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The Effect of in-ovo injection of anthocyanin on post-hatching traits of Ross 308 broiler chicks

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This study investigated the effect of injecting hatching eggs with different anthocyanin concentrations on the morphological and physiological characteristics of one-day hatching chicks at two different times (days 12 and 18) of the incubation period. This study was conducted at Al-Anwar hatchery facility from 11-11-2023 to 2-12-2023. A total of 900 fertilized eggs were used for this study, divided into two groups (450 eggs each), and each group had six treatments with three replicates and 25 eggs for each replicate. The treatments were (NC) negative control, (PC) positive control (NaCl solution), and 50, 60, 70, and 80 ppm of Anthocyanin for T3, T4, T5, and T6, respectively. Results showed that hatching and embryonic mortality percentages were significantly affected in both injected dates in addition to the hatched chick weight in the second period. The percentage of dead piped chicks was significantly low on the 50-ppm anthocyanin level compared to other treatments on day 18 while no significant effect was found on day 12 of the incubation period. The highest recorded chick length was at the level of 50 ppm anthocyanin compared to the control on day 18, while on day 12, the 60 ppm anthocyanin level significantly showed the highest wing length value compared to the control. Both leg length and tonic immobility were significantly affected in both periods, especially when the anthocyanin treatments significantly increased leg length and decreased tonic immobility compared to the control treatments of the second incubation period. Significant differences were obtained between treatments on the appearance and feather condition, leg, and navel status at day 12, while at day 18, only chick activity and appearance and feather condition significantly appeared different effects between treatments.

Keywords: Anthocyanin, hatching chicks, morphological traits, in-ovo feeding

INTRODUCTION

In order to avoid problems of post-hatching chicks and because of the long periods needed to move chicks from the hatchery to the farm, in-ovo injection technology has been found. In ovo (injection) feeding is considered one of the most and latest successful methods have been used to feed embryos during the hatching (most likely days 12-18) and post-hatching periods to improve health and performance [1]. In ovo injection (early feeding) technology using exterior nutrients confirmed the beneficial effect of this method for having good health with better performance for most of the hatching chicks [2]. In-ovo feeding to embryos using nutrients and other additives can stimulate intestinal microflora, which can lower the starvation effects during the past hatching periods because chicks 48-72 h after hatching have different operations, including sorting, counting, vaccination, and transportation without access to the feed [1,3,4,5,6,7,8]. These operations also cause oxidation stress in newly hatching chicks; as a result, using plant extract as nutritional supplementation leads to antioxidant activity for the chicks [9]. Therefore, anthocyanin is the most natural compound commonly found in plants such as Apples, red cabbage, bananas, mangoes, sweet cherries, red corn, green beans, etc. It was also known as polyphenols or flavonoids, can be used as growth promoters and have pharmacological effects against pathogens [10]. Anthocyanin is usually found bonded to sugar molecules by glycoside bonds; thus, when the bond is broken, the sugar will be released, and the remaining part will be called anthocyanidins, which include cyaniding, peonidin and delphinidin compounds [11]. In addition, it was found that Anthocyanins have antioxidant and medicinal effects as well as bioflavonoid phytochemicals, which can assist in removing free radicals [12,13]. Moreover, anthocyanins protect against DNA damage by reducing enzyme inhibition, lipid peroxidation, estrogen activity, anti-inflammatory activity, and enhancing immunity (cytokine production) [14]. [15] mentioned that anthocyanins can reduce intestinal infections of Escherichia coli and improve nutrient absorption by increasing villi length, crypt depth, and mucous membrane thickness.

All the positive aspects of anthocyanins mentioned above can be injected into the eggs as a feed additive or supplement to improve the hatched chicks' performance. Because of the nutrient compounds delivered to the embryo around day 12 or 17 to 18 of the incubation period via air sac or by amnion. Therefore, this study aims to evaluate the effect of injecting hatching eggs on day 12 and 18 with anthocyanins for improving the hatching rate, the weight of hatched chicks, morphological and physical characteristics of the post-hatching chicks.

MATERIALS AND METHODS

This study was approved by the Department of Animal Science Committee – College of Agriculture- AL-Qasim Green University, which was conducted at AL-Anwar Hatchery Institute that belonged to AL-Anwar Poultry Company located in Al-Muradiyah area- Babylon Province. The study lasted for three weeks, from 11-11-2023 to 2-12-2023, to investigate the injection effect of anthocyanin on the hatching eggs (Ross 308) on days 12 and 18 of the incubation period to evaluate the physical and phenotypic characteristics of the newly hatching chicks. This means that on day 12 (450 eggs) were used, and another 450 eggs were used for the 18 days of the incubation period.

Experimental eggs were obtained from the above hatchery, originally imported from the Turkish company Paktavuk. Clean and regular normal-shaped eggs weighed (66 ± 1 g) were selected, while dirty or unnormal eggs were excluded. A total of 900 eggs were individually weighed using a digital scale, which was used for this experiment.

Preparation of the anthocyanin solution

The Violet plant was the only source of anthocyanin preparation, meaning that anthocyanin was the only derivative from the violet plant [16]. Then, the solution was prepared by mixing 1 ml of anthocyanin was diluted with 1 liter of distilled water, then diluted with NaCl solution to obtain a concentration of 10,20,30 ppm.

The dosage used for injecting the hatching eggs was 0.3 ml/egg, while the experimental treatments were divided as follows: each group contained the following dosage:

T1: a control group without injections.

T2: Injecting with NaCl solution (positive group)

T3: Injecting anthocyanin solution at a level of 50 ppm/egg.

T4: Injecting anthocyanin solution at a level of 60 ppm/egg.

T5: Injecting anthocyanin solution at a level of 70 ppm/egg.

T6: Injecting anthocyanin solution at a level of 80 ppm/egg.

Studied traits

The hatching chick's traits were measured based on the methods reported by [8]. Whereas the chick's length and leg length were measured according to [19], while wing length was mentioned by [18], and tonic immobility was referred to [17], while the phenotypic traits were done according to [20,21].

Statistical analysis

Use the statistical analysis according to the design (CRD) [22, 23] and use the mathematical model:

$Y_{ij} = \mu + T_i + e_{ij}$

RESULTS AND DISCUSSION

Hatching chick's performance on day 12

This study showed that in-ovo injection of anthocyanin with 60 ppm/egg (T4) had the lowest hatching percentage, which was not significantly different from T2, T3, and T6, respectively, compared to the control treatment. For the Embryonic mortality percentage, it showed that T3, T4, and T6 were not significantly different from the positive control (T2). In contrast, the negative control T1 and T5 had the lowest values compared to other treatments. Similarly, no significant differences were found between treatments in the chicks' weight (Table 1).

Also, in-ovo injection of anthocyanin did not significantly affect the percentage of piped eggs, live piped chicks, dead piped chicks, and abnormal chicks among all treatments (Table 2).

Moreover, the early feed injection with anthocyanin did not significantly affect the chick length (Table 3). However, other parameters, including wing length, leg length, and tonic immobility, showed significant superiority, whereas the wing length was significantly higher in the anthocyanin treatments (T3, T4, T5, and T6) compared to the control treatments (T1 and T2) ($P \leq 0.05$). The fourth treatment showed the only significant effect on the leg length ($P<0.01$) when anthocyanin was used at 60 ppm/egg compared to other treatments. An early feed injection of anthocyanin did not show any significant difference with control treatments in the tonic immobility except for the third and fifth treatments, which was found to be significantly low ($P<0.01$). The addition of anthocyanin was found to be significantly higher in T4 in wing length, leg length, and tonic immobility, respectively; however, when the anthocyanin levels increased, these values linearly decreased.

The only morphological traits significantly affected by anthocyanin were appearance, feather condition, and leg and navel status. Appearance and feather condition was significantly higher in the first treatment than in the second and fourth treatments. However, no significant differences were found with treatments 3,5 and 6, respectively. The lowest value of the leg status was found in the first treatment, which was not significantly different from treatments 2,3 and 5, but it was still significantly different from treatments 4 and 6. No significant differences were found among all treatments in the navel status trait except for the second treatment was found to be significantly lower ($P<0.01$) than the other one at day 12 (Table 4).

An early feed injection with anthocyanin at 18 days of age found that the hatching percentage did not show significant differences between treatments except for the second treatment, which was found to be significantly low among others. However, the second treatment had the highest value regarding embryonic mortality percentage among the others. The hatched chick's weight was significantly high in T5 and low in T1. At the same time, no significant effect was found between anthocyanin treatments (3,4,5, and 6) and (2, 3,4, and 6) and also between treatments (1,2, 4, and 6), respectively (Table 5).

Dead piped chicks' percentage was the only trait significantly affected ($P<0.05$) among other traits. The positive control treatment (T2) had the highest value compared to (T3), which had the lowest value. At the same time, no significant differences were found between treatments 1,3,4,5 and 6 (Table 6).

Chick length was found to be significantly affected by the anthocyanin injection. The third treatment (50 ppm/egg) had the highest value compared to the control treatments. In contrast, no significant difference was reported between control and other anthocyanin treatments, including T4, T5, and T6. Leg length had significant superiority in the anthocyanin treatments compared to the control treatments. On the other hand, tonic immobility was found to be significantly high in the control treatments in contrast with the anthocyanin treatments (T3…T6) (Table 7).

Table 8 showed that activity and appearance and feather condition were significantly higher ($P<0.05$); ($P<0.01$) in the anthocyanin treatments compared to the positive control treatment (T2); however, no significant difference was found between the adverse control treatment (T1) and the anthocyanin treatments. The anthocyanin treatments did not significantly affect other traits, including the state of yolk retracted inside the abdomen, eye appearance, leg status, navel status, and residual yolk, were not significantly affected.

DISCUSSION

Anthocyanin is a natural component commonly found and derivative from plants. As mentioned, this product has many positive effects on animals without side effects. Therefore, it can show improvements in the production and health of some animal species or might not have that effect based on the animal type or the environmental conditions found during the experiment. As a result, during day 12 of the incubation period in – ovo feeding of the Anthocyanin at high concentrations (T5 and T6) was not significantly different from the control treatment (T1); however, the low concentration did show a significantly low hatching percentage compare to the control. On the other hand, the embryonic mortality with low levels (T3 and T4) of Anthocyanin significantly increased embryonic mortality percentage while the high levels did not (table 1). This means that low levels of anthocyanin decrease the hatching percentage and increase embryonic mortality percentage, while the high levels were equal to the control for both. A study published by [24] reported that in-ovo feeding of prebiotics at day 12 using 3.5 mg of Galactooligosaccharides with ross 308 did not affect hatchability. At the same time, another study by [25] found that in-ovo feeding of prebiotics at day 12 using 1.9 mg of raffinose family oligosaccharides (RFOs) with ross 308 had decreased the hatchability. In-ovo feeding of vitamin C decreases embryonic mortality and increases the hatchability of the newly hatched chicks [26, 27]. It was found that embryonic mortality percentage increased in the anthocyanin treatments compared to the control treatment. The reason is probably that in-ovo injection of the early-stage embryos can stimulate the growth of microorganisms, which, in the end, positively affects intestinal health [28]. The decrease in hatching percentage in the treatment injected with NaCl (T2) could be because of the hyperglycemia caused by the high level of carbohydrates injected into the embryo [1]. [29] reported that saline solution with low carbohydrate level increased hatchability while the high carbohydrate level reduced hatchability in pigeons.

In-ovo injection of anthocyanin did not significantly affect the percentage of piped eggs, live piped chicks, dead piped chicks, and abnormal chicks among all treatments (Table 2). This might be because of the ca percentage in the eggshells, whereas the shell thickness and breaking strength have the lowest pipping percentage and vice versa [30].

Moreover, the early feed injection with anthocyanin did not significantly affect the chick length (Table 3). However, other parameters, including wing length, leg length, and tonic immobility, showed significant superiority. The increase in wing length and leg length, especially in treatments T3 through T6, is definitely due to the early Feeding with Anthocyanin. However, a study published by [31] found that in-ovo injection of betaine and choline on day 12 of the incubation period using 0.25, 0.375, and 0.50 mg on ross 308 did not significantly affect leg and wing length. However, the tonic immobility was decreased in the anthocyanin treatments compared to the controls.

The morphological traits significantly affected by anthocyanin were appearance and feather condition, leg, and navel status, respectively. Appearance and feather condition was significantly higher in the first treatment than in the second and fourth treatments. However, no significant differences were found with treatments 3,5 and 6, respectively. The lowest value of the leg status was found in the first treatment, which was not significantly different from treatments 2,3 and 5, but it was still significantly different from treatments 4 and 6. No significant differences were found among all treatments in the navel status trait except for the second treatment was found to be significantly lower ($P<0.01$) than the other one at day 12 (Table 4).

An early feed injection with anthocyanin at 18 days of age found that the hatching percentage did not show significant differences between treatments except for the second treatment, which was found to be significantly low among others. As mentioned earlier, carbohydrate levels can affect the hatchability percentage, whereas high carbohydrate levels decrease the hatchability percentage and vice versa [1,29]. [32] reported that in-ovo feeding of corticotrophin-releasing hormone (CRH) from day 10-18 of the incubation period using 0.1, 1, or 2 µg of CRH in 100 µl solution in Cobb 500 did not show any significant effect on hatching time or hatchability. However, the second treatment had the highest value regarding embryonic mortality percentage among the others. In-ovo feeding using vitamin C had decreased embryonic mortality and increased hatchability [26,27], which is similar to this study when adding anthocyanin; the hatching percentage increased while the embryonic mortality decreased. The hatched chick's weight was significantly high in T5 and low in T1. [33] observed that in-ovo feeding of 65 ng thyroxine hormone in a volume of 500 µl showed an increase in the hatched chick weight, which is approximately similar to the results of this study when anthocyanin used had increased the chick weight, especially in T5. Many studies have shown that in-ovo feeding is done with compounds such as prebiotics, probiotics, and hormones. Enzymes, drugs, etc., can increase hatched body weight and reduce mortality [1]. [34] reported that in-ovo injection of anthocyanin by 10, 20, and 30 ppm/egg showed a significant increase in the hatching and embryonic mortality percentages and chick weight; however, using a higher amount of anthocyanin in this study did not show significant differences with the control, except for the second treatment.

The percentage of dead piped chicks was significantly higher in the negative control only, which is similar to the study published by [34]; however, the anthocyanin treatments did not show any value, while this study showed values even though it was not significant. In addition, another study by [35] showed no significant effect on the Abnormal chicks' traits when they used zinc methionine injection.

Chick length was found to be significantly affected by the anthocyanin injection. At the same time, the third treatment (50 ppm/egg) had the highest value compared to the control treatments. However, a study reported by [34] showed that the chick length was shorter than the result of this study. The possible reason was that the anthocyanin levels used in their study were low, contrasting with the current study. Meanwhile, approximately similar results were observed for leg length between these two studies. However, tonic immobility showed lower values compared to the study mentioned above when they reported higher values, and the reason is probably that when the level of anthocyanin increased, the tonic immobility decreased and vice versa. Another study by [35] found that in-ovo injection of Zinc Methionine at the levels of 60,80,100 ppm/0.3 ml significantly showed higher chick length compared to the control treatment, which is approximately close to the results of this study, when the anthocyanin treatments showed higher chick length compare to the control treatment as well. Similarly, no significant effect was found in the wing length trait of this study and the study published by [35]; however, it disagreed with the current study in the leg length trait, whereas the injection of anthocyanin significantly increases the leg length while the zinc methionine did not. Researchers found that chick length had a positive effect on productive performance in the future when they observed that an increase of 1 cm in the chick length would predict a weight gain between 113-214 g at the end of the rearing period [36,37,38].

Table 8 showed that activity and appearance, and feather condition were significantly higher ($P<0.05$); ($P<0.01$) in the anthocyanin treatments compared to the positive control treatment (T2); however, no significant difference was found with the negative control. [34] reported that in-ovo injections of 10,20 and 30 mg/egg anthocyanin did not show any effect on activity, while there was an effect on the appearance and feather condition in contrast to the results of the current study. [35] had approximately similar results with [34] when they used in–ovo injection of zinc methionine. However, the zinc methionine study disagreed with the results obtained recently in this study when it showed a significant effect on the following characteristics, including eye appearance, leg status, navel status, and residual yolk, while the current study did not.

CONCLUSION

In-ovo injection is a new technology that has been used in order to improve chicks' health, immunity, production, etc… by using components such as vitamins, minerals, amino acids, hormones, enzymes, drugs, prebiotics, probiotics, synbiotics, herbal products and more. Most studies used in–ovo injection have shown a positive effect on chicks, while others did not. Therefore, this study concluded that using anthocyanin positively affected the 18-day injection period in contrast with the first period. At the same time, the anthocyanin level 70 ppm had the highest hatching percentage, the lowest embryonic mortality percentage, and the highest hatched chick weight, among other treatments in the second period. The lowest dead piped chicks percentage was obtained at the level of 50 ppm anthocyanin when the eggs were injected on day 18 of the incubation period. As mentioned earlier, the second egg injection gave a better result in the chick length, leg length, and tonic immobility compared to the first one and to the control, specifically at the 50ppm anthocyanin levels. The significant and non-significant effects of activity, appearance and feather condition, state of yolk retracted inside the abdomen, eye's appearance, leg status, navel status, and residual yolk of the 18-day in-ovo injection were found to be higher than those values in the 12-day in-ovo injection; whereas, all the anthocyanin levels showed approximately the same results which were significantly higher than the positive control (treatment 2). Therefore, in-ovo feeding at day 18 showed the best results compared to the first one (day 12), and the best anthocyanin levels were 50 and 70 ppm, based on the results obtained in the current study. The study recommended that in-ovo injection (early feeding) of anthocyanin at a concentration of 50 ppm would yield a good result when the eggs were injected on day 18 of the incubation period. This study suggests that anthocyanin can be in-ovo injected into the eggs of other poultry species, such as turkeys and ducks.

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