

## EFFECT OF IN-OVO INJECTION OF BLOOD PLASMA FROM IRAQI LOCAL CHICKEN ON HATCHING TRAITS AND IMMUNE PARAMETERS OF BROILERS HATCHING EGGS

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### ABSTRACT

The current research was conducted to investigate the impact of injecting hatching eggs with varying levels of plasma of local chickens. In this investigation, 180 fertile eggs were divided into three groups. The first group (**P0**) is the control, while the eggs in both the second(**P1**) and third groups (**P2**) were injected with 50 or 100 µl of local chicken blood plasma, respectively. The eggs were injected at the first day of incubation (0 day). After 21 days, the fertility, hatching characteristics, immunological, and physiological properties of the hatched chicks were evaluated. The results revealed no significant variations in the hatching percentage, weight of the hatched chicks, and their relative weight. The physiological blood traits revealed that the third treatment showed a significant rise in red blood cells, hematocrit value, and hemoglobin with a significant decrease in white blood cells. The third treatment showed a considerable rise in the total protein concentration due to the higher globulin protein level, which is vital for immunity, and improved antioxidant glutathione levels. The treatments had no significant effect on the relative weights of the heart, liver, and residual yolk in the intestine at one day of life. Overall, it can be concluded that injecting local chicken blood plasma did not affect hatchability. This was accompanied by an improvement in the immune status of the hatched chicks, as evidenced by a decline in the white blood cell count and a rise in globulins.

**Keywords:** In-ovo injection, plasma, chicken, immunity

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### INTRODUCTION

The practice of injecting hatching eggs is now widely common in hatcheries on a

global level, as it improves the subsequent growth of the chicks, without compromising the development of embryos throughout the embryonic growth stages or the rate of hatching (Ohta *et al.*, 2001). There is a unique method of providing nutrients to the embryo throughout the incubation stage through in-ovo injection (Macalintal, 2012).

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Feeding the embryo before hatching, by injecting nutrients, is predicted to raise the rate of hatching and assist the embryo in hatching effectively, by ensuring the emergence of chicks out of their eggshell, developing the digestive system, and increasing the weight of the body (Uni and Ferket, 2004). Bhanja *et al.* (2012) confirmed that the injection of fertilized eggs with certain nutrients (like vitamins) is supportive in terms of improving the weight of the body throughout the period of rearing. The processes of selection and genetic improvement on broiler hybrids towards increasing their growth and achieving high weights within short periods have led to an increase in mortality rates due to the birds' sensitivity to various diseases, as there is a negative correlation between body weight and the immune response (Qureshi and Havenstein, 1994).

Many methods have been used to enhance the immunity of hatched chicks. Some of them are related to feeding the mothers, while others are directed towards feeding the embryos inside the hatching eggs, which is one of the techniques for early feeding of bird embryos to produce healthy chicks with high productivity (Zhava and Ferkat, 2005). The process of hatching egg injection with nutrients involves introducing various elements into the egg in the form of liquids. The suggested nutrients are acids (Ohta *et al.*, 2001), sugars such as maltose, amino acids, sucrose or dextrin (Uni *et al.*, 2005), vitamins such as folic acid (Ghane-Khoshkebijari *et al.*, 2024), or injecting probiotics, which are also called competitive exclusion cultures (Castañeda Bustillo, 2020).

Due to the lack of studies concerning injecting broiler embryos with the plasma of local chickens, this study was conducted to determine the impact of injection fertilized eggs with various levels of plasma taken from local chickens on some hatching characteristics, the quality of hatched chicks, and their physiological traits.

## MATERIALS AND METHODS

This study was performed at the Poultry Field and Laboratories of the Animal Production Department, College of Agriculture, University of Tikrit, and the objective was to study the influence of injecting hatching eggs with local Iraqi chicken blood plasma on hatching traits and immune parameters.

### Obtaining the Blood Plasma:

Plasma was obtained by collecting blood from a flock of local Iraqi chickens in the farms of the Department of Animal Production. The blood was taken from the wing vein with a volume of 5 ml per bird, and placed in convenient blood collection tubes that contain an anticoagulant (K-EDTA). The blood was collected from 10 randomly selected birds. After allowing the blood to settle, it was centrifuged at 3500 rpm for 15 minutes. After that, the plasma was separated from the cellular components and extracted using a micropipette, placed in clean glass tubes, and subsequently used for egg injection.

**Source of Hatching Eggs:** In this study, 180 fertilized eggs from a 42-week-old Ross 308 broiler breeder flock, reared on the floor with a 9 male:1 female ratio, were used. The eggs were taken from the Al-Mu'tasim fields near Samarra and were kept for only 24 hours at room temperature.

### Egg Treatment:

After obtaining the eggs, they were inspected, and unsuitable eggs, with cracks or fractures, were excluded. The chosen 180 eggs were disinfected using 75% ethanol, labeled, and individually weighed using a sensitive electronic scale with two decimal places. They were randomly divided into three treatments (60 eggs per treatment) with three replicates of 20 eggs each.

### Injection Method and Timing:

The manual method was used with an insulin syringe of 0.5 ml capacity and a 27 G

needle, drilled with a pointed drill to avoid shell cracks. The injection was performed at 0 day of incubation, before placing the eggs in the incubator (Bertin *et al.*, 2009), at a depth of 2-3 mm below the air cell (Weber *et al.*, 2004). After disinfecting with 75% ethanol and wiping with medical cotton, the injection spot was sealed with nail polish (Aljumaily and Taha, 2019), and the eggs were placed in the incubator. An Italian-made automatic incubator, FLEM. was used for the process of incubation.

### Experimental Design:

- Group 1 (P0): No injection.
- Group 2 (P1): Injection of hatching eggs with 50 µl of local chicken blood plasma.
- Group 3 (P2): Injection of hatching eggs with 100 µl of local chicken blood plasma.

### Studied Traits:

**Hatching Percentage:** Hatching percentage was calculated as indicated by Tona *et al.*, (2003).

**Embryonic Mortality Rate:** The stage of embryonic mortality was determined as indicated by Pedroso *et al.* (2006) by breaking unhatched eggs remaining in the incubator after 21 days of incubation. Embryonic mortality was divided into early stage (1-5 days), middle stage (6-14 days), and late stage (15-18 days) by weighing the deceased embryo as per the method of Khalil (2009).

**The Average Weight of The Hatched Chicks:** The hatched chicks were weighed in the hatchery using a sensitive scale (Citizen model Fr-H1200, with 0.01 precision), and the average weight at hatching was calculated for each replicate in the experiment.

### Relative Weight of Hatched Chicks:

It was calculated according to the formula indicated by Reijrink *et al.* (2009):

### Blood Collection and Plasma Extraction:

To analyze the physiological features of the hatched chicks, blood was obtained from five chicks per treatment by cutting the

jugular vein. The blood was deposited in specialized blood collection tubes containing an anticoagulant (K-EDTA) and then separated into two parts:

The first portion was utilized for completing the blood profile tests, including the total number of red blood cells, the number of white blood cells, the hematocrit value, the concentration of hemoglobin, and the constants of the red blood cells. This was performed by following the approach suggested by Campbell (1995). The second phase was performed to collect blood plasma by centrifugation (3000 rounds per minute) for 20 minutes. After that, the plasma was deposited in special plastic tubes and stored until biochemical blood tests were performed, including glucose concentration, cholesterol, total protein, albumin, globulin, AST enzyme, ALT enzyme, and glutathione concentration. All tests were completed using ready-to-use test kits, except for glutathione, which was quantified according to the method specified by Taha (2021).

### Statistical analysis:

The statistical analysis software SAS (2011) was utilized for data analysis. The Complete Randomized Design (CRD) was applied to evaluate the data, and significant differences between treatments were tested using Duncan's Multiple Range Test (1955).

## RESULTS

### Hatching Traits

The results in Table (1) show the impact of injecting the hatching eggs with various concentrations of local chicken blood plasma on some hatching traits of ROSS-308 broiler breeder eggs. There were no significant differences in the percentage of hatching for fertilized eggs. Also, there were no significant differences in the weight of the hatched chicks or their relative weight to the egg.

The results in Table (2) show a significant increase in the total number of red blood cells, hematocrit values, and hemoglobin

concentration in hatched chicks from eggs injected with higher plasma (P2), compared to hatched chicks from eggs injected with plasma (P1) or the untreated control group. Additionally, the treatment of hatching eggs with higher plasma (P2) resulted in a significant decrease in the total number of white blood cells compared to the first and second groups. There were no significant effects of the treatments on the mean

corpuscular volume or the mean concentration of the hemoglobin. The decrease in the number of white blood cells in chicks hatched from eggs injected with chicken blood plasma can be explained by the plasma's effect on the chicks' immune system. Plasma injection may have caused changes in immune balance, leading to a reduction in the production or activity of white blood cells.

**Table 1:** The effect of in-ovo injection of blood plasma from Iraqi local chicken on some hatchability characteristics of hatched chicks.

Traits Treatment	Hatchability %	Weight of chicks/g	Relative weight of hatched chicks %
P0	94.3± 2.96	43.2± 1.36	75.9 ± 2.48
P1	86.0 ± 2.92	43.6± 0.13	74.7± 0.62
P2	85.6 ± 3.05	42.8± 0.58	73.0± 0.47±

- No significant differences between the the means at the probability level ( $p \leq 0.05$ ).
- (P0): No injection. (P1): Injection with 50 µl of local chicken blood plasma/egg. (P2): Injection with 100 µl of local chicken blood plasma/egg.

**Table 2:** Effect of in-ovo injection of blood plasma from Iraqi local chicken on immune parameters and some physiological blood characteristics of hatched chicks.

Treatment Traits	P0	P1	P2
Erythrocytes *10 <sup>6</sup> /µl	1.18 ±0.10 b	1.30± 0.06 b	1.76 ± 0.13 a
Leukocytes *10 <sup>3</sup> /µl	7.26 ±0.66 a	8.57± 0.65 a	3.30 ± 0.66 b
PCV%	26.6 ± 2.84 b	27.6 ± 0.88 b	34.3 ± 1.20 a
Hb g/100 ml	8.0 ± 0.86 b	8.3± 0.26 b	10.4 ± 0.36 a
MCV fml	224.6 ± 9.22	210.4± 5.27	197.4 ± 21.33
MCH	68.0 ± 2.79	63.7 ± 1.59	59.6 ± 6.46

- The different letters in the column refer to statistically significant differences between the averages of the coefficients at the probability level ( $p \leq 0.05$ ). Otherwise, no.
- (P0): No injection. (P1): Injection with 50 µl of local chicken blood plasma/egg. (P2): Injection with 100 µl of local chicken blood plasma/egg.

### Biochemical Blood Traits:

The results in Table 3 show the effect of injecting hatching eggs with different concentrations of local chicken blood plasma on some biochemical blood traits. Regarding serum proteins, a significant increase in total protein concentration in (P2) group, compared to the (P1) group, although there is no significant differences between (P2) group and the control group. For albumin concentration, no significant difference was observed between the treatments and the control group. Although injecting higher plasma volume had a significant rise in the globulin protein concentration, compared to

the first and second treatments, the lower plasma volume recorded a significant decrease in the globulin concentration compared to the control.

The treatments had no significant effect on the glucose and cholesterol concentrations in blood plasma. Similarly, no significant differences were observed in the stress indicators, represented by ALT enzyme activity and glutathione levels. However, AST enzyme activity showed a significant increase in favor of the higher plasma injection (P2), compared to the control group only.

**Table 3:** Effect of in-ovo injection of blood plasma from Iraqi local chicken on immune parameters and some biochemical blood characteristics of hatched chicks.

Treatment	P0	P1	P2
Traits			
Total protein (g/100ml)	4.0 ± 0.21 ab	3.5 ± 0.35 b	4.5 ± 0.24 a
Albumin (g/100ml)	1.8 ± 0.15	1.9 ± 0.08	1.9 ± 0.15
Globulin (g/100ml)	2.2 ± 0.05 b	1.6 ± 0.20c	2.8 ± 0.15 a
Glucose (mg/100 ml)	200.0 ± 10.6	196.6 ± 16.1	216.3 ± 10.2
Cholesterol (mg/100 ml)	182.5 ± 7.2	167.5 ± 10.4	172.4 ± 7.6
AST (IU/L)	91.0 ± 2.08 b	102.3 ± 4.4ab	110.0 ± 7.54a
ALT (IU/L)	81.0 ± 4.40	68.6 ± 5.54	73.3 ± 1.52
Glutathione μmol/l	1.3 ± 0.20b	2.0 ± 0.10a	2.5 ± 0.21a

- The different letters in the column refer to statistically significant differences between the averages of the coefficients at the probability level ( $p \leq 0.05$ ). Otherwise, no.
- (P0): No injection. (P1): Injection with 50 μl of local chicken blood plasma/egg. (P2): Injection with 100 μl of local chicken blood plasma/egg.

### Relative Weights of Certain Vital Organs

The results in Table (4), showed no significant effects on the relative weights of

the heart, liver, and residual yolk in the intestine as a result of treating hatching eggs with local chicken blood plasma.

**Table 4:** Effect of in-ovo injection of blood plasma from Iraqi local chicken on the relative weights of the heart, liver, and remaining yolk of the hatched chicks.

Treatment	Traits		
	Heart %	Liver %	Intestinal residual yolk %
P0	0.72 ± 0.07	2.3 ± 0.06	10.8 ± 1.15
P1	0.80 ± 0.04	2.5 ± 0.11	12.7 ± 1.13
P2	0.71 ± 0.01	2.1 ± 0.14	12.6 ± 0.78

- No significant differences between the means of the coefficients at the probability level ( $p \leq 0.05$ ).
- (P0): No injection. (P1): Injection with 50 μl of local chicken blood plasma/egg. (P2): Injection with 100 μl of local chicken blood plasma/egg.

## DISCUSSION

The effect of in-ovo injection of blood plasma from Iraqi local chicken on some hatchability characteristics of hatched chicks revealed no negative effects of injecting local chicken blood plasma on the hatching percentage, the weight of the hatched chicks, and their vitality (Emam *et al.*, 2022). The vascular system begins to form during the first hours of the second day of incubation, where the heart and red blood cells originate. The heart originates from the mesoderm of the thoracic cavity, initially forming a tube, while red blood cells originate from the extraembryonic mesoderm, starting as clusters called blood islands. These blood islands, then, connect and fuse to form a plexus of blood vessels. The umbilical

mesenteric arteries, branching from the aorta, meet the vascular plexus, forming the circulatory system. This system connects the embryo to the yolk sac in a tightly regulated manner, allowing the heart to start pumping the blood (red blood cells and extracellular fluid) through the arteries and veins, which leads to the complete development and functionality of the heart (Lansford and Rugonyi, 2020).

It has been mentioned above and observed that the most important components are red blood cells, which require two essential elements for their formation: the globin protein and the heme (Xing *et al.*, 2022). Proteins naturally require specific types and numbers of amino acids to form in the ribosomes that are located on the rough

endoplasmic reticulum (Givisiez *et al.*, 2020). Moreover, the presence of iron is necessary. Therefore, it is believed that the increase in the number of red blood cells could be due to the increased availability of amino acids used in building the globin protein.

Regarding the significant increase in the globulin concentration and the decrease in white blood cell numbers in the injection treatments, the plasma may have provide an immune role that impact the development of white blood cells in newly hatched chicks (Taha *et al.*, 2019).

Alhayaly *et al.* (2024a) and Soslau, (2020) observed an immune role for platelets during embryonic stages, which might explain the increased globulin protein concentration and the total protein in the serum of hatched chicks from eggs treated with regular or platelet-rich plasma from local Iraqi chicken blood. Alhayaly *et al.* (2024b) and Al-Hadithi, (2002) previously indicated a significant increase in globulin protein, especially gamma globulin, in the serum of local chickens compared to acclimated chickens.

For the significant increase in red blood cell numbers and related blood traits, Campbell (1995) found that all blood traits usually have a positive correlation on normal growth conditions for birds. Therefore, it is expected that the increased number of red blood cells is the primary reason for the increased PCV and Hb percentages in the second and third treatments with plasma.

Regarding the antioxidant status indicator (glutathione), the availability of amino acids in plasma injection treatments may play a fundamental role in the synthesis of enzymes, which are necessary for the glutathione cycle that demands the activity of glutathione peroxidase. In addition, these treatments might provide the essential amino acids that are necessary for the synthesis of glutathione itself, potentially explain the

elevated glutathione levels in the blood plasma of hatched chicks.

## CONCLUSIONS

Injecting local chicken blood plasma did not have a negative impact on the hatching percentage or the weight of hatched chicks. This was accompanied by an improvement in the immune status of the hatched chicks, evidenced by a decline in the white blood cell count and a rise in the globulins. Additionally, the hatched chicks obtained from plasma-treated eggs showed improvements in red blood cell traits and their indicators.

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## تأثير حقن بلازما الدم المستخلص من الدجاج العراقي المحلي على صفات الفقس والمعلقات المناعية لبيض تفريخ البدارى

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تم إجراء الدراسة الحالية للتحقيق في حقن ببيض الفقس لبدارى التسمين بمستويات متفاوتة من البلازما المستخلصة من دم الدجاج المحلي. في هذا التحقيق، تم استخدام ١٨٠ بيضة خصبة وتم تقسيمها إلى ثلاثة مجموعات. كانت المعالجة الأولى (P0) بمثابة المجموعة الضابطة، بينما تم حقن البيض في كل من المجموعة الثانية (P1) والثالثة (P2) ب ٥٠ و ١٠٠ ميكرو لتر من بلازما دم الدجاج المحلية على التوالي. تم إجراء الحقن في بداية التفريخ عمر ٠ يوم ، وبعد ٢١ يوما ، تم تقييم الخصوبة وخصائص الفقس والخصائص المناعية والفسولوجية للكتاكيت المفرخة ، ولوحظت النتائج التالية: لم تكن هناك اختلافات معنوية في نسبة الفقس ووزن الكتاكيت المفرخة ووزنها النسبي. اما بالنسبة لسماوات الدم الفسيولوجية: أدى حقن الكمية الاعلى من البلازما في المجموعة الثالثة إلى ارتفاع كبير في خلايا الدم الحمراء وقيمة الهيماتوكريت والهيموجلوبين مع انخفاض كبير في خلايا الدم البيضاء. كما أظهر أيضا حقن الكمية الاعلى من البلازما ارتفاعا كبيرا في تركيز البروتين الكلي بسبب ارتفاع مستوى بروتين الجلوبيولين ، وهو أمر حيوي للمناعة، إلى جانب تحسين مستويات الجلوتاثيون المضادة للأكسدة. لم يكن لحقن البلازما تأثير كبير على الأوزان النسبية للقلب والكبد وصفار البيض المتبقي في الأمعاء في اليوم الاول من الفقس. بشكل عام ، يمكن الإستنتاج أن حقن بلازما دم الدجاج المحلية لم يؤثر على قابلية الفقس. ورافق ذلك تحسن في الحالة المناعية للكتاكيت المفرخة، كما يتضح من انخفاض عدد خلايا الدم البيضاء وارتفاع الجلوبيولين. وعليه، فيمكن اعتماد هذه الطريقة لإنتاج كتاكيت ذات كفاءة دموية ومناعية افضل من الكتاكيت العادية.