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Effect of aromatase inhibitors on the productive performance of broiler (Ross 308) chicks

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Abstract
This study was conducted at the poultry farm of the Animal Production Department, College of Agriculture, Al-Qasim Green University, from March 8, 2022, to April 12, 2022, for 35 days. The study aim was to find out the effect of aromatase inhibitors on productive traits from hatching until 35 days of age. A total of 180 chicks were selected from a total of 300 sterile eggs. The chicks were distributed into five treatments with three replicates and 12 chicks per replicate. These treatments were the control (T_1) without any additions or treatments; hatching eggs were sprayed with 1mg/100 ml of aromatase inhibitor solution in the second treatment (T_2); hatching eggs were dipped in 1mg/100 ml of aromatase inhibitor solution in the third treatment (T_3); hatching eggs were sprayed with 2mg/100 ml of aromatase inhibitor solution in the fourth treatment (T_4); hatching eggs were dipped in 2mg/100 ml of aromatase inhibitor solution in treatment five (T_5). The results showed that (LBW) and weight gain (WG) in T_2 was significantly (p<0.05) higher than T_5 in the first week while in week 4, T_2 and T_4 were significantly higher than T_3 in the live body weight, whereas weight gain as higher in T_1 compared to T_3; otherwise no differences were found between other treatments. For feed intake, T_1 showed significant increase compared to T_3 and T_5 in the fourth week while the overall rate showed significant increase in T_1 and T_2 compared to T_3 whereas no differences were found with others. Finally, no significant effect was observed between treatments regarding feed conversion ratios.

Keywords: Broiler, productive performance, aromatase inhibitors

1. Introduction
Meat production in broiler chickens is marked by rapid weight gain and a low feed conversion ratio, making it an efficient system for protein production. However, despite these advantages, a significant challenge persists: a decline in reproductive performance, particularly fertility, during the later stages of the rearing period in breeding flocks. As global demand for protein continues to rise, poultry production must increase accordingly to meet the need for both meat and eggs (Fouad *et al.*, 2020) [8].

The sustained achievement of this particular form of growth depends significantly on reproductive factors like the fertility and hatchability rates of broiler and layer hens. Aromatase is an enzyme that changes testosterone into estrogen. This is one of the most important things that affects male fertility. This change in hormones can throw off the balance of hormones in poultry, which can have bad effects on their reproductive and productive traits. Aromatase inhibitors (AIs) have come to light as promising ways to fight these effects AIs stop the aromatase enzyme from changing testosterone into estrogen. This keeps testosterone levels higher and may even make it easier to get pregnant (Weil *et al.*, 1999; Ali *et al.*, 2017) [2, 20]. Scientists examined into how useful they are for both laying hens and grill chickens. For example, using AIs to change the levels of estrogen in broilers has shown potential for improving their productivity. There is evidence from a variety of studies that this is the right way to go. Ali *et al.* (2017) [2] concluded that giving Ross 308 roosters letrozole, a non-steroidal AI, improved the quality of their semen and raised their plasma testosterone levels, which helped slow down the decline in reproductive

health that comes with age.In the same way, Adeldust *et al.*. (2021) [1] demonstrated that oral letrozole administration in aged Ross 308 breeder roosters significantly enhanced sperm concentration, motility, and testicular histology. These improvements were associated with elevated testosterone and reduced estradiol levels, underscoring the role of AIs in hormonal regulation. In addition to supporting with reproduction, aromatase inhibition has also been linked to greater sperm retrieval rates (Schlegel, 2012) [17]. Several AIs are steroidal, such as Formestane, and some are not, including Letrozole and Anastrozole. Stops aromatase from functioning by attaching to its iron part (Schieweck *et al.*, 1993) [15]. Aromatase is a type of cytochrome P450 enzyme that can be found in the testes, brain, and liver, among other places. It helps change testosterone into estrogen by taking off the 19-methyl group and the Aromatase a cyclic structure Blocking this enzyme raises the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the testosterone/estrogen ratio in the blood, which has a positive effect on reproductive physiology (Smith and Dowsett, 2003; Karnon, 2006) [12, 18]. Some studies suggest that aromatase inhibition may also affect sexual differentiation and feeding behavior, in addition to reproductive traits (Alward, 2016) [3]. The present study aims to evaluate the effects of the aromatase inhibitor anastrozole on the productive performance of broiler chickens from hatching until 35 days of age.

Materials and methods

Experimental treatments

180 chicks were selected from a total of 300 sterile eggs. The chicks were distributed into five treatments with three replicates and 12 chicks per replicate. these treatments are described as follows: the control treatment (T_1) without any additions or treatments; in the second treatment (T_2), hatching eggs were sprayed with 1mg/100 ml of aromatase inhibitor solution; in the third treatment (T_3), hatching eggs were dipped in 1mg/100 ml of aromatase inhibitor solution; in the fourth treatment (T_4), hatching eggs were sprayed with 2mg/100 ml of aromatase inhibitor solution while in the fifth treatment (T_5), hatching eggs were dipped in 2mg/100 ml of aromatase inhibitor solution in treatment five (T_5).

After the chicks arrived at the rearing hall, they were randomly distributed into pens (replicates), and the measurement for each pen was 1 x 1.5 m. The chicks were fed a starter diet (21.29% protein and 3027 kcal/kg feed) from one day of age until the end of the second week of life. They were then replaced with a grower diet (19.34% protein and 3059 kcal/kg feed) until the beginning of the fourth week when they were replaced with a finisher diet (19.22% protein and 3093 kcal/kg feed). Feed and water were provided ad libitum (Table 1), with all ideal conditions for chick growth provided, including temperature, relative humidity, and ventilation, according to the Ross 308 Broiler Guide.

Table 1: Ingredients and nutrients composition of the basal diet of the study, as-fed basis.

Ingredients	Starter Diet (%)	Grower Diet (%)	Finisher Diet (%)
Yellow corn	43.66	42.90	26.24
Soybean	32.38	26.44	24.34
Wheat	19.51	23.61	22.00
Premix*	2.38	2.36	0.00
Wheat bran	0.00	2.36	7.02
Limestone	1.24	1.18	1.08
Sunflower oil	0.28	0.72	2.36
Salt	0.19	0.19	0.19
Choline chloride	0.10	0.09	0.09
Sodium bicarbonate	0.10	0.09	0.05
Monocalcium	0.10	0.00	0.21
Garlic	0.03	0.03	0.02
Lysofort	0.02	0.02	0.02
G21 enzymes	0.01	0.01	0.00
Super 4 Enzymes	0.00	0.00	2.34
Flour	0.00	0.00	14.04
Total	100	100	100

Calculated Nutrient Analysis

Nutrient	Starter	Grower	Finisher
Energy (Kcal/kg)	3027.73	3054.67	3093.47
Protein (%)	21.29	19.34	19.22
Calcium (%)	0.97	0.91	0.79
Methionine + Cysteine (%)	0.85	0.80	0.82
Threonine (%)	0.79	0.71	0.79
Iodine (%)	0.00119	0.00118	0.00122
Zinc (%)	0.08779	0.08702	0.10313
Iron (%)	0.05826	0.05757	0.01957
Phosphorus (%)	0.48000	0.45300	0.39000
Lysine (%)	1.31000	1.17100	1.14700
Methionine (%)	0.50000	0.47700	0.48400
Manganese (%)	0.07634	0.07566	0.11247
Copper (%)	0.01149	0.01138	0.01503

* Premix produced by Provimi 3110 (Jordanian origin) contained 3800 kcal/kg metabolizable energy,, 7% crude protein,, 1.1% fat,, 15% calcium,, 4% lysine,, 11% available phosphorus,, 4.8% sodium., 8.5% methionine., 8.5% methionine + cysteine., 0.55% threonine., 575,000 IU/kg vitamin A., 201,250 IU/kg vitamin D3,, 3000 mg/kg vitamin E., 138 mg/kg vitamin B1., 345 mg/kg vitamin B2., 1840 mg/kg vitamin B3,, 552 mg/mcg vitamin B5., 184 mg/kg vitamin B6,, 46 mg/kg vitamin B9,. 1000 mcg/kg Vitamin B12., 6900 mcg/kg Biotin., 20,000 mcg/kg Choline Chloride., 2760 mcg/kg Iron., 3680 mcg/kg Manganese., 3680 mcg/kg Zinc., 9.2 mcg/kg Selenium., 50 mcg/kg Iodine.

** Based on chemical analysis of the feed according to NRC (1994)

Productive traits

Live body weight (LBW) and weight gain (WG)

The average LBW of each replicate was calculated at the end of each week by weighing all birds in each replicate. The average live weight/ bird was calculated based on the equation provided by (AL-Fayyadh *et al.*, 2011) [5]:

Weekly weight gain rate (g/bird) = Average LBW at the end of the week (g) - Average LBW at the beginning of the week (g).

Feed Intake (FI)

The weekly feed consumption rate for birds in a single replicate was calculated according to (Al-Fayyadh *et al.*, 2011) based on the following formula [5]:

Amount of feed consumed during the period = Amount of feed provided at the beginning of the week (g) - Amount of feed at the end of the week.

Feed Conversion Ratio (FCR)

The feed conversion ratio (FCR) was calculated based on the equation reported by (Al-Zubaidi, 1986) [4]:

No mortality was recorded throughout the experimental period.

Statistical Analysis

The data was analyzed using a complete randomized design (CRD) whereas the significant differences between means compared using Duncan's (1955) [7] multinomial test. The statistical analysis was performed using the SAS program (2012) [16] according to the following mathematical model: Yij = μ + Ti + eij; whereas

- Yij: the value of observation j for treatment i.
- μ: the overall mean of the trait.
- Ti: the effect of treatment i.
- Eij: the random error normally distributed with a mean of zero and a variance of σ2e.

Results

Live body weight (LBW)

Table (2) shows the effect of hatching eggs treated with an aromatase inhibitor on live body weight. In the first week, there was a significant ($p \leq 0.05$) superiority of T_2 compared to T_5. Whereas no significant difference was observed in the second, third, and fifth weeks. However, in the fourth week, significant ($p \leq 0.05$) superiority was observed for both T_2 and T_4 compared to T_3.

Table 2: Effect of treating hatching eggs with an aromatase inhibitor on LBW (mean ±SE).

Treatment	Week 5 (Mean±SE)	Week 4 (Mean±SE)	Week 3 (Mean±SE)	Week 2 (Mean±SE)	Week 1 (Mean±SE)
T1	1834.70±7.40	1238.00±3.23 ab	763.33±4.24	394.46±10.48	140.00±4.50 ab
T2	2002.30±1.47	1251.00±5.70 a	823.33±3.82	400.67±10.66	151.33±2.31 a
T3	1854.30±7.74	1138.90±13.91 b	755.83±6.61	391.14±2.05	140.00±0.57 ab
T4	1963.70±6.41	1253.76±8.18 a	833.47±9.60	410.62±4.03	149.16±6.78 ab
T5	1897.30±10.10	1226.69±5.25 ab	796.47±10.44	380.06±15.35	136.88±3.12 b

Different letters within a column indicates significant differences.. N.S.: Not significant (*) at the level of ($p \geq 0.05$). T_1: Control treatment without treatment, T_2: Spraying with 1 mg/100 ml aromatase inhibitor solution, T_3: Dipping with 1 mg/100 ml aromatase inhibitor solution, T_4: Spraying with 2 mg/100 ml aromatase inhibitor solution, T_5: Dipping with 2 mg/100 ml aromatase inhibitor solution.

Weight Gain (WG)

Table (3) shows the effect of treating hatching eggs with an aromatase inhibitor on weekly weight gain. We found that there was a significant superiority ($p \leq 0.05$) in the first week in T_2 compared to T_5. Also, significant superiority ($p \leq 0.05$) in the fourth week was observed in T_1 compared to T_3. Whereas no significant difference in the second, third, and fifth weeks, and the overall rate was recorded.

Table 3: Effect of treating hatching eggs with an aromatase inhibitor on WG (mean±SE)

Treatment	Overall Rate (Mean± SE)	Week 5 (Mean ±SE)	Week 4 (Mean ±SE)	Week 3 (Mean ±SE)	Week 2 (Mean± SE)	Week 1 (Mean± SE)
T1	1800.8±7.61	596.7±2.05	474.67±1.49 a	368.88±3.85	257.46±9.77	103.16±5.57 ab
T2	1967.3±1.47	751.3±9.20	427.67±9.38 ab	422.67±3.39	249.33±11.07	116.33±2.31 a
T3	1818.5±7.86	715.4±1.64	383.07±2.44 b	364.70±4.51	251.14±1.48	104.16±0.44 ab
T4	1926.7±6.70	709.9±3.75	420.30±4.93 ab	422.85±8.58	261.45±10.63	112.18±5.89 ab
T5	1859.7±9.55	670.6±5.28	430.23±7.97 ab	416.32±9.61	243.17±5.22	99.38±4.47 b

Different letters within a column indicates significant differences. N.S.: Not significant at the ($p \geq 0.05$) level. T_1: Control treatment without treatment. T_2: Spraying with 1 mg/100 ml aromatase inhibitor solution. T_3: Dipping with 1 mg/100 ml aromatase inhibitor solution. T_4: Spraying with 2 mg/100 ml aromatase inhibitor solution. T_5: Dipping with 2 mg/100 ml aromatase inhibitor solution.

Feed Intake (FI)

Table (4) shows the effect of treating hatching eggs with an aromatase inhibitor on feed consumption. It is noted that there was no significant difference in the first, second, third, and fifth weeks of the study. However, it was mentioned that there is a significant superiority ($p \leq 0.05$) in the fourth week in T_1 compared to treatments T_3 and T_5 and also in the overall rate; whereas significant ($p \leq 0.05$) increase was found in treatments T_1 and T_2 compared to T_3.

Table 4: Effect of treating hatching eggs with an aromatase inhibitor on the FI (mean±SE)

Treatment	Overall Rate (Mean ±SE)	Week 5 (Mean ±SE)	Week 4 (Mean ±SE)	Week 3 (Mean ±SE)	Week 2 (Mean ±SE)	Week 1 (Mean ±SE)
T1	2750.84±6.12 a	1000.04±7.91	845.37±8.06 a	441.83±5.04	318.89±4.62	144.71±1.82
T2	2754.50±9.37 a	990.67±1.49	778.17±9.16 ab	517.33±9.40	342.17±4.74	126.17±2.89
T3	2514.26±2.13 b	864.83±1.32	717.83±3.71 b	516.57±8.38	296.41±9.49	118.62±3.11
T4	2681.97±4.63 ab	940.48±5.76	802.30±1.08 ab	517.91±8.43	289.68±4.80	131.60±0.58
T5	2598.74±8.82 ab	933.70±6.28	741.61±2.21 b	545.00±2.30	261.30±5.90	117.13±1.16

Different letters within a column indicate significant differences. N.S.: Not significant at the (p<0.05) level.T_1: Control treatment without treatment.T_2: Spraying with 1 mg/100 ml aromatase inhibitor solution.T_3: Dipping with 1 mg/100 ml aromatase inhibitor solution. T_4: Spraying with 2 mg/100 ml aromatase inhibitor solution.T_5: Dipping with 2 mg/100 ml aromatase inhibitor solution.

Feed Conversion Ratio (FCR)

Table (5) shows the effect of treating hatching eggs with an aromatase inhibitor on the feed conversion ratio. It showed that no significant differences were observed during the study between treatments

Table 5: Effect of treating hatching eggs with an aromatase inhibitor on the FCR (mean±SE)

Treatment	Overall Rate (Mean ±SE)	Week 5 (Mean ±SE)	Week 4 (Mean ±SE)	Week 3 (Mean ±SE)	Week 2 (Mean ±SE)	Week 1 (Mean ±SE)
T_1	1.47±0.07	1.71±0.23	1.78±0.01	1.19±0.07	1.26±0.18	1.41±0.23
T_2	1.37±0.04	1.37±0.13	1.82±0.08	1.21±0.06	1.37±0.06	1.07±0.02
T_3	1.35±0.01	1.17±0.08	1.87±0.09	1.41±0.15	1.17±0.08	1.13±0.03
T_4	1.39±0.11	1.49±0.27	1.92±0.15	1.21±0.07	1.15±0.04	1.17±0.06
T_5	1.33±0.01	1.38±0.05	1.72±0.04	1.31±0.04	1.07±0.04	1.18±0.05

Different letters within a column indicates significant differences. N.S.: Not critical at the (p<0.05) level. T_1: Control treatment without treatment. T_2: Spraying with 1 mg/100 ml aromatase inhibitor solution. T_3: Dipping with 1 mg/100 ml aromatase inhibitor solution. T_4: Spraying with 2 mg/100 ml aromatase inhibitor solution. T_5: Dipping with 2 mg/100 ml aromatase inhibitor solution.

Discussion

The significant improvement in body weight observed in broiler chicks treated with aromatase inhibitors (AIs) may be attributed primarily to increased testosterone levels resulting from the inhibition of estrogen synthesis. Aromatase, the enzyme responsible for converting androgens to estrogens, plays a critical role in hormonal regulation. By blocking this enzyme, AIs enhance circulating testosterone, which in turn promotes muscle protein synthesis and growth. Testosterone is well-known for its anabolic effects on skeletal muscle. Robert *et al.* (1989) [14] highlighted that testosterone increases muscle mass by stimulating muscle protein synthesis. This finding is also supported by Upendram *et al.* (2010) [19], who demonstrated the hormone's key role in muscle hypertrophy and improved body composition. These mechanisms are particularly relevant in broiler chickens, where rapid muscle development is essential for productive performance. Moreover, the developmental timing of hormonal modulation may also influence outcomes. According to Hamadani *et al.* (2013) [11], the availability of nutrients in the early stages of embryogenesis enhances the digestive tract's activity and the secretion of digestive enzymes, which in turn improves nutrient absorption and growth. Relatedly, Emad *et al.* (2017) [9] demonstrated that aromatase inhibitors raised serum testosterone levels in poultry while lowering oestrogen levels, onfirming the idea that hormonal changes affect growth performance. These results are supported by recent research.. As reported by Haider *et al.* (2020), providing Lohmann roosters the non-steroidal AI anastrozole caused their body weight to rise noticeably. Similar to this, Ahmad *et al.* (2023) [6] reported that some medicinal plants have antioxidant compounds that prevent aromatase activity, which in turn increases growth parameters and testosterone production. Furthermore, the use of different hormone-modulating substances, such as artificial androgens, oestrogen antagonists, and AIs, has demonstrated encouraging outcomes in the production of broilers. Younis *et al.* (2023) [21] demonstrated that tamoxifen and boldenone improved productive performance and carcass characteristics in broiler chickens. These treatments led to higher body weight and increased muscle mass. However, they also induced alterations in gonadal histology, raising concerns about potential long-term effects on reproductive function.

Further implications of AI use were examined by Abdulateef *et al.* (2021) [22], who found that administration of AIs during embryonic development influenced sex differentiation, resulting in a higher proportion of male phenotypes. Since male broilers typically exhibit superior growth rates compared to females, this shift could contribute positively to overall flock performance. Nonetheless, these interventions also carry risks, particularly regarding animal welfare and hormonal imbalance. Therefore, aromatase inhibitors enhance productive performance in broiler chickens by elevating testosterone levels, promoting muscle development, and potentially favoring male-biased sex differentiation. While these outcomes present clear advantages for meat production, the possible reproductive and developmental side effects warrant cautious application.

Conclusion

This study demonstrates that spraying hatching eggs with anastrozole an aromatase inhibitor at concentrations of 1 mg/100 mL and 2 mg/100 mL of distilled water positively influenced the productive performance of broiler chicks (Ross 308). Specifically, both concentrations led to notable improvements in the weight gain, body weight, and feed intake when compared to the control groups. Among the tested treatments, the 1 mg/100 mL dosage proved to be the most effective; suggesting that lower concentrations may optimize growth performance without unnecessarily increasing exposure to the compound

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Author's contributions

Haider Alwan, Mohammed K. A. Altamimi and Emad Abdulgabbar Ali contributed to the study's conceptualization, methodology, data conclusion, statistical analysis and drafting of the original manuscript. Salah Mahdi Alsherify and Samaher Saad Hadi supervised the research activities, critically evaluated the manuscript content and performed proofreading and final revisions.

Conflict of interest

The authors declare no conflict of interest.

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