EFFECT OF SEVEN-DAY EGG STORAGE AT ROOM TEMPERATURE ON MATERNAL IMMUNITY TO NEWCASTEL DISEASE AND INFECTIOUS BURSAL DISEASE

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ABSTRACT

Five hundred and forty eggs obtained from SASO-Balady farm at Dakahlia province were used for studying the effect of 7 days egg storage at room temperature on maternal immunity to Newcastle and Infectious bursal diseases. Antibody titers in dam's sera, egg yolk and hatched chicks were measured by ELISA. Seven days storage of eggs at 16-18°C resulted in 8% reduction in hatchability. Also chicks hatched from stored eggs have lower maternal derived antibody than chicks hatched from non-stored eggs. In the same time the immune response to Newcastle oil adjuvant vaccine was lower in chicks hatched from stored eggs than in those hatched from non-stored ones.

INTRODUCTION

Pre-incubation storage condition of chicken eggs affect hatchability and chick quality. The duration of egg storage, temperature, humidity, gaseous environment, and the orientation and positional changes of the eggs are among those conditions that affect hatchability and chick quality **(Proudfoot, 1969, Mayes and Takeballi, 1984, Butler, 1991 and Meijerhof, 1992)**. Decreasing hatchability, increasing embryonic abnormalities and moralities are sequels to increasing storage time **(Merritt, 1964, Arora and Kosin, 1966a,b, Sittman et al., 1971a, b, Whitehead et al., 1985, and Fasenko et al., 1992)**.

Maternally derived antibodies to Newcastle (ND) and Infectious bursal disease (IBD) represent the first line of defense mechanisms in the first few weeks of life. Degree and duration of protection afforded depends mainly on the levels of antibodies transferred from dams to the progeny **(Heller et al., 1977, Lucio and Hitchner, 1979)**.

Few, if any, studies have been published on the effect of egg storage on maternally derived antibodies. The purpose of this study was to examine the effect of pre-incubation storage of egg on maternally derived Newcastle disease and Infectious bursal disease antibodies and the immune

response of hatched chicks to Newcastle oil adjuvant vaccines.

MATERIAL AND METHODS

Egg collection, storage and experimental designs:

On collection day, 20 serum samples were taken from parent SASO-Balady flock to determine Newcastle disease (ND) and Infectious bursal disease (IBD) antibody titers. 540 clean freshly collected eggs were obtained at the same day of serum collection from the SASO-Balady breeder farm (10,000 dams). Ten eggs were selected randomly for bacteriological examination by swabbing from internal egg contents and were cultured on nutrient and MacConkey agar. Plates were incubated at 37°C for 48 hours. Twenty-five eggs were opened on the first day of egg collection for yolk collection while 25 other eggs were subjected for yolk collection after storage for 7 days. The remaining 480 eggs were randomly divided into 2 equal groups. 240 freshly collected grouped eggs were put in automated egg incubators until hatching. Incubation temperature was 37.5°C and relative humidity was 60% in the egg-incubator. The second group (240 eggs) was stored for 7 days in a clean room prior to setting for hatching. Temperatures of the storage room ranged from 16 to 18°C. On hatching day, unhatched eggs were opened, examined and recorded. 135 healthy one day old hatched chicks were selected per each group and were subdivided into 3 equal replicates for further studies. Ten chicks per replicate were slaughtered for serum collection at day 1, 11, and 21 post-hatching. 15 chicks per each replicate were subdivided on day 21 of chicken life into 2 subgroups where 7 chicks/each subgroup were left as non-vaccinated negative controls and the other 8 birds were vaccinated with ND-oil adjuvant vaccine. Each chicks in vaccinated subgroups received 0.5ml of ND inactivated oil adjuvanted vaccine (Phylapest, Sanofi Animal Health, Batch No. 2310H1). Vaccinated and unvaccinated birds were bled from wing vein on day 7 and 21 post-vaecination for separation of sera. Antibody titers were measured by ELISA in diluted yolk samples and in sera that were stored frozen at -20°C for 3-4 weeks before antibodies were measured.

Egg yolk collection and dilution method (KPL manual):

Each egg under investigation was broken into a clean petri-dish. 0.2 ml yolk was collected by placing a tuberculin syringe-tip directly against yolk membrane and quickly drawing the plunger back. Excess yolk material was discarded and the outside of the syringe was wiped by wipe tissues. 1.8 ml of phosphate buffer saline were drawn into syringe and the contents were expelled into test tubes where the contents were redrawn and expelled 3 times by the same tuberculin

syringe. Tubes were sealed and vortexed for 1 minute and stored in —20°C for 3-4 weeks.

ND and IBD antibodies titration:

Serum and diluted yolk samples were taken off from freezer and left at room temperature for thawing and vortexed for 1 minute. In a 96 well plate, 60ul of diluted yolk suspension sample and 6ul of serum samples were added to 240ul and 300ul of ELISA dilution buffer, respectively. 50ul of each of diluted yolk and serum samples were transferred to single ND and/or IBD virus-coated ELISA plate well that was supplied with 50ul dilution buffer. ELISA tests were proceeded with normal KPL-ELISA test procedure. Optical densities were red on Bio-Tek ELX 800 reader and ND and IBD titers were calculated according to the formula given by KPL procedure.

Statistical analysis:

Data were grouped and expressed as means ± S.D. Group means for ELISA antibody titers were subjected to analysis of variance **(Snedecor and Cochran, 1967)** using the general linear models procedure and a software package (SAS, 1987). Significant differences (determined by analysis of variance for treatment groups) were compared using Duncan,s multiple range procedure **(Duncan, 1955)**. All statements of significance were based on the 0.05 level of probability.

RESULTS AND DISCUSION

Poultry have a remarkable ability to transfer antibodies from dams to their progeny through yolk. The higher the maternally derived antibodies, the stronger and longer period of protection is afforded to the progeny. Under field condition, inereasing levels of afforded maternally derived antibodies were done mainly through using oil adjuvant vaccines and live vaccines to vaccinate dams prior to and/or during laying periods.

Because bacterial yolk contamination might affect yolk and maternally derived antibody absorption (Sander et al., 1998), we examined bacteriologically the internal egg contents. Bacteriological examination of yolk and albumin of the 10 freshly collected eggs were negative in both MacConkey and nutrient agar and thus, the test-eggs were assumed to be bacteriologically sterile.

ELISA-antibody titers of parent flocks for ND and IBD were 7196 and 9079, respectively.

The effect of egg storage on hatchability, embryonic abnormalities, mortalities, growth performance and immunity are of great practical interest. In our research, the 7 days storage of chicken eggs at 16-18^{0}C resulted in 8% reduction in hatchability. The maternally derived antibodies for ND and IBD were presented in Fig. 1 and 2.

At 1-day old, chicks hatched from stored eggs had an average ND ELISA titer of 2393 that was significantly lower than the average titers (7039) of chicks hatched from non-stored eggs (Fig. 1). According to the KPL manual, the adequate ND-ELISA protective titers should be $\leq$ 1800. Hundred percent of the one day old chicks hatched from non-stored eggs had antibody titers above the protective levels, versus 43.3% of chicks hatched from stored eggs which had adequate protective titers. Chicks hatched from non-stored eggs continued to have higher antibody titers at day 11, 21 and 28 post-hatching than chicks hatched from stored eggs (Fig. 1).

ND-oil adjuvant vaccines applied to chicks at the age of 21 days resulted in no detectable antibodies 7 days post-vaccination. On day 21 post-vaccination higher antibody responses were detected in chicks hatched from non-stored eggs than in those hatched from stored eggs (Fig. 3). Antibodies to ND were not detected in non-vaccinated negative control groups.

Chicks hatched from stored eggs had 3011 IBD-titers that was significantly lower than the 7771 titers of chicks hatched from non-stored eggs and continued to be lower throughout the test periods (Fig.2). 100% of one day old chicks hatched from non stored egg had higher IBD antibody titers than the suggested KPL-adequate protective titers to IBD ($\geq$ 3000) while only 40% of chicks hatched from stored eggs had adequate protective titers.

To solve the question of whether the reduction of antibodies in one day old chick hatched from 7 day storage eggs was a matter of reduction in antibody transfer from yolk to the chicks or it s a matter of deleterious effect of storage on already existing antibodies in yolk, the yolk antibodies to ND and IBD were measured. ND and IBD antibodies were significantly reduced by the 7th day storage time in yolk (Fig. 1 and 2).

We may conclude that storage of eggs for 7 days at 16-18^{0}C were detrimental for maternally derived antibodies and to the immune responses of hatched chicks to vaccination. If such practice is needed in most of our Balady breeder farms, it may be valuable to vaccinate progeny batched from stored eggs early in their first few days of live and to store the eggs at lower temperature. However, further work is in needs to inforce the last statements.

Fig. 1: EFFECTOF EGG STORAGE ON ND-MATERNALLY DRIVED ANTIBODIES

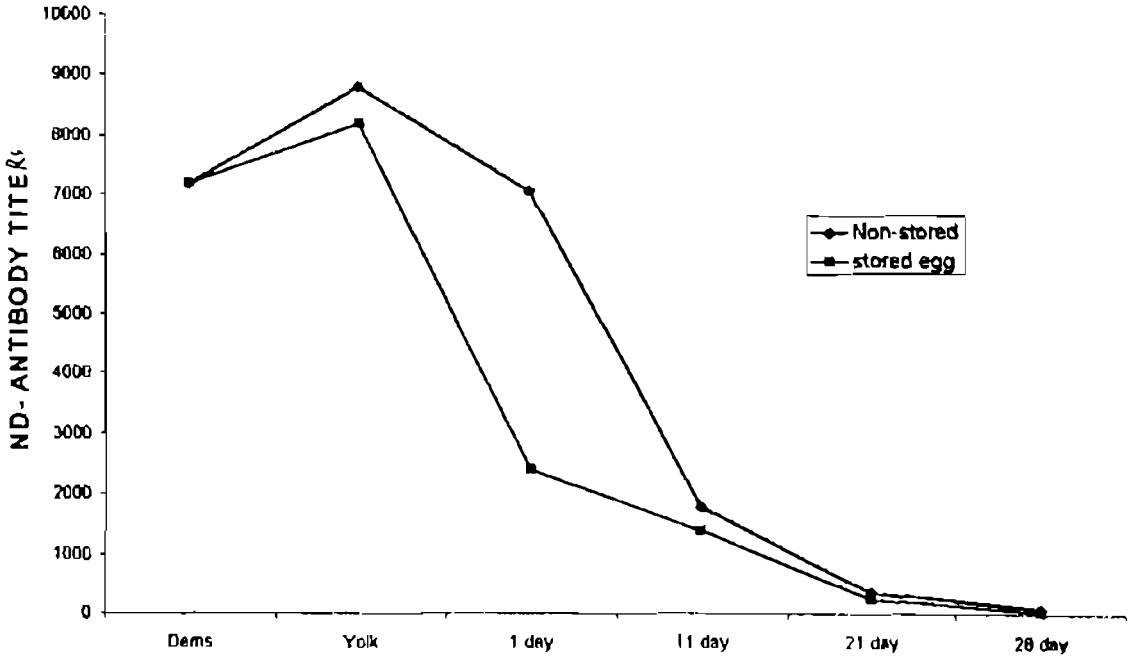


Fig. 2: EFFECT OF EGG STORAGE ON IBD-MATERNAL IMMUNIY

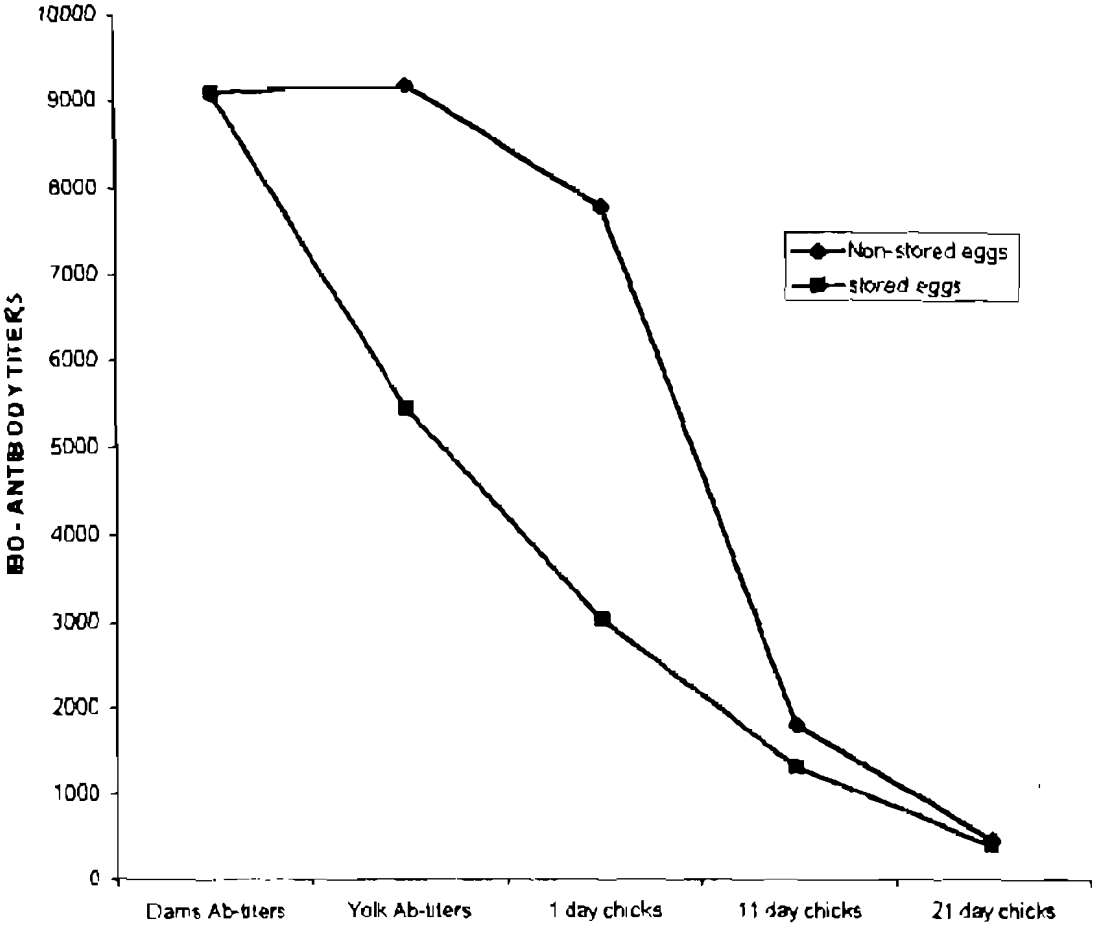
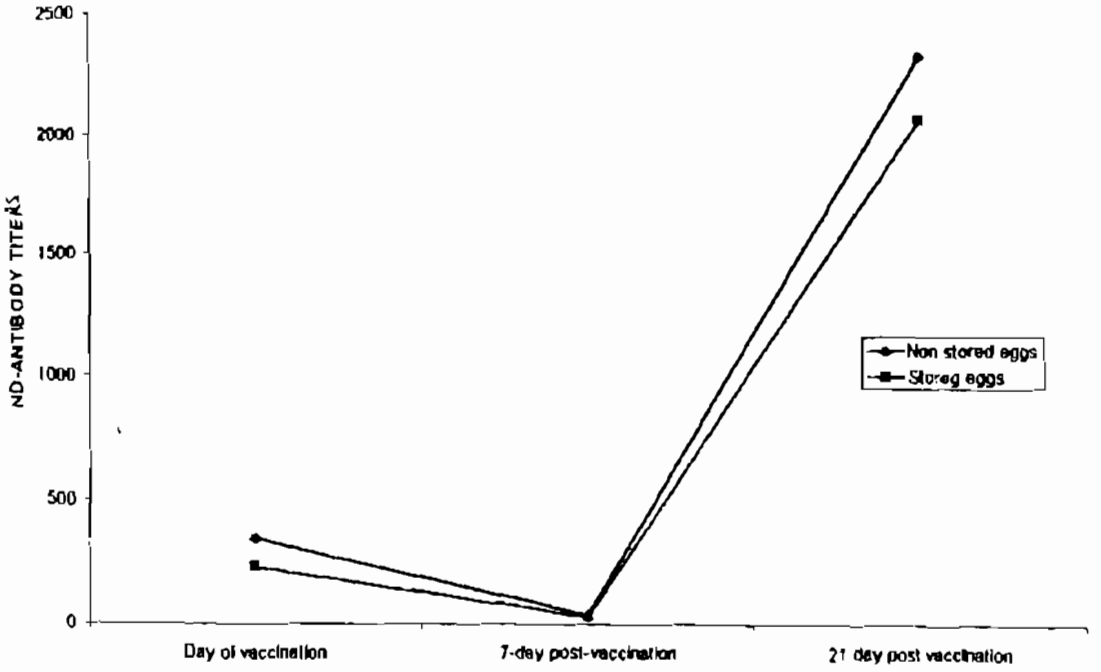


Fig.3: EFFECT OF EGG STORAGE ON ANTIBODY RESPONSE OF HATCHED CHICKS TO ND-OIL ADJUVANT VACCINES



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الملخص العربى

دراسة عن تأثر المناعة الأمية لمرضى النيوكاسل والجمبورو بتخزين بيض التفريخ ٧ أيام

المشتركون فى البحث

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تم إستخدام ٥٤٠ بيضة لدراسة تأثير بقاء البيض فى غرف التخزين (١٦-١٨ درجة مئوية) لمدة ٧ أيام على مستوى الأجسام المناعية الأمية لمرضى النيوكاسل والجمبورو.

أستخدم إختبار الأليزا فى قياس مستوى الأجسام المناعية فى مصل الأمهات وقت تجميع البيض وكذلك لقياس المناعة بصفار البيض قبل وبعد التخزين وفى مصل الكتاكيت الناتجة من البيض الغير مخزن والمخزن.

أشارت النتائج إلى إنخفاض نسب الفقس ٨٪ فى البيض المخزن عن مثيلاتها للبيض الغير المخزن، وانخفاض مستوى الأجسام المناعية لمرضى النيوكاسل والجمبورو فى مصل الكتاكيت الناتجة من البيض المخزن عن مثيلاتها للبيض الغير مخزن. كما تأثرت سلبياً الاستجابة المناعية للتحصين بلقاح النيوكاسل الزيتى فى الكتاكيت الناتجة من البيض المخزن عن مثيلاتها للبيض الغير مخزن.