Asian Journal of

Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS

Asian Journal of Poultry Science

ISSN 1819-3609
DOI: 10.3923/ajpsaj.2016.104.110

Research Article
Effect of Sodium Butyrate on Salmonella Enteritidis Infection in Broiler Chickens

[1]Wafaa A. Abd El-Ghany, [1]M.H. Awaad, [2]S.A. Nasef and [2]A.F. Gaber

[1]Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Animal Health Research Institute, Dokki, Egypt

Abstract
This study was designed to examine the effect of using sodium butyrate encapsulated in palm fat in comparison with enrofloxacin on Salmonella Enteritidis (SE) infection in broiler chickens. Two hundred, day-old broiler chicks were allocated into 5 equal groups (n = 50). Group 1 was kept without challenge or treatment (blank control), Group 2 was fed on sodium butyrate, Group 3 was challenged and treated with enrofloxacin, Group 4 was challenged and treated with sodium butyrate and Group 5 was only challenged (positive control). Challenged groups were orally inoculated with 0.3 mL (1.5×10^8 SE/mL/chick) at the 2nd day of age. Enrofloxacin was given at the 3rd day of age in water (10 mg kg^{-1} b.wt.) for 5 successive days; however, sodium butyrate was added in doses of 1.0, 0.5 and 0.25 kg t^{-1} for starter, grower and finisher ration respectively from day till 5 weeks old. The results revealed no mortalities and decrease in the severity of signs and lesions in treated groups than positive control one. At the 4th week of age, sodium butyrate supplement gave significant ($p\leq0.05$) improvement in body weight, weight gain and feed conversion than others. The re-isolation rate and enumeration of SE were lower in sodium butyrate and enrofloxacin treatments than positive control. In conclusion, sodium butyrate as an acidifier could be used as an environmentally friendly supplement when compared with enrofloxacin for treatment of SE infection in broiler chickens as it could reduce the disease picture severity, improve performance variables and decrease the intestinal colonization.

Key words: Sodium butyrate, Salmonella, chickens, performance

Received: December 14, 2015 Accepted: February 09, 2016 Published: March 15, 2016

Citation: Wafaa A. Abd El-Ghany, M.H. Awaad, S.A. Nasef and A.F. Gaber, 2016. Effect of sodium butyrate on Salmonella enteritidis infection in broiler chickens. Asian J. Poult. Sci., 10: 104-110.

Corresponding Author: Wafaa A. Abd El-Ghany, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salmonella Enteritidis (SE) is still one of the leading causes of foodborne infections in the world, mainly due to the consumption of food prepared from poultry meat and eggs (Rabsch *et al*., 2001). Effective control of pathogens, such as Salmonella, represents a major task to the poultry producers worldwide. Under such circumstances antibiotics as growth promoters in food animal production have been used since 1946 throughout the world (Chowdhury *et al*., 2009). They are used in poultry production to improve performance, to stabilize the intestinal microbial flora and to prevent some specific intestinal pathogens (Jones and Ricke, 2003). In recent years, concerns about antimicrobial resistance have grown, but the main concerns have been focused specifically on resistance within the food supply (Barza, 2002; Cui *et al*., 2005).

Organic acids are among the alternative growth promoters that are already being used in practice for decades and stimulate growth performance in poultry (Dibner and Buttin, 2002; Dibner, 2004). The supplementation of organic acids to poultry diets was shown to suppress the growth of certain species of bacteria, mainly acid-intolerant species, such as Salmonella, *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes* and Campylobacter (Van Immerseel *et al*., 2002) and enhanced nutrient utilization, growth and feed efficiency (Denli *et al*., 2003).

Butyric acid is one of Short Chain Fatty Acids (SCFA), which has higher bactericidal activity when the acid is un-dissociated (Leeson, 2007). It can be used for the treatment of several intestinal bacterial infections like salmonellosis (Van Immerseel *et al*., 2005; Fernandez-Rubio *et al*., 2009). Butyrate, which is a by-product of microbial fermentation of products such as resistant starch is considered to be important for normal development of epithelial cells (Pryde *et al*., 2002; Brouns *et al*., 2002).

This study was designed to examined the effect of using sodium butyrate encapsulated in palm fat when compared with enrofloxacin on disease picture, zootechnical performance variables and intestinal colonization of SE experimentally infected broiler chickens.

MATERIALS AND METHODS

Experimental chickens: Two hundred and fifty five, day-old Cobb broilers (mixed sex) from a local hatchery were used in this study. The birds were housed in battery cages in groups. Birds were provided with water and Salmonella free feed *ad libitum*. The chickens were vaccinated against different diseases according to the vaccination programs usually adopted in Egyptian chicken broiler farms.

Ration: Chickens were fed on commercial balanced ration (Feedmix company) free from any pathogen or medicinal additives. Prior to use, the ration (random sample) was examined for Salmonellae using the standard method. From 1-16 days of age, they received a starter diet (not less than 23% crude protein; 3000 kcal kg^{-1} ME), from 17-28 days of age a grower diet (not less than 21% crude protein; 3100 kcal kg^{-1} ME) and from 29-35 days of age a finisher diet (not less than 19% crude protein; 3150 kcal kg^{-1} ME).

Feed additives: Sodium butyrate encapsulated in palm fat (Admix®30 produced by NUTRI-AD international, Belgium) was used in this trial in the following dietary levels in the test groups; Starter diet: 1 kg t^{-1}, Grower diet: 0.5 kg t^{-1} and Finisher diet: 0.25 kg t^{-1} from day old till the end of experiment (35 days).

Drug used: At the 3rd day of age, oral solution of enrofloxacin 20% was given in a dose of (10 mg kg^{-1} b.wt.) (0.25 mL L^{-1} of the drinking water) as continuously for 5 successive days.

Challenging bacteria: Bacteriologically, serologically and molecularly identified avian strain of SE was obtained from Reference Laboratory for Veterinary Quality Control of Poultry Production (RLQP), Dokki, Egypt. The used strain of SE was grown in 10 mL of buffered peptone water and incubated at 37°C for 24 h. Fresh inoculums of 5×10^8 Colony Forming Unit CFU mL^{-1} were prepared according to Mc Farland standard in normal saline to inoculate 0.3 mL $chick^{-1}$ (Fernandez *et al.,* 2002) at 3 days old chicks.

Experimental design: Two hundred and fifty five, day-old broiler chicks were kept for 5 weeks. At arrival, 5 sacrificed chicks were cultured to confirm their freedom of Salmonellae. Chicks were randomly allocated into 5 equal groups (1-5), consisting of 50 birds each. Group 1 was kept without challenge or treatment (blank control), Group 2 was fed on sodium butyrate, Group 3 was challenged and treated with enrofloxacin, Group 4 was challenged and treated with sodium butyrate and Group 5 was only challenged (positive control).

Measured parameters

Diseases picture: Clinical signs, mortalities and post-mortem lesions were observed and recorded daily after challenge.

Zootechnical performance variables: During the 35 days experimental period, the growth performance parameters of chickens were evaluated by recording body weight in weekly intervals and total feed consumption till the end of study. Weekly and cumulative body weight gains of birds were calculated for each group according to Brady (1968). Feed consumed was recorded daily, the uneaten discarded and Feed Conversion Ratio (FCR) was calculated (total feed: total gain) according to the equation of Ensminger (1980).

Bacterial re-isolation: Liver and caecum were collected from 5 sacrificed birds in the challenged groups at 7 and 19 day of age for SE re-isolation according to ISO 6579 (2002) (Microbiology of feeding stuffs-horizontal method for detection of Salmonella species).

Bacterial enumeration: Five caecal contents from each group at 35 days of age were examined for SE enumeration (Thushani *et al.*, 2003). Decimal dilutions in BPW were prepared and 0.1 mL of each dilution was inoculated by spread plate to XLD in duplicate. These plates were incubated for 18-24 h at 37°C and dark centered colonies were counted as Salmonellae.

Statistical analysis: At the end of experiment, some analyses was done via Statistical Analyses Software (SAS) in the statistical level of 5% according to data gathered from dietary, weight improvement, average of feed conversion rate and weight of rearing period.

RESULTS AND DISCUSSION

The widespread use of antibiotics as 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. To reduce 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 SCFA are considered as potential alternative to antibiotic growth promoter (Van Immerseel *et al.,* 2005) and used for years in poultry to control Salmonella infections (Van Immerseel *et al.*, 2002).

No mortalities were observed in all groups, all over the observation period. No clinical signs were seen in blank and sodium butyrate treated chickens. Clinical signs of depression, ruffling, dullness, watery diarrhea and off food were observed 3 days post-challenge in SE experimentally infected groups. Severe signs were recorded in positive control group, however, the severity of signs was milder in sodium butyrate and enrofloxacin treated groups than un-treated positive control one. Sacrificed chickens of control groups showed no lesions, while SE challenged groups revealed septicaemia, enteritis and congested internal organs, where, the severity of lesions were less pronounced in treated groups.

In the present study, Table 1 clearly demonstrates that enrofloxacin used agains SE in the study significantly ($p\leq 0.05$) improved the body weight of the broilers compared with other groups. Sodium butyrate supplementation showed lower significant ($p\leq 0.05$) body weight at the 1st, 2nd, 3rd and 5th week of age. At the 4th week old, there were no significant differences between groups and infected treated chickens with sodium butyrate showed improvement in this parameter. Abdel-Fattah *et al.* (2008) found that the addition of dietary citric acid, acetic acid or lactic acid improved body weight of broiler chickens compared with control group and Chowdhury *et al.* (2009) found that citric acid supplementation caused significant increase in body weight of broilers. However, Bonos *et al.* (2010) observed no effect on body weight of Japanese quail by addition of acidifiers to diets.

Table 1: Weekly body weight means of chickens in different treatment

	Weeks of age (Mean±SE)				
Groups	1st week	2nd week	3rd week	4th week	5th week
Group 1	147.75 ± 2.15^{ab}	375.70 ± 6.65^{ab}	805.08 ± 11.521^{ab}	1368.14 ± 16.91^{a}	1914.89 ± 32.24^{ab}
Group 2	142.62 ± 2.23^{b}	349.23 ± 8.23^{cd}	766.72 ± 15.52^{bc}	1302.42 ± 20.75^{a}	1853.36 ± 33.68^{ab}
Group 3	149.72 ± 2.09^{a}	$377.58\pm7.83_{a}$	824.40 ± 14.046^{a}	1367.79 ± 21.54^{a}	1947.76 ± 29.37^{a}
Group 4	143.31 ± 2.06^{ab}	327.60 ± 8.74^{b}	728.54 ± 18.57^{c}	1315.63 ± 24.51^{a}	1821.55 ± 40.13^{b}
Group 5	146.68 ± 2.14^{ab}	353.78 ± 8.86^{bc}	795.09 ± 16.33^{ab}	1348.35 ± 23.10^{a}	1898.69 ± 38.51^{ab}

NB: Different litters of columns denote significant variations between means ($p\leq 0.05$), SE: Standard error, Group 1: Group of birds neither challenged nor treated (blank), Group 2: Group of birds treated with sodium butyrate (negative control), Group 3: Group of birds experimentally challenged with SE and treated with enrofloxacin, Group 4: Group of birds experimentally challenged with SE and treated with sodium butyrate, Group 5: Group of birds experimentally challenged with SE (positive control)

Table 2: Weekly body weight gain means of chickens in different treatment

	Body weight gain (g/bird/week) (Mean±SE)					
Groups	0	1st week	2nd week	3rd week	4th week	5th week
Group 1	47.80	99.95±0.28[b]	227.95±0.04[a]	429.38±0.35[b]	563.06±1.07[b]	546.75±0.14[c]
Group 2	46.89	95.73±0.36[c]	206.61±0.22[b]	417.49±0.29[c]	536.70±0.17[e]	550.94±0.18[b]
Group 3	47.70	102.00±0.44[a]	227.86±0.46[a]	446.86±0.36[a]	543.35±0.57[d]	579.97±0.85[a]
Group 4	46.80	96.51±0.28[c]	184.29±0.11[c]	400.94±0.61[d]	587.09±0.56[a]	505.92±1.15[d]
Group 5	46.90	99.78±0.12[b]	207.10±0.05[b]	441.31±0.17[a]	553.26±1.15[c]	550.34±0.53[b]

NB: Different litters of columns denote significant variations between means ($p \le 0.05$), SE: Standard error, Group 1: Group of birds neither challenged nor treated (blank), Group 2: Group of birds treated with sodium butyrate (negative control), Group 3: Group of birds experimentally challenged with SE and treated with enrofloxacin, Group 4: Group of birds experimentally challenged with SE and treated with sodium butyrate, Group 5: Group of birds experimentally challenged with SE (positive control)

Table 3: Weekly feed intake means of chickens in different treatment

	Feed intake (g/bird/week) (Mean±SE)				
Groups	1st week	2nd week	3rd week	4th week	5th week
Group 1	189.96±0.35[c]	404.92±0.086[a]	651.56±0.04[b]	817.77±0.28[c]	954.29±0.29[a]
Group 2	188.20±0.21[c]	370.38±0.034[b]	687.90±0.15[a]	878.00±0.24[a]	989.83±0.19[a]
Group 3	197.56±0.21[b]	404.71±0.09[a]	778.39±0.58[a]	941.00±0.424[b]	981.62±0.22[a]
Group 4	221.20±0.098[a]	356.48±0.07[c]	724.23±0.45[c]	964.05±0.18[b]	882.28±0.41[c]
Group 5	199.56±0.29[b]	363.17±0.26[b]	750.69±0.15[b]	976.11±0.29[b]	929.57±0.04[b]

NB: Different litters of columns denote significant variations between means ($p \le 0.05$), SE: Standard error, Group 1: Group of birds neither challenged nor treated (blank), Group 2: Group of birds treated with sodium butyrate (negative control), Group 3: Group of birds experimentally challenged with SE and treated with enrofloxacin, Group 4: Group of birds experimentally challenged with SE and treated with sodium butyrate, Group 5: Group of birds experimentally challenged with SE (positive control)

The results of average body weight gains of the broiler groups for 5 weeks are summarized in Table 2. The effect of sodium butyrate on the broiler weight gain is consistent with its effect on body weight in this study. Sodium butyrate treated group showed significant ($p \le 0.05$) decrease in the weight gain at 1, 2, 3, 5 weeks of age compared to positive control and enrofloxacin treated groups but in 4 week old, sodium butyrate treated chickens showed an increase in the body weight. In addition, the average daily weight gain at weeks 3, 4 and 5 the birds fed on sodium butyrate showed higher daily weight gains than the control group. Our results corresponds with consequences reported from Mansoub (2011), who reported that up to 0.2% of sodium butyrate increased weight gain of broilers during the first 28 days. In growing and finishing phases, chicks fed partially protected sodium butyrate diet showed better weight gain than chicks fed control. Contrary to the findings of the present study, Antongiovanni *et al.* (2007) and Mahdavi and Torki (2009), who demonstrated that sodium butyrate or colistin sulfate supplementation in starter phase did not affect weight gain, feed intake and feed conversion ratio. Moreover, Leeson *et al.* (2005), Hu and Guo (2007) and Aghazadeh and TahaYazdi (2012) found that butyric acid supplementation had no effect on average weight gain or feed conversion rate.

Table 3 shows the feed intake along 5 weeks experimental period for different groups. Infected and treated chickens with sodium butyrate consumed significantly ($p \le 0.05$) more feed than positive control group as well as the other birds receiving either of the other treatment (enrofloxacin or sodium butyrate) in weeks 1, 3 and 4 of age. Compared with positive control group, birds fed on sodium butyrate alone consumed the same amount of feed in the 1st and 5th weeks of age, decreased in the 2nd week and increased in weeks 3rd and 4th one. Compatible results were with Leeson *et al.* (2005), who detected that feed intake of the birds fed 0.4% butyric acid was decreased compared with birds fed the non-medicated diet during the starter period and birds fed 0.2% butyric acid had similar feed intake to the control birds. Also, Zulkifli *et al.* (2000) demonstrated that broiler chickens fed on probiotic and butyric acid showed decrease in feed intake, but Nezhad *et al.* (2007) and Chowdhury *et al.* (2009) found that addition of citric acid did not affect feed intake in broilers. Pinchasov and Jense (1989) and Hu and Guo (2007) reported that butyric acid, unlike other acids such as propionate, did not depress feed intake. Aghazadeh and TahaYazdi (2012) recorded that total feed intake (0-42 day) was greater in the group fed butyric acid in a dose of 2.5 g kg^{-1} in both starter and grower feed and Panda *et al.* (2009) reported that butyrate up to 0.6% had no adverse effect on feed intake.

The results of feed conversion rate in different groups along the 1st, 2nd, 3rd, 4th and 5th week of age are tabulated in Table 4. Comparing the results of feed conversion rate between blank negative control chickens and those fed on

Table 4: Feed conversion rate means of chickens in different treatment

Groups	Feed conversion rates 1st week	2nd week	3rd week	4th week	5th week
Group 1	1.90±0.005[c]	1.78±0.005[b]	1.52±0.012[d]	1.45±0.017[c]	1.74±0.023[b]
Group 2	1.96±0.017[bc]	1.79±0.017[b]	1.64±0.02[c]	1.64±0.023[b]	1.80±0.012[a]
Group 3	1.93±0.017[c]	1.78±0.012[b]	1.74±0.04[b]	1.73±0.02[a]	1.69±0.017[c]
Group 4	2.20±0.033[a]	1.93±0.005[a]	1.81±0.03[a]	1.64±0.007[b]	1.74±0.02[b]
Group 5	2.00±0.005[b]	1.75±0.011[c]	1.64±0.04[c]	1.76±0.012[a]	1.68±0.011[c]

NB: Different litters of columns denote significant variations between means ($p \leq 0.05$), SE: Standard error, Group 1: Group of birds neither challenged nor treated (blank), Group 2: Group of birds treated with sodium butyrate (Negative control), Group 3: Group of birds experimentally challenged with SE and treated with enrofloxacin, Group 4: Group of birds experimentally challenged with SE and treated with sodium butyrate, Group 5: Group of birds experimentally challenged with SE (positive control)

Table 5: Re-isolation of SE from internal organs (liver and caecum) of experimentally infected chicken at day 19th of age

Organ	No. of examined samples	Group 3 No.	Group 3 %	Group 4 No.	Group 4 %	Group 5 No.	Group 5 %
Caecum	5	0	0	2	40	4	80
Liver	5	0	0	3	60	4	80

Group 3: Group of birds experimentally challenged with SE and treated with enrofloxacin, Group 4: Group of birds experimentally challenged with SE and treated with sodium butyrate, Group 5: Group of birds experimentally challenged with SE (positive control)

Table 6: Most probable number of SE in ceacal content of experimentally infected chicken at 35th day of age

Organ	No. of examined chicken	SE counts Groups 3	Groups 4	Groups 5
Caecum	5	0.5×10^2	8×10^2	1.0×10^3
		0	0	0.5×10^4
		0	0	5.0×10^3
		0	0	0
		0	0	0

Groups 3: Group of birds experimentally challenged with SE and treated with enrofloxacin, Groups 4: Group of birds experimentally challenged with SE and treated with sodium butyrate, Groups 5: Group of birds experimentally challenged with SE (positive control)

sodium butyrate, that there were no significant ($p \leq 0.05$) difference at the 1st and 2nd week, became significantly ($p \leq 0.05$) better at the 3rd, 4th and 5th weeks old. It can be noticed that sodium butyrate treated group showed high feed conversion rate than positive control group and enrofloxacin treated one at the 1st, 2nd, 3rd and 5th week of age, meanwhile, at the 4th week feed conversion rate became more better than others. These results are in agreement with those reported by Leeson *et al.* (2005), Hu and Guo (2007) and Aghazadeh and TahaYazdi (2012), who recorded that diet butyric acid (2-4 g kg^{-1}) had no effect on feed conversion ratio during the period from 0-42 days. the obtained results are incompatible with those reported by Smulikowska *et al.* (2009), who reported that sodium butyrate positively affected feed conversion rate in comparison with the control diet and Panda *et al.* (2009) who found that higher concentration of butyrate i.e., 0.4% in the diet was adequate for optimum body weight gain and feed conversion ratio and those reported by El-Sawy *et al.* (2015) and Taherpour *et al.* (2009) as they concluded that higher levels of sodium butyrate were required for optimum average weight gain and feed conversion rate.

Table 5 shows the results of re-isolation of SE from caecum and liver from infected and treated groups at day 19 of age. It was seen that the highest incidence of SE re-isolation was in positive control group (80%) from both caecum and liver, followed by 40, 60 and 0% and 0% from sodium butyrate and enrofloxacin treatments, respectively.

Considering the results of SE enumeration at the end of the experiment (5 weeks), Table 6 demonstrated that the highest most probable number of SE was in positive control chickens but decreased in both enrofloxacin and sodium butyrate treated ones. Results revealed that sodium butyrate feed supplemented was effective in reducing SE populations in caecal content. These reductions in Salmonella counts in the caecum is important for the microbiological safety of poultry products, because this site and also cloaca represent two common locations in the birds where the bacteria are present 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. Moreover, Zou *et al.* (2010) reported that the populations of Salmonella, *Escherichia coli* and *Clostridium perfringens* in the caecum were decreased by supplementation of sodium butyrate.

Organic acids were shown to lower the pH in the animal intestines and as a result, bacterial growth will be disturbed. The non-ionized (un-dissociated) organic acids can infiltrate the bacterial cell wall and interrupt the normal physiology of certain types of bacteria by disrupting DNA and protein synthesis in the bacteria (Nursey, 1997).

It is commonly that the SCFA diffuse into the bacterial cell in un-dissociated form which is favored by low pH. Inside the bacterial cell the acid dissociates, resulting in reduction of intracellular pH and anion accumulation (Russell and

Diez-Gonzalez, 1997; Van Der Wielen *et al*., 2000). It is proposed that SCFA in the feed will have antibacterial effects in the crop but will have no effects further down in the gastrointestinal tract (Thompson and Hinton, 1997). The SCFA, however, can be impregnated in or coated on micro-pearls, from which they are released slowly during transport in the gastrointestinal tract. In this way SCFA could also reach the small intestine and the caeca, the latter being the predominant site for Salmonella colonization (Anonymous, 1997).

The principle of microencapsulation and continuous slow release of the encapsulated products was recently developed and has potential as a way to target probiotic bacteria as well as chemical compounds to the intestinal environment (Cheu *et al*., 2001; Favaro-Trindade and Grosso, 2002). These results are in agreement with the findings of Van Immerseel *et al*. (2005) who found that coated butyric acid was superior to uncoated butyric acid in reducing Salmonella colonization of the ceca and internal organs of SPF layer chickens shortly after infection with SE.

CONCLUSION

Sodium butyrate as an acidifier could be used as an environmentally friendly supplement when compared with enrofloxacin for treatment of SE infection in broiler chickens as it could reduce the disease picture severity, improve performance variables and decrease the intestinal colonization. Taking in consideration that food safety is probably the biggest issue facing poultry production systems today and preventing contamination of poultry products with food borne pathogens remains a considerable challenge for producers and integrations; sodium butyrate can be of closer scrutiny as it can be part of feeding concept to replace antibiotic growth promoters specially as organic acid compounds do not cause residues in meat and therefore are not harmful to human beings.

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