Influence of exogenous gonadal steroids on pubertal age of hens and internal qualities of eggs

Olufemi M. Alabi

Department of Animal Science and Fisheries Management, Bowen University – Iwo, Nigeria. *Corresponding author: E-mail: olufemi.alabi@bowenuniversity.edu.ng

Citation: Alabi, O. M. (2019). Influence of exogenous gonadal steroids on pubertal age of hens and internal qualities of eggs. *Zhivotnovadni Nauki*, *57*(1), 36-43 (Bg).

Abstract

Ability of egg-type chickens to start laying earlier in life is a desirable trait that commercial poultry farmers always look forward to achieving. However, this may be delayed by some factors such as hormonal imbalance. There is a dearth of information on the use exogenous gonadal steroids to enhance the puberty attainment among egg-type chickens and internal qualities of eggs thereof. 144, 11-weeks old Super Black (SBL) hens and 144, 11-weeks Super Brown (SBR) hens were randomly allotted into four hormonal treatment groups with 36 hens per group each with three replicates in a randomized complete block design. The treatment groups are: T1 – with no hormone (NH; control), T2 – with Estradiol (E₂), T3 – with Progestin (P₄) and T4 – with equal combination of estradiol and progestin (EP). The steroids were injected intramuscularly at 5 mg per kg body weight of the hens. The experiment lasted 17 weeks during which data were collected on average daily feed intake (ADFI), hen-day production (HDP) and mortality rate while ages at puberty and peak production were determined with the HDP. Egg weight and some internal qualities were also measured at puberty and peak production. Data generated were subjected to analysis of variance statistically. All parameters investigated were significantly (P < 0.05) affected by steroidal treatment and strain. Hens (SBR) on P_{4} attained puberty and peaked earlier (149.67 and 175.33 days respectively) than hens on other treatments but with the highest mortality rate (8.33%). The weight and internal qualities of eggs were not affected by the treatments and strain. The results of this experiment suggest that exogenous gonadal steroids can be used to enhance puberty attainment among egg-type chickens in the humid tropics with the best result with the use of progestin.

Key words: age, egg, hens, performance, puberty, sex steroid hormones

Introduction

The words puberty and sexual maturity have been used interchangeably several times in livestock production and there is need to define them and to agree on where and when they can be used for other. According to Hawkins (1995), puberty is the stage in life at which a person's reproductive organs become able to function. This is also known as the pubertal age. Sexual maturity on the other hand is the stage at which the reproductive organ are fully grown or developed. While maturity interprets the development, puberty goes along with the functionality of the reproductive organs. Some organs may develop well but may not be functional. So it is possible to have animals that are sexually matured but may not have reached pubertal age.

While it is possible to assess the pubertal age of both small and large ruminants, swine and rabbits, but to determine the pubertal age for individual birds within a flock of several thousand members is quite difficult to determine (Smith, 1995). However, Egbunnike (2002) and Reddy et al. (2006) described the pubertal age of a flock of hen as the age at which 50% of the birds come into lay. Factors that can affect puberty attainment of egg-type chickens include; strain, nutrition (Cassy et al., 2004); live weight and growth rate (Attia et al., 1993); environmental stress, housing, diseases and hormonal status of the hens (Cassy et al., 2004). Hormones play a major role in the reproductive physiology of mammals and aves and apart from the Growth Hormone (GH) secreted by the anterior pituitary, gonadal hormones such as estrogen and progesterone are well involved in the physiology of egg production (Alabi, 2009). Gonadal hormones are secreted by the gonads (ovaries of females and testes of males). In domestic chickens, ovaries secret estradiol and the granulose cell layer of the mature preovulatory follicle secrets progesterone through a complex pathway and feedback controls as described by Pirsaraei et al. (2008). Injection of female gonadal steroids on hen have been reported to have inductive effects on ovulation (Rangel et al., 2005). Other reproductive hormones that work in conjuction with the gonadal steroids are luteinizing hormone (LH) and gonadotrophin-releasing hormone and follicle stimulating hormone (FSH) (Etches, 1996). Gonadal steroids also influence growth regulation of poultry positively. Oestrogen have been reported to have androgenic effect of female broilers although causing Fatty Liver-Hemorrhagic Syndrome in laying hens and reduces weight gain in male broilers and promotes fat deposition (Decuypere and Byse, 2005).

As a result of the influx of parent stock of egg-type chickens into Nigeria hatchery industries from various sources many cases of late– dropping among pullets were reported. Despite good feeding and vaccination programmes, some pullets were reported to start dropping as late as twenty-five weeks of age hence the need to look inward into the possibility of using exogenous gonadal steroids to enhance the puberty attainment of egg-type chickens.

The issue of hormone residues in meat have generated a lot of concern from consumers perspectives in Europe and USA and have been linked to cases of cancer in humans (Sundlof, 1994; Gandhi and Snedeker, 2000). However, the use of natural substances such as steroids have been reported by several researchers to be beneficial to livestock production without detrimental effect on humans consuming the products thereof as being speculated (Nelson et al., 1970; Preston, 1975; Velle, 1986; Coelho et al., 1988; Ferrando, 1990; Ladokun, 2006; Alabi and Oguntunji, 2011) when used for other things apart from direct growth promotion.

Meanwhile, egg qualities are of paramount interest to the consumers and such characteristics that may influence the acceptability of eggs must be taken care of. Egg qualities may be affected by such as the management of the flock, environmental temperature, relative humidity and hormonal status of the hen (Robert, 2004).

The objective of this work is to investigate the effect of exogenous female gonadal steroids (estrogen and progesterone) on puberty attainment and other performance characteristics of two strains of egg-type chickens in the humid tropics and some qualities of the eggs laid by them.

Material and Methods

Experimental birds and design

The experiment was carried out at the layers unit of the Teaching and Research farm of Bowen University – Iwo, Nigeria. The climatic conditions of the area have been earlier reported by Oguntunji, 2013). A total of 144 SBL and 144 SBR layers were used for the experiment. The pullets (200 for each strain) were purchased from a reputable hatchery in Nigeria at day old and were reared with routine management practices strictly observed. At the 11th week of age, the birds were randomly allotted to the treatment groups for each of the strains. The treatment groups were as follows: Treatment 1 - Noexogenous hormone (Control); NH, Treatment 2 - Exogenous Estradiol benzoate, E.; Treatment 3 – Exogenous Progesterone, P₄; Treatment 4 – Exogenous Estradiol + Progesterone; EP.

Each strain was divided into four treatment groups of 36 pullets. Each treatment group was further subdivided into 3 groups (replicates) of 12 pullets in a randomized complete block design. All the birds were housed in conventional battery cages.

Hormones administration and general management

The birds were allowed to adjust for 1 week after which the exogenous hormones were administered. At that 12th week of age and weekly thereafter till 15th week of age, the hormones were administered intramuscularly in the thigh. The hormone injections were obtained from licensed pharmacy shop and the dosage per pullet was 5 mg per kilogram live weight as described by Wayne and Liu (2004) and Stephanie et al. (2005). The administration was carried out between the hours of 7.00 am and 8.00 am on each day. Feed and water were given ad libitum. Initially the birds were given grower mash up to the 18th week of age after which they were given layers mash up to the end of the experiment. General routine management practices in term of vaccination and medication were strictly observed.

The experiment lasted for 17 weeks, during which data was collected in respect of daily feed intake, mortality rate and hen-day production (HDP). HDP data was used to determine the respective age in days at which each treatment group attained 50% egg production as earlier described by Egbunnike (2002) while the age at which each group attained the highest level of HDP was taken as their ages at peak production.

Meanwhile, the HDP was calculated using the formula:

$HDP = \frac{\text{Number of eggs produced per day}}{\text{Stock position}}$

Internal egg qualities measurements

From the age of first drop, analyses for some external and internal qualities started on biweekly basis with six eggs per replicate in random collection. External quality parameters such as Egg weight (EW), Egg length (EgL), Egg width (EgW) while the internal qualities investigated were shell thickness (ST), albumen weight (AW), albumen height (AH), yolk weight (YW), yolk height (YH), yolk length (YL) were measured while Haugh unit and Yolk index were calculated as described below:

Haugh unit: (Hu%) = 100 Log AH + 7.57-1.7 $EW^{0.37}$ (Eisen et al., 1962)

Yolk index:
$$(YI) = \frac{Yolk height}{Yolk length}$$

Statistical analyses

All the data generated were subjected to analysis of variance using the General Linear Models program of SAS 8.02 (SAS, 2004). Treatment means where significant were separated using Duncan option of the same software while the significance level was set at P < 0.05.

Results and Discussion

Daily Feed Intake

Table 1 shows the performance of the egg type chickens administered with exogenous female hormones with respect to feed intake. Feed intake among the two strains no significant (P > 0.05) difference was observed between the ADFI of birds on E₂ and EP but they differed significantly (P < 0.05) from NH and P₄ while significant (P < 0.05) difference was also observed between birds on NH and P_{A} . For SBL strain, the range was from 94.08 g (NH) to 99.38 $g(P_{\lambda})$ while for SBR strain, the range was from 90.75 g (NH) to 96.55 g (P_A). Furthermore, strain effect was also observed on the values of ADFI. In the entire treatment group, SBL strain had higher ADFI significantly (P < 0.05) than the SBR strain. The strain x treatment (strain-treatment interaction) effects showed that SBR strain on NH had the lowest ADFI of 90.75 g while SBL strain on P_4 had the highest value of 99.38 g. The results agree with the findings of Bauman et al., 1982; Vivat et al. (1992); Hull and Harvey (2001); Liu and Bacon (2005); Sun et al. (2006) who reported that anabolic steroids influence the feed intake of chickens by enhancing nutrients metabolism and tissues absorptive rate. From the results, birds on exogenous P_4 the highest ADFI followed by those given EP and E₂ across the two strains. This result corroborates the claim that

			Treatments			
Parameters	Strains	NH	E ₂	P_4	EP	SEM
ADFI (g)	IBL IBR SEM	94.08 ^{cx} 90.75 ^{cy} 0.70	96.15 ^{bx} 93.60 ^{by} 0.68	99.38 ^{ax} 96.55 ^{ay} 0.65	96.45 ^{bx} 93.75 ^{ay} 0.71	0.50 0.55
Age at puberty (days)	IBL IBR SEM	164.33ª× 160.33ªy 3.50	157.67 ^{bx} 154.67 ^{by} 2.50	152.67 ^{cx} 149.67 ^{cy} 2.50	157.33 ^{bx} 153.67 ^{by} 3.15	4.50 4.30
Age at peak production (days)	IBL IBR SEM	189.67 ^{ax} 187.33 ^{ay} 2.00	182.33 ^{bx} 178.33 ^{ay} 3.50	177.67 ^{cx} 175.33 ^{by} 3.00	182.67 ^{bx} 177.67 ^{ay} 3.50	4.50 4.20
Mortality rate (%)	IBL IBR	0.00 2.78	5.55 5.55	8.33 8.33	5.55 5.55	

Table 1. Effect of exogenous estradiol and progestin on performance characteristics of hens

abc: means in the same row with different superscript are significantly different (p < 0.05)

xy: means in the same column with different superscript are significantly different (p < 0.05)

ADFI = Average Daily Feed Intake, NH = No Gonadal steroid, E₂ = Estradiol, P₄ = Progestin, EP = Estradiol + Progestin

exogenous hormones can be used to stimulate feed intake in chickens. However, combination of estrogen and progesterone doesn't produce synergetic effect on parameters measured. Furthermore in all the treatment groups, SBR strain consumed lower feed than SBL. This variation might be due to genetic differences between the two strains as reported by Bruggeman (2006) and Joly (2008).

Age at Puberty

According to Figure 1, both exogenous hormones and strain had influence on the age at puberty (AP) of the two strains. In the two strains, no significant (P > 0.05) difference was observed on AP of birds on E, and EP but they differed significantly (P < 0.05) from those on NH and P_{A} while birds on NH and P_4 also differed significantly (P < 0.05). For SBL strain, the range was from 152.67 days (P_A) to 164.33 days (NH) while for SBR strain, the range was from 149.67 days (P_{A}) to 160.33 days (NH). In all the treatment groups SBR strain attained puberty significantly (P < 0.05) earlier than the SBL strain. The strain x treatment effects showed that SBR strain on P_{A} attained puberty earlier at 149.67 days significantly (P < 0.05) than others while SBL strain on NH attained puberty lately at 164.33 days of age. Exogenous gonadal hormones obviously influenced the age at which the hens attained puberty. This is in consonance with the work of Nakada et al. (1994) who affirmed that progesterone injection influenced the plasma concentration of Leutenizing hormone (LH) and Follicle stimulating hormone (FSH) and therefore inducing ovulation and subsequent egg development. Apart from gonadal hormones, administration of growth hormone (GH) can also accelerate puberty among hens (Hull and Harvey, 2001).

Age at Peak Production

Age at peak production followed the same trend with age at puberty (Figure 2). This agrees with the finding of Adeyinka et al. (2006) that exogenous P_4 can be used to improve egg production in hens. The strain effect revealed that SBR strain is superior to SBL strain with respect to these parameters.

Mortality

From Table 1, the trend of the mortality rate in the two strains agrees with the findings of Shanawany (1982) and Yang et al. (1998) that birds that start egg production earlier are likely to have higher mortality rate as a result of prolapse of the oviduct. Although, the result for mortality did not follow particular trend, SBL strain on NH had no mortality, SBR on NH had 2.78%,

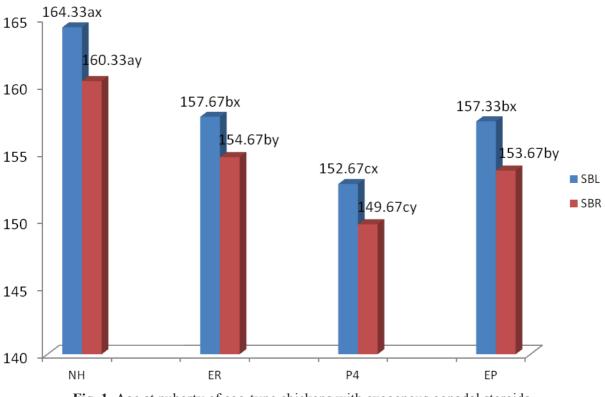


Fig. 1. Age at puberty of egg-type chickens with exogenous gonadal steroids

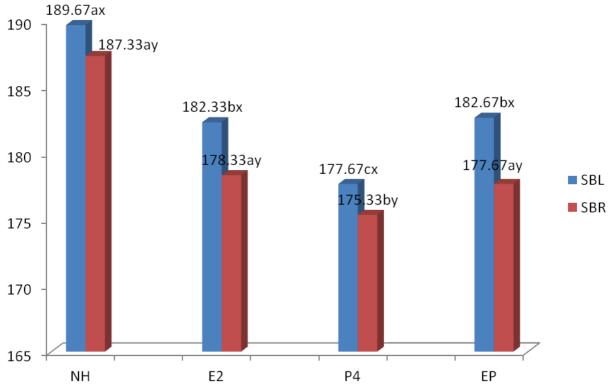


Fig. 2. Age at peak production of egg-type chickens with exogenous gonadal steroids

SBR and SBL on E_2 including SBR and SBL on EP had 5.55%, while SBL and SBR strain on P_4 had 8.33%.

External and Internal Egg qualities

Table 2 shows the results of the external and internal qualities of eggs from hens administered with female gonadal hormones. Egg weight was influenced by the exogenous hormones. No significant (P > 0.05) difference was observed between the egg weight of birds on E₂ and EP but they differed significantly (P < 0.05) from those on NH and P_4 while the EW of those on NH and P_{A} differed significantly (P < 0.05). The range of the egg weight was from 41.97 g (P_{4}) to 47.51 g (NH). The above results agree with the findings of Hocking (1987); Williams et al. (1992); Joseph and Oduntan (1999) that exogenous hormones will induce ovulation in chickens but the resulting egg will be smaller in size. The egg shell thickness (ST) was also influenced by the exogenous hormones. No significant (P > 0.05) difference was noted between the birds on exogenous hormones but they differed significantly (P <0.05) from those in the control group. This result corroborate the findings of Hansen and Beck (1998) that gonadal hormones aid calcium deposition during egg shell formation among chickens. Other egg quality parameters investigated were not significantly (P > 0.05) influenced by the exogenous hormones and this is in alignment with the findings of Choprakarn et al. (1989) that holding conditions of the hen prior to lay affect the albumen height and Haugh unit of eggs significantly rather than any other factor.

Conclusion

Attainment of puberty by layers strains of chickens can be enhanced with the use of exogenous female gonadal hormones preferably progesterone as can be inferred from the results of this study without negative effect on the desirable internal qualities of eggs.

References

Adeyinka, F. D., Adeyinka, L. A., Eduvie, L. O., Jokthan, G. E., & Orunmuyi, M. (2006). Effect of progesterone secretion on Egg production in the Grey Helmet Guinea Fowl (*Numida meleagris galleata*). Proc. 31st Ann. Cong. Nig. Soc. for Ani. Prod. 12th -15th March 2006 Zaria: 126-130.

Table 2. Effect of Exogenous (Gonadal Steroids on Internal Qualities of Eggs laid by hens
	Treatments

	rieathents									
Parameters	NH	E ₂	P_4	EP	SEM					
Egg weight (g)	49.50ª	46.70 ^b	43.15°	46.85 [⊳]	2.08					
Egg length (cm)	5.08ª	4.84 ^b	5.07ª	4.90 ^b	0.10					
Egg width (cm)	4.06ª	3.70°	3.80 ^b	3.85 ^b	0.10					
Shell thickness (mm)	0.43 ^b	0.46 ^b	0 47 ^b	0.47 ^b	0.04					
Albumen weight (% of EW)	63.48	3.47	63.95	63.65	0.62					
Albumen height (cm)	8.25 ^b	8.40ª	7.95°	8.20 ^b	0.10					
Yolk weight (% of EW)	23.65	23.75	23.86	23.88	0.34					
Yolk height (cm)	1.46	1.45	1.45	1.46	0.05					
Yolk length (cm)	3.65	3.70	3.74	3.80	0.17					
Yolk Index	0.40	0.39	0.39	0.38	0.01					
Haugh Unit (%)	73.15	74.25	74.55	74.35	0.16					

abcd: Means with different superscript are significantly different (p < 0.05)

SEM: Standard Error of Mean

 $NH = no hormone; E_2 = estradiol; P_4 = progestin; EP = estradiol + progestin$

Alabi, O. M. (2009). Physiological Response of Eggtype Chickens to Different Intensive Housing systems and exogenous hormones.*Ph.D Dissertation submitted* to Department of Animal Science. University of Ibadan, Nigeria.

Alabi, O. M. & Oguntunji, A. O (2011). Blood Chemistry and Performance Characteristics of Meat-Type Male Chickens in Response to Exogenous Testosterone Enanthate. *International Journal of Current Research and Review;Vol.3.*No(4): pp: 76-82. http://www.ijcrr.com

Attia, Y. A., Yamani, K. A., Burke, W. H. & Jensen, L.S. (1993). Daily energy allotment and reproductive performance of broiler breeder males. *Poultry Science*, *72*(1), 42-50.

Bauman, D. E., Eisemann, J. H., & Currie, W. B. (1982, July). Hormonal effects on partitioning of nutrients for tissue growth: role of growth hormone and prolactin. In *Federation proceedings* (Vol. 41, No. 9, pp. 2538-2544).

Cassy, S., Metayer, S., Crochet, S., Rideau, N., Collin, A., & Tesseraud, S. (2004). Leptin receptor in the chicken ovary: potential involvement in ovarian dysfunction of ad libitum-fed broiler breeder hens. *Reproductive Biology and Endocrinology*, 2(1), 72.

Choprakarn, K., Salangam, I., & Tanaka, K. (1988). Laying performance, egg characteristics and composition in indigenous hen. *J. Nat.Res.Council. Thailand*.30:1-17.

Coelho, J. F. S., Galbratith, H. & Topps, J. H. (1988). The effect of a combination of trenbolone acetate and $n17\beta$ -oestradiol on the performance, carcass composition and blood characteristics of castrated lambs. *Anim. Prod.* 36:360-365.

Decuypere, E., & Buyse, J. (2005). Endocrine control of postnatal growth in poultry. *The Journal of Poultry Science, 42*(1), 1-13.

Egbunnike, G. N. (2002). Effect of crude inhibin on the attainment of puberty and ovarian activities in layers in the humid tropics. *Proc.* 7th Ann. Conf. of Anim. Sc. ASAN of Nig. (ASAN) Abeokuta 16-19 September 2002: 41-43.

Eisen, E. J., Bohren, B. B., & McKean, H. E. (1962). The Haugh unit as a measure of egg albumen quality. *Poultry Science*, *41*(5), 1461-1468.

Etches, R. J. (1996). The ovary. In Reproduction in Poultry, Ed. R. J. Etches. Cambridge: Cab Int.: 125-166.

Ferrando, R. (1990). Methods of measuring hormone levels in animal products. In: *Steroid analysis. Muskin Groner and Kirk (Eds), Lea and Ferbiger, New York.*

Ghandi, R., & Snedeker, S. M. (2000). Consumer concerns about hormones in food. Fact Sheet #37. Cornell Cooperative Extension. Cornell University Program on Breast Cancer and Environmental Risk Factors in New York State.

Hansen, K. K., & Beck, M. M. (1998). Heat stress, estrogen implants and calcium uptake in duode-

num of hens. *Poultry report.coop ext. Univ.of Nebraska* (46):70-75.

Hawkins, J. M. (1995). Puberty and Maturity: in (Joyce. Ed) The oxford mini-reference dictionary. Oxford Univ. Press: 252-327.

Hocking, P. M. (1987). Nutritional interactions with reproduction in birds. *Proceedings of the Nutrition Society*, *46*(2), 217-225.

Hull, K. L., & Harvey, S. (2001). Growth hormone: roles in female reproduction. *Journal of Endocrinol- ogy*, *168*(1), 1-23.

Joly, P. (2008). Factors that influence Egg weight: How to change it to meet market requirement. *Isa Genetics* .*Rev.* (2): 18-28.

Ladokun, A. O. (2006). Exogenous hormonal regulation of growth rate, blood chemistry and fertility in pigs. *Ph. D dissertation submitted to the department.of Anim. sc. Univ.of Ibadan, Nigeria.*

Liu, H. K., & Bacon, W. L. (2005). Changes in egg production rate induced by progesterone injection in broiler breeder hens. *Poultry science*, *84*(2), 321-327.

Nakada, T., Koja, Z., & Tanaka, K. (1994). Effect of progesterone on ovulation in the hypophysectomised hen. *British poultry science*, *35*(1), 153-156.

Nelson, J. A., Strucker, R. F., & James, R. (1978). Estrogen contents of some plants. *J .Toxicol. Environ. Health, 24*:325-338.

Oguntunji, A. O. (2013). *Phenotypic and biochemical characterization of the Nigerian Muscovy ducks. Nigeria: Bowen University, Iwo, Osun State* (Doctoral dissertation, Ph. D. Thesis).

Onagbesan, O. M., Metayer, S., Tona, K., Williams, J., Decuypere, E., & Bruggeman, V. (2006). Effects of genotype and feed allowance on plasma luteinizing hormones, follicle-stimulating hormones, progesterone, estradiol levels, follicle differentiation, and egg production rates of broiler breeder hens. *Poultry science*, *85*(7), 1245-1258.

Pirsaraei, Z. A., Shahneh, A. Z., Zaghari, M., Zamiri, M. J., & Mianji, G. R. (2008). Effect of testosterone and growth hormone injection before puberty on follicles size, rate of egg production and egg characteristics of the Mazandaran Native breeder hens. *African Journal of Biotechnology*, 7(17). 3149-3154

Preston, R. L. (1975). Biological responses to estrogen additives in meat producing cattle and lambs. *Journal of animal science*, *41*(5), 1414-1430.

Rangel, P. L., Lassala, A., & Gutierrez, C. G. (2005). Testosterone immunization blocks the ovulatory process in laying hens without affecting ovarian follicular development. *Animal reproduction science*, *86*(1-2), 143-151.

Reddy, I. J., David, C. G., & Raju, S. S. (2006). Inter sequence pause days, egg production, steroid and luteinizing hormone in domestic hen (Gallous domesticus) immunized against cprolactin. *International Journal of Poultry Science*, 5(5), 420-427.

Roberts, J. R. (2004). Factors affecting egg internal quality and egg shell quality in laying hens. *The Journal of Poultry Science*, *41*(3), 161-177.

Shanawany, M. M. (1982). The effect of ahemeral light and dark cycles on the performance of laying hens—(a review). *World's Poultry Science Journal*, *38*(2), 120-126.

Smith, A. J. (1995). Poultry. *The Tropical Agriculturists*. CTA. (Ed.Rene Coste). Macmillan Publisher, London.

Stephanie, M. C., Elizabeth, A., & Johnson, P. A. (2005). Progesterone and estradiol levels and the sex of resulting egg from progesterone-induced ovulations in domestic chickens. *Journal.of Animal.Science*, *82*: 98-102.

Sun, J. M., Richards, M. P., Rosebrough, R. W., Ashwell, C. M., McMurtry, J. P., & Coon, C. N. (2006). The relationship of body composition, feed intake, and metabolic hormones for broiler breeder females. *Poultry science*, *85*(7), 1173-1184.

Sundlof, S. F. (1994). Human health risks associated with drug residues in animal-derived foods. *Journal of Agromedicine*, *1*(2), 5-20.

Velle, W. (1986). Endogenous anabolic agents in farm animals. In: Anabolic agents in Anima. Prod. (Eds. F. C. Lu and J. Rendel) Environmental quality and safety supplement, vol. (5), 159-170.

Vivat, V., Cohen-Tannoudji, J., Revelli, J. P., Muzzin, P., Giacobino, J. P., Maltier, J. P., & Legrand, C. (1992). Progesterone transcriptionally regulates the beta 2-adrenergic receptor gene in pregnant rat myometrium. *Journal of Biological Chemistry*, 267(12), 7975-7978.

Wayne, L. B., & Liu, H. (2004). Progesterone injections induce a polycystic ovarian follicle syndrome (PCOF) in turkey hens. *Proc.10th conf. on Anim. Rep.Developmen*, 50-54.

Williams, J., Sharp, P. J., & Goddard, C. (1992). The Effects of Growth Hormone on Ovarian Follicular Growth in Domestic Hen. *J. Reprod. Fertil*, *59*.

Yang, J., Long, D. W., & Bacon, W. L. (1998). Effect of exogenous progesterone on luteinizing hormone secretion in domestic turkey hens at different reproductive states. *General and comparative endocrinology*, *110*(3), 337-345.

SAS (2004). Statistical Analysis System. *Institute User's Guide*. SAS Institute, Cary, N.O.