IJAS Iranian Journal of Applied Animal Science

The Effect of *in ovo* Injection of Aromatase Inhibitors on the Performance of Broilers

Research Article

T. Mokarrami[1], B. Navidshad[1*], N. Hedayat Evrigh[1] and F. Mirzaei Aghjehgheshlagh[1]

[1] Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, Iran

Received on: 22 Apr 2017
Revised on: 19 Jan 2018
Accepted on: 31 Jan 2018
Online Published on: Mar 2020

*Correspondence E-mail: bnavidshad@uma.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

Change in sex ratios for broiler chickens, has many economic benefits of the poultry industry. The gonads in chick embryo at an early stage have the potential to convert to both sexes (male and female). Sexual differentiation is due to aromatase expression in the left gonad in 5-6 days of the embryonic period and the production of estrogen from testosterone. Administration of aromatase inhibitors prevents the synthesis of estrogen in genetically female birds and produce males with female genotype. In this study, the effects of *in ovo* injection of 0.1 mg mushroom extract, nettle extract and fadrozole hydrochloride as aromatase inhibitors on the fifth day of incubation period on hatchability, embryo mortality and performance of hatching chicks were examined. The results of this experiment showed that there were no significant differences in hatchability, embryo mortality during incubation and the weight of hatching chicks. Daily feed intake and body weight gain in chickens hatched from mushroom extract and fadrozole hydrochloride groups were higher than the two other groups ($P<0.05$). Also, *in ovo* injection of a mushroom extract, nettle extract or fadrozole hydrochloride had no effect on the dressing percentage and internal organ weights.

KEY WORDS aromatase inhibitors, broiler chickens, fadrozole hydrochloride, mushroom extract, nettle extract, sexual differentiation.

INTRODUCTION

Changing the sex ratios and increasing the male broiler production, has great economic benefits of the poultry industry. Many researchers have reported the difference in growth rates between male and female broiler chickens (Laseinde and Oluyemi, 1994; Henrry and Burke, 1998). 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). There are faster growth and better FCR in males than female broilers (Clinton, 1998). Laseinde and Oluyemi 1994 reported a significant difference in weight gain and feed consumption between two sexes of broilers.

Verapeen and Driver (2000) observed a significant difference in economic return of male and female broilers reared separately.

They reported that the body weight gain and the weight of different parts of the carcass in males were heavier than females. The final body weight is probably the most important economic factor in broilers.

Another important economic factor in the broiler industry is the carcass characteristics and especially the abdominal fat content. Certainly, male broiler performance traits are superior to females (Broadbent *et al.* 1981; Engku an Noraziah, 2000). The superiority of male broilers to females for body weight reaches to 20 percent in 57 days of age (Engku, 1984).

Gonads in chick embryo at an early stage have the potential to convert to both sexes (male and female). The sexual differentiation is a result of expression of aromatase enzyme in left gonad at 5-6 d of the embryonic period and the production of estrogen from testosterone (Yoshida *et al*. 1996; Shimada, 1998).

In birds, sex steroids and also anti- müllerian hormone is naturally involved in sex differentiation; and androgen and estrogen are resulted in male and female sexes, respectively. The P450 aromatase enzyme (arom P450) has been identified as a key enzyme in the synthesis of estrogen (Shimada, 1998). Administration of aromatase inhibitors is resulted in inhibition of synthesis of estrogen (the hormone responsible for the ovarian structure and the secondary sex characteristics) in females and production of males with female genotype (Shimada and Saito, 2000). The non-synthetic anti-aromaitases are natural biochemical compounds which are found in fungi, vegetables, fruits and other plants. Flavonoids are natural anti-aromatase compounds that are abundantly found in plants (Sumpter and Fostier, 1996; Grove, 1981). Interest in the flavonoids in cancer diseases, especially breast cancer is because of the same aromatase inhibitors that decreases the biosynthesis of estrogen. Several flavonol glycosides have been identified in the aerial parts of nettle (Chaurasia and Wichtl, 1987) which has anti-aromatase and anti-breast cancer properties. The results of studies on the use of edible mushrooms for the treatment and prevention of cancer, especially breast cancer showed that polyunsaturated fatty acids such as linoleic acid and alpha-linolenic acid act as the main aromatase inhibitors (Chen *et al*. 2006). *In ovo* techniques are used to embryo feeding (Gholami *et al*. 2015; Pender *et al*. 2017; Abousaad *et al*. 2017). Due to the clear advantages of rearing male broilers than females and also with regard to the saving time, energy and labor in flocks with higher growth rate, the aim of this study was to compare the effects of *in ovo* injection of a mushroom extract, nettle extract and fadrozole hydrochloride as aromatase inhibitors on broiler performance.

MATERIALS AND METHODS

Maceration method was used to prepare the nettle extract (Azwanida, 2015). In brief, 400 g of dried nettle was crushed to a diameter of 1 millimeter and added to an aluminum foil covered Erlenmeyer flask containing 50% ethyl alcohol and kept in room temperature for 72 hours. The mixture was stirred one a day. After 72 hours, for initial purification, the mixture was passed through a net so that the soluble material was separated from the pulp. Subsequently, Watten filter paper was used three times continuously to obtain an insoluble material free solution.

The solution was concentrated using a vacuum vaporizer. The dry matter of nettle extract was determined to be 0.706 g/mL. The same procedure was used to prepare mushrooms extract from 400 grams of dried mushroom. The dry matter of the dry matter of nettle extract was determined to be 0.706 g/mL and of extract was determined to be 0.763 g/mL.

All procedures of this expeiment were approved by the animal welfare committee of animal science department of UMA university, Iran. First, a pre-test was performed to determine the appropriate dosage of *in ovo* injection of plant extracts. Based on the results of the pre-test, 0.1 mL solution containing 500 micrograms of dry matter of mushroom and nettle extracts was injected in the main experiment. The fadrozole hydrochloride was injected at 0.1 mL dosage containing 100 ppm. In the main trial, 300 fertilized eggs from Ross® 308 broiler breeder were individually weighed and distributed into five groups with 4 replicates each. The treatment was as follows: 1: the control group or not injected eggs, 2: the eggs injected with 0.1 mL distilled water, 3: the eggs injected with 0.1 mL of nettle extract, 4: the eggs injected with 0.1 mL mushroom extract, and 5: the eggs injected with 0.1 ml fadrozole hydrochloride.

First, the eggs were placed in the incubator, and on the fifth day of incubation the eggs were injected into the air cells using insulin syringes. Before injecting, the egg shell disinfected using 70% ethanol and after the injection the melted paraffin was used to block the pore. Eggs were placed for 18 days in the setter and 3 days in the hatchery conditions with temperature and humidity recommended standards and in 21 days of incubation the unhatched eggs were broken to determine the embryo mortality during the incubation period and the step of embryo mortality was determined based on fetal growth according to the guidelines of Aviagen (2014).

Before distributing the chickens into the groups, the sex of chickens was determined from wing features, and the numbers of female and male birds in each cage were recorded. The male Ross® 308 chicks have a short primary feather on the wing than the females (Aviagen, 2014).

The chickens were reared for 6 weeks. The chicks were divided to 4 treatments (including chicks from eggs injected with mushroom extract, nettle extract, fadrozole hydrochloride and control), with 6 replicates, 10 chicks per replicate.Due the lack of significant difference between the hatchery traits between the control eggs and the eggs injected with distilled water, just the control group was included in the production period trial. The diets were similar for all treatments. The diets were formulated using the WUFFDA software for starter (1 to 10 days), grower (11 to 24 days) and finisher (25 to 42 days) phases of rearing period, based on the nutrient requirements of Ross 308 broil-

ers. The components and chemical analysis of the diets are presented in Table 1.

Feed intake, body weight and FCR of birds were recorded per cage for all three phases of the rearing period. At 42 days of age, 2 birds from each cage were randomly selected and slaughtered. Live weight, carcass weight and the weight of the edible parts of the carcass were measured. Statistical analysis was carried out using the procedure generalized linear model (GLM), SAS (2002) statistical software and the means were compared using Duncan's multiple range test at the 5% level.

RESULTS AND DISCUSSION

The effect of experimental treatments on the incubation parameters

Effects of *in ovo* injection of anti-aromatase agents on the incubation parameters are given in Table 2. *In ovo* injection of anti-aromatase compounds did not affect the incubation parameters of embryo mortality rate, hatchability and hatched chick's live weight in the experimental groups. Some previous reports also confirm no negative impact of anti-aromatase on the hatchability and the weight of hatching chicks.

Matsushita *et al*. (2006) also reported that *in ovo* injection of an aromatase inhibitor, atrazine has no effect on the hatchability of the fertilized eggs. Mohammadrezaei *et al.* (2012) concluded that *in ovo* injection of fadrozole hydrochloride and insulin-like growth hormone has no effect on hatchability. Mafi (2012) reported that *in ovo* injection of nettle root extract to make changes in the sex of broilers had no significant effect on hatchability, embryo mortality and body weight of the hatched chicks. According to previous reports, there is no difference in the body weight of one day old, male and female broilers and the differences appears in the next weeks of rearing period (Marks, 1985; Marks,1986; Marks, 1987; Burke, 1992).

In ovo injection of anti-aromatase compounds and chicks performance

The effects of *in ovo* injection of a mushroom extract, nettle extract and fadrozole hydrochloride on the performance traits of broilers have provided in Table 3. There are differences ($P<0.05$) in terms of daily feed intake and body weight gain between the groups receiving anti-aromatase agents and the control group in different phases of rearing periods; and the differences increased with increasing the age of the birds. In the starter period (0-10 days), the differences in the daily feed intake, daily gain and FCR between the experimental groups were not significant.

In the grower phase (11-24 days), the best daily weight gain was observed in the fadrozole hydrochloride treated group and the differences with the nettle extract treated group and the control group were significant ($P<0.05$). The mushroom extract treated group showed superiority to the control group ($P<0.05$), however, no significant difference observed between the nettle extract treated group and the control group.

The finisher phase feed intake (25-42 days) in fadrozole hydrochloride and mushroom extract treated groups were significantly higher than the control and the nettle extract treated groups ($P<0.05$).

The highest daily weight gain during the finisher phase was observed in the fadrozole hydrochloride treated group, such that the difference with the nettle extract and control groups were significant ($P<0.05$).

The mushroom extract group has a higher daily weight gain than the control group ($P<0.05$). In the whole rearing period (0-42 days), the most daily intake was observed in the fadrozole hydrochloride treated and mushroom extract treated group with 107.00 and 105.75 g/b/d, respectively, as compared with the control and the nettle extract treated group with 96.98 and 99.45 g/b/d, respectively ($P<0.05$).

On the other hand, the highest daily weight gain was related to the fadrozole hydrochloride treated and the mushroom extract treated group with 62.88 and 61.02 g/b/d, respectively; and the difference with the control and the nettle extract treated group (55.33 and 56.80 g/b/d, respectively) were significant ($P<0.05$). No significant differences were found in the FCR of experimental groups during the whole rearing period.

The differences observed in the performance of chicks can attributed to the sex effects (Broadbent *et al*. 1981; Engku and Noraziah, 2000). Laseinde and Oluyemi 1994 reported a significant difference in weight gain and feed consumption in male birds as compared to females. In the experiment Mohammadrezaei *et al.* (2012), *in ovo* injection of fadrozole hydrochloride and fadrozole hydrochloride with insulin-like growth factor significantly increased feed intake and body weight of broilers compared with the control group, but the FCR was not affected. Mottaghitalab and Razani (2005) also reported that fadrozole hydrochloride injection improves FCR and growth rate compared to the control group. Mafi (2012) in a similar trial showed that the male broilers hatched from *in ovo* injected eggs with nettle extract had a higher body weight gain.

The significant differences have also reported between body weight of turkey chicks following *in ovo* injection of fadrozole hydrochloride and the control group (Burke and Henry, 1999).

Table 1 The ingredients and the chemical composition of the basal experimental diet

Ingredient (%)	Starter (0-10 days)	Grower (11-24 days)	Finisher (24-42 days)
Corn	48.33	50.86	54.11
Soybean meal	42.52	39.34	35.31
Fat	4.67	5.71	6.64
Dicalcium phosphate	1.87	1.72	1.65
Limestone	1.1	1	0.93
Vitamin premix[1]	0.25	0.25	0.25
Trace mineral premix[2]	0.25	0.25	0.25
Methionine	0.37	0.31	0.3
Lysine	0.23	0.15	0.15
Salt	0.36	0.36	0.36
Salinomaysin 12 %	0.05	0.05	0.05
Calculated analysis (%)			
Metabolizable energy (kcal/kg)	3000	3100	3200
Crude protein	23	21.5	20
Calcium	0.98	0.9	0.85
Available phosphorus	0.49	0.45	0.43
Na	0.16	0.16	0.16
Lysine	1.44	1.3	1.2
Methionine	0.71	0.6	0.16
Methionine + cystine	1.08	0.99	0.94

[1] Each kg of vitamin premix provided: vitamin A: 900 IU; vitamin D: 2000 IU; vitamin E: 18 IU; vitamin K: 2 mg; vitamin B_1: 1.8 mg; vitamin B_2: 6.6 mg; vitamin B_6: 3 mg; vitamin B_{12}: 15 µg; Niacin: 30 mg; Pantothenic acid: 10 mg; Biotin: 0.1 mg; Folic acid: 1.25 mg and Choline chloride: 200 mg.
[2] Each kg of trace mineral premix provided: Fe: 50 mg; Cu: 10 mg; Mn: 100 mg; Zn: 85 mg; I: 0.8: mg and Se: 0.2 mg.

Table 2 The effects of *in ovo* injection of anti-aromatase agents on the hatchery parameters of eggs

Item	Control[1]	Control[2]	Mushroom extract	Nettle extract	Fadrozole	SEM	P-value
Initial average weight of eggs in incubator (g)	62.26	62.49	62.11	62.25	63.03	0.479	0.684
The average egg weight on 18 d of incubation (g)	54.25	54.04	53.79	53.27	53.82	0.386	0.493
Chicks hatchling weight (g)	41.04	41.31	41.51	41.57	41.07	0.470	0.894
Fertile eggs hatchability %	81.46	80.30	81.81	77.15	80.59	2.043	0.823
0-2 day embryo mortality	3.71	5.38	3.85	3.71	3.45	1.188	0.965
3-5 day embryo mortality	1.79	1.79	1.67	3.71	3.45	1.083	0.881
6-11 day embryo mortality	1.92	3.45	5.51	5.79	1.78	1.094	0.436
12-17 day embryo mortality	5.49	3.45	3.45	3.71	3.57	1.155	0.939
18-21 day embryo mortality	5.63	5.63	3.71	5.93	7.14	1.904	0.964

[1] Control: not injected eggs.
[2] Control: the eggs injected with 0.1 mL distilled water.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effect of *in ovo* injection of anti-aromatase agents on the performance of the hatching broilers

Growth phases	Control	Mushroom extract	Nettle extract	Fadrozole	SEM	P-value
Feed intake (g/bird/d)						
Starter	27.49	28.77	28.72	28.07	0.816	0.654
Grower	75.18	79.64	77.34	82.19	1.892	0.086
Finisher	152.55^{b}	170.69^{a}	156.05^{b}	172.12^{a}	4.682	0.014
Total	96.98^{b}	105.75^{a}	99.45^{b}	107.00^{a}	2.107	0.008
Weight gain (g/bird/d)						
Starter	18.83	20.46	19.22	19.69	0.678	0.384
Grower	47.92^{c}	52.62^{ab}	48.89^{bc}	54.13^{a}	0.464	0.020
Finisher	81.37^{c}	90.98^{ab}	84.01^{bc}	94.65^{a}	2.510	0.004
Total	55.33^{b}	61.02^{a}	56.80^{b}	62.88^{a}	1.236	0.001
Feed conversion ratio						
Starter	1.47	1.41	1.49	1.42	0.023	0.058
Grower	1.57	1.52	1.58	1.52	0.024	0.143
Finisher	1.87	1.88	1.86	1.82	0.026	0.380
Total	1.75	1.74	1.75	1.70	0.019	0.287

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of *in ovo* injection of anti-aromatase agents on the carcass and internal organ relative weights of broilers

Item	Control	Mushroom extract	Nettle extract	Fadrozole	SEM	P-value
Carcass	65.57	62.37	64.48	64.18	0.602	0.182
Leg	19.38	18.32	18.29	18.47	0.298	0.211
Breast	30.69	29.19	27.99	29.31	0.622	0.321
Abdominal fat	1.02	1.17	0.88	1.03	0.352	0.385
Heart	0.49	0.49	0.45	0.49	0.083	0.408
Liver	1.85	1.91	2.11	2.03	0.225	0.318
Spleen	0.10	0.08	0.10	0.09	0.112	0.813
bursa of fabricius	0.12	0.13	0.14	0.11	0.175	0.739

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results of the present study confirm previous reports on the effects of *in ovo* injection of anti-aromatase compounds to change the gender of birds and thus improve the production performance. In this study, the fadrozole hydrochloride and mushroom extract treated groups had comparable performance traits that reflects the impact of this plant extract on the sex differentiation.

Effects of treatments on carcass weight and internal organs

According to Table 4, *in ovo* injection of a mushroom extract, nettle extract and fadrozole hydrochloride did not influence the dressing percentage and internal organ relative weights.

Mafi (2012) reported that *in ovo* injection of nettle root extract did not affect the weight of carcass and different parts of the carcass. Mottaghitalab and Razani (2005) also reported comparable values for dressing percentage, abdominal fat weight, breast weight, thigh weight and the weight of other body parts in birds hatched from eggs *in ovo* injected with different levels of fadrozole hydrochloride and the control group. However, Merkley *et al*. (1980) concluded that there are significant differences in different parts of the carcass between male and female broiler chicks, so that females had bigger breasts and smaller thighs compared to males (Broadbent *et al*. 1981; Grey *et al*. 1982; Shahin *et al*. 1996).

CONCLUSION

The anti-aromatase compounds by creating a sex change and increase the ratio of males in flock improved the average production traits. Comparing the results of the *in ovo* injection of mushroom extract and fadrozole hydrochloride clearly shows that the plant extract has been as effective as the fadrozole hydrochloride to change the sex ratios and improved broiler production traits.

ACKNOWLEDGEMENT

This article was funded by the University of Mohaghegh Ardabii Research Fund, Ardabil, Iran.

REFERENCES

Abousaad S., Lassiter K., Piekarski A., Chary P., Striplin K., Christensen K., Bielke L.R., Hargis B.M., Dridi S. and Bottje W.G. (2017). Effect of *in ovo* feeding of dextrin-iodinated casein in broilers: II. Hatch window and growth performance. *Poult. sci.* **96,** 1478-1484.

Aviagen. (2014). Ross 308: Broiler Nutrition Specification. Aviagen Ltd., Newbridge, United Kingdom.

Azwanida N.N. (2015). A Review on the extraction methods use in medicinal plants, principle, strength and limitation. *J. Med. Aroma Plants.* **4,** 196-201.

Broadbent L.A., Wilson B.J. and Fisher C. (1981). The composition of the broiler chicken at 56 days of age: Output, components and chemical composition. *British Poult. Sci.* **22,** 385-290.

Burke W.H. (1992). Sex differences in incubation length and hatching weights of broiler chicks. *J. Poult. Sci.* **71,** 1933-1938.

Burke W.H. and Henry M.H. (1999). The effects of *in ovo* administration of testosterone or an antiandrogen on growth of chick embryos and embryonic muscle characteristics. *Poult. Sci.* **78,** 1 006-1013.

Chaurasia N. and Wichtl M. (1987). Flavonoglycosides from *Urtica dioica*. *J. Planta Med.* **53,** 432-434.

Chen S., Oh S., Phuny S., Hur G., Jingye L., Lingkwok S., Gayle E., Belury M., Adams L. and Dudley W. (2006). Anti-aromatase activity of phytochemicals in White Buttom Mushrooms (*Agaricus bisporus*). *J. Cancer Res.* **66,** 24-32.

Clinton M. (1998). Sex determination and gonadal development: A bird's eye view. *J. Exp. Zool.* **281,** 457-465.

Engku A. (1984). Carcass yield of broilers. *MARDI Res Bull*. **12,** 107-115.

Engku A. and Noraziah M. (2000). Straight-run vs sex-segregated production of broilers. Pp. 129-130 in Proc. 22nd Malaysian Soc. Anim. Prod. Ann. Conf., Kota Kinabalu, Malaysia.

Gholami J., Qotbi A.A.A., Seidavi A.R., Meluzzi A., Tavaniello S. and Maiorano G. (2015). Effects of *in ovo* administration of betaine and choline on atchability results, growth and carcass characteristics and immune response of broiler chickens. *Italian J. Anim. Sci*. **14,** 187-192.

Grey T., Robinson D. and Jones J.M. (1982). Effect of age and sex on the eviscerated yield, muscle and edible offal of a commercial broiler strain. *British Poult. Sci.* **23,** 289-298.

Grove 1.F. (1981). Volatile compounds from the mycelium of the mushroom *Agaricus bisporus*. *J. Phytochem.* **20,** 2021-2022.

Henrry M.H. and Burke W.H. (1998). Sexual dimorphism in broiler chicks embryos and embryonic muscle development in late incubation. *Poult. Sci.* **77,** 728-736.

Laseinde E.A. and Oluyemi J.A. (1994). Effect of sex separation at the Finisher phase on the comparative growth performance, carcass characteristics and breast muscle development between male and female broiler chicken. *Nigerian J. Anim. Prod.* **21,** 11-18.

Mafi M. (2012). Manipulation of chicken sex differentiation using *in ovo* injection of Stinging Nettle (*Urtica dioica*) root extract. MS Thesis. Giulan Univ., Rasht, Iran.

Marks H. (1985). Sexual dimorphism in early feed and water intake of broilers. *J. Poult. Sci*. **64,** 425-428.

Marks H.L. (1986). The role of water intake on sexualdimorphism for early growth of broilers. *J. Poult. Sci*. **65,** 433-435.

Marks H.L. (1987). Sexual dimorphism in broilers following periods of equal water and feed intake. *J. Poult. Sci*. **66,** 381-389.

Matsushita S., Yamashita J., Iwasa T., Tomita T. and Ikeda M. (2006). Effects of *in ovo* exposure to imazalil and atrazine on sexual differentiation in chick gonads. *J. Poult. Sci.* **85,** 1641-1647.

Merkley J., Weinland B., Malone G. and Chaloupka G. (1980). Evaluation of five commercial broiler crosses. 2. Eviscerated yield and component parts. *Poult. Sci.* **59,** 1755-1760.

Mohammadrezaei M., Toghyani M., Eghbalsaied S., Gheisari A. and Esmaeili M. (2012). Growth performance and sex differentiation in broiler chickens submitted to *in ovo* injection of fadrozole and insulin-like growth factor I. Pp. 1039-1042 in Proc. Nutr. Feed Technol. Conf., which city??? Brazil.

Mottaghitalab M. and Razani K. (2005). Egg treatment with anti-aromatase: Effects on the chicks male: Female ratio, and their economic performance. *Iranian J. Agric. Sci*. **36,** 375-383.

Pender C.M., Kim S., Potter T.D., Ritzi M.M., Young M. and Dalloul R.A. (2017). *In ovo* supplementation of probiotics and its effects on performance and immune-related gene expression in broiler chicks. *Poult. Sci.* **96,** 1052-1062.

SAS Institute. (2002). SAS®/STAT Software, Release 9.3. SAS Institute, Inc., Cary, NC. USA.

Shahin K., Ibrahim S. and Elfaham A. (1996). The effects of breed and sex on carcass composition and tissue distribution of chickens. *Indian J. Anim. Sci.* **66,** 504-510.

Shimada K. (1998). Gene expression of steroidogenic enzymes in chicken embryonic gonads. *J. Exp. Zool*. **281,** 450-456.

Shimada K. and Saito N. (2000). Molecular mechanisms of sex determination and sex differentiation. *Japanese Poult. Sci.* **37,** 3-11.

Sumpter J.P. and Fostier A. (1996). Effects offlavonoids on aromatase activity an *in vitro* study. *J. Steroid Biochem. Mol. Biol.* **57,** 215-223.

Yoshida K., Shimada K. and Saito N. (1996). Expression of P450 (17α) hydroxylase and P450 aromatase genes in the chicken gonad before and after sexual differentiation. *Gen. Comp. Endocrinol.* **102,** 233-240.

Verapeen D.S. and Driver M.F. (2000). Separate sex growing of Ross 208 Broilers and effects on broiler performance and carcass quality. *Sci. Technol. Res. J.* **4,** 145-152.