Effect of Mesenteric Injection at Different Hatching-Window Intervals on the Physiological Traits of Broiler Chickens

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Abstract

The experiment was conducted at Al-Anwar Poultry Farm, at Al-Muradiya district Babylon Province, for 35 days (March 1–April 5, 2024), and aims to determine the effect of mesenteric injection at different hatching window (HW) intervals on the antioxidant traits in the blood serum of broiler chickens. A total of 600 unsexed one-day-old chicks (Ross 308) (45±1.5 g) were allocated into ten factorial experiment treatments (2×5). The first factor was HW (150 chicks hatched at 493 h and 150 chicks hatched after 493 h), and the second factor was mesenteric injection administration method (mesenteric injection with electrolyte solution, mesenteric injection with water, intra-crop gavage with electrolyte solution, intra-crop gavage with water, and no treatment), with 3 replicates per treatment and 20 chicks per replicate. The results showed a significant interaction between HW and mesenteric injection on antioxidant traits in broiler chicks. Chicks hatched before 493 h and receiving mesenteric electrolyte injection recorded the highest serum malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) levels (32.94 and 52.27 µmol/L), with increases of 15–30% compared to other treatments. This group also exhibited the highest catalase (CAT) activity (23.59 µmol/L) and superoxide dismutase (SOD) levels (39.96 µmol/L), indicating enhanced resistance to oxidative stress. Chicks hatched after 493 h with mesenteric water injection or oral water drenching also showed improved antioxidant parameters, but to a lesser extent. The control and mesenteric water groups had lower values, highlighting the role of electrolytes and water administration in improving ionic and water balance, reducing oxidative stress, and enhancing the activity of key antioxidant enzymes in broiler chicks.

Keywords: Electrolytic solution, Mesenteric injection, Hatching windows, Serum Biochemical Indices.

Introduction

Hatching windows (HW) are defined as the time between the first hatched chicks and the last which is called 'take-off'; this process happens between 461 h to 510 h of incubation period [1]. High variance of HW occurred due to different causes: breeder age, breeder feed, eggs weight, eggs characteristics, eggs storage, and finally incubation conditions [2]. The vast time between first and last hatched chicks causes overwhelming stress to the hatchery employer because of different percentages of hatched chicks along the HW. Additionally, the quality of chicks was different, so, many hatcheries' strategies were taken to overcome this

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problem [3]. The weak performance and welfare occurred in early hatched chicks due to spending more than 30 h in hatchery conditions (air temperature 37 C, RH of 80% and air polluted with down), this situation leads to lose about 10% of body weight in comparison to 1-3% loss of body chicks weight hatched 2 h before take-off [4].

Electrolytic component of newly hatching chicks was altered after hatch due to long stay of hatching chicks in hatcher machines [5], so certain ions (Na+, K+ and Cl-) offers an objective measure and real-time insight into physiological status of single chicks [6], and they were considered strong ions because of their effect on the acid-base balance of body fluids [7].

The mesenteric region supports intra-abdominal organs through a combination of vascular connections and peritoneal-fascial attachment [8,9]. demonstrated that the yolk sac-Jejunum/Ileum complex is a highly active region of mesentery enclosing the vitelline duct, vitelline artery, umbilical vein, and yolk, and is contiguous with both Jejunum/Ileum, so, injection (i.e. ions) into mesenteric region could distribute throughout the mesentery in apposition with organs including the aorta and the pericardium and heart before being absorbed into their respective organs including heart, lung, and kidney [10].

According to the previous facts, this experiment was conducted to assess the ability of mesenteric injection with electrolytic solution to mitigate the effect of hatching windows on Antioxidant properties.

Material and Methods

Electrolytic Solution (ES):

An electrolytic solution (ES) was prepared according to [11], via dissolving 3.5 g of NaCl, 1.5 g of KCl and 2.9 g of $Na_3C_6H_5O_7$ in 100 ml flask of de-ionized H_2O as thinner to achieve 1.1372 *N* of whole ES, the entire ES was then autoclaved within 2 days to prepared to inject into mesentery region of newly hatched chicks, the same concentrate of electrolytic solution was used via intra-crop administration with plastic tube connected to 0.5 ml syringe.

Experimental Design:

A total of six hundred unsexed one day old chicks (ROSS 308) (45±1.5 g) were used for this trail, they were distributed into 10 5) , the first main ×factorial treatments (2 factor (300 chicks) was an hatching windows threshold (150 chicks hatch at 493 h and 150 chicks hatch after 493 h of incubation period), the second main factor was administration method with 120 chicks for each (via mesenteric injection with 0.5ml of electrolytic solution, ml de-ionized 5mesenteric injection with 0. H_2O ,intra-crop gavage administration with ml of electrolytic solution, intra-crop 50. ml of de-5gavage administration with 0. ionized H_2O, and none injected or gavage administration), 20 chicks were used for each replicate and 3 replicates for each

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treatment (60 chicks per treatment). Feed and water were free access (*ad libitum*), illumination and other directions of broiler rearing were used according to the ROSS 308 guide [12].

Blood Collection and Analysis:

Blood samples were collected at the ages of one day and eleven days. Two birds were selected from each replicate, ensuring their live weights were as close as possible to the average live weight for that replicate, with the selection done randomly. The birds were deprived of food for twenty-four hours but allowed to drink water. Thereafter, the live weight of each bird was measured, and then they were slaughtered by cutting the jugular vein and left to bleed out in a vertical position. Two milliliters (2 ml) of blood were collected in standard glass tubes (Bijou bottles) for the biochemical composition analysis of the serum, according to the procedures outlined in the research of Kohn [13] and Mouldin et al. [14]. The diets used in the experiment were illustrated in Table 1 and had an optimum requirement as a nutrition specification for the breeding company [12].

Statistical Analysis:

To assess the significant effect of main factors and their interactions on selected parameters, firstly, every percentage data was transformed to arc sine, then all transformed data were analysed using Two-Way ANOVA analysis. The analysis was applied by using the General Linear Model (GLM) procedure of statistical software package SAS version 9.1[15], *P-values* less than 0.05, 0.01, and 0.001 were considered to be significant for the main effect. Results were presented as *mean/ SEM (pooled)*.

Table 1. The diets used in the experiment

Ingredients	Experimental diets (%)		
	Starter (1-10 days)	Grower (11-21 days)	Finisher (22-35 days)
Corn	52.8	58.65	62.4
Wheat	10	10	10
Soybean (48%protein)	29.8	24	20.5
Protein concentrate*	5	5	5
Sunflower oil	0.3	0.3	0.3
Limestone	1.14	1.21	1.22
Dicalcium phosphate	0.5	0.35	0.2
Methionine	0.17	0.17	0.13
Lysine	0.19	0.22	0.15
Salt (NaCl)	0.1	0.1	0.1
Total	100	100	100
Calculated Chemical Composition**			
Crud protein (%)	21.94	19.66	18.29
ME (Kcal/kg feed)	2940	2995	3035

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Lysine (%)	1.39	1.26	1.11
Crude fibre (%)	2..73	2.64	2.58
Calcium (%)	0.9	0.88	0.83
Meth + Cyc (%)	1.03	0.97	0.9
Available phosphor	0.44	0.41	0.38

The protein concentrate utilized in the present study was of the Brocon type, originating * from the Netherlands. Each kilogram of the concentrate provides 40% crude protein, 5% crude fat, 2.20% crude fiber, 5% calcium, 4.68% available phosphorus, 3.85% lysine, 4.12% methionine + cystine, 0.42% tryptophan, 2017 kcal/kg of metabolizable energy, and 1.70% threonine. Moreover, it contains a balanced mixture of vitamins and trace minerals formulated to meet the nutritional requirements of poultry for these essential elements.

The chemical composition of the diet was determined in accordance with the NRC [16] ** standards.

Results and Discussion

The effect of injection type and chick removal time in HW on Antioxidant properties in the blood serum of broiler chicks at one day of age:

The data in Table 2 indicate a significant effect of the interaction between the two experimental factors on serum MDA content at one day of age. A significant superiority was observed in the treatments of removing chicks before 493 hours with water drenching and removing chicks after 493 hours with mesenteric water injection, as they achieved the highest means (40.51 and 39.67 μmol/L), respectively, compared with the other interaction treatments. It is also noted that the treatment of removing chicks after 493 hours with mesenteric electrolyte injection significantly outperformed the treatment of removing chicks before 493 hours under the control group, with an increase of 61.05%.

The remaining treatments shared in the significant superiority and were ranked from highest to lowest as follows: removing chicks before 493 hours with electrolyte drenching, followed by removing chicks after 493 hours with electrolyte drenching combined with mesenteric electrolyte injection, which topped the significant differences; then removing chicks before 493 hours with mesenteric electrolyte injection; followed by removing chicks before 493 hours with mesenteric water injection and removing chicks after 493 hours under the control group; then removing chicks after 493 hours with water drenching; and finally, the treatment of removing chicks before 493 hours under the control group, which came at the bottom of the significance scale. The removal factor alone did not significantly affect serum MDA content.

The significant interaction effect on serum MDA may be attributed to the significant influence of the injection factor. A significant effect was observed for the injection treatments in this trait, as the electrolyte drenching treatment recorded the highest mean (35.94 μmol/L) compared with the lowest mean in the control

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treatment (18.42 µmol/L), representing a percentage increase of 48.74%. The significance levels of the treatments were as follows: mesenteric water injection and mesenteric electrolyte injection had lower significance than electrolyte drenching, which was followed by water drenching, while the control treatment exhibited the lowest significance level.

This increase may be explained by the fact that mesenteric injection or water drenching temporarily altered the internal fluid and electrolyte balance, triggering a defensive oxidative response. Although water may improve hydration status and restore ionic balance, the injection process itself is considered a stress stimulus that can transiently elevate MDA. This assumption was supported by Akbarian et al. [17], who demonstrated that heat and environmental stress in poultry increase MDA concentrations as an indicator of lipid peroxidation whenever internal homeostasis is disturbed. Similarly, Xu et al. [18] and Kohrogi et al. [19] reported that physiological stress, early injection, handling, or immune stimulation cause significant increases in MDA levels due to elevated reactive oxygen species (ROS) production and reduced efficiency of antioxidant enzymes such as SOD and GPx.

Conversely, the findings of this study differ from those of Mohamed et al. [20], who reported a decrease in MDA levels in broiler blood when provided with ionized water, from 4.13 to 2.79 nmol/mL. Al-Enzy et al. [21] similarly found that the addition of sodium chloride (NaCl) significantly reduced lipid peroxidation markers (MDA, PV, FFA) in broiler chickens.

Table 2. Influence of Injection Method and Hatch-Window Removal Time on Serum Antioxidant Traits in One-Day-Old Chicks.

Treatments(1)		MDA (µmol/L)	CAT (µmol/L)	GSH-Px (µmol/L)	SOD (µmol/L)
Hatching windows factor					
Before 493 h		28.93	27.08b	37.45b	35.20
After 493 h		31.04	32.42a	42.97a	33.34
P-value (0.05)		0.1385	0.0008	0.0001	0.1394
Administration factor					
Control		18.42c	24.87b	40.04ab	24.73e
MI with H_2O		32.17ab	28.62b	40.00ab	45.22a
MI with ES		32.35ab	24.48b	38.28b	39.43b
Intra-crop with H_2O		31.05 b	45.98a	42.07a	28.83d
Intra-crop ES		35.94a	24.80b	40.66a	33.14c
P-value (0.05)		0.0001	0.0001	0.0233	0.0001
Interaction (hatching windows × administration)					
Before 493 h	Control	13.58 e	28.27 c	40.80 a	24.31 c
	MI with H_2O	24.67cd	20.64 d	34.93 c	44.47a
	MI with	29.83bc	15.57 d	33.11 c	42.98a

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After 493 h	ES				
	Intra-crop with H_2O	40.51a	36.88 a	39.52 ab	26.91 bc
	Intra-crop ES	36.08ab	34.03 ab	38.90 b	28.96 bc
	Control	23.27cd	21.47d	39.28cd	25.15de
	MI with H_2O	39.67a	36.60b	45.08a	45.97a
	MI with ES	34.87ab	33.40bc	43.45ab	35.88bc
	Intra-crop with H_2O	21.60d	55.08a	44.62a	30.76cd
	Intra-crop ES	35.81ab	15.57d	42.43 abc	28.96de
P-value (0.05)		0.0001	0.0001	0.0001	0.0001
Total mean		2145.98	29.99	29.75	40.21
SEM(2)		13.2716	1.6666	2.2121	0.7451

1- Experimental treatments: The hatching window (before 493 hours and after 493 hours) represents the first factor, while the injection factor (control, mesenteric water, mesenteric electrolyte, water drenching, electrolyte drenching) represents the second factor.

2- SEM represents the Standard Error of the Mean.

3- Different letters within the same column indicate significant differences among treatment means at 0.05 and 0.01 probability levels.

A significant effect was observed in the interaction between the two experimental factors on serum CAT enzyme content, as the treatment of removing chicks after 493 hours combined with water drenching significantly outperformed all other interaction treatments by achieving the highest mean value (55.08 µmol/L). It is noted that the interaction treatment of removing chicks before 493 hours under the control group did not differ significantly from the treatment of removing chicks after 493 hours with mesenteric electrolyte injection, with mean values of 28.27 and 33.40 µmol/L, respectively.

The remaining treatments shared in the significant superiority and arranged in descending order as follows: removing chicks before 493 hours with water drenching and removing chicks after 493 hours with mesenteric water injection, which topped the significance scale; followed by removing chicks before 493 hours with electrolyte drenching and removing chicks after 493 hours with mesenteric electrolyte injection; then removing chicks before 493 hours under the control group; and finally, the treatments of removing chicks after 493 hours under the control group, removing chicks before 493 hours with mesenteric water injection, removing chicks before 493 hours with mesenteric electrolyte injection, and removing chicks after 493 hours with electrolyte drenching, which occupied the lowest significance levels.

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The previous significant superiority may be attributed to the significant effect of chick removal time (before vs. after 493 hours), which significantly influenced serum CAT levels. The treatment of removing chicks after 493 hours achieved the highest mean value (32.42 μmol/L), representing a 16.47% increase.

The significant interaction effect on CAT content also resulted from the significant effect of the injection factor, as injection treatments showed clear differences. Water drenching significantly achieved the highest mean value (45.98 μmol/L), compared with the lowest means recorded in the control, mesenteric water, mesenteric electrolyte, and electrolyte drenching treatments (24.87, 28.62, 24.48, and 24.80 μmol/L, respectively), with percentage increases of 45.91%, 37.75%, 46.57%, and 46.06%.

The effect of chick removal time and activity is administration method on CAT attributed to their influence on the ionic and water balance in the body, which enhances the activity of antioxidant enzymes such as catalase. CAT (CAT) plays a key role in breaking down hydrogen peroxide ($H_2 O_2$), a reactive oxygen species (ROS). Excess accumulation of ROS triggers oxidative stress. CAT prevents hydrogen peroxide from reacting with oxygen in the presence of iron-binding compounds, thereby reducing the formation of highly toxic hydroxyl radicals (OH) and other harmful species [22,23].

A significant interaction effect was also observed on serum GSH-Px content, as the treatments of removing chicks after 493 hours with mesenteric water injection and removing chicks after 493 hours with water drenching significantly recorded the highest means (45.08 and 44.62 μmol/L), respectively.

The treatment of removing chicks before 493 hours under the control group did not differ significantly from removing chicks after 493 hours with mesenteric electrolyte injection, with mean values of 40.80 and 43.45 μmol/L. The remaining treatments were ranked as follows from highest to lowest significance: removing chicks after 493 hours with mesenteric electrolyte injection, followed by removing chicks after 493 hours with electrolyte drenching, then removing chicks before 493 hours under the control group, followed by removing chicks before 493 hours with water drenching and removing chicks after 493 hours under the control group, then removing chicks before 493 hours with electrolyte drenching, while removing chicks before 493 hours with mesenteric water or mesenteric electrolyte injection recorded the lowest significance levels. The significant superiority is attributed to the significant effect of chick removal time, as removing chicks after 493 hours significantly produced the highest mean (42.97 μmol/L), with a percentage increase of 12.84%.

The significant interaction effect on GSH-Px content is also attributed to the injection factor. Water drenching and electrolyte drenching significantly achieved the highest means (42.07 and 40.66 μmol/L), compared with the lowest mean recorded in mesenteric electrolyte injection (38.28 μmol/L), with percentage increases of 9.00% and 5.85%. The control

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and mesenteric water treatments followed, while mesenteric electrolyte injection recorded the lowest significance level.

These results differ from Nuengjamnong and Angkanaporn [24], who reported no significant effect on serum GSH-Px in Arbor Acres broilers. The present results may be attributed to enhanced oxidative stress resistance in chicks removed after 493 hours, as these chicks may face environmental challenges such as heat, dehydration, or nutritional stress. Additionally, water and electrolyte supplementation improve ionic balance, boosts immunity, and enhances organ function by increasing GSH-Px activity, a key antioxidant enzyme protecting cells from oxidative damage [25,26].

A significant interaction effect was found for serum SOD content, as the treatments of removing chicks after 493 hours with mesenteric water injection and removing chicks before 493 hours with mesenteric water and mesenteric electrolyte injection significantly recorded the highest means (45.97, 44.47, and 42.98 µmol/L), compared with other treatments. The treatment of removing chicks after 493 hours with mesenteric electrolyte injection significantly outperformed removing chicks before 493 hours under the control group, with a 32.24% increase.

The remaining treatments ranked in descending order as follows: removing chicks after 493 hours with mesenteric electrolyte injection, followed by removing chicks after 493 hours with water drenching, then removing chicks before 493 hours with electrolyte drenching, removing chicks after 493 hours with electrolyte drenching, removing chicks before 493 hours with water drenching, removing chicks after 493 hours under the control group, and finally removing chicks before 493 hours under the control group, which recorded the lowest significance level. Chick removal time alone did not significantly affect SOD content.

The significant interaction effect is primarily due to the injection factor, as mesenteric water injection significantly achieved the highest mean (45.22 µmol/L), compared with the lowest recorded value in the control group (24.73 µmol/L), with a percentage increase of 45.31%. Mesenteric electrolyte injection followed, then electrolyte drenching, water drenching, and lastly the control group.

These results are consistent with Parandoosh et al. [27], who reported significant interactions between injection and water supplementation on SOD activity in serum. Water administration enhances SOD activity by improving hydration and cellular metabolism, while dehydration increases ROS formation and elevates SOD demand [28,29]. Electrolytes further support ionic balance within cells, enhancing SOD efficiency in converting superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) [30,31].

Effect of Injection Type and Chick Pull Time on Serum Antioxidant Parameters at 11 Days of Age:

The data in Table 3 indicate a significant interaction effect between the two experimental factors on serum MDA content at 11 days of age. The interaction treatment of chicks hatched before 493

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hours combined with the control group showed a significant superiority, recording the highest mean value (32.94 µmol/L) compared with the other interaction treatments. It is also noted that this same interaction treatment significantly outperformed the treatment of chicks hatched after 493 hours with mesenteric electrolyte injection, with a percentage increase of 29.05%. The remaining interaction treatments shared significant differences, arranged from highest to lowest as follows: chicks hatched after 493 hours with mesenteric water injection ranked first, followed by chicks hatched after 493 hours with oral water drenching and oral electrolyte drenching, then chicks hatched after 493 hours with the control treatment, followed by chicks hatched before 493 hours with mesenteric electrolyte injection, then chicks hatched before 493 hours with oral electrolyte drenching, followed by chicks hatched before 493 hours with mesenteric water injection and oral water drenching. Finally, the treatment of chicks hatched after 493 hours with mesenteric electrolyte injection ranked at the bottom of the significance scale. It is also observed that HW factor alone did not have a significant effect on serum MDA content.

The significant interaction effect on MDA content may be attributed to the significant influence of the chick injection factor, as a significant effect of the injection treatments was observed for this trait. The control treatment showed a significant superiority, recording the highest mean value (30.32 µmol/L) compared with the lowest mean recorded by the mesenteric electrolyte injection treatment (25.48 µmol/L), representing a percentage increase of 15.96%. The mesenteric water injection, oral water drenching, and oral electrolyte drenching treatments followed the control treatment in significance, while the mesenteric electrolyte injection treatment showed the lowest significant value. This indicates that injection played a major role in improving ionic and water balance in the body, thereby reducing oxidative stress. These findings agree with Mohamed et al. (2025), who reported that water drenching reduced MDA levels in broiler blood at 37 days of age. They also agree with Nuengjamnong and Angkanaporn (2015), who reported that supplementing electrolytes reduced MDA levels in Arbor Acre broilers. Conversely, Livingston et al. (2022) reported no significant effect of electrolyte supplementation (sodium chloride and sodium bicarbonate) on MDA levels in heat-stressed broilers.

Serum CAT content also showed a significant effect of the interaction between the two experimental factors. The interaction treatments of chicks hatched before 493 hours with mesenteric electrolyte injection and those hatched before 493 hours with oral electrolyte drenching showed significant superiority, recording the highest means of 23.59 and 23.34 µmol/L, respectively, compared with the other interaction treatments. It was also noted that chicks hatched after 493 hours with mesenteric electrolyte injection were statistically equal to chicks hatched before 493 hours with the control treatment, with mean values of 12.70 and 17.57 µmol/L, respectively. The remaining treatments followed in descending order of significance, starting with chicks hatched after 493 hours with the control treatment,

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followed by chicks hatched before 493 hours with the control treatment, then chicks hatched before 493 hours with oral water drenching. These were followed by chicks hatched after 493 hours with oral water drenching, mesenteric water injection, and oral electrolyte drenching. Finally, the treatments of chicks hatched after 493 hours with mesenteric electrolyte injection and chicks hatched before 493 hours with mesenteric water injection ranked lowest in significance at 11 days of age.

The observed significant differences may be attributed to the significant effect of HW (before or after 493 hours) on CAT enzyme levels, as chicks hatched before 493 hours significantly outperformed those hatched after 493 hours, recording the highest mean value of 18.71 µmol/L, representing a percentage increase of 17.69%.

The significant interaction effect for CAT may also be due to the significant influence of the chick injection factor. The control, mesenteric electrolyte injection, and oral electrolyte drenching treatments showed significant superiority, recording the highest means of 18.75, 18.02, and 18.96 µmol/L, respectively, compared with the lowest mean recorded by the mesenteric water injection treatment (13.12 µmol/L), representing percentage increases of 30.02%, 27.19%, and 30.80%, respectively. Oral water drenching showed a significance level below those of the control, mesenteric electrolyte, and oral electrolyte treatments, while mesenteric water injection recorded the lowest significant value. The data in Table 3 indicate a significant interaction effect between the two experimental factors on serum MDA content at 11 days of age. The interaction treatment of chicks hatched before 493 hours combined with the control group showed a significant superiority, recording the highest mean value (32.94 µmol/L) compared with the other interaction treatments. It is also noted that this same interaction treatment significantly outperformed the treatment of chicks hatched after 493 hours with mesenteric electrolyte injection, with a percentage increase of 29.05%. The remaining interaction treatments shared significant differences, arranged from highest to lowest as follows: Chicks hatched after 493 hours with mesenteric water injection ranked first, followed by chicks hatched after 493 hours with oral water drenching and oral electrolyte drenching, then chicks hatched after 493 hours with the control treatment, followed by chicks hatched before 493 hours with mesenteric electrolyte injection, then chicks hatched before 493 hours with oral electrolyte drenching, followed by chicks hatched before 493 hours with mesenteric water injection and oral water drenching. Finally, the treatment of chicks hatched after 493 hours with mesenteric electrolyte injection ranked at the bottom of the significance scale. It is also observed that HW factor alone did not have a significant effect on serum MDA content.

The significant interaction effect on MDA content may be attributed to the significant influence of the chick injection factor, as a significant effect of the injection treatments was observed for this trait. The control treatment showed a significant superiority, recording the highest mean value (30.32 µmol/L)

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compared with the lowest mean recorded by the mesenteric electrolyte injection treatment (25.48 µmol/L), representing a percentage increase of 15.96%. The mesenteric water injection, oral water drenching, and oral electrolyte drenching treatments followed the control treatment in significance, while the mesenteric electrolyte injection treatment showed the lowest significant value. This indicates that injection played a major role in improving ionic and water balance in the body, thereby reducing oxidative stress. These findings agree with Mohamed et al. (2025), who reported that water drenching reduced MDA levels in broiler blood at 37 days of age. They also agree with Nuengjamnong and Angkanaporn (2015), who reported that supplementing electrolytes reduced MDA levels in Arbor Acre broilers. Conversely, Livingston et al. (2022) reported no significant effect of electrolyte supplementation (sodium chloride and sodium bicarbonate) on MDA levels in heat-stressed broilers.

Serum CAT content also showed a significant effect of the interaction between the two experimental factors. The interaction treatments of chicks hatched before 493 hours with mesenteric electrolyte injection and those hatched before 493 hours with oral electrolyte drenching showed significant superiority, recording the highest means of 23.59 and 23.34 µmol/L, respectively, compared with the other interaction treatments. It was also noted that chicks hatched after 493 hours with mesenteric electrolyte injection were statistically equal to chicks hatched before 493 hours with the control treatment, with mean values of 12.70 and 17.57 µmol/L, respectively. The remaining treatments followed in descending order of significance, starting with chicks hatched after 493 hours with the control treatment, followed by chicks hatched before 493 hours with the control treatment. Chicks hatched before 493 hours with oral water drenching. These were followed by chicks hatched after 493 hours, which received oral water drenching, mesenteric water injection, and oral electrolyte drenching. Finally, the treatments of chicks hatched after 493 hours with mesenteric electrolyte injection and chicks hatched before 493 hours with mesenteric water injection ranked lowest in significance at 11 days of age.

The observed significant differences may be attributed to the significant effect of HW (before or after 493 hours) on CAT enzyme levels, as chicks hatched before 493 hours significantly outperformed those hatched after 493 hours, recording the highest mean value of (18.71 µmol/L), representing a percentage increase of (17.69%).

The significant interaction effect for CAT may also be due to the significant influence of the chick injection factor. The control, mesenteric electrolyte injection, and oral electrolyte drenching treatments showed significant superiority, recording the highest means of 18.75, 18.02, and 18.96 µmol/L, respectively, compared with the lowest mean recorded by the mesenteric water injection treatment (13.12 µmol/L), representing percentage increases of 30.02%, 27.19%, and 30.80%, respectively. Oral water drenching showed a significance level below those of that control, mesenteric electrolyte, and oral electrolyte treatments, while mesenteric

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water injection recorded the lowest significant value.

Table 3. Influence of Injection Method and Hatch-Window Removal Time on Serum Antioxidant Traits in 11-Day-Old Chicks.

Treatments[1]		MDA (µmol/L)	CAT (µmol/L)	GSH-Px (µmol/L)	SOD (µmol/L)
Hatching windows factor					
Before 493 h		26.99	18.71a	47.24a	31.86
After 493 h		28.66	15.40b	41.53b	30.40
P-value (0.05)		0.1759	0.0109	0.0009	0.5074
Administration factor					
Control		30.32 a	18.75 a	47.40 a	33.21 a
MI with H_2O		28.08 ab	13.12 b	41.23 b	34.30 a
MI with ES		25.48 b	18.02 a	44.39 ab	31.29 ab
Intra-crop with H_2O		27.32 ab	16.42 ab	41.10 b	31.24 ab
Intra-crop ES		27.93 ab	18.96 a	47.83 a	25.59 b
P-value (0.05)		0.05	0.0275	0.0217	0.05
Interaction (hatching windows × administration)					
Before 493 h	Control	32.94 a	17.57 abcd	46.72 abc	34.68 a
	MI with H_2O	24.40 cd	11.77 d	43.01 bc	39.96 a
	MI with ES	27.59 abcd	23.59 a	45.86 abc	31.21 ab
	Intra-crop with H_2O	24.36 cd	17.29 bcd	48.34 ab	31.13 ab
	Intra-crop ES	25.65 bcd	23.34 a	52.27 a	22.32 b
After 493 h	Control	27.69 abcd	19.94 abc	48.07 ab	22.32 ab
	MI with H_2O	31.75 ab	14.47 cd	39.45 cd	28.64 ab
	MI with ES	23.37 d	12.70 d	42.91 bc	31.38 ab
	Intra-crop with H_2O	30.28 abc	15.54 cd	33.85 d	31.36 ab
	Intra-crop ES	30.21 abc	14.34 cd	43.39 bc	28.86 ab
P-value (0.05)		0.0001	0.0036	0.0002	0.0004
Total mean		2145.98	27.82	17.05	44.39
SEM[2]		13.2716	0.6699	0.7329	0.9408

1- Experimental treatments: The hatching window (before 493 hours and after 493 hours) represents the first factor, while the injection factor (control, mesenteric water, mesenteric electrolyte, water drenching, electrolyte drenching) represents the second factor.

2- SEM represents the Standard Error of the Mean.

3- Different letters within the same column indicate significant differences among treatment means at 0.05 and 0.01 probability levels.

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The data for blood serum GSH-Px content indicate a significant interaction effect between the two experimental factors. The treatment involving chick removal before 493 hours combined with electrolyte drenching showed a significant superiority, recording the highest mean value of 52.27 µmol/L compared with the other interaction treatments. No significant differences were observed between the treatment of chick removal before 493 hours with the control and the treatment of chick removal after 493 hours with mesenteric electrolyte injection, with mean values of 46.72 and 42.91 µmol/L, respectively.

The remaining treatments also demonstrated significant differences, arranged in descending order from highest to lowest: chick removal after 493 hours with mesenteric water, followed by chick removal after 493 hours with water drenching and electrolyte drenching, then chick removal after 493 hours with the control and chick removal before 493 hours with mesenteric electrolyte injection, followed by chick removal before 493 hours with electrolyte drenching, then chick removal before 493 hours with mesenteric water and water drenching, and finally chick removal after 493 hours with water drenching, which ranked last in significance.

The aforementioned significant superiority may be attributed to the significant effect of the chick removal factor (before vs. after 493 hours), which significantly influenced GSH-Px content. Chicks removed before 493 hours showed significantly higher means (47.24 µmol/L) with a percentage increase of 12.08%.

The significant interaction effect in GSH-Px may also be due to the significant influence of the injection factor. A significant effect was observed among injection treatments, where the control and electrolyte drenching treatments recorded the highest means (47.40 and 47.83 µg/L, respectively) compared with the lowest means observed in mesenteric water and water drenching treatments (41.23 and 41.10 µg/L, respectively), with percentage increases of 13.01% and 14.07%. The mesenteric electrolyte treatment followed at a slightly lower significance level, while mesenteric water and water drenching ranked lowest. These findings agree with Nuengjamnong and Angkanaporn [24], who reported that supplementation with sodium, potassium, and bicarbonate increased GSH-Px levels in Arbor Acre broilers.

Moreover, a significant interaction effect was observed between the two experimental factors in blood serum SOD content at 11 days of age. The treatments involving chick removal before 493 hours with mesenteric water and the control recorded the highest means (39.96 and 34.68 µmol/L, respectively) compared with the other treatments. The treatment involving chick removal before 493 hours with the control was statistically similar to chick removal after 493 hours with mesenteric electrolyte injection, with mean values of 34.68 and 31.38 µmol/L, respectively.

These were followed by a group of treatments sharing similar significance levels and collectively superior to the remaining treatments. In descending order, these included: chick removal after 493

hours with mesenteric electrolyte injection and water drenching, chick removal before 493 hours with mesenteric electrolyte injection and water drenching, chick removal after 493 hours with electrolyte drenching and mesenteric water, and the control. The treatment involving chick removal before 493 hours with electrolyte drenching ranked lowest.

The hatching-window factor, in both levels, had no significant effect on SOD The significant interaction effect content. for SOD appears to be primarily influenced by the injection factor. The control and mesenteric water treatments showed significant superiority, recording the highest mean values (33.21 and 34.30 µmol/L, respectively), compared with the lowest mean observed in the electrolyte drenching treatment (25.59 µmol/L) with percentage increases of 22.94% and 25.39%, respectively. The mesenteric electrolyte and water drenching treatments followed at moderate significance levels, while electrolyte drenching ranked lowest. These results agree with Mohamed et al. [20], who reported that water drenching increased total antioxidant capacity (TAC), including SOD activity, in broilers at 37 days of age.

Conclusion

According to the obtained results, the interaction treatments led to significant improvement in GSH-Px levels compared with other treatments. Removing chicks before 493 hours combined with electrolyte drenching recorded the highest values. Moreover, both control and electrolyte drenching treatments showed a clear enhancement in GSH-Px activity compared with mesenteric and water Similarly, significant drenching treatments. responses were observed in SOD levels after 11 days, where the interaction of early chick removal with mesenteric water and control achieved the highest means. Injection factors also showed a pronounced effect, as control and mesenteric water treatments outperformed electrolyte drenching. Overall, water and electrolyte applications contributed to improving antioxidant enzyme activity in broiler chicks.

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