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Aromatase inhibitors: Their application in commercial broiler chick production

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Abstract
Difference in body weight between male and female broiler birds at marketing age is quite common. Male broiler birds attain marketing body weight earlier than female birds, thereby reducing feed costs. *In ovo* injection of aromatase inhibitors into hatching broiler eggs at 6.5 days of incubation resulted in a higher proportion of hatched male chicks. This is because aromatase inhibitors caused the female embryos to develop a male phenotype without any change in the genotype. Aromatase inhibitors did not have any effect on the male embryos. There was no change in the genotype or phenotype of male chicks. Aromatase inhibitors occur naturally in tomato, garlic mushroom and other food substances. Chemical aromatase inhibitors like clomiphene citrate, tamoxifen, atrazine, imazalil and fadrazole have been studied.

Keywords: Aromatase inhibitors, sexual differentiation, chick embryos

Introduction
It is common knowledge that the male of broiler chicken is heavier than it`s female counterpart. The live weight difference between them at marketing age is approximately 400-500gm. Given this difference in the body weight at marketable age, the male broiler bird attains marketable body weight a few days earlier than the female bird, thereby, resulting in a substantial financial savings due to lesser feed consumption. Verapeen and Driver (2000) [16] confirmed this finding in their study (Table 1) and found a significant difference in the economic returns of male and female broilers when reared separately. Many researchers have reported the difference in growth rates between male and female broiler chickens. (Laseinde and Oluyemi, 1994; Henry and Burke, 1998) [6, 5]. The body weight gain, feed consumption and feed conversion ratio (FCR) in male broilers is better than the female birds or herds of mixed sexes (Aviagen, 2014) [1].
There are faster growth and better FCR in males than female broilers (Clinton, 1998) [3]. Laseinde and Oluyemi 1994 [6] reported a significant difference in weight gain and feed consumption between two sexes of broilers.

Sex Differentiation in Birds / Chicken
In birds, sex determination is dependent on the sex steroids (androgens and estrogens) and Anti Mullerian Hormone (AMH). If the developing embryo is male, then the embryonic testes will produce AMH. This causes the Mullerian or female ducts to disappear, hence the term Anti Mullerian Hormone, whist testosterone produced by the testes causes the male or Wolfian ducts to survive. Though both male and female embryonic gonads (testes and ovaries) produce AMH at the high levels, regression of Mullerian ducts occurs only in male embryos. In the female chicken, Estrogen, the C18 steroid hormone is responsible for the development of ovarian structure and secondary sexual characters like smaller comb, finer and shorter legs, longer and straight tail and more feathering along the center of the back.
In birds, males have ZZ chromosomes (homogametic), females are ZW (heterogametic), unlike in mammals (Matsushita *et al*., 2006) [8]. The W chromosome in the female genotype controls early Aromatase (s) enzyme synthesis and leads to estrogen synthesis (Shimada, 1998, 2002) [13]. Aromatase is a cytochrome P450 enzyme or a group of enzymes that catalyzes a critical step in the conversion of androgens like testosterone (C 19 steroids) into estrogens (C 18 steroids), a process called Aromatization. Aromatase production and expression is controlled by a gene. Estrogens and their receptors are important for sexual differentiation to determine female gender. Chick`s embryonic gonads are bi-potential at an early stage (Shimada, 2002) [14].

Like other bird species, female chickens will only develop the left gonad into a functional ovary (Romanoff, 1960) [11]. During female sexual development, the left gonad differentiates into a single ovary and oviduct, while the right is idle and regresses resulting in generation of the regular female phenotype (Lin *et al*., 1995) [7]. During sexual differentiation in female, testosterone will be converted to estrogen in response to Aromatase enzyme expression in the left gonad at 6.5 days of incubation (Shimada, 1998; Yoshida *et al*., 1996) [13, 17]. In male birds, both gonads will develop into testes (Shimada, 1998) [13]. Low levels of P450 Aromatase expression because of lack of estrogen receptors in the right gonad causes a shortage of estrogen synthesis in males (Nakabayashi *et al*., 1998; Shimada 1998; Yoshida *et al*., 1996) [13, 17]. The expression of Aromatase gene in females begins around day 5-6 of incubation, prompted by the synthesis of estrogen (Zhang *et al*., 1998). To create a functional left ovary, differentiation between right and left ovary in females specifically depends on the estrogen receptor expression in the left gonad (Bruggeman *et al*., 2002) [2]. Sexual differentiation between female and male depends on lack of Aromatase enzyme and estrogen, though the estrogen receptor is available in males before sexual differentiation (Smith *et al*, 1997) [15]. Both male and female chicken embryos are capable of androgen synthesis, but only female embryos can create estrogen during the brief time period before sexual differentiation of gonads. Thus, the expression of P450 Aromatase mRNA in the female chicken embryo has a critical role in early stage of estrogen production.

Table 1: Effects of rearing method on live weight and weight gain (gram) of broilers reared to 18 weeks of age

Live weight (g/week)				
Rearing methods	4	8	12	18
	*	*	*	*
Separate males	343.33^{b}	1316.67^{b}	2633.33^{b}	3433.33^{b}
Mixed sex	405.00^{a}	1366.67^{a}	2300.00^{a}	3166.67^{a}
Separate females	339.33^{c}	1166.67^{c}	2133.00^{c}	2766.67^{c}
SEM	23.12	8.33	47.10	74.52
Weight gain (g/week)				
	*	*	*	*
Separate males	303.33^{b}	1276.67^{b}	2593.33^{b}	3393.33^{b}
Mixed sex	368.33^{a}	1330.00^{a}	2263.33^{a}	3130.00^{a}
Separate females	306.00^{c}	1133.33^{c}	1933.33^{c}	2733.33^{c}
SEM	23.87	8.50	48.86	73.22

*: $p<0.05$, a, b, c, means with different superscript within a column are significantly different

Aromatase Inhibitors (AIs)

Aromatase inhibitors were first designed and synthesized independently by the three research groups of Cecil Robinson, O`Neal Johnston and Doug Covey and were used to treat breast cancer in human patients. Tamoxifen, an AI was used to treat breast cancer after which FDA (USA) has approved other AIs like Aromasin. Anastrozole was patented in 1987 and was approved for medical use in 1995.It is on the World Health Organization's list of essential medicines. Anastrozole is available as a generic medication. In 2017, it was the 258th most commonly prescribed medication in the United States with more than one million prescriptions.

In poultry industry, Aromatase Inhibitors have found application in the commercial broiler operations, where in, their ovo injection of hatching eggs altered the sex ratio in broilers in such a way that more number of male broiler chicks were produced resulting in increased profits to broiler chicken farmers due to their inherent ability to attain early body weight gains. AIs prevent the synthesis of estrogen in genetically female birds and produce males with female genotype.

Aromatase inhibitors are present in many naturally occurring foods like Nettle plant, Grape seed extract, green chillies, limes and lemons, mushrooms, oranges, radish, oats, tomatoes, garlic, green tea, coffee etc.

Chemical Aromatase Inhibitors have also been used in commercial broiler chicken where they have been found to alter the sex ratio of the embryos by producing more number of male chicks. Some of the chemical Aromatase inhibitors that have been studied were Clomiphene, Tamoxifen, Imazalil, Atrazine and Fadrozole.

Salwan M Abdulateef *et al*., (2021) [12], investigated the effect of *In ovo* exposure to the Aromatase inhibitors from Tomato and Garlic extract on sexual differentiation and embryonic development in chicken embryos. They injected Tomato extract, Garlic extract, mixed tomato and garlic extract into the albumen of the experimental eggs and found that there was a significant increase in the percentage of male chicks compared to control treatments. *In ovo* injection of Tomato extract showed the highest production of male chicks (91.66%), Garlic extract injection showed 79.16% and mixed Garlic and Tomato extract showed a male chick percentage of 91.66. Control 1 showed 41.66% of male chicks whilst Control 2, where Distilled water was injected *In ovo* showed 45.83% of male chicks. (Fig.1)

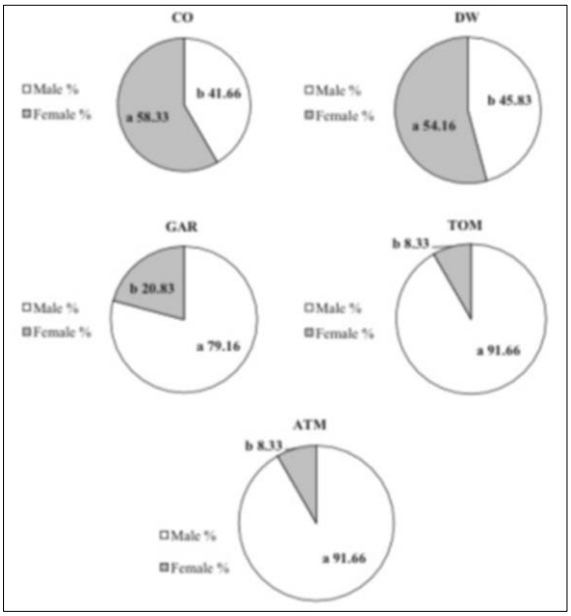

Fig 1: The effect of *In ovo* exposure to aromatase inhibitors on the percentage of female to male of broiler chickens. SEM. 9.12 to make and 9.12 to female: Mean; 70.0 to make and 30.0 to female a and b; mean in the same rows with different superscripts differ significantly probability value 0.01and 0.05. "Control 1:no. injection(ICOR): control 2:distalished water (DM, 0.1ml/egg): garlic extract (GAR, 0.1mg/egg); tomato extract (TOM, 0.1mg/egg); and garlic and tomato mixed extract (ATM, 0.1mg/egg). *Injected into eggs in 5 days incubation

Fazli *et al*., (2015) [4] studied the effect of *In ovo* injection of Aromatase Inhibitors Clomiphene citrate, Tamoxifen, Tomato extract and Garlic extract on sex differentiation in broiler chicks. They found that *In ovo* injection of broiler hatching eggs with natural substance like Tomato and Garlic extracts caused the highest percentage of male chicks, with 70.97% for Tomato extract, 80.43% for Garlic extract, Tamoxifen produced 61.34% male chicks, Clomiphene producing 57.62% male chicks. The percentage of male chicks in the control group was 53.98. (Table 2).

Table 2: Effect of garlic and tomato extracts, tamoxifen, and clomiphene citrate on hatchability, male ratio, and one-day-old weight of broiler chickens.

Treatment	Hatchability (%)	Male (%)	1-old weight (g)
DW (control)	77	53.98	35.8
GAR	68	80.43	37
TOM	72	70.97	35.6
TMX	77	61.34	36.1
CLC	67	57.62	34.3
Pr >F	0.1201	0.0145	0.1076

Means within columns with different superscripts differ significantly (p<0.05)
DW, distilled water, GAR, garlic extract, TOM, tomato extract, TMX, tamoxifen, CLC, clomiphene citrate.

Mokarrami *et al*., (2020) [9] worked on the *In ovo* injection of mushroom extract, nettle extract and Fadrozole hydrochloride into the broiler hatching eggs and observed an increase in the ratio of males. Comparing the results of the *In ovo* injection of mushroom extract and Fadrozole hydrochloride showed that the plant extract has been as effective as Fadrozole hydrochloride to change the sex ratios.

Matsushita *et al*., (2006) [8] observed that Aromatase inhibitors alter the sex ratio by increasing the number of male chicks. They have examined the effects of Atrazine and Imazalil, two commonly used pesticides, on sexual differentiation in chickens. In Atrazine exposed female chicks, the left gonad had normal ovary like structures, however, the right gonad didn't regress but persisted. Atrazine inhibited the regression of right gonad in female chicks, which then produced testosterone and AMH, thereby causing disappearance of Mullerian ducts and the consequent development of a chick with male phenotype and female genotype.

In Imazalil exposed female chicks the left gonad had a portion containing seminiferous tubules like structures that are normally present in testes of males. The remaining right gonad had testes like structures. Their results suggested that Imazalil partially inhibits female gonadal Aromatase activity thereby partially inhibiting the differentiation of female gonads.

Broiler hatcheries in India may initiate the use of Aromatase inhibitors for the benefit of broiler chicken farmers in earning more profits.

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