**Review of Literature**

**2.1. Economic importance of *Salmonellae*:**

**Cowden *et al.*, (2003)** found outbreaks of salmonellosis, involving cases of infection with *Salmonella* Enteritidisphage types (PT) 5c and 6a.

**Rabsch *et al*., (2003)** said that the animal-derived food products continue to be the most important source for human cases of salmonellosis in the United States. *Salmonella* Typhimurium, the most frequently isolated serotype from human cases of disease in the United States (23% ) of cases reported between 1987 and 1997, is also the most frequently isolated serotype from beef carcasses (14% of isolations) and raw ground pork (18% of isolations). *Salmonella* Typhimuriumis the third most common isolate from chicken carcasses (10% of isolations), raw ground beef (15% of isolations) in the United States However, some *Salmonella* serotypes frequently isolated from patients are infrequently isolated from food sources *Salmonella* Enteritidis, the second most frequently isolated serotype from patients (21% of cases reported between 1987 and 1997).

**Hensel (2004)** reported that *Salmonella enterica* can cause disease in humans and a variety of animals; other serovars are highly restricted to a specific host. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea and vomiting, to typhoid fever, a life threatening infection.

**Myint (2004)** mentioned that *Salmonella* alone affects about 1.4 million people each year in the United States with about 16,000 hospitalizations and more than 500 deaths annually according to (CDC). *Salmonellae* were responsible for zoonotic disease transmission.

**Berrang *et al.*, (2006)** stated that the *Salmonella* was frequently as a cause of food- illness.

**Revolledo *et al*., (2006)** mentioned that *Salmonella* infections were mainly asymptomatic in poultry but associated with widespread human illness from this source. Therefore, there is continuing interest in finding ways of preventing flock infection and, hence, contamination of poultry products with *Salmonellae*. This study considers aspects of *Salmonella* carriage in poultry and host interactions that may be exploitable in the future to improve existing control measures. These include factors involved in colonization of the gastrointestinal.

**Papezova *et al*., (2008)** isolated *Salmonella* *enterica*. subsp. *Enterica* originating from poultry and poultry products were responsible for the vast majority of human gastrointestinal disorders in Europe.

**Ruiz Lopez *et al*., (2008)** said that *Salmonella* is considered to be the main cause of human intestinal infections originating from the consumption of chicken meat and eggs contaminated with this bacterium.

**Dunkley *et al.*, (2009**) mentioned that food borne *Salmonella*, continues to be a major cause of salmonellosis with *Salmonella* Enteritidisand *Salmonella* Typhimurium considered to be responsible for most of the infections.

**O'Flanagan *et al.,* (2009)** said that an outbreak of gastroenteritis has affected residents of the UK, Republic of Ireland and Finland since Feb. 2008 is presented. *Salmonella* Agonais the suspected cause of the outbreak. The source of the causative agent is thought to be contaminated meat products manufactured by an Irish food company.

**Valadez *et al.*, (2009**) stated that *Salmonella enterica* is a major food-borne pathogen of world-wide concern. Sensitive and rapid detection methods to assess product safety before retail distribution were highly desirable since *Salmonella* is most commonly associated with poultry products.

**Freitas Neto *et al*., (2010)** mentioned that salmonellosis was a worldwide disease caused by bacteria of the genus *Salmonella* currently, there are over 2,500 identified serovars of *Salmonella* a reduced number of these serovars, about eighty, are implicated in most animals and human diseases. Most cases of salmonellosis in humans are associated with the consumption of contaminated food products such as beef, pork, poultry meat, eggs, vegetables, juices and other kind of foods.

**Shahet *et al.,* (2011)** reported that *Salmonella enteric* serovars Enteritidis (*Salmonella* Enteritidis*)* was a major cause of food-borne gastroenteritis in humans worldwide. Poultry and poultry products are considered the major vehicles of transmission to humans.

**Babu *et al*., (2012)** reported that *Salmonella* Enteritidis (SE) is one of the leading causes of food-borne salmonellosis, and macrophages play an essential role in eliminating this pathogen.

**2.2. Incidence of *Salmonellae* in chickens:**

**Bailey and Scott (1994)** detected *Salmonellae* in hatcheries, 17% in egg shell, 21% in chick’s rinses and 5% in intestines at 0 day.

**Edel *et al.*, (1994)** isolated *Salmonella* Enteritidis from 2 out 351 flocks of hens which produced hatching eggs. *Salmonella* Enteritidisaccounted for 33.5% of human cases of salmonellosis.

**Sharma *et al.*, (1996)** isolated *Salmonella* Typhimurium from visceral organs in an outbreak in a flock of 1385 chicks. Mortality rate increased to 92.8% within all days.

**Terregino *et al.*, (2000)** determined causes of mortality of broiler chicks during the first 3 weeks of life. The mortality rates were 4.52% during the first breeding cycle and 3.12% in the second one; the commonest causes of mortality found in the first week were omphalitis, yolk sac infections and septicemia. *E.*coli and *enterococcus* spp. were the most prevalent agents isolated. Diseases of complex or unknown etiology such as sudden death syndrome and baby chick nephropathy were also observed.

**Roy *et al.*, (2002)** examined 4745 samples from poultry, poultry environment and poultry products in 1999 and 2000 from the Pacific Northwest (USA). 569 of samples were *Salmonella* positive (11.99%).

**Purushothaman *et al.*, (2003)** isolated 65 *Salmonella* strains from poultry and poultry environment, reveled 21 serotypes from poultry farms in India.

**Guerin *et al.,* (2005)** found that *S*. Typhimurium, *S*. Heidelberg, *S*. Hadar, *S*. Kentuckey and *S*. Thompson were the most frequently isolated serovars, approximately 60% of the *S*. Typhimurium were isolated from cattle, whereas over 90% of the *S*. Heidelberg, *S*. Hadar, *S.* Kentucky and *S*. Thompson were isolated from chickens. There was an increasing trend in isolates from chickens, cattle and pig and decreasing trend in isolates from turkey.

**Nógrády *et al.* (2008)** recorded that in Hungary, the rate of occurrence of *Salmonella* Infantis has increased in the past years both in the poultry industry and in humans.

**Michael *et al.* (2006)** states that *S*. Agona played an important role in Brazil as causative agent of salmonellosis in food producing animals in particularly in pigs and poultry as well as in humans.

**Pieskus *et al*., (2008)** reported that the incidence of *Salmonella* in conventional broiler farms was 29% in Lithuania, 20% in Italy and 11% the Netherlands, while in Germany *Salmonella* was not detected. *Salmonella* was isolated from organic broiler flocks in Italy (18.1%) and in Netherlands (3.7%). The authors indicated that *S*. Enteritidis and *S*. Typhimurium dominated in Lithuanian broiler flocks while *S*. Infantis and *S*. Javiana were predominant in the Netherlands. *S*. Hadar and *S*. Heidelberg seemed to be prevalent in Italy.

**Akhtar *et al.,* (2010)** revealed that overall serovars *S*. Enteritidis prevalence rate in 206 *Salmonella* positive samples were 75.24% (155). Out of 58 isolates of *Salmonella* recovered from human stool samples, 44 (75.86%) were *S*. Enteritidis. Isolation frequency of *S*. Enteritidis from total isolates (148/206) in poultry sources was 111/148(75%), which indicated the zoonotic potentional of *S*. Enteritidis in Pakistan.

**Kumari *et al*., (2013)** tested 134 dead poultry birds collected from 23 different farms suspected to be infected with *S*. Gallinarum. Mortality pattern revealed that maximum mortality occurred in 1-2 week aged birds. Out of 23 *Salmonella* isolates, 19 samples were identified as *S*. Gallinarum (9, 12) and 4 samples as *Salmonella* Enteritidis (9, 12: gm).

**Gong *et al*., (2014)** isolated 323 *Salmonella enterica* strains from 3,566 cloacal swab samples of 51 poultry farms in seven regions of 12 provinces of China between 2006 and 2012. revealed 9.8% in chickens (167/1,706).Overall, *S.* Typhimurium was the most detected serovar; among the individual species, *S.* Pullorum was most isolated from chickens, *S*. Enteritidis was most common in ducks, *S.* Typhimurium was most common in geese and pigeons, and *S. enterica* serovar Saintpaulwas most common in turkeys.

**2.3. Incidence of *Salmonellae* in Egypt:**

**Aziz El-Din *et al*., (1992)** examined 2216 samples of dead in shell embryos, freshly dead baby chicks, broilers and freshly dead adult layers collected from different locatities of sohag province. A total of 20 salmonella species could be isolated, 7 strains proved to be salmonella gallinarum pullorum. the incidence of salmonella gallinarum pullorum isolate was the follows Tema (0.86%), Dar-El-Salam (0.71%), Gerga (0.62%), Akmim (0.47%), El-Balina (0.43%), and Sohag (0.20%) while no salmonella gallinarum pullorum could be isolated from Tahta, Gohina, El-Maragha, Sakolta and El-Menshah localities.Concerning the type of samples the incidence of salmonella gallinarum pullorum was the follows:Dead in shell embryos (0.43%). Baby chicks (0.29%) and Broilers (0.34%) while no salmonella gallinarum pullorum could be isolated from adult chickens.

**Ibrahim (2005)** recovered 41 isolates of*Salmonella***,** 19 were recovered from feed and 22 from poultry. *S*. Montevideo, *S*. Agona, *S*. Blockely, *S*. Hadar and *S*. Virchow were the most important serovars recovered from feed, while *S*. Agona, *S*. Arizona, *S*. Cerro, *S*. Enteritidis, *S*. Gallinarum Pullorum and *S*. Typhimurium were from poultry.

**Ammar *et al.,* (2010)** isolated *S*. Enteritidis from broiler chickens, chickens meat and food poisoning patient in Dakhlia Governorate, Egypt.

**Hazem (2010)** examined bacteriologically 150clocalswabs were obtained different chickens farms to recover the organism. The percentage of positivity for *Salmonella* was 12.6%. serotyping using available antisera revealed that 19 isolates were identified as *S*. Typhimurium (5), *S.* Heidelberg (2), *S*. Pullorum (6) and *S*. Enteritidis (6).

**Osman *et al*., (2010)** found that Mortality rates after the 1st week of infection with the four serovars(*Salmonella* Kentucky, *Salmonella* Typhimurium, *Salmonella* Shubra and *Salmonella* Enteritidis), only *S*. Typhimurium and *S.* Enteritidis produced a fulminate infection (88%) in each of the chicks and mice, The lowest mortality was seen in chicks and mice inoculated with S. Kentucky (40% and 44% respectively,). Over 40% mortality was observed in SPF chicks (45.8%) and BALB/c mice (60%) inoculated with *Salmonella* Shubra. The lethality of *S*. Typhimurium in mice was 66% while *S.* Enteritidis was lethal to chicks by 72%. Analysis of the level of fecal shedding of the four serovars from both infected SPF chicks and infected BALB/c mice revealed that, *S.* Typhimurium was consistent in being the highest in reisolation rate throughout the 7 days of observation while, *S.* Enteritidis comes second in reisolation rate. A variable reisolation picture was noticed from *S.* Kentucky and serovar Shubra throughout the experimental period. The highest level of reisolation was on the 1stday post infection (DPI) recorded as 60%, 40% and 20% from the serovars Enteritidis, Typhimurium, Shubra and Kentucky, respectively.

**El-Baz and Younis (2011)** examined 152 chicken samples from Dakahlia Governorate in two seasons cold (winter and spring) and hot (summer and autumn) seasons .Salmonellae were isolated from (27) out of (152) chickens that were examined (17.8%) with high incidence during hot seasons (25%) other than in the other seasons (10.5%), The rate of recovery of Salmonellae from the different internal organs showed that high recovery rate was from liver, caecum, spleen and then small intestine as the follow (12.5%), (8.55%), (7.24%) and (3.29%), respectively,the results of serological serotyping revealed that Salmonella Typhimurium 13.8% (21 isolates) and Salmonella Kentucky 3.95% (6 isolates) from the examined samples and S.Typhimurium and S.Kentucky were isolated from livers while S.Typhimurium is the only serovare which isolated from small intestine, caecum and spleen.

**Wafaa *et al.,*** **(2012)** recorded that there were different *Salmonella*serovars including *S*.Enteritidis, *S*. Infantis, *S*.Chiredzi, *S*.Kentucky, *S*. Typhimurium and *S*. Tsevie circulating in broiler chicken farms in Kalubia Governorate, Egypt and the most prevalent ones were *S*. Enteritidis and *S*. Typhimurium.

**Rabie *et al.,* (2012)** found that prevalence of *Salmonella* spp. was (14%), (4%) and (10%) in broiler chickens, raw chickens meat and patient, respectively in Toukh, Kalubia Governorate, Egypt.

**Ibrahim *et al.,* (2013)** reported that the prevalence of *Salmonella* from poultry in 2009 and 2010 in Beni-Suef governorate, Egypt. Cloacal swabs were collected from poultry (150 broilers, 50 breeders, 50 layers, 50 turkeys,50 ducks and 30 litter samples).The recovered *Salmonella* strains were found belonging to *S*. Kentucky, *S*. Typhimurium and *S*. SaintPaul. The obtained results demonstrated that the occurrence of *Salmonella* spp. accounted for 16.66, 10.0, 2.0, 6.0 and 2.0% in broilers, breeders, layers, ducks and turkeys, respectively.

**Ezzat *et al*., (2014)** examined 1000 samples collected from 200 broiler chickens (40 apparently healthy, 80 diseased chickens and 80 freshly dead broiler chickens). Results revealed the presence of 37 out of 200 chickens (18.5%) *Salmonella* species isolates, representing: 3 from apparently healthy chicken (7.5%), 21 from diseased chickens (26.25%) and 13 from freshly dead broiler chickens (16.25%).

**Ibrahim *et al*., (2014)** found that the incidence of Salmonellae among imported chicks was 11.67% compared to 21.67% among local chicks using conventional cultural isolation methods. Salmonella newport (S. newport) showed the highest incidence rate in imported chicks, while Salmonella enteritidis and Salmonella typhimurium were frequently detected in local chicks.

**Abd-Elghany *et al*., (2015)** Tested 200 chicken samples collected from Mansoura, Egypt.Salmonella was detected in 16% (8/50), 28% (14/50), 32% (16/50) and 60% (30/50) of whole chicken carcasses, drumsticks, livers and gizzards, respectively, with an overall prevalence of 34% (68/200) among all samples. One hundred and sixty-six isolates were identified biochemically as Salmonella, and confirmed genetically by PCR, based on the presence of invA and stn genes. The spvC gene, however, was detected in only 25.3% (42/166) of the isolates. Isolates were serotyped as Salmonella Enteritidis (37.3%), S. Typhimurium (30.1%), S. Kentucky (10.8%), S. Muenster (8.4%), S. Virchow (4.8%), S. Anatum (4.8%), S. Haifa (1.2%), and four were non-typable.

**Abdel-Maksoud *et al*., (2015)** Found that the detection rates of Salmonella were 60%, 64% and 62% in chicken parts, skin, and faeces, respectively, whereas the egg yolks and eggshells were uniformly negative. Salmonella Kentucky and S. Enteritidis serotypes comprised 43.6% and 2.6% of the isolates, respectively, whilst S. Typhimurium was absent.

**Abd El-Tawab *et al*., (2015)** isolated Salmonellae from 579 birds (348 chickens, 104 ducks, 30 turkeys, 50 quail, 30 pigeons and 17 geese) from 4 Egyptian Governorates.The Samples collected from internal organs (liver, cecum, spleen and heart). Sixty-three (10.9%) out of 579 birds were found positive while 516 (89.1%) birds were negative for Salmonella isolation. The number and percentage of positive chickens, ducks, turkeys, quails, pigeons and geese were 43 (12.4%), 10 (9.6%), 3 (10%), 5 (10%), 2 (6.7%) and 0 (0%) respectively.In this study, S. Typhimurium, S. Apeyeme, S. Kentucky, S. Daula, S. Newport, S. Tamale, S. Molade, S. Colindale, S. Lexington, S. Bargny, S. Enteritidis, S. Papuana, S. Labadi, S. Santiago, S. Magherafelt, S. Rechovot, S. Takoradi, S. Angers and S. Shubra were isolated from chickens. While S. Inganda, S. Infantis and S. Larochelle were isolated from ducks but S. Virchow and S. Vejle were isolated from turkeys. S. Shangani and S. Jedburgh were isolated from quails while S. Alfort and S. Wingrove were isolated from pigeons.

**Barbour *et al*.,(2015)** showed that S. Enteritidis and S. Typhimurium were predominant in poultry along with other non-typhoid strains, namely S. Infantis, S. Kentucky, S. Tsevie, S. Chiredzi, and S. Heidelberg.

**2.4. Pathology of *Salmonellae*:**

**Rettger (1900)** was one of the earliest who described a septicemia and septicaemic picture among chickens with isolated *Salmonella*, the same author in his further studies **(1909)** reported fatal septicemia in chickens accompanied with “white diarrhea”.

**Suganuma** **(1960)** described the histopathological changes due to pullorum disease in field cases which might have been complicated by other bacterial and/or viral agents.

**Chishti *et al.*, (1985)** described pathological changes of the chickens due to *Salmonella* species infection in young birds as enlarged and congested liver, spleen and kidneys. Livers may have a white necrotic focuses of 2-4 mm in diameter. The yolk sac content may show non absorption with its contents appear caseous and creamy.

**Buchholz and Fairbrother (1992)** recorded splenomegaly, and gray necrotic foci with peticheal hemorrhages in the lungs, with pale discolored livers in *Salmonella* infected birds.

**Wong *et al.*, (1996)** reported that in the peracute reaction of *Salmonella* infectiononly severe vascular congestion in various organs especially liver, spleen and kidney. While in acute and subacute cases, there is multifocal necrosis of hepatocytes with accumulation of fibrin and infiltration of heterophils mixed with few lymphocytes and plasma cells. While in chronic cases they reported large nodules in the heart, with chronic venous congestion in the liver and intestinal fibrosis. The spleen showed congestion, fibrinous exudation of vascular sinuses and severe hyperplasia of the mononuclear phagocytic system cells. They described the ceca of young chickens with extensive necrosis of the mucosa and submucosa, with accumulation of necrotic debris mixed with fibrin and hetrophils in the lumen. The heart showed necrosis of myfibers with infiltration of hetrophils mixed with lymphocytes and plasma cells.

**Henderson *et al.*, (1999)** recorded that flocks suffered from *Salmonella* infection with respiratory signs may have white nodules in the lungs, cardiac muscles and pancreas. They added that presence of necrotic nodules in the heart may lead to further pathological changes as venous congestion of the liver and ascitis. The pericardial sac becomes thick and the pericardial fluid is yellow serofibrinous exudates. The author also observed similar nodules in the muscles of proventiculous and gizzard. The ceca may contain caseous core. Swollen joints were also observed.

**Pennycott and Duncan (1999)** noticed unabsorbed yolk sac, pneumonia, hepatitis and typhlocolitis as the most common lesions accompanied *Salmonella* infections.

**2.5. Experimental infection with *Salmonella*:**

**Barrow *et al.,* (1998)** inoculated chickens orally with 109 CFU of *S.* Enteritidis phage type 4 strain P125/09 at 4 days of age and treated immediately afterwards by administration of enrofloxacin, in the drinking water (0.5 ml/liter), virtually eliminated this organism from the elementary tract. However, an initially quinolone-sensitive *E.* Coli flora present in the bird’s feaces was rapidly replaced by a quinolone-resistant flora which persisted after withdrawal of the medication.

**Van Immerseel *et al*., (2004)** used 5 groups of 20 chickens and given feed with no supplement or feed supplemented with acetic acid (0.24%) or formic acid (0.22%), or propionic acid (0.27%) as film-coated microbeads or butyric acid (0.15%) as spray-cooled microcapsules. The groups were challenged with 5x103 CFU *S.* Enteritidisat d 5 and 6 post-hatch, butyric acid-impregnated microbeads in the feed, however, resulted in a significant decrease of colonization by *S.* Enteritidisin the ceca but not in liver and spleen.

**Van Immerseel *et al*., (2005)** compared powder form and coated butyric acid in their ability to reduce *Salmonella* colonization of ceca and internal organs after infection of young chickens with *Salmonella* Enteritidis. Four groups of 25 SPF layer chickens were given feed either supplemented with powder form butyric acid, coated butyric acid, a combination of powder form and coated butyric acid (all groups received a total of 0.63 g of butyric acid/kg) or non-supplemented feed. The SPF layer chickens were orally infected with 106 cfu of *S.* Enteritidis. Coated butyric acid significantly decreased cecal colonization 3 d post-infection compared with control chickens, and powder form butyric acid had no effect. To study long-term shedding and colonization of *Salmonella* in broilers given coated butyric acid as feed additive (0.63 g of active product butyric acid/kg), 10 Ross broiler chickens were infected at d 5 with 105 cfu of *S.* Enteritidis. The group of broilers receiving coated butyric acid had a significantly lower number of broilers shedding *Salmonella* bacteria, but cecal colonization at slaughter age was equal for both groups. It is concluded that, butyric acid decreases cecal colonization shortly after infection, decreases fecal shedding, and as a consequence, decreases environmental contamination by *S.* Enteritidis infected broilers.

**Puyalto and Locatelli (2009)** used 2 groups of 100; one group (control) received a standard diet and the second group (GU-BP) received the same diet with the addition of Gustor BP 70 (butyric acid) at the rate of 1.3 kg/t. On day 5 20% of the birds in each group were orally inoculated with 105 CFU of *S.* Enteritidis. *Salmonella* infection in the control group increased gradually with 36, 74 and 100% of birds infected on days 9, 27 and 41. In contrast, the GU-BP group showed a significant (P<0.001) reduction of *Salmonella* infection with 16, 20 and 6% of birds infected on days 9, 27 and 41.

 **Rubio *et al*., (2009)** used 3 groups of fifty 1-d-old broilers each were fed the following diets: T0 = standard broiler diet (control); T1 = diet supplemented with 0.92 g/kg of an additive with free sodium butyrate (Gustor XXI B92); and T2 = diet supplemented with 0.92 g/kg of an additive with sodium butyrate partially protected with vegetable fats (Gustor XXI BP70). 20% percent of the birds were orally infected with *Salmonella* Enteritidis at d 5 pos-thatching. Both butyrate-based additives showed a significant reduction (*P* < 0.05) of *Salmonella* Enteritidis infection in birds from d 27 onward. However, the partially protected butyrate additive was more effective at the late phase of infection. Partially protected butyrate treatment successfully decreased infection not only in the crop and cecum but also in the liver. There were no differences in the spleen. These results suggested that sodium butyrate partially protected with vegetable fats offers a unique balance of free and protected active substances effective all along the gastrointestinal tract because it is slowly released during digestion.

**Hosseini *et al*., (2011)** used 150 one-day-old chicks (Ross 308) were assigned to 5 experimental groups (30 birds per group) including control and 4 treatment with different hatchery probiotic administration methods comprised of *in ovo* injection, oral gavage, spray and vent lip application. All chickens were challenged by 8 Log CFU SE using oral gavage one day after administration of probiotics. So administration of probiotics reduced the number of colonized chicks, compared with control group. Decline was non significant 1 day after PC, but after PC in day 7 was significant. The most number of infected chicks was observed in control group, and the lowest was observed in vent lip method.

**Wafaa *et al*., (2012)** used three hundred and ten, day-old Hubbard broiler chicks.Ten chicks were sacrificed and cultured to screen their absence of Salmonellae. Three hundred birds were divided into 4 groups. Chickens in group (1) were kept as blank control negative non infected-non treated birds, while those of group (2) were challenged non treated birds. Group (3) was vaccinated intramuscularly by the autogenous bacterin at the first day of age in a dose of 0.2 ml/bird and boostered as a second dose at 10 days of age in a dose 0.5 ml/bird, however, group (4) was given a commercial probiotic preparation as 1 gm/4 liter of the drinking water from the first day of age and continued for 5 successive days. All birds in groups 2, 3, and 4 were challenged orally by 0.5 ml containing 109 CFU/ml *S.* Enteritidis at 20 days of age. The results showed that the both the bacterin and the probiotic are equally effective in reducing signs, mortalities, gross lesions, the shedding rate and the re-isolation of *S.* Enteritidis and also increasing in the performance of chickens. The effect of the bacterin and the probiotic was significant when compared with the infected non treated chickens. In conclusion, double doses of locally prepared autogenous *S.* Enteritidis bacterin and the probiotic preparation were effective and safe methods for prevention of *S.* Enteritidis infection in broiler chickens.

 **Cerisuelo *et al*., (2014)** used 480 1d-old male broilers distributed into 5 treatments (8 pens per treatment and 12 birds per pen). Dietary treatments consisted of the addition of different doses of EO (Essential Oil) (0 mg/kg, control; 50 mg/kg, EO50 and 100 mg/kg, EO100) or a combination of EO with 1 g/kg of sodium-butyrate (B; EO50 + B, EOB50 and EO100 + B, EOB100) to a basal diet. All birds were orally infected with 108 CFU of *Salmonella* Enteritidis on d 7 of study. No differences were observed on growth performance among treatments. At slaughter, *Salmonella* contamination (positive samples) in cecum was lower in birds fed EOB50 compared with the other treatments, whereas birds fed the control diet showed the highest colonization rates. The pH of the cecal content was not different among treatments. Thus, EO or its combination with sodium-butyrate did not affect growth performance. However, a clear effectiveness of these products was observed in *Salmonella* control, especially when low doses of EO were combined with sodium-butyrate (EOB50).

**Junior *et al*., (2014)** assessed the efficacy of blends of organic acids and oregano extracts in controlling *Salmonella* Enteritidis persistence in the crop and ceca of broiler chicken. A total of 105 1d-old male broiler breeder chicks were randomly distributed into 5 different treatments according to the supply of referred blends and *Salmonella* Enteritidis challenge. *Salmonella* Enteritidis was inoculated directly in the crop of birds at the 15 d of age. Treatments were un supplemented unchallenged, un supplemented challenged, supplemented through water and challenged, supplemented through feed and challenged, and supplemented through feed and water and challenged. Use of the additives in feed and water and in water alone efficiently controlled *Salmonella* shedding and reduced cecal persistence.

**2.6. Organic acids in chickens:**

 **Paster (1979)** observed that organic acids or volatile fatty acids (VFA) were originally added to animal feed to inhibit fungal growth.

**Hinton and Linton (1988)** noticed that *In vivo* studies examining the effect of VFAs on the gastrointestinal microflora intestinal pathogens have most often used formic acidand propionic acid.

**Izat *et al*., (1990)** determined that species that have been shown to be sensitive to various organic acids include pathogens of both human and animal concern such as *Salmonella* and *E.* Coli*.*

**Cherrington *et* *al*., (1991)** thought that undissociated VFAs were penetrate the cell membrane of bacteria and dissociate in the more alkaline cytoplasm, hereby increasing the inward leak of protons so that efflux of protons is not rapid enough to alkalinize the cytoplasm.

**Corrier *et al.*, (1995)** said that competitive exclusion studies in poultry implicated these same compounds (organic acids) in the cecae of chickens with reduced *Salmonella* counts.

**Ricke (2003)** examined *in* *vitro* the ability of VFAsto inhibit the intestinal microflora of poultry and confirmed that they do have antibacterial activity. Although the exact mechanisms by which VFAs inhibit intestinal microflora are unclear.

**Van Immerseel *et al.,* (2003)** showed that the ability of *Salmonella* Enteritidisto invade chicken epithelial cells *in vitro* was influenced by the VFAs. The promising results obtained from research has resulted in the development of a number of commercial products, however more research is required to improve their effectiveness. Of particular interest was the phenomenon of acid tolerance, including the stress response and virulence factors which allow for resistance in more acidic environments.

**2.7. Butyric acid in chickens:**

**Van Immerseel *et al*., (2004)** revealed that (SCFA) are widely used as feed additives in poultry for the control of pathogenic bacteria, such as *Salmonella* Enteritidis. Results showed that butyric acid-impregnated microbeads in the feed, however, resulted in a significant decrease of colonization by *S.* Enteritidisin the ceca but not in liver and spleen.

**Leeson *et al*., (2005)** mentioned that short-chain fatty acids such as butyrate are considered potential alternatives to antibiotic growth promoters.

**Van Immerseel *et al*., (2005)** compared powder form and coated butyric acid in their ability to reduce *Salmonella* colonization of ceca and internal organs shortly after infection of young chickens with *Salmonella* Enteritidis.

**Panda *et al*., (2009)** studied the effect of graded levels of butyric acid (butyrate) on performance, gastrointestinal tract health and carcass characteristics in young broiler chickens. Subsequently, 4 experimental diets were formulated to contain 0.05% furazolidone or 0.2, 0.4 and 0.6% butyric acid. Each diet was fed at random to 8 replicates of 6 chicks each throughout the experimental period (0-5 wk). The results showed that 0.4% butyrate in the diet was similar to antibiotic in maintaining body weight gain and reducing *E.* Colinumbers but superior for feed conversion ratio. No added advantage on these parameters was obtained by enhancing the concentration of butyrate from 0.4 to 0.6% in the diet. Feed intake and mortality were not influenced by the dietary treatments. The villus length and crypt depth in the duodenum increased significantly in all the butyrate treated diets irrespective of the level tested. It is concluded that 0.4% butyric acid supplementation maintained performance, intestinal tract health, and villi development in broiler chickens.

**Puyalto and Locatelli (2009)** demonstrated a negative correlation between *Enterobacteria* and levels of volatile fatty acids, such as butyric acid. However, the free form of this acid is difficult to maintain. A slow-release formulation (Gustor BP 70) has been developed which releases sodium butyrate in the distal sections of the digestive tract, where it acts as a natural growth promotor and also reduces the level of pathogenic bacteria, especially *Salmonella*.

**Rubio *et al*., (2009)** revealed that sodium butyrate is a sodium salt of a volatile short-chain fatty acid (butyric acid) used to prevent *Salmonella* Enteritidis infection in birds.

**Herrera *et al*., (2011)** studied the replacement of the antibiotic growth promoter (bacitracin zinc 30 ppm) with sodium butyrate (300 g/ton) in the diet. Results in 24 weeks of experimentation were similar between treatments (P > 0.05), According to information obtained in 24 weeks with 32 week old hens, the addition of sodium butyrate to feed as a substitute for the growth promoter (bacitracin zinc), was similar in the productive performance and egg quality.

**Zhang *et al*., (2011)** investigated the effects of dietary sodium butyrate on the growth performance and immune response of broiler chickens. In exp 1, 240 1-d-old chickens were allocated into 4 dietary groups (0, 0·25, 0·50 or 1·00 g sodium butyrate/kg) with 6 replicates each. In exp 2, 120 1-d-old chickens were fed a control diet (without sodium butyrate) or 1·00 g sodium butyrate/kg diet. Half of the chickens fed on each diet were injected intra-peritoneally with 0·5 g/kg body weight of *Escherichia* Coli lipopolysaccharide at 16, 18 and 20 d of age. The results indicated that dietary sodium butyrate supplementation can improve the growth performance in chickens under stress and that this may be used to moderate the immune response and reduce tissue damage.

**Aghazadeh and TahaYazdi (2012)** evaluated the effects of butyric acid (BA) levels and wheat form (WF) on the performance of broiler chickens, 320 day-old Ross 308 broiler chicks were randomly distributed among 32 floor pens. with four levels of BA (B1: 0g BA/kg in both starter and grower feed; B2: 2.5 g BA/kg in both starter and grower feed; B3: 2.5 g BA/kg in starter and 1 g BA/kg in grower feed; and B4: 2.5 g BA/kg in starter and 0 g BA/kg in grower feed) and two forms of wheat (whole (WW) vs. ground (GW)) were used. Dietary supplementation with BA had no effect on average weight gain (AWG) or feed conversion ratio (FCR) in the starter, grower/finisher and over whole (0 - 42 d) trial periods. However, birds consumed more when the diet was supplemented with butyrate (B2) relative to the control and other experimental diets during 0 - 42 d, but this increase was not associated with improved AWG or FCR as compared with that of the control. The length of the entire gut was augmented by BA and WW feeding.

**Ortiz *et al*., (2013)** used a total of 120 one-day-old Cobb chicks were allocated at random to 4 experimental treatments (T1: Control; T2: Control + 3kg/Tm 70% Na Butyrate protected with palm stearine; T3: Control + 3kg/Tm 70% Na Butyrate protected with PFAD (Palm Fatty Acids Distillate) sodium salt; T4: Control + 7kg/Tm Na Butyrate coated with palm stearine) 3 replicates/treatment. BW, average daily gain (ADG), average daily feed intake (ADFI) and FCR were recorded for the 0-42d. Broilers receiving T3 had higher final body weight (FBW) and better ADG than control diet. Also ADFI was higher in T3 than T1. Animal receiving butyrate (T2+T3+T4) had better performance in terms of FBW, ADG and ADFI. It was conclude that butyrate enhanced zootechnical parameters.

**Cerisuelo *et al*., (2014)** investigated the effect of a specific blend of essential oils EO and a combination of this blend of EO with sodium-butyrate on growth performance and *Salmonella* colonization in broilers. No differences were observed on growth performance among treatments. At slaughter, *Salmonella* contamination (positive samples) in cecum was lower in birds fed combination compared with the other treatments. So, EO or its combination with sodium-butyrate did not affect growth performance. However, a clear effectiveness of these products was observed in *Salmonella* control, especially when low doses of EO were combined with sodium-butyrate.

**Chamba *et al*., (2014)** evaluated the effect of partially protected sodium butyrate (PSB) on performance, digestive organs, intestinal villi and *E.* Coli development in broilers chickens. Nine hundred twenty four one-day-old mixed Cobb chicks were divided in 3 treatments with 7 replicates each in a randomized block design. T1 was a control diet without any growth promoter, T2 was the control diet plus colistin at 100,000 IU/kg body weight and T3 was the control diet with PSB at 700 ppm. There were no significant differences on performance among all treatments in starter phase (1-14d). Chicks fed PSB in grower (15-28 d) and finisher phases (29-42 d) had the highest weight gain and the best feed conversion ratio. Jejunal villi of birds fed PSB and colistin at 42 days were higher than those in birds fed the control diet. These data indicate that partially protected sodium butyrate and colistin improves performance, colistin as an antibiotic growth promoter and PSB by improving intestinal villi development in broilers chickens.

**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.

**Jiang *et al*., (2014)** investigated the effects of micro-encapsulated sodium butyrate (MSB) on oxidative stress and apoptosis induced by dietary corticosterone (CORT) in the intestinal mucosa of broiler chickens.

**Zhou *et al*., (2014)** reported that butyric acid is a major short chain fatty acid (SCFA), produced in the gastrointestinal tract by anaerobic bacterial fermentation, that has beneficial health effects in many species including poultry.

**Matis *et al*., (2015)** evaluated the influence of butyrate on insulin signaling in chickens because butyrate is produced during microbial fermentation in the large intestine of birds, and butyrate is widely used as a feed additive in animal production.

**van den Borne *et al*., (2015)** found that butyrate can be a very good alternative to antimicrobial growth promoters. Effective dietary application requires coating because the majority of uncoated butyrate is purportedly absorbed before reaching the proximal small intestine.

**2.8. Butyric acid in Egypt:**

**Abdel-Mageed (2012)** observed that using dietary butyric at a level of 0.2% in Japanese quail diets containing sub-optimal energy and protein levels helped in reducing microflora count, particularly pathogens and in turn, improving quail performance and immunity.

**Awaad** ***et al.,* (2014)** noticed lowering average lesion scoring indicated the efficacy of sodium butyrate encapsulated in palm fat in lowering damage associated with necrotic enteritis. The recorded mortality and enumeration of intestinal *C.* Perfringens were lower in treated groups vs. their non-treated ones.

**Kamal and Ragaa (2014)** used two hundred broiler chicks divided into 4 groups (50 birds) of each. The control (T1) group were fed the basal diet whereas 3% butyric acid (T2), 3% fumaric acid (T3) and 3% lactic acid (T4). Broiler chicken fed diets supplemented with organic acids had significantly (*p* < 0. 0 5) improved body weight gains and feed conversion ratio. No effect (*p* < 0. 0 5) on cumulative feed consumption was observed. Broiler chicken fed acidified diets had better immune response as indicated by a higher serum globulin level than the control.

**Tony and Hamoud (2014)** concluded that exogenous administration of slow-release sodium butyrate was capable of improving performance, enhancing immunity and disease resistance in broiler chickens.

**El-Sawy *et al.,* (2015)** showed that administration of yeast beta-glucan or sodium butyrate significantly improved average weight gain and feed conversion ratio. Significant improvement in length and width of intestinal villi in comparison with the control group was observed.

**2.9. Effect of feed additives on *Salmonellae* virulence genes:**

**Durant *et al*., (2000)** found that the invasion process of *Salmonella* Typhimuriumto invade the intestinal mucosal cells requires genes encoded on the *Salmonella* pathogenicity island 1 (SPI1). Two transcriptional activators, HilA and InvF, encoded in SPII regulate the expression of invasion genes in response to environmental stimuli such as osmolarity, oxygen tension, and pH. During its pathogenic life cycle, *Salmonella* Typhimurium is also exposed to short-chain fatty acids (SCFA), especially acetate, propionate, and butyrate, in the intestinal lumen. The effects of SCFA on the expression of hilA and invF-lacZY transcriptional fusions were examined to determine the potential role of SCFA in the pathogenesis of *Salmonella* Typhimurium. The pH-dependent manner of induction suggests that entry of SCFA into the cell was necessary for induction. We speculate that SCFA may serve as an environmental signal that triggers the expression of invasion genes in the gastrointestinal tract.

**Lawhon *et al*., (2002)** found that the invasion process required genes of *Salmonella* pathogenicity island 1 (SPI-1). BarA, a sensor kinase postulated to interact with the response regulator SirA, is required for the expression of SPI-1 invasion genes.found that acetate restored the expression of invasion genes in the barA mutant.These results suggest that the rising concentration of acetate in the distal ileum provides a signal for invasion gene expression.Two other short-chain fatty acids (SCFA), propionate and butyrate, present in high concentrations in the caecum and colon, had effects opposite to those of acetate: neither restored invasion gene expression in the barA mutant, and both, in fact, reduced expression in the wild-type strain. Further, a combination of SCFAs found in the distal ileum restored invasion gene expression in the barA mutant, whereas colonic conditions failed to do so and also reduced expression in the wild-type strain. These results suggest that the concentration and composition of SCFAs in the distal ileum provide a signal for productive infection by *Salmonella*, whereas those of the large intestine inhibit invasion.

**Gantois *et al*., (2006)** found that butyrate down-regulated the expression of 19 genes common to both serovars by a factor of twofold or more, and 17 of these genes localized to the *Salmonella* pathogenicity island 1 (SPI1). These included the SPI1 regulatory genes *hilD* and *invF*.

**Boyen *et al*., (2008)** found that some frequently used short- (SCFA) and medium-chain fatty acids (MCFA) are able to alter virulence gene expression and decrease *Salmonella* Typhimurium colonization and shedding in pigs using well established and controlled *in vitro* and *in vivo* assays. Expression of virulence gene fimA was significantly lower when bacteria were grown in LB-broth supplemented with sub-MIC concentrations of caproic or caprylic acid. Expression of hilA and invasion in porcine intestinal epithelial cells was significantly lower when bacteria were grown in LB-broth containing sub-MIC concentrations of butyric acid or propionic acid and caproic or caprylic acid .So coated butyric acid decreased the levels of faecal shedding and intestinal colonization of *Salmonella* Typhimurium, but had no influence on the colonization of tonsils, spleen and liver. Uncoated fatty acids, however, did not influence fecal shedding, intestinal or tonsillar colonization in pigs. In conclusion, supplementing feed with certain coated fatty acids, such as butyric acid, may help to reduce the *Salmonella* load in pigs.

**Haghighi *et al*., (2008)** investigated *Salmonella* pathogenicity islands (SPI-1) gene expression and the pathogenicity of quinolone-resistant *Salmonella*. mRNA expression levels of the *invA* and *avrA* genes, located in SPI-1, in quinolone-susceptible and quinolone-resistant Salmonella strains were determined using real-time fluorescent quantitative reverse transcription-polymerase chain reaction (RT-PCR). Twenty-five quinolone-resistant Salmonella mutants were derived from quinolone-susceptible strains by multiple-passage selection through increasing concentrations of ciprofloxacin *in vitro*, while an additional 15 strains were quinolone-resistant *Salmonella* clinical isolates. Sequence analysis showed no gene deletion or point mutations of nine SPI-1 genes (including *invA* and *avrA*) occurred in either the selected or clinical quinolone-resistant strains, while a single gyrA point mutation (S83F) was observed in all 40 quinolone-resistant strains. The mRNA expression levels of *invA* and *avrA* were significantly decreased (P<0.005) in quinolone-resistant strains (clinically acquired or experimentally selected in vitro), compared to the quinolone-susceptible strains. The resistant strains also had a slower growth rate combined with decreased epithelial cell invasion and intracellular replication in epithelial cells and macrophages. The results suggest that quinolone-resistance may be associated with lower virulence and pathogenicity than in quinolone-susceptible strains.

**Johny *et al*., (2012)** investigate the efficacy of feed supplemented with caprylic acid (CA), 1-d-old straight-run broiler chicks on *Salmonella* Enteritidis invasion. The cell invasion study revealed that CA reduced invasive abilities of all *Salmonella* Enteritidis strains by ~80%. Gene expression studies indicated that CA downregulated (*P* < 0.001) *Salmonella* invasion genes *hilA* and *hilD*. and potentially reduces the pathogen’s ability to invade intestinal epithelial cells by downregulating key invasion genes, *hilA* and *hilD*.

**Hung *et al*., (2013)** found that propionate, a fatty acid abundant in the intestine of animals, repressed *Salmonella* pathogenicity Island 1(SPI1) at physiologically relevant concentration and pH, reducing expression of SPI1 transcriptional regulators and consequently decreasing expression and secretion of effector proteins, leading to reduced bacterial penetration of cultured epithelial cells.

**2.10.Enrofloxacin used in chickens:**

**Humbert *et al*., (1997)** studied the efficacy of three decontamination treatments: enrofloxacin either with or without the movement of birds to a clean area, and enrofloxacin combined with movement of birds and a competitive exclusion treatment. The control group remained untreated. Two hundred and forty, four-week-old laying birds naturally infected with *Salmonella* Enteritidis PT33. The results demonstrated that although antibiotic therapy, the movement of birds into a clean house and competitive exclusion, either combined or not, had some efficacy in reducing infection levels.

**Barrow *et al.,* (1998)** reported that experimentally-infected chickens with (SE) and treated by enrofloxacin at commercially recommended concentrations in the drinking water virtually eliminated this organism from the alimentary tract.

**Seo *et al*., (2000)** indicated that the use of enrofloxacin10 mg/kg of enrofloxacin in drinking water daily for 10 days, followed by normal avian gut flora by two doses beginning at 10 and 8 wk of age, could aid the elimination of SE from young chicks persistently infected at an early age. The combined treatment, compared with enrofloxacin alone, protected chickens from re-infection by 40%.

**Gammaz (2007)** compared between the effects of probiotics and antibiotics as enramycin and enrofloxacin on the broiler performance. The average feed intake, body weight, weight gain, feed conversion ratio and feed efficiency were measured. The results showed that all treated groups showed lower feed intake than the control group except enrofloxacin treated birds showed higher feed intake than control. In the same time all treated birds revealed higher body weight and weight gain except enrofloxacin treated birds showed lower level than control group. Feed efficiency and feed conversion ratio were more efficient in all treated birds except that of enrofloxacin treated birds which were less efficient than the control and treated groups.

**Sureshkumar *et al*., (2013)** evaluated the impact of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in Newcastle disease virus vaccinated broiler chicken after pulsed water medication. Experimental birds were administered with enrofloxacin at recommended therapeutic dose 10mg/Kg body weight, through drinking water for five consecutive days from 43rd to 47th day of age. There was no significant change in body weight, cumulative feed intake, feed efficiency and behaviour in enrofloxacin administered groups. The results indicated that enrofloxacin did not have any appreciable effect on broiler's performance.