The Effect of *Bacillus Subtilis* Spores - Based Probiotic and Florfenicol on the Colonization of *Salmonella Enteritidis* in Intestine of the Broilers

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Abstract: The current study investigated the effect of dietary supplementation of *Bacillus* spores based probiotic and drinking water supplementation of florfenicol on *Salmonella enteritidis* colonization in the intestine of broiler chicks, also recorded the clinical signs which appeared during the experiment. A total of 220 broiler chicks (one day old, Ross) free from *Salmonella* infection divided into 5 equal groups, the broilers were then subjected to the following treatments:1st group was given *Salmonella enteritidis*, *Bacillus* spores based probiotic and florfenicol, 2nd group was given *Salmonella enteritidis* and florfenicol, 3rd group was given *Salmonella enteritidis* and *Bacillus* spores based probiotic. 4th group was given *Salmonella enteritidis* only and used as positive control group. 5th group was given neither *Salmonella enteritidis* nor treatments and used as negative control group. The statistical analysis for results by Duncan multiple range test and ANOVA test showed that, there was a significant difference at $p<0.05$ between groups using *Bacillus* spores based probiotic, florfenicol, combination of both treatments and positive control group in colony counts of *Salmonella enteritidis* in intestine of broiler chicks. Furthermore, the results showed that broiler chicks fed with probiotic supplements had a minimal viable colony count of *Salmonella enteritidis* bacteria in the intestinal tract. *Bacillus* spores based probiotic fed birds was able to resist *Salmonella enteritidis* infection with few mild clinical signs. Improved resistance to other bacterial disease is expected from the supplementation of *Bacillus* spores based probiotic in the formulation of feeds for broilers.
[M.M. Hashem; A. Ramdan, H.Y. El Zorba Soad A. Abd El Wanis and A.M. El Mahdy **Antigenotoxic The Effect of *Bacillus Subtilis* Spores - Based Probiotic and Florfenicol on the Colonization of *Salmonella Enteritidis* in Intestine of the Broilers.** Researcher. 2012;4(2):77-85]. (ISSN: 1553-9865). http://www.sciencepub.net. 15

Key Words: *Bacillus* spores based probiotic, florfenicol, *Salmonella enteritidis,* broiler chicks.

1. Introduction

The poultry industry has developed in several areas such as nutrition, genetics, and management to maximizing the efficiency of growth performance and meat yield. However, nowadays, the poultry industry has focus more attention towards addressing public concern for environmental and food safety. Animals including poultry are vulnerable to potentially pathogenic microorganisms such as *Escherichia coli, Salmonella sp., Clostridium perfringens and Campylobacter sputorum.* pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to de-conjugating effects of bile acids (Engberg et al., 2000), this leads to depressed growth performance and to increased incidence of disease. Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathology (Waldroup et al., 1985). Their usefulness has seldom been contested, it is their relatedness with similar antibiotics used in human medicine and the possibility that their use may contribute to the pool of antibiotic resistant bacteria that causes concerns (Philips, 1999). In the light of that situation, the feed manufacturers and the animal growers have been actively looking to an efficacious alternative to antibiotic. Probiotics are the most promising alternative to antibiotics. Probiotics are viable microbial additives which assist in the establishment of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes (Green and Sainsbury, 2001). Probiotics can be comprised of a single or multiple bacterial species, generally containing species of *Bacillus, bifidobacterium, enterococcus, E. coli, Lactobacillus, Lactococcus, Streptococcus,* and a variety of yeast species (Patterson and Burkolder, 2003).

Probiotics should have certain characteristics to be beneficial as a direct-fed microbial. For instance, the probiotic should not be hydrolyzed or absorbed by enzymes or tissues of the host. Probiotics should have the ability to selectively enrich for one or a limited number of intestinal bacteria that are beneficial to the host. Furthermore, probiotics should be able to beneficially alter the activities of the intestinal micro biota. Finally, probiotics should beneficially stimulate the host's immune system (Simmering et al., 2001).

Florfenicol, a structural analogue of thiamphenicol possessing a wide spectrum of activity against both gram positive and gram negative bacteria including *Pasturilla*, *Salmonella, E coli* and *Staphylococcus aureus*.

The mechanism of antibacterial activity of florfenicol is similar to that of chloramphenicol and thiamphenicol, through inhibition of bacterial protein synthesis at the 50s ribosomal subunit (Syriopoulou et al., 1981 and Cannon et al., 1990).

Most serotypes of *Salmonella* are placed in the paratyphoid group which is described as the *Salmonella* serotypes other than *S. pullorum, S. gallinarum,* and *S. Arizona* (Ashton, 1990). *Salmonella enteritidis* (SE) has classified as one of the paratyphoid group. Paratyphoid infections have received significant attention over the last decade because poultry constitute a significant source of *Salmonella* that can induce food-borne illness in humans (St. Louis et al., 1988).

This study aims are to evaluate the antimicrobial effect of *Bacillus* based probiotics in comparison with the antimicrobial effect of florfenicol against *Salmonella enteritidis* in the intestine of broiler chicks. In addition too, record the clinical signs on the chicks in different groups during the experiment.

2. Materials and Methods

A) Materials

Bacillus Spores Based Probiotics

Bacillus subtilis 4×10^9 CFU/g (brand name *Gallipro* strain deposit no. DSM 17299 .the probiotic was obtained in 5 kg multi-layered paper bags with plastic insert from Biochem Misr ltd, Heliopolis, Cairo, Egypt).

Florfenicol

florfenicol (chemical formula $c_{12}h_{14}cl_2fno_4s$), is the fluorinated derivative of thiamphenicol, in which the hydroxyl group at c-3 has been replaced with fluorine (the drug was obtained as *banflor* brand name from Marcyrl pharmaceutical industries, Egypt, Obour city. Each 100 ml of the drug contain 10 gram florfenicol in a concentration of 10% florfenicol).

Salmonella Enteritidis

Reference strain of *Salmonella enteritidis* ATCC 13076.(Mast company ,UK)

Chickens

Two hundred and twenty apparently healthy broiler one day old Ross chicks were used in this experiment obtained from El-Wadi Company for Poultry.

Ration

Chicks fed on commercial balanced ration free from any pathogen or medicinal additives obtained from Al Qahira Company for poultry.

B) Methods

Experimental Design

At the beginning 20 (1 day old) chicks, a reprehensive sample from the used ration and wheat straw which used as bedding will be examined to make sure that the chicks, ration and bedding are free from *Salmonella* pathogen, the rest of the chicks will be divided into equal groups each group contain 40 chicks (n=40).

The chicks in the first group were received florfenicol from first day of life (10% florfenicol) continuously in drinking water in a dose 15 mg/kg b.wt. (Shen et al , 2003), probiotic (*Bacillus subtilis* 4×10^9) continuously in ration in a dose 200 gm/1 ton (Mokhtari et al , 2010) and the dose of *Salmonella Enteritidis* (10×10^6) in a dose 0.1 cm^3 (Ishola and Holt ,2008) given orally for each chick after 48 hours from administration of the previous 2 treatments.

The chicks in the second group were received florfenicol from first day of life (10% florfenicol) continuously in drinking water in a dose 15 mg/kg b.wt. and *Salmonella Enteritidis* (10×10^6) in a dose 0.1 cm^3 which given orally for each chick after 48 hours from administration of florfenicol .

The chicks in the third group were received probiotic from first day of life (*Bacillus subtilis* 4×10^9) continuously in ration in a dose 200 gm/1 ton and *Salmonella Enteritidis* (10×10^6) in a dose of 0.1 cm^3 which given orally for each chick after 48 hours from administration of the probiotic.

The chicks in the fourth group (standard group) were received *Salmonella Enteritidis* ($10\times10^{6)}$ in a dose 0.1 cm^3 given orally for each chick.

The fifth group (control group) were received neither treatments nor *Salmonella Enteritidis.*

5 chicks from each group were slaughtered at the 6, 9, 12,15and 18 for enumeration of *Salmonella enteritidis*.

The clinical signs were recorded for each group during the first 18^{th} day of the experiment.

Analytical Procedure

***Salmonella Enteritidis* infective dose preparation**

It was carried out according to Ishola and Holt, (2008).

***Salmonella Enteritidis* detection**

It was carried out according to ISO 6579, (2002).

***Salmonella Enteritidis* Enumeration:**

It was carried out according to Thushani et al., (2003).

Statistics analysis:

All data were statistically analyzed using ANOVA test and Duncan Multiple Range test

3. Results

Bacillus subtilis spores based probiotic, florfenicol in a dose of 200g/ton feed and 15 mg/kg b.wt. respectively and combination were given orally for 18 days where *salmonella* colony count in the caecum of the chicks at different groups was carried out.

Oral administration of *Bacillus subtilis* spores based probiotic in a dose of 200g/ton feed decrease total *Salmonella* colony count in the caecum of chicks in group 3 compared to positive control group. (Table 1 and 2)

Oral administration of florfenicol in a dose of 15 mg/kg b.wt. decrease total *Salmonella* colony count in the caecum of chicks in group 2 compared to positive control group. (Table 1 and 3)

Oral administration of *Bacillus subtilis* spores based probiotic and florfenicol in a dose of 200g/ton feed and 15 mg/kg b.wt. respectively decrease total *Salmonella* colony count in the caecum of chicks in group 1 compared to positive control group . (Table 1 and 4).

Table 1: Results of *Salmonella enteritidis* colony count at 6, 9,12,15 and 18 days of age in caecum of chicks infected with *S.enteritidis* in oral dose o.1 cm^3/chick at 3rd day of life & not treated (Group 4) (n=5).

	Day6	Day9	Day12	Day15	Day18
Minimum	$1.5*10^8$	$1.3*10^9$	$3.8*10^{10}$	$1*10^8$	$1.7*10^9$
Maximum	$2.6*10^8$	$2.9*10^9$	$5.6*10^{10}$	$2.1*10^8$	$3.1*10^9$
Mean	$2.06*10^8$	$2*10^9$	$4.7*10^{10}$	$1.6*10^8$	$2.3*10^9$
Standard Error	$0.229*10^8$	$0.268*10^9$	$0.346*10^{10}$	$0.207*10^8$	$0.251*10^9$

Table 2: Results of *Salmonella enteritidis* colony count at 6, 9,12,15 and 18 days of age in caecum of chicks infected with *S.enteritidis* in oral dose o.1 cm^3/chick at 3rd day of life & treated with probiotic in daily dose of 200G/ton feed from day one till day 18 (Group 3) (n=5).

	Day6	Day9	Day12	Day15	Day18
Minimum	$6.7*10^9$	$5.2*10^{10}$	$2.3*10^7$	$1.7*10^5$	$1.2*10^4$
Maximum	$9*10^9$	$7.6*10^{10}$	$4.5*10^7$	$3.7*10^5$	$2.7*10^4$
Mean	$7.9*10^9$	$6.3*10^{10}$	$2*10^7$	$2.8*10^5$	$1.8*10^4$
Standard Error	$0.445*10^9$	$0.490*10^{10}$	$0.288*10^7$	$0.373*10^5$	$0.270*10^4$

Table 3: Results of *Salmonella enteritidis* colony count at 6, 9, 12, 15 and 18 days of age in caecum of chicks infected with *S.enteritidis* in oral dose o.1 cm^3/chick at 3rd day of life & treated with florfenicol in daily dose of 15mg/kg b.wt. from day one till day 18 (Group 2) (n=5).

	Day6	Day9	Day12	Day15	Day18
Minimum	$1*10^6$	$1*10^7$	$2.3*10^8$	$1.6*10^7$	$1.6*10^3$
Maximum	$1.2*10^6$	$1.2*10^7$	$4.5*10^8$	$2.6*10^7$	$4.4*10^3$
Mean	$1.1*10^6$	$1.1*10^7$	$3.3*10^8$	$2.1*10^7$	$3*10^3$
Standard Error	$0.045*10^6$	$0.024*10^7$	$0.409*10^8$	$0.179*10^7$	$0.489*10^3$

Table 4: Results of *Salmonella enteritidis* colony count at 6, 9, 12, 15 and 18 days of age in caecum of chicks infected with *S.enteritidis* in oral dose o.1 cm^3/chick at 3rd day of life & treated with combination of probiotic and florfenicol in daily dose of 200G/ton feed and 15mg/kg b.wt. respectively from day one till day 18 (Group 1) (n=5).

	Day6	Day9	Day12	Day15	Day18
Minimum	$1*10^{10}$	$2.4*10^{11}$	$1*10^7$	$1.2*10^6$	$1*10^4$
Maximum	$1.4*10^{10}$	$4.2*10^{11}$	$1.2*10^7$	$3.1*10^6$	$1.2*10^4$
Mean	$1.2*10^{10}$	$3.4*10^{11}$	$1.1*10^7$	$2*10^6$	$1.1*10^4$
Standard Error	$0.071*10^{10}$	$0.329*10^{11}$	$0.045*10^7$	$0.332*10^6$	$0.045*10^4$

Statistical analysis for results

The state analytical result using ANOVA test at $p<0.05$ significant value, Duncan multiple range test at $p<0.05$ was showing that there were significant difference in group 4 (control) between each of day 6, day 9, and day 12, on the other hand there were significant difference between day 6 and day 18, while there were no significant difference between day6 and day 15 within the same group.

In group 2, group 3 and group 1 there were significance difference between day 6, day 9, day 12, day 15 and day 18 within each group. It may be attributed to the different treatments used in these groups (table 5).

It was shown in the same table that there was significant differences between control group (g4) and treated ones within day 6, day 12, day 15 and day 18 with 2 exceptions at day 12 between group 4 (control).

Furhternore group 3, between group 2, group 3, group 1 at day 18 there were no significant difference within the fore mentioned groups.

Table 5: Statistical analytical results of *Salmonella enteritidis* count of the experimentally infected and treated chicks (Group 3, 2 and 1) and infected and none treated chicks (Group 4) at day 6, 9,12,15 and 18 of age (mean±SE,n=5)

Group / Day	Group 4	Group 3	Group 2	Group 1
Day 6	$2.06 \times 10^8 \pm 0.229 \times 10^8$ a	$7.90 \times 10^9 \pm 0.445 \times 10^9$ d	$1.10 \times 10^6 \pm 0.045 \times 10^6$ b	$1.20 \times 10^{10} \pm 0.071 \times 10^{10}$ d
Day 9	$2.00 \times 10^9 \pm 0.268 \times 10^9$ b	$6.30 \times 10^{10} \pm 0.490 \times 10^{10}$ e	$1.10 \times 10^7 \pm 0.024 \times 10^7$ c	$3.40 \times 10^{11} \pm 0.329 \times 10^{11}$ e
Day 12	$4.70 \times 10^{10} \pm 0.346 \times 10^{10}$ c	$2.00 \times 10^7 \pm 0.288 \times 10^7$ c	$3.30 \times 10^8 \pm 0.409 \times 10^8$ d	$1.10 \times 10^7 \pm 0.045 \times 10^7$ b
Day 15	$1.60 \times 10^8 \pm 0.207 \times 10^{8\,a}$	$2.80 \times 10^5 \pm 0.373 \times 10^5$ b	$2.10 \times 10^7 \pm 0.179 \times 10^7$ c	$2.00 \times 10^6 \pm 0.332 \times 10^6$ c
Day 18	$2.30 \times 10^9 \pm 0.251 \times 10^9$ b	$1.80 \times 10^4 \pm 0.270 \times 10^4$ a	$3.00 \times 10^3 \pm 0.489 \times 10^3$ a	$1.10 \times 10^4 \pm 0.045 \times 10^4$ a
F-Calculated	169.542#	124.687#	141.613#	121.34#

Significant at $P < 0.05$ using ANOVA test a, b, c, d insignificant difference between similar litters using Duncan Multiple Range test at $P < 0.05$

Clinical signs

Table 6: Displayed clinical signs in chicks at different groups of the experiment.

	Group 1 Treated with both drugs	**Group 2** Treated with florfenicol	**Group 3** Treated with probiotic	**Group 4** Infected, not treated	**Group 5** Not infected, not treated)
Ruffled feathers	+ve	+ve	+ve	+ve	-ve
Emaciation	-ve	-ve	-ve	+ve	-ve
Lameness	-ve	+ve	-ve	+ve	-ve
Drowsy	-ve	-ve	-ve	+ve	-ve
Dropping wings	-ve	-ve	-ve	+ve	-ve
Somnolence Closed eye	-ve	-ve	-ve	+ve	-ve
Anorexia	-ve	-ve	-ve	+ve	-ve
Profuse watery diarrhea	+ve	+ve	+ve	+ve	-ve
Dehydration	-ve	-ve	-ve	+ve	-ve
Pasting of the vent	+ve	+ve	+ve	+ve	-ve

Figure 1: Chicks not infected nor treated {group5} show no clinical signs

Figure 2: Chicks infected with *Salmonella enteritidis* and not treated {group 4} show profuse watery diarrhea

Figure 3: Chicks infected with *Salmonella enteritidis* and treated with Bacillus spores based pribiotic {group 3} show profuse watery

Figure 4: Chicks infected with *Salmonella enteritidis* and treated with florfenicol {group 2} show profuse watery diarrhea

Figure 5: chicks infected with *Salmonella enteritidis* and treated with both treatments {group 1} show profuse watery diarrhea

4. Discussion

The *Salmonella enteritidis* count mean values in caecum of chicks in group 4 (infected with *Salmonella enteritidis*) at day 6, day 9, day 12, day 15, and day 18 were 2.06×10^{8}CFU±0.229×10^{8} CFU, 2×10^{9} CFU±0.268×10^{9} CFU, 4.7×10^{10} CFU±0.346×10^{10} CFU , 1.6×10^{8} CFU±0.207×10^{8} CFU, 2.3×10^{9} CFU±0.251×10^{9} CFU respectively. (Table 1)

The *Salmonella enteritidis* count in infected and treated chicks with probiotic (group 3) were recorded in table 2 it was revealed that at the 6th day the mean values of *Salmonella* count was 7.9×10^{9} CFU±0.445×10^{9} CFU with a minimum value 6.7×10^{9} CFU and maximum value 9×10^{9} CFU. (Table 2)

The *Salmonella enteritidis* count mean values in caecum of chicks in group 2 (infected with *Salmonella enteritidis* and treated with florfenicol) at day 6, day 9, day 12, day 15, and day 18 were 1.1×10^{6} CFU ±0.045×10^{6} CFU, 1.1×10^{7}

CFU±0.024×10^7 CFU, 3.3×10^8 CFU±0.409×10^8 CFU, 2.1×10^7 CFU±0.179×10^7 CFU, 3×10^3 CFU±0.489×10^3 CFU respectively. (Table 3)

The highest *Salmonella enteritidis* mean count of the infected and treated chicks with combination of both treatments (g1) was recorded at 9th day 3.4×10^{11} CFU ±0.329×10^{11} CFU with a minimum value 2.4×10^{11} CFU and maximum value 4.2×10^{11}CFU while the lowest mean count of *Salmonella* of the same group was recorded in the 18th (1.1×10^4 CFU ±0.045×10^4 CFU with a minimum value 1×10^4 CFU and maximum value1.2×10^4 CFU .The day 12 and day 15 showed relatively same mean count ranged between 10^7-10^6 CFU. (Table 4)

Table 6 illustrated the typical clinical signs of chicken infected with ***Salmonella enteritidis*** which were ruffled feathers , emaciation , lameness , drowsy ,dropping wings , somnolence ,closed eye , anorexia ,profuse watery diarrhea , dehydration ,and pasting of the vent (group 4),

It was shown negative clinical signs in group 5 which was neither infected nor treated; it was shown that the profuse watery diarrhea was recorded in all groups under study (group 4, group3, group2 and group1).

The florfenicol treatment succeeded to eliminate some of clinical signs as emaciation, drowsy, dropping wings, somnolence, closed eye, anorexia and dehydration.

It was noticed that group 3 (treated with probiotic) and group 1 (treated with both treatments) failed to eliminate ruffled feathers, profuse watery diarrhea and pasting of the vent from the typical form of clinical signs illustrated in group 4.

Vanderhoof (2001) review the concept of probiotics as a viable therapeutic modality in the treatment of gastrointestinal disease. The antibiotics used for the hope of growth stimulation affect the gut micro flora, which results in the reduction of the resistance to infection caused by certain bacteria. Sub-therapeutic antibiotics not only influence intestinal microbial populations and activities but also affect animal metabolism and specifically alter intestinal function (Anderson et al., 2000).

It was recognized that there was in chicken a natural resistant to *Salmonella* infection developed with the establishment of a mature intestinal flora.

The significance of normal indigenous intestinal micro-flora, especially the anaerobes, in protecting the host against pathogenic transient bacteria such as *Salmonella typhimurium* was demonstrated in mice as well as with *Vibrio cholera* in guinea pigs .the caecal cultures inhibited the growth of *Salmonella typhimurium in vitro*, and they forecasted the use of these cultures as a preventive *in vivo* treatment (Royal and Mutimer, 1972).

Nurmi and Rantala (1973) introduced the method of "competitive exclusion" (ce) to increase the resistance of young chicks to *Salmonella* infection by inoculating them orally with intestinal content from adult birds. They demonstrated that orally inoculation of 1–2 day old chicks with a 1:10 dilution of normal intestinal contents from healthy adult birds one day prior to oral challenge with *S. infantis* resulted in 77 % of birds free from infection. This study was the basis for further development of the competitive exclusion methods.

Successful probiotic colonization depends on the survival and stability of the probiotic strain, specificity of the strain relative to host, dose and frequency of administration, health and nutritional status of the host, effect of age, stress and genetics of the host (Mason et al., 2005).

In this study the presence of the probiotic inhibit *Salmonella* and disturbance of the intestinal micro biota with the antibiotics can increase susceptibility to infection but addition of probiotic increase resistance to infection this result agreed with those reported by (Stavric and Kornegay, 1995 and Rolfe, 2000).

Concerning the antibiotics or generally the antimicrobial character or antimicrobial activity of bioactive compounds produced by *Bacillus* species .there were full discussion reviewed by (Stein, 2005 and Mobolaji, 2009) among the genus *Bacillus, B. subtilis* produces a broad spectrum of bioactive lipopeptides which have a great potential for biotechnological and biopharmaceutical applications. The characteristic structure of lipopeptides is a fatty acid combined with an amino-acid moiety. Several lipopeptides have potent antibiotic activity and have been the subject of several studies on the discovery of new antibiotics. The list includes surfactin, produced by *B. subtilis,* the most powerful biosurfactant known to date (Peypoux et al., 1999). these compounds have many pharmacological activities: antibacterial, antifungal, antiviral, and antimycoplasma properties; inhibition of the fibrin clot formation and hemolysis (Cameotra and Makkar ., 2004); formation of ion channels in lipid bilayer membranes (Sheppard et al ., 1997); antitumour activity against ehrlich's ascites carcinoma cells (Cameotra and Makkar ., 2004); and inhibition of the cyclic adenosine 3,5-monophosphate phosphodiesterase. This explain the reduction of *Salmonella enteritidis* count through out the study after addition of *Bacillus subtilis* as probiotic feed additives and this result agreed with that reported by (Monteiro et al ., 2005) .

the genus *Bacillus* encompasses a number of bacteriocinogenic species, such as *B. subtilis* which produces subtilin, (Jansen and Hirschmann, 1944) and subtilosin (Zheng and Slavik, 1999), the

objective of this study is to use *Bacillus subtilis* as probiotic; to get the potential antimicrobial activity of a bioactive compound produced by *Bacillus subtilis* as mentioned above; was achieved in reduction of *Salmonellae* count in caecum of experimental chicks.

Florfenicol resistance has emerged over the past few years in multidrug-resistant *Salmonella enterica* serovars typhimurium, mainly of definitive phage type (dt) 104, agona and paratyphi b (Boyd et al., 2001 and Cloeckaert et al., 2000) the flor gene, responsible for florfenicol resistance, showed 97% identity to pp-*flo,* and was located in these serovars within a chromosomal cluster of antibiotic resistance genes as part of a genomic island of 43 kb called

sgi1 *(Salmonella* genomic island 1) (Boyd et al., 2001) other antibiotic resistance genes accounted for the remaining resistances of the multidrug-resistance pattern, i.e. resistance to ampicillin, streptomycin spectinomycin, tetracyclines and sulphonamides. Florfenicol resistance conferred by flor*,* located either on plasmids or on the chromosome, has also been reported in *Escherichia coli* strains isolated from cattle and poultry (Cloeckaert et al., 2000 and White et al., 2000) a flor gene variant (95% nucleotide identity) was recently identified on plasmid r55 from *Klebsiella pneumoniae,* initially described in the 1970s as conferring non-enzymic chloramphenicol resistance, thus suggesting that spread of the flor gene may have occurred a long time before the introduction of florfenicol (Cloeckaert et al., 2001) spread of florfenicol-resistant strains may have occurred prior to the introduction of florfenicol, through the use of chloramphenicol or other unrelated antibiotics, and selection of strains where flor is associated with other antibiotic resistance genes, either on plasmids or on the chromosome, as in the case of multidrug-resistant *S. enterica* serovar typhimurium dt 104.

The formentioned data about the resistance of florfenicol explained the significance decrease in *Salmonella* mean count from day 6 to day 18, the presence of few counts at day 18 (3×10^3 CFU±0.489×10^3 CFU) compared with (2.3×10^9 CFU±0.251×10^9 CFU) at the same day for control group infected non treated with florfenicol may be attributed to the resistance of experimentally used *Salmonella enteritidis* which fully discussed previously and this agreed with that reported by (Meunier et al., 2003).

So in this study the *Bacillus subtilis* was used as alternatives probiotic in experimentally farmed organic chickens this is agreed with (Al-sultan, 2003; Wekhe et al., 2007; Ghazala and Ali, 2008).

Conclusion

The present research confirmed (1) the usage of the bacillus spores based probiotics in the ration of the experimental chicks from day 1 reduce the severity degree of clinical signs and decrease the salmonella enteritidis count in the caecum in comparison with standard group (group 4) (2) the usage of the florfenicol in drinking water from day 1 reduce the severity degree of clinical signs and decrease the salmonella enteritidis count in the caecum in comparison with standard group(group 4)

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