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The effect of evaluating the effectiveness of injecting hatching eggs with different levels of allicin and nano-allicin on hatching characteristics and some physiological indicators of broiler chicks

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Abstract

This study was conducted in two parts. eggs were injected, As for the second experiment In it, the hatched chicks from the first experiment were raised . results of this experiment showed:

Significant differences were observed between treatments in the percentage of dead embryos, with treatment T3 recording the highest percentage for this trait, at 3%, compared to treatment T5.Chick vitality was observed to be two seconds higher in treatments T2, T5, and T6, compared to broiler chick vitality in treatments T1, T3, and T4, which was one second higher.At 35 days of age, significant differences ($P<0.05$) were observed between all treatments. There was a significant decrease ($P<0.05$) in glucose concentration in treatments T2, T3, T4, T5, and T6 compared to treatment T1. There was a significant decrease ($P<0.05$) in protein concentration in treatment T1. There was a significant increase ($P<0.05$) in globulin concentration in treatments T4, T5, and T6 compared to treatment T1. On the first day of the chicks' life, significant differences ($P<0.05$) occurred, as the cholesterol concentration increased significantly in treatments T2 and T5 compared to treatment T4, and there was a significant decrease ($P<0.05$) in the triglyceride concentration in treatment T3, and the VLDL concentration increased significantly ($P<0.05$) in treatment T5, and significant differences ($P<0.05$) occurred in treatment T5 compared to treatment T1 in the LDL concentration.

Keywords: Allicin, broiler, hatching eggs , hatching characteristics, biochemical characteristic , lipoprotein.

I. Introduction

Today, the use of antibiotics in poultry feed faces serious criticism. However, there are some important factors that limit the use of antibiotics, such as drug resistance in bacteria and drug residues in meat. These effects have led to the elimination or severe reduction of their use in many countries due to concerns about the development of antibiotic-resistant human pathogenic bacteria and legislative measures to limit their use in many other countries (Cervantes, 2006) (Michard, 2008). Therefore, attempts have been made to find other alternatives of natural origin. Intra-oval injection of exogenous substances may be a technique for enhancing chick performance (Kadam et al., 2013). Many years ago, the intra-ovum injection technique was first introduced to inoculate broiler embryos (Ricks et al., 1999). It was then used to deliver nutrients to embryos. Poultry has a limited supply of nutrients for embryo development (Uni et al., 2012). Therefore, the ability to mobilize nutrients and inhibitors is also scarce to produce the embryo's needs,

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resulting in poor embryo growth, reduced hatchability, and poor chick quality. This deficiency is also solved by providing additional sources of essential nutrients and antioxidants via intra-ovum administration (Urso et al., 2015). Nowadays, feeding eggs with antioxidants throughout the incubation period may enhance the antioxidant status of chicken embryos (El-Saadany et al., 2019) and post-hatching developmental stages (Yigit et al., 2014). Recently, there has been an increasing trend towards the use of phytochemicals (such as polyphenols, isoflavones, and carotenoid compounds) obtained from natural sources due to their Their potential antioxidant activities (Alagawnay et al., 2022) (Wani et al., 2022) (Madkour et al., 2024), nanobiotechnology has been used to improve the growth performance and nutritional status of broiler chickens. These nanoparticles are typically absorbed in larger quantities from the gastrointestinal tract and exert enhanced biological effects in the target tissues of the animals. These nanoparticles are efficiently absorbed and reach essential organs via the blood (Handford et al., 2014) (Peters et al., 2016). Allicin is an organosulfur compound extracted from garlic, a species of the Alliaceae family (Eric, 1985). Allicin has a distinctive pungent odor and exhibits antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Bautista et al., 2005). Its mechanism of action is as an antioxidant or anti-stress agent, such as free radical scavenging. Allicin hydrolyzes to form 2-propene sulfenic acid, which is capable of binding free radicals and acting as an anti-stress agent (Vaidya et al., 2009; Block et al., 2010). Therefore, this study aimed to demonstrate the effect of injecting broiler hatching eggs with different levels of allicin and nano-allicin on hatching characteristics and some physiological indicators of broiler chicks.

II. Materials and methods:

- Birds and Dietary treatment:

This study was conducted in two parts. The first experiment was conducted at the Al-Jaflawi Poultry Hatchery in Babil Governorate from November 5, 2024, to November 26, 2024, where eggs were injected. The second experiment was conducted at the Abu Ghraib Poultry Farm in the Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, from November 26, 2024, to December 31, 2024, for a period of 42 days. The hatched chicks from the first experiment were raised. The birds were randomly distributed into six treatments, 45 birds per treatment, with three replicates (15 birds per replicate). The treatments were divided as follows:

1) T1: No injection (negative control).
2) T2: Injection of 0.3 ml/egg with saline (NaCl) (positive control).
3) T3: Inject hatching eggs with 2.5% nano-allicin at a dose of 0.3 ml/egg.
4) T4: Inject hatching eggs with 5% nano-allicin at a dose of 0.3 ml/egg.
5) T5: Inject hatching eggs with 2.5% allicin at a dose of 0.3 ml/egg.
6) T6: Inject hatching eggs with 5% allicin at a dose of 0.3 ml/egg.

All treatments were given water and feed ad libitum throughout the experiment period and the feed components were according to the broiler requirements guide, Rose 308, Table (1).

HPLC Analysis (High-Performance Liquid Chromatography)

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High-performance liquid chromatography (HPLC) analysis was performed on the allicin powder sample to confirm its purity. The resulting chromatogram showed a single, prominent peak at a retention time of 4.18 minutes. This peak represented 100% of the total chromatogram area, indicating that the sample contained only a single compound, without impurities or other compounds under the approved separation conditions. This indicates that the allicin used has a high degree of purity, enhancing its reliability in further biological or chemical studies (Zhou et al., 2020).

- Hatching characteristics::

The hatching percentage for the treatments was calculated by extracting the percentage for each treatment according to (Khattab et al., 1992). As for the weight at hatching (g), the hatched chicks for each treatment were weighed in the hatchery.

-Biochemical Characteristics

Blood samples were collected from birds at two days and 35 days of age.

Blood serum analyses were performed at Al-Shifa Laboratory in Baghdad using an automated blood chemistry analyzer manufactured by Xian Yima. The analysis included glucose, cholesterol, triglycerides, low-density lipoproteins, total protein, albumin, and globulin. The absorption coefficient was measured using a spectrophotometer at various wavelengths ranging from 400 to 600 nanometers for each of the aforementioned tests, according to the instructions included with the kits for each test, which were supplied by the French company BIOLABO.

As for high-density lipoproteins (HDL), very-low-density lipoproteins (VLDL), and low-density lipoproteins (LDL), they were estimated according to: (Grove, 1979) (Burstein et al., 1980).

Table 1. Percentage composition of the experimental diets

Types of diets	Starter diet%	Grower Diet%	Finisher Diet%
Corn	47.7	45.7	58.6
Wheat	10	15.2	4.1
Soybean Meal [1] (48% crude protein)	33	28.9	26.2
Protein Concentrate *	5	5	5
Sunflower Oil	2	3.2	4.1
Limestone	1.1	1.1	1.2
Dicalcium Phosphate	0.7	0.5	0.4
Na Cl	0.3	0.2	0.2
Vitamin and Mineral premix	0.2	0.2	0.2
Total	100	100	100
Chemical Calculated Values[3]			
Crude Protein %	23.14	21.66	20.7
M.E. Kcal/ Kg Diet	3000.5	3103.7	3204.65
Crude Fiber %	2.7	2.7	2.5

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Lysine, %	1.32	1.21	1.13
Methionine+ Cysteine	0.88	0.84	0.81
Ca %	0.94	0.88	0.89
Available P%	0.48	0.44	0.47

* use the Dutch protein concentrate, Barcon-5 Special W, which contains 40% crude protein, 2107 kcal of metabolizable energy, 2.2% crude fiber, 5% crude fat, 4.2% calcium, 4.68% available phosphorus, 3.7% methionine, and 3.85% lysine.
* Soybean meal of Argentine origin contains 48% crude protein.
[3]Based on (NRC, 1994).

- Statistical analysis

The Statistical computations were done using SAS software program (SAS, 2012) to explore the influence of treatment. Duncan's multiple range test (1955) to compassion between means. The statistical model was as follows:

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

Where:

Yij = Dependent variable.

μ = Overall mean.

T_i= Effect of treatment (Control and 3 treatments).

e_{ij} = Error term.

Estimate the correlation of coefficient between H/L ratio and characters of egg quality variables.

III. Results and Discussion:

Figure (1) shows the evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano allicin on the hatching rate of broiler chicks. No significant differences were observed between the studied treatments and the control treatment in the hatching rate.

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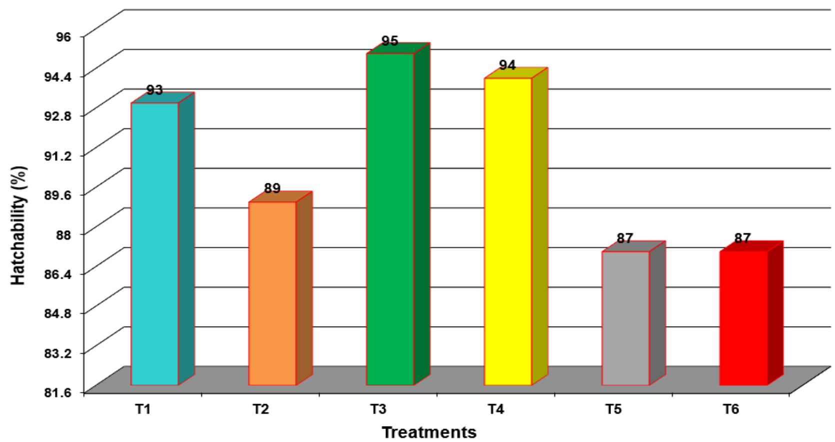

Figure 1: Evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano-allicin on the hatchability of broiler chicks.

It is noted from Figure (2) that the effectiveness of injecting hatching eggs with different levels of allicin and nano allicin in the percentage of dead embryos of broiler chicks was evaluated. Significant differences were observed between the treatments, as treatment T3 recorded the best percentage for this trait, 3%, compared to treatment T5, which reached 12%.

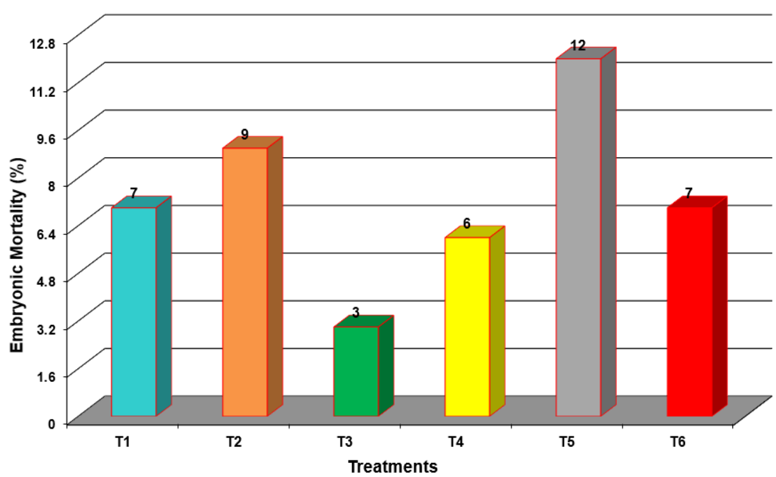

Figure 2: Evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano-allicin on the percentage of embryo deaths in broiler chicks.

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It is noted from Figure (3) the effect of the studied treatments on the percentage of crushed eggs, as the maximum percentage was 2% in treatments T2 and T6, followed by treatment T5, while it was non-existent in treatments T1, T3 and T4 in this study, until the differences between the treatments were not significant.

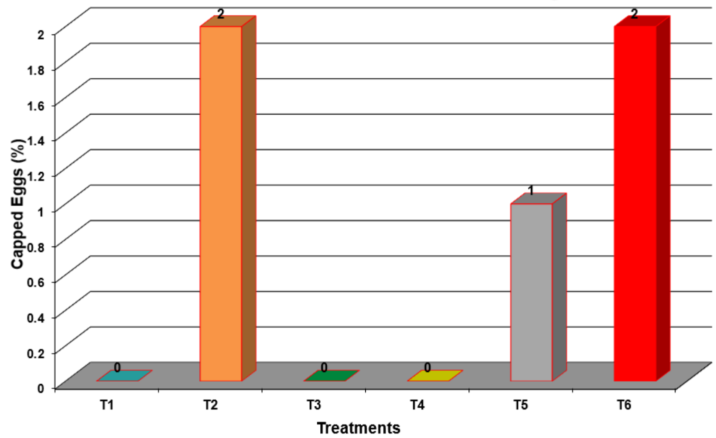

Figure 3: Evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano-allicin on the percentage of broiler eggs hatched.

Figure (4) shows the evaluation of the vitality of broiler chicks treated with injecting hatching eggs with different levels of allicin and nano allicin. It was observed that the vitality of the chicks was 2 seconds in treatments T2, T5 and T6, which is higher than the vitality of the broiler chicks in treatments T1, T3 and T4, which reached 1 second.

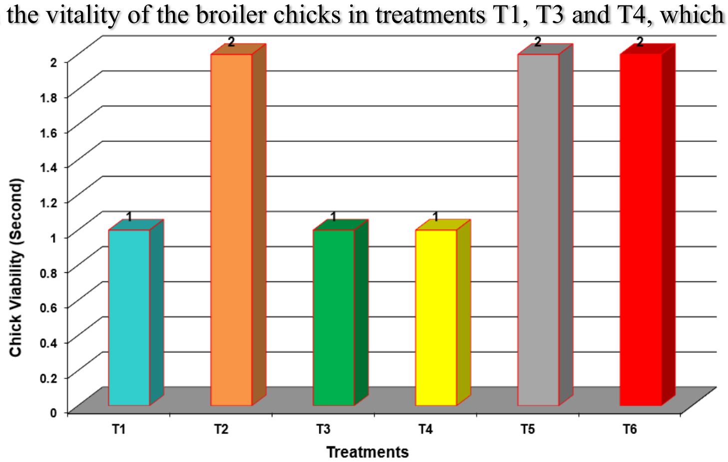

Figure 4: Evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano-allicin on the vitality of broiler chicks.

Table (2) shows the effect of the studied treatments on the biochemical characteristics on the first day of the chicks' life when evaluating the effectiveness of injecting hatching eggs with different levels of Allicin and nano Allicin for

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broiler chickens on the biochemical characteristics. There were no significant differences in all the studied characteristics on the first day of life between all treatments and the control treatment.

Table (2) : Evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano allicin for broiler chickens on biochemical characteristics on the first day of chicks' life.

Mean ± Standard error				
Treatments	Glucose (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Glubulin (g/dl)
T1	132.70 ± 4.33	3.54±0.26	1.18±0.29	1.71±0.32
T2	121.99 ± 9.92	3.19±0.11	1.17±0.46	1.57±0.26
T3	118.42 ±9.52	3.63±0.21	0.69±0.12	2.25±0.36
T4	124.13±5.17	3.63±0.12	1.10±0.36	1.83±0.32
T5	127.20±9.21	3.56±0.23	0.62±0.03	2.20±0.18
T6	117.12±18.10	3.46±0.03	0.95±0.15	1.79±0.12
Significant	N.S	N.S	N.S	N.S

The means with different letters within the same column are significantly between them
($P<0.05$) *, NS= No significant

While Table (3) showed the effect of the studied treatments on the biochemical characteristics at the age of 35 days, there were significant differences ($P<0.05$) between all treatments, as it was noted that there was a significant decrease ($P<0.05$) in the glucose concentration in treatments T2, T3, T4, T5, T6 compared to treatment T1, and a significant decrease ($P<0.05$) in the protein concentration occurred in treatment T1 compared to treatments T4, T5.
No significant differences were observed between treatments T1, T2, T3, T6 for the same trait. Likewise, no significant differences were observed between treatments T2, T3, T4, T5, T6. No significant differences were observed in albumin concentration among all treatments. A significant increase ($P<0.05$) was observed in globulin concentration in treatments T4, T5, T6 compared to treatment T1. No significant differences occurred between treatment T1 and treatments T2, T3. Likewise, no significant differences occurred between treatments T2, T3 and treatments T4, T5, T6 for the same trait.

Table (3): Evaluation of the effectiveness of injecting hatching eggs with different levels of Allicin and Nano-Allicin on the biochemical characteristics of broiler chickens at 35 days.

Mean ± Standard error				
Treatments	Glucose (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Glubulin (g/dl)
T1	155.34± 8.17a	3.92± 0.07c	1.04±0.10	1.93± 0.13b
T2	109.77± 19.61b	4.13± 0.12bc	0.97±0.13	2.10± 0.09ab
T3	100.81± 7.33b	4.29± 0.15abc	0.98±0.13	2.19± 0.23ab
T4	97.84± 8.82b	4.78± 0.18ab	0.70±0.02	2.81± 0.10a
T5	105.51± 9.89b	4.95± 0.49a	0.89±0.15	2.67± 0.41a

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T6	92.98± 10.02b	4.54± 0.16abc	0.68±0.07	2.71± 0.18a
Significant	*	*	N.S	*

The means with different letters within the same column are significantly between them
(P<0.05) *, NS= No significant

Table (4) shows the effect of the studied treatments on lipoproteins on the first day of the chicks' life when evaluating the effectiveness of injecting hatching eggs with different levels of allicin and nano allicin for broiler chickens on lipoproteins. There were significant differences (P<0.05) as cholesterol concentration increased significantly in treatments T2, T5 compared to treatment T4, while no significant differences appeared between treatments T1, T3, T6 and treatments T2, T4, T5.

There was also a significant decrease (P<0.05) in the concentration of triglycerides in treatment T3 compared to treatments T2, T5, T6, and no significant differences were found between treatments T1, T4 and T2, T3, T5, T6. The same table shows a significant increase (P<0.05) in the concentration of VLDL in treatment T5 compared to treatments T3, T4, and significant differences (P<0.05) occurred in treatment T5 compared to treatment T1 in the concentration of LDL, and no significant differences were found between treatments T2, T3, T4, T6. At the same time, no significant differences were found between the treatments in the concentration of HDL.

Table (4): Evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano allicin for broiler chickens on lipoprotein levels on the first day of chicks' life.

Mean ± Standard error					
Treatments	Cholestrol (mg/dl)	Triglycride (mg/dl)	HDL	VLDL	LDL
T1	97.13±9.65ab	88.88±1.21 abc	44.17±9.56	17.77±0.24abc	6.73±1.00b
T2	110.88±6.37a	91.04±3.89 ab	43.25±4.28	18.21±0.78ab	9.89±0.94 Ab
T3	97.72±4.39ab	73.07±7.43 c	45.16±1.72	14.61±1.48 c	9.31±0.11 ab
T4	88.94±5.04b	76.92±5.07 bc	44.40±11.05	15.38±1.01bc	7.85±1.45 ab
T5	115.92±7.10a	99.31± 5.59a	42.20±0.82	19.86±1.11 a	10.61±0.88 a
T6	104.71±3.91ab	92.89±6.33 ab	50.71±4.34	18.57±1.26ab	8.69±0.81 ab
Significant	*	*	N.S	*	*

The means with different letters within the same column are significantly between them
(P<0.05) *, NS= No significant

Table (5) shows the evaluation of the effectiveness of injecting hatching eggs with different levels of Allicin and Nano Allicin for broiler chickens in lipoproteins at day 35. There was a highly significant decrease (P<0.01) in cholesterol concentration in treatments T3, T4, T5, T6 compared to treatment T1, and cholesterol concentration increased in

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treatment T2 compared to treatment T4. No significant differences appeared between treatments T1 and T2, and no differences were observed between treatments T2, T3, T5, T6, and there were no differences between treatments T3, T4, T5, T6 for the same trait.

A highly significant increase (P<0.01) in triglyceride concentration was observed in treatments T1 and T2 compared to treatments T4, T5, T6. No significant differences were observed between treatment T3 and the remaining treatments. The same table shows a highly significant difference (P<0.01) in HDL concentration increase in treatment T6 compared to treatments T1, T4. No significant differences were observed between treatments T1, T2, T3, T4, and T5, T6. The high morale (P <0.01) occurred in the VLDL concentration, as its concentration increased in T1, T2 compared to the T4, T5, T6 transactions, and moral differences did not occur between these transactions and T3 transactions, and a moral height (P <0.05) occurred in the LDL concentration in T1 transaction compared to T2 treatment and no moral differences occur between these two transactions and the rest Experience transactions.

Table (5): Evaluation of the effectiveness of injecting hatching eggs with different levels of allicin and nano allicin for broiler chickens on lipoprotein at 35 days.

Mean ± Standard error					
Treatments	Cholestrol (mg/dl)	Triglycride (mg/dl)	HDL	VLDL	LDL
T1	122.43± 7.68a	123.63± 6.14a	36.48±5.50 c	24.72±1.22a	10.08±2.31a
T2	110.05±0.70ab	110.72±11.54a	46.05±0.79 bc	22.14±2.30a	4.18±0.20 b
T3	92.24±17.37bc	100.48±7.62ab	46.36±2.34 bc	20.09±1.52ab	8.02±1.86 Ab
T4	61.75± 4.84c	83.96±6.80b	43.80±2.70 c	16.79± 1.36b	7.32±0.30 ab
T5	83.53±9.38bc	78.27±6.64b	57.45±5.26 ab	15.65±1.32 b	9.18±1.61 Ab
T6	87.25±6.06bc	83.86±3.48b	60.00±4.62 a	16.77±0.69 b	7.79±1.46 ab
Significant	**	**	**	**	*

The means with different letters within the same column are significantly between them
(P<0.05) *, NS= No significant

Several studies have demonstrated the cholesterol-lowering effect of garlic in broiler and layer chickens (Ismail et al., 2021) (Elbaz et al., 2022) (Motamedi and Taklimi, 2014) (Omer et al., 2019) (Ao et al., 2010). Enzymes contained in garlic may play a role in regulating lipid metabolism, enhancing the activities of enzymes that stimulate biliary cholesterol secretion, and reducing the partial absorption of dietary cholesterol (Al-Massad et al., 2018).

Diallyl polysulfides from garlic extract can protect cell membranes from lipid peroxidation (Horie et al., 1992). In addition, the essential oils in garlic and other plants can scavenge oxygen free radicals by reducing malondialdehyde (MDA) levels and enhancing superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels (Pourali et al., 2014) (Chen et al., 2009) (Lin et al., 2017). In addition, organosulfur compounds in garlic can treat liver damage by reducing the release of pro-inflammatory hepatic cytokines and enhancing antioxidant activity by inhibiting the expression of cytochrome P450 2E1 (Guan et al., 2018) (Lai et al., 2014). This explains the results obtained.

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IV. Conclusion:

Injecting hatching eggs with different levels of allicin and nano-allicin affected hatchability and some physiological parameters of broiler chicks. Improvements were observed in some injection treatments compared to negative and positive control treatments, and a significant improvement in blood parameters was observed in injection treatments compared to negative and positive control treatments at 35 days of age. Improvements in lipid profiles were also observed in injection treatments at 35 days of age.

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