Effect of Synbiotic on immune response of experimentally infected broiler chickens with *E.coli* and salmonella

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ABSTRACT

A total of 100 diseased living chickens were collected from different farms in Gharbia Governorate and were subjected to clinical and postmortem examination as well as for isolation and identification of *E. coli* and salmonella from tissue samples including spleen, heart, liver, intestine and lungs . The predominant isolates were *Salmonella Entertidis* and *E.coli O78*.An experiment was conducted on 120 one day old chicks (avian 48) then divided into 6 groups to evaluate the effect of supplementation of20g /1000 bird/day in drinking water Synbiotic (Poultry star®)on immune response of normal and experimentally infected chicks with salmonella and *E.coli* . First group is acontrol negative group (G1), second group Synbiotic supplemented group (G2), third group infected with *Salmonella Entertidis* (G3) , fourth group infected with *Salmonella Entertidis* and supplemented with Synbiotic (G4), fifth group infected with *E.coli O78* (G5) and the last group infected with *E.coli O78* and supplemented with Synbiotic (G6). Significant decrease in phagocytic activity , phagocytic index and weight of bursa of Fabricius, thymus and spleen in infected groups were observed, but significant increase in HI titer, phagocytic activity , phagocytic index and weight of bursa of Fabricius,thymus and spleen in Synbiotic supplemented groups were observed .

KEYWORDS: Broilers, Synbiotic, Salmonella. *E.coli*. Immune response.

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1. INTRODUCTION

Enteric disorders are one of the most important groups of diseases that affect poultry and cause high economic losses in many areas worldwide due to increased mortality rates, decreased weight gain, increased medication costs, and increased feed conversion rates (Hafez, 2011). Salmonella species and *E.coli* are the two most important food-borne pathogens of public health interest incriminated in poultry meat worldwide (Adeyanju and Ishola, 2014). Therefore, for salmonella and *E.coli* control, there were a wide range of antimicrobials but antimicrobial resistance has emerged as a global public health problem in recent years (Harrison & Lederberg, 1998). For that, there is a worldwide attempt to reduce antibiotic usage, because of its residues in meat, development of resistant bacteria, and imbalance of normal microflora (Sorum & Sunde, 2001). So that, it was important to find alternatives to antibiotics. Among the many purported alternatives to the use of antibiotics are the incorporation of either probiotics, prebiotics or Synbiotic into feed and/or drinking water. Probiotics had antagonistic effect through secretion of substance that inhibit the growth and development of pathogenic bacteria (Mazmanian et al., 2008). Prebiotics exerts its effect by binding to pathogens in the intestinal lumen and therefore block the adhesion of those bacteria to the epithelial cells (Spring et al., 2000). Synbiotic stimulate beneficial bacteria and improve the health of gut (Elijah and Ruth, 2012). Therefore, the aim of this work is to evaluate the influence of supplementation of commercial Synbiotic, for 35 days old

experimentally infected broiler chickens with salmonella and *E.coli* on immune response and immune organs.

2. MATERIAL AND METHODS

2.1.Samples collection

A total of 100 (10-30 days old) diseased living chickens were collected from different farms in Gharbia Governorate and were subjected to clinical and postmortem examination as well as for isolation and identification of *E.coli* and salmonella from tissue samples including spleen, heart, liver, intestine and lungs .The samples were transported in ice boxes and transferred to the laboratory. Collected samples were cultured within a time limit.

2.2.Bacteriological examination of salmonella and E. coli

2.2.1. Salmonella

Samples were pre-enriched in buffered peptone water 1/10 dilution (W/V). Incubation is carried out at 37 °C ± 1°C for 18 ± 2hrs. From the pre-enrichment culture, 0.1 ml was transferred to a tube containing 10 ml of the Rappaport Vassiliadis broth and then incubated at 41.5ºC ± 1ºC for 24 ± 3hrs.The broth cultures were plated onto selective agar plates media to identify and observe gross colony morphology usingXylose Lysine Desoxycholate agar (XLD) , Brilliant Green agar, Salmonella – Shigella agar media, Hektoen Enteric agar plates and MacConkey's agar media and incubated at 37.0 ± 1 °C for 24 ± 3hrs (ISO 6579, 2002).

2.2.2. E.coli

Samples were pre-enriched into buffer peptone water and incubated at 37˚C for 18 ±2 hrs under aerobic condition .A loopful from the broth of each sample was streaked onto MacConkey's agar and Eosin Methylene Blue agar. The inoculated plates were incubated at 37 ºC for 24 hrs. (Quinn et al., 2002). The Salmonella and *E.coli* isolates were subjected to different biochemical tests such as sugar fermentation test, Indole production ,Christener's urea agar test, Methyl-Red and Voges-Proskauer (MR-VP) test (Quinn *et al*., 2002)

2.3. Serogrouping and serotyping of isolates

Ten isolates of *E.coli* were serogrouped according to Edwards and Ewing (1972) and five Salmonella isolates were serotyped according to Kauffman (1973).

2.4.Experimental design

2.4.1. Experimental chicks

The present experiment was conducted on 120 healthy one-day-old broiler chicks (avian 48). Broiler chickens were divided intosix equal groups, each group contained 20 birds. All birds were subjected to the ordinary vaccination program for broilers against New Castle and Gumboro diseases. All birds were fed balanced commercial starter, growing and finishing rations . Fresh and clean drinking water was fed ad-libtium. The birds were housed in floor – pen (0.1 m2 / bird), in clean well ventilated separate experimental rooms throughout the period of experiment. Birds were divided into 6 equal groups: group one (G1) control group; non-treated normal chicks, group two (G2) normal chicks treated with Poultry star®, group three (G3) *Salmonella Entertidis* infected group; group four (G4) *Salmonella Entertidis* infected and supplemented with Synbiotic, group five (G5) *E.coli* O78 infected group and group six (G6) *E.coli* O78 infected and supplemented with Synbiotic. Chicks of group (G3) and (G4) infected with *Salmonella Entertid* isorally (10^8 viable microorganisms/bird) at the 8th day old according to Johny et al., (2012). Chicks of group (G4) and (G6) infected with *E.coli O78* orally (10^8 viable microorganisms / bird) at the 14th day old according to El- Boushy et al .,(2006)

2.4.2. Synbiotic(Poultry star®)

Composed of probiotics (*Enterococcus sp., Bifidibacterim sp., Pedicoccus sp.* and *Lactobacillus sp.*).The product contains minimum of 5×10^{11} CFU/Kg and prebiotics

(fructo- oligosaccharides) in a dose of 20g /1000 bird /day in drinking water.

2.4.3. Blood samples

Blood was collected from wing vein (cutaneous brachial vein) from 3 birds of each group at 21, 28 and 35 days old and divided into heparinized blood (blood was collected on heparin (20 IU/ml)for measurement of phagocytic activity and serum (blood was collected in plain clean well-dried centrifuge tube for HI (Hemagglutination Inhibition) test against ND (New-Castle virus).

2.4.4. Immunological studies

2.4.4.1.Determination of phagocytic activity and phagocytic index (Richardson and Smith, 1982)

2.4.4.2.Estimation of humeral immunity

By using HI test against ND using the standard micro plate system (King and Seal, 1998).

2.4.4.3.Weighting of the immune organs

Three birds were randomly chosen from each group and slaughtered at 35days old. Bursa of Fabricius, thymus and spleen were removed from each bird and weighted separately.

2.4.5. Statistical analysis

Data are represented as mean ± SE (standard error). One-way analysis of variance (ANOVA) - Tuky test was used to compare the mean values of the various groups at a significance level of $P \leq 0.05$ by using SPSS 20 (2011).

3. RESULTS

3.1.Identification of the isolated Salmonella and E.coli

On XLD, salmonella appeared as smooth colonies with black center while onto Salmonella-Shigella agar, it appeared pale colored colonies indicated non lactose fermenting with or without black centers while onto MacConkey's agar appeared as pale, colorless smooth, transparent and raised colonies. On the other hands, it gave negative results on Christener's urea agar and Voges-Proskauer test and positive results on Triple sugar iron agar, Methyl-Red test and Simmon's Citrate test. On MacConky's agar, *E.coli* appeared as pink streaks (lactose fermenter colonies) while onto EMB agar showed metallic green sheen colonies. Biochemically, Negative results were recorded on Triple sugar iron agar, Voges-Proskauer test, Christener's urea agar but positive results on Methyl-Red test and indole production test.

3.2. Incidence of Salmonella and E.coli in chickens

Salmonella was isolated from diseased living chicks from liver, heart and spleen with a percentage of 3%, 1% and 1%, respectively. Five isolates of salmonella were isolated from 100 sample. *E.coli* was isolated from diseased living chicks from liver, intestine, heart, spleen and lungs with a percentage of 15%, 12%, 10%, 8%and 7 % respectively. Fifty-two isolates of *E.coli* were isolated from 100 sample.

3.3. Sero grouping and serotyping results

Five salmonella isolates were serologically identified as: (3) *Salmonella Enteritidis,* (1) *Salmonella Charity and(*1) *Salmonella Remiremont.* Ten *E.coli* isolates were also serologically identified as: (5) *E.coli*O78 ,(3) *E.coli* O157 and (2) Un-typed *E.coli*

3.4. Immunological parameters

3.4.1. Phagocytic percent and phagocytic index

There was significant increase in phagocytic percent and phagocytic indexin G2 which were 34.20±1.21, 32.93 ±0.41 and 33.36±0.66 and 4.46±0.33, 3.9±0.30 and 3.43±0.08 respectively during the 3rd ,4thand 5th week when compared with G1. However, there was significant decrease in phagocytic percent and phagocytic index in G3 (22.20±1.21, 21.16±0.60 and 21.46±0.37 and 1.53±0.12, 1.63±0.18 and 1.43±0.13 respectively) and G5 (23.5 ±

1.21, 21.53±0.71 and 23.10±0.60 and 1.66±0.08, 1.56±0.12 and 1.46±0.06 respectively) when compared with G1during the 3rd, 4thand 5th week. A significant increase in phagocytic activity and phagocytic index were observed in G4 (29.13±0.63, 29.23±1.15 and 27.16±0.66 and 2.93±0.06, 2.66±0.16 and 2.16±0.16 respectively) and G6 (28.23±0.50, 27.13 ±0.63 and 28.20±0.55 and 3.17±0.57, 2.56±0.29 and 2.3±0.05 respectively when compared to G3 and G5 during the3rd, 4thand 5th week (Table 1 & 2).

3.4.2. Haemagglutination inhibition (HI) test

There was a significant increase in antibody titer against NDin G2 (5.66±0.33, 5.00 ±0.57 and 5.66±0.33) when compared with control group. However, there wasno significant difference in antibody titer in G3 and G5 during the 3rd, 4th and 5th week. A significant increase in antibody titer were observed in G4 (4.33±0.33, 4.33±0.33 and 4.66±0.33) and G6 (4.66±0.33, 3.66±0.33 and 4.33±0.66) when compared to G3 and G5 during the 3rd, 4th and 5th week (Table 3).

3.4.3. Weighting of the immune organs

There was significant increase in weight of bursa of Fabricius, thymus and spleen in G2 (3.00±0.14, 9.00±0.37 and 3.73±0.12 respectively) when compared with G1. However, there was a significant decrease in weight of bursa of Fabricius, thymus and spleen in G3 (1.43±0.03, 3.80±0.35 and 1.76±0.03 respectively) when compared with G1 and significant decrease in group G5 (1.66±0.08, 4.46±0.31and 2.00±0.05 respectively) when compared with G1 during the 5th week. A significant increase in weight of bursa of Fabricius, thymus and spleen were observed in infected and supplemented with Synbiotic groups (Table, 4) where G4 were (1.96±0.03, 6.40±0.70 and 2.63±0.12 respectively)and G6 were (2.23±0.13, 6.66±0.12 and 2.76±0.14 respectively) when compared to G3 and G5.

Table (1): Effect of Synbiotic on phagocytic percent in experimentally infected broiler chickens with *E.coli* and Salmonella (Means ±S.E.).

Age	G1	G2	G3	G4	G5	G6
3rd week	29.46±0.68^{b}	34.20±1.21^{a}	22.20±1.21^{c}	29.13±0.63^{b}	23.5±1.21^{c}	28.23±0.50^{b}
4th week	29.20±0.60^{b}	32.93±0.41^{a}	21.16±0.60^{c}	29.23±1.15^{b}	21.53±0.71^{c}	27.13±0.63^{b}
5th week	28.86±1.21^{b}	33.36±0.66^{a}	21.46±0.37^{c}	27.16±0.66^{b}	23.10±0.60^{c}	28.20±0.55^{b}

Means ± S.E. With different superscript (a, b, c, d) within the same column are significantly different at $p<0.05$. G1: control group, G2: Synbiotic supplemented group G3: group infected with salmonella G4: group infected with salmonella and supplemented with Synbiotic G5: group infected with *E.coli* G6: group infected with *E.coli* and supplemented with Synbiotic.

Table (2): Effect of Synbiotic on phagocytic index in experimentally infectd broiler chickens with *E.coli* and Salmonella (Means ±S.E.)

Age	G1	G2	G3	G4	G5	G6
3rd week	3.10±0.20^{b}	4.40±0.33^{a}	1.53±0.12^{c}	2.93±0.06^{b}	1.66±0.08^{c}	3.17±0.57^{b}
4th week	2.80±0.1^{b}	3.90±0.30^{a}	1.63±0.18^{d}	2.66±0.16^{b}	1.56±0.12^{c}	2.56±0.29^{b}
5th week	2.66±0.28^{b}	3.43±0.08^{a}	1.43±0.13^{c}	2.16±0.16^{b}	1.46±0.06^{c}	2.30±0.05^{b}

Table (3): Effect of Synbiotic on HI antibody titer against ND vaccine in experimentally infected broiler chickens with *E.coli* and Salmonella (Mean ± S.E.)

Age	G1	G2	G3	G4	G5	G6
3rd week	3.00±0.57^{bcd}	5.66±0.33^{a}	2.00±0.57^{d}	4.33±0.33^{abc}	2.66±0.33^{cd}	4.66±0.33^{ab}
4th week	2.66±0.33^{bc}	5.00±0.57^{a}	2.33±0.33^{bc}	4.33±0.33^{a}	1.33±0.33^{c}	3.66±0.33^{ab}
5th week	2.33±0.33^{b}	5.66±0.33^{a}	2.00±0.57^{b}	4.66±0.33^{a}	2.33±0.33^{b}	4.33±0.66^{a}

Table (4): Effect of Synbiotic on weight of immune organs of experimentally infected broiler chickens with *E.coli* and Salmonella (Means ±S.E.)

Age	G1	G2	G3	G4	G5	G6
BF	2.30 ± 0.17^{b}	3.00 ± 0.14^{a}	1.43 ± 0.03^{d}	1.96 ± 0.03^{bc}	1.66 ± 0.08^{cd}	2.23 ± 0.13^{b}
Thymus	7.06 ± 0.50^{b}	9.00 ± 0.37^{a}	3.80 ± 0.35^{c}	6.40 ± 0.70^{b}	4.46 ± 0.31^{c}	6.66 ± 0.12^{b}
Spleen	2.80 ± 0.15^{b}	3.73 ± 0.12^{a}	1.76 ± 0.03^{c}	2.63 ± 0.12^{b}	2.00 ± 0.06^{c}	2.76 ± 0.14^{b}

4. DISCUSSION

In the current study, out of 100 samples from chickens were found to be positive to salmonella (5 %) and this result was nearly agreed with Hassan et al., (2003). The recovery of salmonella species from internal organs of the examined chickens were higher from liver followed by heart and spleen 3%, 1%, and 1% respectively. This result was nearly in coordinating with Chaiba*et al.,* (2009) who isolated a higher level of salmonella from liver (11.11 %). Five salmonella *i*solates which serologically examined were (3) *Salmonella Enteritidis*, (1) *Salmonella Chairty*, (1) *Salmonella Remiremont* and this result was nearly agreed with Dahal, (2007). Concerning to *E.coli* isolation, 52 out of 100 samples from chickens were found to be positive with a percentage of 52 %, this result was nearly in coordinating with Roy *et al.*,(2012).The *E.coli* isolates were higher from liver followed by intestine ,heart , spleen and lungs 15%, 12%, 10%, 8%and 7 % respectively. This result was agreed with Sharada et al., (2010). Ten *E.coli* isolates were examined serologically and showed (5) *E.coli* O78, (3) *E.coli* O157, (2) Untyped *E.coli* and this result was nearly agreed with Roshdy et al., (2012) who revealed that *E.coli* O78 The most commonly detected *E. coli* serogroup from different organs of chicken. There was a significant increase in phagocytic percent and phagocytic index in G2 when compared with G1 during the 3rd, 4th and 5th week. The obtained results were in agreement with El-Sissi and Mohamed (2011) who examined the effect of the Synbiotic on peripheral blood mononuclear cells and found that phagocytic % & index of broiler chickens exhibited significant increase when compared with control group. Also, there was a significant increase in G4 and G6 when compared to G3 and G5.This due to probiotics act on macrophages activity in a dose dependent manner and explained by El-Sissi and Mohamed (2011) .There was a significant decrease in phagocytic % & index in G3 and G5 when compared with G1 during the3rd ,4th and 5th week. These results were in agreement with Hegazy et al., (2010) who mentioned that *E. coli* infection cause impairment of polymorphonuclear leukocytes (PMNLs) function, decreased phagocytic activity and ineffective opsonization. There was significant increase in antibody titer in G2 when compared with G1 during the 3rd, 4th and 5th week. In addition, there was a significant increase in G4 and G6 when compared to G3 and G5. The obtained results were in agreement with El-Sissi and Mohamed (2011) who reported that Synbiotic improve the HI antibody titers for NDV and this due to binding of structural components of commensal bacteria to Toll-like receptors (TLRS) expressed on the surface of macrophage and dendritic cells in the lamina propria may lead to their activation and differentiation. Upon its activation, they promote the activation and differentiation of different subsets of other immune system cells, leading to the production of cytokines such as IL4, IL10 and transforming growth factor ß that are important for antibody production and isotype switching (Di Giacinto et al., 2005). There was no significant difference in antibody titer in G3 and G5 when compared with G1 during the 3rd, 4th and 5th week. This occurred due to the antigenic stimulation, which accompany variety of

infectious and hepatic diseases (Thrall, 2004). Measurement of immune organ weight is a common method for evaluation of immune status in chickens (Heckert et al., 2002). There was a significant increase in weight of bursa of Fabricius, thymus and spleen in G2 when compared with G1 during the5th week. In addition, a significant increase in weight of bursa of Fabricius, thymus and spleen were observed in G4 and G6 when compared to G3 and G5. The obtained results were in agreement with Huang *et al.,* (2007) who revealed that Synbiotic cause significant increases in the absolute weight of the immune organs (thymus and bursa) and numerical increase in the spleen weight. However, there was significant decrease in weight of bursa of Fabricius, thymus and spleen in G3 and G5 when compared with G1 during the 5th week. The obtained results were in agreement with Sadeghi et al., (2013) who reported that salmonella challenging depressed the immune organ growth. Low bursa weight could be interpreted as an indicator of low immune activity because it is a major lymphoid organ in poultry and this decrease in the immune tissue weight produces an effect on immune cell phenotypes, immune cell proliferation, and antibody production (Ghaderi –Joybari et al., 2014).

5. CONCLUSION

From the fore mentioned results , it could be concluded that Synbiotic (Poultry star®) had clear impact in increasing immune response in normal and infected broiler chickens and advise to apply it on a wider scale in the poultry field.

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