**Discussion**

The widespread use of antibiotics as therapeutic agents and growth promoters result in the 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 **(DuPont and Steele, 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 feed acidifiers which has contributed immensely to the minimization of pathogens. Butyric acid is one of short chain fatty acids (SCFA), which has higher bactericidal activity when the acid is un-dissociated **(Zhou *et al*., 2014)**. It has been that used for years in poultry to control intestinal bacterial infection like salmonellosis**(Van Immerseel *et al.,* 2005)**.

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 **(Awaad *et al.*, 2014)**; sodium butyrate encapsulated in palm fat can be of closer scrutiny as it can be part of feeding concept to replace antibiotic growth promoters **(Lückstädt, 2003)** specially as organic acid compounds do not cause residues in meat, and therefore are not harmful to human beings **(Sterzo *et al*., 2007)**.

Therefore, in the current study, we investigated the efficacy of using encapsulated sodium butyrate in comparison with enrofloxacin for reducing (SE) in broiler chickens.

No mortalities were observed in all groups under the conditions of our study. Signs of depression, ruffling, dullness, watery diarrhea and off food were seen in (SE) challenged groups which were milder in the treated groups than positive control one. Sacrificed chickens of (SE) challenged groups showed septicaemia, enteritis and congested internal organs where the severity of lesions was less pronounced in treated groups.

Here, sodium butyrate did not affect on body weight in broilers compared with other groups. The obtained results are compatible with those reported by **Bonos *et al.* (2011)** who observed no effect on body weight of Japanese quails by addition of acidifiers to diets. Whereas **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. **Chowdhury *et al.* (2009)** reported that citric acid supplementation as an acidifier induced significant increase on body weight in broiler chickens.

It was found that at 4th week of age ,chicks fed sodium butyrate diet showed better weight gain than chickens in control. Result of this experiment corresponds with consequences reported by **Mansoub (2011)** who found that up to 0.2% of sodium butyrate in diet increased weight gain during the first 28 days. Contrary to the findings of the present study, **Antongiovanni *et al*. (2007); Liu (2009) and Mahdavi and Torki (2009)** recorded 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 *et al.* (2012)** stated that butyric acid supplementation had no effect on average weight gain or feed conversion rate.

In this work, it was noticed that birds fed sodium butyrate consumed less than positive control group ones. This result is compatible with that reported by **Leeson *et al.* (2005)** who demonstrated that feed intake of birds fed 0.4% butyric acid was decreased compared with birds fed non-treated diet during the starter period, and birds fed 0.2% butyric acid had similar feed intake to the control birds. Also, **Pinchasov and Jensen (1989); Hu and Guo (2007) and** **Panda *et al.* (2009)** reported that butyric acid, unlike other acids such as propionate, did not depress feed intake. **Aghazadeh *et al.* (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 in both starter and grower feed. On the other hand, **Zulkifli *et al.* (2000)** observed that broiler chickens fed on probiotic and butyric acid showed decrease in feed intake. Moreover, **Nezhad *et al.* (2007)** **and** **Chowdhury *et al.* (2009)** found that addition of citric acid did not affect feed intake in broilers.

Sodium butyrate treatment at 4 weeks old showed better FCR than positive control and enrofloxacin treated groups. Our results in agreement with those reported by **Smulikowska *et al.* (2009)** who demonstrated that sodium butyrate positively affected FCR in comparison with the control diet and **Panda *et al.* (2009)** who found that higher concentration of butyrate (0.4%) in the diet was adequate for optimum body weight gain and FCR. Additionally, **Taherpour *et al.* (2009) and El-Sawy *et al.* (2015)** concluded that higher levels of sodium butyrate were required for optimum average weight gain and FCR. Contrary results by **Leeson *et al.* (2005) and Hu and Guo (2007)** who detected that diet with butyric acid (2 to 4 g/kg) had no effect on FCR during 42 days. **Aghazadeh *et al.* (2012)** demonstrated that butyric acid supplementation did not improve FCR.

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 **(Brons *et al*. 2002 and Pryde *et al*., 2002)**.Butyrate appears to play a role in development of the intestinal epithelium and recognized as the most effective source of energy for epithelial cells proliferation resulting in improvement of zootechnical performance parameters **(Mroz *et al.*, 2006)**.

In the current study, the efficacy of microencapsulated sodium butyrate for reducing (SE) infection was investigated. Re-isolation of (SE) from liver and caecum of experimentally infected chickens at day 19th of age revealed 60% and 40% respectively in sodium butyrate supplemented group as compared with 80% in infected untreated group (positive control). Reduction in (SE) enumeration in the cecum of sodium butyrate supplemented group at the end of the experiment (35th day of age) is important for the microbiological safety of poultry products, as this site and cloaca represent two common locations in the birds where the bacteria are present in high numbers **(Cerquetti and Gherardi, 2000 and Li *et al.*, 2003**). This might be due to the continuous slow release of the acidifier **(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 caeca and internal organs of SPF layer chickens shortly after infection with (SE), **Van Immerseel *et al.* (2004)** who indicated significant reduction of *Salmonella* in the caeca of birds fed organic acids and **Cox *et al.* (1994)** who showed that butyric acid in particular was effective in reducing *Salmonella* colonization of the intestine. Similarly, **Zou *et al.* (2010)** demonstrated that *Salmonellae*, *Escherichia* Coli and *Clostridium* Perfringens populations in the caecum were decreased by supplementation of sodium butyrate.

The auspicious effect of acidifiers over the organism is due to the better adhesion of the lactic acid bacteria to the intestinal epithelium in comparison with the pathogenic bacteria and stooping their implementation over the mucus membranes of the intestine **(Awaad *et al.*, 2011)**.

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)**.

The pathogenesis of salmonellosis depends upon a large number of factors controlled by an array of genes those synergies into the actual virulence of *Salmonella* **(Murugkar *et al.*, 2003)**. Nucleic acid based diagnostic techniques are being employed for the detection of various gene-encoded virulence factors **(Prager *et al.*, 1995 and Rahman *et al.*, 2000)**.Accordingly; *in vivo* assay using conventional PCR was conducted to express these genes in the used (SE) strain after its re-isolation from livers of experimentally infected birds (7th day post-inoculation) from groups treated or untreated with sodium butyrate. Three virulence genes were assayed including; *Salmonella* invasion (*inv*A) *Salmonella* enterotoxin (*stn*), and plasmid encoded fimbrial (*pef*A) genes. Strains that re-isolated from untreated group detected all these genes with an incidence of 100%. While modulation and deletion of *pef*A or *stn* genes were recorded in sodium butyrate treated strains with an incidence of 33.33%. **Durant *et al.* (2000)** reported that SCFA modulated the expression of the *hil*A and *inv*F genes of *S.* Typhimurium. **Boyen *et al.* (2008)** found that some frequently used SCFA and medium-chain fatty acids were able to alter virulence gene expression and decrease *S*. Typhimurium colonization and shedding in pigs using well established and controlled *in vitro* and *in vivo* assays. Modulation of these virulence genes in the present work might explain the antibacterial effect of sodium butyrate; a conclusion that could be partially confirmed by reports of **Baloda *et al.* (1983)** and **Chopra *et al.*, (1987)**. who mentioned that *Salmonella* induced diarrhoea is a complex phenomenon involving several pathogenic mechanisms including production of enterotoxin which is mediated by *stn* gene. **Thorns *et al.* (1996)** have shown that *pef* genes product play an important role in bacterial adhesion to the epithelial cells. **Csiko *et al.* (2014)** found that butyrate, a commonly applied feed additive in poultry nutrition, can modify the expression of certain genes, including those encoding cytochrome P450 (CYP) enzymes.

Dissociated internal butyrate can affect *Salmonella* virulence in a variety of manners. *In vitro* cell invasionof epithelial cells can be suppressed (decreased hilA, invF and sipC expressions) when butyrate andpropionate, but not acetate, were added to the culturemedia **(Porter and Curtiss, 1997 and**  **VanImmerseel *et al.*, 2003 and 2004)**. **Lawhon *et al.* (2002)** explained the bacterial suppressor effect of acids on the basis of changes in *Salmonella* Pathogenicity Island (SPI-1) expression. It has been found that SPI-1 contains *Salmonella* virulence genes arranged in operonsrequired to invade epithelial host cells during early stages of infection. These genes are transcriptionally regulated by the HilA protein, encoded by a gene of the SPI-1 pathogenic island **(Durant *et al.*, 2000)**.

Electron microscopy examination of (SE) strains (SEM and TEM) re-isolated from the caeca of broiler chickens (with or without sodium butyrate supplementation) could reveals some changes, according our conditions of examination. SEM of samples isolated from sodium butyrate treated group revealed some degraded and broken cells and the bacterial population looked aged to some extent. While the bacterial cells looked normal in untreated group (control positive). TEM of samples isolated from treated group showed transparent “lipids like” bodies, while normal features was observed in the untreated group. Further investigations are necessary to understand the association, if any, between sodium butyrate treatment and detected changes in bacterial cells.These results are similar to concept of **Belguith *et al*.,(2009)** Who observed morphological changes in treated *Salmonella* with Aqueous garlic extract (AGE).AGE-treated bacteria showed a remarkable lysis of the cell membrane and nonhomogeneous disposition of cytoplasmic materials, in contrast to control bacteria with intact cell membranes.and **Martínez-Arámburu *et al*.,(2015)** detected that the sodium salts of ferulic and caffeic acids produced a bacteriostatic or bactericidal effect depending on the dose and the cells of *S.* Typhimurium *and L.* Monocytogenes exposed to hydroxycinnamic salts in sublethal conditions experienced morphological changes, such as elongated microstructures.

The histomorphometric observations of the gut revealed that (SE) challenged untreated group showed severe necrosis of enterocytes with massive heterophils infiltration in lamina propria. On the other hand; (SE) challenged sodium butyrate treated group showed intact enterocytes with increased number of goblet cells besides folding of intestinal surface with mild inflammatory reaction involving the lamina propria. Parallel results were obtained by **Brons *et al.* (2002**) **and Pryde *et al.* (2002)** who mentioned that butyrate appears to play a role in the development of the intestinal epithelial cells. **Frankel *et al.* (1994)** and **El-Sawy *et al*. (2015)** found that sodium butyrate increased the villus height and crypt depth of the jejunum. Furthermore, **Jiang *et al.* (2014)** investigated the effects of micro-encapsulated sodium butyrate on oxidative stress and apoptosis induced by dietary corticosterone in the intestinal mucosa of broiler chickens. The protective effect of sodium butyrate supplementation in birds challenged with (SE) was reflected on intestinal histomorphometric parameters as the presence of microbial load like (SE) caused significant reduction in villous height, villous height: crypt depth ratio and significant increase in crypt depth that is not related to increase villous height as shown but related to increased cell turn over and sloughing induced by *Salmonella*. Accordingly; sodium butyrate has a protective effect against fast tissue turn over induced by (SE) and relatively and partially increased villous height and villous height: crypt depth ratio as compared with sodium butyrate non challenged group that achieving significant increase in intestinal parameters and the final results reflected on villous height: crypt depth ratio to be similar to un-challenged un-treated group (normal histological structure of intestinal villi). These results indicated that sodium butyrate supplementation achieved a beneficial effect on intestinal histomorphometric parameters by increasing villous height and villous height: crypt depth ratio which in turn positively might reflect on nutrient digestion and absorption and finally the body weight. Also, sodium butyrate supplementation increased crypt depth that was beneficial whereas crypt depth is considered as a progenitor cells for villous epithelium and this may be assumed to be related to the demand for increase of the villous height and hence total increase in gut surface area. **Mallo *et al.* (2011)** evaluated the influence of sodium butyrate on the animal performance, energy and protein digestibility of the diet and villi development. The lengths and widths of the villi were affected by the addition of butyrate in the diet. **Scheppach *et al.* (1995)** recorded that SCAFA stimulate the proliferation of normal crypt cells, improving healthy tissue turnover and maintenance.

It was concluded that inclusion of butyrate in the diet improves the digestibility of energy and protein by increasing intestinal absorption surface. **Chamba *et al.* (2014)** evaluated the partial protection of sodium butyrate on intestinal villi and *E.* Coli development in broiler chickens. They mentioned that jejunal villi of birds fed sodium butyrate and colistin at 42 days of age were higher than those in birds fed the control diet indicated by improved performance, by improving intestinal villi development in broilers chickens. In contrary with ours, those reported by **Biagi *et al.* (2007) and Claus *et al.* (2007)** who demonstrated insignificant effect of sodium butyrate on small intestinal epithelium of healthy pigs and chickens.

In conclusion; the used microencapsulated sodium butyrate in the present investigation had a positive effect on performance , bactericidal action against SE as it reduced its enumeration in caecum and liver and altered its morphology as well as modulated some of its virulence genes. It also played a positive role in the development of intestinal epithelial cells.