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The Impact of Sodium-Butyrate Microencapsulated in Balm Fat in Impedance of Colisepticaemia in Broiler Chickens

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Abstract: *The objective of this study is to determine the effect of sodium butyrate microencapsulated in balm fat (NaB) on performance, clinical and pathological pictures, virulence gene expression and electromicroscopy of E. coli infection (colisepticaemia) in broiler chickens. One day-old chickens (n=90) allotted into 3 equal groups (1-3) with 3 replicates of 10 each were used in this study. Duration of the experiment extended from one day of age up to slaughter (35 days). The birds fed mash diet for 3 phases: starter (1-14 days), grower (15-28 days) and finisher (29-35 days). Birds of group1 (T) fed on NaB in the following dietary levels; starter diet: 1 kg/ton, grower diet: 0.5 kg/ton and finisher diet: 0.25 kg/ton. Chickens of groups 2 (PC) and 3 (NC) fed on plain ration. For experimental induction of colisepticaemia; experimented chickens of groups T and PC were individually infected by crop gavages with 4.5×10^{8} CFU/ml/bird of E. coli serogroup O78 in PBS for 2 successive days. Birds of group 3 were kept without infection.*

NaB supplementation to colisepticaemic broiler chickens improved performance, reduced virulence genes by cPCR and rt-qPCR analysis. It also reduced both gross and histopathological lesion scores, and altered electromicroscopic profile of the inoculated E. Coli.

Conclusively; NaB treatment could play a positive role in impedance of colisepticaemia in Broiler Chickens.

Key words: *Sodium butyrate microencapsulated in balm fat, chicken performance, colisepticaemia, E. coli electromicroscopy, E. coli virulence genes.*

Abbreviations: *C. perfringens=Clostridium perfringens, FCR=Feed conversion ratio, BW=Body Weight, BWG= Body Weight Gain, PC=Positive control, FI=Feed intake, NC=Negative control, GIT=Gastrointestinal tract, AGP=Antibiotic growth promoters, PI=Post infection, BF=Bursa of Fabricus, T group= supplemented with diet contained Sodium butyrate, PBS =Phosphate buffered saline, TEM=Transmission Electron Microscope, RCMB=Regional Center for Mycology and Biotechnology, GALT=Gut associated lymphoid tissues, CFU=Colony forming units, APEC= Avian pathogenic Escherichia coli, SCFA= Short chain fatty acids.*

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I. Introduction

Avian collibacillosis is a complex syndrome characterized by multiple organ lesions that is a major cause of morbidity, mortality, and condemnation of carcasses in the poultry industry **(Allan *et al.*,1993),** specially colisepticemia **(Ewers *et al.*, 2003).** Antibiotics were widely used in control of *E. coli* in poultry but after long time of usage antibiotic resistance has been developed **(Blanco *et al.*, 1997).** Antibiotic resistance for *E. coli* developed as a result of the misuse of antibacterial drugs resulting in appearance of resistant strains **(Lerbech *et al.*, 2014).** Antibiotic resistance became a widely distributed problem in human and veterinary field **(Habrun *et al.*, 2010).** AGP have been used for decades to improve poultry performance with low cost of implementation and ease adding to feed and water (**Fernandez-Rubio et al., 2009**). However, currently concern about possible antibiotic residues and resistance has arisen restrictions of antibiotics use in poultry **(Jan et al., 2007; Saberfar et al., 2008).** Because of this fact, industry and researchers have had to look for natural alternatives such as oily plant extracts (**Mitsch *et al*., 2004),** yeast cell walls **(Gajewska *et al*., 2012)**, probiotics **(Dankowiakowska *et al*., 2013; Patterson and Burkholder, 2003)** and prebiotics **(Hajati and Rezaei, 2010).** As the issue of controlling poultry enteropathogens without the use of AGP is becoming a big challenge; natural alternative concepts based on natural ingredients for GIT integrity and antibacterial action became highly commendable **(Awaad *et al.*, 2014).** Because of their pH-reducing and antimicrobial effects; acidifiers appeared as one of the most feasible and functional alternative to AGP **(Lückstädt, 2003).** SCFA are promissory and potentially alternatives to AGP **(Adil *et al.*, 2011).** The auspicious effect of acidifiers over the organism is due to

the better adhesion of the lactic acid bacteria to GIT epithelium in comparison with the pathogenic bacteria, and stopping the implementation of those bacteria over the mucous membranes of the intestine **(Awaad *et al.*, 2014).** Butyrate (SCFA) is the most important energy source of the colonocytes (**Roediger, 1982**). It regulates the proliferation and differentiation of the GIT epithelium (**Gálfi, and Neogrády, 2002)** Butyrate improves the balance of the intestinal microflora which can influence the health of the host animal or the human host (**Candela *et al.*, 2010).**

Due to butyrate selective antimicrobial action on most enteric pathogens **(Fernández-Rubio *et al.*, 2008),** the present work was dedicated to elucidate the effect of supplementation of NaB encapsulated in palm fat on experimentally induced colisepticaemic broiler chickens with determination of its possible effect on APEC virulence genes and electromicroscopic profile as well.

II. Materials And Methods

Sodium butyrate (NaB):

NaB encapsulated in palm fat which is fat coated sodium of alimentary fatty acid produced by NUTRI-AD International, Belgium was used in this trial. Its ingredient is n-Butyric acid sodium salt 30±2%. NaB supplementation in ration was given at a dosages according to the manufacturer's recommendations (starter diet: 1 kg/ton, grower diet: 0.5 kg/ton, and finisher diet: 0.25 kg/ton).

Experimental birds:

One-day-old male Arbor Acres Plus broiler chickens (n=90) were assigned at random into 3 equal experimental groups (1-3) of 30 birds each assigned into 3 replicates. The birds kept on deep litter pens (2*1 m) with 10 birds per replicate. All chickens were vaccinated against ND at the 7^{th} and 21^{st} day of age using live Hitchner B1 and La Sota strain vaccines, respectively. Live infectious bursal disease vaccine (IBD 228-E vaccine) was administrated at the 14^{th} day of age. Drinking water method was used as a route of administration of the live vaccines. On 10^{th} day of age; 0.5 ml inactivated avian influenza vaccine (H5N1) was injected subcutaneously in the back of chickens neck. The diets used were formulated to meet the nutrient requirements of the broiler chickens during starter, grower, and finisher periods according to the National Research Council. Semduramicin was added to all rations at a concentration of 25 ppm as a coccidiostat. No antibiotics were administrated in water or feed, for the whole experimental period (35 days). Birds had free access to feed and water.

Experimental design :

Birds of 1^{st} group (T group) supplemented with diet contained NaB. While those of 2^{nd} (PC) and 3^{rd} (NC) groups fed on standard diet without NaB supplementation . At d 14 and 15 of age; chickens of groups 1 and 2 were inoculated by crop gavages with 4×10^{8} CFU/ml/bird of *E. coli* serogroup O78 in PBS for 2 successive days. The used strain of *E. coli* has been isolated from cases of chicken colisepticaemia **(Morsy, 2015).**

Measured parameters:

1-Productive performance :

Chicken performance response variables were determined according to **North (1984).** Weekly individual BW was measured on all birds. FI (g/d/bird), FCR (g feed/g live BWG), and mortality rate were measured for each replicate. Dead birds were weighed to include their weights in the feed conversion estimates.

2-Gross lesion scoring:

Necropsy was adopted on 6 randomly selected birds (2 birds/replicate) of T and PC groups at the end of the experiment (35 days) for demonstrating macroscopic lesion scoring of colisepticemia. Lesion scoring was performed using the following scale after **Morsy (2015):**

Airsacculitis:0=no lesions, 1=thickened, 2=frothy, 3= opaque, 4= caseated..

Perihepatitis:0=no lesions, 1=thickened, 2=thin fibrinous layer, 3=thick and extensive layer of fibrinous exudates, 4=thick layer of caseated material.

Pericarditis:0= no lesions, 1= thickened (cloudy), 2= severe thickening with myocarditis, 3=filled with watery or thick yellow (milky) pericardial fluid, 4= completely cirrhotic heart.

Salpingitis:0= no lesions, 1=dilated oviduct with water content, 2= dilated oviduct with caseous exudates.

3-Related organ weight:

At the end of experiment (35^{th} day), 6 birds from each group (2 birds/replicate) were slaughtered and the internal organs including liver, heart, gizzard, intestine, thymus, spleen and BF were weighed. The related

organ weights were calculated by the following formula: Related organ weight=organ weight (g) × 100 ÷ Live body weight (g).

4-Histopathological assay:

Specimens including; heart, liver, spleen, cecum, thymus glands, BF and cecal tonsils were collected from randomly sacrificed 3 chickens/group (1 bird/replicate) at the end of the experiment, fixed in 15% buffered formalin and paraffin-embedded sections were stained with Hematoxylin and Eosin **(Bancroft *et al*. 1996)** and scored for histopathological lesions according to the method described by **Rosales *et al.* (1989)**.

5-Gene expression analysis:

DNA from the re-isolated *E. coli* strains from heart of infected NaB treated and untreated birds was extracted using commercially available kit, QIAamp DNA Mini Kit (Qiagen – Germany – GmbH), (Cat. # 51304) according to the manufacturer's instructions. The virulence genes were amplified using their specific oligonucleotide primers for *E. coli* virulence genes supplied from metabion (Germany) or Biobasic (Canada) using cPCR. Oligoneucleotide primers sequences, target gene, amplicon sizes and cycling conditions during cPCR are illustrated in **Table 4**. The amplification products were photographed by agarose gel electrophoreses **(Sambrook *et al*., 1989)**. Gel documentation system and the data were analyzed through computer software **(Livak and Schmittgen, 2001)**. The results clarified that the studied *E. coli* strain contained only the following 6 virulence genes; *iss, iutA, iroN, papC, ompA and fimH*. Accordingly; rt-qPCR was performed to estimate the fold change values of different genes of *E. coli* re-isolated from NaB treated birds related to the genes of *E. coli* re-isolated from PC group. Amplification curves and CT values were determined by the stratagene MX3005P software to estimate the variation of gene expression on the RNA of different samples, the CT of each sample was compared with that of the control group according to the "ΔΔCt" method stated by **Yuan *et al*. (2006**).

6-Electron Microscopy Examination:

At d 17 of age, liver specimens of 3 birds of T and PC groups were subjected to *E. coli* re-isolation. The re-isolated strains and the originally used strain were subjected to TEM examination for detection of any morphological alteration. The re-isolated *E. coli* were streaked on agar plates at 37°C and single colonies from freshly streaked agar plates were incubated at 37°C to mid-logarithmic phase. Samples were processed according to **Bozzola and Russell (1999).** Briefly; specimens were fixed by immersion in 1% potassium permanganate solution for 5 min at room temperature. Fixed specimens were then washed in distilled water three times (15 min for each step). Specimens were then dehydrated using a graded series of ethanol (30, 50, 70, 90, and 100%). After dehydration, specimens were maintained in pure propylene and then transferred to epoxy embedding resin. After that, these specimens were placed in capsules that contained an embedding medium and heated at 60°C for about 48 h. Specimen sections were double stained in uranyl acetate and lead citrate. The stained sections were examined using a JEM-1010. TEM (JEOL Ltd, Tokyo, Japan) was operated at accelerating voltage of 80 KV at the electromicroscopy unit of RCMB; Al-Azhar University, Cairo, Egypt.

Statistical analysis:

One-way analysis of variance was adopted using SAS software general liner models procedure **(SAS Institute, 2000).** The main factor was NaB supplementation as a mean effect. Mean values assessed for significance using Duncan's multiple range tests. Statements of statistical significance are based upon $P \leq 0.05$.

III. Results And Discussion

The mortality rate reached 10% in *E. coli* infected groups (T and PC). Our results showed negative effect of NaB supplementation on BW, BWG and FCR of chickens in the first two weeks. However, from the 3rd week until the end of experiment the FI was reduced and the FCR improved with significant overall FCR at 5th week of age ($P \leq 0.05$) **(Table 1).** Our obtained results could be explained as the functionality of intestines of day old chicks and the activity of the digestive enzymes are not sufficiently developed (**Ravindran, 2003**). Accordingly; fat-coated NaB did not emulsified and released completely **(Noy and Sklan, 1994; Leeson and Summers, 2001).** Thus the digestion of feed and absorption of nutrients did not completely accomplished, that lead to lowered BWG and poor FCR during the starter period of chicken growth in response to fat-coated NaB and the carry-over effects might affect overall performance of birds **(Ahsan *et al.*, 2016)**. **Table 2** compares the organ body weight ratio of T and PC groups including heart, liver, gizzard, intestine, BF, thymus glands and spleen. There was a significant increase in liver and thymus glands together with numerical increase in other organs in T group vs. PC group ($P \leq 0.05$).

Many investigators recorded that NaB supplementation during starter phase of diets had no effect on average BWG, FI or FCR (**Leeson *et al.*, 2005; Hu and Guo, 2007; Antongiovanni *et al*., 2007; Liu, 2009; Mahdavi and Torki, 2009; Aghazadeh *et al.*, 2012; Wu et al., 2018**). Contradictory findings have been

mentioned by other investigators who reported that partially coated NaB in broiler diet significantly increased the FI and BWG (**Mansoub, 2011; Hernandez *et al.*, 2013; Chamba *et al.*, 2014**). Parallel findings to our results had been obtained by many investigators in studying the efficacy of Na-butyrate encapsulated in palm fat in chickens under stress (challenged with *E. coli* or *Clostridium perfringens)* **(Taherpour *et al.*, 2009; Panda *et al.*, 2009; Smulikowska *et al.*, 2009; Zhang *et al*., 2011; El-Sawy *et al.*, 2015; Eshak *et al.*, 2016)**. The positive effect of NaB encapsulated in palm fat on performance variables could be used alternatively to AGP which confirms the results of **Awaad *et al.* (2011)** who reported that usage of protected organic acids, in poultry nutrition, could be an efficacious tool to replace AGP and showed that their use is considered a novel and effective alternative to antibiotics in broiler chickens. **Gauthier (2002)** concluded that organic acids could be a powerful tool in maintaining the health of poultry GIT, thus improving their production performances.

Macroscopic and microscopic lesion scores of colisepticaemia in T and PC groups are illustrated in **Table 3**. Significant reduction in macroscopic lesion score of airsacculitis , perihepatitis, pericarditis and peritonitis has been obtained in T group vs. PC group with an average lesion score of "0.04" in T group vs. "1.96" in PC group ($P \leq 0.05$). Histopathological alterations involving parenchymatous and immune organs including polyserositis of spleen, liver and pericardium, beside the immune suppression that characterized by lymphocytic depletion of splenic, bursal and thymic follicles with fibrinous exudation in splenic parenchyma revealed significant reduction of these lesions in T group vs. PC group with an average lesion score of "0.55" in T group vs. "1.94" in PC group respectively ($P \leq 0.05$). Histopathological lesions are shown in **Figs 1 and 2**.The liver exhibited centrilobular hepatocellular necrosis that could be resulted from severe myocardial weakness and loss of contractile myocardial tone caused by severe myocarditis induced by *E. coli;* with the overall results passive congestion in liver and severe centrilobular hypoxia induced by passive hepatic congestion resulted in necrosis in the centrilobular hepatocytes. NaB possed anti-inflammatory effect with modulation of cell-mediated immunity represented by the disappearance of polyserositis and myocarditis. Adminstration of NaB resulted in milder microscopic lesions, significant decrease in thymic cortical depletion and BF lymphoid depletion. Moreover; it had marked immunostimulatory effect of cecal tonsils that showed lymphoid activation and mitosis of lymphoid elements that accord with those reported by **Vanhoutvin *et al.* (2009)** who mentioned that butyric acid or its sodium salt mediated the immune response. Our results are on line with findings of **Sikandar (2017)** who reported on the greater sized thymus medulla and germinal center area in spleen of NaB treated chickens with the increasing trend of observed bursal parameters that may effectuate the systemic immune systems in broilers. Additionally, **Friedman and Bar-Shira, (2005)** reported that butyric acid is considered as the prime enterocyte energy source, necessary for the development of GALT. Our recorded lesion scores in colisepticaemic chickens supplemented with NaB linked well with observations of **Kwan and Ricke (2005)** who referred that the highest bactericidal efficacy of NaB against the acid-intolerant species such as *E. coli* and *Salmonella spp.* is the selective stimulation of beneficial gut bacteria. **Van Deun *et al.* (2008)** mentioned that NaB is a selective bactericidal agent due to its activity of lowering the pH of crop and gizzard and upper part of intestine, controlling harmful bacteria such as Salmonella spp., *E. coli* and *Campylobacter jejuni*. Eventually; our obtained results might attributed to the fact that organic acids have properties of lowering the intestinal pH, enhancing protein digestion, influencing intestinal cell morphology, stimulating pancreatic secretions, acting as a substrate for the intermediary metabolism, improving the retention of many nutrients (e.g. chelating minerals), increase intestinal integrity and influencing the electrolyte balance in the feed and intestine **(Gauthier, 2002; Zhang et al., 2011).**

For gene expression analysis; cPCR has been adopted to determine the nine virulence genes of *E. coli* (*iss, iutA, iroN, tsh, papC, KpsMTII, ompA, hly and fimH).* The studied *E. coli* strain (serogroup O78) proved to have only 6 genes *(iss, iutA, iroN, papC, ompA, fimH).* Performing rt-qPCR to estimate the degree of gene expression downregulation in the re-isolated *E. coli* colonies from heart of infected T and PC chicken groups was performed. The relative expression of target genes was determined compared to a standard reference gene (*16S rRNA*), and the method utilized for the analysis of relative changes in the mRNA expression of a target genes was that of **Livak and Schmittgen (2001).** Our final results revealed that NaB resulted in downregulation of all tested virulence genes *(iss, iutA, iroN, papC, ompA, fimH)* of *E. coli* re-isolated from T group vs. that re-isolated from PC group with a range of 0.1571 to 0.3610 **(Table 5 and Figs. 3, 4).** *E. coli* is a commensal bacterium of the bird's intestinal tract, but it can invade different tissues resulting in systemic symptoms (colibacillosis). This disease occurs only when the *E. coli* infecting strain presents virulence factors (encoded by specific genes) that enable the adhesion and proliferation in the host organism. **Rodriguez-Siek et al., (2005)** demonstrated that 9 (*cvaC, iroN, iss, iutA, sitA, tsh, fyuA, irp-2* and *ompT*) of a total of 38 studied genes occur more frequently in APEC. **Ahmed *et al.* (2013)** characterized the virulence genes of APEC isolated from septicemic broilers in Egypt at the molecular level and found that among 91 non-repetitive *E. coli* isolates; 73 (80.2%) carried three or more of the APEC virulence genes *iroN, ompT, iss, iutA,* and *hlyF*. **Hussein *et al.* (2013)** In their study on 219 *E. coli* isolates from 84 poultry flocks in Egypt reported that more than 90% of the

total APEC examined possessed *iroN*, *ompT*, *hlyF*, *iss*, and *iutA*. Therefore; one of the most common mechanisms of NaB as an antibacterial agents is to affect on the expression of these genes.

Transmission electron microscopy (TEM) examination of *E. coli* reisolated from liver of broiler chickens (with or witout NaB treatment) showed signs of induced cells damage and reduction in size **(Fig. 5).** The use of butyric acid in poultry nutrition is quite well accepted due to the reduction of pH that limits the development of pathogens and helps in the digestion of proteins, this could be attributed to its mode of action as: Once NaB reaches the stomach of the bird, it quickly release sodium ion and, due to the low pH, butyrate is rapidly converted to the undissociated form, termed the butyric acid. This form is the one responsible for the antimicrobial activity, as the butyric acid is strongly lipophilic and can diffuse across the membranes of bacteria (**Jankowskia *et al.*, 2012)**. Inhibition of pathogenic bacteria by organic acids is by penetration (non-ionized) into lipophilic bacterial cell wall, dissociation at neutral cytosolic pH and releases anions and protons that cause lethal accumulation of anions affecting purine bases **(Choi et al., 2000)**, denatures essential enzymes **(Roe et al., 2002)** and results in bacterial cell death. **Awaad *et al.* (2016)** on supplementation of NaB to *Salmonella enteritidis* infected broiler chickens reported that TEM of samples isolated from treated group showed transparent lipids like bodies, while normal features were observed in the untreated group.

In conclusion; supplementaion of NaB microencapsulated in palm fat to colisepticaemic broiler chickens resulted in improving in their productive performance variables and reduction of maroscopic and microscopic lesions as well. It had a bacteriocidal action against *E. coli* as it downregulated its virulence genes and altered its morphology. Eventually; NaB treatment could play a positive role in impedance of colisepticaemia in Broiler Chickens.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests

References

[1]. Adil S., G. Banday, M. Bhat, M. Salahuddin, T. Ahmad Raquib and S. Shanaz, 2011. Response of broiler chicken to dietary supplementation of organic acids. J. Central Eur. Agric., 12: 498-508.

[2]. Aghazadeh, A. M. and TahaYazdi, M. (2012). Effect of butyric acid supplementation and whole wheat inclusion on the performance and carcass traits of broilers. South Africa African Journal of Animal Sciences, 42 (3): 241-248.

[3]. Ahmed, A. M.; Shimamoto, T.; Shimamoto, T.(2013): Molecular characterization of multidrug-resistant avian pathogenic Escherichia coli isolated from septicemic broilers.International Journal of Medical Microbiology; 2013. 303(8):475-483.

[4]. Ahsan, U., Cengiz, Ö, Raza, I., Kuter, E., Chacher, M., Iqbal, Z., . . . Çakir, S. (2016). Sodium butyrate in chicken nutrition: The dynamics of performance, gut microbiota, gut morphology, and immunity. World's Poultry Science Journal, 72(2), 265-275. doi:10.1017/S0043933916000210

[5]. Allan, B.J., Van den Hurk, J. V. and Potter, A.A. (1993). Characterization of E. coli isolated from cases of avian colibacillosis. Can. J. Vet. Res.; 57(3): 146-151.

[6]. Antongiovanni, M.; Buccioni, A.; Petacchi, F.; Leeson, S.; Minieri, S.; Martini, A. and Cecchi, R. (2007). Butyric acid glycerides in the diet of broiler chickens: effects on gut histology and carcass composition. Italian Journal of Animal Science, 6: 19-25.

[7]. Awaad M. H. H., A. Atta, Basma Shalaby, H. B. Gharieb, M. Elmenawey, K. Madian, Eman, F. Farag and Amal, N. Awad (2014). The Efficacy of Na-Butyrate Encapsulated in Palm Fat on Experimentally Induced Necrotic Enteritis and Enumeration of intestinal resident Clostridium perfringens in Broiler Chickens. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372.Volume 7, Issue 1 Ver. III, PP 40-44 www.iosrjournals.org www.iosrjournals.org.

[8]. Awaad M. H. H., A. M. Atta, M. Elmenawey, B. Shalaby, G. A. Abdelaleem, K. Madian, K. Ahmed, D. Marzin, G. Benzoni and D.K. Iskander (2011). Effect of Acidifiers on Gastrointestinal Tract Integrity, Zootechnical Performance and Colonization of Clostridium Perfringens and Aerobic Bacteria in Broiler Chickens. Journal of American Science, 7(4):618-628]. (ISSN: 1545-1003). http://www.americanscience

[9]. Awaad M.H.H., Wafaa A. Abd El-Ghany, S.A. Nasef, Medhat S. El-Halawany, Faten F. Mohamed and Ahmed F. Gaber (2016). Effect of Na-butyrate Supplementation on Electromicroscopy, Virulence Gene Expression Analysis and Gut Integrity of Experimentally Induced *Salmonella enteritidis* in Broiler Chickens.Asian Journal of Poultry Science 10(3):126-133. ISSN 1819-3609 DOI: 10.3923/ajpsaj.2016.126.133.

[10]. Bancroft, J.D., Stevens, A. and Turner, D.R. (1996). Theory and Practice of Histological Techniques. 4th Ed. New York, Churchill, Livingstone

[11]. Blanco J.E., Blanco M., Mora A. & Blanco J. 1997. Production of toxins (enterotoxins, verotoxins and necrotoxins) and colicins by Escherichia coli strains isolated from septicemic and healthy chickens: relationship with in vivo pathogenicity. J. Clin. Microbiol. 35:2953-2957

[12]. Bozzola J. J. and Russell L. D. (1999): Electron microscopy: Principles and Techniques for Biologists.

[13]. Candela, M., Maccaferri, S., Turroni, S., Carnevali, P. and Brigidi, P. (2010) Functional intestinal microbiome, new frontiers in prebiotic design. Int. J. Food Microbiol.,140: 93-101.

[14]. Dankowiakowska, A., I. Kozlowska and M. Bednarczyk, 2013. Probiotics prebiotics and symbiotics in poultry. Mode of action limitation and achievements. J. Central Eur. Agric., 14: 467-478.

[15]. El-Sawy, A. F.; El-Maddawy, Z.; Ibrahiem, H. S. and Bo-Ghazel, S. (2015): The growth promoting effect of *Beta-glucan* in comparison with sodium butyrate in broiler chicks. Animal and Veterinary Sciences, 44 (1): 23-3

[16]. Eshak M. G., M. A. Elmenawey, A. Atta, H. B. Gharib, B. Shalaby and M. H. H. Awaad (2016). The efficacy of Na-butyrate encapsulated in palm fat on performance of broilers infected with necrotic enteritis with gene expression analysis. Veterinary World, Vol.9, EISSN: 2231-0916 www.veterinaryworld.org.

[17]. Ewers, C., Janssen, T. and Wieler, L.H. 2003. Avian pathogenic Escherichia coli (APEC). Berl MunchTierarztl Wochenschr, 116(9-10): 381-395.

[18]. Fernandez-Rubio, C., C. Ordonez, J. Abad-Gonzalez, A. Garcia-Gallego, M. Pilar Honrubia, J. Jose Mallo and R. Balana-Fouce, 2009. Butyric acid-based feed additives help protect broiler chickens from Salmonella enteritidis infection. Poult. Sci., 88: 943-948.

[19]. Fernández-Rubio, C., Ordónez, C., Abad-González, J., Garcia-Gallego, A., Pilar Honrubia, M., Jose Mallo, J. and Balana-Fouce, R. (2008) Butyric acid based feed additives help protect broiler chickens from Salmonella enteritidis infection. Poult. Sci., 88: 943-948.

[20]. Friedman A, E Bar-Shira (2005). Effect of nutrition on development of immune competence in chickens gut associated lymphoid system. WPSA, Proc. 15thEur. Symp. Poult. Nutr. 247-255.

[21]. Gajewska, J., J. Riedel, A. Bucka, J. Zabik and M. Michalczuk, 2012. Influence of prebiotics and butyric acid on the composition of intestinal microflora of broiler chickens. Annals of Warsaw University of Life Sciences-Sggw Anim., 51: 47-53.

[22]. Gálfi, P. and Neogrády, S. (2002) The pH-dependent inhibitory action of n-butyrate on gastrointestinal epithelial cell division. Food Res. Int., 34: 581-586.

[23]. Gauthier, R. (2002) Intestinal health, the key to productivity (The case of organic acids). Scientific precongress Avicola IASA. XXVII Convencion ANECA-WPDC, Puerto Vallarta, Jal. Mexico, 30 April 2002.

[24]. Habrun B. , G. Kompes , Ž. Cvetnic, S. Špičić , M. Benić , and M. Mitak (2010). Antimicrobial sensitivity of of *Escherichia coli, Salmonella spp., Pasteurella multocida, Streptococcus suis, and Actinobacillus pleuropneumoniae* isolated from large pig breeding farms in Croatia. VETERINARSKI ARHIV 80 (5), 571-583, 2010

[25]. Hajati H. and M. Rezae (2010). The application of Prebiotics in poultry production. International Journal of Poultry Sci. 9,3:298-304.

[26]. Hernandez, J., Afanador, G., Ariza-Nieto, C. and Avellaneda, Y. (2013) Evaluation of coated and powder sodium butyrate in diets for broilers reared with reused litter during a commercial production cycle. Journal of Animal Science 91(E-Suppl. 2): 335.

[27]. Hussein,A. H. M.; Ghanem, I. A. I.; Eid, A. A. M.; Ali, M. A.; Sherwood, J. S.; Li, G. W.; Nolan, L. K.; Logue, C. M. (2013): Molecular and phenotypic characterization of Escherichia coli isolated from broiler chicken flocks in Egypt. Avian Diseases; 2013. 57(3):602-611.

[28]. Jan, I.S., Y.H. Ko, S.Y. Kang and C.Y. Lee, 2007. Effect of a commercial essential oil on growth performance. digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed Sci. and Tech., 134: 304-315.

[29]. Kwan YM, Ricke SC (2005). Induction of acid resistance of Salmonella typhimurium by exposure to short chain fatty acids. Appl. Environ. Microbiol. 64(9) : 3458 – 3463.

[30]. Leeson, S. and Summers, J.D. (2001) Nutrition of the chicken 4th Ed. Guelph, Ontario, Canada: University Books.

[31]. Leeson, S., Namkung, H., Antongiovanni, M. and Lee, E.H. (2005) Effect of butyric acid on the performance and carcass yield of broiler chickens. Poultry Science 84: 1418-1422.

[32]. Lerbech AM, Opintan JA, Bekoe SO,Ahiabu M-A, Tersbøl BP, et al. (2014) Antibiotic Exposure in a Low-Income Country: Screening Urine Samples for Presence of Antibiotics and Antibiotic Resistance in Coagulase Negative Staphylococcal Contaminants. PLoS ONE 9(12): e113055.

[33]. Liu, L.(2009). Effects of sodium butyrate on growth digestion, intestinal histology and immunity in broiler chickens. Tesis de Master Nanjing Agricultural College, 8315.

[34]. Livak, K. J. 1., Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Methods. Dec; 25(4):402-8

[35]. Lückstädt, C. (2003). Biotronic–solutions for modern livestock production. Biomin Newsletter, 3: 1-4.

[36]. Mahdavi. R. and M. Torki (2009). Study on usage period of dietary protected butyric acid on performance, carcass characteristics, serum metabolite levels and humoral immune response of broiler chickens. J. Anim. Vet. Adv., 8:1702-1709.

[37]. Mansoub, N. H. (2011). Comarative effect of butyric acid, probiotic an garlic on performance and serum composition of broiler chickens. Adv. Environ. Biol., 11:507-511.

[38]. Mitsch, P., K. Zitterl-Eglseer, B. Kohler, C. Gabler, R. Losa and I. Zimpernik, 2004. The effect of two different blends of essential oil components on the proliferation of Clostridium perfringens in the intestines of broiler chickens. Poult. Sci., 83: 669- 675.

[39]. Morsy, E.A.(2015). Further studies on bacterial diseases causing high mortality in broiler baby chicks. Ph D thesis, Faculty of Veterinary Medicine, Cairo University.

[40]. North, M.O. (1984) Broiler, roaster, and capon management.In: Commercial Chicken Production Manual. 3rd ed. Ch. 20.The AVI Publishing Company Inc., Westport Connecticut.p387.

[41]. NOY, Y. and SKLAN, D. (1994) Enzyme secretion and small intestinal passage time in the young chick. Proceedings 9th European Poultry Conference, Glasgow, UK. pp. 451-452.

[42]. Patterson, J.A. and K.M. Burkholder, 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci., 82: 627-631.

[43]. Ravindran, V. (2003) Development of digestive function in neonatal poultry: physiological limitations and potential. Proceedings of the 15th Annual Australian Poultry Science Symposium, Sydney, Australia, pp. 1-7.

[44]. Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, and L. K. Nolan. 2005. Characterizing the APEC pathotype. Vet. Res. 36:241–256.

[45]. Roe AJ, C O'Byrne, D Mclaggan, IR Booth (2002). Inhibition of Escherichia coli growth by acetic acid: a problem with methionine biosynthesis and homocysteine toxicity. Microbiol. 148(pt 7) : 2215-2222. https://doi. org/10.1099/00221287-148-7-2215.

[46]. Roediger W. E.W. (1982). Utilization of nutrients by isolated cells of the rat colon. Gastroenterology 83(2):424-429.

[47]. Rosales, A.G., Villegas, P., Lucent, P. D., Fietcher, D. J., Mohamed, M. A. and Brown, J. (1989). Isolation, identification and pathogenicity of two field strains of infectious bursal disease virus. Avian Diseases, 33: 35-41.

[48]. Saberfar, E., B. Pourakbari, K. Chabokdavan and F. Taj Dolatshahi, 2008. Antimicrobial Susceptibility of Escherichia coli Isolated from Iranian Broiler Chicken Flocks, 2005-2006. J. Appl. Poult. Res., 17: 302-304.

[49]. Sambrook, J.; Fritscgh, E.F.;and Mentiates (1989): Molecular coloning. A laboratory manual. Vol !., Cold spring Harbor Laboratotry press, New York.

[50]. SAS® (2000). User's Guide, Release 8th ed. (Cary, NC, SAS Institute).

[51]. Sikandar Arbab , Hafsa Zaneb, Muhammad Younus, Saima Masood, Asim Aslam, Farina Khattak, Saima Ashraf, Muhammad Shahbaz Yousaf, and Habib Rehman(2017) . Effect of sodium butyrate on performance, immune status, microarchitecture of small intestinal mucosa and lymphoid organs in broiler chicken. Asian-Australas J Anim Sci Vol. 30, No. 5:690-699

[52]. Smulikowska, S., J. Czerwiniski, A. Mieczkowska and J. Jankowiak (2009).The effect of fat coated organic acid salts and a feed enzyme on growth performance, nutrient utilization, microflora activity and morphology of small intestine in broiler chickens. J. Anim. Feed Sci., 18: 478-489.
[53]. Taherpour, K. H. Moravej, M. Shivazad, M. Adibmoradi and B. Yakhchali (2009). Effects of dietary probiotic and butyric acid glycerides on performance and serum composition in broiler chickens. Afr. J. Biotechnol.,8:2329-2334.
[54]. Van Deun, K., Haesebrouck, F., Van Immerseel, F., Ducatelle, R. and Pasmans, F. (2008) Short chain fatty acids and L-lactate as feed additives to control Campylobacter jejuni infections in broilers. Avian Pathology 37: 379-383.
[55]. Vanhoutvin, S.A., Troost, F.J., Hamer, H.M., Lindsey, P.J., Koek, G.H., Jonkers, D.M., Kodde, A., Venema, K. and Brummer, R.J. (2009) Butyrate-induced transcriptional changes in human colonic mucosa. *PLoS One,* 4: e6759.
[56]. Wu W, Xiao Z, An W, Dong Y, Zhang B (2018) Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers. PLoS ONE 13(5): e0197762. https://doi.org/10.1371/journal. pone.0197762
[57]. Yuan, J.S.; Reed, A.; Chen, F. and Stewart, C.N. (2006): Statistical analysis of real-time PCR data. BMC Bioinformatics 2006, 7:85.Adil S., G. Banday, M. Bhat, M. Salahuddin, T. Ahmad Raquib and S. Shanaz, 2011. Response of broiler chicken to dietary supplementation of organic acids. J. Central Eur. Agric., 12: 498-508.
[58]. Zhang, W.H., Gao, F., Zhu, Q.F., Li, C., Jiang, Y., Dai, S.F.and Zhou, G.H. (2011) Dietary sodium butyrate alleviates the oxidative stress induced by corticosterone exposure and improves meat quality in broiler chickens.*Poult. Sci.,*90: 2592-2599.

Table 1 . Results of productive performance

BW						
Chicken gr.	1W	2Ws	3Ws	4Ws	5Ws	Overall
T	40.367 [a] ±0.17	183.15 [a] ±3.19	879.28 [a] ±18.02	1302.96 [a] ±29.11	1967.22 [a] ±29.01	1967.22 [a] ±29.01
PC	40.733 [a] ±0.23	189.36 [a] ±1.75	928.81 [a] ±21.65	1364.37 [a] ±20.8	1969.26 [a] ±45.04	1969.26 [a] ±45.04
FI						
T	144 [a] ±3.06	378.6 [a] ±4.62	689.5 [a] ±30.28	883.8 [a] ±26.90	1149.7 [a] ±79.90	3245.6 [a] ±47.29
PC	146.3 [a] ±1.86	374.2 [a] ±4.21	750.3 [a] ±28.10	926.7 [a] ±25.91	1161.7 [a] ±47.46	3359.2 [a] ±81.30
BWG						
T	142.8 [a] ±3.02	270.5 [a] ±2.76	425.7 [a] ±18.44	423.7 [a] ±12.40	664.3 [a] ±46.88	1926.9 [a] ±29.01
PC	148.6 [a] ±1.52	290.1 [a] ±3.71	449.4 [a] ±16.50	435.6 [a] ±11.82	604.9 [a] ±24.24	1928.5 [a] ±44.81
FCR						
T	1.01[a]±0.012	1.40[a]±0.004	1.62[b]±0.001	2.09[b]±0.004	1.73[b]±0.002	1.68[b] ± .006
PC	0.98[b]±0.001	1.29[b]±0.002	1.67[a]±0.002	2.13[a]±0.002	1.92[a]±0.003	1.74[a]± .005

Data reported as mean ± SEM. Different superscript letters in each row represent existence of significant differences. (p < 0.05)

Table 2. Organ body weight ratio.

Groups	Organs						
	Heart %	Liver %	Gizzard %	Intestine %	Bursa %	Thymus %	**Spleen%**
T	0.65 [a] ±.051	2.32[b]±.072	2.95 [a] ±.218	6.96 [a] ±0.132	0.15 [a] ±0.013	0.34[a]±0.070	.13 [a] ±.030
PC	0.44 [a] ±.158	3.21[a]±.122	3.18 [a] ±.293	6.12 [a] ±0.794	0.12 [a] ±0.058	0.15[b]±0.138	.12 [a] ±.027

Data reported as mean ± SEM. Different superscript letters in each row represent existence of significant differences. (p < 0.05)

Table 3: Macroscopic and Microscopic lesion score of different organs among treated groups

Lesion score	***Broiler chicken groups***	
	PC group	***T group***
Macroscopic lesions		
Airsacculitis	2.17[b]±0.70	0.17[a]±0.17
Perihepatitis	2.00[b]±0.77	0.00[a]±0.00
Pericarditis	1.83[b]±0.83	0.00[a]±0.00
Peritonitis	1.83[b]±0.83	0.00[a]±0.00
Sum of lesion score	7.83	0.17
Average lesion score	1.96	0.04
Microscopic lesions		
Splenic lymphoid depletion	2.21[b]±0.69	1.64[a] ±0.92
Inflammation of splenic capsule	2.33 [b] ±0.57	0.00[a] ±0.0
Bursal lymphoid depletion	2.40 [b] ±0.50	1.51[a] ±1.01
Cecal tonsils lymphoid depletion	2.50 [b] ±0.52	0.12[a] ±0.35
Thymic lymphoid depletion	1.60[a] ±0.50	1.47[a] ±0.51
Perihepatitis	3.00 [b] ±0.00	0.00[a] ±0.00
Hepatocellular necrosis	1.66 [b] ±0.50	0.57[a] ±0.53
Cholangiohepatitis	0.00[a] ±0.00	0.20[a] ±0.44
Pericarditis	2.33 [b] ±0.57	0.00[a] ±0.00
Myocarditis	1.33 [b] ±0.57	0.00[a] ±0.00
Sum of lesion score	19.36	5.51
Average lesion score	1.94	0.55

±SD(*p* ≤0.05).

Table 4. Oligoneucleotide primers sequences, target gene, amplicon sizes and cycling conditions during cPCR.

Target gene	Primer sequence	Amplicon segment (bp)	Amplification (35 cycles)				Final extension	Reference
			1ry denat	2ry denat	Annealing	Extension		
iss	F:ATGTTATTTTCTGCCGCTCTG R:CTATTGTGAGCAATATACCC	266 bp	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec	72˚C 7 min.	Yaguchi *et al*., 2007
fimH	F:TGCAGAACGGATAAGCCGTGG R:GCAGTCACCTGCCCTCCGGTA	508 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 7 min.	Ghanbarpour and Salehi, 2010
Tsh	F:GGT GGT GCA CTG GAG TGG R:AGT CCA GCG TGA TAG TGG	620 bp	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Delicato *et al*., 2003
iutA	F:GGCTGGACATGGGAACTGG R:CGTCGGGAACGGGTAGAATCG	300 bp	94˚C 5 min.	94˚C 30 sec.	63˚C 30 sec	72˚C 30 sec.	72˚C 7 min.	Yaguchi *et al*., 2007
iroN	F:ATC CTC TGG TCG CTA ACT G R:CTG CAC TGG AAG AAC TGT TCT	847 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	Ewers *et al*., 2007
hly	F:AACAAGGATAAGCACTGTTCT GGCT R:ACCATATAAGCGGTCATTCCC GTCA	1177 bp	95˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 1 min.	72˚C 12 min.	Piva *et al*., 2003
papC	F:tgatatcacgcagtcagtagc R:ccggccatattcacataa	501 bp	95˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec	72˚C 10 min.	Wen-jie *et al*., 2008
kpsMT II	F:CAGGTAGCGTCGAACTGTA R:CATCCAGACGATAAGCATGAG CA	280 bp	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Ewers *et al*., 2007
ompA	F:AGCTATCGCGATTGCAGTG R:GGTGTTGCCAGTAACCGG	919 bp	95˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 1min.	72˚C 12 min.	

Table 5. Expression analysis of *E. coli* virulence genes (rt-qPCR).

Gr	*16S rRNA*	*iutA*		*papC*		*ompA*		*Iss*		*iroN*		*Fim H*	
			Fold change		Fold change		Fold change		Fold change		Fold change		Fold change
	CT	**CT**		**CT**		**CT**		**CT**		**CT**		**CT**	
T	19.91	22.70	0.3610	23.16	0.1638	22.91	0.1571	24.01	0.2146	22.90	0.3487	22.48	0.2045
PC	20.31	21.63		20.95		20.64		22.19		21.78		20.59	

T =Group of birds challenged and supplemented with NaB.

PC= Group of birds challenged and not supplement with any additives (Positive control).

16S rRNA gene was set as reference gene for all genes analyzed.

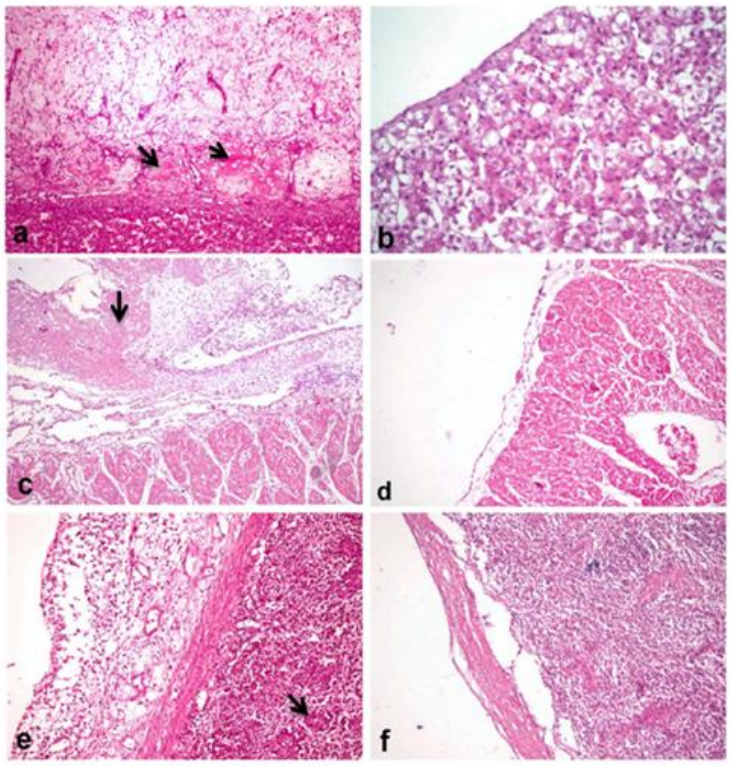

Fig.1: Histological section from different organs of PC group (a,c & e) vs. T group (b,d & f):
a)Liver showing a marked thickening of the perihepatic capsule with fibrin(arrow) and edema with heterophils mixed with mononuclear cell infiltration and proliferation of fibrovascular tissue (X 200).
b)Liver showing normal histological structure of perihepatic capsule and vacuolization of underlying hepatocytes (X400).
c)Heart showing dense eosinophilic fibrinous exudate infiltration in the pericardial tissue (arrow) with sloughing of lining mesothelium and extensive heterophils infiltration. Notice the extension of inflammatory reaction into the underlying myocardium that showed myocarditis and intermuscular edema (X100).
d)Heart with normal pericardium histological structure. Notice the intact flattened mesothelial lining of pericardium and normal thickness of subpericardial connective tissue(X200).
e)Splenic capsule showed thickening of capsule , sloughing of lining epithelium, fibrin exudation and heterophils infiltration with organization and reticuloendothelial hyperplasia (arrow)(X200).
f)Spleen showed normal thickness of splenic capsule with reticuloendothelial hyperplasia (X200).

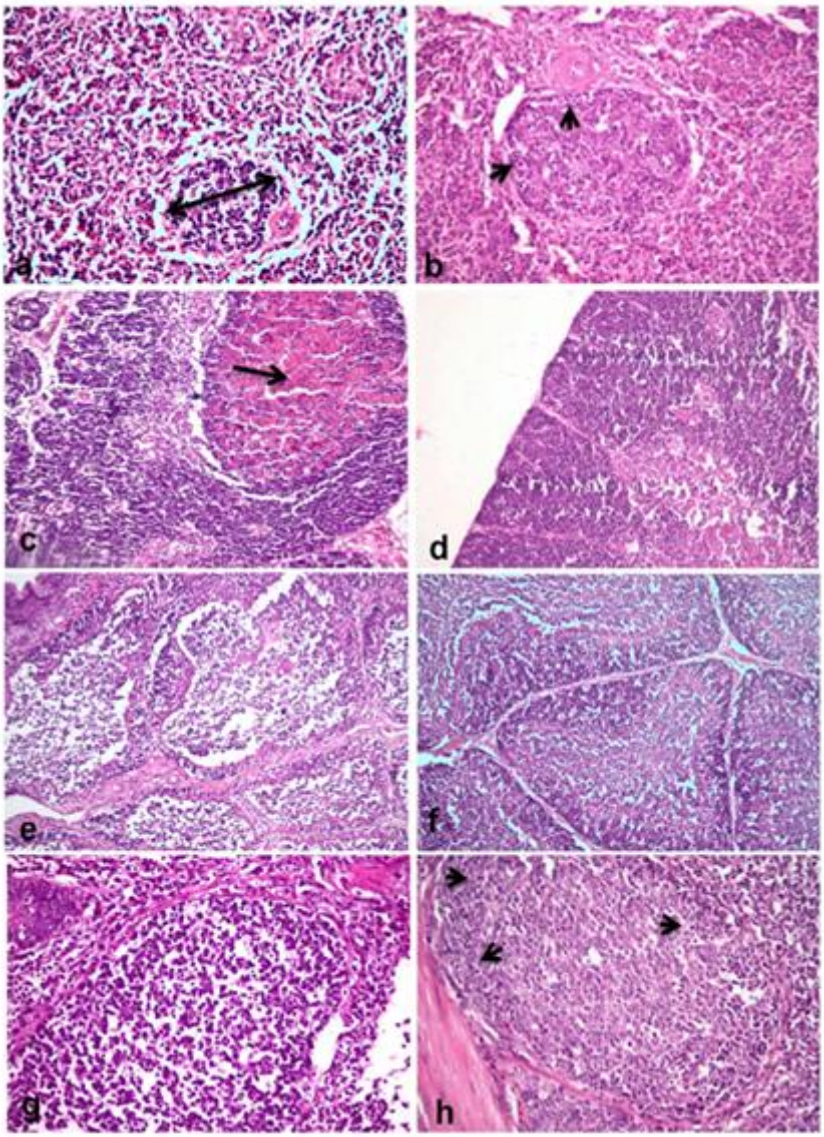

Fig.2: Histological section from immune organs of PC group (a,c,e & g) vs. T group (b,d,f & h):
a)Spleen showing lymphoid follicular atrophy (double head arrow) and depletion and hyperplasia of reticuloendothelial cells (X 400).
b)Spleen showing mild lymphocytic depletion with mitosis of lymphoid elements(arrow) (X400) .
c)Thymus showed cortical hemorrhage (arrow) and depletion (X100).
d)Thymus showing normal cellular population of thymocytes of cortex (X100).
e)Bursa showing interfollicular fibrosis with marked cortical and medullary depletion (X400).
f)Bursa showed moderate lymphocytic depletion of medulla (X400).
g)Cecal tonsils showing karyopyknosis and depletion of lymphoid elements (X400).
h)Cecal tonsils showing activation and mitosis of lymphoid elements (arrow) (X400).

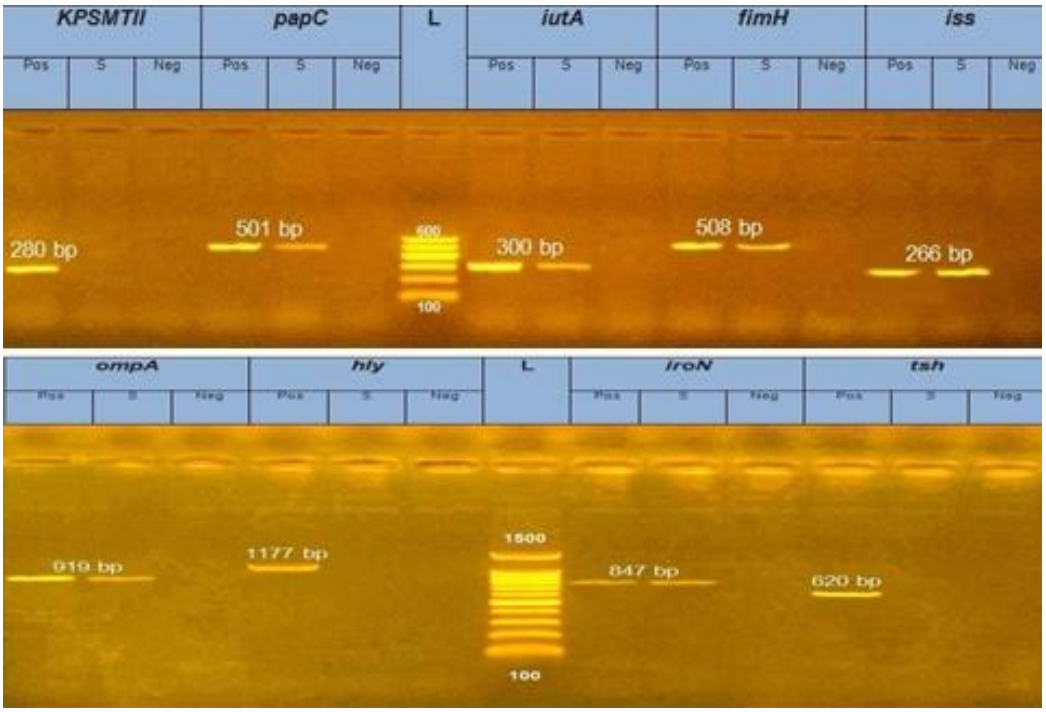

Fig.3 Results of cPCR: Positive amplifications at 280 bp, 501 bp, 300 bp, 508 bp, 266 bp, 919 bp and 1177 bp fragments detected for *KPSTII, papC, iutA, fimH, iss, ompA, ompA, hfy* and *iron* virulence genes respectively. All genes were detected in *E. coli* positive strain and *E. coli* serogroup O78 except in *KPSTII, hly and tsh* genes that could only be detected in positive strain.

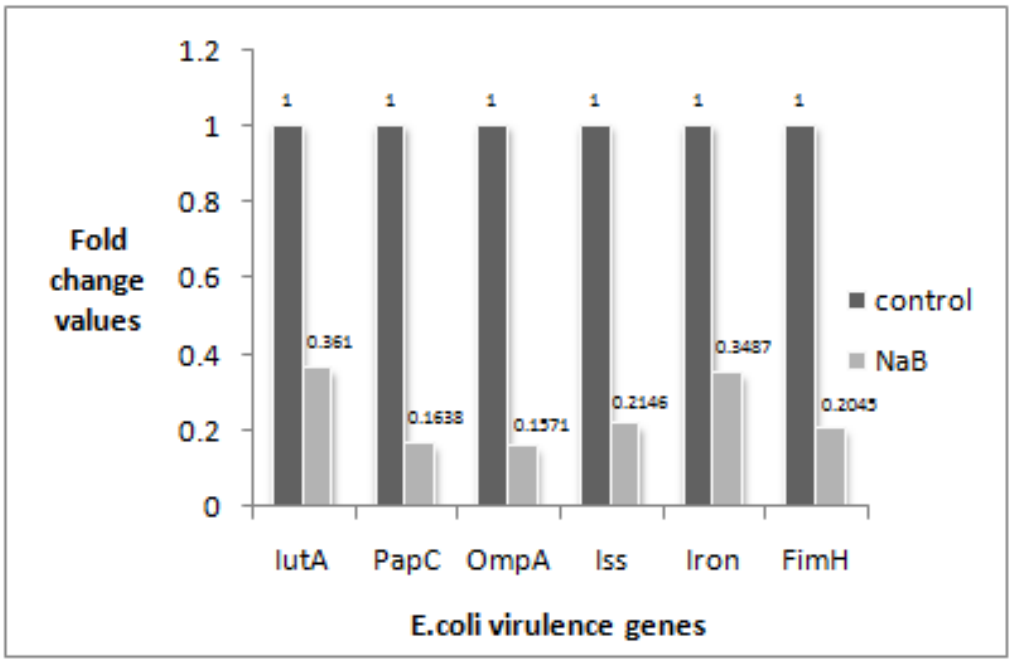

Fig. 4 . Expression analysis of *E. coli* genes by rt-qPCR. Showing the fold change values of different genes related to the genes of control group.

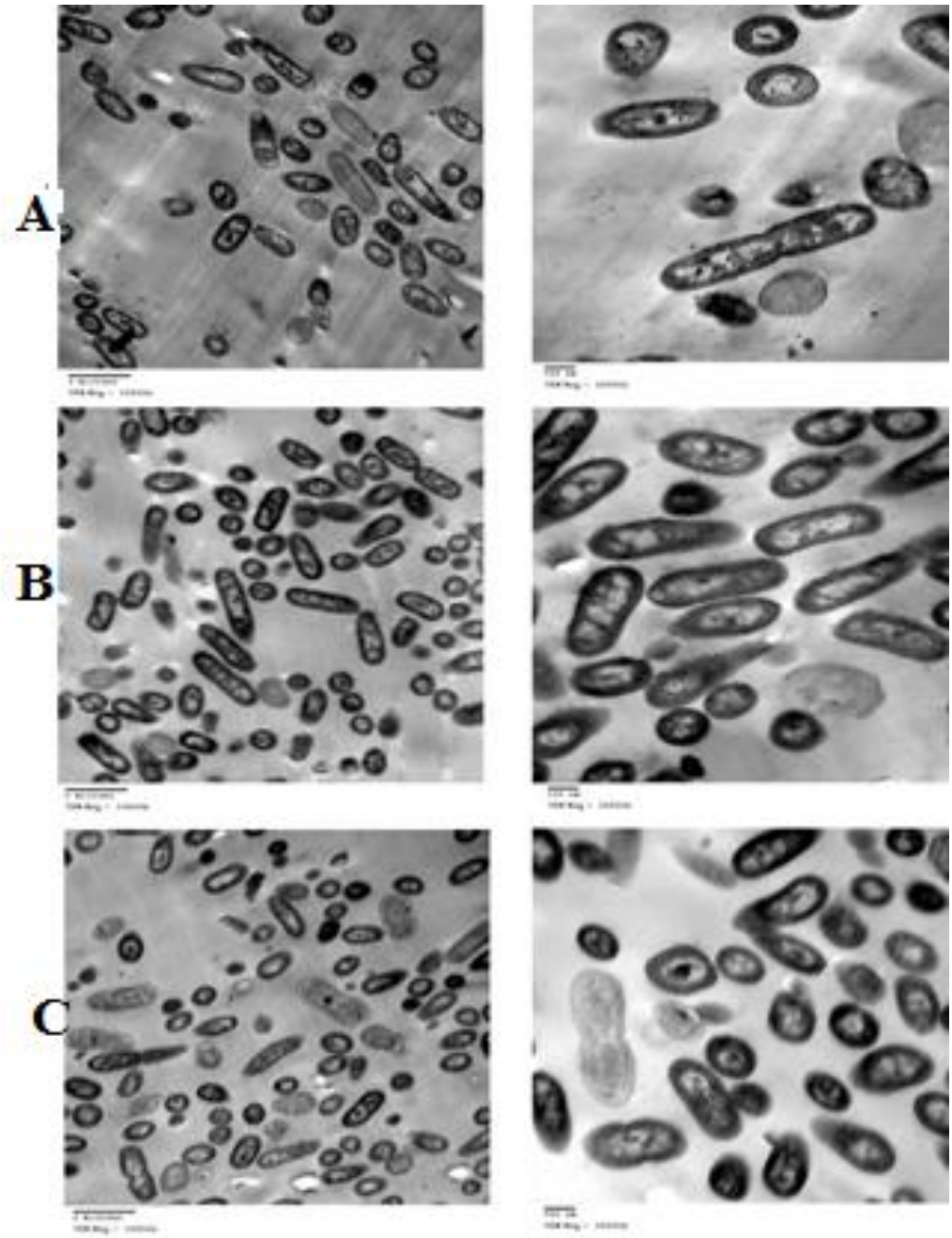

Fig. 5. TEM of examined *E. coli* isolated from the liver of chicken broilers after 17 days PI.
A) TEM of thin sections of original *E. coli* strain grown at 37°C.
B) TEM of thin sections of *E. coli* colonies re-isolated from PC group.
C) TEM of *E. coli* colonies re-isolated from T group. Notice the signs of induced cells damage and reduction in size of group C.
Scale bars are 2 µm (left column) and 0.5 µm (right column), respectively.

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