

## DETECTION OF INSULIN SECRETION AND LIPID PROFILE FOR CHROMIUM-SUPPLEMENTS AGAINST H5 INFLUENZA VACCINATION IN LAYERS

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### ABSTRACT

This study was aimed to determine whether chromium (Cr III) supplements have a beneficial effect on the insulin secretion and lipid profile of layers vaccinated against H5 avian influenza (AI). The study included serological parameter by ELISA kit and biochemical parameter for lipid profile test as well as to compare between highly pathogenic avian influenza (HPAI) recombinant (rH5N1) vaccine and classical (cH5N8) vaccine. The study was designed as follow: {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: is negative control with no vaccination or supplementation}. Levels of insuline at 21 days old there were showed a significant increase in G3 and G5 as compared with another groups. At 42 days old, G3, G4 and G5 revealed the highest levels as compared with another groups. Similarity results for 60 days old groups. The results of lipid profile test at 60 days old showed the lowest level of cholesterol in G5 as compared to another groups and lowest levels of vLDL in G3, G4 and G5. However, there were no significant differences in triglycerides (Tri.Gs), HDL and LDL levels in all groups. In conclusion, Cr III supplementation can improve insulin secretion in layers vaccinated with AI H5 vaccines and it has a positive impact on lipid profile.

**Key words:** pancreas activity, cholesterol, triglycerides, recombinant and classical vaccine.

طالب وعلوي

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الكشف عن إفراز الأنسولين وصورة الدهون في الدجاج البياض المدعم بالكروم والمحصن بلقاح إنفلونزا الطيور H5

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المستخلص

هدفت هذه الدراسة إلى تحديد ما إذا كان مكمل الكروم الثلاثي (Cr III) له تأثير مفيد على إفراز الأنسولين وصورة الدهون في الدجاج البياض المحصن بلقاحات إنفلونزا الطيور (H5). تضمنت الدراسة معيار مصلي وهو (ELISA) ومعيار كيميويحيوي يسمى اختبار صورة الدهون وكذلك للمقارنة بين لقاحي إنفلونزا الطيور شديد العدوى المؤتلف (rH5N1) والتقليدي (cH5N8). صممت الدراسة على النحو التالي: {G1: أعطيت rH5N1 فقط؛ G2: أعطيت cH5N8 فقط؛ G3: أعطيت rH5N1 مع Cr III؛ G4: أعطيت cH5N8 مع Cr III؛ G5: أعطيت Cr III فقط؛ G6: سيطرة سلبية بدون تطعيم أو مكمل}. أظهرت مستويات الأنسولين عند عمر 21 يوماً زيادة معنوية في G3 و G5 مقارنة بالمجموعات الأخرى. في عمر 42 يوماً، كشفت G3 و G4 و G5 عن أعلى المستويات مقارنة بالمجموعات الأخرى. كانت النتائج مشابهة في عمر 60 يوماً. أظهرت نتائج اختبار مستوى الدهون في عمر 60 يوماً أدنى مستوى للكوليسترول في G5 مقارنة بمجموعات أخرى وأدنى مستويات من vLDL في G3 و G4 و G5. ومع ذلك، لم تكن هناك فروق معنوية في مستويات الدهون الثلاثية و HDL و LDL في كل المجموعات. نستنتج من هذه الدراسة إنه يمكن لمكمل Cr III تحسين إفراز الأنسولين في الطبقات المحصنة بلقاحات إنفلونزا الطيور H5 ولها تأثير إيجابي على صورة الدهون.

الكلمات المفتاحية: نشاط البنكرياس، كوليسترول، دهون ثلاثية، اللقاح المؤتلف والتقليدي.

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## INTRODUCTION

Poultry production is a crucial industry that provides a significant source of protein for human consumption (2,6). However, infectious diseases, such as avian influenza, pose significant challenges to the poultry sector, resulting in reduced bird health and productivity (3,13,27). Vaccination is one approach used to control avian influenza in poultry flocks (5,7,28). However, vaccination can have a negative impact on the metabolic health of birds, including reduced insulin secretion and altered lipid metabolism (8). The crucial function that Cr III, an important trace metal, plays in glucose and lipid metabolism, insulin sensitivity, and pancreatic function in both humans and animals (4,24). In individuals with diabetes and the metabolic syndrome, supplementing with Cr III has been shown to enhance glycemic control and insulin sensitivity (10,12). Additionally, High-density lipoprotein (HDL) levels are reportedly elevated and triglyceride and cholesterol levels are decreased when Cr III supplementation is used (19,26). This research sought to determine how Cr III supplementation affected insulin secretion and lipid profile in layers vaccinated against H5 influenza vaccines. The results of the study could have significant implications for the poultry industry as it highlights a potential strategy to improve the metabolic health of birds, reduce economic losses, and improve food security.

## MATERIALS AND METHODS

### Study design

A total of 300 (Lohmann Brown-Classic) layer chicks are divided into six groups and raised from 1 to 60 days old. They were divided randomly into six groups; each group contained 50 chicks and was distributed as follows:

**G1:** Fifty chicks were given the (rH5N1) vaccine at dose (0.1 ml) subcutaneously (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 0.1 g/Kg) 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 0.1 g/Kg) supplement in water for the rest of their lives.

**G5:** Fifty chicks are fed with (Cr III 0.1 g/Kg) 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 poultry farm in district of Al-Hira/ province of Al-Najaf, the rooms was 2.5×2×2 m for each separated group. It was exactly 60 days in the interval of (17 of February up to 18 of April 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<sup>®</sup> B.E.S.T.), and inactivated vaccines avian influenza cH5N8 (Animal Vaccine<sup>®</sup>) 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 maternal immunity against avian influenza. At the ages of 21, 42, and 60 days, five blood samples were drawn randomly from each group for the detection of insulin secretion by the ELISA kit (Sunlong Biotech). Five blood samples was drawn randomly at the ages of 60 days for the detection of lipid profile test.

### 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 (23). (Analysis of Variance-ANOVA).

## RESULTS AND DISCUSSION

**Results of comparison between difference groups in concentration levels of insulin secretion:** The results of concentration levels of insulin secretion showed that at 21 days old in G5 and G3 (0.6662 ±0.11 and 0.6312 ±0.07) µIU/L respectively, were the highest (P≤0.05) followed by G4 and G2 respectively and then followed by G1 and G6 respectively as in

Table (1). At 42 days old, levels in G5, G3 and G4 ( $0.9332 \pm 0.12$ ,  $0.8842 \pm 0.06$  and  $0.7722 \pm 0.05$ )  $\mu\text{IU/L}$  respectively, were the highest followed by G6, G1 and G2 ( $0.1852 \pm 0.04$ ,  $0.1422 \pm 0.04$  and  $0.1132 \pm 0.04$ )  $\mu\text{IU/L}$  respectively, with statistically significant difference at the levels of ( $P \leq 0.05$ ). At 60 days old, levels in G5, G3 and G4 ( $1.2962 \pm 0.13$ ,  $1.1612 \pm 0.08$  and  $0.8442 \pm 0.05$ )  $\mu\text{IU/L}$  respectively, were the highest followed by G1, G2 and G6 ( $0.0662 \pm 0.01$ ,  $0.0472 \pm 0.01$  and  $0.0322 \pm 0.01$ )  $\mu\text{IU/L}$  respectively, with statistically significant difference at the levels of ( $P \leq 0.05$ ). While the results among ages, there was no significant difference when comparing the results of concentration levels of insulin secretion in G1, G2, G4 and G6. G5 was increased gradually among times at 21, 42 and 60 days old ( $0.6662 \pm 0.11$ ,  $0.9332 \pm 0.12$  and  $1.2962 \pm 0.13$ )  $\mu\text{IU/L}$  respectively, G3 was also increased gradually among times at 21, 42 and 60 days old ( $0.6312 \pm 0.07$ ,  $0.8842 \pm 0.06$  and  $1.1612 \pm 0.08$ )  $\mu\text{IU/L}$  respectively, both G5 and G3 were with statistically significant difference at the levels of ( $P \leq 0.05$ ). The insulin level in this study was increased in treated groups compare to control group. This increasing was specifically in vaccinated groups, which was significantly higher than the other treated groups and this result is similar to the findings of Qaid and Abdelrahman, (21) who found that insulin

regulates the metabolism of proteins, lipids, and carbohydrates, encouraging the uptake of amino acids and protein synthesis in addition to the utilisation of glucose. Although, insulin data reported is in accordance with Lu *et al.* (16); Moghetti and Tosi (20) they demonstrated that insulin levels decrease dramatically with aging and increased proportionally with age. A prior research also looked at the potential involvement of insulin as a growth agent in chick embryos. Additionally, the presence of the Cr III supplement in the organometallic molecule known as glucose tolerance factor (GTF) allows it to potentiate the action of insulin, which in turn has a significant impact on metabolism (15). Also Vincent (29) agree with study who demonstrated that four chromic ions must first be firmly bound by the oligopeptide low-molecular-weight chromium-binding protein (chromodulin) for the oligopeptide to achieve the conformation necessary for binding to the insulin receptor's tyrosine kinase active site. The metal-saturated oligopeptide chromodulin can bind to an insulin-stimulated insulin receptor, stimulating the receptor's tyrosine kinase activity, and binding chromic ions in response to an insulin-mediated chromic ion flux. Therefore, it seems that chromodulin contributes to an autoamplification process in insulin signaling (22).

**Table 1. Comparison between difference groups in concentration levels of insulin secretion**

G/ DAYS	Mean $\pm$ SE of Insulin $\mu\text{IU/L}$			LSD value
	21 days old	42 days old	60 days old	
G1	$0.287 \pm 0.02$ B a	$0.1422 \pm 0.04$ B a	$0.0662 \pm 0.01$ B a	0.217 NS
G2	$0.342 \pm 0.05$ AB a	$0.1132 \pm 0.04$ B a	$0.0472 \pm 0.01$ B a	0.307 NS
G3	$0.6312 \pm 0.07$ A b	$0.8842 \pm 0.06$ A ab	$1.1612 \pm 0.08$ A a	0.382 *
G4	$0.5472 \pm 0.09$ AB a	$0.7722 \pm 0.05$ A a	$0.8442 \pm 0.05$ A a	0.311 NS
G5	$0.6662 \pm 0.11$ A b	$0.9332 \pm 0.12$ A ab	$1.2962 \pm 0.13$ A a	0.381 *
G6	$0.2862 \pm 0.04$ B a	$0.1852 \pm 0.04$ B a	$0.0322 \pm 0.01$ B a	0.292 NS
LSD value	0.328 *	0.377 *	0.412 *	---

Means with different big letters in the same column and small letters in the same row are significantly different. \* ( $P \leq 0.05$ ). 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 in lipid profile at 60 days old:** The results of cholesterol showed that levels in G6 (115

$\pm 6.1$ ) mg/dl were the highest followed by G1, G2, G4 and G3 ( $114 \pm 6.3$ ,  $113 \pm 4.9$ ,  $92 \pm 3.8$  and  $89 \pm 2.8$ ) mg/dl respectively, then G5

(85 ±2.3) mg/dl, with statistically significant difference at the levels of  $P \leq 0.05$  (Table 2). The results of LDL showed that levels in G1, G6 and G2 (47 ±2.5, 46 ±2.6 and 45 ±2.6) mg/dl respectively, were the highest followed by G4, G5, and G3 (28 ±1.7, 21 ±0.95 and 20 ±1.5) mg/dl respectively, with statistically highly significant difference at the levels of  $P \leq 0.01$  (Table 2). There was no significant difference when comparing the results of TriGs, HDL and LDL in all groups. The results of total cholesterol were agreement with study by Sahin *et al.* (22), Moeini *et al.* (19) and Habibian *et al.* (9), they discovered that chicks that received vaccinations and a diet enriched with CrIII had lower serum cholesterol. Broiler chicken meat's cholesterol level may be decreased using additional Cr III. According to Mir *et al.* (18), broiler chicken diets containing 10% flaxseed lowered the fat percentage and cholesterol content of the muscles in the breast and thighs, and Cr III supplementation gradually decreased these factors. Additionally, the current meta-analysis revealed that while supplementary Cr III had no discernible impact on the relative weight of the liver, which in birds serves as the primary site for the production of fatty acids, cholesterol, and phospholipids, and belly fat, it had a positive impact on the blood lipid profile. As well as Marmett and Nunes (17)

Found that the hepatic enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase has been proven to be suppressed by chromium, which lowers fat production. Although, the effects of Cr III supplementation (200, 400, and 800 g/kg diet) as CrPic on broiler chickens were examined by Lee *et al.* (14) in two experiments. They discovered that CrPic at the dose of 200 g/kg diet decreased the blood cholesterol concentration at 3 weeks in the first experiment, but the blood LDL, HDL, and triglycerides concentrations did not change. While the results was disagreement with study by Xiao *et al.* (30) and Toghyani *et al.* (25) who found that the administration of Cr III to the chickens diet have no effect on cholesterol levels and Triglyceride was not significantly affected in treated groups. Also the results by Moeini *et al.* (19) and Habibian *et al.* (9) who found that the levels of Tri.Gs. was not affected by vaccination or addition of Cr III to the diet. The results for HDL and Serum LDL was in accordance with results obtained by Akbari and Toriki (1); Jahanian and Rasouli (11) showed that the level of HDL and VLDL was not affected by Cr III administration and vaccination, while, LDL levels was reduced in chickens feed with Cr III supplement. Finally, a number of these research findings indicated that the effects of Cr III supplements on the blood lipid profile of broilers varied

**Table 2. Comparison between difference groups in lipid profile at 60 days old**

G/ Types	Mean ± SE of lipid profile (mg/dl)				
	Cholesterol	Tri.Gs	HDL	VLDL	LDL
G1	114 ±6.3 AB	45 ±2.6	58 ±2.9	9 ±0.42	47 ±2.5 A
G2	113 ±4.9 AB	50 ±2.8	58 ±2.9	10 ±0.63	45 ±2.6 A
G3	89 ±2.8 AB	55 ±2.7	58 ±2.7	11 ±0.68	20 ±1.5 B
G4	92 ±3.8 AB	55 ±2.7	53 ±2.4	11 ±0.68	28 ±1.7 B
G5	85 ±2.3 B	45 ±2.6	55 ±2.6	9 ±0.42	21 ±0.95 B
G6	115 ±6.1 A	55 ±2.7	58 ±2.9	11 ±0.68	46 ±2.6 A
LSD value	27.53 *	12.07 NS	6.81 NS	3.79 NS	11.47 **

Means with different big letters in the same column. \* ( $P \leq 0.05$ ), \*\* ( $P \leq 0.01$ ). 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

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