STUDIES ON SOME FACTORS AFFECTING ACQUIRED IMMUNITY IN CHICKENS

By

AHMED HASSAN MOHAMED HARIDY

B.Sc. Agric. Sci. (Poultry Production), Fac. Agric., Cairo Univ., 1996
M.Sc. Agric. Sci. (Avian Physiology), Fac. Agric., Cairo Univ., 2003

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SUPERVISION SHEET

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Date / /

دراسات على بعض العوامل التى تؤثر على المناعة المكتسبة فى الدجاج

رسالة مقدمة من

أحمد حسن محمد هريدى
بكالوريوس فى العلوم الزراعية (إنتاج دواجن)- كلية الزراعة – جامعة القاهرة، ١٩٩٦
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للحصول على درجة

دكتور الفلسفة

فى

العلوم الزراعية
(علوم دواجن)

قسم الانتاج الحيوانى
كلية الزراعة
جامعة القاهرة
مصر

٢٠١٠

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Name of Candidate: Ahmed Hassan Mohamed Haridy **Degree:** Ph.D.
Title of Thesis: Studies on Some Factors Affecting Acquired Immunity in Chicken
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ABSTRACT

Experiment 1. One-day-old grandparent chicks were used to determine the effect of vaccination programs and immunomodulation by prebiotic (IM-104) or probiotics (protexin) on grandparents immune response, maternal antibody transfer to progeny and day-old-chicks produce without detectable maternal antibody. At 21 weeks of age, 184 hens and 20 males were randomly chosen from female line females and female line males. These birds were randomly divided into four groups. Group 1. Vaccinated at 21 and 42 weeks of age using tetravalent emulsion inactivated vaccine against (IBDV + NDV + IBV + Reo) and treated with prebiotic at 42 weeks of age. Group 2. Vaccinated at 21 and 42 weeks of age using the same tetravalent emulsion inactivated vaccine and treated with probiotics at 42 weeks of age. Group 3. Vaccinated at 21 and 42 weeks of age using the same tetravalent emulsion inactivated vaccine. Group 4. Vaccinated only at 21 weeks of age using tetravalent emulsion inactivated vaccine and served as a control group. Blood samples were collected from 184 grandparent hens every three weeks from 42 till 66 weeks of age to determine antibody titers against NDV, IBV, IBDV and Reo virus. Hatching eggs of grandparents were collected separately from each group three times a day. To detect maternal antibody titers, blood samples were collected from 184 day-old chicks.

Experiment 2. At 51 weeks of grandparents age, 184 chicks (46 chicks from each group) were used to determine the effect of vaccination programs and immunomodulators on profile of anti-NDV, anti-IBDV, anti-IBV and anti-Reo maternal antibodies decline. Blood samples were collected from 40 chicks at 1, 3, 5, 7, 9, 11, 13 and 15 days of age. ELISA was used to evaluate, humoral titers, maternal antibody titers and profile of maternal antibody decline.

The current results indicated that, in general, the revaccinated and supplemented with a prebiotic (IM-104) or a probiotic (Protexin) groups had significantly higher antibody titers against the four studied viruses as compared to those not revaccinated hens. The revaccinated groups produced chicks with significantly higher maternal antibody titers and lower percentage of chicks without detectable maternal antibodies (UMA chicks) than those produced from the fourth group. The relative level of maternal antibodies that was transmitted from dams to their progeny, represent about 41.1 – 51.2 % from that of their dams. Theses result explained that maternal antibody declined with chicks' age. This finding may be attributed to normal catabolism and increasing in body weight that induces increasing in blood volume which cause maternal antibody dilution.

Key words: poultry, immune response, maternal antibody, immunomodulator.

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عنوان الرساله: دراسات على بعض العوامل التى تؤثر على المناعة المكتسبة فى الدجاج
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المستخلص العربى

التجربة الأولى الهدف من هذه الدراسة هو تحديد تأثير برامج التحصين المختلفة وكذلك المنشطات المناعية (البريبيوتيك أوالبروبيوتيك) على كل من الأستجابة المناعية فى الجدود و المناعة الأمية المنقولة للكتاكيت عمر يوم . تم تقسيم ١٨٤ جده (إناث خط الإناث) و ٢٠ جد (ذكور خط الإناث) إلى أربعة مجاميع و ذلك على عمر 21 أسبوع : المجموعة الأولى تم تحصينها ضد مرض النيوكاسيل ومرض الإلتهاب الشعبي المعدي ومرض الجمبورو ومرض الريو باللقاح الزيتي الخامل عند عمر ٢١ و٤٢ أسبوع و كذلك تمت معاملتها بالبريبيوتيك (IM-104) فى ماء الشرب بمعدل ٤ مل لكل لتر ماء و لمدة ٧ ايام عند الأسبوع ٤٢. المجموعة الثانية تم تحصينها ضد الأمراض السابقة عند عمر ٢١ و٤٢ أسبوع و كذلك تمت معاملتها بالبروبيوتيك (Protexin) فى ماء الشرب بمعدل ٢ جم لكل لتر ماء و لمدة ٧ ايام عند الأسبوع ٤٢ . المجموعة الثالثة تم تحصينها ضد الأمراض السابقة عند عمر ٢١ و٤٢ أسبوع . أما المجموعة الرابعة فتم تحصينها ضد هذه الأمراض مرة واحدة فقط عند عمر ٢١ أسبوع . بداية من الأسبوع ٤٢ تم تجميع عينات الدم من الجدود مرة كل 3 أسابيع حتى الأسبوع ٦٦ وذلك لتقدير الأجسام المناعية المتكونة ضد الأمراض تحت الدراسة . تم تجميع البيض الناتج من كل مجموعة منفصلا وتم إرساله إلى معمل التفريخ وبعد تفريخه تم أخذ عينات الدم من الكتاكيت عمر يوم لتقدير المناعة الأمية ضد الأمراض السابق ذكرها.
التجربة الثانية عند الاسبوع ٥١ من عمر الجدود تم أخذ عدد ١٨٤ كتكوت من الكتاكيت الناتجة بواقع ٤٦ كتكوت من كل مجموعة وذلك بهدف تحديد تاثير برامج التحصين المختلفة وكذلك المنشطات المناعية (البريبيوتيك والبروبيوتيك) على نمط انخفاض المناعة الأمية مع تقدم عمر الكتاكيت. تم تجميع عينات الدم من عدد ٤٠ من الكتاكيت عند عمر ١ و ٣ و ٥ و ٧ و ٩ و ١١ و ١٣ و ١٥ يوم من عمر الكتاكيت.
تم تقدير مستوى الأجسام المناعية في الجدود وتقدير مستوى المناعة الأمية في الكتاكيت الناتجة منها بإستخدام تقنية ELISA .
و أشارت النتائج إلى الاتى : كان مستوى الأجسام المناعية فى المجموعات التى أعيد تحصينها عند الأسبوع ٤٢ و التى تمت معاملتها بالبريبيوتيك أو البروبيوتيك أعلى معنويا عن المجموعة التى لم يعاد تحصينها .
كان مستوى الأجسام المناعية الأمية أعلى معنويا فى الكتاكيت عمر يوم الناتجة من المجموعات التي أعيد تحصينها فقط و التى تمت معاملتها بالبريبيوتيك أو البروبيوتيك عن الكتاكيت الناتجة من المجموعة التي لم يعاد تحصينها و كذلك كانت نسبة الكتاكيت عمر يوم والتى لم يظهر بها أجسام مناعية أمية والناتجة من المجموعات الثلاث الأولى أقل معنويا عن مثيلتها الناتجة من مجموعة المقارنة .
حصلت الكتاكيت على مناعة أمية عند عمر يوم تعادل نسبتها من ٤١.١% - ٥١.٢% من المناعة الموجودة في امهاتها.
تنخفض المناعة الامية فى الكتاكيت مع العمر ويرجع هذا الى حدوث هدم طبيعى لبروتينات الدم ومنها الجلوبيولين المناعى و كذلك زيادة وزن الكتكوت تؤدى الى زيادة حجم الدم بالتالى يحدث تخفيف للأجسام المناعية فى الدم.

الكلمات الداله: الدواجن ، الاستجابه المناعيه ، المناعه الاميه ، المحفزات المناعيه

DEDICATION

I am especially indebted to my beloved great Father who taught me how to be a good man with any success in my life. Finally and by no means least, I am especially grateful to my beloved Mother, Sisters, wife, daughter and my son for their patience and moral support throughout this study.

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CONTENT

List of Abbreviations

ACH	Achyranthan, a low-molecular-weight polysaccharide
AE	Avian encephalomyelitis
AIV	Avian influenza virus
ALV	Avian leukosise virus
ANV	Avian nephritis virus
APC	Antigen preseuting cell
APS	Astragalan, a high-molecular-weight polysaccharide
BF	Burse of fabricious
BSA	Bovine Serum Albumin
CAA	Chicken Anemia Agent
CAV	Chicken Anemia Virus
ChGG	Chicken gamma globulin
CD	Cluster of Differentiation
cIg	Immunoglobulin containing cells
CO	Corn oil
CTL	Cytotoxic T lymphocyte
D.W	Drinking water
E.	Eimeria species
ED	Embryonation day
E.D	Eye droop
EDS	Egg Drop syndrome
EID	Embryonic incubation day
ELISA	Enzyme linked immunosorbant assay
FC	Fragment of crystallization
FPV	Fowl pox virus
GALT	Gut-associated lymphoid tissue
GICs	Glandular cells
GM-CSF	Granulocyte/macrophage-colony stimulating factor
HA	Haemagglutinin
HE	Haemorrhagic enteritis
HI	Haemagglutination inhibition
HVT	Herpes virus turkey

IBDV	Infectious Bursel Disease Virus
IBV	Infectious Bronchitis Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN	Interferon
IL	Interlukens
I/M	Intramuscularly
ISGNAS	An International Study Group on Antimicrobial Strategies
Kcal	Kilo calories
LAK	Lymphokine-activated killer cells
LPS	Lipopolysaccharide
MAB	Maternal Antibodies
MDA	Maternally derived antibodies
MDV	Marek's disease virus
ME	Metabolizable energy
MGF	Mammalian granulocyte-colony stimulating factor
MHC	Major histocompetability complex
MOS	Manan Oligo saccharide
NK	Natural killer cell
NO	Nitric oxide
ORT	Ornithobacterium RhinoTracheitis
PBS	Phosphate buffer solution
PFC	Haemolytic plaque forming cells
PH	Post hatching
P.I.	Postimmunization
ppm	Part per million
ReoV	Respiratory enteric orphan virus
REV	Reticuloendotheliosis virus
S. enteritis	Salmonella enteritis
S/C	Subcutaneous
SECs	Superficial epithelial cells
SIG	Surfce immunogloboline
SO	Sunflower oil
SPF	Spicofic pathogen free
SRBC	Sheep Red Blood Cell
TC	Cytotoxic T-cell

TCR	T-cell receptor
TH	Tcell helper
TNF	Tumor necrosis factor
TRT	Turkey Rhinotracheitis
TT	Tetanus toxoid
UMA	Unmaternaled antibody
VA	Viral arthritis

INTRODUCTION

Representatives of all the classes of vertebrates have the capacity to react immunologically, but it is only in relation to birds and mammals that our knowledge of the extent of this capacity is substantial. Immunity is highly developed in birds, both cellular immunity, displayed for example in tissue graft rejection reactions, and serological immunity; the fowl being a particularly good producer of antibodies. It has been known that passive immunity to tetanus toxin is transmitted from mother to offspring in the fowl. The newly hatched chick is, in fact, well equipped with maternal immunity. Moreover it is known that immunity is transmitted from mother to young in many other species of birds. Clearly the transmission of passive immunity can occur only by way of the egg and there is ample evidence that it does so by way of the yolk, and the white of the egg (Rogers, 1970).

The immune system of newly hatched chick is only partially mature and therefore is not capable of providing complete protection against pathogens upon its first encounter with the external environment after hatching. Innate immune mechanisms seem to be fully functional in the neonate but optimal adaptive immune responses only develop during the first few weeks after hatching (Fred Davison *et al*., 2008). The protection of newly hatched chicks against pathogenic organisms depends on immunity acquired passively from their parents, as well as development of active cellular and humoral immune defenses by chicks. Maternal immunity in the form of antibodies is transferred to the chick through the egg. In chickens, serum IgG from the hen is

actively transferred into the egg yolk during folliculogenesis. This IgG is absorbed into the circulation of the chick during development, reaching a peak just prior to hatching. Immunoglobulins M and A are accumulated in the hen's oviduct secretions, then incorporated into the albumin protein of the egg (Nancy *et al.*, 1996).

Although control of diseases in chickens is attainable through vaccination, chicks aged 21 days or younger may respond poorly to vaccination because their immune system has not matured (Schwartz, 1994). Furthermore, the presence or absence of maternal antibody can contribute to a maternal effect for viability of progeny, especially during the early period after hatch. (Nordskog and Pevzner, 1977; Pinard and van der Zijpp, 1993). Higher antibody in 1-day-old broilers resulted in few vaccine-induced reactions, less vaccine virus shed, and decreased duration of vaccine-induced immunity from vaccination. 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 (Prabhakar, 2002).

It is thought that the application of immune stimulators with vaccine could improve the efficacy of vaccination (Kong *et al*., 2006). Prebiotics substance has been defined as a non-digestible feed ingredient that beneficially affects the host by selectively stimulating the immunity and neutralizing the toxin. The more common and well studied prebiotic are Lipopolysaccharides (Avanee Choudhari *et al*., 2008).