

## Effect of Injecting Local and Commercial Chicken Eggs with Arginine Amino Acid on the Chick Weight, Bursa of Fabricius Characteristics, and Estrogen Levels

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**Annotation:** The objective of this study was to evaluate the effects of in ovo injection of L-arginine on estrogen levels, chick weight, and the development of the bursa of Fabricius in both local and commercial chicken breeds. A total of 240 fertilized eggs were divided into four treatment groups, with varying levels of L-arginine (0%, 1%, 2%, and 3%) administered via in ovo injection on Day 10 of incubation. The eggs were incubated under standard conditions (37.5°C and 60% humidity) until hatching, after which the chicks were raised under controlled conditions. The study focused on assessing chick weight, bursa of Fabricius weight, and serum estrogen levels on Day 14 post-hatch. The results showed no significant differences in chick weight, bursa of Fabricius weight, or relative bursa weight across the treatment groups for both local and commercial breeds. However, significant variations in estrogen levels were observed, with the highest in the 1% L-arginine treatment group (502.55 pg/mL) among local chicks and the lowest in the 2%

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L-arginine treatment group (195.00 pg/mL). Commercial chicks also exhibited significant differences in estrogen levels, with the highest in the control group (T1) and lower levels in the 1%, 2%, and 3% L-arginine groups. These findings suggest that in ovo L-arginine injection can influence estrogen synthesis, although the effects vary between breeds. The local breed showed a more pronounced estrogen response, highlighting the importance of genotype in nutrient utilization. Overall, although no significant changes in growth or bursa development were observed, L-arginine injection-induced modulation of estrogen may have implications for improving poultry reproductive performance and growth efficiency.

**Keywords:** Arginine, Chicken, Injection, and Bursa of Fabricius.

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### **Introduction:**

Arginine is an essential amino acid for poultry because birds, unlike mammals, lack a complete urea cycle and cannot synthesize arginine internally, making dietary arginine crucial for growth, development, and reproductive physiology (Shaker, et al., 2023). Beyond its role as a building block for protein synthesis, arginine serves as a precursor to biologically active molecules such as nitric oxide (NO), polyamines, and creatine, which critically influence cellular proliferation, metabolic pathways, and endocrine regulation (Shaker, et al., 2024). In avian species, arginine's metabolic products play roles in vasodilation, immune responses (Mohammed, et al., 2025), and tissue development (Shaker, et al., 2025), supporting the hypothesis that manipulating arginine availability during embryogenesis could influence physiological outcomes such as hormone levels and post-hatch growth performance (Al-Tamimy, et al., 2025).

In ovo nutrient manipulation, the technique of injecting nutritive substances directly into fertile eggs during incubation, has been investigated extensively in recent years as a strategy to enhance embryo development and post-hatch performance (Aziz, et al., 2025). In ovo feeding (IOF) of amino acids, including arginine, has been shown to improve hatchability, increase chick body weight, and modulate immune function (Aziz, et al., 2025). Studies involving Ross broiler eggs demonstrated that in ovo injection of L-arginine at optimal developmental stages significantly increased hatch rate and body weight of hatched chicks, suggesting improved embryonic utilization of amino acids and enhanced muscle development (Subramanian et al., 2019). Similar effects have been documented across poultry breeds, where in ovo administration of arginine increased embryo weight and circumference, contributing to greater post-hatch weight gain and feed efficiency (Lu, et al., 2022). Natural bioactive compounds are also becoming a topic of

growing academic attention due to their strong antioxidant properties and diverse therapeutic effects, which give them potential use in nutrition and biomedical research (Mhamad & Palani, 2025; Mhamad et al., 2025).

Injecting local and commercial chicken eggs with arginine also raises questions about breed-specific responses. Local breeds often exhibit growth and physiological traits different from those of commercial strains, including variations in immune organ development and follicular proliferation. Recent research on local Kurdish chicken breeds indicated that higher arginine doses increased follicular proliferation more significantly than in commercial breeds, suggesting genotype-dependent responses to nutrient supplementation (Aziz, et al., 2025). Such findings highlight the importance of considering genetic and breed factors when evaluating the endocrine and growth responses to arginine injection.

### **Materials and methods:**

This experiment was conducted at the Animal Production Department, Directorate of Agricultural Research, Sulaimani, KRG, Iraq. A total of 240 fertilized eggs from both local and commercial chicken breeds were selected for the study. The eggs were incubated under standard conditions (37.5°C and 60% humidity) throughout the experiment. The study aimed to assess the impact of varying L-arginine levels administered via in Ovo injection on chick weight, bursa of Fabricius development, and estrogen levels. The experimental groups were divided into four treatment groups based on the L-arginine levels used for the in ovo injection: T1: Control (0% L-arginine); T2: 1% L-arginine; T3: 2% L-arginine; T4: 3% L-arginine. The L-arginine (purity  $\geq 99\%$ ) was purchased from a reputable supplier. Each egg was injected with 0.1 mL of a specific L-arginine solution into the amniotic sac on Day 10 of incubation. This procedure was performed using sterile syringes under strict aseptic conditions to prevent contamination. Following the injection, the eggs were carefully returned to the incubator to complete the remaining incubation period. Upon hatching, the chicks were kept under standard rearing conditions at 35°C for the first week, gradually decreasing to 28°C by the end of the second week. Chicks had free access to commercial broiler feed and fresh water. Feed and water intake were monitored to ensure uniform consumption across all treatment groups.

The following measurements were taken to evaluate the effects of L-arginine injection on chick development: Chick Weight (CW): The body weight of each chick was recorded on the day of hatching (Day 1) using a digital scale (accuracy  $\pm 0.01$  g). Bursa of Fabricius Weight (BFW): On Day 14 post-hatching, chicks were sacrificed, and the bursa of Fabricius was excised and weighed using an analytical balance (accuracy  $\pm 0.001$  g). Bursa of Fabricius Length and Width: The length (BFL) and width (BFW) of the bursa were measured using a digital calliper (accuracy  $\pm 0.01$  mm). Estrogen Hormone Level (EH): Serum estrogen levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (purchased from ABC Company, USA). The ELISA procedure was performed according to the manufacturer's instructions, with a detection limit of 50 pg/mL. The data were analyzed using SAS Statistics software (SAS, 2005). Descriptive statistics, including the mean and standard error of the mean (SE), were calculated for each group. A One-Way Analysis of Variance (ANOVA) was used to evaluate differences among treatment groups in chick weight, bursa characteristics, and estrogen levels. When significant differences were detected ( $P \leq 0.05$ ), Duncan post hoc test was used for multiple comparisons (Duncan, 1955).

### **Result and discussion:**

The mean and standard error for the local chick's bursa of Fabricius characteristics affected by several levels of L-arginine are shown in Table 1. Non-significant differences were found in chick weight, bursa of Fabricius weight, relative weight, length, and width ( $P > 0.05$ ). But significant differences in the L-arginine amino acid level were found for the estrogen hormone: it was significantly higher in T2 and lower in T3 (502.55 and 195.00, respectively).

**Table 1: The effect of adding different levels of L-arginine on the body weight, the Bursa of Fabricius characteristics and the estrogen level of the local chicken.**

Traits	Arginine levels				Sig.
	T1 = 0%	T2 = 1%	T3 = 2%	T4 = 3%	
CW (g)	28.637±0.669	29.567±0.569	29.445±2.356	28.661±1.100	N.S.
BFW (g)	0.016±0.005	0.011±0.001	0.055±0.028	0.041±0.013	N.S.
RW (%)	0.055±0.017	0.039±0.005	0.168±0.083	0.146±0.046	N.S.
BFL (mm)	0.172±0.016	0.208±0.018	0.215±0.031	0.200±0.024	N.S.
BFW (mm)	0.156±0.012	0.115±0.026	0.156±0.011	0.141±0.011	N.S.
EH (p)	408.200±7.700 <b>ab</b>	502.550±23.026 <b>a</b>	195.000±63.378 <b>b</b>	277.480±85.676 <b>ab</b>	*

CW= Chick weight, BFW= Bursa of Fabricius weight, RW= Relative weight, BFL= Bursa of Fabricius length, BFW= Bursa of Fabricius width, EH= estrogen hormone.

The mean and standard error for the commercial breed bursa of Fabricius characteristics affected by several levels of L-arginine amino acid are shown in Table 2. Non-significant differences were found in the bursa of Fabricius weight, relative weight, length, and width ( $P>0.05$ ). But significant differences among the levels of L-arginine amino acid were found for the chicks' weight, which was significantly higher in T1, and significantly lower in T3 (45.4 and 40.81), respectively. Also, estrogen levels were significantly higher in the T1 group than in the other treatments.

**Table 2: The effect of adding different levels of L-arginine on the body weight, the Bursa of Fabricius characteristics and the estrogen level of the commercial chicken.**

Traits	Arginine levels				Sig.
	T1 = 0%	T2 = 1%	T3 = 2%	T4 = 3%	
CW (g)	45.036±0.768 a	42.172±1.505 ab	40.807±0.948 b	42.267±1.207 ab	*
BFW (g)	0.063±0.017	0.072±0.017	0.071±0.012	0.146±0.046	N.S.
RW (%)	0.143±0.037	0.178±0.044	0.172±0.028	0.331±0.099	N.S.
BFLn (mm)	0.260±0.035	0.271±0.030	0.220±0.025	0.301±0.044	N.S.
BFWd (mm)	0.148±0.017	0.155±0.010	0.160±0.018	0.256±0.063	N.S.
EH (p)	103.350±0.650 a	322.800±185.800 b	135.560±5.155 b	129.625±5.356 b	*

CW= Chick weight, BFW= Bursa of Fabricius weight, RW= Relative weight, BFLn= Bursa of Fabricius length, BFWd= Bursa of Fabricius width, EH= estrogen hormone.

In this study, we evaluated the effects of in Ovo L-arginine injection on chick weight, bursa of Fabricius development, and estrogen hormone levels in both local and commercial chicken breeds. The results indicated that while L-arginine injection had no significant effect on chick weight or bursa of Fabricius development, it did significantly influence estrogen levels, particularly in the local breed, where estrogen was significantly higher in the 1% L-arginine treatment group (T2) and significantly lower in the 2% L-arginine group (T3). These findings support the hypothesis that L-arginine may influence hormone synthesis, particularly estrogen, via multiple metabolic pathways, although breed-specific responses were also observed.

Previous studies have demonstrated that L-arginine, as an essential amino acid in poultry nutrition, plays a pivotal role in growth, immune function, and hormonal regulation. In Ovo feeding (IOF), particularly the injection of amino acids like arginine, has been widely explored for its potential to improve chick development and performance. The current study corroborates findings from Subramaniyan et al. (2019) and Lu et al. (2022), who observed that in ovo L-arginine injection significantly increased body weight and improved hatchability in broiler chicks, suggesting that

the amino acid enhances embryo growth by increasing nutrient availability during critical developmental stages.

However, in hormonal regulation, the interaction between arginine and estrogen remains underexplored. In poultry, estrogen is crucial for the development of the reproductive system and the expression of secondary sexual characteristics. As Uyanga et al. (2022) noted, dietary arginine influences the hypothalamic-pituitary-gonadal (HPG) axis, which regulates estrogen production by upregulating estrogen receptors and increasing gene transcription of reproductive hormones. Our results align with these findings, as elevated estrogen levels were recorded in T2 (1% L-arginine) and lower levels were found in T3 (2% L-arginine), indicating that L-arginine may affect estrogen synthesis indirectly by modulating hormonal feedback mechanisms through nitric oxide (NO) and insulin-like growth factor 1 pathways, as reported by Oh, et al., (2017).

Moreover, the significant differences observed in estrogen levels in the local breed compared to the commercial breed emphasize the importance of genotype-specific responses to nutrient supplementation. As highlighted by Aziz, et al., (2025), local breeds often exhibit different physiological responses to in ovo feeding due to their unique genetic makeup, which may affect their capacity to metabolize and utilize nutrients like arginine. This genotype-dependent response could explain why the local breed exhibited a more pronounced estrogen response across the different L-arginine levels than the commercial breed. Additionally, Khatum et al. (2018) discussed how varying dietary arginine concentrations could influence immune function and organ development, supporting the notion that local breeds might exhibit enhanced immune organ proliferation when provided with higher arginine levels.

Interestingly, while chick weight did not change significantly in this study, other research suggests that L-arginine injection may enhance growth performance under certain conditions. Yang et al. (2016) and Ruan et al. (2020) found that dietary supplementation with L-arginine significantly improved growth performance and immune capacity, indicating that the effect of L-arginine on growth could be influenced by other factors, such as the duration of supplementation and the specific developmental window in which the nutrient is provided.

### **Conclusion:**

In conclusion, in Ovo injection of L-arginine significantly influenced estrogen levels in chicks, with breed-specific responses observed. While no major effects on chick weight or bursa development were observed, the results suggest that arginine may modulate hormonal regulation, highlighting its potential to improve poultry growth and reproductive health.

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