Impact of Symbiotic on the Immune Response of Broiler Chickens Against NDV and IBV Vaccines

Ashgan F. El-Sissi and Samah H. Mohamed

Department of Immunology, Animal Health Research Institute, Dokki, Giza, Egypt

Abstract: To evaluate the effect of symbiotic, (0.5 or 1 kg/Ton of feed), on innate and humeral immune responses of broiler chickens for routine vaccination with Newcastle disease virus (NDV) and Infectious bronchitis virus (IBV) vaccines. A total of 180 one-day old chicks (Hubbard local breed) were divided into 3 groups 60 chicks each. The first group consumed normal broiler diet without any additives and served as control group, groups 2&3 consumed diets contained 0.5 or 1 kg/Ton of symbiotic for 42 days experimental period. The administration of symbiotic to broiler chickens early in life increased significantly (p<0.05) the phagocytic activity, lysozyme activity and nitric oxide levels in a dose dependent manner and improved the oxidative state by increasing glutathione (GSH) and decreasing malondialdehyde (MDA). High concentration of symbiotic improves the antibody response to NDV and IBV vaccines.

Key words: Symbiotic • Immune response • Broiler chickens • NDV • IBV • Vaccines

INTRODUCTION

Probiotics have been defined as a live microbial feed supplement that can beneficially affect the intestinal microbial balance, resulting in improved body weight gain and reduce mortality in broiler chickens [1, 2]. Prebiotics are defined as non digestible, but fermentable, food ingredients as oligosaccharides (B-glucans, mannans, inulin-type fructans) that beneficially affect the host by selectively stimulating the growth and activity of one or limited number of species of probiotics bacteria in colon. Symbiotic are defined as a combination of a probiotic and a prebiotic, aimed to increase the survival activity of probiotics in vivo and stimulating indigenous bifido bacteria and lactobacilli [3]. Choct et al. [4] reported that supplementary digestive enzymes as amylase, cellulase, beta-glucanase, hemicellulase improved nutrient digestibility, reduced small intestinal fermentation and increased cecal fermentation in chickens. Mohnl et al. [5] found that the symbiotic had a comparable potential to improve broiler performance as Avilamycin (an antibiotic growth promoter).

Colonization of chicken intestine by commensal bacteria is an ongoing process which begins immediately after hatch and is established by week 2 post hatch [6]. It is possible that commensal bacteria, which interact closely with cells within the chicken gut associated lymphoid tissue (GALT), play a role in the development of immune response. It has been demonstrated that the chicken GALT reaches its functional maturity by week 2 post hatch [7]. By this time, the chicken GALT encompasses cells of the immune system, including T&B cells, macrophages and natural killer (NK) cells [8]. Early colonization of intestines of 1day-old chicks by a probiotic containing Lactobacillus acidophilus and Bifidobacterium bifidum, resulted in a significant enhancement of the immune response [9]. Lactobacillus and bifidobacterium are the genera most frequently used as probiotic [10].

Oral administration of probiotic have been used to improve immunity to infectious agents by colonization in the gastrointestinal tract, activating immunocytes, promoting the endogenous host defense mechanisms and modulating the systemic and mucosal immune system [11]. Moreover, it has been shown that probiotics stimulate different subsets of immune system cells to produce cytokines, which in turn play a role in the induction of immune response [12, 13].

Few information are available regarding the effect of adding symbiotic product to broiler diets on the immune status of broiler chickens. Based on this concept, the present study was designed to evaluate the effect of symbiotic on innate and humeral immune responses to routine vaccination with NDV and IB virus vaccines, as well as oxidant/antioxidant balance.
MATERIALS AND METHODS

Symbiotic used is a commercial combination of Lactobacillus, Bifido bacteria, oligosaccharides derived from yeast cell wall with some digestive enzymes amylase, cellulase, beta-glucanase, hemicellulase and maltodextrin.

Experimental Design: One hundred and eighty, 1-day-old broiler chicks of both sexes (Hubbard local breed) were obtained from a local hatchery and divided into 3 groups 60 chicks each:

Group (1): Chicks fed on normal diet and kept as control.

Group (2): Chicks fed on normal diet mixed with symbiotic 0.5 kg/Ton (low dose)

Group (3): Chicks fed on normal diet mixed with symbiotic 1 kg/Ton (high dose)

All groups were vaccinated by bivalent NDV-IBV vaccine at 7th day of age, then by Lasota vaccine at 21st day of age.

Blood Samples: Heparinized blood samples were taken (5 samples/group) at 5th & 12th day post 1st & 2nd vaccination for separation of mononuclear cells used in phagocytic activity and at the end of the experiment for detection of malondialdehyde & glutathione.

Blood samples for serum separation were taken from all groups (5 samples/group) at 2nd day, 1st & 2nd week post 1st vaccination and 2nd day, 1st, 2nd and 3rd week post 2nd vaccination, to measure antibody against NDV & IBV vaccine and for measurement of lysozyme and nitric oxide.

Evaluation of Innate Immunity

Assay of Phagocytosis: The test was performed according to Bos and Souza [14] with some modification briefly; peripheral blood mononuclear cell layer was collected, washed and resuspended in RPMI-1640 supplemented with 15% FCS. Then monolayer of macrophages was obtained by seeding 1ml 5×10^6 mononuclear cells in culture and staining chambers with cover slip and incubated for 1hr at 37° in 5% co2 and 99% humidity. Non adherent cells were removed by washing 3 times, then after incubation for 24 hrs, the adherent macrophages were incubated at the same condition with 1 ml Candida Albicans (10^7/ml RPMI with 15%FCS), washed 3 times, fixed and stained. Finally count 100 macrophages to determine % of phagocytic macrophages (number of phagocytic macrophages/total number of macrophages) and phagocytic index (number of macrophages engulf ≥3 Candida spores/total no of phagocytic macrophages).

Lysozyme Assay: Lysozyme activity was measured by agarose gel plate lyses assay according to Peeters and Vantrappen [15]. Briefly, Lysoplates were prepared by dissolving 1% agarose in 0.06 mPBS at pH 6.3 in which Micrococcus lysodeikticus (50 mg/100 ml agarose) had been dispersed. Then 25 µl of serum samples and standard lysozyme were added in each well. After 18 hours the cleared zones diameter were measured. The concentration of lysozyme was obtained from logarithmic curve prepared using standard lysozyme solution.

Nitric Oxide Assay: Carried out according to Jose et al. [16] and Yang et al. [17] Briefly 100µl of serum sample was mixed with 80µl of 375mM ZnSO4 and 120µl of 275 Mm NaOH, then centrifuged at 13000 rpm for 20 min to remove proteins. Supernatant was obtained and added to 400 µg of Cu plated Cd, then shook for 2.5h at room temperature after adding 100µl of 0.2 M glycine buffer. 100µl Supernatant was added into 96-well ELISA plate then added 100µl of Griess reagent. The optical density was determined at 545 nm with an ELISA plate reader. Nitric oxide concentration was calculated from standard curve using NaNO2.

Evaluation of Humeral Immune Response

Detection of Antibodies Titer to ND: Using Haemagglutination inhibition test (HI) according to Beard [18] with chicken RBCs and 4 units of NDV antigen, then geometric mean titers were calculated.

Detection of Antibodies Titer to IBV Vaccine: Using Infectious Bronchitis Virus Antibody Test ELISA kits (BioChek) according to the manufacture’s instruction.

Detection of Malondialdehyde and Glutathione: Malondialdehyde was measured according to Ohkawa [19] and glutathione was measured according to Ellman et al. [20] at the end of experiment.

Statistical Analysis: Data obtained were statistically analyzed using analysis of variance and comparing between groups were performed using least significant difference (LSD) at P<0.05 according to Petrie and Watson [21] and computerized using SPSS (1999).
RESULTS AND DISCUSSION

Recent researches and development of symbiotic products have been increasingly focused on functional benefits including resistance to gastrointestinal bacterial infection and improved immune status in broiler chicks. The consumption of a probiotic in combination with a suitable prebiotic (symbiotic) can result in synergistic effects [22].

In this study, we examined the effect of the symbiotic on Peripheral blood mononuclear cells, the phagocytic % & index of broiler chickens (Table 1 & Photo1) exhibited significant increase in groups (2) and (3) compared to control group at 5th day post 1st & 2nd vaccination. Also at 12th day post 2nd vaccination in group (3), receiving 1 kg/Ton (high concentration). These results agree with previous findings [23, 24, 25] which recorded that probiotic including LAB increases the activities of phagocytes, also with Shimada et al. [26] who reported that probiotics act on macrophages activity in a dose dependent manner. The activities of phagocyte may be explained as, the bacterial cell or bioactive peptide released during fermentation by lactic acid bacteria activate immune response through a dynamic interaction with specific Toll-like receptors on the surface of macrophage (it was known that the phagocytosis by macrophages is Toll-like receptors dependent) this interaction between host cells and pathogens or their structural components may play a critical role in the early innate immune response. The activation of the TLRs starts signaling cascades that involve the activation of proteins and transcription factors inducing the secretion of proinflammatory & effectors cytokines which farther activate macrophage cells [27].

Lysozyme was known to be one of Lysosomal enzyme which attacks mucopeptide in cell walls of various bacteria and a member of innate humeral factors that elaborated from polymorph nuclear and mononuclear cells [28]. Our results (Table 2) showed significant increase at 2nd day post 1st vaccination in group 3 and at 2nd weeks post 2nd vaccination in groups 2, 3 and 2 with control group. These results are in agreement with Schiffrin et al. [23] and Weir [29] who recorded that, the probiotic including LAB increases the activities of lysozyme due to activation of phagocytic macrophage.

Regarding to nitric oxide level in serum (Table 2) there is significant increase at 2nd day post 1st and 2nd vaccination in groups (2, 3). Nitric oxide is generated during immune and inflammatory response, it is involved in innate immunity as a toxic agent towards infectious organisms and can induce or regulate death and function of host immune cells [30]. It is produced at high levels by macrophages through its activation [31].

Concerning to humeral immune response, high dose of symbiotic (group 3) improve the HI antibody titers for NDV and ELISA antibody (OD) for IBV comparing with that of control group. Figures 1 and 2 while the result of
Table 1: Effect of the dietary supplementation of symbiotic on phagocytic %& index of Peripheral blood mononuclear cells of broiler chickens

<table>
<thead>
<tr>
<th>Parameter/time</th>
<th>Phagocytic %</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>5th day post 1st vac.</td>
<td>54±1.67a</td>
<td>59±0.33ab</td>
</tr>
<tr>
<td>12th day post 1st vac.</td>
<td>53±1.22</td>
<td>54±3.18b</td>
</tr>
<tr>
<td>5th day post 2nd vac.</td>
<td>55±1.86a</td>
<td>61±0.33bc</td>
</tr>
<tr>
<td>12th day post 2nd vac.</td>
<td>54±1.67a</td>
<td>56±0.98ab</td>
</tr>
<tr>
<td>LSD</td>
<td>4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Means with different capital letters are significant different between groups.
Means with different small letters are significant different between time intervals.

Table 2: Effect of the dietary supplementation of symbiotic on Serum lysozyme (µg/ml) and nitric oxide (µmol/ml) of broiler chickens

<table>
<thead>
<tr>
<th>Parameter/time</th>
<th>Lysozyme</th>
<th>Nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>2nd day post 1st vac.</td>
<td>9.27±1.23a</td>
<td>11.12±0.58b</td>
</tr>
<tr>
<td>1st week post 1st vac.</td>
<td>18.76±1.87b</td>
<td>20.93±1.59a</td>
</tr>
<tr>
<td>2nd day post 2nd vac.</td>
<td>23.7±0.87b</td>
<td>26.85±4.27a</td>
</tr>
<tr>
<td>1st week post 2nd vac.</td>
<td>32.72±2.18a</td>
<td>32.75±2.52a</td>
</tr>
<tr>
<td>2nd week post 2nd vac.</td>
<td>33.96±2.19a</td>
<td>43.68±3.97ab</td>
</tr>
<tr>
<td>LSD</td>
<td>5.41</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Means with different capital letters are significant different between groups.
Means with different small letters are significant different between time intervals.

Table 3: Effect of the dietary supplementation of symbiotic on Glutathione and Malondialdehyde

<table>
<thead>
<tr>
<th>Parameters/groups</th>
<th>Glutathione Mmol/L</th>
<th>Malondialdehyde Mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>3.82±0.09a</td>
<td>16.19±0.02a</td>
</tr>
<tr>
<td>Group 2</td>
<td>4.43±0.05b</td>
<td>10.63±0.31b</td>
</tr>
<tr>
<td>Group 3</td>
<td>4.49±0.09b</td>
<td>11.23±0.34b</td>
</tr>
</tbody>
</table>

Means with different small letters are significant different between groups.

Regarding to the effect of symbiotic on oxidant and antioxidant status, there is a significant increase in GSH level and decrease in MDA level in 2&3 groups comparing to control group (Table 3). Our results may be due to antioxidative activity of probiotic bacteria Lactobacillus, Bifido bacteria and oligosaccharides (major constituents of symbiotic), these results were in agreement with studies that described antioxidative activity of bifidobacteria and lactobacilli \[37, 38\] and are confirmed by Kai Truusala et al. \[38\] who showed that the administration of the probiotic significantly reduce MDA values Also by Hutt et al. \[40\] That showed increase of GSH after the consumption of the symbiotic.

In conclusion, this study provides evidence that the oral administration of symbiotic (low & high doses) to broiler chickens early in life enhances innate immunity which represented by significantly increase phagocytic activity, lysozyme activity and nitric oxide in dose dependent manner. The administration of symbiotic in high dose improve humeral immune response represented by increase antibody response to NDV and IBV vaccines. The administration of symbiotic in both doses improved.
the oxidative state of broiler chickens by increase GSH and decrease MDA due to antioxidative activity of the used symbiotic constituents.

REFERENCES


