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Efficacy of Some Selected Antimicrobial Substances in Prevention of Enteric Bacterial Infection in Broiler Chicks

Iheukwumere, I. H[1]. Uneze, B. C.[2] Ejike, C. E.[3]
1.Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria
2.Department of Medical Laboratory Science, Faculty of Health Science, Nnamdi Azikiwe University, Awka, Nnewi Campus
3.Department of Medical Microbiology, Chukwuemeka Odumegwu Ojukwu University

Abstract
This study was carried out to investigate the efficacy of autogenous bacterin from enteric bacteria isolated from chicken feeds, commercially prepared probiotic, conventional antibiotics as well as *Zingiber officinale* extract in prevention of enteric bacterial infection in broiler chickens. A total of 1,536 samples of different brands of chicken feeds were collected and screened for the presence of enteric bacteria using pour plate technique.The pathogenic potentials of the isolates on chickens were investigated by challenging the chickens orally using 0.5 ml of the inoculum (10^8cells/ml). The efficacy of locally prepared autogenous bacterin (AB), commercially prepared probiotics (CP), autogenous bacterin plus probiotics (ABCP), ciprofloxacin (CPX), Oxytetracycline (OXY) and *Zingiber officinale* (ZO) extract were investigated using *in vivo* method. The titer of antibodies produced by the vaccinated chickens was determined using micro agglutination test. *Escherichia coli* O157:H7 SS52 (EC), *Salmonella* ser. Typhimurium U288 (ST), *Escherichia coli* SEC470 (ES), *Salmonella* ser. Enteritidis YU39 (SY) and *Salmonella* ser. Enteritidis FM366 (SE) were isolated from the feed samples. There were significant obvious pathological signs and lesions in the internal organs of the infected non-protected chickens, which decreased significantly ($P<0.05$) when the chicks were protected with CP, ZO, CPX, OXY and ABCP. The internal organs of the infected non-protected chickens showed high viable mean plate counts (VMPCs) and these were significantly ($P<0.05$) decreased when protected with the antimicrobial agents, of which the VMPCs of ABCP were the least. Moreover, the serological investigation revealed an improvement in the titer of antibiotics after vaccination and probiotic treatment. The tested antimicrobial agents have proved to be safe and effective against the isolates, of which ABCP showed the most pronounced activity.
Keywords: Enteric Bacteria, autogenous Bacterin, *Zingiber officinale*, ciprofloxacin, probiotic, Oxytetracycline,

INTRODUCTION

Chicken diseases have contributed significantly to increase in mortality rate and economic losses in the chicken industry. As a result, antibiotics, sometimes at sub-therapeutic concentrations, are often included in feed given to chickens to prevent disease, reduce mortality and morbidity, enhance feed conversion efficiency and improve growth rates (Oguttu *et al.,* 2008). However, the use of antibiotics in chicken feeds is not totally safe. One of the main concerns is the development of antibiotic resistant bacteria (Oguttu *et al.,* 2008). The rampant use of antibiotics in chicken production has resulted in the development and maintenance of populations of antibiotic-resistant gram-negative enteric bacilli in the intestinal tracts of these chickens and their products (Oguttu *et al.,*2008). The clinical significance of these phenomena is that selective pressure for resistance caused by using antibiotics may result in multiple antibiotic resistance and these antibiotic resistant bacteria are known to be transmissible from chicken to man (Oguttu *et al.,* 2008). The use of naturally produced antimicrobial agents without any adverse effects on human health to inhibit the proliferation of pathogenic bacteria in chicken feed is a more congenial option to overcome the problems associated with feed contamination (Tharmanaj and Shah, 2009).

The secondary metabolites such as phytochemicals produced by medicinal plants and organic acids, short chain fatty acids, hydrogen peroxide, reuterin, diacetyl, bacterixins and bacteriocin-like inhibiting substances are some of the metabolic products of *Lactobacillus* species suggested to have potential antimicrobial effects (Tharmaraj and Shah, 2009). The importance of probiotics mainly *Lactobacillus* species in chicken feeds and growth performance in chickens have equally been documented by other researchers (Shah and Dave, 2002; D' Mello, 2006). Despite different methods of control attributed to enteric bacterial infections (Wafaa *et al.,* 2012; Ali *et al.,* 2014), enteric bacteria mainly *E. coli* and *Salmonella* species remain the primary causes of reported food poisoning worldwide (Ali *et al.,* 2014).This study was carried out to investigate the efficacy of autogenous bacterin from enteriac bacteria isolated from chicken feeds, commercially prepared probiotic, conventional antibiotics as well as *Zingiber officinale* extract in the prevention of enteric bacterial infection in broiler chickens.

MATERIALS AND METHODS

Collection of Samples: A total of 1536 commercially produced poultry feed samples were aseptically collected from three major chains of distributors; wholesalers, retailers and consumers. The feed types which included X (756 samples), Y (756 samples) and Z (756 samples) were aseptically collected from twenty-one (21) major towns located within the twenty-one (21) Local Government Areas of Anambra State. One cup of the feed sample was aseptically collected from each feed type by randomly collecting one Table spoon of the feed sample from each bag containing the feed type. The feed samples were mixed and homogenized to generate a representative sample for each feed type. The representative feed samples were collected from Broiler starter (128 samples), Grower mash (128 samples), Broiler finisher (128 samples) and Layer mash (128 samples) for each feed type (X, Y and Z) using aluminum foil. The samples were carefully labeled, classified based on the sources of collection and transported to the laboratory for analysis within 1 h.

Culture and Isolation of Enteric Bacteria: This was carried out using the modified method of Arotupin *et al*. (2007). One gram (1.0g) of each sample was first measured and dissolved in 10ml of sterile distilled water prior to serial dilution. One milliliter aliquot was aseptically transferred into a sterile test tube containing 9.0 ml of the diluent (distilled water) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the sample was plated on *Salmonella-Shigella* agar (SSA/Biotech) for *Salmonella* and *Shigella* species and MacConkey agar (MA/Biotech) for coliforms and non coliforms. All the plates in triplicates were incubated inverted at 44.5˚C for 24 h for *E. coli* and 37˚C for 24 h for other enteric bacteria.

Characterization and Identification of the Isolates: The isolates were subcultured on nutrient agar (Biotech), incubated invertedly at 37˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Arotupin *et al.,* 2007), biochemical reactions (Arotupin *et al.,* 2007; Uwaezuoke and Ogbulie, 2008) and molecular characterization (Habtamu *et al.,* 2011; Gabriela *et al.,* 2014). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Preparation of plant materials: The fresh rhizomes of *Zingiber officinale* were collected from cultivated land at Uli in Ihiala L.G.A of Anambra State, Nigeria. The sample was authenticated by Ukpaka C. J, a botanist in Biological Science Department, Faculty of Sciences, Anambra State University, Uli. The rhizomes of *Zingiber officinale* were harvested and dried under shade at room temperature for 14 days. The dried rhizomes were ground to powdered form using sterile electric grinder. Twenty gram of the sample was macerated with distilled water for 72h. The mixture was filtered using Whatman No 1 filter paper. The extract was concentrated by evaporating to dryness at room temperature in a steady air current (Iheukwumere *et al*., 2012).

Determination of extract value of the plant materials: The concentration of the extract was determined by evaporating 1.0 g of the extract in an evaporating dish of known weight in an oven to dryness and weighed. The dish containing the residue was allowed to cool and weighed. The weight of the residue was obtained by subtracting the weight of the empty dish from the weight of the dish and residue. The above method was done in duplicate (Iheukwumere *et al*., 2012)

Preparation of the test samples of the plant extract for *in vivo* study: In this study the concentration of 500mg/ml of the extract was used to screen for the antimicrobial activity. This was done by using the modified method of Iheukwumere *et al*. (2012). Here, 2.5 g of the extract was dissolved in 5.0 ml of peptone water.

Preparation of probiotics for *in vivo* antibacterial assay: The probiotic used in this experiment was a commercially prepared probiotic (*Lactobacillus acidophilus* and *Streptococcus thermophilus)* plus potassium, vitamin A, E and K, riboflavin and thiamine. This product was manufactured by Bomac Vets Company, U.SA. The probiotic was giving in the drinking water in a dose of 1g/l of the drinking water, for a period of 14 days for the chicks and 2 g/l for period of 14 days for adult layers

Preparation of local bacterin (bacteria vaccine) from the isolated bacteria: This was carried out by the modified method of Wafaa *et al.* (2012). The bacterin was prepared from the pure cultures of the isolates. The isolates were grown on nutrient agar at 37˚C for 24 h. Growth was harvested in normal saline and inactivated with 1% formal saline at room temperature for 24 h. Using McFarland matching tube, washed concentrates of inactivated bacteria were suspended in normal saline to contain 10^8 cells/ml. The sterile bacterin was obtained by adding equal volume of incomplete Ferund's adjuvant to the adjusted washed concentrate of inactivated bacteria and kept at refrigerator until used. The bacterin was given to the experimental chicks at the first day of age in dose of 0.2 ml/chick and boostered as a second dose at 7 days of age in a dose of 0.5 ml/chick. The bacterin in the two shots was given intramuscularly (IM) in the thigh muscles.

Quality control tests on the prepared bacterin: The prepared bacterin was tested for purity, complete inactivation, sterility and safety according to the Standard International Protocols as described by the British Veterinary Codes (Wafaa *et al*., 2012).

Purity test: The test was done before inactivation of the isolates. It was done to confirm that the broth culture of the isolates did not contain any contamination by other organisms before inactivation. This was done by sub culturing the broth into MacConkey agar and *Salmonella-Shigella* agar and incubated at 37˚C for 24 h. The

resulting colonies were aseptically streaked on Nutrient agar and incubated at 37˚C for 24 h. The colonies were Gram stained, examined and finally confirmed using their unique biochemical reactions.

Complete inactivation test: This was carried out to ensure that the isolates were completely inactivated. The MacConkey agar and *Salmonella-Shigella* agar were inoculated with the bacterin, incubated at 37˚C for 48 h. No visible growth of isolates was seen.

Sterility test: The prepared bacterin was confirmed to be free from any fungal contaminants by inoculating it into Sabouraud Dextrose Agar (SDA) plates and incubated at room temperature for 7 days.

Safety test: Nine (9), day-old broiler chicks were inoculated intramuscularly (IM) with a large dose of the prepared bacterin (ten-fold of the normal bacterin dose). The chicks were observed daily for seven (7) successive days for any signs of local reactions, clinical signs or deaths.

***In vivo* Activities of the Antimicrobial Substances against the Enteric Bacterial Isolates:** A total of eighty-two (82 chicks) were purchased. At arrival, randomly two chicks were sacrificed and then examined bacteriologically to prove their freedom from the isolates. The chickens were grouped into six (6) groups which include: group A, B, C, D, E and F. Group A contained five (5) broiler chicks, groups B, C,D, E and F contained fifteen (15) broiler chicks each, and the treatments to the groups were as follows: **Group A:** Blank control (only distilled water) for the chicks period of fourteen (14) days; **Group B:** Antibiotics (ciprofloxacin/oxytetracycline), 0.25g/L for the chicks for a period of seven (7) days; **Group C:** Medicinal plant (*Zingiber officinale* aqueous extract), 1.0 ml (500 mg/L)/L of distilled water for the chicks for period of fourteen (14) days; **Group D:** Probiotics. 1g/L for the chicks (14 days); **Group E:** Vaccination; The first day of age, a dose of 0.2ml/chick and boostered as a second dose at 7 days (0.5 ml/chick) for the chicks; **Group F:** Vaccination plus probiotics i.e. those vaccinated were allowed to feed on probiotic for fourteen (14) days. The experimental chickens were then exposed to the isolates via oral route after 14 days of age. The chickens were carefully monitored for a period of 4 weeks.

Detection of micro agglutination antibody titers in the sera of the broiler chicks after vaccination with locally prepared bacterin (bacteria vaccine): Just before the first dose of the bacterin (zero hour), the chickens were randomly selected and their blood were collected. Also just before the second booster dose of the isolates, another blood were collected. After the second vaccination, the blood samples from the birds were also collected at a period of fourteen (14) days. The blood samples were allowed to separate. The separated sera were used against the isolates for agglutination reaction and the antibody titer against the isolates were determined and recorded. (Wafaa *et al.,* 2012).

Examination of experimented chickens: The administered chickens were carefully observed for the obvious pathological signs of the challenged organisms for period of 4 weeks, the protection rates of inhibitory substances were determined, and the chickens were sacrificed and gross examination of the morphologies of the internal organs and intestines were carried out. Also, the internal organs (Liver, Lung, Spleen and Heart) were harvested and some portions of these organs were cultured on MacConkey agar and *Salmonella-Shigella* agar, and incubated at 37˚C for 24 h for *Salmonella* species and at 44.5˚C for 24 h for *E. coli.* The suspected colonies were identified morphologically and biochemically (Wafaa *et al.,* 2012). The remaining portions of the organ were subjected to histopathological examination (Dashe *et al.,* 2013).

Statistical Analysis

The results of the data generated were expressed as mean ± standard deviation (SD). The statistical analysis of data generated from protective study was carried out using chi-square at 95% confidence limit (Wafaa *et al.,* 2012). The statistical analysis of other valuable data generated from this study was examined using SPSS package program version 20.0. Data were analyzed by one-way Analysis of Variance (ANOVA) to determine the significant difference of the mean values at 95% confidence limit. Pair wise comparison of mean was done by Least Significant Difference (LSD) (Wafaa *et al.,* 2012, Dashe *et al.,* 2013)

RESULTS

Number of Samples that Showed Positive Results: Out of 1536 chicken feed samples collected from major towns located within the twenty-one (21) Local Government Areas of Anambra State, 934 (60.81%) samples were positive to enteric bacteria (Table1).

Characterization and identification of the Isolates from Feed Samples: The morphological characteristics of the isolates are shown in Table 2. Isolates 5, 7 and 11 were isolated from *Salmonella-Shigella* agar (SSA) and they exhibited similar morphological characteristics on SSA plates. In addition, isolates E and G exhibited similar morphological characteristics on MacConkey agar (MA) plates. The isolates were further characterized using their biochemical and molecular characteristics as shown in Tables 3 and 4 respectively.

Micro agglutination Antibody Titers in the Sera of the Broiler Chicks after Vaccination with Locally Prepared Bacterin (Bacteria Vaccine): The result of micro agglutination antibody titres in the sera of broiler chicks after vaccination with locally prepared bacterin is shown in Table 5. On the zero day (before first vaccination dose), the antibody titer values (ATVs) of sera samples collected from the test and control was zero.

On the 7th day (before booster vaccination dose), four-fifth ($^4/_5$) of the chicks vaccinated against *E. coli* O157:H7 SS52, two-fifth ($^2/_5$) of the chicks vaccinated against *S.* ser. Typhimurium U288 and three-fifth ($^3/_5$) of the chicks vaccinated against *S.* ser. Enteritidis FM366 had their maximum ATVs $^1/_{40}$. There was no ATV recorded for non-vaccinated chicks on the 7th day. On the 14th day (before challenge), one-fifth ($^1/_5$) of the chicks vaccinated against *E. coli* O157:H7 SS52 had maximum ATV $^1/_{640}$ while one-fifth ($^1/_5$) recorded maximum of ATV $^1/_{80}$. One-fifth ($^1/_5$) of the chicks vaccinated against *S.* ser. Typhimurium U288 had maximum ATV $^1/_{320}$ while one-fifth ($^1/_5$) had maximum ATV $^1/_{40}$. Two-fifth ($^2/_5$) of the chicks vaccinated against *S.*ser. Enteritidis FM366 had maximum ATVs $^1/_{320}$ while one-fifth ($^1/_5$) had maximum ATV $^1/_{40}$. There was no ATV recorded from non-vaccinated chicks on the 14th day. Also the ATVs against *E. coli* O157:H7 SS52 was slightly higher than ATVs of *S.* ser, Typhimurium U288 and *S.*ser. Enteritidis FM366.

Total Mean Viable Plate Counts of Challenge Isolates from the Internal Organs of Chickens Administered Different Antimicrobial Substances: The total mean viable plate counts of challenge isolates from the internal organs of chickens administered different antimicrobial substances are shown in Tables 6 and 7. The results revealed that there was no visible growth observed in the internal organs of those chickens administered Ciprofloxacin (Cpx), Oxytetracycline (Oxy), Vaccination (Bacterin) and vaccination plus commercially prepared probiotic (PRO). Also, no visible growth was observed in the liver of those chicks administered *Z. officinale* extract (Table 6). The total mean viable plate counts (TMPCs) from the liver of the chicks administered *Z. officinale* extract and PRO against *S.* ser. Typhimurium U288 and *S.* ser. Enteritidis FM366 were significantly ($P<0.05$) lower than the TMPCs from the liver of the infected chicks (Table 7). The study also revealed that the TMPCs from the internal organs administered *Z. officinale* extract were significantly ($P<0.05$) lower than the TMPCs from the internal organs of those chicks administered PRO. There was no visible growth observed in the organs from non-infected (normal) chicks. The inhibitory substances showed more protection to the internal organs of the chicks against *E. coli* O157:H7 SS52 than *Salmonella* species.

Histological Features of Internal Organs of chickens Infected by the Isolates after Oral Administration of Different Antimicrobial Substances: The pathological features of internal organs of chickens infected by test isolates after oral administration of different antimicrobial substances are shown in Tables 8 and 9. The histopathological examination of the internal organs of the protected chickens against the test enteric bacteria revealed that *Z. officinale* extract gave complete protection to the liver and partial protection to the lungs and heart against *E. coli* O157:H7 SS52. Also, partial protection was giving to the liver by *Z. officinale* extract against *Salmonella* species. Vaccination (Bacterin) gave reasonable protection to the liver against *E. coli* O157:H7 SS52 and *S.* ser. Enteritidis FM366, but partial protection to the lungs against *E. coli* O157:H7 SS52. Ciprofloxacin and Oxytetracycline offered reasonable protection to the liver and heart against *E. coli* O157:H7 SS52 and *Salmonella* species, but the lungs of those chickens administered Ciprofloxacin were normal with spotty haemorrhage in the lumen of the air sacs. Commercially prepared probiotic offered reasonable protection to the liver against *E. coli* O157:H7 SS52 but the lungs and heart were not protected against *E. coli* O157:H7 SS52. Also, there was no reasonable protection offered by the commercially prepared probiotic to the liver against *Salmonella* species. Vaccination (Bacterin) plus commercially prepared probiotic offered reasonable protection to the liver, lungs and heart against the enteric bacteria.

Table 1: Types and sources of chicken feed samples that was positive to enteric bacteria

Type of feed	Positive sample (%)	Negative sample (%)	Total (%)
X	294 (57.42)	218(42.58)	512(33.33)
Y	312(60.94)	200(39.06)	512(33.33)
Z	328(64.06)	184(35.94)	512(33.33)
Total	934(60.81)	602(39.19)	1536(99.99)

Table 2: Morphological characteristics of the isolates from chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Appearance on agar plate	Red colony on MA	Red colony on MA	Colourless with black center on SSA	Colourless and dark at the center on SSA	Colourless and dark at the center on SSA
Edge	Entire	Entire	Entire	Entire	Entire
Size (mm)	1.00	1.20	2.20	1.40	1.60
Consistency	Soft	Soft	Soft	Soft	Soft
Optical property	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Slightly raised	Convex	Slightly raised	Slightly raised	Slightly raised
Pigmentation	–	–	–	–	–
Gram Reaction	–	–	–	–	–
Shape	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+

SSA = *Salmonella-Shigella* Agar
MA = MacConkey Agar
+ = Positive – = Negative

Table 3: Characteristics and identities of the enteric isolates from the chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Indole production	+	+	–	–	–
Hydrogen Sulphide	–	–	+	+	+
Ornithine decarboxylase	–	–	–	–	–
Methyl Red	+	+	+	+	+
Voges-Proskauer	–	–	–	–	–
Citrate Utilization	–	–	+	+	+
Catalase	+	+	+	+	+
Urease	–	–	–	–	–
Glucose	+	+	+	+	+
Maltose	+	+	+	+	+
Dulcitol	–	–	+	+	+
Lactose	+	+	–	–	–
Xylose	+	+/–	+/–	+	+
Arabinose	+	+	+	+	–
Inositol	–	–	+	–	–
Mucate	–	–	–	+	+

E – *Escherichia coli* G – *Escherichia coli*
5 – *Salmonella* species 7 –*Salmonella* species
11 – *Salmonella* species + = Positive – = Negative

Table 4: Molecular identities of the isolates

Isolate	Max score	Total score	Query Cover	Gap	Identity	Accession Number	Description
E	2856	2967	100%	0%	100%	CO010304.1	*Escherichia coli* strain 0157:H7 str SS52 Complete genome
G	1297	1297	100%	0%	96%	CP007594.1	*Escherichia coli* strain SEC470 Complete genome
5	2193	4386	100%	0%	98%	CP003836.1	*Salmonella enterica* subsp. enterica serovar Typhimurium str U288 Complete genome
7	660	660	100%	0%	96%	NG03836.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str FM366 Complete genome
11	2844	2844	100%	0%	100%	CP011428.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str YU39 Complete genome

Table 5: Micro agglutination antibody titres in the sera of broiler chicks after vaccination with locally prepared bacterin

Isolate	Day	Interval	Total	Antibody Titers of the chick's serum at different dilutions						
				0		40				
					20		80	160	320	640
	0	BFVD	5	0	0	0	0	0	0	0
EC	7	BBVD	5	0	1	4	0	0	0	0
	14	BC	5	0	0	0	1	1	2	1
	0	BFVD	5	0	0	0	0	0	0	0
ST	7	BBVD	5	0	3	2	0	0	0	0
	14	BC	5	0	0	1	2	2	1	0
	0	BFVD	5	0	0	0	0	0	0	0
SE	7	BBVD	5	0	2	3	0	0	0	0
	14	BC	5	0	0	1	1	1	2	0
	0	BFVD	5	0	0	0	0	0	0	0
C	7	BBVD	5	0	0	0	0	0	0	0
	14	BC	5	0	0	0	0	0	0	0

EC — *E. coli*O157:H7 SS52, ST — S. ser. Typhimurium U288, SE — S.ser. Enteritidis FM366, C — Control, BFVD — before First Vaccination Dose, BBVD — Before Booster Vaccination Dose, BC — Before Challenge

Table 6: Total mean viable plate counts of *E. coli* O157:H7 SS52 re-isolated from the internal organs of the chicks administered different antimicrobial substances

Protection	Liver ($x10^8$CFU/g)	Lungs ($x10^8$CFU/g)	Heart ($x10^8$CFU/g)
ZO	0.00 ± 0.00	4.00 ± 1.73	6.00 ± 1.53
Pro	0.00 ± 0.00	7.00 ± 1.53	11.00 ± 1.73
Cpx	0.00 ± 0.00	1.00 ± 0.00	0.0 ± 0.00
Vac	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00
Vac + Pro	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C_1	28.00 ± 3.61	24.00 ± 3.61	39.00 ± 4.58
C_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

ZO — *Zingiber officinale,* Pro — commercially prepared probiotic, Cpx — Ciprofloxacin, Vac — Vaccination, C_1 — Infected chicken without protection, C_2 — Normal chicken

Table 7: Total mean viable plate counts of *Salmonella* species re-isolated from the liver of the chickens administered different antimicrobial substances

Protection	ST ($x10^8$CFU/g)	SE ($x10^8$CFU/g)
ZO	7.00 ± 1.00	4.00 ± 1.73
Pro	11.00 ± 2.65	14.00 ± 2.00
Oxy	0.00 ± 0.00	0.00 ± 0.00
Vac	0.00 ± 0.00	0.00 ± 0.00
Vac + Pro	0.00 ± 0.00	0.00 ± 0.00
C_1	36.00 ± 4.63	43.00 ± 1.73
C_2	0.00 ± 0.00	0.00 ± 0.00

ZO — *Zingiber officinale,* Pro — commercially prepared probiotic,
Cpx — Ciprofloxacin, Vac — Vaccination, C_1 — Infected chicken without protection,
C_2 — Normal chicken
ST------- *S.* ser. Typhimurium U288 SE---------- *S.* ser. Enteritidis FM366

Table 8: Histological features of internal organs of chicks infected by *E.coli* O157:H7 SS52 after oral administration of different antimicrobial substances

Protection	Liver	Lungs	Heart
Zingiber officinale	Prominent central vein	Multifocal necrosis and congestion of portal artery	Slight alteration of the heart architecture and disintegration of muscle fibres
Vaccination (Bacterin)	Prominent central vein with infiltration of mononuclear cells (MNC)	Congestion and distention of the blood vessels, and filling of alveoli with mild blood	Mild focal congestion with infiltration of mononuclear cells
Ciprofloxacin	Prominent central vein and multifocal necrosis	The lungs are intact with intact bronchioles and spotty haemorrhage in the lumen of the air sacs	High degree of proliferation of mononuclear cells around interstices
Commercially synthesized probiotic	Intact parenchymal cells and some necrosis	Blood congestion and oedema in the alveoli	Distortion of the oblique fibres
Vaccination plus Probiotic	Prominent central vein and multifocal necrosis	Intact bronchioles and the lumen of the air sacs	Mild focal congestion and mild infiltration of mononuclear cells
Control	Normal liver morphology with prominent central vein	Normal lungs with intact bronchioles and alveoli	Normal heart morphology with intact myofibres and oblique fibres

Table 9: Histological features of internal organs of chicks infected by *Salmonella* species after oral administration of different antimicrobial substances

Protection	***S.*ser. Typhimurium U288**	***S.*ser. Enteritidis FM366**
Liver (ZO)	Massive congestion of central vein and sub massive necrosis	Slight congestion of central vein, slight necrosis and loss of hepatocyutes
Liver (Vac)	Congestion of central vein	Prominent central vein with infiltration of mononuclear cells
Liver (Oxy)	Mild infiltration of mononuclear cells in the central vein	Slight congestion of the central vein with mononuclear cells infiltrates
Liver (Pro)	Enlargement of sinusoids with deposit of granules	Sub massive necrosis and infiltration of red blood cells in the liver
Liver (Vac + Pro)	Prominent central vein and non-prominent multifocal necrosis	Vacoulation of hepatocytes though the liver appears normal
Liver (control)	Normal morphology	Normal morphology

ZO — *Zingiber officinale*extract, Pro — commercially synthesized probiotic,
Oxy — Oxytetracycline, Vac — Vaccination (Bacterin), Vac + Pro — Vaccination plus commercially synthesized probiotic

DISCUSSION

The presence of enteric bacteria in the feed samples could be traced from the management practices of mill, dust, feed ingredients, and transportation of the feeds, poor handling and sanitary conditions attributed to the feed samples. Similar findings were reported by many researchers (Immersed *et al.,* 2002; Jones and Richardson, 2004; Alshawabkeh, 2006; Maciorowski *et al.,* 2007). Researchers had shown that animal housing and transportation of equipments can also harbour enteric bacteria and this contributes to the contamination of chicken feeds (Primm, 2008). Maciorowki *et al.* (2007) also stated that the high prevalence and high populations of enteric bacteria in animal wastes was evidence that manure could be a principal source of enteric pathogens to chicken industry. Chicken feeds contaminated by enteric bacteria pathogenic to humans can contribute to human food-borne illness through the feed-food-human chain. This shows that the production of chicken feeds requires microbiological safety regulations to escape microbial contamination of the product. Similar deduction was drawn by different researchers (Davies and Wales, 2010; Chowdhuri *et al.,* 2011; Fredrick and Huda, 2011).

Reasonable antibody titer values were generated after the 14th day (before challenge). Barrow and Lovell (2001) and Olabisi and Peter (2008) reported on production of high level of serum IgG after oral inoculation of *S.* serovar Enteritidis in layer chickens. Other researchers also reported the enhancement of immune response against *S.* serovar Enteritidis infected birds through vaccination using locally prepared bacterin (Methner and Steinbach, 2007; Okamura *et al.,* 2004; Davies and Breslin, 2004; Pakpinyo *et al.,* 2008).

The absence of growth observed in the internal organs administered ciprofloxacin, Oxytetracycline, vaccination and vaccination plus commercially prepared probiotics supports the findings of Wafaa *et al.* (2012). Several researchers have documented that the frequency of enteric bacteria re-isolation from the internal organs was significantly reduced in protected chickens (Khan *et al.,* 2003; Okamura *et al.,* 2005; Radwan *et al.,* 2007). Penha *et al.* (2009) and Priyantha (2009) found that vaccination of chickens with bacterin induced significant reduction of organ colonization after re-infection of the chickens.

The reducing effect of probiotics on the colonization of enteric bacteria was studied comprehensively by several researchers. It was reported that probiotics maintained or increased the normal intestinal flora which are normally found in the intestinal tract of hatched chicken and these flora can exclude enteric bacteria colonization (Mead, 2000; Seo *et al.,* 2000; Wafaa *et al.,* 2012). The absence of visible growth of enteric bacteria 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 protection rate of vaccination plus commercially prepared probiotics was maximum against the enteric bacteria studied. Feberwee *et al.* (2001) has proved that enteric bacterin was highly protective for broiler or layer chickens when using clinical signs, mortalities and post-mortem lesions as criteria for measuring the protective index.

The maximum protection achieved by vaccinating those chickens 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 unfavourable P^H for the growth of the enteric bacteria pathogens (Alkoms *et al.,* 2000; Johansen *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 probiotic on the immune response indicates the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The direct effect might be related to the stimulation of lymphatic tissue (Kabir *et al.,* 2004), whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract or through the reduction of enteric bacteria 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

This study has revealed the presence of *Escherichia coli*O157:H7 SS52*, E. coli* SEC470 *Salmonella* serovar Typhimurium U288, *Salmonella* serovar Enteritidis FM366 and *Salmonella* Enteritidis YU39 in the chicken feed samples, of which *E. coli* SEC470 and S. serovar Enteritidis YU39 recorded very low counts from the studied samples. The *in vivo* study of the susceptibility patterns of these organisms to both natural and synthetic antibiotics showed safe and pronounced activities of *Zingiber officinale* extracts, ciprofloxacin, oxytetracycline and locally prepared *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 bacterins (vaccination), but the bacterins together with commercially prepared probiotics proved to be most effective. From this study, it could be concluded that the use of locally prepared bacterins in double doses together with probiotic preparation are most effective and safe methods of preventing *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 infections in chickens. It should be taken into consideration that the bacterins together with probiotics must go in parallel with bio-security measures and good management practices to eradicate *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 infections in chicken flocks.

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