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Some studies on probiotics on humoral and cellular immunity in broiler chickens

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Abstract:

This study aimed to investigate the effect of a monospecies commercial probiotic (pediococcus acidilactici) on cellular and humoral immune response of broiler chickens for routine vaccination against New castle disease (ND) and Avian influenza (AI). A total of 160, one day broiler chickens were divided into 4 equal groups; 40 chickens for each. Chickens of group (1) was served as negative control. Chickens of group (2) were fed on commercial ration supplemented with probiotic (pediococcus acidilactici) 1 Kg / ton of ration. Chickens of group (3) were fed on commercial ration supplemented with probiotic 1 Kg / ton and vaccinated with inactivated NDV and AI - H5N1 vaccines. Chickens of group (4) were given inactivated ND and AI - H5N1 vaccine. The results showed that probiotics supplementation stimulate both humoral and cell- mediated immune response. Chickens supplemented with probiotic and vaccinated showed significant increase in HI titers against NDV at 5th weeks compared to vaccinated chickens and against (AI) at 21 days post-vaccination among groups. Lysozyme activity revealed significant increase in probiotic chickens versus non treated ones at 1st and 5th weeks of age. Chicken supplemented with probiotic (G2) showed significant increase in phagocytic percent of macrophage and index at 1st, 2nd and 5th weeks as compared to control. Protection rate against challenge with NDV reached 90 % in chickens supplemented with probiotic and vaccinated while chickens vaccinated only revealed 60 % protection rate. It was concluded that probiotic (pediococcus acidilactici) proved to be able implement humoral and cell- mediated immune response.

Key words: probiotics- chickens- humoral immunity- cellular immunity- NDV- Avian influenza (AI) - vaccines.
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Introduction

Poultry industry has always been confronted with challenges in the form of various diseases. The major economic losses are due to infectious diseases which could be caused by viruses, bacteria, fungi, protozoa, and the cost of preventive medication. This led to increased use of antibiotics in the poultry industry for prophylactic, therapeutic and growth promotion purposes. The presence of antibiotic residues in poultry meat and eggs may have deleterious effects on human consumers. The residues of antibiotics can cause resistance of human flora and pathogenic microbes to those groups of antibiotics. Moreover, cross-resistance to antibiotics used in the therapy of humans and other animals could also result **(Edens, 2003; Pelicano *et al.*, 2004).** Probiotics defined as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. **Kafilzadeh *et al.* (2013)** However, according to the currently

However, according to the currently adopted definition by Food and Agriculture Organization and World Health Organization FAO -WHO **(2001),** probiotics are: live microorganisms which when administered in adequate amounts confer a health benefit on the host. Advantage of a probiotic is that it neither has any residues in animal production nor exerts any antibiotic resistance by consumption. Therefore, a lot of researchers have partially replaced antibiotics with probiotics as therapeutic and growth promoting agents.

It was reported that probiotics have a good impact on the poultry performance **(Mountzouris *et al.*, 2007),** improve microbial balance, synthesize vitamins **(Fuller, 1989),** decrease pH and release bacteriocins **(Rolfe, 2000),** improve feed consumption in layers and broilers **(Nahashon *et al.*, 1994).** In modern broiler management, preventive measures are taken to control of such diseases and bacterial enteritis, which reduce feed utilization and live performance characteristics. Probiotic feed additives are frequently used for this purpose **Zohair (2006).** Bacterial resistance to antibiotics and the risk of residues in poultry products for human consumption have encouraged the use of probiotic microorganisms **(Patterson and Burkholder, 2003).**

Administration of probiotics influences the development of the immune response **(McCracken and Gaskins, 1999)** and stimulate different cell sets to produced cytokines, which play a role in the induction and regulation of the immune response **(Lammers *et al.*, 2003).**

The present study was planned to investigate the effects of a monospecies commercial probiotic (pediococcus acidilactici) on cellular and humoral immune response of broiler chickens for routine vaccination with NDV and AI vaccine.

Material and Methods

Probiotic: (Bactocell ME®) was obtained from Egavit company contains a minimum of 10^{10} CFU / g of viable pediococcus acidilactici. It added at rate 1 Kg / ton of food.

Experimental design:

A total of 160 one day broiler chickens were divided into 4 equal groups.All groups were kept in separate pens and fed on commercial ration ad libitum.

Group (1): chickens served as negative control.

Group (2): chickens were fed on commercial ration supplemented with probiotic **(Bactocell)®** 1 Kg / ton.

Group (3) : chickens were fed on commercial ration supplemented with probiotic **(Bactocell)®** 1 Kg / ton and vaccinated with NDV and inactivated H5N1 Avian influenza (AI) vaccines.

Group (4) : chickens were vaccinated with NDV and inactivated H5N1 Avian influenza (AI) vaccine (Intervet International BV Boxmeer-Holland) by subcutaneous route at 14th day of age.

Blood samples:

1- Heparinized blood samples for the assay of phagocytic activity of peripheral blood monocytes at 1st, 2nd 3rd and 4th weeks of age.

2- Clotted blood for serum sample for detection of antibody titer against ND

virus and AI virus by Haemagglutination inhibition (HI) and lysozyme concentration.

Evaluation of humoral immune response:

HI test:

Detection of HI antibodies titer to ND virus using (HI) the test was carried out according to **Majiyagbe and Hitchner (1977)** with chicken 1% RBCs and 4 units of NDV antigen. Results were calculated as log2 titres.

Evaluation of cell-mediated immune response:

-Measurement of Lysozyme activity by agarose cell lysis assay: according to **Schltz (1987)** using *Micrococcus lysodeikticus* (500 mg/1 liter) in 1 % agarose the concentration of lysozyme was obtained from logarithemic curve prepared using standard lysozyme solution.

-Phagocytic activity of peripheral blood monocytes using *candida albicans*: according to **Richardson and smith (1981);** and **Barry and John (1988)** as modified by **El-Enbwaay (1990).** Briefly peripheral blood mononuclear cell layer was collected, washed and resuspended in RPMI supplemented with 20% FCS in 10^7 /ml and incubated for 1 hour at 37° in humidified co_2 5 % in cell culture and staining chamber (CCSC) containing sterile rounded cover slips to prepare monolayer of adherent cells the cells were incubated for 24 hrs. After washing 3 times 1 ml *Candida Albicans* (10^6/ml RPMI) were added and incubated for 1 hour in the same condition after washing 3 times, the cover slips fixed and stained. Finally count 200 macrophages to determine percent of phagocytic macrophages (number of phagocytic macrophages / total number of macrophages including non-ingesting cells × 100) and phagocytic index (number of macrophages engulf ≥ 3 Candida spores/total no of phagocytic macrophages.

Challenge test:

At the end of experiment 10 birds from each group were challenged with a velogenic viscerotropic strain of ND (VVND) virus characterized previously. The challenge dose was$10^{6.8}$ EID_{50}/ ml /bird by intramuscular injection then kept under close observation for further 2 weeks for clinical signs, mortality and lesion scoring.

Statistical Analysis:

Collected data of were analyzed for mean and standard error and were statistically analyzed by conducting analysis of variance (ANOVA) test for least significant Difference (LSD) for determination of the significance between means at $P< 0.05$ according to **Petrie and Watson (1999).**

Result

Effect of probiotics on Humoral immune response

Determination of HI antibody levels in chicken sera against ND virus: Maternal antibodies to ND virus were decreased gradually by time to be undetected (zero) at 3 weeks and 4th weeks in control negative (G1) and probiotic treated chickens (G2); respectively. Vaccinated chickens (G4) showed significant increase HI - titers

on 1st, 2nd, 3rd and 4th weeks as compared to control (G1). Chickens supplemented with probiotic and vaccinated with NDV (G3) showed significant increase at 5th weeks as compared to vaccinated chickens (G4), probiotic treated (G2) and control (G1).

HI - antibody log_2 titers in chicken sera against AI - (H5N1): There is no significant difference in HI titers against AI among all groups at 9 days post-vaccination. While chickens supplemented with probiotic and AI vaccine (G3) showed higher AI - HI titers at 21 days post-vaccination.

Effect of probiotics on chicken cell mediated immune response:

Serum lysozyme activity: Positive results of the serum lysozyme activity was determined by the presence of clear zone around wells. The results in chickens supplemented with probiotic (G2) revealed significant increase in lysozyme activity at 1st, 2nd and 5th week as compared to control (G1). Also Chickens supplemented with probiotic and vaccinated against ND vaccine (G3) and chickens vaccinated only (G4) showed significant increase at 1st and 5th weeks compared to control (G1).

Phagocytic activity: Chicken supplemented with probiotic (G2) showed significant increase in phagocytic percent and index at 1st, 2nd and 5th weeks as compared to control (G1) (Table 1). Chickens vaccinated against ND (G4) revealed significant increased phagocytic percent and index at 1st, 2nd and 3rd weeks as compared to control. Chickens supplemented with probiotics vaccinated (G3) showed significant increase in phagocytic % at 1, 2, 3 and 5th weeks compared to control and at 3rd weeks compared to probiotic group (G2) also phagocytic index at 1st and 5th weeks compared to control.

Table (1): Effect of oral supplementation of probiotic on phagocytic activity of chicken groups.

G	1st week		2nd week		3rd week		5th week	
	Ph%	Index	Ph%	Index	Ph%	Index	Ph%	Index
G1	A 62±1.4	A 0.13±0.07	A 65±1.7	A 0.17±0.09	A 69±1.6	A 0.16±0.01	A 69±2.9	A 0.17±0.01
G2	a 69±1.9	a 0.19±0.05	a 73±2.1	a 0.23±0.02	aB 79±2.1	B 0.21±0.01	a 77±3.01	a 0.25±0.02
G3	a 71±1.3	a 0.23 ± .02	a 73±3.1	b 0.21 ± .02	abc 88±1.9	0.23±.02	a 83± 2.1	ab 0.27±.02
G4	a 71±1.2	a 0.24±.01	a 76±1.7	aB 0.28±.02	ac 76±2.4	ab 0.33±0.06	76±2.2	B 0.19±0.02

The mean difference is significant at 0.05 level. Small letters indicate significantly different between groups
against capital letters in the same vertical column (using ANOVA test).
G1: Negative control group.
G2: Supplemented with probiotic not vaccinated.
G3: Supplemented with probiotic and vaccinated.
G4: Vaccinated only.

Challenge test: Chickens supplemented with probiotic and vaccinated with NDV vaccine (G3) showed 90 % protection rate, while chickens vaccinated only with NDV vaccine (G4) revealed 60 % protection rate. On other hand both negative control group (G1) and chickens supplemented with probiotic (G2) expressed 0 % protection.

Discussion

It is possible that, for enhancing host immune response may be attributed to probiotic induce colonization in the gastrointestinal tract, activating immunocytes, promoting the endogenous host defense mechanisms and modulating the systemic and mucosal immune system **(Dalloul *et al* ., 2003)**.Moreover, binding of structural components of Commencal bacteria to Toll-like receptors (TLRS) expressed on the surface of macrophage 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) and Mohamadzadeh *et al.* (2005).** The effect of probiotic (pediococcus acidilactici) in broiler chickens in mean antibody titers against ND vaccine exhibited significantly higher antibody titers than other groups. Maternal antibodies were gradually declined this decline was delayed in the chickens supplemented with probiotic (G2) at 4 week of age, while in control group decline at 3 weeks. Our results were supported by the finding of **Zulkifli *et al.* (2000), Kabir *et al.* (2004), Khaksefidi and Ghoorchi (2006), Sohail *et al.* (2010) and Hassan *et al.* (2012)** whom reported significant increase in antibody titers against NDV vaccine in chickens received probiotic. This result can be explained by probiotics may modulate the systemic immune response of antibodies against antigens in poultry **(Mathivanan and Kalaiarasi., 2007; Apata., 2008).** Moreover, **Haghighi *et al.*, (2005)** showed that the supply of probiotics increase the blood and intestinal antibodies against different poultry antigens.

Our result disagree with those of **Bitterncourt *et al.* (2014)** who reported non-significant difference in ND antibody titers.

In the present study, HI titer in group (3) which received probiotic and vaccine was significantly higher than other groups. These findings are in agreement with those reported by **Zulkifli *et al.* (2000), Dalloul *et al.* (2003), Hassan *et al.* (2012) and El-Baky (2013).**

A significant elevation of serum lysozyme activity in (G2) chickens supplemented with probiotic as compared to control. Our result agree with **Tarakanov *et al.* (2006)** who used (Microcycol probiotic) to determine its effects on the immunological status it was shown that lysozyme activities increased with increasing dosages of Microcycol.

Phagocytic activity of macrophage (phagocytic % and index) was higher in chickens supplemented with probiotic compared to control as well as chickens vaccinated and supplemented with probiotic. Our result agree with **Tarakanov *et al.* (2006)** who recorded phagocytic activities increased with increasing dosages of Microcycol in groups received probiotics, there were significant increases in the counts and activities of lymphoblasts, macrophages and dendritic cells. **Perdigon *et al.* (1991)** obtained protection against

intestinal pathogens by inducing the increase of phagocytic activity of peritoneal macrophages in mice treated with a probiotic. Our result disagree with **Bitterncourt *et al.* (2014)** who found that no statistical difference in phagocytic activity in chickens treated with probiotics compared to control.

In the current study chickens supplemented with probiotic and vaccinated (G3) exhibited 90% protection while chickens vaccinated only (G4) revealed 60% protection against NDVs. Similar result obtained by **Wang *et al.* (2013)** indicated that probiotic microorganism inhibits influenza viruses by at least two mechanisms, direct physical interaction and strengthening of innate defense at the cellular level. **Lee *et al.* (2013)** reported that Sublingual administration of Lactobacillus rhamnosus enhanced protection against influenza virus infection by enhancing mucosal secretory IgA production, and T and NK cell activity. Moreover, interleukin (IL)-12 levels in the lungs increased significantly.

Our results proved that usage of probiotics (pediococcus acidilactici) was of value in improvement of chicken immune response to used inactivated ND and AI vaccine.

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الملخص العربى

هدفت هذه الدراسة إلى تقصى تأثير البروبيوتك (بيديوكوكس اسيديلاكتيسى) على المناعة الخلوية والسائلة فى دواجن التسمين بعد إجراء التحصين الدورى للنيوكاسل والأنفلونزا عدد 160 كتكوت عمر يوم قسمت إلى أربع مجموعات متساوية 40 كتكوت لكل لمجموعة. المجموعة الأولى ضابطة سالبة والمجموعة الثانية تم تغذيتها على عليقة مضافا إليها البروبيوتك (بيديوكوكس اسيديلاكتيسى) 1 كجم / طن والمجموعة الثالثة تم تغذيتها بالعليقة مضافا إليها البروبيوتك بنفس التركيز وتحصينها ضد النيوكاسل والأنفلونزا والمجموعة الرابعة تم تحصينها فقط ضد النيوكاسل والأنفلونزا .

أوضحت النتائج أن إضافة البروبيوتك ينشط كلا من المناعة الخلوية والسائلة. أظهرت الكتاكيت المحصنة وتم إعطائها البروبيوتك زيادة معنوية فى الأجسام المناعية المضادة لفيروس مرض النيوكاسل عند 5 أسابيع من العمر وعند 21 يوم بالنسبة للتحصين ضد الإنفلونزا مقارنة بالجموعة المحصنة فقط.

أظهرت الكتاكيت المعطاة بروبيوتك إرتفاع معنوى فى نشاط الليسوزيم عند الأسبوع الأول والخامس من العمر. كما أظهرت زيادة معنوية فى قدرة الخلايا الإبتلاعية الأكولة(phagocytic activity) فى الأسبوع الأول والثانى والخامس من العمر .

بالنسبة لإختبار التحدى بالعدوى الصناعية بفيروس مرض النيوكاسل شديد VVNDV أظهرت المجموعة المعطاة البروبيوتك والمحصنة باللقاح ضد فيروس مرض النيوكاسل نسبة حماية 90% بينما المجموعة المحصنة فقط أظهرت نسبة حماية 60%.

نستنتج أن البروبيوتك (بيديوكوكس اسيديلاكتيسى) قادر على تحسين الإستجابة المناعية السائلة والخلوية فى الدجاج.