



International Journal of Bioscience and Biochemistry

ISSN Print: 2664-6536
 ISSN Online: 2664-6544
 Impact Factor: RJIF 5.4
 IJBB 2025; 7(1): 01-04
www.biosciencejournal.net
 Received: 01-10-2024
 Accepted: 05-11-2024

Mohammed Khalil Ibrahim Al-Saeedi
 College of Environmental
 Sciences, Al-Qasim Green
 University, Iraq

Ameer Shamkhi Noor
 College of Engineering,
 Al-Qasim Green University,
 Iraq

Hashim Hadi Al-Jebory
 Agriculture College,
 Al-Qasim Green University,
 Iraq

Corresponding Author:
Mohammed Khalil Ibrahim Al-Saeedi
 College of Environmental
 Sciences, Al-Qasim Green
 University, Iraq

Effect of in ova injection annatto seed extract on some blood biochemical traits of broiler chicks (Ross 308)

Mohammed Khalil Ibrahim Al-Saeedi, Ameer Shamkhi Noor and Hashim Hadi Al-Jebory

DOI: <https://dx.doi.org/10.33545/26646536.2025.v7.i1a.89>

Abstract

Medicinal plants contain many active substances that act as antioxidants as part of the requirements of sustainable development and environmentally friendly green farming technologies. This study was conducted at Al-Jaflawi Poultry Hatchery from November 22, 2024 to November 25, 2024 to study the effect of injecting hatching eggs with aqueous extract of annatto seeds on some biochemical characteristics of broiler chicks exposed to post-hatching nutritional fasting. 300 fertilized eggs were used divided to four groups, each group 75 eggs, and injected with aqueous extract of annatto seeds at 18 days of embryo age. The groups were G1 is free, G2, G3, and G4 *In ovo* injected with 0.1, 0.2, and 0.3 ml aqueous extract of annatto seeds / egg in amniotic sac. The results were: significant decrease for G2, G3, and G4 groups compared to G1 group in cholesterol at 12 hours, significant decrease for G2, G3, and G4 groups on G1 group in triglyceride at 12 hours, significant decrease for G3, and G4 groups on G1 group in glucose at hatching and 12 hours, significant improvement in Glutathione peroxidase (GSH-PX) for *In ovo* injection groups compared to G1 group.

Keywords: Medicinal plants, antioxidants, sustainable development, green farming technologies, annatto seeds, aqueous extract

Introduction

The time it takes for the chicks to move from the hatchery to the rearing hall, which could take two or three days, is considered one of the stress factors due to the chicks being exposed to nutritional starvation, which may affect the subsequent productive performance of the chicks as well as the immune status (Kadhim *et al.*, 2021) ^[13]. The technology of injecting hatching eggs came to deliver nutrients to chicken embryos (Al-Saeedi *et al.*, 2022) ^[23]. By scavenging free radicals, binding metals, or removing reactive oxygen species that would otherwise cause oxidation, antioxidants are chemical substances that, when present in small amounts, can postpone or prevent oxidative damage (Huang *et al.*, 2005) ^[11]. Since antioxidants are the primary means of scavenging reactive oxygen species, their action is necessary to stop the oxidative chain reactions that these species are causing (Augusto *et al.*, 1995) ^[4]. When given to feeds, antioxidants stop or slow down the oxidation of macromolecules like lipids, nucleic acids, and carbohydrates (Ratnam *et al.*, 2006; Al-Jebory and Ibrahim, 2021) ^[17, 2]. Antioxidants can be introduced during processing or they can be found naturally in foods. Antioxidants help preserve meat's nutritional value and prolong its shelf life in addition to preserving its quality (Reische *et al.*, 2002) ^[18]. Because natural antioxidants are highly effective at reducing and protecting against the attack of reactive oxygen species on cell components like DNA, proteins, and lipids, their inclusion in the diet may have positive health effects for consumers (Su *et al.*, 2007) ^[22]. The chemical components bixin and norbixin, which are categorized according to their structural characteristics under carotenoid derivatives known as apocarotenoids, are responsible for the orange-red hue of annatto seeds (Hari *et al.*, 1994) ^[9]. The crude oil pigment extracted is called bixin which is soluble in water and is called norbixin. The solubility of carotenoids in water is poor (Smith Nigle, 2005) ^[21]. Bixin was first isolated by Boussingault in 1825 and its molecular formula is (C₂₅H₃₀O₄). Bixin is almost unique in the carotenoid family and is an unsaturated hemi-carotenoid C₂₅ diapocarotenoid, which can function as a pigment in a number of different chemical structures. Bixin is the first carotenoid to exhibit geometric isomerism (Lima *et al.*, 2001) ^[14].

Because of the aforementioned, the current study is to investigate the effects of injecting hatching eggs with varying concentrations of annatto seed aqueous extract on some physiological traits of broiler chicks exposed to feed fasting.

Materials

In order to investigate the impact of injecting hatching eggs with varying concentrations of aqueous extract of annatto seeds on certain biochemical characteristics of broiler chicks exposed to nutritional fasting, this experiment was carried out at Al-Jaflawi Poultry Hatchery from November 22, 2024, to November 25, 2024. At 18 days of embryo age, 300 fertilized eggs were employed and split into four treatments, each consisting of 75 eggs that were injected with an aqueous extract of annatto seeds. G1 was left free, while G2, G3, and G4 were injected *In ovo* with 0.1, 0.2, and 0.3 milliliters of annatto seed/egg aqueous extract in the amniotic sac. exposing chicks to a 12-hour fast following hatching, as per Kadhim *et al.* (2021) [13].

Using a pre-made test kit (Kit) from the French company Biolabo, the level of cholesterol in the blood serum was determined based on Franey and Elias (1968) [8]. Using a

pre-made analysis kit (Kit) from the French company Biolabo, the level of triglycerides in the blood serum was mg/100 ml of blood. Using a kit developed by the Spanish business LINEAR and based on Henry *et al.* (1982) [10], the amount of glucose in the blood serum was determined in milligrams per 100 milliliters of blood. enzyme glutathione peroxidase (GSH-PX) using Sedlak and Lindsay's (1968) methodology. based on the manoldihyde (MDA) concentration as stated by Beuge and Aust (1978) [5].

Apply the mathematical model and statistical analysis in accordance with the design (CRD) (SAS, 2012: Duncan, 1955) [19, 7]: $Y_{ij} = \mu + T_i + e_{ij}$

Results and Discussion

Lipid profile

Table 1: shown the effect of groups in cholesterol, at hatching there were no significant effect between groups, while at 12 hours after hatching significant ($p \leq 0.05$) decrease for G2, G3, and G4 groups compared to G1 group. Table 2: shown the change in triglyceride, at hatching there were no significant effect between groups, so as at 12 hours significant ($p \leq 0.05$) decrease for G2, G3, and G4 groups compared to G1 group.

Table 1: Effect of in ova of annatto seed extract in cholesterol of broiler chicks (Ross 308)

Treatments	Cholesterol at hatching	Cholesterol at 12 hours
G1	155.78±9.32	139.01±3.00 a
G2	151.13±8.13	122.49±2.15 c
G3	156.73±5.06	131.33±2.90 b
G4	155.78±4.91	124.21±2.21 c
P- value	N.S	*
Letter-differentiated means differ significantly at 0.05		

Table 2: Effect of in ova of annatto seed extract in triglyceride of broiler chicks (Ross 308)

Treatments	Triglyceride at hatching	Triglyceride at 12 hours
G1	115.66±2.49	119.67±5.43 a
G2	114.60±2.07	98.11±2.75 b
G3	112.77±4.57	96.59±1.57 b
G4	116.07±4.92	118.95±2.03 a
P- value	N.S	*
Letter-differentiated means differ significantly at 0.05		

Glucose

Table 3: shown the effect of study in glucose, at hatching significant ($p \leq 0.05$) decrease for G3, and G4 groups

compared to G1, and G2 groups, so as at 12 hours significant ($p \leq 0.05$) decrease for G2, G3, and G4 groups compared to G1 group.

Table 3: Effect of in ova of annatto seed extract in glucose of broiler chicks (Ross 308)

Treatments	Glucose at hatching	Glucose at 12 hours
G1	157.72±1.52 a	276.80±4.20 a
G2	157.19±0.89 a	222.55±1.45 b
G3	150.18±1.81 b	225.49±3.47 b
G4	142.23±2.36 c	237.13±2.16 b
P- value	*	*
Letter-differentiated means differ significantly at 0.05		

Antioxidant status

Table 4: shown the impact of groups in GSH-PX and MDA, in GSH-PX when hatching significant ($p \leq 0.05$) increase for

G2, G3, and G4 groups compared to G1 group, there were no significant effect between groups in GSH-PX at 12 hours and in MDA.

Table 4: Effect of in ova of annatto seed extract in Glutathione peroxidase (GSH-PX) and mannodihyde (MDA) of broiler chicks (Ross 308)

Treatments	GSH-PX at hatching	GSH-PX at 12 hours	MAD at hatching	MDA at 12 hours
G1	152.82±1.61 d	13.29±6.05	2.36±0.20	5.36±2.24
G2	165.27±4.0 c	140.13±7.14	2.46±1.23	4.36±5.28
G3	182.93±3.25 b	139.26±5.10	2.89±3.00	4.10±3.78
G4	199.16±4.48 a	138.26±8.00	2.01±2.78	5.08±2.50
P- value	*	N.S	N.S	N.S
Letter-differentiated means differ significantly at 0.05				

The exposure of chicks to nutritional fasting causes them to be exposed to oxidative stress, which is what we notice through the increase in glucose, cholesterol and triglyceride values and the decrease in glutathione peroxidase levels in the control treatment compared to the injection treatments. The significant improvement in these traits in the annatto injection treatments is because tocopherols, tocotinols, and carotenoids are present in annatto seeds, in addition to the fact that the seeds are a good source of vitamin E, which enhances its action in preventing the oxidation process because this vitamin is considered a natural antioxidant (Appiah-Nkansah, 2011) [3]. Because vitamin E lowers oxidative stress, it lessens the deleterious effects of corticosterone, which is released from the adrenal cortex and helps the body produce glucose from non-carbohydrate sources so it can use it as fuel (Cuong and Chin, 2014) [6]. The possession of annatto seeds Annatto has antioxidant activity against oxidative stress and contains protein and essential and non-essential amino acids for G3 and G4 annatto addition coefficients by protecting proteins by free radicals resulting by oxidative stress, thus increasing protein building and preventing protein breakdown and its use in building body tissues, in addition to annatto seeds containing vitamin A, which works to improve the secretion of thyroxine hormone, which has an important role in the process of protein and fat metabolism in the feed (Liu *et al.*, 2005) [15]. Additionally, anatoto seeds contain tocopherols and tocotinols, which are important for preventing and eliminating free radicals from oxidative stress, boosting the immune system, and improving the body's metabolism. They also protect the cell membrane from oxidation and stop free radicals from damaging DNA and active enzyme sites (Ng *et al.*, 2004; Ibrahim and Al-jebory, 2020) [16, 12]. It is also possible that the injection of annatto seed extract into the hatching eggs was swallowed by the fetus through the amniotic sac and absorbed through the intestine to play its role as an antioxidant in the fetus's body.

Conclusion

According to the current study, injecting hatching eggs with an aqueous extract of annatto seeds enhanced the antioxidant levels and increased the chicks' resistance to fasting.

References

- Jafar M, Al-Jebory HH, Al-Saeedi MKI. Effect of *In ovo* injection of lysophospholipid in hatching traits, chick's quality, and chicks physical traits of broiler (Ross 308). *Adv Anim Vet Sci.* 2024;12(7):1206-1213.
- Al-Jebory HH, Ibrahim MK. Effect of adding bee propolis to diet on productive performance of broiler chickens. *Indian J Ecol.* 2021;48(15):203-207.
- Appiah-Nkansah E. Carotenoid eggs from layers fed with annatto (*Bixa orellana*) concentrate for the health-conscious consumer [PhD thesis]. Kumasi (GH): Kwame Nkrumah University of Science and Technology; 2011. p. 182.
- Augusto O, Hix S, Morais MS, Vasquez-Vivar J. Free radical reactions: Formation of adducts with biomolecules and their biological significance. *Ciênc Cult.* 1995;47:280-287.
- Beuge JA, Aust SD. Estimation of serum malondialdehyde level. In: Hoffee PA, Jone ME, editors. *Methods in Enzymology.* New York (NY): Academic Press; 1978. p. 1-10.
- Cuong TV, Chin KB. Effects of annatto (*Bixa orellana* L.) seeds powder on physicochemical properties, antioxidant and antimicrobial activities of pork patties during refrigerated storage. *J Food Sci.* 2014;36(4):476-486.
- Duncan DB. Multiple ranges test and multiple F-test. *Biometrics.* 1955;11:1-42.
- Franey RJ, Elias A. Serum cholesterol measurement based on ethanol extraction and ferric chloride-sulfuric acid. *Clin Chem Acta.* 1968;21:255-293.
- Hari RK, Patel TR, Martin AM. An overview of pigment production in biological systems: functions, biosynthesis, and applications in the food industry. *Food Rev Int.* 1994;10:49-70.
- Henry RJ, Sobel C, Kim J. Determination of uric acid. In: Tietz NW, editor. *Fundamentals of Clinical Chemistry.* London (UK): W.B. Saunders Company; 1982.
- Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J Agric Food Chem.* 2005;53(6):1841-1856.
- Ibrahim MK, Al-Jebory HHD. Study of the effect of bee propolis on some biochemical, immunological traits and intestinal microflora of broiler chickens (Ross 308). 1st Scientific International Virtual Agricultural Conference, IOP Conf Ser: Earth Environ Sci. 2020;553(1):012022. doi:10.1088/1755-1315/553/1/012022.
- Kadhim AH, Al-Jebory HH, Ali MA, Al-Khafaji FR. Effect of early feeding (*In ovo*) with nano-selenium and vitamin E on body weight and glycogen level in broiler chickens exposed to fasting conditions. 4th International Conference for Agricultural and Sustainability Sciences, IOP Conf Ser: Earth Environ Sci. 2021;910(1):012009. doi:10.1088/1755-1315/910/1/012009.
- Lima LRP, Oliveira TT, Nagem TJ, Pinto AS, Stringheta PC, Tinoco ALA, *et al.* Bixina, norbixina, and quercetina and their effects on lipid metabolism in rabbits. *Braz J Vet Res Anim Sci.* 2001;38(4):196-200.
- Liu MJ, Wang Z, Wong RN, Wu Q. Diosgenin induced cell cycle arrest and apoptosis in human leukemia K562

- cells with the disruption of Ca²⁺ homeostasis. *Cancer Chemother Pharm.* 2005;55(1):79-90.
16. Ng MH, Choo YH, Ma AN, Chuah CH, Hashim MA. Vitamin E (tocopherol, tocotrienol, and tocotrienol) in palm oil. *Lipids.* 2004;39(10):1031-1035.
 17. Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar RM. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *Natl Inst Pharm Educ Res.* 2006;113(3):189-207.
 18. Reische DW, Lillard DA, Eitenmiller RR. Antioxidants. In: Akoh CC, Min DB, editors. *Food lipids: chemistry, nutrition, and biotechnology.* 2nd ed. New York (NY): Marcel Dekker; 2002. p. 507-534.
 19. SAS. *Statistical Analysis System, Users Guide.* Version 9.1. Cary (NC): SAS Inst Inc.; 2012.
 20. Sedlak J, Lindsay RH. Estimation of total protein-bound and non-protein sulphhydryl groups in tissue with Ellman's reagent. *Anal Biochem.* 1968;25:192-205.
 21. Smith NJH. *Geography of crop plants* [Internet]. Gainesville (FL): Department of Geography, University of Florida; 2005. Available from: <https://www.geo.ufl.edu>
 22. Su L, Yin J, Charles D, Zhou K, Moore J, Yu L. Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon, and oregano leaf. *Food Chem.* 2007;100(3):990-997.
 23. Al-Saeedi MK, Al-Jebory HH, Abood MH. Progress phenotypic traits of hatched chicks and growth indicators of broiler chicks fed embryonically with zinc methionine. *Arch Razi Inst.* 2022;77(6):2139-2145.