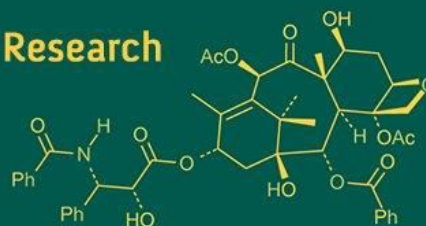


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(1): 01-03
www.biochemjournal.com
Received: 02-10-2024
Accepted: 06-11-2024

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Enhanced of in ova injection of annatto seed extract in administration hatching traits of broiler (Ross 308)

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DOI: <https://doi.org/10.33545/26174693.2025.v9.i1a.3400>

Abstract

This study was conducted at Al-Jaflawi Poultry Hatchery/Babil Governorate from November 22, 2024 to November 25, 2024 to study the effect of injecting hatching eggs with different levels of aqueous extract of annatto seeds on some hatching characteristics of broiler chicks exposed to nutritional fasting. The experimental groups were the first group without injection and the second, third and fourth groups injected with 0.1, 0.2, and 0.3 from aqueous extract of annatto seeds into the amniotic sac. The results showed, significant increase for T₄ in hatching ratio, significant increase in embryo mortality for T₁ compared other groups, the T₂ and T₃ was the heights significantly in chicks weight at hatching and 12 hours after hatching, while the in ova injection groups was the best in glycogen level in the breast muscle, liver and heart.

Keywords: Al-Jaflawi poultry hatchery, Babil governorate, hatching eggs, aqueous extract

Introduction

Poultry meat is considered one of the most important sources of protein food for the world's population, and the use of antioxidants can improve the productive efficiency of broilers (Al-Jebory *et al* 2023 a,b,c,d; Al-Jebory *et al.*, 2021; Ajafar *et al.*, 2024) [4, 5, 6, 7, 3, 9]. Recently, Hatched chicks are exposed to fasting for a long period that may reach 48-72 hours due to the counting, sorting and vaccination of chicks against diseases, which may expose them to stress, loss of energy and exposure to oxidative stress (Kadhim *et al.*, 2021) [18], research has focused on the use of natural antioxidants because consumers prefer foods containing natural ingredients rather than synthetic ones due to their health benefits, in addition to improving the nutritional value and quality of meat (Fasseas *et al.*, 2008; Al-jebory *et al.*, 2021) [16, 18]. Antioxidants from natural sources have been widely used either in folk medicine or as traditional food ingredients or additives in various recipes (Chan *et al.*, 2011; Shahidi and Ambigaipalan, 2015) [12, 21]. Another reason for using natural antioxidants is to avoid carcinogenic substances due to the side effects associated with long-term consumption of some synthetic antioxidants. Annatto is a perennial shrub of the Bixaceae family and the genus Bixa, native to the tropical forests of central and South America and Brazil. Annatto contains thirty-five components, with bixin and norbixin appearing to be the largest components. Bixin is more abundant than norbixin, accounting for 2-5% of the seed, but the content can reach more than 7% of the dry weight of the seeds (Dallorso, 2008) [13]. Other components of Achiote include: acetone, eotene, tomentholic acid, carotenoids, methyl ester, bixin, apocarotenoids, and orelin. In addition to these compounds, it also contains fatty acids (Enciso *et al.*, 2009) [15]. In the leaves we find: Bixaganene, ishwano (essential oil) among other mono and flavonoids, 7-apigenin bisulfate, 7-luteolin bisulfate, apigenin bisulfate, and lactones. Carotenoids include: bixin, norbixin, orelin, β -carotene, cryptoxanthin, methylbixin, zeaxanthin, lutein, vitamins (A, B, D), proteins, sugars, cellulose, lipids, calcium, iron, phosphorus, acetone, alkaloids and phytolic acid (Huaman *et al.*, 2009) [17]. In the seeds we find: carotenoids expressed as provitamin A (1000 - 2000 IU/g of dry seeds), among which are: bixin, betabixin, methylbixin, norbixin, orelin, zeaxanthin, β -carotene, lutein, and cryptoxanthin. They also contain sodium bixinate, achiote, tomentholic acid, pectin, proteins, tannins, and a sesquiterpene hydrocarbon, ischauran (seed flower essence).

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The seeds also contain silica, potash, a high phosphorus content, a low calcium content, and a high protein content that includes adequate levels of tryptophan and lysine, but low levels of methionine, isoleucine, lysine, phenylalanine, and threonine (Enciso *et al.*, 2009) [15]. Therefore, the current study aims to inject hatching eggs with aqueous extract of annatto seeds and study its effect on hatching characteristics of broilers.

Materials

This experiment was conducted at Al-Jaflawi Poultry Hatchery from November 22, 2024 to November 25, 2024. to study the effect of injecting hatching eggs with different levels of aqueous extract of annatto seeds on some hatching characteristics of broiler chicks exposed to nutritional fasting. The experimental groups were: T₁ is free, T₂, T₃, and T₄ injected with 0.1, 0.2, and 0.3 ml aqueous extract of annatto seeds / egg in amniotic sac. Exposing chicks to a 12-hour feeding fast after hatchery according to (Kadhim *et al.*, 2021) [18]. The traits were measured on the day of hatching, where the hatching characteristics were measured according to (Ajafar *et al.*, 2024) [1]. Glycogen was also measured in the heart, liver and muscles according to (Al-Saedi *et al.*, 2024).

Use the statistical analysis according to the design (CRD) (SAS, 2012: Duncan, 1955) [20, 14] and use the mathematical model: $Y_{ij} = \mu + T_i + e_{ij}$

Results and Discussion

1. Hatching traits

Noted in table 1: Effect of treatments in hatching percentage the T₄ treatment increase significantly ($p \leq 0.05$) on other treatments and T₂, and T₃ increase compared to T₁, while in mortality percentage the T₁ treatment increase significantly ($p \leq 0.05$) compared injection treatments.

Table 1: Effect of in ova of annatto seed extract in hatching traits

Treatments	Hatching percentage %	Mortality percentage %
T ₁	84.10±1.00 c	15.90±3.00 a
T ₂	86.75±1.50 b	13.25±0.26 b
T ₃	85.46±0.25 b	14.34±1.00 b
T ₄	87.43±1.24 a	12.37±0.36 c
P- value	*	*
Means with different letters, different significantly at ($p \leq 0.05$).		

Table 2: shown the impact of study in chicks weight, at hatching the T₂, and T₃ increase significantly ($p \leq 0.05$) on T₁, while at 12 hours after hatching the T₂, and T₃ increase significantly ($p \leq 0.05$) on T₁, and T₄, the T₄ increase on T₁.

Table 2: Effect of in ova of annatto seed extract in chicks weight (gm)

Treatments	Weight at hatching	Weight at 12 hours
T ₁	43.52±0.37 b	41.90±0.10 c
T ₂	44.45±0.04 a	43.37±0.37 a
T ₃	44.20±0.45 a	43.47±0.47 a
T ₄	44.03±0.73 ab	42.40±0.60 b
P- value	*	*
Means with different letters, different significantly at ($p \leq 0.05$).		

2. Glycogen

Table 3: shown the effect of study in glycogen level of liver, heart, and breast muscle. In liver, the T₃ treatment came height significantly ($p \leq 0.05$) compared to T₁, T₄, and T₂,

and T₄ increase on T₁ treatment. In heart (table 3) the T₃ treatment was height significantly ($p \leq 0.05$) compared to other treatments, T₄ increase significantly on T₁, and T₂. At same time in breast muscle, the T₄ treatment was height significantly ($p \leq 0.05$) compared to other treatments, while T₂, and T₃ increase on T₁ treatment.

Table 3: Effect of in ova of annatto seed extract in liver glycogen

Treatments	Liver glycogen
T ₁	36.42±4.02 c
T ₂	39.32±4.18 ab
T ₃	40.15±1.63 a
T ₄	38.48±2.09 b
P- value	*
Means with different letters, different significantly at ($p \leq 0.05$).	

Table 4: Effect of in ova of annatto seed extract in heart glycogen

Treatments	Heart glycogen
T ₁	11.15±0.84 c
T ₂	14.95±0.95 c
T ₃	24.98±1.83 a
T ₄	21.85±2.32 b
P- value	*
Means with different letters, different significantly at ($p \leq 0.05$).	

Table 5: Effect of in ova of annatto seed extract in breast muscle glycogen

Treatments	Breast muscle glycogen
T ₁	15.41±1.88 c
T ₂	20.34±1.61 b
T ₃	22.94±1.25 b
T ₄	29.44±0.44 a
P- value	*
Means with different letters, different significantly at ($p \leq 0.05$).	

The improvement in hatching characteristics and the improvement in the level of glycogen in the heart, liver and chest muscle of the hatched chicks in the treatments of injection of the aqueous extract of annatto seeds compared to the first treatment may be due to the seeds containing phenolic substances that act as natural antioxidants that improve the hatchability and growth of the chicks, which is reflected in the weight of the chicks upon hatching (Alasalvar *et al.*, 2001) [2]. Annatto seeds also contain high levels of carotenoids of the type's bixin and norbixin, and these two types can be stored in the liver and then can be converted into vitamin A when needed. Vitamin A improves the secretion of thyroxine hormone by acting on the thyroid gland, and thyroxine hormone has an important role in the metabolism of proteins and fats in the feed. In addition, annatto seeds contain active substances of phenolic compounds and flavonoids that enhance antioxidants in the body, which improved the level of glycogen in the body, indicating that they are not exposed to Hatched chicks are stressed (Ambrosio *et al.*, 2006; Braz *et al.*, 2007) [10, 11]. The exposure of chicks to fasting is an obvious matter and occurs in the hatchery as a result of waiting for the chicks after hatching to dry and then carrying out the counting and sorting operations, vaccination and transfer to the rearing halls, which exposes them to stress and this affects the immunity of the chicks and the growth rate (Kadhim *et al.*, 2021) [18]. The improvement of the annatto extract injection treatments may be due to the annatto seeds containing tocopherols and tocotinols, which work to develop the

immune response and its positive reflection on improving the ratio of heterogeneous cells to lymphocytes, in addition to protecting the cell membrane from oxidation and protecting the active enzyme sites and DNA from free radical damage. Therefore, protecting the cell membranes and not exposing them to oxidation led to an increase in the activity of oxidation enzymes and thus an increase in the number of red blood cells. This increase led directly to an increase in the percentage of hemoglobin and hemoglobin (Ng *et al.*, 2004) ^[19].

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