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Synergistic Effects of Probiotics and Autogenous Bacterin against *Salmonella enterica* Serovar Typhimurium Strain U288

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Abstract

Salmonellosis, a disease caused by pathogenic strains of *Salmonella*, has been reported to have an essential degree in morbidity and mortality of humans (especially farmers) and poultry resulting in low productivity. This study was undertaken to evaluate the synergistic effects of probiotics and autogenous bacterin against *Salmonella enterica* serovar Tyhimurium strain U288 isolated from different poultry feed collected from Orlu-west Local Government Area of Imo State. Different types of feed samples were aseptically collected and screened for the presence of *Salmonella enterica* serovar Typhimurium using standard microbiological techniques. The pathogenic potentials of the organism in broiler chicks were investigated by challenging the chicks orally using 0.5ml of the inoculum (10^8cells/ml).The protective effects of locally prepared autogenous bacterin (B), commercially prepared probiotics (P) and autogenous bacterin plus commercially prepared probiotics (BP) were investigated using *in vivo* technique. The study revealed significant ($p<0.05$) pathological features and lesions in the liver and spleen of the infected chicks. The mean plate counts were significantly ($p<0.05$) recorded more in the liver than spleen. The *in vivo* study showed that P, B and BP showed pronounced activity against the tested isolates of which BP proved to be more effective. Therefore the combination of autogenous bacterin and probiotics exhibited synergistic activity, and was effective in preventing infection caused by *S. enterica* serovar Tyhimurium U288.

Keywords: *Salmonellosis, Salmonella, Probiotics, Autogenous bacterin, Synergism*

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Introduction

Chicken feed is food for farm poultry, including chickens, ducks and other domestic birds. They are referred to as complete feeds because they are produced to contain all the proteins, energy, vitamins minerals and other nutrients necessary for proper growth, egg production and health of the birds. Feeds ranges from starters, layers, growers to finishers. Before the twentieth century, poultry were mostly kept on general farms, and foraged for much of their feeds, eating insects, grain spilled by cattle and horses around the farm. The quantity of feed and nutritional requirements of the feed depending on the weight and age of the poultry, their rate of growth, their rate of egg production, the weather and the amount of nutrition the poultry obtained from foraging. This results in a wide variety of food formulations (Heuser, 2000). Healthy poultry require a sufficient amount of proteins and carbohydrates, along with the necessary vitamins, dietary minerals and an adequate supply of water. Lactone fermentation of feed can aid in supplying vitamins and minerals to poultry. The feed must remain clean and dry. Contaminated feed can infect poultry and damp feed encourages fungal growth. Mycotoxin for example is one of the most common and certainly most underreported causes of toxicoses in poultry. Diseases can be avoided with proper maintenance of feed and feeder. A feeder is a device that supplies feed to the poultry (Heuser, 2000).

Salmonella is a genus of rod-shaped, gram-negative bacteria. They are non-spore formers and are predominantly motile with cell diameter between 0.7−1.5$_{u}$m, length of about 2−5$_{u}$m and have peritrichous flagella. They belong to the Enterobacteriaceae family which causes one of the most common enteric (intestinal) infections: salmonellosis (Miller *et al.,* 2005). The two species of *Salmonella* are *Salmonella enteric and Salmonella bongori*. *Samonella enterica* is further divided into six sub-species which are *arizone, diarizone, enteric, houtenae, indica, salamae* and each of them includes over 2500 serovars (Su and Chiu, 2007). In the 19th century, the causative agent of typhoid was identified which eventually became known as *Salmonella* and smith first isolated *Bacillus cholerasuis,* now called *Salmonella enterica* (*S. enterica*) sub-species *enteric* serovar *Cholerasuis,* from swine diagnosed with hog cholera (Starr *et al.,* 1995). While smith was the first to actually identify the organism, Salmon was credited the discovery which came to bear his name. In any case, today the number of known strains of the bacteria totals over two thousand (Behravesh*et al.,* 2008). *Salmonella enterica* sub-species are found worldwide in allwarm blooded animals and in the environment.

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*Salmonella bongori*is restricted to cold- blooded animals particularly reptiles. It grows best at a temperature of 6−46^{0}C (43−115ºF), optimum produce hydrogen sulfide which can readily be detected by growing them on media containing ferrous sulfate. *Salmonella* serovar *Pullorum* causes infections in warm blooded animals. It can be introduced into a flock by wild birds, animals and flies.

Fowl typhoid is caused by one of two poultry adapted strains of *Salmonella* bacteria, *Salmonella* serovar Gallinarum. This can cause mortality in birds of any age, broiler parents and brown-shell egg layers are especially susceptible. The route of infection is oral or via the navel/yolk. Transmission may be Trans ovarian or horizontal by faecal-oral contamination, egg-eating even in adults. *Salmonellosis* is one of the most common causes of food poisoning, it occurs in animals and humans. In both cases, it is an enteric disease of varying severity, usually involving diarrhea. With poultry, however, most *salmonella* infections are without symptoms. The commonest serotype causing diseases in humans are *Salmonella serovar enteritis* and *Salmonella serovar* Typhimurium (Su and Chiu, 2007).

Salmonella is a major microbial hazard in animal feed. *Salmonella* can persist for long periods in a wide range of materials. The lack of uniformity involved in *Salmonella* contamination and the large volumes of feed produced make accurate assessments of feed contamination rates difficult. *Salmonella* control principles maybe divided into three broad categories: effort to prevent contamination from entering the facility, work to reduce microbial multiplication within the plant, and procedures designed to kill the pathogen. Preventing contamination also involves controlling dust, managing the flow of equipment and humans, reducing Salmonella multiplication in feed manufacturing facilities involves discovering microbial growth niches and reducing conditions that lead to growth. Killing *Salmonella* may involve thermal processing or recontamination after thermal processing. Chemical additions to control *Salmonella* in feed primarily involve the use of products containing organic acid, formaldehyde, or a combination of such compounds. The use of autogenous bacterin is also another method used to control *Salmonella* infection in broiler chicks (Su and Chiu, 2007).

Antibiotics play very important roles in controlling and treatment of *salmonella* infections, in a situation in which antibiotics are needed, ampicillin or amoxicillin are the best choices (Miller *et al.,* 2005). Ceftriaxone, cefotaxime, or flouroquinolones are effective option for antimicrobial-resistant strains. Cephalosporin is recommended for animals at high risk of invasive disease. But misuse and frequent use of antibiotics have led to *Salmonella* drug resistance. Autogenous bacterin is also useful in protecting the animals from the infections, it is a killed bacterial vaccine created from the disease-causing organism. This vaccine has served as a means of protecting the immune system of the animals against specific infections. It reduces the rate of diseases and death in animals (Sherrill *et al.,* 1999). In areas where there is a lack of antibiotics or vaccines, may result in high rate of diseases and mass deaths.

Many researchers have studied different ways of synergistic effects of probiotics and autogenous bacterin against inositol negative motile *Salmonella* species such as Barrow *et al.* (2007) stated that "Therefore the main form of controlling the presence of *Salmonella* sp. in poultry production is related to biosecurity measures and vaccination, associated with the right use of antibiotics, prebiotics and probiotics". Van immerseel *et al.,* (2005) stated "because the level of protection offered by live vaccine strains depend on the administration route". In Nigeria, the importance of controlling moulds and mycotoxins in feeds is widely known and practiced, but the control of bacteria is less well understood and frequently overlooked (Maciorowski *et al.,* 2007). Though hygiene program with the use of a long-acting chemical treatment on the poultry feed is the only way to minimize spread of the infections. This work has been designed to check the synergistic effects of probiotics and autogenous bacterin against *Salmonella enterica* serovar Typhimurium strain U288 isolated from the chicken feed.

Materials and Methods

Isolation and Characterization of Salmonella enterica serovar Typhimurium

Ten folds serial dilution was carried out on each of chicken feed samples and 1.0 mL was aseptically taken from the third test tube and poured plated into the *Salmonella Shigella* Agar and incubated at 37°C for 48 h. After 48 h incubation, the grown colonies were sub-cultured, characterized and identified using their colony descriptions, microscopic and biochemical characteristics (Iheukwumere *et al*., 2018).

Procurement of Chicks

A total of eighteen (18) day old chicks of mixed-sex obtained from Ausonic farm at Ihiala, Anambra State were used for this study. The chicks were kept in separate, thoroughly cleaned and disinfected cages and provided with feeds and water frequently.

Inoculation into the chicks

This was carried out using the method of Wafaa *et al*. (2012). Broth culture of the isolate was centrifuged at 3000 r.p.m for 10 minutes. The sediment was diluted with sterile phosphate buffer saline (PBS) and adjusted to the 10^8CFu/ml using 0.5 McFarland matching Standard which is (0.6ml of 1% $BaCl_2.2H_20$ + 99.4ml of 1% concentration of H_2SO_4). Then the chicks were orally infected using 0.5 ml of the prepared inoculum.

Examination of infected chicks

The infected chicks were carefully observed for the obvious pathological signs of the organism challenged for a period of fourteen (14) days. The number of deaths was also observed. After fourteen (14) days, the infected chicks were sacrificed and gross examination of their internal organs morphologies was carried out using the methods of Wafaa *et al.* (2012).

Re-isolation of the organism from the infected organs

The internal organs of the infected chicks were harvested and portions were aseptically macerated in peptone water and serially diluted using ten-fold serial dilution. Samples were inoculated into *Salmonella Shigella* Agar (S.S.A) and incubated at 37ºC for 24 hours using the methods of Wafaa *et al.* (2012).

Protection of Chicks

This was carried out using the modified method of Wafaa *et al.,* (2013). Autogenous bacterin was used for the study.

Synergistic Effects of Autogenous Bacterin

A total of eighteen (18) day old chicks were used for this study. In addition, autogenous bacterin prepared from the pure culture of *Salmonella enterica* serovar Typhimurium strain U288 was also used for this study.

Preparation of autogenous bacterin

This was carried out by the method of Wafaa *et al.,*(2012). The isolate was grown on nutrient broth at 37ºC for 24 h. The culture was centrifuged at 3000 r. p.m. for ten (10) minutes and the supernatant was decanted. The sediment was washed with normal saline and suspended into 1% formal saline at room temperature for 24 hours. The sterile autogenous bacterin was obtained by adding an equal volume of incomplete Ferund's adjuvant to adjusted washed concentrate of inactivated bacterium and kept at refrigerator until when used. The autogenous bacterin was given to the experimental chicks at first day in a dose of 0.2ml/chick and boostered at a second dose at 7days in dose of 0.5ml/chick. The autogenous bacterin in the two shots was given subcutaneously through the thigh.

Experimental Design

This was carried out using the method of Wafaa *et al.,* (2012). The chicks were grouped into three (3) groups which include groups A, B and C. Each group contained six chicks each. The treatments to the group were as follows: Group A was intramuscularly administered autogenous bacterin; 0.2 ml/chick for the first dose and boostered on the 7th day with 0.5ml/chick and then challenged with 0.5ml of test organism after 14 days. Group B was Infected with 0.5 ml of test organism without protection. Group C was given only distilled water. The experimental chicks were carefully monitored for a period of 2 weeks for any obvious pathological signs.

Detection of the Humoral Immune Response

Just before the first dose of the autogenous bacterin (zero hours), the chicks were randomly selected and their blood was collected. Also just before the second booster dose, another blood sample was also collected on 14th day. The blood samples were allowed to separate. The separated sera were used against the isolate for agglutination reaction using micro agglutination titre techniques. The serum collected from the chicks was serial diluted using two-fold serial dilution. Then 0.1µL of the diluted serum ($^1/_{20}$, $1/_{40}$, 1/80, $^1/_{160}$ and$^1/_{320}$) was deposited on the wells of the micro filter and aseptically mixed with 0.1µL of the test isolate. This was incubated at 37ºC for 90 minutes. The agglutination results and titer value was recorded. This was repeated after 7 days (before booster dose) and 14 days (before challenge) and this is in accordance with the methods of Wafaa *et al.* (2012).

Examination of Protected Chicks: The protected chicks were carefully observed for the obvious pathological signs of the administered test organism for a period of 2 weeks, the protection rates of the inhibitory substances were determined, and the chicks were sacrificed and gross examination of the morphologies of internal organs and intestine was carried out. Also, the internal organs were harvested and some portions of these organs were cultured on *Salmonella Shigella* agar and incubated at 37ºC for 48 h. The counts were taken and the colonies were identified morphologically and biochemically (Wafaa *et al.,* 2012).

Statistical Analysis

The data obtained in this study were presented in tables and figures. Their percentages were also calculated. The sample means and standard deviations of some of the analytical data were also calculated. Chi-square (x^2) was used to determine the significance of the sample sources. The significance of the prevalence of the isolates in the studied samples was determined at 95% using one-way analysis of variance (ANOVA). A pairwise comparison was carried out using the student "t" test.

Results

Micro agglutination antibody titers generated from the sera of broiler chicks after vaccination with prepared autogenous bacterin and probiotics are shown in Table 1. On the first day (Before first vaccination dose), the antibody titer values (ATVs) of sera samples collected from the test and control chicks were zero. On the seventh day (before booster dose), ($^1/_6$) of the chicks vaccinated with the autogenous bacterin and probiotics had maximum ATVs $^1/_{320}$whereas$^3/_6$ and $^2/_6$ of the remaining vaccinated chicks recorded $^1/_{80}$ and $^1/_{160}$titre values respectively. On the 14th day (before challenge), $^4/_6$ and $^3/_6$ of the remaining vaccinated chicks recorded $^1/_{160}$ and $^1/_{320}$ respectively. There was no ATV value recorded from non-vaccinated chicks after 14th days. The obvious pathological signs of the challenged isolate in the infected chicks are shown in Table 2. The chicks infected with the test organism without protection recorded significant ($p<0.05$) obvious pathological signs of the test organism which was significantly ($p<0.05$) reduced in those chicks administered autogenous bacterin, probiotics and bacterin with probiotics. No obvious pathological sign was recorded among the control (non-infected chicks). The gross pathological lesions of the internal organs of the infected chicks are shown in Table 3. The chicks infected with the test organism without protection recorded significant ($p<0.05$) gross pathological lesions which were significantly ($p<0.05$) reduced in those chicks administered autogenous bacterin, probiotics and bacterin with probiotics. No obvious pathological sign was recorded among the control (non-infected chicks).

The mean organ/bodyweight of experimental chicks administered autogenous bacterin and probiotics is shown in Table 4. The organ/body weights were more in the liver of the infected chicks. The organ/body weights of the experimental chicks administered autogenous bacterin and probiotics was more in the spleen and less in the liver. The spleen of the infected chicks without protection was not developed so the organ weight was not taken.

The total mean viable plate count of the studied isolates from the internal organs of the experimental chicks administered autogenous bacterin and probiotics is shown in Table 5. The counts were significantly ($p<0.05$) more in the liver and less in the spleen of the infected chicks without protection. The counts were significantly ($p<0.05$) reduced in the protected chicks with autogenous bacterin and probiotics.

The protection rate of autogenous bacterin and probiotics against the test organism is shown in Table 6. All the infected chicks protected with autogenous bacterin and probiotics were significantly ($p<0.05$) protected. No death occurred. The experimental chicks showed 100% protection.

Table 1: Antibody titers in the sera of the broiler chicks protected with autogenous bacterin and probiotic

Isolate	Day	Interval	Total	Antibody titers of the chick's serum at different dilutions					
					20	40	80	160	320
T1	0	BFVD 6		6	0	0	0	0	
	7	BBVD 6		6	6	3	2	0	
	14	BC	6		6	6	6	4	2
T2	0	BFVD 6		0	0	0	0	0	
	7	BBVD 6		6	6	6	2	4	
	14	BC	6		6	6	6	3	3
Control	0	BFVD 6		6	0	0	0	0	
	7	BBVD 6		6	0	0	0	0	
	14	BC	6		6	0	0	0	0

BFVD = Before First Vaccination Dose; BBVD = Before Booster Vaccination Dose; BC= Before Challenge.

Table 2: Obvious pathological signs of challenged isolates in the infected chicks

	N = 4				
Pathological signs	**B**	**P**	**BP**	**C_1**	**C_2**
Diarrhea	0	0	0	3	0
Respiratory distress	0	1	0	3	0
Weakness	0	1	0	3	0
Anorexia	0	0	0	1	0
Dysentery	0	0	0	2	0
Alopecia	0	0	0	0	0
Death	0	0	0	0	0

N =Total number of chicks; B = Bacterin vaccination; C1 = Infected chicks without protection; C2 = Normal chicks; P = Protection with probiotics; BP = Protection with bacterin and probiotics.

Table 3: Gross pathological lesions of the internal organs of the infected chicks

			N = 4		
Gross lesion	**B**	**P**	**BP**	**C_1**	**C_2**
Liver Oedema	0	1	0	3	0
Liver Haemorrhage	0	0	0	3	0
Perihepatitis	0	1	0	3	0
Congestion	1	2	1	3	0
FAI	1	0	1	3	0
Enterocolitis	0	0	0	2	0
Spleen deformation	0	0	0	ND	0

N =Total number of chicks; B = Bacterin vaccinated chicks; C1 = Infected chicks without protection; C2 = Normal chicks; ND = Not developed; FAI = Fluid accumulation in the intestine; P= Protection with probiotics; BP= Protection with bacterin and probiotics.

Table 4: Mean organ/body weights of the experimental chicks

O/B	L/B	S/B
B	0.02 ±0.01	1.10±0.01
P	0.03±0.01	1.30±0.01
BP	0.02±0.01	I.01±0.01
C_1	0.04±0.02	ND
C_2	0.02±0.01	1.00±0.02

O/B = Organ/Body weight; L/B = Liver/Body weight; S/B = Spleen/ Body weight; B = Bacterin protection before infection; P = Probiotics protection before infection; BP = Bacterin and probiotics protection before infection; C1 =Infection without protection; C2 = Normal chicks; ND = Not developed.

Table 5: Total mean viable plate counts of the studied isolates from the internal organs of infected chicks administered autogenous bacterin and probiotics

Protection	Spleen (cfu/g)	Liver (cfu/g)
B	7.00±5.70	11.00±9.86
P	11.00±0.00	14.00±0.00
BP	0.00±0.00	0.00±0.00
C_1	19.00±7.30	25.00±6.50
C_2	0.00±0.00	0.00±0.00

B = Bacterin vaccinated chicks; P = Probiotics protected chicks; BP = Bacterin and probiotics protected chicks; C1 = Infected chicks without protection; C2= Normal chicks.

Table 6: Protection rate of autogenous bacterin and probiotics against inositol negative motile Salmonella species

Protection	N	D	M (%)	S	P (%)
B	4	0	0	4	100
P	4	0	0	4	100
BP	4	0	0	4	100
C_1	4	0	0	4	0^d
C_2	4	0	0	4	100^a

B = Bacterin vaccinated chicks; P = Probiotics protected chicks; BP =Bacterin and probiotics protected chicks; C1= Infected chicks without protection; C2 = Normal chicks; N = Total number of chicks; D= Number of death; M =Mortality rate; S =Number survived; P = Protection rate; 100a= No protection; 0d= Control positive.

Discussion

The maximum titer value attained by tested *Salmonella* species bacterin supported the findings of many researchers (Davies and Breslin, 2004; Okamura *et al.,* 2004).

The *in vivo* study was carried out to determine the protection rate of locally prepared autogenous bacterin (B), commercially prepared probiotics (P) and locally prepared autogenous bacterin plus commercially prepared probiotics (BP). The absence of growth observed in the internal organs administered BP supports the findings of Wafaa *et al.* (2012). Several other researchers have documented that the frequency of *Salmonella* species re-isolated from the internal organs was significantly reduced in the protected chickens (Khan *et al.,* 2003; Radwan, 2007). The significant decrease in TMPCs showed by the internal organs from those chickens administered BP corroborated to the findings of other researchers (Barbour *et al.,* 2003; Wafaa *et al.,* 2012). The competitive exclusion mechanism exhibited by probiotics against the pathogen *Salmonella* species was comprehensively studied by several researchers. From this result, it was reported that probiotics maintained or increased the normal intestinal flora which are normally found in the intestinal tracks of hatched chicken and these flora can exclude *Salmonella* species colonization (Mead, 2000; Wafaa *et al.,* 2012). The absence of visible growth of *Salmonella* species observed in non- infected (normal) day-old chicks supports the finding of Magdelena *et al.* (2011), who reported that during the first 3 days of life, chicken was protected from incoming antigens by increased expression of β-defensins (gallinacins 1,2,4 and 6), which made the chicks germ-free.

The maximum protection achieved by vaccinating those chicks fed with diet supplemented with commercially prepared probiotics could be attributed to the synergistic effects of the two substances. The bacterin activated and boosted the humoral and cellular components of immune response (Wafaa *et al.,* 2012) whereas the probiotics produced lactic acid that created unfavorable pH for the growth of the *Salmonella* species pathogens (Alkoms *et al.,* 2000; Johasen *et al.,* 2004). The probiotics also compete with the pathogens (Wafaa *et al.,* 2012) and produced bacteriocin that was toxic to the enteric bacteria (Pascual *et al.,* 2009). The positive effect of feeding diet containing probiotics on the immune response indicates the enhancement of the formulating bacteria on the acquired immune response exerted by T and B lymphocytes. The direct effect might be related to the stimulation of lymphatic tissue, whereas the indirect effect may occur via changing the microbial population of the lumen of the gastrointestinal tract or through the reduction of *Salmonella* species pathogen colonization. Shoeib *et al.* (2007) reported that the bursa of probiotic treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Christensen *et al.* (2002) suggested that some of these effects were mediated by cytokines secreted by immune cells stimulated with vaccination and probiotic bacteria. On the other hand, vaccinating chickens fed with diet supplemented with probiotics has beneficial effects for chicks, particularly during the first days of life.

Conclusion

The *in vivo* study of synergistic effect of probiotics and autogenous bacterin against *Salmonella enterica* serovar Typhimurium strain U288, showed safe and pronounced activities with locally prepared autogenous bacterin (B) and commercially prepared probiotics (P), but locally prepared autogenous bacterin plus commercially prepared probiotics (BP) proved to be most effective.

References

Alcoms, H.L., Skytta, E., Saaela, M., Maltila, S.T., Latra, K.K. and Helender, I.M. (2000). Lactic acid permeabilizes Gram-negative bacteria by disturbing the outer membrane. *Applied Environmental Microbiology* **66**: 2001–2005

Alshawabkeh, K.M. (2006). Occurrence of *Salmonella* on poultry feed in Jordan. *Jordan Journal of Agricultural Sciences* **2**(2): 46–50

Agbor, T.A. and McCormick, B.A.(2011). *Salmonella* effectors: important players modulating host cell function during infection. *Cell Microbiology* **13**:1858–1869.

Barrow, P.A (2007). *Salmonella* infections: immune and non-immune protection with vaccines. *Avian pathology* **36** (1):1–13.

Barbour, E.K., Frerichs, W.M., Nabbut, N.H., Poss, P.E. and Brinton, M.K. (2003). Evaluation of bacterins containing three predominant phage types of *Salmonella* Enteritidis for prevention of infection in egg laying chickens. *American Journal of Veterinary Resource* **54**: 1308–1309

Behravesh, C.B. (2008). Salmonellosis in control of communicable diseases manual, 19th edition. American public health association. pp. 535–540.

Butcher, G.D and Miles, R.D. (1995). Minimizing microbial contamination in feed mills producing poultry feed. P. 93.

Brenner, F.W., Villar, R.G., Angulo, F.J., Tauxe, R. and Swaminathan, B.(2000). *Salmonella* nomenclature. *Journal Clinical Microbiology* **38**(7):2465-2467.

Carter, J.D. and Hudson, A.P. (2009).Reactive arthritis: clinical aspects and medical management. *Rheum Disease Clinical North America* **35**(1): 21-44.

Breslin, M., Venables, L.A., Springing, K.A., Cooles, S.W., and Woodward, M.J. (2002). A laboratory study of an inactivated bivalent iron

restricted *Salmonella* enteritidis challenges in chickens. *Veterinary Microbiology* **89**: 167−179.

Christensen, H.R., Frokraer, H. and Pestka, J.J. (2002).*Lactobacillus* differently nodulate expression of cytokines and maturation surface markers in murine dendritic cells. *Journal of Immunology* **186**: 171−178

Davies, R. and Breslin, M. (2004). Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella*Enteritidis had been used. *Avianpathology***33**: 135−146.

Hardy, A. (1999). "Food, hygiene, and the laboratory.A short history of food poisoning in Britain."*The Journal of the Society for the Social History of Medicine.* **12**(2):293–311.

Heuser, G. (2005). Feeding Poultry. Norton Creek press, Philadelphia. Sixteenth Edition, pp. 527–626.

Iheukwumere, I. and Umedum, C. (2013). Effects of Gossypiumhirsutum leaf extracts on Gram negative bacteria isolated from cervix of females with unexplained infertility. *African Journal of Science* **14**(1): 3261−3270.

Immerseel, F.V., Cauwerts, K., Devriese, L.A., Haesebrouck, F. and Ducatelle, R. (2002). Feed additives to control *Salmonella* in poultry.*World's Poultry Science* **58**: 431−443

Johansen, S.A., Griffith, R.W., Wesley, I.V. and Scanes, C.G. (2004).*Salmonella enteric* serovar *Typhimurium* colonization of the crops in the domestic turkeys: Infleunce of probiotics and probiotic treatment (*Lactobacillus acidophilus*). *Avian Discovery* **48**: 279 −286

Jones, F.T. and Richardson, K.E. (2004).*Salmonella* in commercially manufactured feeds. *Poultry Science* **83**: 384 −391

Khan, A.A., Nwaz, M.S., Khan, S. and Sernigella, C.E. (2009). Detection of multi drug resistant *Salmonella by multiplex polymerase chain reaction. FEEMS Microbiology Letter***182**: 355−360

Jones, F.T. (2011). A review of practical *salmonella* control measures in animal feed. *Journal of Applied Poultry Resources***20** 102−113.

Jepson, M.A. and Clark, M.A. (2001). The role of M cells in *Salmonella* infection. *MicrobesInfection*.**3**:1183–1190.

Maciorowski, K., Jones, F., Pillai, S. and Ricke, S. (2004).Incidence, sources and control of food borne *Salmonella spp*.In poultry feeds.*Worlds Poultry of Science Journal***60**: 446−457.

Magdalena, C., Helena, H., Marta, M., Hana, Franstisek, S. and Ivan, R. (2011). Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica*seronarenteritidis.*Infection*. Journal of Infection and Immunity **7** (79): 2755−2763

Mead, G.C. (2000). Prospects of competitive exclusion treatment to control *Salmonella species* and other food borne pathogens in poultry. The *Veterinaryjournal* **59**: 111−123

Miller,S. and Pegues, D. (2001).*Salmonella spp.* Including *salmonella typhiiIn* Mandell, Doughlas and Bennet's principles and practice of infectious diseases, sixth edition, PP. 581−584.

Okamurah, H., Lillehoj, S., Rayburne, R.B., Babu, U. and Heckert, R. (2004). Cell mediated immune responses to a killed Enteritidis vaccine. Lymphocyte proliferation, T- cell changes and Interluekin – 6 (IL-6), IL-1, IL-2, AND IFN- (gamma) production.*Complement Immunology and Microbiology of Infectious Diseases***27:** 255−277.

Pascual, M., Hugas, M., Badiola, J.I. Monfort, J.M. and Garriga, M. (2009). *Lactobacillus salivarius* CC2197 prevents *Salmonella* Enteritidis colonization in chickens. *Applied Environmental Microbiology* **65**: 4981–4986.

Radwan, H.M. (2007). Studies on *Salmonella* infection in chicken flocks. Masters in Vetinary Science, Thesis in Faculty of Veterinary Medicine, Department of Avian and Aquatic, Animal Medicine, Alexander University, Egypt, pp. 89−103

Radcliff, J. (2006). Pathogenic control in feed mills; feed processing and quality control technical reports series. Pp. 45–49.

Ryan, K. and Ray, C. (2004). Sherris Medical Microbiology, McGraw Hill, New York.PP. 25−45.

Sherill, D., Charles, E.B., David, J.H. and Robert, J.E. (1999). Field observation with *Salmonellaenteritidis*bacterin. *Aviandiseases* **43** (4): 664–669.

Shoeib, H.K., Sayed, A.N., Sotony, S.A. and AAbdelGhaffar, S.K. (2007). Response of broiler chicks to probiotics (pronifer) supplementation. *Journal of Assuit Veterinary Medicine***36**: 103−116.

Scallan, E., Hoekstra, R.M., Angulo, F.J, Tauxe, R.V., and Widdowson, M.A. (2011). Foodborne illness acquired in the United States, major pathogens. *Emerging Infectious Diseases*. **17**(1):7-15.

Su, L.H. and Chiu, C.H. (2007).*Salmonella*: clinical importance and evolution of nomenclature. *Chang Gung Medical Journal* **30** (3):210-219.

Wafaa, A.A., Soumaya, S.A., Hatem, M.E. and Rehab, E.D. (2012). A trial to prevent *Salmonella*Enteritisinfection in broiler chickens using autogenous bacterin compared with probiotics preparation. *Journal* of *Agricultural Science***4** (5): 91−108.

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