Effect of injecting a mixture of amino acids through the mesenteric tissue and hatching time on the physiological traits of broiler

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Abstract

The current study was designed to investigate the influence of mesenteric injection of an amino acid solution on the ameliorative effects of hatching times against the physiological and antioxidant traits of broiler chickens (ROSS 308) and to determine whether amino acid injection could mitigate the physiological and oxidative effects associated with delayed hatching. The first major variable was hatching time intervals, where 180 chicks were hatched within 493 hours of incubation, while the rest 180 chicks were hatched after 493 hours of incubation period. The second experimental factor was injection treatment, namely, mesenteric injection of 0.5 ml of amino acids (IAA), mesenteric injection of 0.5 ml of deionized water (IWA), and no injection. There were twenty chicks per replicate and there were three replicates for each treatment group, thus sixty chicks in total per treatment. The results showed significant interactions between hatching window and mesenteric injection for several traits studied ($P \leq 0.05$). IAA mesenteric injection significantly decreased total protein (TP) levels to 2.94 and 2.87 g/100 ml before and after 493 h, respectively, and globulin (GLB) levels to 1.549 and 1.542 g/100 ml, respectively ($P \leq 0.05$), whereas albumin (ALB) was not significantly affected. Regarding antioxidant traits, IAA injection before 493 h resulted in higher SOD and GSH-PX levels (39.96 and 43.01 µmol/L, respectively). Hatching time significantly affected SOD, with higher levels before 493 h than after 493 h (34.49 vs. 29.61 µmol/L; $P \leq 0.05$), while injection type significantly affected MDA and SOD ($P \leq 0.05$). The interaction between hatching time and injection type significantly affected MDA, CAT, GSH-PX, and SOD ($P \leq 0.05$). Regarding serum lipid traits, hatching time significantly affected TG and VLDL ($P \leq 0.05$), while injection type significantly affected Chol, HDL, and LDL ($P \leq 0.05$). The interaction significantly affected Glu, Chol, HDL, TG, VLDL, and LDL ($P \leq 0.05$). Mesenteric amino acid injection improved antioxidant capacity and lipid metabolism in broiler chicks, particularly in early-hatched birds.

Keywords: Amino acids, Mesenteric injection, Hatching windows, Serum Biochemical Indices.

Introduction

The variation in the hatching times of newly hatched chicks is considered one of the most important challenges in broiler chicken rearing, due to its impact on the chicks' characteristics and, consequently, the subsequent production performance of broiler chickens [1]. This variation in hatching times within the same hatch is referred to as the "hatching window" [2], which ranges from 24 to 36 hours. The hatching window is influenced by several factors, including the age of the hens, the duration of egg storage, and egg weight, as well as the type of incubator, along with other minor factors [3].

Hatching chicks at different times results in uneven or delayed commencement of the newly hatched chicks from the same starting point, as well

as nutritional deficiencies. Chicks experience dehydration, particularly when transferring from the hatchery to the rearing rooms over an extended period. This process negatively impacts the subsequent production performance of broiler chickens [4].

The electrolyte balance of newly hatched chicks may be altered after hatching due to prolonged retention in the hatcher machines [5]. Therefore, amino acids are essential for maintaining the physiological balance of each chick, playing a pivotal role in regulating the acid-base balance of body fluids [6]. They also contribute to the maintenance of various cellular functions by influencing ion composition in cell membranes and supporting biochemical reactions within the body [7].

The mesenteric region supports intra-abdominal organs through a combination of vascular connection and peritenon-fascial attachment [8], demonstrated that the yolk sac–jejunum/ileum complex is a highly active mesenteric region that encloses the vitelline duct, vitelline artery, umbilical vein, and yolk. This region is contiguous with the jejunum and ileum, suggesting that substances injected into the mesenteric region, such as amino acids, may be distributed throughout the adjacent mesenteric tissues. These tissues are in close apposition to organs, including the aorta, pericardium, and heart, potentially facilitating the subsequent absorption of amino acids by organs such as the heart, lungs, and kidneys [10].

Based on the previous findings, this experiment was conducted to assess the ability of mesenteric injection with amixture of amino acids to mitigate the effect of hatching windows on the physiological traits of broiler.

Material and Methods

BIO-AMINO FORT INJ Solution (BS):

The BIO-AMINO FORT INJ solution, an aseptic injection solution, was used to replenish essential amino acids and minerals in the chicks. This solution was manufactured by Bio-Pharmachemic in Vietnam and is specifically formulated for mesenteric administration in newly hatched chicks. The amino acid solution was administered using a sterile 1 mL insulin syringe.

Experimental Design:

A total of three hundred sixty unsexed one day old chicks (ROSS 308) (45±1.5 g) were used for this trial, they were distributed into 6 factorial treatments (2× 3) , the first main factor (360 chicks) was an hatching windows threshold (180 chicks that hatched before 493 h and 180 chicks that hatched after 493 h of incubation), the second main factor was administration method with 120 chicks (ROSS 308) for each (via mesenteric injection with 0.5 ml of amino acids, mesenteric injection with 0.5 ml de-ionized H_2O, and Non-injected control group), 20 chicks were used for each replicate and 3 replicates for each treatment (60 chicks per treatment). Feed and water were free access (ad libitum), illumination and other directions of broiler rearing were used according to ROSS 308 guide [11].

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 feed for 6–12 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 Kelly and Alworth [12] and Lebedev et al [13]. The diets used in the experiment met the recommended nutritional requirements of the breeding company [11].

Statistical Analysis:

To assess the significant effect of main factors and its interactions on selected parameters, firstly, every percentage data was transfer to arc sine, then all transformed data were analysed using Two-Way ANOVA analysis. The analysis was applied by using General Linear Model (GLM) procedure of statistical software package SAS version 9.1 [14], 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*).

Results and Discussion

Table 1 Shows that there was a significant effect of the interaction between the two experimental factors on total protein content in the blood at 11 days of age, as the interaction treatment of chick pull time after 493 hours with the control significantly recorded the highest mean value of (3.23 g/100 ml) compared with the other interaction treatments. It is also observed that the interaction treatment of chick pull time before 493 hours with the control was not significantly different from the treatment of chick pull time after 493 hours with IAA, recording mean values of (3.12 and 2.87 g/100 ml), respectively. The remaining treatments shared significant superiority and were arranged accordingly from the highest to the lowest, where the treatments of chick pull time before 493 hours with the control, chick pull time after 493 hours with IWA, and chick pull time before 493 hours with IWA showed the highest significant superiority. Finally, the two treatments of chick pull time before 493 hours with IAA and chick pull time after 493 hours with IAA ranked at the end of the significance scale. The table also shows that hatch window and injection factors did not significantly affect serum total protein content. It is also observed from the table that hatch window, injection, and their interaction did not significantly affect serum albumin content. The stability of serum albumin levels may indicate that hepatic protein-synthetic function was not markedly affected by the treatments. These results agree with the study of Elwan et al. [15], who did not observe any significant effect of amino acids on serum albumin content.

As for blood globulin content, a significant response to the interaction between the two experimental factors was observed. The treatment of chick pull time before 493 hours with the control significantly achieved the highest mean value of (1.763 g/100 ml) compared with the other interaction treatments. The control treatment before 493 h showed a higher value compared with the amino acid treatment after 493 h (1.763 vs. 1.542

g/100 ml, respectively). The remaining treatments showed significantly higher values and were arranged accordingly from the highest to the lowest, including the treatments of chick pull time after 493 hours with the control and IWA, the interaction treatment of chick pull time before 493 hours with IAA, and the interaction treatment of chick pull time after 493 hours with IAA. The interaction treatment of chick pull time before 493 hours with IWA recorded the lowest value. It is also noted that the pull-time factor at both levels did not significantly affect serum globulin content.

The significant superiority of the control treatment in total protein and globulin levels may be related to differences in amino acid availability and utilization during the early post-hatch period. Amino acids provide substrates for hepatic protein synthesis and the production of circulating proteins, including globulins, which are closely associated with humoral immune functions. During the early growth stage, cells are more responsive to nutritional stimulation, making adequate amino acid availability particularly important for protein synthesis, immune development, and nutrient utilization. This agrees with what was indicated by Madej et al [16], who stated that early nutrition effectively contributes to improving the health of newly hatched chicks by reducing dehydration and stress levels and stimulating the growth of gut-associated lymphoid tissues, thereby enhancing bird immunity and efficiency in utilizing nutrients during this sensitive stage of life, which is positively reflected in total protein and globulin levels.

The results also agree with what was reported by Mohamed et al [17], in their study on broiler chickens, where they explained that supplying ionized water caused an increase in total protein and globulin concentrations in blood serum. Our results are also consistent with the study of Abou-Elkhair et al [18], which indicated that supplementation of low crude protein diets with a mixture of amino acids (threonine, tryptophan, and valine) linearly increased serum total protein and globulin values. The Significantly higher values of the interaction treatments in blood globulin content may be explained by its association with the vital role of the method of treatment administration (in IWA, and IAA) in enhancing the immune response of the bird. Injection, particularly with water, may facilitate the rapid absorption and direct delivery of substances into the bloodstream, bypassing gastrointestinal digestion and degradation, thereby enhancing globulin formation [19]. On the other hand, amino acids act to maintain effective homeostasis and enhance protein synthesis in tissues in addition to improving immune response [20], [21]. In contrast to our results, the study of Saleh et al [22], indicated that the addition of amino acids at moderate to high levels to chicken diets had no significant effect on total protein, albumin, and globulin levels.

Table 1. Effect of Injection Type and Chick Pull Time During the Hatch Window on Serum Protein Parameters of Broiler Chicken at 11 Days of Age.

treatments[1]		TP (g/100 ml)	ALB (g/100 ml)	GLB (g/100 ml)
Main effect of hatching time				
Before 493 h		3.054	1.407	1.646
After 493 h		3.044	1.513	1.531
Probability		0.9008	0.0610	0.1689
Main effect of injection type				
Without		3.094	1.496	1.597 ab
Water		3.072	1.372	1.699 a
Amino acid		2.981	1.512	1.469 b
Probability		0.4445	0.0804	0.0433 *
Interaction between hatch pull time and injection type				
Before 493 h	**Control**	3.12 ab	1.353	1.763 a
	Water	3.00 ab	1.620	1.419 b
	Amino acid	2.94 b	1.391	1.549 ab
After 493 h	**Control**	3.23 a	1.596	1.631 ab
	Water	3.10 ab	1.487	1.626 ab
	Amino acid	2.87 b	1.323	1.542 ab
P-value		0.0379 *	0.3137	0.049 *
Total mean		3.049	1.460	1.588
SEM [2]		0.037	0.03	0.40

1- Experimental Design: The hatching window (prior to 493 hours and after 493 hours) was considered the first factor, whereas the injection treatment (control, mesenteric water, mesenteric amino acids) was regarded as the second factor.

2- SEM: Values are presented as the Standard Error of the Mean.

3- Statistical Significance: Means within the column bearing different superscripts differ significantly at ($p \leq 0.05$).

The data presented in Table (2) indicate a significant effect of the interaction between the two experimental factors on serum malondialdehyde content at 11 days of age. The treatments of chick pull time before 493 hours with the control and chick pull time after 493 hours with the control significantly recorded the highest mean values of (32.79 and 31.75 µmol/L), respectively, compared with the remaining interaction treatments. It was also observed that there were no significant differences between the treatment of chick pull time before 493 hours with the control and the treatment of chick pull time after 493 hours with IAA. The remaining treatments shared significant superiority and were arranged accordingly from the highest to the lowest, where the treatments of chick pull time after 493 hours with IAA and IWA showed the highest significant superiority. Finally, the treatments of chick pull time before 493 hours with IWA and IAA ranked at the end of the significance scale. It is also noted that the pull-time factor at both levels did not significantly affect serum malondialdehyde content.

The significant interaction effect on serum malondialdehyde content may be attributed to the significant superiority observed in the chick injection factor. A significant effect of injection treatments was noted in this trait, as the IWA treatment significantly recorded the highest mean value of (31.79 µmol/L) compared with the lower mean values of the IAA and control treatments, with significant

increases of (13.43 and 21.01%), respectively. This increase may be explained by the fact that in mesenteric injection temporarily altered the fluid and electrolyte balance within the body, leading to activation of a defensive oxidative response. Although water may improve hydration status and restore ionic balance, the injection process itself is considered a stress stimulus that may temporarily elevate malondialdehyde levels. This was indicated by Akbarian et al. [23], in their study on the effects of heat and environmental stress in chickens, where any disturbance in homeostasis increased malondialdehyde concentration as an indicator of lipid oxidation. Xu et al [24], and Kohrogi et al [25], also explained that physiological stress, early injections, transportation, or immune stimulation cause significant increases in malondialdehyde concentrations due to elevated production of reactive oxygen species (ROS) and reduced efficiency of antioxidant enzymes such as SOD and GPx. This is also supported by Mohamed et al [17], who reported that water supplementation reduced MDA levels in the blood of chickens at 37 days of age.

On the other hand, El-Tarabany et al [26], reported that improving amino acid balance and antioxidant properties in broiler chickens leads to oxidative stability and reduced lipid peroxidation associated with elevated MDA. Elwan et al [15], also demonstrated that amino acids, including methionine and cysteine, improved antioxidant status through increasing glutathione and antioxidant capacity, which was reflected in reduced oxidative stress in the resulting chicks. In contrast, Saleh et al. [22], indicated that chicks fed low crude protein diets with high amino acid levels (LPHA) recorded significantly ($P<0.05$) higher hepatic malondialdehyde (MDA) content.

Serum catalase enzyme content also showed a significant effect of the interaction treatments between the two experimental factors. The treatment of chick pull time after 493 hours with IWA significantly achieved the highest mean value of (22.21 µmol /L) compared with the remaining interaction treatments. It was observed that the interaction treatment of chick pull time before 493 hours with the control was not significantly different from the treatment of chick pull time after 493 hours with IAA, recording mean values of (13.24 and 17.30 µmol/L), respectively. The remaining treatments shared significant superiority and were arranged accordingly from the highest to the lowest, where the treatments of chick pull time after 493 hours with IAA and chick pull time before 493 hours with IWA showed the highest significant superiority. Finally, the treatment of chick pull time after 493 hours with the control and the treatments of chick pull time before 493 hours with the control and IAA ranked at the end of the significance scale.

The table also shows that hatch window, injection, and their interaction did not significantly affect serum catalase enzyme content. The effect of both chicks pull time and method of administration on catalase enzyme activity may be attributed to the influence of these factors on ionic and water balance within the body, thereby contributing to enhanced antioxidant enzyme activity represented by catalase and improving the body's ability to resist oxidative stress. CAT is considered one of the most important antioxidant enzymes, as it plays a major role in catalyzing the

decomposition of hydrogen peroxide (H_2 O_2), which is considered a reactive oxygen species. Oxidative stress may be stimulated in the body when the concentration of these radicals exceeds the physiological limit. More importantly, catalase contributes to preventing the reaction of hydrogen peroxide with oxygen under the influence of iron-binding compounds, thereby reducing the production of more toxic hydroxyl radicals (OH) and other radicals [27], [28].

The catalase enzyme response may also be associated with the role of amino acids in improving the physiological and immune status of the bird and reducing oxidative damage. Khatlab et al [29], indicated that amino acid supplementation caused a reduction in catalase enzyme activity and an increase in SOD level, attributing the decrease in catalase activity to increase NO production and cellular damage in the jejunum. In contrast, the increase in SOD activity represented an attempt to compensate for the increase in free radicals and reduce oxidative damage [30]. Likewise, Ogbuagu et al [31], reported that supplementation with amino acids, including L-serine, increased blood catalase enzyme levels. This indicates that the effect of amino acids on the antioxidant system depends on the type of amino acid added to the diet, its biological nature, and the level of oxidative stress to which the birds are exposed.

Table 2. Effect of Injection Type and Chick Pull Time During the Hatch Window on Serum Antioxidant Parameters of Broiler Chicken at 11 Days of Age

treatments[1]		MDA (µmol/L)	CAT (µmol/L)	GSH-PX (µmol/L)	SOD (µmol/L)
Main effect of hatching time					
Before 493 h		28.32	15.74	40.44	34.49 a
After 493 h		27.96	16.23	43.09	29.61 b
Probability		0.8354	0.7605	0.1599	0.0311 *
Main effect of injection type					
Without		25.11 b	13.76	43.75	36.89 a
Water		31.79 a	16.31	40.76	31.16 b
Amino acid		27.52 b	17.87	40.79	28.11 b
Probability		0.0097 **	0.1237	0.3242	0.0072 **
Interaction between hatch pull time and injection type					
Before 493 h	**Control**	32.79 a	13.24 b	41.76 ab	36.05 ab
	Water	21.74 b	16.93 ab	47.29 a	29.76 ab
	Amino acid	24.41 b	11.77 b	43.01 ab	39.96 a
After 493 h	**Control**	31.75 a	14.47 b	39.45 b	28.64 b
	Water	27.77 ab	22.21 a	36.55 b	27.46 b
	Amino acid	28.39 ab	17.30 ab	42.52 ab	30.43 ab
P-value		0.0379 *	0.0002 **	0.050 *	0.0498 *
Total mean		3.049	28.14	15.98	41.76
SEM [2]		0.037	0.94	0.84	0.97

1- Experimental Design: The hatching window (prior to 493 hours and after 493 hours) was considered the first factor, whereas the injection treatment (control, mesenteric water, mesenteric amino acids) was regarded as the second factor.

2- SEM: Values are presented as the Standard Error of the Mean.

3- Statistical Significance: Means within the column bearing different superscripts differ significantly at ($p \leq 0.05$).

It is evident that serum glutathione peroxidase enzyme content was significantly affected by the interaction between the two study factors. The treatment of chick pull time before 493 hours with IWA significantly recorded the highest mean value (47.29 µmol/L) compared with the remaining interaction treatments. It was also observed that the treatment of chick pull time before 493 hours with the control showed no significant difference from the treatment of chick pull time after 493 hours with IAA, recording mean values of (41.76 and 42.52 µmol/L, respectively). Among the remaining treatments, chick pull time before 493 hours with IAA, chick pull time after 493 hours with IAA, and chick pull time before 493 hours with the control showed higher mean values, whereas the treatments involving chick pull time after 493 hours with the control and IWA occupied the lowest significant rank. The interaction between hatch window and injection type was significant at the ($p \leq 0.05$).

Furthermore, a significant interaction response between the two experimental factors was observed in serum superoxide dismutase enzyme content. The treatment of chick pull time before 493 hours with IAA significantly recorded the highest mean value (39.96 µmol/L) compared with the remaining interaction treatments. The treatment of chick pull time before 493 hours with the control did not differ significantly from the treatment of chick pull time after 493 hours with IAA, with mean values of (36.05 and 30.43 µmol/L, respectively). Among the other interaction treatments, chick pull time before 493 hours with IWA also showed a higher mean value, whereas the treatments involving chick pull time after 493 hours with the control and IWA occupied the lowest significant rank. This significant interaction may be related to the significant effect of chick pull time on serum superoxide dismutase enzyme content, with the treatment before 493 hours recording a higher mean value (34.49 µmol/L) than the treatment after 493 hours (29.61 µmol/L), corresponding to a percentage increase of (14.14%).

The significant interaction effect on serum superoxide dismutase enzyme content may also be attributed to the significant superiority observed in the chick injection factor. A significant effect of injection treatments was observed in this trait, as the control treatment significantly achieved the highest mean value of (36.89 µmol/L) compared with the lower mean values of the IWA and in IAA treatments, which were (31.16 and 28.11 µmol/L), respectively, with percentage increases of (15.53 and 23.80%). The significant superiority in serum glutathione peroxidase and superoxide dismutase enzyme contents, particularly in the IAA treatments, may also be attributed to the vital role of amino acids in enhancing immune system efficiency and improving antioxidant capacity in birds. Increased ROS production during immune cell activation raises the requirement for glutathione, while increased glutathione synthesis requires the availability of amino acids involved in its formation. Mak et al. [32], observed increased glutathione levels during T-cell activation, and de novo glutathione synthesis, rather than recycling from GSSG, was found to be important for T-cell differentiation and functions [33]. Therefore, previous studies have demonstrated that amino acids such as

glutamine, arginine, and threonine play important roles in supporting immune function and maintaining intestinal integrity in broiler chickens. Gottardo et al [34] reported that dietary amino acid supplementation influenced the immune response of broilers challenged with Eimeria spp. and *E. coli*, while Tan et al [35] found that supplemental L-arginine attenuated intestinal mucosal disruption in broilers subjected to a coccidial challenge. In addition, Khatlab et al [29] reported that free methionine supplementation alleviated intestinal oxidative stress in broiler chickens, whereas Maroufyan et al [36] indicated that methionine and cysteine contribute to immune function and resistance to Eimeria infection. These findings support the physiological importance of amino acids in immune and intestinal functions; however, the present study differs from these studies in the route of amino acid administration, as amino acids were administered by mesenteric injection rather than dietary supplementation.

The increase in glutathione peroxidase and superoxide dismutase enzyme activities in some interaction treatments may also be attributed to the fact that these enzymes represent the antioxidant defense line in the body. Improvement in the immune and physiological status of birds enhances their efficiency in eliminating free radicals and reducing oxidative damage. Chrystal et al [37] and Hilliar et al [38] explained that amino acid metabolism, digestibility, and absorption are affected by the health status of birds, and that supplementation may contribute to improving physiological stability and reducing pathological and stress-related effects, which is positively reflected in the biological and enzymatic indicators associated with health status and resistance to oxidative stress.

Table 3 shows that indicated a significant interaction response between the two study factors in serum glucose content at 11 days of age. The interaction treatment of chick pull time after 493 hours with IWA recorded the highest value of (171.73 mg/100 ml) compared with the remaining interaction treatments. It was also observed that there were no significant differences between the interaction treatments of chick pull time before 493 hours with the control and early pull chick after 493 hours with IAA, recording mean values of (160.17 and 164.32 mg/dL), respectively. The higher serum glucose concentration observed in the control treatment may be related to differences in nutrient availability and metabolic utilization during the early post-hatch period. Glucose represents an important energy substrate for newly hatched chicks, and its circulating concentration may vary according to energy requirements, nutrient utilization, and metabolic adaptation during early growth. Therefore, the higher glucose concentration observed in the control treatment may reflect differences in endogenous energy metabolism and glucose utilization among the experimental treatments. The remaining treatments differed significantly and were ranked accordingly from the highest to the lowest mean value, where the treatments of chick pull time after 493 hours with IAA and chick pull time before 493 hours with the control showed the highest significant superiority, followed by the treatment of chick pull time before 493 hours with IWA. Finally, the treatments of chick pull time after 493 hours with the control and

chick pull time before 493 hours with IAA occupied the lowest significant rank.

Table 3 also showed that hatch window, injection, and their interaction did not significantly affect serum glucose content. These findings are consistent with those reported by Jassim and Aqeel [39], which showed that water increased blood glucose levels in broiler chickens. This may be attributed to the fact that water administration, as part of in injection substances, improves fluid balance within the body, thereby enhancing the absorption of essential nutrients such as glucose. Water also helps improve digestion and nutrient absorption through the intestines, leading to increased blood glucose concentration [17], [40].

A significant interaction effect between the two experimental factors was also observed in serum cholesterol content. The interaction treatment of chick pull time after 493 hours with IWA significantly recorded the highest mean value of (162.31 mg/dL) compared with the remaining interaction treatments. It was also observed that there were no significant differences between the interaction treatments of chick pull time before 493 hours with the control and chick pull time before 493 hours with IAA, recording mean values of (154.46 and 160.67 mg/dL), respectively. This was followed by a group of treatments that shared the same significant superiority rank and significantly exceeded the remaining treatments. Their mean values were arranged from the highest to the lowest as follows: chick pull time after 493 hours with IAA, chick pull time before 493 hours with IWA and the control, and chick pull time after 493 hours with the control. Finally, the treatment of chick pull time before 493 hours with IAA ranked at the end of the significance scale. It is also noted that the hatch window factor at both levels did not significantly affect blood cholesterol content.

The significant interaction effect on cholesterol content may be attributed to the occurrence of significant superiority in the chick injection factor levels. A significant effect of injection treatments was observed in this trait, as the IWA treatment significantly recorded the highest mean value of (164.28 mg/dL) compared with the IAA treatment, which recorded the lowest mean value of (142.46 mg/dL). The control treatment shared significance with both the superior and inferior treatments. These results may agree with what was reported by Soltan and Hussein [41], who stated that supplementation of broiler diets with tryptophan at levels of 0.20%, 0.25%, and 0.30% reduced total lipid and plasma cholesterol levels. Saleh et al [22], also reported that amino acid supplementation in chicken diets did not show a significant effect on glucose and cholesterol levels. Similarly, Abou-Elkhair et al [18], did not observe any significant effect on cholesterol or glucose among treatments supplemented with amino acids. This variation in cholesterol response may be attributed to differences in the type of amino acid used, dietary protein level, and the balance between cholesterol synthesis in the liver and its utilization in cell membrane and hormone formation processes, making the effect of amino acids on cholesterol inconsistent and dependent on the nutritional and physiological conditions of the bird.

Table 3. Effect of Injection Type and Chick Pull Time Within the Hatch Window on Serum Lipid and Glucose Parameters Traits of Broiler Chickens at 11 Days of Age.

Treatments		Glu (mg/dL)	Chol (mg/dL)	HDL (mg/dL)	TG (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)
Main effect of hatching time							
Before 493 h		152.23	153.76	41.46	94.91 a	18.98 a	93.32
After 493 h		149.01	155.82	37.96	76.46 b	15.29 b	102.57
Probability		0.6022	0.8029 NS	0.3021	0.0017 **	0.0016 **	0.2576
Main effect of injection type							
Without		142.35	157.65 ab	34.52 b	89.93	17.98	105.13 a
Water		156.16	164.28 a	40.08 ab	76.98	15.39	108.89 a
Amino acid		153.34	142.46 b	44.52 a	90.15	18.03	79.90 b
Probability		0.634	0.0443 *	0.0491 *	0.0968	0.0965	0.010 **
Interaction between hatch pull time and injection type							
Before 493 h	**Control**	160.17 ab	154.46 ab	37.19 ab	112.61 a	22.52 a	94.74 b
	Water	151.22 b	154.98 ab	44.63 a	69.03 b	13.80 b	96.54 b
	Amino acid	124.79 c	144.52 b	41.47 ab	83.58 b	16.71 b	86.34 b
After 493 h	**Control**	131.47 c	151.82 ab	38.36 ab	76.93 b	15.38 b	98.07 ab
	Water	171.73 a	162.31 a	45.72 a	88.55 b	17.71 b	98.87 ab
	Amino acid	164.32 ab	160.67 ab	30.87 b	83.44 b	16.69 b	113.10 a
P-value		0.0379 *	0.0483 *	0.0447 *	0.0411 *	0.0029 **	0.0027 **
Total mean		3.049	150.62	154.79	39.71	85.69	17.13
SEM (2)		0.037	3.06	4.12	1.74	3.35	0.67

1- Experimental Design: The hatching window (prior to 493 hours and after 493 hours) was considered the first factor, whereas the injection treatment (control, mesenteric water, mesenteric amino acids) was regarded as the second factor.

2- SEM: Values are presented as the Standard Error of the Mean.

3- Statistical Significance: Means within the column bearing different superscripts differ significantly at ($p \leq 0.05$).

It is evident from the table that there was a significant effect of the interaction between the two experimental factors on HDL content in serum. The treatments of chick pull time after 493 hours with IWA and chick pull time before 493 hours with IWA significantly recorded the highest mean values of (45.72 and 44.63 mg/100 ml), respectively, compared with the remaining interaction treatments. It was also observed that the treatment of chick pull time before 493 hours with the control did not differ significantly from the treatment of chick pull time after 493 hours with IAA, recording mean values of (37.19 and 30.87 mg/100 ml), respectively. The remaining treatments shared significant superiority and were arranged in descending order from the highest to the lowest, where the treatments of chick pull time before 493 hours with IAA, chick pull time after 493 hours with the control, and chick pull time before 493 hours with the control showed the next levels of significant superiority. Finally, the treatment of chick pull time after 493 hours with IAA recorded the lowest significant value. It is also noted that the pull-time factor at both levels did not significantly affect serum HDL content.

The significant interaction effect on HDL content may be attributed to the significant effect of the chick injection factor, as injection treatments showed a significant influence on this trait. The IAA

treatment significantly recorded the highest mean value of (44.52 mg/100 ml) compared with the lowest mean value in the control treatment (34.52 mg/100 ml), with a percentage increase of (22.46%), while the IWA treatment showed an intermediate level between them.

Furthermore, the table also indicates a significant interaction effect between the two experimental factors on triglycerides and very low-density lipoproteins (VLDL), where the treatment of chick pull time before 493 hours with the control significantly recorded the highest mean values of (112.61 and 22.52 mg/100 ml), respectively, compared with the remaining interaction treatments. It was also observed that the treatment of chick pull time before 493 hours with the control significantly outperformed the treatment of chick pull time after 493 hours with IAA by (25.90% and 25.88%), respectively. The remaining treatments followed a descending order of significance, including the treatments of chick pull time after 493 hours with IWA, chick pull time before 493 hours with IAA, chick pull time after 493 hours with IAA, and the control treatment, while the treatment of chick pull time before 493 hours with IWA occupied the lowest significant rank. It is also noted that injection levels did not significantly affect serum triglycerides and VLDL content.

The previous significant differences may be attributed to the significant effect of chick pull time before and after 493 hours, as this factor significantly influenced serum triglycerides and VLDL, where the treatments before 493 hours recorded the highest mean values of (94.91 and 18.98 mg/100 ml), respectively.

These results are also in agreement with Abou-Elkhair et al [18], who observed a significant reduction in triglycerides in amino acid-supplemented treatments compared with the control, attributing this effect to reduced feed intake, which led birds to utilize body fat as an energy source, thereby reducing serum triglyceride levels [42]. Several studies [18], [22], [43], also reported that broilers fed low-protein diets supplemented with amino acid mixtures (threonine, tryptophan, and valine) showed reduced blood triglycerides. Xie et al. [44], also reported that dietary tryptophan supplementation influenced glycolipid metabolism-related parameters in broiler chickens, with the effects varying according to dietary protein level. These findings indicate that amino acid supplementation may influence lipid metabolism in broilers, although the response may depend on the amino acid provided and the nutritional context.

Regarding low-density lipoprotein (LDL) content, a significant interaction response between the two experimental factors was observed. The treatment of chick pull time after 493 hours with IAA significantly recorded the highest mean value of (113.10 mg/100 ml) compared with the remaining interaction treatments. It was also observed that this treatment significantly exceeded the treatment of chick pull time before 493 hours with the control by (16.23%). The remaining treatments were arranged in descending order of significance, starting with the treatments of chick pull time after 493 hours with the control and in IWA, while the treatments of chick pull time before 493 hours with IWA, the control, and IAA occupied the lowest significant ranks. It is

also noted that the hatch window factor did not significantly affect serum LDL content.

The significant interaction effect on LDL may be attributed to the significant effect of the injection factor, as injection treatments significantly influenced this trait. The IWA and control treatments recorded the highest mean values of (108.89 and 105.13 mg/100 ml), respectively, compared with the lowest mean value in the IAA treatment (79.90 mg/100 ml), with percentage increases of (26.62% and 23.99%). These findings are consistent with Shihab et al. (2019), who reported that water administration increased LDL levels in quail blood.

This increase in LDL may also be explained by the role of amino acids in regulating lipid and cholesterol metabolism, as elevated levels of certain amino acids, particularly tryptophan, may stimulate hepatic lipid and cholesterol synthesis through effects on insulin secretion and metabolic pathways. Xie et al [44], reported that tryptophan supplementation in low-protein diets increased serum triglycerides and cholesterol levels, while Wang et al [45], also found that increased tryptophan levels elevated total plasma cholesterol in broiler chickens.

Conclusion

The study concluded that mesenteric injection with either the amino acid mixture (IAA) or water (IWA) produced several physiological, biochemical, and antioxidant responses in broiler chickens compared with the other treatments. Mesenteric IAA was associated with improved antioxidant status, particularly in SOD and GSH-Px, and generally lower concentrations of some lipid fractions, including LDL and TG. These effects may be related to the role of amino acids in supporting antioxidant defense and regulating lipid metabolism. In contrast, mesenteric IWA was associated with increased CAT activity and HDL concentration but also with higher Chol and LDL levels. These findings indicate that the physiological and biochemical responses to mesenteric injection varied according to the injected solution, with the amino acid mixture showing effects on antioxidant and lipid-related parameters that differed from those observed following water injection.

References

[1] Such, N., Schermann, K., Pál, L., Menyhárt, L., Farkas, V., Csitári, G., & Dublecz, K. 2023. The hatching time of broiler chickens modifies not only the production traits but also the early bacteriota development of the ceca. Animals, 13(17): 2712.

[2] Boyner, M. 2023. A flying start: Adapted hatching and post-hatch feeding in broiler chickens. Acta Universitatis Agriculturae Sueciae, 2023(38).

[3] Abioja, M. O., Ojoawo, H. T., Akinjute, O. F., & Logunleko, M. O. 2023. Influence of egg storage length and orientation on hatching traits and spread of hatch in Transylvanian naked neck chickens. Journal of Animal Physiology and Animal Nutrition, 107(4): 1083–1092.

[4] Kalaba, Z. M., Ismail, F. S. A., & Kassem, M. F. 2023. Physiological differences, physical characteristics, and some plasma parameters on chicks

hatched from multi stage incubator system versus single stage incubator system. Journal of Animal and Poultry Production, 119–124.

[5] Wilcox, C. H., Sandilands, V., Mayasari, N., Asmara, I. Y., & Anang, A. 2024. A literature review of broiler chicken welfare, husbandry, and assessment. World's Poultry Science Journal, 80(1): 3–32.

[6] Lee, J. T., Rochell, S. J., Kriseldi, R., Kim, W. K., & Mitchell, R. D. 2023. Functional properties of amino acids: Improve health status and sustainability. Poultry Science, 102(1): 102288.

[7] Alabi, T., & Adedokun, S. 2025. Amino acid nutrition in poultry: A review. Animals, 15(22): 3323.

[8] Rahdari, A., & Hamidi, F. 2025. The effect of intraperitoneal injection of Glycyrrhizin on central regulation of food intake in broilers injected with LPS. British Poultry Science, 66(1): 124–130.

[9] Ling, G., Wang, L., Rui, F., Li, Z., Wang, J., Ren, K., Zhou, X., Ghartey-Kwansah, G., Xu, M., & Jones, O. 2017. Transportation of liquid crystal and CaCO3 vaterite crystal in chicken embryo and early postnatal development. Molecular Crystals and Liquid Crystals, 647(1): 373–384.

[10] Wang, L., Xu, M., Jones, O., Zhou, X., Li, Z., Sun, Y., Zhang, Y., Bryant, J., Ma, J., & Isaacs, W. B. 2020. Use of the mesentery and peritoneum to facilitate the absorption of yolk sac nutrients into viscera of the developing chick: Novel function for this organ. Mesentery and Peritoneum, 4:1-12.

[11] Aviagen. 2026. ROSS 308 Broiler performance objectives. https://aviagen.com/tmea/brands/ross/products/ross-308.

[12] Kelly, L. M., & Alworth, L. C. (2013). Techniques for collecting blood from the domestic chicken. Lab animal, 42(10), 359–361.

[13] Lebedev, S., Kazakova, T., Marshinskaia, O., & Grechkina, V. (2023). The assessment of serum trace element levels as the diagnostic biomarkers of functional state of broiler chickens. Veterinary world, 16(7), 1512–1519.

[14] SAS. 2004. SAS Users Guide: Statistics Version 6th ed (6th ed). SAS Institute Inc.

[15] Elwan, H. A. M., Elnesr, S. S., Xu, Q., Xie, C., Dong, X., & Zou, X. 2019. Effects of in ovo methionine-cysteine injection on embryonic development, antioxidant status, IGF-I and TLR4 gene expression, and jejunum histomorphometry in newly hatched broiler chicks exposed to heat stress during incubation. Animals, 9(1): 25.

[16] Madej, J. P., Graczyk, S., Bobrek, K., Bajzert, J., & Gaweł, A. 2024. Impact of early posthatch feeding on the immune system and selected hematological, biochemical, and hormonal parameters in broiler chickens. Poultry Science, 103(3): 103366.

[17] Mohamed, A., Khalil, M., Soliman, F., & El-Sabrout, K. 2025. The effect of drinking ionized water on the productive performance, physiological status, and carcass characteristics of broiler chicks. Animals, 15(2): 229.

[18] Abou-Elkhair, R., Ahmed, H., Ketkat, S., & Selim, S. 2020. Supplementation of a low-protein diet with tryptophan, threonine, and valine and its impact on growth performance, blood biochemical constituents, immune parameters, and carcass traits in broiler chickens. Veterinary World, 13(6): 1234.

[19] Wang, S., Wang, P., Thompson, R., Ostrikov, K., Xiao, Y., & Zhou, Y. 2023. Plasma-activated medium triggers immunomodulation and autophagic activity for periodontal regeneration. Bioengineering & Translational Medicine, 8(4): 1–14.

[20] Min, Y. N., Liu, S. G., Qu, Z. X., Meng, G. H., & Gao, Y. P. 2017. Effects of dietary threonine levels on growth performance, serum biochemical indexes, antioxidant capacities, and gut morphology in broiler chickens. Poultry Science, 96(5): 1290–1297.

[21] Ospina-Rojas, I. C., Murakami, A. E., Duarte, C. R. A., Eyng, C., Oliveira, C. A. L., & Janeiro, V. 2014. Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. British Poultry Science, 55(6): 766–773.

[22] Saleh, A. A., Amber, K. A., Soliman, M. M., Soliman, M. Y., Morsy, W. A., Shukry, M., & Alzawqari, M. H. 2021. Effect of low protein diets with amino acids supplementation on growth performance, carcass traits, blood parameters and muscle amino acids profile in broiler chickens under high ambient temperature. Agriculture, 11(2): 185.

[23] Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., & De Smet, S. 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. Journal of animal science and biotechnology, 7(1): 37.

[24] Xu, H. L., Li, H., Bao, R. K., Tang, Y. X., Elsherbeni, A. I. A., Gharib, H. B. A., & Li, J. L. 2022. Transport Stress Induced Cardiac NO-NOS Disorder Is Mitigated by Activating Nrf2/HO-1/NQO1 Antioxidant Defense Response in Newly Hatched Chicks. Frontiers in veterinary science, 9: 938826.

[25] Kohrogi, R., Shimamoto, S., Nakashima, K., Sonoda, D., Ohtsuka, A., & Ijiri, D. 2020. Effects of delaying post-hatch feeding on lipid peroxidation and antioxidant enzyme mRNA expression in the pectoralis major muscle of newly hatched chicks. Animal science journal Nihon chikusan Gakkaiho, 91(1): e13327.

[26] El-Tarabany, M. S., Ahmed-Farid, O. A., Nassan, M. A., & Salah, A. S. 2021. Oxidative stability, carcass traits, and muscle fatty acid and amino acid profiles in heat-stressed broiler chickens. Antioxidants, 10(11): 1725.

[27] Wang, W., Zhu, J., Cao, Q., Zhang, C., Dong, Z., Feng, D., Ye, H., & Zuo, J. 2022. Dietary catalase supplementatio n alleviates deoxynivalenol -induced oxidative stress and gut microbiota dysbiosis in broiler chickens. Toxins, 14(12): 830.

[28] Tang, M., Fang, R., Xue, J., Yang, K., & Lu, Y. 2022. Effects of catalase on growth performance, antioxidant capacity, intestinal morphology, and microbial composition in yellow broilers. Frontiers in Veterinary Science, 9: Article 802051.

[29] Khatlab, A. D. S., Del Vesco, A. P., de Oliveira Neto, A. R., Fernandes, R. P. M., & Gasparino, E. 2019. Dietary supplementation with free methionine or methionine dipeptide mitigates intestinal oxidative stress induced by Eimeria spp. challenge in broiler chickens. Journal of Animal Science and Biotechnology, 10(1): 58**.**

[30] Çam, Y., Atasever, A., Eraslan, G., Kibar, M., Atalay, Ö., Beyaz, L., & Liman, B. C. 2008. Eimeria stiedae: Experimental infection in rabbits and the effect of treatment with toltrazuril and ivermectin. Experimental Parasitology, 119(1): 164–172.

[31] Ogbuagu, N. E., Ayo, J. O., Aluwong, T., & Akor-Dewu, M. B. 2023. L-serine modulates activities of antioxidant enzymes and behavioral responses in broiler chickens subjected to feed restriction during the hot-dry season. Journal of Veterinary Behavior, 60: 1–9.

[32] Mak, T. W., Grusdat, M., Duncan, G. S., Dostert, C., Nonnenmacher, Y., Cox, M., & Brenner, D. 2017. Glutathione primes T cell metabolism for inflammation. Immunity, 46(4): 675–689.

[33] Lian, G., Gnanaprakasam, J. R., Wang, T., Wu, R., Chen, X., Liu, L., & Wang, R. 2018. Glutathione de novo synthesis but not recycling process coordinates with glutamine catabolism to control redox homeostasis and directs murine T cell differentiation. eLife, 7: e36158.

[34] Gottardo, E. T., Burin Junior, Á. M., Lemke, B. V., Silva, A. M., Busatta Pasa, C. L., & Muller Fernandes, J. I. 2017. Immune response in Eimeria sp. and E. coli challenged broilers supplemented with amino acids. Austral Journal of Veterinary Sciences, 49(3): 175–184.

[35] Tan, J., Applegate, T. J., Liu, S., Guo, Y., & Eicher, S. D. 2014. Supplemental dietary L-arginine attenuates intestinal mucosal disruption during a coccidial vaccine challenge in broiler chickens. British Journal of Nutrition, 112(7): 1098–1109.

[36] Maroufyan, E., Kasim, A., Yong Meng, G., Ebrahimi, M., Teck Chwen, L., Mehrbod, P., & Soleimani Farjam, A. 2013. Effect of dietary combination of methionine and fish oil on cellular immunity and plasma fatty acids in infectious bursal disease challenged chickens. The Scientific World Journal, 2013(1): 531397.

[37] Chrystal, P. V., Moss, A. F., Khoddami, A., Naranjo, V. D., Selle, P. H., & Liu, S. Y. 2020. Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. Poultry Science, 99(3): 1421–1431.

[38] Hilliar, M., Keerqin, C., Girish, C. K., Barekatain, R., Wu, S. B., & Swick, R. A. 2020. Reducing protein

and supplementing crystalline amino acids, to alter dietary amino acid profiles in birds challenged for subclinical necrotic enteritis. Poultry Science, 99(4): 2048–2060.

[39] Jassim, E. Q., & Aqeel, C. H. 2017. Effect of alkaline water and/or magnetic water on some physiological characteristic in broiler chicken. Journal of Entomology and Zoology Studies, 5(5): 1643–1647.

[40] Shibata, M., Takahashi, T., Kozakai, T., Shindo, J., & Kurose, Y. 2023. Development of active jejunal glucose absorption in broiler chickens. Poultry Science, 102(8): 102804.

[41] Soltan, M. E., & Hussein, E. A. 2017. Effect of different levels of tryptophan supplementation on growth performance and some blood constituents of Sinai Bedouin chicks. Egyptian Journal of Nutrition and Feeds, 20(3): 493–502.

[42] Nukreaw, R., & Bunchasak, C. 2015. Effect of supplementing synthetic amino acids in low-protein diet and subsequent re-feeding on growth performance, serum lipid profile and chemical body composition of broiler chickens. The Journal of Poultry Science, 52(2): 127–136.

[43] Abdallah, A. A. E., Mahmoud, M. A., Khalifah, M. M., El-Deken, M. R., Abou-Shehema, B. M., Farag, M. E., & Yossef, M. S. 2025. Effect of supplementation with valine, isoleucine, and tryptophan amino acid on growth performance, carcass traits, and nitrogen excretion of broiler chickens fed low-protein diets. Journal of King Abdulaziz University: Meteorology, Environment and Arid Land Agriculture, 35(1): 71–90.

[44] Xie, K., Feng, X., Zhu, S., Liang, J., Mo, Y., Feng, X., Ye, S., Zhou, Y., Shu, G., Wang, S., Gao, P., Zhu, C., Fan, Y., Jiang, Q., & Wang, L. 2024. Effects of tryptophan supplementation in diets with different protein levels on the production performance of broilers. Animals, 14(13): 1838.

[45] Wang, B., Min, Z., Yuan, J., Zhang, B., & Guo, Y. 2014. Effects of dietary tryptophan and stocking density on the performance, meat quality, and metabolic status of broilers. Journal of Animal Science and Biotechnology, 5(1): 44.