IMMUONOLOGICAL AND BIOCHEMICAL EVALUATION OF AVIAN INFLUEZA VACCINES SUPPLEMENTED WITH CHROMIUM IN LAYERS

M. F. Talib A. H. Ulaiwi
Researcher Prof.
Coll. Vet. Med., University of Baghdad
E-mail: amjed.h@covmuobaghdad.edu.iq

ABSTRACT

This study was aimed to investigate the immune response of the vaccinated chickens through avian influenza virus antibody (AIV-Ab) titers as well as evaluate liver function. The study measure of immunological parameters, namely the hemaglutinine inhibition test (HI) and a biochemical parameter called the liver function test, as well as a comparison between the highly pathogenic avian influenza (HPAI) recombinant (rH5N1) vaccine and the classical (cH5N8) vaccine. The study was designed as follows: {G1: given rH5N1 vaccine only; G2: given cH5N8 only; G3: given rH5N1 with Cr III supplement; G4: given cH5N8 with Cr III supplement; G5: given Cr III supplement; G6: negative control with no vaccination or supplementation}. The titer of AIV-Ab at 21 days old showed a significant increase in G3 as compared with other groups. At 42 and 60 days old, G3 and G4 revealed the highest levels as compared with another groups. The results of ALT enzyme levels at 30 and 60 days old showed the lowest levels in G3 and G5 as compared to other groups. Additionally, there was a significant decrease in AST enzyme levels in all groups except G6 at both ages 30 and 60 days old. In conclusion, Cr III supplementation can improve the immune response against AI vaccines and has a positive impact on liver health.

Key words: HI Test, antibody titer, liver functions test.

التقييم المناعي والكيميوحيوي للقاحات انفلونزا الطيور المعززة بالكروم في الدجاج البياض

محمد فاضل طالب أمجد حسين عليوي
باحث أستاذ
كلية الطب البيطري – جامعة بغداد

المستخلص

هدفت هذه الدراسة إلى التحقق من الاستجابة المناعية للدجاج الملقح من خلال عيار الأجسام المضادة وكذلك لتقييم وظائف الكبد. تضمنت الدراسة معيار مناعي وهو اختبار (HI) ومعيار كيميوحيوي يسمى اختبار وظائف الكبد وللمقارنة بين لقاح أنفلونزا الطيور شديدة العدوى المؤتلف (rH5N1) واللقاح الكلاسيكي (cH5N8). صممت الدراسة على النحو التالي: {G1: أعطيت لقاح rH5N1 فقط؛ G2: أعطيت cH5N8 فقط؛ G3: أعطيت rH5N1 مع مكمل الكروم الثلاثي؛ G4: أعطيت cH5N8 مع مكمل الكروم الثلاثي؛ G5: أعطيت مكمل الكروم الثلاثي؛ G6: سيطرة سلبية بدون تطعيم أو مكملات}. معيارية الاجسام المضادة في عمر 21 يومًا أظهر زيادة كبيرة في G3 مقارنة بالمجاميع الأخرى. في عمر 42 و60 يومًا، كشفت G3 وG4 عن أعلى المستويات مقارنة بالمجاميع الأخرى. أظهرت نتائج مستويات (ALT) عند عمر 30 و60 يومًا أدنى المستويات في G3 وG5 مقارنة بالمجاميع الأخرى. كذلك كان هناك انخفاض كبير في مستويات (AST) في كل المجاميع باستثناء G6 في كل من عمر 30 و 60 يومًا. يستنتج ان مكملات الكروم الثلاثي قادرة على تحسين الاستجابة المناعية ضد لقاحات إنفلونزا الطيور ولها تأثير إيجابي على صحة الكبد.

الكلمات المفتاحية: فحص HI، معيارية الاجسام المضادة، اختبار وظائف الكبد

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INTRODUCTION

Avian influenza is a viral infection that poses a significant threat to human and poultry populations worldwide due to its highly contagious and zoonotic nature (10,14,32). Vaccination has been demonstrated to be a successful method to prevent the transmission of the disease, and both classical high pathogenic avian influenza (HPAI) and recombinant vaccines have been developed for this purpose (7,11,12). Chromium III (Cr III), an essential trace element, has been demonstrated to possess immunomodulatory properties that can enhance the immune response in animals (3,15,39). Specifically, it has been shown to increase cytokine production and lymphocyte proliferation, making it a promising candidate as a vaccine additive (10,14). One frequently employed technique for assessing the immunological response to avian influenza vaccination is the HI test (1,4,18). Furthermore, changes in ALT/AST levels can indicate potential liver toxicity associated with vaccine additives, including Cr III (2,6,5,34). Therefore, it is critical to investigate the immunological and biochemical effects of Cr III supplement, to guarantee that avian influenza vaccinations are both effective and safe (19,20,21). This study aimed to estimate the immune response of highly pathogenic avian influenza vaccines rH5N1 and cH5N8, evaluate the effects of Cr III on the general immune response of avian influenza vaccines, and investigate the reaction between HPAI vaccines and Cr III supplements on liver activities.

MATERIALS AND METHODS

Study design

A total of three hundred of one day layer chicks for the (Lohmann Brown-Classic) one day old, were adopted. They were purchased from a licensed hatchery and divided into six equal groups housed in a separated room. The experiment lasted for 60 days as follows:

G1: Fifty chicks were given the (rH5N1) vaccine at dose (0.1 ml) S/C only two times (1, 30 days old).

G2: Fifty chicks were given the (cH5N8) vaccine at dose (0.1 ml) S/C only two times (1, 30 days old).

G3: Fifty chicks were given the vaccine (rH5N1) vaccine at dose (0.1 ml) S/C only two times (1, 30 days old), with (Cr III 1 g/L) in water for the rest of their lives.

G4: Fifty chicks were given the (cH5N8) vaccine at dose (0.1 ml) S/C only two times (1, 30 days old), with (Cr III 1 g/L) supplement in water for the rest of their lives.

G5: Fifty chicks are fed with (Cr III 1 g/L) supplement in water for the rest of their lives.

G6: Fifty chicks (control negative) were not supplemented or vaccinated.

Experimental location

This experiment was carried out at a poultry farm in the district of Al-Hira, province of Al-Najaf; the cages were 2.5×2×2 for each separated group. It was exactly 60 days in the interval from 17/2 – 18/4/2022.

Vaccination

Inactivated oil emulsion vaccine containing H5 HA of avian influenza virus rH5N1 strain generated by Baculovirus Expressed System Technology (B.E.S.T.) and a whole inactivated LaSota strain of Newcastle disease virus (Volvac® B.E.S.T.), and inactivated vaccines avian influenza cH5N8 (Animal Vaccine®) were given in (1, 30 day -old) with dose (0.1 ml) S/c.

Serum sampling

Five blood samples were withdrawn randomly from each group after 72 hr. of hatching to HI-test titer estimation of AIV maternal immunity. At the ages of 21, 42 and 60 days, five blood samples were drawn randomly from each group for the detection of AI immune response by the HI-test. Blood was drawn randomly at the ages of 30 and 60 days for the detection of liver enzymes ALT/AST levels.

Statistical analysis

The Statistical analysis system software has been done to determine the influence of variables on the study parameters. Significant comparisons among means were performed using the LSD test (29). (Analysis of Variance-ANOVA).

RESUITS AND DISCUSSION

Titer of AIV-Ab haemagglutination inhibition (HI) test

The results of HI test showed that at 21 days old high level of AIV-Ab in G3 (1024 ±63.8) when compared to the results of other groups with a statistically highly significant difference at the levels of ($P \leq 0.01$). There was no significant difference among G4,G1 and G2

respectively. On the other hand G5 and G6 showed lower levels (281.6 ±11.4 and 256.0 ±13.7 respectively) when compared to the results of other groups as in Table (1). At 42 days old, G3 and G4 revealed the highest (P≤0.01) followed by G2 and G1 (512.0 ±23.6 and 358.4 ±15.6 respectively). Additionally, G5 (256.0 ±13.7) was higher than G6 (115.2 ±7.2). At 60 days old, results of G3 (1638.4 ±65.2) and G4 (1469.4 ±55.8) were the highest (P≤0.01) followed by G1 (921.6 ±27.4) and then followed by G2 (716.8 ±23.8). Moreover, G5 (204.8 ±10.4) was higher than G6 (89.6 ±4.6). While the results among ages, there was no significant difference when comparing the results of AIV-Ab in G5. On the other hand, there was an increase with statistically highly significant difference when comparing the results of G1, G2, G3 and G4 at 21 days old with results of same groups at 42 days old. Results of these groups at 60 days old, also showed increase with statistically highly significant difference (P≤0.01) when comparing the results at 42 days old. Results of G6 showed decrease in the levels with a statistically highly significant difference between the levels at 21 days old when compared with the levels at 42 days old and 60 days old. The findings show that Cr III considerably increased immunity with recombinant vaccines as compared to the classical vaccine. In vaccinated groups, this immunity was significantly and particularly boosted, which compared to the other vaccinated groups, was much greater. In accordance with the control group, the H5N1 infection did not prompt a significant immune response. These results are similar to results obtained by Singh *et al.* (30) that showed the vaccines based on the conserved nucleoprotein and matrix protein especially (recombinant vaccines) primarily those determined by hemagglutination inhibition (HI), are the accepted surrogate measures of immune protection, recombinant vaccines have been shown to induce a cross-protective cell-mediated immune response, thereby reducing the morbidity associated with the disease. Also, Cheng *et al.* (9) and Srinivasan *et al.* (33) found that Cr III stimulates innate immunity. Also T lymphocyte, macrophage, and heterophil activity rises as a consequence of Cr III's immunomodulatory action, and antibody production also rises, increasing the potential of the humoral response (13,38). The influenza virus's negatively charged binding receptors may bind to positively charged proteins through oligosaccharides on the surfaces of host cells decreasing the viral titer and likely impairing virus entrance, humoral immunity (HI) levels of experimental chicken groups that were exposed to the AIV virus (H5N1) recombinant vaccines and were given a protective treatment of Cr III in their water supply (17). Inhibition of the virus-receptor hemagglutination mechanism prevents virus penetration into the host cell (17). The results obtained (22,23) recombinant vaccine was both quick-acting and efficient when birds produced antibody responses after vaccination and they shed noticeably less flu virus when challenged with a natural flu strain, suggesting that the birds would be less likely to spread infection. Even when birds received a lower dosage, high amounts of protective antibodies were still created. In contrast Inactivated vaccine (H5N8), Infected hens responded by expressing both cellular and humoral immune responses after receiving the H5N8 vaccine. Result showed (24,25,35) found increased cellular antiviral response, activation of natural killer cells, and proliferation of T helper 1 cells (IL-2) are all effects of H5N8. This result is similar to result of Chanthavanich *et al.* (8,2) and Rockman *et al.* (28) who found that (IL-2, IL-8, and IFN) were upregulated in response to the H5N8 vaccination. All of these elements may improve the ability of hens that have survived to handle infections. Infection with influenza viruses like H5N1 and H5N6 is characterized by an increase in IL-6 and IL-8, as well as interferons, which are factors in apoptosis (6,27). The H5N8 inactivated vaccines were found to induce humoral immune responses with higher titers of IgG, HI, NI and MN antibodies, with the H5N8 inactivated vaccine eliciting the highest antibody titers. The HI antibodies play an indispensable role in preventing viral infections. As well as higher HI antibody responses were produced by the H5N8 inactivated vaccination against homologous H5N8 viruses or other H5 viruses (H5N1 clade 1 and H5N6 clade 2.3.4.4d). This is consistent

with previous studies, Lee *et al.* (16) found that the H5N1 inactivated vaccine was unable to even induce NI and MN antibodies against H5N8 virus, indicating that H5N1 vaccine might not produce cross-neutralizing antibodies against the H5N8 virus.

Unexpectedly, the homologous HI antibodies stimulated by H5N8 classical vaccine were a little less than those who were stimulated by H5N1 inactivated recombinant vaccines. Since phenotypic diversity is one of the characteristics of H5 viruses, the antigenicity of viruses in various subclades varies widely, and genetic changes across strains may result in antigenic modification, resulting in different HI activities among viruses (6,36). Due to the lesser immunogenicity of H5 HA compared to H1 HA, prior research has also shown that the H5N8 vaccine could elicit robust seroprotective HI titers with several doses and adjuvants.

Table 1. Titer of AIV-Ab Haemagglutination inhibition (HI) test.

G/ DAYS Unit Ab.	Mean ± SE of AIV-Ab. 21 days old	42 days old	60 days old	LSD value
G1	409.6 ±18.1 B b	358.4 ±15.6 BC b	921.6 ±27.4 B a	182.36 **
G2	358.4 ±16.5 B b	512.0 ±23.6 B b	716.8 ±23.8 C a	169.07 **
G3	1024 ±63.8 A b	819.2 ±27.4 A b	1638.4 ±65.2 A a	252.34 **
G4	486.4 ±21.5 B b	716.8 ±19.4 A b	1469.4 ±55.8 A a	184.05 **
G5	281.6 ±11.4 C a	256.0 ±13.7 CD a	204.8 ±10.4 D a	92.33 NS
G6	256.0 ±13.7 C a	115.2 ±7.2 D ab	89.6 ±4.6 D b	119.51 **
LSD value	171.23 **	155.42 **	198.94 **	---

Means with different capital letters in the same column and small letters in the same row are significantly different. *(P≤0.05) **(P≤0.01). G1: rH5N1 vaccine only. G2: cH5N8 vaccine only. G3: rH5N1 vaccine with Cr III supplement. G4: cH5N8 vaccine with Cr III supplement. G5: Cr III supplement only. G6: (control negative) were not supplemented or vaccinated.

Comparison between difference groups and ages in Liver enzyme ALT

The results of ALT showed that at 30 days old levels in G5 and G3 respectively were the lowest (P≤0.01) when compared to other groups, followed by G4 and G1 respectively, and then followed by G6 and G2 respectively as in Table (2). At 60 days old, levels in G3 and G5 with same result (0.8 ±0.5) were the lowest (P≤0.05), followed by G1 and G4 respectively. G6 and G2 respectively were the highest (P≤0.05). There was no significant difference when comparing the results of ALT in all groups among different ages.

Comparison between difference groups and ages in liver enzyme AST

The results of AST showed that at 30 days Level in G5 (113 ±5.7) was the lowest (P≤0.01) when compared to other groups, followed by G2 and G1 respectively, levels in G6 (181 ±9.7) were the highest with (P≤0.01) as in Table (3). At 60 days old, levels in G6 (201 ±9.4) were the highest (P≤0.05) followed by G2, G1, G4, G5 and G3 respectively with lowest (P≤0.05). There was no significant difference when comparing the results of AST in all groups among different ages. The results of liver enzymes ALT and AST in this study were decreased in supplemented by Cr III groups compare to the un supplemented and control group. Zhu *et al.* (40) revealed that the liver is recognized as the primary organ for the metabolism, detoxification, and biological elimination of Cr III and other heavy metals. It also assists in the metabolism and elimination of other heavy metals and foreign substances. Hepatic injury is characterized by turbidity and the degeneration of central venous hepatocytes, and the severity of hepatic injury is strongly connected with the exposure to the poison. As well as, the structure and function of the liver are damaged under the action of

chromium ions (33,37). Also the AI vaccines showed mode of action effect on liver enzyme this agree with Sitohy *et al.* (31) revealed the recombinant vaccination of chickens by H5N1 lead to total reduction on levels chicken liver enzyme. This study concluded that the rH5N1 avian influenza vaccine showed a rapid onset and provided higher levels of protection compared to the cH5N8 vaccine. The supplementation of water-soluble Cr (III) improved the general immune response and liver health status.

Table 2. Comparison between difference groups and ages in Liver enzyme ALT.

G/ DAYS U/L	Mean ± SE of ALT 30 days old	60 days old	LSD value
G1	1.3 ±0.15 B a	1.7 ±0.25 AB a	0.287 NS
G2	1.8 ±0.26 A a	2.0 ±0.09 A a	0.304 NS
G3	0.8 ±0.05 C a	0.8 ±0.05 C a	0.179 NS
G4	±0.06 B a	1.4 ±0.18 BC a	0.326 NS
G5	0.6 ±0.02 C a	0.8 ±0.5 C a	0.266 NS
G6	1.9 ±0.18 A a	2.1 ±0.08 A a	0.287 NS
LSD value	0.498 *	0.603 **	---

Means with different capital letters in the same column and small letters in the same row are significantly different *(P≤0.05) **(P≤0.01). G1: rH5N1 vaccine only. G2: cH5N8 vaccine only. G3: rH5N1 vaccine with Cr III supplement. G4: cH5N8 vaccine with Cr III supplement. G5: Cr III supplement only. G6: (control negative) were not supplemented or vaccinated.

Table 3. Comparison between difference groups and ages in Liver enzyme AST.

G/ ADYS U/L	Mean ± SE of AST 30 days old	60 days old	LSD value
G1	146 ±6.7 B a	155 ±6.4 B a	16.44 NS
G2	148 ±8.1 B a	157 ±8.1 B a	15.97 NS
G3	122 ±5.8 BC a	129 ±6.3 B a	11.27 NS
G4	132 ±6.5 BC a	139 ±6.7 B a	13.08 NS
G5	113 ±5.7 C a	134 ±6.0 B a	23.54 NS
G6	181 ±9.7 A a	201 ±9.4 A a	20.09 NS
LSD value	27.69 **	31.74 *	---

Means with different capital letters in the same column and small letters in the same row are significantly different. *(P≤0.05). **(P≤0.01).). G1: rH5N1 vaccine only. G2: cH5N8 vaccine only. G3: rH5N1 vaccine with Cr III supplement. G4: cH5N8 vaccine with Cr III supplement. G5: Cr III supplement only. G6: (control negative) were not supplemented or vaccinated.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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