Influence of sodium butyrate on salmonella infection in broiler chicks

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A B S T R A C T

One hundred and ten 1-day-old broiler chickens were obtained from commercial breeder farm and kept under strict hygienic measures and were proved to be salmonella free. The birds were divided into 5 equal groups. The first group was kept as negative control. The second group was infected with (1×10^8) *Salmonella entertidis* intra crop at 8th day of age. The third group was given Sodium butyrate 0.98 mg/mL orally in drinking water from the first day till the end of experiment at 40th day of age. The fourth group was treated with Sodium butyrate from the 1st day as in the 3rd group and infected with *Salmonella enteritidis* at 8th day of age. The fifth group was infected with inoculum containing (1×10^8) *Salmonella entertidis* intra crop at 8th days of age and treated Sodium butyrate 0.98 mg/mL in drinking water for 5 successive days after appearance of signs. Blood and serum samples were collected from each group at 14, 21 and 28 days of age for estimation of macrophages phagocytosis, malondialdehyde (MDA), Superoxide dismutase (SOD), total protein, albumin, globulin and Nitric Oxide (NO). Live body weight and feed intake were recorded for each repetition on days 7, 14, 28 and 35. Reisolation of inoculated organism from the muscle, liver, and two ceci of experimentally infected chicks was carried out and confirmed by PCR. Tissue specimens from intestine were collected for histopathological examination. The results showed significant increase in total protein and globulin level in groups 2, 3 and 4. MDA showed significant decrease in group 2 and 4 in the first week, on the other hand MDA showed significant decrease in group 2 and 5 in the second week, but decrease in group 2 only in the third week. SOD showed significant decrease in group 2 in the first week, but in the second week, there were decrease in groups 2, 4 and 5. While SOD showed significant increase in groups 3, 4 and 5 in the third week. NO showed significant increase in groups 2 and 3 in the first week but in the second week there was significant increase in NO level in groups 2, 3 and 4 but in the third week the results revealed significant increase in groups 3 and 4 only. Macrophage showed significant decrease in groups 2 and 5. Phagocytic activity showed significant decrease in group 2 and significant increase in group 3. Body weight showed significant increase in group 3 during the second and the third week of experiment, but in the fourth and fifth week there was significant decrease in group 2 and increase in group 3. Histopathological examination revealed that Group 3 (sodium butyrate**)** showed high absorptive surface from tall and thick intestinal villi and hyper activation of intestinal crypts and proliferation of villous enterocytes. The results indicated that sodium butyrate can be used as antioxidant so improve the growth performance in chickens under stress and this may be attributed to enhancing the immune response and reduce tissue damage. It was concluded that sodium butyrate can be used in control and prevention of salmonella infection in chickens.

Keywords: sodium butyrate**,** salmonella, antioxidants, phagocytosis, chickens

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1. INTRODUCTION

Poultry industry is one of the most important sectors providing high quality protein for human consumption all over the world. Also, challenging the poultry industry to find alternative means of control diseases as excessive use of antibiotic either for therapeutic or protective purposes led to the appearance of bacterial resistant strains (Azza et al., 2012). Salmonella is a facultative intracellular pathogen infecting wide range of hosts (Ogunleye et al., 2009). *Salmonella enteritidis* is one of the most salmonella serotype in poultry products that associated with human salmonellosis (Haiqi et al., 2013) and consider an important international public health and economic problem resulting in syndromes such as enteric fever, bacteremia, focal infection, and enterocolitis. Therefore, human health protection by the elimination of food borne pathogens from food animals and their products has become very important for all sectors of the food production chain (Thirabunyanon and Thongwittaya, 2012). Salmonella species are members of the family Enterobacteriaceae. They are rod - shaped gram-negative, facultative organisms. It is considered

one of the dangerous zoonotic diseases causing severe economic losses in both human and animal resources. Most bacterial pathogens including pathogenic *salmonella* species contains multiple virulence properties that make them able to invade and survive within the host and ultimately cause a disease (Bowe et al., 1998). PCR has been demonstrated to be a very specific and sensitive method for the detection of Salmonellae. InvA gene of Salmonella contains sequences unique to this genus and has been proved to be a suitable PCR target with a potential diagnostic application (Jamshidi et al., 2009). Butyric acid is one of the short chain fatty acids, which has higher bacterial activity when the acid is un-dissociated (Antongiovanni et al., 2007). It can be used for the treatment of several intestinal bacterial infections like Salmonellosis (Fernandez-Rubio et al., 2009; Van Immerseel et al., 2005).

This study was designed to examine the effect of using sodium butyrate on intestinal colonization of *Salmonella enteritidis* experimentally infected broiler chicken.

2. Materials and Methods

2.1. Drug

Sodium butyrate is a drug produced and distributed by Care Pharma Groups. Each 1ml contain 100 gm Sodium butyrate and given orally for chickens by 0.98mg⁄ml drinking water per day.

2.2. Challenging bacteria:

Salmonella enteritidis strain (obtained from Laboratory of Bacteriology, Department of Food Hygiene, Animal Health Research Institute, Dokki, Egypt). Four to five isolated colonies of the tested strain were picked up by a sterile inoculating loop and inoculated in tubes of sterile peptone water 0.1% (5 ml in each) and were then incubated at 37°C/24 hrs. (Saeed and Tariq, 2005). From this culture, dilutions up to 10^{10} were plated on XLD agar to determine the cell concentration. The cell count was adjusted to 10^8 cfu/ml for Salmonella entritidis (Kantachote and Charernjiratrakul, 2008).

2.3. Experimental design

Chickens: One hundred and ten 1-day-old broiler chickens were obtained from commercial breeder farm were kept under strict hygienic measures and given ration and water *ad libitum.* Before infection, a random sample which included 10 birds was scarified for postmortem and bacteriological examination to prove that these birds are salmonella free. The remaining birds were *divided* into 5 groups each of 20 chicks. The first group was kept as negative control. The second group was infected with inoculum containing (1×10^8) *Salmonella entertidis* intracrop at 8th day of age (Johny et al., 2012). The third group was given Sodium butyrate 0.98 mg/mL orally in drinking water from the first day till the end of experiment at 40 day of age. The fourth group was treated and infected as group two and three. The fifth group was infected with inoculum containing (1×10^8) *Salmonella entertidis* intracrop at 8th day of age and administrated with Sodium butyrate 0.98 mg/mL orally in drinking water for 5 successive days after the clinical signs appeared.

2.4. Vaccination:

All groups were vaccinated against Newcastle disease with Hitchiner B1 at the 7th day and LaSota at 21th day of age and vaccinated against Gumboro disease (IBD) at 13th day of age.

2.5. Sampling:

Blood samples were collected from each group at the end of experiment. Each blood sample was divided into three portions. The 1st portion was collected with anticoagulant for measuring macrophage and phagocytosis. The 2nd portion was collected on anticoagulant for estimation of antioxidant stress (malondialdehyde MDA and Superoxide dismutase SOD) in plasma. The 3rd portion was collected without anticoagulant for serum separation for measuring, Total protein, albumin and globulin and Nitric Oxide (NO).

2.6. Hematological examinations:

Macrophage was estimated according to Gordon (1999). Phagocytic assay was performed according to Woldeniwet and Rowan (1990).

2.7. Biochemical analysis:

Serum total protein was evaluated according to (Henry 1974). Albumin was determined according to Doumas (1971). lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS), using malondialdehyde (MDA) as standard by the method of Beuge and Aust (1978). Superoxide dismutase (SOD) was determined by method described by (de Cavanagh et al., 1995).

2.8. Measurements:

Data collection was based on 100% of experimental population. The method of weight was conducted on a group basis in each of seven repetitions. Live body weight, feed intake and chick mortality were recorded for each repetition on days 7, 14, 28 and 35. Adjusted feed conversion

was calculated as feed consumed divided by gain weight of live birds plus dead or cull birds (kg feed/kg of gain).

2.9. Isolation and identification of Salmonella enteritidis:

Isolation of Salmonella was carried out according to the technique described formerly by Quinn et al. (2002). Reisolation of inoculated organism from internal organs (liver and 2 ceci) of experimentally infected birds was recorded. All groups were kept under observation post infection to record the clinical symptom and mortality rate. The samples were taken aseptically from 3 organs from each bird (muscls, liver, two ceci) at age 21 day and 35 day. All samples were inoculated into nutrient broth and incubated at $37C^0$ for 24 hours, from previously prepared broth 1ml was aseptically transferred to each 10 ml selenite F broth and incubated at $43C^0$ for 18 hrs. Loopful of enrichment culture was streaked onto selective plating media, Xylose lysine desoxy cholate (XLD) agar plates then incubated at 37 °C for 24 hrs. Suspected colonies were purified and identified by different biochemical reactions according to Quninn et al., (2002).

2.10. Microscopic examination

It was carried out according to Cruickshank et al. (1975).

2.11. Serological identification of salmonellae:

It was carried out according to Kauffman- white scheme as described by Kauffmann (1973).

2.12. Methods of PCR technique (Olivera et al., 2003):

DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^OC for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit. Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in table (1). PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reactions were performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH). Electrophoresis was done in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the PCR products were loaded in each gel slot. A Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles) Secondary denaturation	Annealing	Extension	Final extension	References
invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	(Oliveira et al., 2003)
sefA	GCAGCGGTTACTATTGCAGC TGTGACAGGGACATTTAGCG	310	94˚C 5 min.	94˚C 30 sec.	52˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	(Akbarmehr et al., 2010)

2.13. Pathological examination:

Postmortem examination was done immediately after slaughtering and tissue specimens from intestine were collected and fixed in 10% neutral buffered formalin. They were routinely processed by standard paraffin embedding technique. Section at 4 microns, stained with Hematoxylin and Eosin (Bancroft and Gamble, 2002).

2.14. Statistical Analysis:

All data were analyzed by the GLM procedure of SAS 9.1 (SAS Institute, 2003)**.** Dunnett's test was used to compare the means. All statements of significance were based on $p < 0.05$.

3. RESULTS

In the present study, a total of 150 samples were collected from broiler chicken muscles, Livers and ceci fifteen organs of each group at age 21 day, 35 day for Salmonella reisolation. Cultural and morphological results using (XLD) as a cultivating media , the resulted Salmonella appeared as smooth colonies with black centers. Microscopically, Salmonella isolates appeared as Gram negative, non-spore forming short rods shaped by using Gram's stain. Table(3) showed reisolation of *Salmonella enteritidis* in G2 (positive control), the incidence of *Salmonella enteritidis* from muscles, liver, two ceci ,were 100% at 21,35 of age, while G**4** which prophylactic with sodium butyrate the incidence of *Salmonella enteritidis* from muscles, liver, two ceci were 0,20% , 40% at 21,35 days of age while in G5 which treated with sodium butyrate for successive 5 days the incidence of *Salmonella enteritidis* from muscles, liver, two ceci were 0,40% ,60% respectively at age 21 day but the incidence was 20%, 60%, 80% respectively at age 35 day. Molecular identification of inv A and Sef A genes of the used Salmonella strain are showed in Figure (A)

Table (4) explain no mortalities were observed in group G1, G3, G4 all over observation period while mortality rate in G2 was 80% due to infection with *salmonella enteritidis* without treatment, while in G5 the highest morality rate is appear at 5th week. There were significant increase in Total protein level in groups 2,3 and 4 during the first week of infection. Albumin level revealed significant decrease in group 2 and slight increase in groups 3 and 4. Globulin showed significant increase in groups 2,3 and 4. In the second week, Total protein showed non-significant changes in groups 2, 3 and 4, Albumin revealed significant decrease in groups 2,4 and 5 but increase significantly in group 3, Globulin showed significant increase in groups 2, 3,4 and 5 (table 6). MDA showed significant decrease in group 2 and 4 in the first week , on the other hand MDA showed significant decrease in group 2 and 5 in the second week , but decrease in group 2 only in the third week . SOD showed significant decrease in group 2 in the first week, but in the second week there were decrease in groups 2, 4 and 5, while SOD revealed significant increase in groups 3,4 and 5 in the third week.

NO showed significant increase in groups 2 and 3 in the first week but in the second week there were significant increase in NO level in groups 2,3 and 4 but in the third week the results revealed significant increase in groups 3 and 4 only (table 7). Macrophage showed significant decrease in groups 2, 5, phagocytic activity showed significant decrease in group 2 and significant increase in group 3 (table 8). Body weight showed significant increase in group 3 during the second and the third week of experiment, but the fourth and fifth week the results revealed significant decrease in group 2 and increase in group 3 (table 9).

P.M. lesion: *Salmonella enteritidis* showed air saculitis, fibrinous heart, enlarged liver with multifocal hemorrhage, enlarged kidneys, spleenitis and the intestinal blood vessels appeared congested and engorged with blood. Gross lesions in groups 4 and 5 showed less severity when compared to group 2.

Histopathological results: Group 1 (control): Intestinal coats, villous length and thickness appeared normal (Fig.1). Group 2 (salmonella infection) resulted the intestinal villi were disorganized with intense destruction of its superficial tips beside mucosal and submucosal mononuclear cells aggregations (Fig.2). Group 3 (sodium butyrate **)** showed high absorptive surface from tall and thick intestinal villi and hyper activation of intestinal crypts and proliferation of villous enterocytes (Fig. 3). Mild proliferations of GALF could be seen. The remaining intestinal coats were normal. Group 4 (Salmonella + sodium butyrate**)** appeared the majority of villi retain their villous length and width with mild intestinal damage from desquamation of villous enterocytes and leukocytic infiltration mainly in mucosa and submucosa beside hyperplasic intestinal crypts epithelium at the base of intestinal villi (Figs. 4 and 5). Group 5 (salmonella + sod + drug 5 days) showed regeneration of villous enterocytes from proliferation intestinal crypts resulting in high and thickened intestinal villi with absence of intervillus spaces was seen. Villus fusions and greater absorptive surface were common with hyperplasic GALF beside minimal intestinal damage (Figs. 6, 7 and 8)**.**

4. DISCUSSION

Salmonella is the most common agents of food borne diseases and poultry products still the main source of *salmonella enteritidis* associated with food borne infections in humans (Setta et al., 2012). The widespread use of antibiotics as

Table (2) collected sample for salmonella reisolation

Age of collection	Group No.	No. of examined birds	Collected samples			Total Sample
			Muscles	Liver	Two ceci	
21 day	G1	5	5	5	5	15
	G2	5	5	5	5	15
	G3	5	5	5	5	15
	G4	5	5	5	5	15
	G5	5	5	5	5	15
35 day	G1	5	5	5	5	15
	G2	5	5	5	5	15
	G3	5	5	5	5	15
	G4	5	5	5	5	15
	G5	5	5	5	5	15
Total		50	50	50	50	150

Table (3) Reisolation of S. enteritidis from infected chickens at 21 and 35 days of age

Age of birds	Group No.	No. of examined birds	S. enteritedis positive samples					
			Muscle		Liver		2 caeci	
			No	%	No	%	No	%
21 day	G1	5	0	0	0	0	0	0
	G2	5	5	100	5	100	5	100
	G3	5	0	0	0	0	0	0
	G4	5	0	0	1	20	2	40
	G5	5	0	0	2	40	3	60
35 day	G1	5	0	0	0	0	0	0
	G2	5	5	100	5	100	5	100
	G3	5	0	0	0	0	0	0
	G4	5	0	0	1	20	2	40
	G5	5	1	20	3	60	4	80
Total		50	11	22	17	34	21	42

Table (4) Mortality rate

Group NO	No of dead chicks					Total	
	W1	W2	W3	W4	W5	No	%
G1	0	0	0	0	0	0	0
G2	0	5	6	3	2	16	80
G3	0	0	0	0	0	0	0
G4	0	0	0	0	0	0	0
G5	0	1	0	1	2	4	20

Table (5): Serotypes of isolated Salmonellae

Strains	Serology		
		O antigen	H antigen
			H1 H2
S.Entritidis		1,9,12	g, m

Table (6) Effect of Sodium butyrate and Salmonella infection on protein profiles

Age	1st week			2nd week			3rd week		
Groups	Total protein (g⁄dl)	(Albumin g⁄dl)	(Globulin g⁄dl)	(Total protein g⁄dl)	(Albumin g⁄dl)	Globulin (g⁄dl)	Total protein	(Albumin g⁄dl)	(Globulin g⁄dl)
1	5.88±0.04b	5.05±0.03a	0.83±0.05c	6.00±0.05a	5.07±0.03a	0.83±0.04b	6.13±0.02a	5.17±0.02a	0.96±0.03a
2	5.90±0.05ab	4.91±0.04b	1.15±0.07a	6.06±0.05a	4.99±0.01b	0.98±0.02a	6.21±0.08a	5.18±0.03a	1.03±0.03a
3	5.94±0.04ab	5.03±0.05ab	0.91±0.06b	5.98±0.03ab	5.08±0.04a	0.94±0.05a	6.23±0.11a	5.31±0.02a	0.92±0.02a
4	6.06±0.06a	5.03±0.04ab	0.87±0.04b	5.93±0.04ab	4.97±0.02b	0.96±0.04a	6.17±0.06a	5.35±0.03a	0.82±0.02b
5				5.89±0.05b	4.95±0.03b	0.95±0.05a	6.05±0.12a	5.29±0.02a	0.75±0.12b

Values are means ± standard error. Mean values with different letters at the same column differ significantly at ($p < 0.05$) by using LSD .

Table (7) Effect of Sodium butyrate and Salmonella infection on oxidative enzymes. n=5

Group	1st week			2nd week			3rd week		
	MDA mol⁄ml	SOD mol⁄ml	NO mol⁄L	MDA mol⁄ml	SOD mol⁄ml	NO mol⁄L	MDA mol⁄ml	SOD mol⁄ml	NO mol⁄L
1	10.64±0.05a	12.18±3.12a	18.60±0.32c	10.50±0.13ab	14.67±0.90a	23.63±1.10b	10.00±0.09a	5.67±1.50b	25.25±0.43c
2	10.21±0.16b	4.28±1.72b	21.84±0.76b	10.13±0.20b	8.91±3.23b	26.48±0.85a	9.30±0.33b	4.94±1.21b	24.10±0.72c
3	10.76±0.24a	15.24±6.23a	25.24±0.64a	10.68±0.10a	13.08±2.30a	26.55±1.71a	9.60±0.23a	8.14±2.23a	26.70±1.64b
4	10.34±0.14b	15.64±4.67a	19.72±0.76c	10.63±0.12a	5.22±2.12c	26.88±1.51a	9.70±0.16a	8.54±2.21a	29.98±1.76a
5				9.50±0.43c	8.69±1.82b	22.33±0.65b	9.93±0.06a	8.67±0.43a	24.80±0.53c

Values are means ± standard error. Mean values with different letters at the same column differ significantly at ($p < 0.05$) by using LSD. NO= nitric oxide

Table (8) Effect of Sodium butyrate and Salmonella infection on macrophage and phagocytosis at 28 day of age. (n=5)

Items	Groups				
	1	2	3	4	5
Macrophage CFU/mL	23.28±0.73b	16.05±0.61c	28.80±0.40a	26.93±0.82a	16.20±1.12c
Phagocytic activity%	40.73±1.75b	25.81±1.52c	45.99±0.21a	39.13±1.21b	38.20±1.78b
Phagocytic index	1.79±0.16a	1.30±0.09b	1.75±0.15a	1.25±0.07b	1.15±0.03b

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p < 0.05$) by using LSD.

Table (9) Effect of Sodium butyrate and Salmonella infection on body weight

Age	Groups					
	G 1	G 2	G 3	G 4	G 5	*P* value
W 2	0.437 ± 0.063a	0.401 ± 0.049a	0.494 ± 0.060b	0.401 ± 0.071a	0.399 ± 0.036a	0.002*
W 3	0.798 ± 0.159a	0.731 ± 0.059a	0.935 ± 0.089b	0.801 ± 0.151a	0.768 ± 0.088a	0.006*
W 4	1.291 ± 0.204a	1.104 ± 0.231b	1.488 ± 0.125c	1.297 ± 0.096a	1.286 ± 0.189a	0.001*
W 5	1.779 ± 0.069a	1.573 ± 0.219b	1.989 ± 0.108c	1.764 ± 0.087a	1.694 ± 0.060a	0.001*

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p < 0.05$)

Table (10) Effect of Sodium butyrate and Salmonella infection on body weight gain (gm./bird/week.

Groups / Age	1	2	3	4	5
W1	125	125	125	125	125
W2	271	191	320	231	229
W3	356	370	445	400	369
W4	489	373	553	496	518
W5	491	459	501	466	408

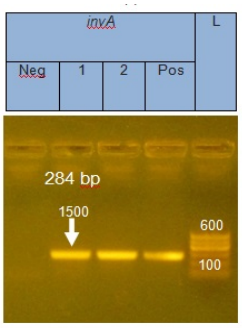
Table (11) Effect of Sodium butyrate and Salmonella infection on feed intake means (gm/ bird)

Groups / Age	1	2	3	4	5
W1	237	237	237	237	237
W2	485.09	345.7	537.6	390.39	412.2
W3	583.84	610.5	676.4	632	616.23
W4	801.96	656.48	851.62	843.2	896.14
W5	854.34	826.2	851.7	829.43	734.4

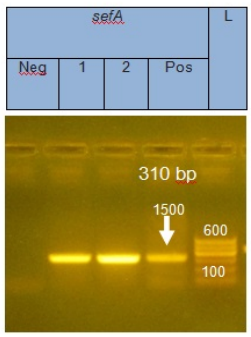
Table (12) Effect of Sodium butyrate and Salmonella infection on Feed conversion rate

Groups / Age	1	2	3	4	5
W1	1.9	1.9	1.9	1.9	1.9
W2	1.79	1.81	1.68	1.69	1.8
W3	1.64	1.65	1.52	1.58	1.67
W4	1.64	1.76	1.54	1.7	1.73
W5	1.74	1.8	1.68	1.78	1.8

Figure (A): Molecular identification of inv A and Sef A genes of the used Salmonella strain: Agarose gel electrophoresis showing virulent genes of Salmonella enteritids.



Neg : negative control
Pos : positive control L : DNA ladder (marker)100-600
invA positive for both samples at 284bp



Neg : negative control
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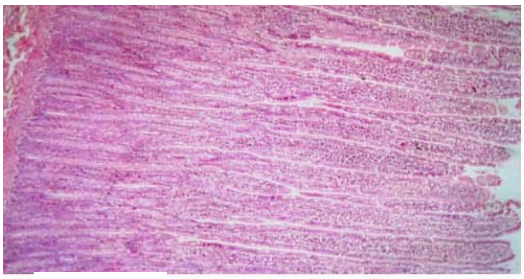

Fig. (1)

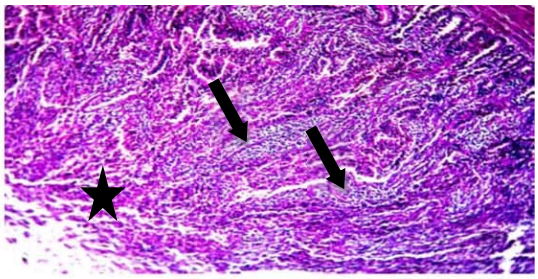

Fig. (2)

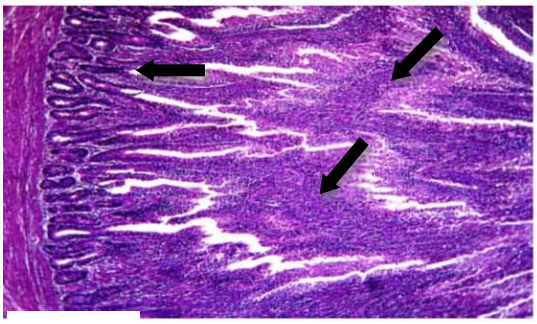

Fig. (3)

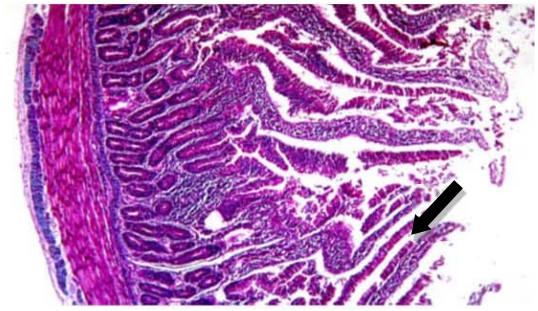

Fig. (4)

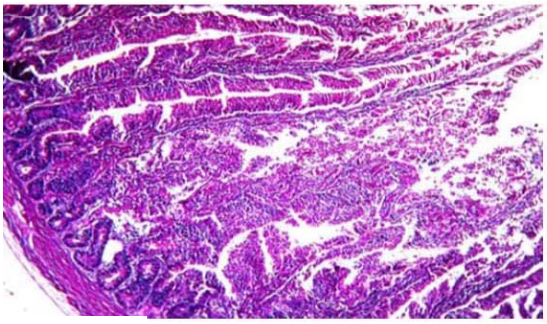

Fig. (5)

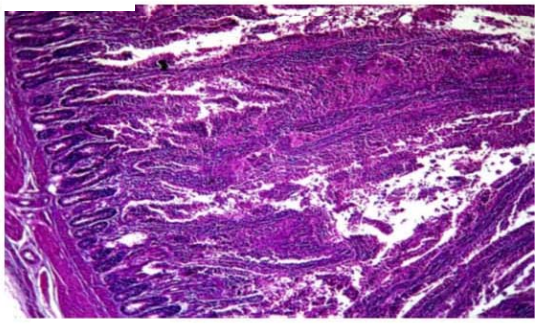

Fig. (6)

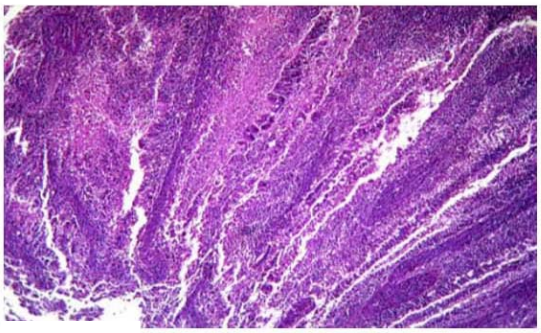

Fig. (7)

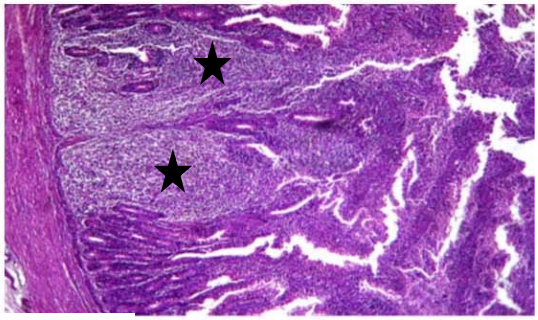

Fig. (7)

Fig (1): Intestine of chicken negative control showing normal intestinal coats with villous length and width. H&E (X 300). **Fig (2):** Intestine of chicken (Salmonella infection) showing intense destruction for upper portion of intestinal villi (star) with mucosal and submucosal lymphocytic aggregations (arrows). H&E (X 300). **Fig (3):** Intestine of chicken (sodium butyrate) showing high absorptive surface from tall and thick intestinal villi (thick arrow) and hyper activation of intestinal crypts and proliferation of enterocytes (thin arrow). H&E (X 300). **Fig (4):** Intestine of chicken (salmonella + sodium butyrate) showing slightly tall and disorganized villi (thick arrow) suffering mild mucosal and submucosal leukocyte aggregation (thin arrows) and mild intestinal damage beside activation of intestinal crypts. H&E (X 300). **Fig (5):** Intestine of chicken (salmonella +sodium butyrate) showing greater regeneration of intestinal villi from proliferation of intestinal crypts. H&E (X 300). **Fig (6):** intestine of chicken (salmonella +sodium butyrate 5 days) showing tall and broad intestinal villi, partial desquamation of villous enterocytes (arrow) and active intestinal glands. H&E (X 300). **Fig (7):** Intestine of chicken (salmonella +sodium butyrate 5 days) showing severe proliferations of intestinal epithelium with greater villous length and width (villous fusion). H&E (X 300). **Fig (8):** Intestine of chicken (salmonella +sodium butyrate 5 days) showing intense hyperplasia of GALF (star) and slightly damage in villous enterocytes. H&E (X 300).

therapeutic agents and growth promoters resulting in development of resistant population of bacteria which made their subsequent use for therapy difficult and result in occurrence of antibiotic residues in the poultry products (Du Pont and Steels, 1987) . The direction towards the use of environmentally friendly alternatives as natural control method has been emerged. The reduction of the risk factors associated with enteropathogens, one of these alternatives is addition of organic acids (feed acidifiers) which has contributed immensely to the minimization of the pathogens coinciding. The short Chain Fatty Acid are considered as potential alternative to antibiotic growth promoter and used for years in poultry to control Salmonella infections (Van Immerseel et al., 2005; Van Immerseel et al., 2002). Several mechanisms may explain beneficial effects of butyrate as examples: control of pathogenic bacteria and improved performance, increased digestibility and reduced oxidative stress and inflammatory processes (Fernandez-Rubio et al., 2009; Guilloteau et al., 2010; Mallo et al., 2012; Panda et al., 2009; Van Immerseel et al., 2004), however, there are still details to be clarified in poultry.

The aim of this work was to study uses of sodium butyrate as antibacterial against *Salmonella enteritidis* which cause severe problem in poultry. In the present study , Table(3) showed reisolation of *Salmonella enteritidis* in G2 (positive control), the incidence of *Salmonella enteritidis* from muscles, liver, two ceci ,were 100% at 21, 35 of age, while G4 which was prophylactic with sodium butyrate, the incidence of *Salmonella enteritidis* from muscles, liver, two ceci were 0,20% , 40% at 21,35 days of age while in G5 which treated with sodium butyrate for successive 5 days the incidence of *Salmonella enteritidis* from muscles, liver, two ceci were 0,40% ,60% respectively at age 21 day. But the incidence was 20%, 60%, 80% respectively at age 35 day. It was seen that sodium butyrate was effective in reducing S.E. populations in caecal content. These reductions in Salmonella in the caecum is important for the microbiological safety of poultry products, because this site is common location in the birds where the bacteria are localized in high numbers (Cerquetti and Gherardi, 2000; Li et al., 2003; Van Immerseel et al., 2004). Compatible findings were seen with Cox et al. (1994), who showed that butyric acid in particular was effective in reducing Salmonella colonization of the intestine. Table (4) explain no mortalities were observed in group G1, G3, G4 all over observation period while mortality rate in G2 was 80% due to infection with *salmonella enteritidis* without treatment, while in G5 the highest morality rate is appear at 5th week due to stop of treatment with sodium butyrate-this result is agree with Fernandez-Rubio et al. (2009).

The current investigation showed an increase in total protein and globulin level in the infected group 2 while albumin significantly decreased comparing with control one at first week post challenge. The increase in total protein level may be attributed to increase globulin due to either antigenic stimulation of infectious agent or associated with development of liver disease (Azza et al., 2012). Also Xie et al. (2000) stated that *S. typhimurium* LPS treated birds resulted in increased levels of blood protein concentration due to an altered production of proteins related to the acute phase response as known in other species. Once the intracellular localization is achieved, *Salmonella* starts an extensive proliferation. Healthy birds recruit macrophages and lymphocytes B and T 24 h after infection. Macrophages engulf bacteria within the intestinal wall, which is the beginning of the systemic phase (Ricke, 2003). *Salmonella* is an intracellular bacterium that survives and multiplies inside the parasitophorous vacuoles of macrophages of internal organs such as the liver and spleen (systemic phase). This internal localization allows *Salmonella* to evade the host immune response (Beal and Smith, 2007). Macrophages, as part of the innate immune system, are also very adept in killing pathogenic microbes by phagocytosis and through the production of antimicrobial products like nitric oxide ((MacMicking et al., 1997). The results of this work showed significant decrease in macrophage, this result may have attributed to the rapid killing of host macrophages and the resulting inflammatory process would likely recruit more phagocytic cells to the site of the initial infection. This scenario could result in an ideal situation for the Salmonella bacteria to then infect more host cells but not to kill, to hide and replicate inside avoiding the host animal immune system.

NO are well-known as antimicrobial chemicals produced by macrophages in response to infection (Setta et al., 2012). In our study *Salmonella enteritidis* infection resulted in increased nitric oxide and at 1st and 2nd week post infection (PI) comparing with the control group. MDA showed significant decrease in group 2 and 4 in the first week, on the other hand MDA showed significant decrease in group 2 and 5 in the second week, but decrease in group 2 only in the third week. SOD showed significant decrease in group 2 in the first week, but in the second week there were decrease in groups 2, 4 and 5, while SOD revealed significant increase in groups 3,4 and 5 in the third

week The alteration in antioxidant indices by sodium butyrate, including the amount of MDA, GSH, and antioxidative enzymes, suggest an improvement in the level of oxidative stress in the intestinal mucosa cells, which may result in improved wound healing. All these results suggest that the mechanism by which butyrate promotes wound healing may not be fully due to the antioxidant stress (Song et al., 2011).

Our in vitro data suggest that sodium butyrate improves the intestinal tight junction and depresses permeability by improving antioxidant ability, a mechanism distinct from the previous studies (Sunkara et al., 2011). The effect of sodium butyrate on body weights in birds challenged with *salmonella enteritidis*. Body weights and body weight gains were significantly decreased in G2 (challenged with *salmonella enteritidis*) from 2 to 5 weeks of age comparing with all experimental groups. Birds that were protected with sodium butyrate (G3) showed significant increase in BW and BWG and lower FCR ($P < 0.05$) compared to the control group. Butyric acid benefits are caused by its effects on promoting intestinal epithelium cell development and modulating intestinal symbiotic bacteria growth (Antongiovanni et al., 2007). It reduces pathogenic bacteria growth and increases beneficial ones. However, groups (4, 5) showed improvements at ($P < 0.05$) in BW and BWG. It is evident form these results that birds protected with sodium butyrate exhibited higher body weights body weight gains and food conversion rate among all groups all over this trial. The results indicated that sodium butyrate supplementation can improve the growth performance in chickens under stress and this may be attributed to moderate the immune response and reduce tissue damage (Zhang et al., 2011), high absorptive surface from tall and thick intestinal villi and hyper activation of intestinal crypts and proliferation of villous enterocytes. There is an indication from the present study of modification to villi development relative to birds protective and treated with sodium butyrate (Hu and Guo, 2007). Sakata (1987) showed that infusion of butyrate into fistulated rats increased the proliferation of crypt cells in both the small and large intestines. Sharma et al. (1995) suggested that the effect on crypt cell growth may reflect changes in the gut microflora, which is known to be a major modulator of epithelial cell activity. Sodium butyrate is a preferred source of energy for the enterocytes. This will result in better development of the intestinal villi, and also in stronger gut lining. Leeson et al. (2005) found numerically longer villus in the duodenum of birds receiving butyric acid in the diets than control birds. The lengths and widths of the villus were also positively affected by the addition of butyrate in the diet.

From this trial and previous research, it was concluded that Na butyrate can be used in treatment and prevention of salmonella infection in chickens as it improves the digestibility of energy and protein by increasing intestinal absorption surface.

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