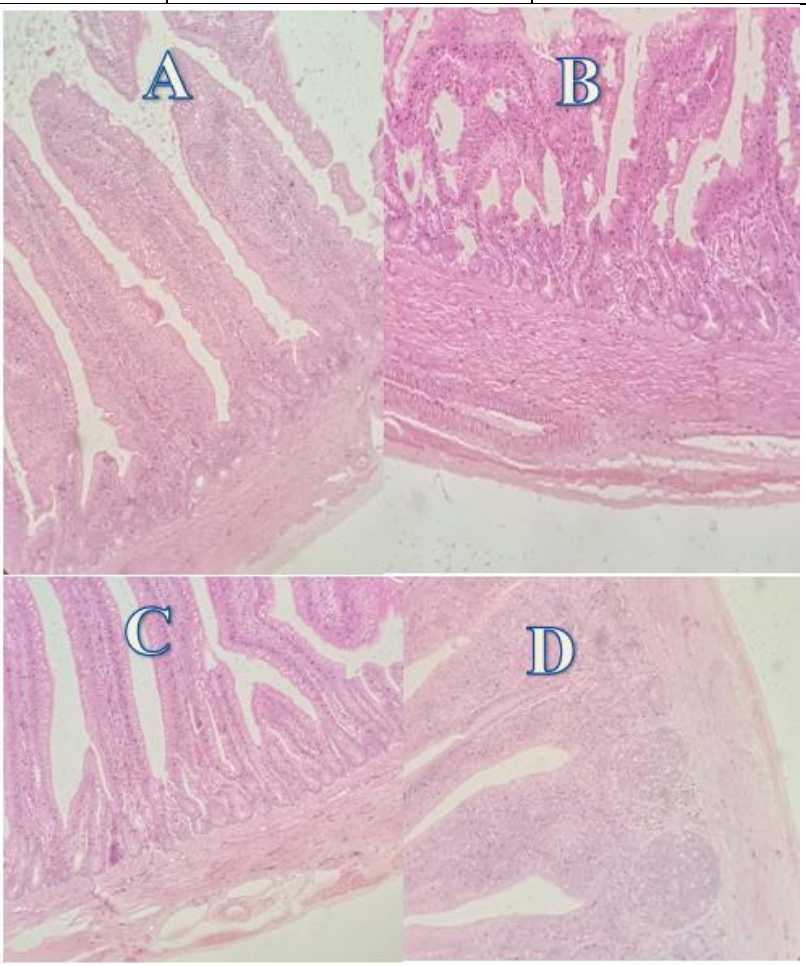

Figure 17: Effect of Enterococcus.Faecium (15 x 10^9 CFU /kg) on chicken's intestine of normal prophylaxis and treated groups after infection with clostridia perfringens type "A" (1.9×10^9 organism/ml) on 19th day of broilers

Along with increasing villi length, there were non-significant degenerative changes in the anatomy of all groups, the cecum examination showed an increase in the lymphatic element after infection. Histopathological examination and lesion scoring; Samples were randomly chosen and averaged. Sample treatments were blinded for lesion scoring (Score scale: 0 = normal; 1 ≤ 25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100%). **[39]** reported that dietary supplementation of E. faecium increased gut microvilli and influenced immune organ development and

mucosal structure chickens. They also showed that dietary supplement of E. faecium had a pronounced effect on genes expression related to the intestinal tissue development and epithelium maturation, and genes responsible for digestion and absorption of nutrients. Regarding the intestinal histopathological lesion scoring (degeneration and congestion), prophylactic and therapeutic groups showed significant improvement of lesions compared with positive control group. C. perfringens infection markedly increased gut gross pathological and histopathological lesion scores, promoted liver C. perfringens invasion, and elevated jejunal mucosal lysozyme activities [39], and it was agreed to our results. **[44]** also reported that dietary supplementation of E. faecium increased the villus height in jejunum and ileum of broilers, which could enhance the digestive and absorptive capacity of the intestinal tract because of a higher absorptive surface area, up-regulation of brush border enzymes and enhancing nutrient transport mechanisms. Both C. perfringens and total coliform counts were significantly the lowest in the prophylactic and treated groups. Furthermore, prophylactic, and treated groups, respectively showed significant improvement in the count when compared positive control group. The relative length of the jejunum and ileum in the control group on day 35 was significantly shorter than that in other groups. Adding E. faecium significantly reduced the relative length of the cecum on day 21 and that agreed by **[50]** who found probiotic E. faecium increased villus height and decreased the crypt depth in jejunum, compared with the control group. Therefore, it seems that dietary E. faecium probiotic could play a positive role in the small intestinal morphology of broilers

4. Conclusion

E.Faecium (M74) was able to significantly lesser the C. perfringens – induced Necrotic enteritis and its consequences on the growth performance, hematological picture, liver and kidney functions, imuunological profile in addition to its positive impacts on the bacterial burden and histopathological picture of the gut intestine of the infected broilers. Taking into account the antibiotic resistance problem, E.Faecium (M74) could be used as one of the best alternative to antibiotics in dealing with C. perfringens (NE) with special references to the preference of prophylactic regimen than the therapeutic one.

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