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ROLE OF LACTOFERRIN DURING EMBRYOGENESIS IN EMBRYONIC DEVELOPMENT AND BLOOD BIOCHEMISTRY OF FAYOUMI CHICKS

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ABSTRACT : An experiment was carried out to study the influences of in ovo lactoferrin (Lf) injection at 7 days of embryogenesis on the development of the embryo, blood biochemistry, haematology, and antioxidant status in Fayoumi chicks. On day 7 of incubation, six hundred fertile eggs obtained from a Fayoumi chicken flock were randomly assigned to four groups of 150 eggs each, with each group further subdivided into 5 replicates of 30 eggs. The first group, un-injected eggs (the control), The second group was injected with 0.1 ml saline, and the third and fourth groups were injected with saline (0.1 ml) containing 50 and 100 µl Lf respectively. In ovo injection of 100 µl Lf significantly augmented the chick weight, thymus %, and bursa% at hatch day. In ovo Lf injection considerably (p < 0.05) reduced overall counts of bacteria and fungi, as well as the count of harmful bacteria, including Shigella, Coliform, and Salmonella. The values of total protein, globulin, albumin, High-Density Lipoprotein (HDL) and antioxidant indices except Glutathione Peroxidase (GSH-px) in the serum were significantly augmented in the Lf-treated groups compared with the control group. Blood hematology, serum creatine, uric acid, triglycerides, and Low-Density Lipoprotein (LDL) not impacted by *in ovo* Lf injection. Lf groups showed significantly (p < (0.05) lower serum total cholesterol, Aspartate Aminotransferase (AST) (p < 0.001), and Alanine Aminotransferase (ALT) (p < 0.05) than the control group. In conclusion, in ovo administration of Lf at 7 days of embryogenesis positively influences the embryo development, blood biochemistry, antioxidant status, and microbial analysis of residual volk sac in Fayoumi chicks.

Key words: antioxidant, blood biochemistry, lactoferrin, poultry

1. INTRODUCTION

Producing healthy chicks under ideal, controlled environmental conditions is a necessity. One of the study areas that have received a lot of attention lately is the in ovo feeding technique for chickens. It is a process based on injecting liquid solutions with carbohydrates, amino acids, and different substances into the poultry embryos during the incubation stage (Saeed et al., 2019; Shehata et al., 2024). Applications of in ovo feeding during incubation serve important functions various such as enhancing hatching, chicks vaccination, increasing digestive capacity and intestinal development, improving muscle growth, immune system, and skeletal system, reducing early embryo mortality, boosting body weight and feed efficiency, as well as reducing difficulties linked to oxidative stress and infections in poultry production (Jochemsen and Jeurissen, 2002; Tako et al., 2004; Bhanja et al., 2004). During the incubation process, different immunological components, including lactoferrin, can be injected directly into the egg to create new uses.

Lactoferrin is a glycosylated iron-containing globular protein (Wang et al., 2019). According to Ashraf et al. (2024), this molecule has a molecular weight of 80 kDa and is made up of roughly 690 amino acids. Bovine lactoferrin (bLF) has received a lot of interest due to its ability to promote growth and other beneficial multi-factorial biological features (Artym and Zimecki, 2023). According to modern research, it can growth by improving nutrient boost absorption and having immune-modulating properties (Abd El-Hack et al., 2023). Lactoferrin's ability to bind iron enhances its nutritional value, which helps to preserve the health of the birds (Wang et al., 2019). At the same time, because of its potent antibacterial properties, lactoferrin lowers the pathogenic load (Drago-Serrano et al., 2018). In addition to promoting the proliferation of advantageous bacteria and possessing the immediate effect of iron defense on pathogens, LF has positive

effects such as antiviral, anti-inflammatory, antimicrobial, and antiparasitic activity (Arslan et al., 2021). LF's antibacterial, antiinflammatory, and antioxidant qualities are strongly linked to its iron chelation action (Demir et al., 2025). Furthermore, LF is a well-known modulatory protein due to its capacity to control immunological responses and cell signaling pathways (Demir et al., 2025). According to Chen et al. (2015), LF the capacity to prevent has neurodegeneration and protect dopaminergic cells from oxidative stress.

Therefore, the purpose of this study was to examine the influence of *in ovo* lactoferrin injection at 7 days of embryogenesis on the development of the embryo, blood biochemistry, haematology, and antioxidant status in Fayoumi chicks.

2. MATERIALS AND METHODS

The experimental work was carried out at El-Azab Poultry Research Station, Fayoum, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Egypt.

2.1. Egg incubation, experimental procedure and application

Eggs were normally incubated under optimal incubation conditions in an automatic incubator. A total of 600 viable eggs from a flock of 56-week-old Fayoumi chickens were randomly allocated into four groups of 150 eggs each, with each group further subdivided into 5 replicates of 30 eggs. The first one (T1) was without injection as a control, the 2nd (T2) was injected with 0.1 ml NaCl 0.75%, the 3^{rd} (T3) and 4^{th} (T4) groups were injected with saline (0.1 ml) containing 50 and 100 µl Lf/egg, respectively. Egg injection procedure into the air sac was carried out on the seventh day of embryonic development. Bovine lactoferrin was purchased from HYGINT Pharmaceutical Company, Alexandria, Egypt.

2.2. Chicks weight at hatch Relative organs weight

The body weight of chicks on hatch day was recorded. Six chicks per group were slaughtered to record the weights of residual yolk, heart, liver, left lung, and small intestine and then expressed as % of the bird's weight as follows:

Relative organ weight (%) = $\frac{\text{Absolute weight of organ (g)} \times 100}{\text{Live body weight (g)}}$

2.3. Blood sample collection and laboratory analyses

At hatch time, blood samples from six chicks per group were randomly collected after slaughtering into two tubes. The first (without an anticoagulant) tube was centrifuged for 10 min at 3000 rpm to separate the serum that was saved at $-80 \circ C$ for laboratory analyses. In order to measure total blood hematology, the second tube contained whole blood along with an anticoagulant. Hematological parameters assessed included hemoglobin concentration and packed cell volume. Serum levels of several antioxidants, including glutathione (GSH), superoxide dismutase (SOD), glutathione-S-transferase (GST), and glutathione peroxidase (GSH-Px) were assessed. Additionally, using commercial kits (Bio-diagnostic Company, Giza, Egypt), an automated system (Shimadzu, Japan) was used to spectrophotometrically determine the serum concentrations of creatine, albumin, total protein, uric acid, lipid profile (total cholesterol, triglycerides, LDL, and HDL), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT).

2.4. Microbiological Examination

In residual yolk sac contents, total counts of fungi, total counts of bacteria, Coliform, Shigella, and Salmonella counts were determined following the method described by Rezaee et al. (2021).

2.5. Statistical analysis

IBM SPSS Statistics for Windows (IBM SPSS 22) was used to analyze all data using a one-way ANOVA according to the following general model: $Yij = \mu + Ti + eij$

Where: Yij: observed value μ : overall mean Ti: treatment effect (i: (1 to 4) eij: random error. Post hoc Duncan's multiple range tests were used to compare means for significance. The criterion for statistical significance was set at P <0.05.

3. RESULTS

3.1. Chick weight and relative weight of some internal organs

The influences of in ovo Lf injection on chick weight, residual yolk sac weight, and relative weight of organs of hatched chicks are displayed in Table 1. In ovo injection of 100 Lf at 7 days of incubation significantly increased the chick weight at hatch day compared to the control groups. Influences of in ovo Lf injection on the seventh day of incubation on the relative weight of organs (gizzard, heart, small intestine, and kidney) and the residual yolk did not display any significant differences among the treatments. The group injected with both Lf levels at 7 days of incubation had significantly (p <0.05) higher thymus % than other groups. Only the high concentration affected the bursa %.

3.2. Assessment of hematological and biochemistry blood parameters

The influences of *in ovo* Lf injection at 7 days of incubation on serum constituents are presented in Table 2. The values of total protein, albumin, and globulin, in the serum were significantly increased in the Lf-treated groups at 7 days of incubation compared with the control group. *In ovo* Lf injection at 7 days of incubation did not consistently affect uric acid compared with the control group. Lf groups showed significantly lower serum creatine, AST (p < 0.001), and ALT (p < 0.05) than the control group (Table 2).

Table 3 shows the effects of the Lf injection at 7 days of incubation on blood hematology and the serum lipid profile. The current study's outcomes showed that, in comparison to the control and NaCl groups. Results indicated that the administration of Lf in egg on the 7th day of incubation showed an insignificant effect on values of Hb and PCV compared to the control group. Furthermore, the Lf groups at two levels had significantly lower serum total cholesterol levels (p <0.05) and higher serum HDL levels. On the other hand, serum triglycerides, and LDL levels did not significantly change between any of the groups. The impacts of in ovo Lf injection at 7 days of incubation on the antioxidant indices of hatched Fayoumi chicks are shown in Table 4. Serum GSH, SOD, and GST activity were all elevated in the Lf injection groups (50 and 100 μ l/egg) in comparison to the control group (p < 0.001). However, Serum GSH-px activity did not change significantly among all groups.

3.3. Microbiological examination of the residual yolk sac.

Table 5 lists the impact of *in ovo* administration of Lf at 7 days of incubation on pathogenic microorganisms in the residual yolk sac. Groups treated with Lf at 7 days of incubation had considerably lower overall counts of bacteria and fungi (p < 0.001) than groups that were not treated. Additionally, compared to the control group, the *in ovo* administration of Lf at 7 days of incubation significantly reduced the count of *Shigella*, *Coliform*, and *Salmonella* (p < 0.001).

4. DISCUSSION

Embryonic growth in poultry can be controlled through in ovo feeding of nutrients and natural bioactive components ((El-Kholy et al., 2021; Saeed et al., 2019). According to El-Kholy et al. (2022), the in ovo injection of these chemicals affects the physiological status of broiler embryos both before and after hatching, resulting in increased post-hatch growth. According to noteworthy literature, bovine LF the improves broiler chickens' development performance (Gao et al., 2021; Saeed et al., Because LF improves growth 2023). performance, it can be employed as a natural growth stimulant in broiler chickens (Hassan et al., 2024). Similar studies have previously demonstrated higher body weight and weight increase in groups treated with LF (Enany et al., 2017; Abd El Monsef et al., 2024).

Lf can encourage the growth and development of a range of tissues by functioning as a growth factor (Naot et al., 2005). Lactoferrin is a bioactive protein and may play a vital role in embryonic development. In the current study, *in ovo* administration of 100 Lf significantly improved the chick weight at hatch day. Thus, in ovo Lf administration may be essential for enhanced embryonic development. Most of the development of the immune system is complete by the late embryonic phase (Eren et al., 2016). Chickens' immune systems can develop and mature more quickly if they are exposed to substrates early. The development of the lymphoid organ is essential for a sufficient immune response. Following an in ovo injection of Lf (100 µl/egg), the relative thymus and bursa of Fabricius weight were increased in the current investigation. Increased thymus and Fabricius weight relative to bursa may be a positive sign of improved avian immunity (Elnesr et al., 2019). It shows that Lf affects cellular proliferation and the weight of lymphoid organs.

In the current study, in ovo Lf administration improves the antioxidant indices in the serum. Furthermore, to shield cells from oxidative damage, LF is essential for inhibiting reactive oxygen species (ROS) (Sokolov et al., 2020). Hassan et al. (2024) displayed that LF-treated groups had significantly reduced MDA levels and significantly greater levels of SOD, CAT, and GSH. The higher antioxidant activity in the LF-treated birds may be attributed to its role in decreasing the intracellular ROS and levels and controlling cellular MDA antioxidant enzymes. Furthermore, LF's capacity to lower lipid peroxidation through iron sequestration and its iron-binding qualities may be linked to elevated CAT and SOD activities (Abd El Monsef et al., 2024). Lf's capacity to chelate iron ions seems to be connected to its antioxidant action (Iglesias-Figueroa et al., 2019).

Lactoferrin is essential for maintaining the normal physiological homeostasis linked to the emergence of clinical diseases (Ashraf et al., 2024). Chen et al. (2024) elucidated that higher levels of bLF regulate abnormalities in the kidneys and liver. Farid et al. (2021) revealed that Lf can maintain physiological homeostasis by regulating pro-inflammatory responses. Assaraj et al. (2018) clarified that Lf has a better protective impact on liver damage. In the current study, in ovo Lf administration improves liver function by reducing liver enzymes. Chen et al. (2024) stated that supplementation with bLF effectively controlled AST and ALT. Hassan et al. (2024) exhibited that LF-treated birds had significantly lower AST, ALT, uric acid, creatinine. These and results are corroborated by another study that claims that LF supports hepato-renal functioning by lowering tissue damage, protein loss, and enzyme release (Abd El Monsef et al., 2025). In harmony, El-Sherbeny et al. (2023) found that LF significantly reduced tissue damage, protein loss, and enzyme release, as evidenced by increased serum TP, globulin and albumin, and decreased levels of AST, ALT, and creatinine. Assaraj et al. (2018) found that Lf has immunomodulatory and liver-preventive effects in rats treated with it, as evidenced by increased gamma globulin levels.

Besides antioxidant indices. another important indicator to measure the health status of chicks is the serum lipid profile. Faridvand et al. (2017) pointed out that Lf decreased LDL, triglyceride, and total cholesterol values and increased HDL values in high-cholesterol-fed rats. Additionally, Lf has a favorable influence on plasma cholesterol levels and delays hepatic lipid accumulation in mice (Takeuchi et al., 2004). Moreover, Lf exerts a beneficial impact on lipid metabolism and diminishes the levels of total cholesterol and LDL (Jańczuk et al., 2022). Antioxidants from Lf can decrease triglyceride and total cholesterol levels in the blood (Jusni et al., 2022). Collectively, these findings underscore the multifaceted role of Lf in lipid regulation and oxidative balance, supporting its potential application in enhancing health and preventing metabolic disorders in animals.

Lactoferrin has a distinct structure and strong antibacterial and immunomodulatory properties associated with the transferrin family (Abd El Monsef et al., 2024). In the current study, *in ovo* Lf administration reduced the overall counts of bacteria and fungi, as well as the count of harmful bacteria in the residual yolk sac. Lactoferrin

leads to particular immune responses and is a strong antibacterial component against a variety of infections (Ashraf et al., 2024). One way to boost avian immunity and reduce their vulnerability to infectious disease is to use immuno-stimulants. Appelmelk et al. (1994) displayed that lactoferrin, a cationic protein, has both bacteriostatic and bactericidal properties. Also, Lf can prevent the bacteria from entering cells, allowing the early prevention of infection. The lactoferrin's bactericidal influence inhibits pathogenic bacteria by interacting directly with the lipopolysaccharide of Gram-negative bacteria (Farnaud and Evans, 2003). Furthermore, LF's bacteriostatic action is primarily carried out by breaking down the peptidoglycans in the bacterial cell wall, which alters the membrane permeability and causes cell lysis (Sahin et al., 2016), Its antimicrobial activity appears to be connected to iron deprivation by eliminating a crucial substrate needed for bacterial growth (Giansanti et al., 2016). Few studies have examined LF's possible efficacy against parasites, particularly coccidia, despite the fact that many have emphasized its preventive qualities as an antibacterial and antifungal agent. LF dramatically changed the caecal microbial populations in chicken birds and had a major antibacterial mpact on multidrug-resistant strains of Escherichia coli (Saeed et al., 2023). LF exhibits bactericidal effects by disrupting the outer membrane of Gram-negative bacteria, as as immunoregulatory effects by well reducing the release of interleukin-1 (IL-1), IL-2 and tumor necrosis factor (TNF) and improving the cytotoxicity of monocytes and natural killer cells (Caccavo et al. 2002).

CONCLUSION

In ovo injection of Lf at 7 days of embryogenesis elicited significant positive effects on the embryo development, blood biochemistry, antioxidant status, microbiological evaluation of the residual yolk sac in Fayoumi chicks on the hatch day. The Lf (100 μ l/egg) had the best results among the injected doses.

Items	Control	NaCl	Lf 50 µl	Lf 100 µl	SEM ¹	P-value
Chick weight (g)	33.11 ^b	32.67 ^b	35.30 ^{ab}	36.98 ^a	0.98	0.021
Gizzard (%)	4.32	5.85	5.03	4.16	0.16	0.190
Liver (%)	3.27	3.48	3.01	2.96	0.11	0.165
Heart (%)	0.92	0.85	0.80	0.75	0.42	0.546
Small Intestine (%)	3.74	4.87	4.08	3.43	0.09	0.213
Bursa (%)	0.23 ^b	0.22 ^b	0.29 ^b	0.35 ^a	0.04	0.182
Thymus (%)	0.31 ^b	0.33 ^b	0.45 ^a	0.54^{a}	0.02	0.112
Residual yolk (%)	13.65	12.55	11.76	12.76	0.14	0.871
Kidney (%)	0.58	0.66	0.68	0.69	0.75	0.432

Table (1): Effect of Lf *in ovo* injection on chick weight and relative weight of organs in newly hatched chicks

^{a, b} letters in the same row indicate significant differences between values with different letters (at $P \le 0.05$). ¹Pooled SEM. Lf =Mean Lactoferrin

Table (2): Effect o	of Lf in ovo injection	on blood biochemical	l in newly hatched	chicks

1Items	Control	NaCl	Lf 50 (µl)	Lf 100 (µl)	SEM2	P-value
Total Protein (g/dL)	4.38b	4.08b	6.05a	5.83a	0.38	0.00
Albumin (g/dL)	3.97b	3.74b	5.78a	5.99a	0.25	0.00
Globulin(g/dL)	0.96b	0.64c	1.48a	1.62a	0.04	0.00
Uric acid(mg/dL)	42.43	44.73	44.03	44.36	1.09	0.18
Creatine(mg/dL)	1.23b	1.58a	1.09b	1.02b	0.07	0.00
AST(IU)	66.54a	60.42a	51.22b	50.17b	2.96	0.00
ALT(IU)	41.37a	38.72a	31.62b	28.37b	1.35	0.04

a, b, c letters in the same row indicate significant differences between values with different letters (at $P \le 0.05$). 1Aspartate Aminotransferase (AST); Alanine Aminotransferase (ALT) 2Pooled SEM. Lf =Mean Lactoferrin.

Table	e (3): Effect of Lf in	ovo injection or	n the blood	hematology	and the	serum lipid	profile in
newly	hatched chicks						

1Items	Control	NaCl	Lf 50	Lf 100	SEM2	P-value
			(µl)	(µl)		
Blood hematology						
Hb (mg/dL)	11.49	11.36	13.36	12.43	0.34	0.17
Packed cell volume (%)	35.33	34.71	40.31	36.63	1.03	0.23
Serum lipid profile						
Cholesterol (mg/dL)	125.64a	125.36a	109.44b	112.19b	2.87	0.04
Triglycerides(mg/dL)	163.83	166.22	159.56	162.71	5.68	0.93
HDL (mg/dL)	42.13b	48.67b	68.35a	70.12a	2.27	0.01
LDL (mg/dL)	56.93	64.32	60.35	62.14	3.76	0.12

a, b letters in the same row indicate significant differences between values with different letters (at $P \le 0.05$). 1H. B= Hemoglobin; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein 2Pooled SEM. Lf =Mean Lactoferrin.

¹ Items	Control	NaCl	Lf 50(µl)	Lf 100 (µl)	SEM ²	P-value
GSH(mg/dL)	4.68 ^b	5.43 ^{ab}	6.37 ^a	7.43 ^a	0.11	0.00
SOD (U/mL)	122.76 ^b	128.42 ^b	258.01 ^a	269.52 ^a	19.19	0.00
GST (U/L)	181.44 ^b	196.41 ^b	287.45 ^a	301.73 ^a	11.28	0.00
GPx (mU/mL)	1763.75	1662.38	1964.70	2020.43	99.08	0.76

Table (4): Effect of Lf in ovo injection on antioxidant indices in newly hatched Chicks

^{a, b} letters in the same row indicate significant differences between values with different letters (at $P \le 0.05$). ¹GSH: Glutathione; SOD: Superoxide Dismutase; GST: Glutathione S-Transferase; GPx: Glutathione Peroxidase

²Pooled SEM. Lf =Mean Lactoferrin.

Table (5):Effect of Lf *in ovo* injection on microbial analysis of residual yolk sac in newly hatched chick

Items	Control	NaCl	Lf 50	Lf 100	SEM ²	P-value
			(µl)	(µl)		
Total counts of bacteria ($\times 10^7$ cfu g ⁻¹)	244.30 ^a	199.12 ^b	122.13 ^c	109.23 ^d	11.36	0.00
Total counts of fungi ($\times 10^6$ cfu g ⁻¹)	83.30 ^a	52.81 ^b	49.40 ^b	29.30 ^b	5.36	0.00
Salmonella ($\times 10^3$ cfu g ⁻¹)	46.02 ^a	39.44 ^a	24.40 ^b	21.50 ^b	1.74	0.00
Shigella (×10 ³ cfu g ⁻¹)	53.20 ^a	26.20 ^b	19.60 ^b	14.83 ^b	4.42	0.00
Coliform Group ($\times 10^5$ cfu g ⁻¹)	77.20 ^a	55.20 ^b	50.40 ^b	29.03 ^c	2.97	0.00

^{a, b, c, d} letters in the same row indicate significant differences between values with different letters (at $P \le 0.05$). ¹Pooled SEM.

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الملخص العربي

دور اللاكتوفيرين خلال التكوين الجنيني في تطور الأجنة والكيمياء الحيوية للدم في كتاكيت الفيومي

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تم إجراء تجربة لدراسة تأثيرات حقن اللاكتوفيرين داخل البيضة في اليوم السابع من تكوين الجنين على تطور الجنين والكيمياء الحيوية للدم وأمراض الدم والحالة المضادة للأكسدة في كتاكيت الفيومي. شملت الدراسة استخدام 600 بيضة مخصبة تم الحصول عليها من قطيع دجاج الفيومي، حيث تم توزيع البيض عشوائيًا على أربعة معاملات في اليوم السابع من الحضانة كل معامله تحتوي علي 5 مكررات وفي كل مكرر عدد 30 بيضه مخصبه ، كالتالي: بيض غير محقون (مجموعه التحكم)، وبيض محقون بـ 0.1 مل من المحلول الملحي، وبيض محقون بـ 0.1 مل من المحلول الملحي المحتوي على 50 و100 ميكرولتر من اللاكتوفيرين علي التوالي.

أدى حقن 100 ميكرولتر من اللاكتوفيرين داخل البيضة إلى زيادة كبيرة في وزن الكتاكيت ونسبة الغدة الزعترية ونسبة جراب فابريشيوس عند الفقس. كما أن حقن اللاكتوفيرين داخل البيضة أدى إلى انخفاض في العدد الإجمالي للبكتيريا والفطريات، بالإضافة إلى تقليل أعداد البكتيريا الضارة، بما في ذلك الشيغيلا والكوليفورم والسالمونيلا.

لوحظت زيادة معنوية في قيم البروتين الكلي والجلوبيولين والألبومين و HDL ومؤشرات مضادات الأكسد ه باستثناء GSH-px في مصل الدم في المجموعات المعالجة باللاكتوفيرين مقارنةً بمجموعة التحكم. ومع ذلك، لم يكن هناك تأثير لحقن اللاكتوفيرين داخل البيضة على مؤشرات الدم، وحمض اليوريك والدهون الثلاثية و LDL في مصل الدم. أظهرت مجموعات اللاكتوفيرين انخفاضًا كبيرًا في الكوليسترول الكلي في الدم والكرياتين وإنزيمي AST و ALT مقارنةً بمجموعة التحكم.

ختامًا، فإن إعطاء اللاكتوفيرين داخل البيضة في اليوم السابع من تكوين الجنين يؤثر إيجابيًا على نمو الجنين والكيمياء الحيوية للدم والحالة المضادة للأكسدة والتحليل الميكروبي لكيس المح في كتاكيت الفيومي