

## STIMULATION OF ACTIVE AND MATERNAL HUMORAL IMMUNE RESPONSE BY BOOSTER RE-VACCINATION AND IMMUNOMODULATOR IN CHICKEN

By

A. M. M. Atta, F. R. Mohamed, H. B. A. Gharib, A. M. Abdo and  
A. H. Haridy\*,

Dept. of Anim. Production, Fac. of Agric., Cairo Univ., Giza, Egypt.

\*Cairo Grandparent Company

Received: 21/03/2010

Accepted: 08/04/2010

**Abstract:** *A total number of 184 hens (female of female line) and 20 cocks (male of female line) of Hubbard grandparent used in this experiment to determine the effect of booster re-vaccination and immuno-modulation by pharmaceuticals prebiotic agent (IM-104) or probiotics (protexin) on humoral and maternal immune response. At 21 weeks of age, birds were randomly divided into four groups (46 female and 5 male each). All birds were vaccinated at 21 weeks of age using inactivated tetravalent oil based vaccine (IBDV + NDV + IBV + Reo). The first three groups were revaccinated at 42 weeks of age using the same vaccine. The first group was supplemented with 4 ml IM-104 / 1 liter of drinking water, while the second group was supplemented with 2 gm protexin / 1 liter in drinking water, whereas, the third group provided plain water (without supplementation) . The fourth group, served as a control group, neither revaccinated nor provided any supplementation. Blood samples and hatching egg were collected separately from each group every six weeks started from 42 till 66 weeks of age. To detect maternal antibody titer, blood samples were collected from day-old chicks.*

*The current results indicated that, in general, the grandparent's hens revaccinated and supplemented with prebiotic (IM-104) or probiotic (protexin) had significantly higher antibody titer against the studied virus as compared to the only revaccinated or not revaccinated hens.*

*The first three groups produced chicks with significantly higher maternal antibody titer and lower percentage of chicks without detectable maternal antibodies (unprotected chicks) than those counterparts produced from the fourth group. The relative level of maternal antibody, which*

*transmitted from dams to their progeny, represent about 41.1 – 51.2 % from that of dams.*

*Generally, It could be concluded that a booster re-vaccination with inactivated vaccines and supplementation with prebiotic (IM-104) or probiotic (protexin) of hens, during egg production period, was a useful tool to keep the hens' antibody titers in high levels. These high levels resulted in producing chicks with high maternal antibody titers and it minimized the number of unprotected chicks.*

## INTRODUCTION

It is known that, high antibodies tittered hens produce high maternal antibodies tittered chicks. Thus, it becomes obvious that the goal should be to make the hen's titer as high and as uniform as possible (Fast, 2002). Gharib *et al.* (2006) suggested that booster re-vaccination of grand-parent hens during the production period, with inactivated vaccine, resulted higher specific antibody titer for hens as well as produced chicks with higher maternal antibody titer and less percentage of chicks without detectable maternal antibodies.

It is thought that the application of immune stimulators, such as prebiotic and probiotic with vaccine could improve the efficacy of vaccination (Kong, *et al.*, 2006). One of the major prebiotic used in poultry industry is Lipopolysaccharides (LPS). Lipopolysaccharides is large molecules consisting of a lipid and a polysaccharide joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, act as endotoxins, elicit strong immune responses. Release of some interleukins such as IL-1, IL-6, or tumor necrosis factor (TNF) by macrophages *in vivo* can be induced with bacterial endotoxins such as *Escherichia coli* endotoxin (LPS) (Henk, *et al.*, 1998).

Probiotics, which means "for life" in Greek, has been defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Patterson, and Burkholder, 2003). Farnell *et al.* (2006) reported that probiotics bacteria can significantly improve heterophil oxidative burst and degranulation in broilers and they may also play a significant role in potentiating the innate immune response. They also indicated that, Probiotics are nonpathogenic bacteria that can promote bird health by reducing pathogen colonization. These reductions are attributed to competitive exclusion, increased volatile fatty acid production and potentiation of the immune system. Probiotics can significantly increase the humoral immune response. A more rapid response is observed in

phagocytes, resulting in enhanced phagocytosis, killing, degranulation, and oxidative burst. It is feasible that modification of the avian gastrointestinal microflora with probiotic bacteria could affect the innate immune response (Farnell, *et al.*, 2006).

This study was carried out to determine whether booster re-vaccination, with inactivated vaccines, against some viral diseases, and supplementation with prebiotic or probiotic of grand-parent hens, during their production period, would improve the active and passive humoral immune response or not.

## MATERIALS AND METHODS

The present experiment was carried out at Cairo Poultry Grandparent Company farms, hatchery and laboratory, Wade Elfargh, 6 of October, Egypt.

A total number of 184 hens (female of female line) and 20 cocks (male of female line) of Hubbard grandparent used in this experiment. The birds were grown together under the same condition, in semi-closed house, and subjected to the same vaccination program (Table 1), according to the vaccination program outlined in the Hubbard grandparents manual (Hubbard Breeder Company, 2005). At 21 weeks of age, birds were randomly divided into four groups (46 female and 5 male each). All groups were vaccinated at 21 weeks of age using inactivated tetravalent oil based vaccine (IBDV + NDV + IBV + Reo virus).

The first three groups were booster re-vaccinated at 42 weeks of age using the same inactivated tetravalent oil based vaccine. The first group supplemented with 4 ml IM-104 / 1 litter in drinking water, while the second group supplemented with 2 gm protexin / 1 litter in drinking water, whereas, the third group was provided with plain water (without supplementation) . Both supplementations were provided for continuous seven days (started three days before revaccination, day of vaccination and continued three days after revaccination). The fourth group neither revaccinated nor provided any supplementation and served as a control group. All birds were not vaccinated with any live vaccine during the egg production stage. The birds were received a commercial breeder ration (17% crude protein and 2800 Kcal ME/kg). Feed was provided with restriction consumption, according to procedure outlined in the Hubbard grandparent manual (Hubbard Breeder Company, 2005), while, water was provided *ad-libitum*. At 21 weeks of age, 14.5 hours of light were provided daily and then the light period increased 30 minutes every other week, until it was fixed at 17 hours daily.

The blood samples were collected via wing vein every six weeks, started five days post vaccination of 42 weeks of age till 66 weeks of age, to determine the humoral immune response against (IBDV + NDV + IBV + Reo virus) vaccine. Hatching eggs were collected separately from each group three times a day during 7 to 10 days after collection of blood samples. All eggs were sprayed with 5% Vircon-S sanitizing saline and stored under 18°C for 7 days. After that, eggs were set in a forced air incubator that provided 37.5°C and 60% relative humidity (R.H.) in the setter and 36.9°C and 80% R.H in the hatchers. At day 18 of incubation all eggs were candled to discard infertile (clear) eggs and dead embryos. The remaining fertile eggs were transferred to hatching basket and returned to hatchers. At hatching day, chicks produced (50 per group) were sent to the lab and blood samples were collected from killed chicks to determine maternal antibody titer against NDV, IBV, IBDV and Reo virus. Sera were separated and stored at -20 °C until the time of antibody detection.

#### **Antibody titer:**

Enzyme linked immunosorbant assay (ELISA) were used to evaluate humoral and maternal antibody titers against NDV, IBV, IBDV and Reo virus vaccine.

#### **Pharmaceutics prebiotic agent (IM-104) and Protexin**

Pharmaceutics prebiotic agent (IM-104) contain cell wall of *Propionibacterium acnes*, Lipopolysaccharide from *E. coli*, Thiomersal, and Excipient (Calier laboratories Company)

Protexin is a probiotics contain: 1-*Streptococcus faecium*; 2-*Streptococcus thermophilus*; 3-*Lactobacillus plantarum*; 4-*Lactobacillus casei*; 5-*Lactobacillus bulgaricus*; 6-*Lactobacillus acidophilus*; 7-*Bifidobacterium bifidum*; 8-*Aspergillus oryzae*; and 9-*Torulopsis spp.*(Protexin Animal Health, 2010).

#### **Statistical analysis**

One way analysis of variance for the data was done using SAS General Linear Model procedures (SAS, 1999). The main factor was the re-vaccination and supplementation with prebaiotic (IM-104) or probiotic (protexin) effect. The significance level was set at 5%. All percentage values were transferred to arc-sine before the analysis (Snedecor and Cochran, 1980). The differences between means were tested by multiple ranges tested according to Duncan (1955).

**Immune Response, Booster Re-Vaccination, Immunomodulator.**

**Vaccination program:**

**Table (I) vaccination program for grandparent flocks**

Age	Vaccines	Method of use
One day	Infectious bronchitis (IB H 120)	E.D **
4 days	Marek's disease by HVT-rispens mixed in the rispens diluent	S/C*
9 days	Reo virus ( live ) Newcastle disease (ND clone 30)	S/C E.D
15 days	Infectious bursal disease	D.W***
21 days	Newcastle disease (ND clone 30)	E.D
25 days	Infectious bursal disease	D.W
32days	Infectious Bronchitis (IB Ma 5)	E.D
45 days	Newcastle disease (ND-oil based ) Newcastle disease (ND clone 30) Reo virus ( live )	I/M**** E.D S/C
9 weeks	Fowl pox	Wing web
11 Weeks	Newcastle disease (ND clone 30) Chicken anemia agent (CAA)	Coarse- spray Wing web
12 weeks	Avian Encephalomyelitis (AE)	D.W
13 weeks	Reo (oil based ) Turkey rhino trachitis ( TRT live ) Infectious laryngo trachitis (ILT live )	I/M E.D E.D
16 weeks	Infectious bronchitis (IB Ma 5) Egg drop syndrome ( EDS oil based )	E.D I/M
18 weeks	Newcastle disease (ND clone 30)	Spray
19 weeks	Turkey rhino trachitis (TRT oil based )	I/M
21 weeks	( Reo virus + NDV + IBDV + IBV MIX oil based )	I/M
42weeks	Birds of groups A, B and C revaccinated using tetraivalent vaccine ( Reo virus + NDV + IBDV + IBV MIX oil based )	I/M

S/C\* = Subcutaneous

E.D\*\* = Eye droop

D.W\*\*\* = Drinking water

I/M\*\*\*\* = intramuscularly

## RESULTS AND DISCUSSION

### I-Antibody Titers of Grandparent Hens

In general, results reported in Table 2 showed that at 42 weeks of age no differences ( $P>0.05$ ) were observed between groups in antibody titer against IBDV, NDV, IBV and Reo virus. While, booster re-vaccination (the first three groups) using tetravalent inactivated vaccine at 42 weeks of age, significantly ( $P<0.05$ ) improved the antibody titer against the previous virus as compared to control group during 48 weeks of age and thereafter. These results are in agreement with the findings of Fast (2002), Charles *et al.* (2005) and Gharib *et al.* (2006). Fast (2002) stated that the killed-type vaccine enhanced the immune response by increasing the stability of the vaccine and stimulating the immune system longer. These results may be due to the high immunogenicity of the inactivated vaccine (Rahman *et al.*, 2002). Robert and Erin (1997) and Gharib *et al.* (2006) concluded that, an important tool that can be used, to achieve uniform high titers, is to vaccinate the breeders twice with inactivated vaccines.

The current results demonstrated that supplementation with IM-104 and booster re-vaccination of birds (group I) exerted another improvement in antibody titer against all viruses under study, but with no significant ( $P>0.05$ ) differences in most ages as compared to the rest groups. The significant ( $P<0.05$ ) improvement due to the IM-104 supplementation were observed at 48 weeks of age in IBDV antibody titer, in NDV antibody titer at 54 weeks of age, in IBV titer at all studied ages, and in Reo virus antibody titer at 48 weeks of age. These results may be due to that IM-104 has a marked immune stimulant effect, as seen by the increase in the number of haemolytic plaque-forming cells producing antibodies against sheep erythrocytes (Gallego-Olivella, *et al.*, 1997). Chen, *et al.* (2003) also documented that polysaccharides are considered as a natural immune stimulants that have been shown to promote the secretion of cytokines and antibodies, as well as enhance the function of natural killer cells, T and B lymphocytes. Henk, *et al.* (1998) and Leshchinsky and Klasing, (2003) reported that, release of Interleukin-1, Interleukin-6, or Tumor Necrosis Factor by macrophages *in vivo* can be induced with bacterial endotoxins such as *Escherichia coli* endotoxin (LPS).

The current results also indicated the benefits of using probiotics and booster re-vaccination to improve the titer of NDV, IBV, IBDV and Reo virus vaccine. At 54 weeks of age the second group which supplemented with probiotic (protexin) shows the highest titer against Reo virus vaccine, while at 66 weeks of age it has a higher titer as compared to the first and fourth groups.

Also the second group had higher ( $P < 0.05$ ) titer against IBDV at 60 weeks of age than forth group (control). However probiotic did not exert any benefits ( $P > 0.05$ ) on antibody titer against IBDV and NDV as compared to groups 1 and 3. Generally, after 42 weeks of age, the control group (forth group) presented the lowest ( $P < 0.05$ ) titer of NDV, IBV, IBDV and Reo virus vaccine against other treated groups during all studied periods.

AamirGhafoor, *et al.*, (2005) found that Protexin treated chicks have a higher titer against Avian Influenza Virus and no mortality post challenge as compared to the cyclophosphamide treated and untreated chicks. Farnell, *et al.*, (2006) reported that Probiotics bacteria can significantly improve heterophil oxidative burst and degranulation in broilers and they may also play a significant role in potentiating the innate immune response.

## **2-Maternal Antibody Titers**

As shown in Table (3), the level of maternal antibody titers is dam dependant. In general, revaccinated dams produced one-day-old-chicks with higher ( $P < 0.05$ ) specific titer as compared to those produced from control group. This superiority ( $P < 0.05$ ) extended from 48 to 66 weeks of age. The present results also explained that using IM-104 and protexin improved ( $P < 0.05$ ) the transmission of maternal antibody titer from dams to progeny.

The advantage of using oil emulsion vaccine in parent and grandparent stock has been documented. The results of Prabhakar, *et al.* (2002) indicated that birds receiving a booster dose of vaccine before the onset of laying, both serum as well as yolk antibody titer increased and in turn enhanced the maternal antibody levels in the progeny during the susceptible period. Giambrone and Closser (1990) reported that maternal antibody was higher in chicks from the younger breeders given the inactivated vaccine, and also maternal antibody was higher in chicks from older breeders given continual live vaccines. Also Perera *et al.* (1996) indicated that replacement fowls should be revaccinated to keep high level of maternal antibodies in progeny.

Devajani-Deka, *et al.* (2001) Found that polysaccharide stimulate production of antibodies after immunization with live fowl pox vaccine. Progeny chicks showed a significantly higher level of maternal antibodies on the day of hatching compared to that from control group. Manoharan, *et al.* (2004) also reported that, polysaccharide improved maternal antibody transfer to chicks. Devajani-Deka, *et al.* (2004) remarked that, use of polysaccharide preparation resulted in a significantly higher antibody levels against IBDV. The maternal antibody levels in respective progeny were also

higher as compared to the antibody titres progeny produced from the untreated group. Shashidhara and Devegowda (2003) found that antibody responses against IBDV were higher in the lipopolysaccharide group. Maternal antibody titers in progeny were also influenced by this supplementation.

The results in Table (4) explained that some chicks hatched without detectable maternal antibodies (unprotected chicks) against one or more virus disease studied. In general, revaccinated groups during production stage produced a lower ( $P < 0.05$ ) number of unprotected chicks. The dams of the first three groups produced almost same number of unprotected chicks against IBDV, NDV, IBV and Reo virus. The transfer of maternal immunoglobulin G (IgG) to the yolk and nestling was investigated in the budgerigar. Specific antibodies to avian polyomavirus and NDV were detected in 82% of yolk extracts of eggs from seropositive hens (Phalen, *et al.*, 1995). Boa Amponsem *et al.*, (1997) found that when parent chickens immunized by Sheep Red Blood Cell two times, they produced 90 % of one-day-old chicks with detected specific maternal antibody. Whereas the ratio of day-old chicks without maternal antibody rises to 20 % when parent flocks immunized only one time. Corkish *et al.*, (1994) reported that the maternal antibody was present in 81% of one day old chicks and it may have affected the colonization of the organism and the ability to isolate *Salmonella enteritidis*.

In the present study, the comparison between antibody titers of dams and that of one-day-old-chicks (Table 5) explained that maternal antibody titers represent about 41.1-51.2 % from that of dams. The relative maternal antibody titers of the fourth group were significantly lower than those of other groups against all virus disease studied. Gharaibeh *et al.*, (2008) quantified antibody titer against 10 different pathogens from the collected serum samples, and the percentage of maternal antibodies transferred was also calculated. The results showed a variation in the level of antibodies transferred among the pathogens tested pathogens. The transfer percentages were 4.3, 19.5, 25.5, 38.6, 73.6, 6.9, 32.4, 22.4, 29.2, and 32.8 for avian encephalomyelitis virus, avian influenza virus, chicken anemia virus, infectious bronchitis virus, infectious bursal disease virus, laryngotracheitis virus, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, Newcastle disease virus, and Reo virus, respectively.

The results of this work may be used in commercial farms to predict the antibody titer in one day-old chicks as a function of their dams' antibody titers. Hamal, *et al.*, (2006) found that IgY levels in the dams' plasma or eggs could be



## Immune Response, Booster Re-Vaccination, Immunomodulator.

used as a direct indicator of maternal antibody transfer to the chicks' circulation, with an expected percentage transfer of approximately 30%.

In conclusion, it could be suggested that the booster re-vaccination, with tetravalent inactivated vaccine, and supplementation, with immunomodulator (IM-104 or protexin), of the grand-parent hens, during production period, was a useful tool to keep the hens' antibody titers in high levels.

**Table (2):** Influence of booster re-vaccination and immunomodulator (IM-104 or protexin) on antibody titer against oil emulsion vaccine of IBDV, NDV, IBV and Reo virus.

Week of Age	Virus disease vaccine	Rev. + IM-104	Rev. + protexin	Rev.	Control
42	IBD	12585.7 ± 322.5 <sup>a</sup>	12323.7 ± 322.5 <sup>a</sup>	12365.0 ± 322.5 <sup>a</sup>	12310.0 ± 322.5 <sup>a</sup>
48		22809.4 ± 286.4 <sup>a</sup>	22102.4 ± 283.3 <sup>ab</sup>	21801.3 ± 283.3 <sup>b</sup>	10914.3 ± 283.3 <sup>c</sup>
54		20605.7 ± 249.3 <sup>a</sup>	20668.8 ± 246.6 <sup>a</sup>	20600.2 ± 243.9 <sup>a</sup>	9169.9 ± 252.2 <sup>b</sup>
60		16933.3 ± 297.1 <sup>a</sup>	16654.9 ± 297.1 <sup>a</sup>	16421.9 ± 293.7 <sup>a</sup>	7673.8 ± 300.5 <sup>b</sup>
66		13287.7 ± 300.2 <sup>a</sup>	13401.8 ± 300.2 <sup>a</sup>	13257.0 ± 296.8 <sup>a</sup>	6294.8 ± 303.7 <sup>b</sup>
42	NDV	9917.0 ± 294.9 <sup>a</sup>	9820.0 ± 294.9 <sup>a</sup>	9879.0 ± 294.9 <sup>a</sup>	9940.0 ± 294.9 <sup>a</sup>
48		14999.6 ± 223.4 <sup>a</sup>	14421.9 ± 220.9 <sup>a</sup>	14650.4 ± 220.9 <sup>a</sup>	9072.6 ± 220.9 <sup>b</sup>
54		14327.1 ± 224.1 <sup>a</sup>	13601.8 ± 221.6 <sup>b</sup>	13012.2 ± 219.2 <sup>b</sup>	7669.8 ± 226.7 <sup>c</sup>
60		12433.5 ± 267.0 <sup>a</sup>	12217.7 ± 267.0 <sup>a</sup>	12370.7 ± 264.0 <sup>a</sup>	6458.3 ± 270.1 <sup>b</sup>
66		10053.1 ± 289.1 <sup>a</sup>	10053.1 ± 289.1 <sup>a</sup>	9998.4 ± 285.8 <sup>a</sup>	4845.7 ± 292.4 <sup>b</sup>
42	IBV	8871.8 ± 286.0 <sup>a</sup>	8979.3 ± 286.0 <sup>a</sup>	8838.9 ± 286.0 <sup>a</sup>	8464.2 ± 286.0 <sup>a</sup>
48		13589.2 ± 331.4 <sup>a</sup>	11945.0 ± 327.8 <sup>b</sup>	12029.3 ± 327.8 <sup>b</sup>	7326.7 ± 327.8 <sup>c</sup>
54		11760.4 ± 307.6 <sup>a</sup>	10581.6 ± 304.1 <sup>b</sup>	10825.5 ± 300.8 <sup>b</sup>	6512.0 ± 311.1 <sup>c</sup>
60		10575.3 ± 287.9 <sup>a</sup>	9967.6 ± 287.9 <sup>a</sup>	8834.1 ± 284.7 <sup>b</sup>	5442.9 ± 291.3 <sup>c</sup>
66		7624.7 ± 299.2 <sup>a</sup>	7179.6 ± 299.2 <sup>ab</sup>	6738.0 ± 295.9 <sup>b</sup>	4249.5 ± 302.7 <sup>c</sup>
42	Reo	8918.0 ± 193.3 <sup>a</sup>	8832.9 ± 193.3 <sup>a</sup>	9084.7 ± 193.3 <sup>a</sup>	8886.9 ± 193.3 <sup>a</sup>
48		13601.3 ± 220.2 <sup>a</sup>	12610.4 ± 217.8 <sup>b</sup>	12567.2 ± 217.8 <sup>b</sup>	7684.0 ± 217.8 <sup>c</sup>
54		14804.5 ± 212.7 <sup>b</sup>	15398.6 ± 210.3 <sup>a</sup>	14585.4 ± 208.0 <sup>b</sup>	6627.3 ± 215.2 <sup>c</sup>
60		12004.6 ± 200.5 <sup>a</sup>	12142.4 ± 200.5 <sup>a</sup>	12511.0 ± 198.2 <sup>a</sup>	5679.0 ± 202.8 <sup>b</sup>
66		9607.5 ± 182.2 <sup>b</sup>	10171.3 ± 182.2 <sup>a</sup>	9855.5 ± 180.1 <sup>ab</sup>	4322.7 ± 184.3 <sup>c</sup>

a,b,c\* Values with different superscripts within age and virus disease vaccine are significantly different ( $p < 0.5$ ).

\*\* Rev.: Revaccinated with tetravalent inactivated vaccine against IBDV, NDV, IBV and Reo virus, at 42 weeks of age.

**Table (3):** Influence of booster re-vaccination and immunomodulator (IM-104 or protexin) on maternal antibody titer against oil emulsion vaccine of IBDV, NDV, IBV and Reo virus.

Week of Age	Virus disease vaccine	Rev.+ IM-104	Rev. + protexin	Rev.	Control
42	IBDV	6054.3 ± 324.3 <sup>a</sup>	6125.2 ± 324.3 <sup>a</sup>	5910.0 ± 324.3 <sup>a</sup>	5963.6 ± 324.3 <sup>a</sup>
48		9329.5 ± 388.3 <sup>a</sup>	9318.6 ± 388.3 <sup>a</sup>	9007.1 ± 388.3 <sup>a</sup>	4976.7 ± 388.3 <sup>b</sup>
54		9146.3 ± 375.2 <sup>a</sup>	9051.8 ± 375.2 <sup>a</sup>	9127.4 ± 375.2 <sup>a</sup>	4236.8 ± 375.2 <sup>b</sup>
60		7621.7 ± 332.1 <sup>a</sup>	7913.6 ± 332.1 <sup>a</sup>	7318.4 ± 332.1 <sup>a</sup>	3653.6 ± 332.1 <sup>b</sup>
66		6034.3 ± 318.5 <sup>a</sup>	6085.4 ± 318.5 <sup>a</sup>	5925.7 ± 318.5 <sup>a</sup>	2877.8 ± 318.5 <sup>b</sup>
42	NDV	4796.6 ± 256.9 <sup>a</sup>	4190.3 ± 256.9 <sup>a</sup>	4592.6 ± 256.9 <sup>a</sup>	4380.7 ± 256.9 <sup>a</sup>
48		6971.8 ± 330.4 <sup>a</sup>	5922.5 ± 330.4 <sup>b</sup>	6472.4 ± 330.4 <sup>ab</sup>	4311.8 ± 330.4 <sup>c</sup>
54		6898.1 ± 310.3 <sup>a</sup>	6815.2 ± 310.3 <sup>a</sup>	6918.3 ± 310.3 <sup>a</sup>	3594.9 ± 310.3 <sup>b</sup>
60		6106.4 ± 299.5 <sup>a</sup>	5675.8 ± 299.5 <sup>a</sup>	5827.5 ± 299.5 <sup>a</sup>	3117.6 ± 299.5 <sup>b</sup>
66		4692.4 ± 257.4 <sup>a</sup>	4790.8 ± 257.4 <sup>a</sup>	4389.0 ± 257.4 <sup>a</sup>	2340.7 ± 257.4 <sup>b</sup>
42	IBV	3962.1 ± 274.7 <sup>ab</sup>	4045.6 ± 274.7 <sup>a</sup>	3217.1 ± 274.7 <sup>b</sup>	4293.9 ± 274.7 <sup>a</sup>
48		6365.0 ± 312.7 <sup>a</sup>	5971.2 ± 312.7 <sup>a</sup>	4884.4 ± 312.7 <sup>b</sup>	3753.1 ± 312.7 <sup>c</sup>
54		5120.6 ± 267.8 <sup>a</sup>	5034.3 ± 267.8 <sup>a</sup>	5443.4 ± 267.8 <sup>a</sup>	3289.6 ± 267.8 <sup>b</sup>
60		4716.3 ± 253.2 <sup>a</sup>	4382.0 ± 253.2 <sup>ab</sup>	3759.0 ± 253.2 <sup>b</sup>	2944.2 ± 253.2 <sup>c</sup>
66		3135.0 ± 209.0 <sup>a</sup>	2822.2 ± 209.0 <sup>ab</sup>	2336.9 ± 209.0 <sup>b</sup>	2290.7 ± 209.0 <sup>b</sup>
42	Reo	4196.4 ± 231.1 <sup>a</sup>	3947.1 ± 231.1 <sup>a</sup>	4041.0 ± 231.1 <sup>a</sup>	4179.4 ± 231.1 <sup>a</sup>
48		5869.2 ± 247.0 <sup>a</sup>	5545.8 ± 247.0 <sup>a</sup>	5676.0 ± 247.0 <sup>a</sup>	2930.1 ± 247.0 <sup>b</sup>
54		6519.8 ± 278.6 <sup>a</sup>	6417.5 ± 278.6 <sup>a</sup>	6574.6 ± 278.6 <sup>a</sup>	2704.5 ± 278.6 <sup>b</sup>
60		5286.0 ± 235.9 <sup>a</sup>	5460.7 ± 235.9 <sup>a</sup>	5477.9 ± 235.9 <sup>a</sup>	2288.2 ± 235.9 <sup>b</sup>
66		4114.8 ± 201.2 <sup>a</sup>	4042.5 ± 201.2 <sup>a</sup>	3982.4 ± 201.2 <sup>a</sup>	1809.5 ± 201.2 <sup>b</sup>

a,b,c\* Values with different superscripts within age and virus disease vaccine are significantly different (p < 0.5)

\*\* Rev.: Revaccinated with tetravalent inactivated vaccine against IBDV, NDV, IBV and Reo virus, at 42 weeks of age.

**Table (4):** Influence of booster re-vaccination and immuno-modulator (IM-104 or protexin) on percentage of chicks produced without detectable maternal antibodies titer against oil emulsion vaccine of IBDV, NDV, IBV and Reo virus.

Virus disease vaccine	Rev.+ IM-104	Rev. + protexin	Rev.	Control
IBDV	5.7 ± 0.50 <sup>b</sup>	6.5 ± 0.50 <sup>b</sup>	6.2 ± 0.50 <sup>b</sup>	7.9 ± 0.50 <sup>a</sup>
NDV	6.2 ± 0.55 <sup>b</sup>	6.5 ± 0.55 <sup>b</sup>	6.9 ± 0.55 <sup>b</sup>	8.7 ± 0.55 <sup>a</sup>
IBV	6.0 ± 0.55 <sup>b</sup>	6.2 ± 0.55 <sup>b</sup>	6.0 ± 0.55 <sup>b</sup>	7.9 ± 0.55 <sup>a</sup>
Reo	6.2 ± 0.48 <sup>b</sup>	6.2 ± 0.48 <sup>b</sup>	6.5 ± 0.48 <sup>b</sup>	8.7 ± 0.48 <sup>a</sup>

a,b,c\* Values with different superscripts within virus disease are significantly different (p < 0.5).

\*\* Rev.: Revaccinated with tetravalent inactivated vaccine against IBDV, NDV, IBV and Reo virus, at 42 weeks of age.

**Table (5):** Influence of booster re-vaccination and immuno-modulator (IM-104 or protexin) on percentage of maternal antibodies transferred from dams to progeny against oil emulsion vaccine of IBDV, NDV, IBV and Reo virus.

Virus disease vaccine	Rev.+ IM-104	Rev. + protexin	Rev.	Control
IBDV	43.7 ± 0.76 <sup>b</sup>	44.8 ± 0.76 <sup>ab</sup>	44.2 ± 0.76 <sup>b</sup>	46.5 ± 0.76 <sup>a</sup>
NDV	47.0 ± 0.95 <sup>a</sup>	45.8 ± 0.95 <sup>a</sup>	46.4 ± 0.95 <sup>a</sup>	47.3 ± 0.95 <sup>a</sup>
IBV	44.8 ± 1.20 <sup>b</sup>	46.1 ± 1.20 <sup>b</sup>	42.9 ± 1.20 <sup>b</sup>	51.2 ± 1.20 <sup>a</sup>
Reo	43.8 ± 0.74 <sup>a</sup>	43.2 ± 0.74 <sup>ab</sup>	43.0 ± 0.74 <sup>ab</sup>	41.1 ± 0.74 <sup>b</sup>

a,b,c\* Values with different superscripts within virus disease are significantly different (p < 0.5).

\*\* Rev.: Revaccinated with trivalent inactivated vaccine against IBDV, NDV, IBV and Reo virus. at 42 weeks of age

## REFERENCES

- AamirGhafoor, Shamoan Naseem, M. Younus ,and Jawad Nazir, 2005.** *Immuno-modulatory Effects of Multistrain Probiotics (Protexin™) on Broiler Chicken Vaccinated Against Avian Influenza Virus (H9).* *International Journal of Poultry Science* 4 : 777-780
- Boa Amponsem, K., E. A. Dunnington, and P. B. Siegel, 1997.** *Antibody transmitting ability of hens from lines of chickens differing in response to SRBC antigen.* *Brit. Poultry Sci.*, 38: 480-484.
- Calier laboratories Company, 2010.** <http://www.calier.es/eng/index.html>
- Charles, F.; Jean, G.; and Frederic, L.(2005).** *Assessment of Newcastle disease vaccination of houbara bustard breeders (Chlamydotis Undulata Undulata).* *J. of Wildlife diseases.* 41:768-774.
- Chen, H. L., D. F. Li, B. Y. Chang, L. M. Gong, J. G. Dai, and G. F. Yi, 2003.** *Effects of Chinese herbal polysaccharides on the immunity and growth performance of young broilers.* *Poult-Sci.* 82: 364-70
- Corkish, J. D., R. H. Davies, C. Wray, and R. A. J. Nicholas, 1994.** *Observations on a broiler breeder flock naturally infected with Salmonella enteritidis phage type 4.* *Vet. Rec.*, 134: 591-594.
- Devajani-Deka., G. N. Dutta, and B. C. Das, 2001.** *Studies on immunostimulating effect of Stresroak and Levamisole upon maternal immunity against Fowl pox.* *Indian-Journal-of-Poultry-Science.* 36: 329-331

- Devajani-Deka., D., K. Sarma, G., and N. Dutta, 2004.** *Assessment of immunomodulatory effect of Stresroak and Levamisole on the maternal immunity against infectious bursal disease by indirect ELISA. Indian-Journal-of-Poultry-Science.* 39: 301-303
- Duncan, N.B.1955.***Multiple range and multiple F test. Biometrics,* 11-42
- Farnell, M. B., A. M. Donoghue, F. Solis de los Santos, P. J. Blore, B. M. Hargis, G. Tellez, and D. J. Donoghue, 2006.***Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. Poultry Science* 85:1900–1906
- Fast, J., 2002.** *Maternal antibody transfer. Canadian poultry consultants Ltd. www.canadianpoultry.com.*
- Gallego-Olivella J., J. M. Ruiz-Martin M, and E. Fadura-Torru.** 1997. *Study of the immunostimulating effect of IM-104 in mice. Immunol Med Microbiol.* 19 :331-3.
- Gharaibeh, S., K. Mahmoud, and M. Al-Natour, 2008.** *Field evaluation of maternal antibody transfer to a group of pathogens in meat-type chickens. Poultry-Science.* 87: 1550-1555
- Gharib, H. B. A., A. M. M. Atta, F. R. Mohamed, and A. H. Haridy, 2006.** *Assessment of booster re-vaccination of grand-parent hens with inactivated vaccine during egg production. Egypt poult. Sci.* 26: 1567-1585
- Giambrone, J. J., and j. Closser, 1990.** *Effect of breeder vaccination on immunization of progeny against Newcastle disease. Avian-Diseases.* 34: 114-119
- Hamal, K R., S. C. Burgess, I. Y. Pevzner, and G. F. Erf, 2006.** *Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. Poultry-Science.* 85: 1364-1372
- Henk, K., Parmentier Mechteld Walraven, and Mike G. B. Nieuwland, 1998.** *Antibody responses and body weights of chicken lines selected for high and low humoral responsiveness to sheep red blood cells. 1. Effect of Escherichia coli Lipopolysaccharide. Poultry Science* 77:248-255
- Hubbard Breeder company 2005.** *Hubbard grandparent manual. Hubbard farms, Walpole, New Hampshire 03608. USA. [www.hubbardbreeders.com](http://www.hubbardbreeders.com)*

- Kong, X.F., Y. L. Hu, Y. L. Yin, G. Y. Wu, R. Rui, D. Y. Wang, and C. B. Yang, 2006.** *Chinese herbal ingredients are effective immune stimulators for chickens infected with the newcastle disease virus. Poultry Science 85:2169–2175*
- Leshchinsky, T. V., and K. C. Klasing, 2003.** *Profile of chicken cytokines induced by lipopolysaccharide is modulated by dietary  $\alpha$ -tocopheryl acetate. Poultry Science 82:1266–1273*
- Manoharan, S., S. Ramesh, M. Parthiban, A. Koteeswaran, N. D. J. Chandran, M. and R. Reddy, 2004.** *Effect of a poly herbal ingredient on day old chick quality by feeding in parent flocks. International-Journal-of-Poultry-Science. 3: 773-778*
- Patterson, J. A., and K. M. Burkholder, 2003.** *Application of prebiotics and probiotics in poultry production. Poultry Science 82:627–631*
- Perera, C. L., J. Noda, S. Cuello, P. Alfonso, A. Nunez, and I. Acosta, 1996.** *Use of ELISA to detect maternal and post-vaccination antibodies to infectious bursal disease in the offspring of vaccinated replacement fowls. Revista de Salud Animal., 18: 151-154.*
- Phalen, D. N., V. G. Wilson, and D. L. Graham, 1995.** *Failure of maternally derived yolk IgG to reach detectable concentrations in the sera of nestling budgerigars (*Melopsittacus undulatus*). Avian-Diseases. 39: 700-708*
- Prabhakar, T. G., G. Ravikumar, H. Taylor, A. Koteeswaran, G. Rajavelu, 2002.** *Serum and egg yolk antibodies in chickens immunized with hydropericardium syndrome vaccine. Cheiron. 31: 140-142.*
- Protexin Animal Health, 2010.**  
*<http://www.protexin.com/animal/product.php?id=16>*
- Rahman, M.M.; Bari, A.S.; Islam, M.R.; Giusuddin, M.; Alam, J.; Sil, G.C.; and Rahman, M.M. (2002).** *Evaluation of maternal and humoral immunity against Newcastle disease virus in chicken. Int. J. of poult. Sci., 5:161-163.*
- Robert, L.O.; and Erin, B. (1997).** *Maternal Antibodies: Some of the factors influencing their transmission. Technical report. Hubbard farms, walpole, New Hampshire 03608. USA. [www.hubbardbreeders.com](http://www.hubbardbreeders.com).*
- SAS, Statistical Analysis System, 1999.** *SAS/STAT User's Guide Release 8 edn. SAS Institute Inc, Cary, NC. 1028pp.*

Sendecor, G. W., and W. G. Cochran, 1980. *Statistical method. Six<sup>th</sup> edition. The Iowa state University press, Ames, Iowa, USA.*

Shashidhara, R G., and G. Devegowda, 2003. *Effect of dietary mannan oligosaccharide on broiler breeder production traits and immunity. Poultry-Science. 82: 1319-1325*

### الملخص العربي

## تشريط الاستجابة المناعية المصلية الفعالة و الأمية بإعادة التحصين و استخدام المحفزات المناعية في الدجاج

عبدالرحمن محمد عطا - فاطمه رسمي محمد - حسن بيومي على غريب - علاء الدين محمد - احمد حسن هريدي\*

قسم الإنتاج الحيواني - كلية الزراعة - جامعة القاهرة

\*شركة القاهرة لجنود الدواجن

الهدف من هذه الدراسة هو تحديد مدى الاستفادة من إعادة التحصين أثناء فترة الإنتاج وكذلك استخدام المحفزات المناعية (البريبيوتيك والبروبيوتيك) و تأثير ذلك على كل من الاستجابة المناعية في الجنود و المناعة الأمية المنقولة للكناكيت عمر يوم . تم تقسيم ١٨٤ جده (إناث خط الإناث) و ٢٠ جد (ذكور خط الإناث) على عمر ٢١ أسبوع إلى أربعة مجاميع: المجموعة الأولى تم تحصينها ضد مرض النيوكسيل ومرض الالتهاب الشعبي المعدي ومرض الجمبورو ومرض الريو بلقاح الزيتي الخامل عند عمر ٢١ و ٤٢ أسبوع و كذلك تمت معاملتها بالبريبيوتيك (JM-104) في ماء الشرب بمعدل ٤ مل لكل لتر ماء عند الأسبوع ٤٢. المجموعة الثانية تم تحصينها ضد الأمراض السابقة عند عمر ٢١ و ٤٢ أسبوع و كذلك تمت معاملتها بالبروبيوتيك (Protexin) في ماء الشرب بمعدل ٢ جم لكل لتر ماء عند الأسبوع ٤٢. المجموعة الثالثة تم تحصينها ضد الأمراض السابقة عند عمر ٢١ و ٤٢ أسبوع. أما المجموعة الرابعة (مجموعة المقارنة) فتم تحصينها ضد هذه الأمراض مرة واحدة فقط عند عمر ٢١ أسبوع. بداية من الأسبوع ٤٢ تم تجميع عينات الدم من الجنود مرة كل ٦ أسابيع حتى الأسبوع ٦٦ و ذلك لتقدير الأجسام المناعية المتكونة ضد الأمراض تحت الدراسة. تم تجميع البيض الناتج من كل مجموعة منفصلة و تم إرساله إلى معمل التفريخ و بعد تفريخه تم اخذ عينات الدم من الكناكيت عمر يوم لتقدير المناعة الأمية ضد الأمراض السابق ذكرها.

و أشارت النتائج إلى الاتي: كان مستوى الأجسام المناعية في المجموعات التي أعيد تحصينها عند الأسبوع ٤٢ و التي تمت معاملتها بالبريبيوتيك أو البروبيوتيك أعلى معنويًا عن المجموعة التي أعيد تحصينها فقط و المجموعة التي لم يعاد تحصينها في معظم الأعمار المدروسة. كان مستوى الأجسام المناعية الأمية أعلى معنويًا في الكناكيت عمر يوم الناتجة من المجموعات التي أعيد تحصينها و التي تمت معاملتها بالبريبيوتيك أو البروبيوتيك عن الكناكيت الناتجة من المجموعة التي لم يعاد تحصينها و كذلك كانت نسبة الكناكيت عمر يوم والتي لم يظهر بها أجسام مناعية أمية و الناتجة من المجموعات الثلاث الأولى أقل معنويًا عن مثيلتها الناتجة من المجموعة الرابعة (مجموعة المقارنة).

و من خلال هذه النتائج يتضح ان إعادة تحصين الجنود مع استخدام المحفزات المناعية مثل البروبيوتيك و البريبيوتيك أثناء فترة الإنتاج يؤدي إلى الحفاظ على مستوى أجسام مناعية مرتفع وكذلك إنتاج كناكيت أمهات ذات مناعة أمية مرتفعة و يقلل من نسبة الكناكيت الناتجة بدون مناعة أمية.