ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 13 (9): 504-509, 2014
ISSN 1682-8356

Effect of Turmeric Powder to Estriol and Progesterone Hormone Profile of Laying Hens During One Cycle of Ovulation

Tyas Rini Saraswati[1], Wasmen Manalu[2], Damiana Rita Ekastuti[2] and Nastiti Kusumorini[2]
[1]Department of Biology, Faculty of Sains and Mathematic, Diponegoro University, Tembalang Campus, Semarang-50275, Semarang, Indonesia
[2]Department of AFF, Faculty of Veterinary Medicine, Bogor Agricultural Institute, IPB Campus, Darmaga, Bogor-16680, Bogor, Indonesia

Abstract: This research was designed to study the profile of the hormones estriol, progesterone, in the blood during a cycle of ovulation in controlled laying hens and laying hens that treated by giving the turmeric powder. Determination of hormone profile was done on laying hens. Six laying hens aged 20 month were divided into two experimental unit. Each experimental unit consist of three laying hens i.e., P0: control (not supplemented with turmeric powder), P1: turmeric powder supplementation at a dose of 405 mg/laying hens/day for a month. Blood was drawn via jugular vein by 4 mL. Blood sampling performed every 2 h starting after hen lay eggs during a cycle of ovulation, then retrieved and analyzed the serum levels of estrogen and progesterone. The data graphed to determine the profile of the hormones estrogen, progesterone and compared between the controlled and the treated with turmeric powder. The results showed that administration of turmeric powder can shorten the cycle of ovulation about 5 h 35 min. Turmeric powder did not affect the pattern of fluctuations in the hormone progesterone. There are 4 progesterone peaks during the first cycle of ovulation both in controlled laying hens and laying hens that treated with turmeric powder. There was a change to the hormone estriol fluctuations. In controlled laying hens takes four peaks hormone estriol in an ovulatory cycle, whereas in laying hen treated with turmeric powder was 1 hormone estriol peak occurring approximately 15 h before ovulation.

Key words: Estriol, laying hens, progesterone, turmeric powder

INTRODUCTION

In birds, as in other vertebrates, reproduction is controlled by the hypothalamic-pituitary-gonadal axis with each component secreting specific neuropeptides or hormone (Bedecarrats *et al*., 2009). Ovulation cycle is regulated by hormonal mechanisms (Kumar and Sait, 2011). The hormone estriol is one of reproductive steroid hormones (Golzieher and Castracane, 2008). Estrogen synthesis occurs in the cells of theca and granulosa cells of the ovary (Wojtysiak and Kapkowska, 2005). Cholesterol is a steroid hormone precursor, the formation through a series of enzymatic reactions (Levi *et al*., 2009). Estrogen production in small follicles (Wojtysiak and Kapkowska, 2005). Its associated with the enzymes in the theca layer that plays a role in the biosynthesis of steroid as P450 aromatase converts testosterone into estrogen. The small follicles and ovarian stroma containing more than 50% of aromatase that produces 85% of estrogen (Amstrong, 1984).

Hundreds of follicles has potential becomes an egg that containing egg yolk. Ovarian follicular hierarchy is a series of follicles with a different diameter. Ovulation is reached after follicles mature (Buchanan *et al*., 2002). Most of the follicles are not ovulate, but many follicles participate in producing steroid hormones from ovaries. In the early stages of follicular development, small follicles begin to produce estrogen and androgen (Etches, 1996). When follicles start to form yolk, estrogen production in this follicle begins decline (Amstrong, 1984). For several hours before ovulation, only the largest follicle produce progesterone. Increase concentrations of progesterone will stimulate secretion of GnRH by the hypothalamus. GnRH secretion into the portal vessels of the hypothalamus initiate gonadotropin pituitary LH secretion and increase the secretion of gonadotropins. Level increased of LH in the blood stimulates the secretion of progesterone. Positive feedback loop between progesterone and LH will result preovulation yolk that causes tearing of the follicle. Release of yolk from the follicle at ovulation occurs when stigma ripped (Etches, 1996; Doi *et al*., 1980). The release of the yolk (the process of ovulation), is the major controlling factor influencing the subsequent steps in the formation and laying of the egg.

Female reproductive tract in the form of an ovum in the oviduct will receive 15 min after ovulation and will provide

Corresponding Author: Tyas Rini Saraswati, Department of Biology, Faculty of Sains and Mathematic, Diponegoro University, Tembalang Campus, Semarang 50275, Semarang-Indonesia

the right environment for fertilization and albumin secretion, eggshell membrane, eggshell and cuticle on concentric layers around the ovum. Oviposition occurs after calcified eggs and require coordination with muscular activity (Etches, 1996). Ovarian response to gonadotropin concentrations enhancement by increasing estrogen production. Estrogen circulating in the liver, enter by diffusion and stimulates the synthesis vitellogenin (Levi *et al*., 2009). Vitellogenin circulated to the surface layer of the growing oocytes. Selectively, vitelogenin will be captured by the receptor, then the process of endocytosis occurred cytoplasmic translocation forming yolk bodies together with the proteolytic cleavage of a subunit vitellogenin yolk lipoprotein, lipovitellin and fosvitin. The existence vitellogenin shows yolk lipoprotein accumulation in the oocyte.

Continuous activity of the liver in the synthesis vitellogenin can induce liver degeneration resulting in liver damage (Saraswati *et al*., 2013a). Turmeric that has a hepatoprotective effect, are compounds that prevent damage in liver (Sengupta *et al*., 2011; Labban, 2014). Curcumin is the main compound in turmeric, which has a scavenger of free radicals, antioxidants and anti-inflammatory (Farghaly and Hussein, 2010; Salama *et al*., 2013; Singh *et al*., 2011; Steffi and Srinivasan, 2014). The content of curcumin in turmeric powder at 7.97% (Saraswati *et al*., 2013b). Mechanism of curcumin as an anti-inflammatory is inhibin the production of prostaglandins by inhibiting the activity of cyclooxygenase-2 (Adelin *et al*., 2013; Tan *et al*., 2009). Curcumin modulates cell growth and response of various types of immune cells (Sengupta *et al*., 2011; Jagetia and Aggarwal, 2007). Turmeric also contains phytoestrogens. Role of phytoestrogens in turmeric powder can stimulate synthesis vitellogenin (Saraswati *et al*., 2013b). Phytoestrogens can bind to estrogen receptors (Harris *et al*., 2005; Morito *et al*., 2001; Nasri *et al*., 2014). Dietary phytoestrogens produce large changes in plasma levels vitellogenin (Turker and Bozcaarmutlu, 2009; Fischer *et al*., 2012). Improvement of liver function and phytoestrogens will stimulate increased synthesis vitellogenin (Saraswati *et al*., 2013b). Vitellogenin carried by the blood stream to the ovaries to follicle's growth (Elnagar and Elhady, 2009). Uptake of large amounts of yolk lipoproteins into developing follicles involves binding of the yolk precursors to a specific VTG/VLDL receptor on the oocyte surface and transport across the cell membrane via receptor-mediated endocytosis (Han *et al*., 2009; Koc and Akbulut, 2012). The mature follicles will be ovulated into tractus reproductive. Based on the above, how changes in profile of the hormone estriol and progesterone in laying hens supplemented with turmeric powder.

The purpose of this study was to determine the profile of the hormone progesterone and estriol on the ovulation cycle, both in laying hens controls and treated turmeric powder.

MATERIALS AND METHODS

Experimental design: This study used turmeric powder (*Curcuma longa* Linn) and six laying hens, age 20 weeks. Its acclimated for a week. Experimental animals were divided into two groups, each group consisted of three laying hens. The first group as control (P:0) not given turmeric powder). The second group (P1) was turmeric powder supplemented with a dose of 405 mg/laying hens/day for a month. Before treatment, six laying hens acclimatized for two weeks in an individual cage. During the experiment, feed and drinking water were provided *ad libitum*.

Methods

Blood sampling and serum preparation: After egg-laying hens, blood is drawn through the jugular vein as much as 4 mL. Blood sampling done the day after the administration of turmeric powder is stopped and started when egg-laying hens. Blood sampling performed every two hours for an ovulatory cycle. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 min. Remove the clot by centrifuging at 2000 rpm for 20 min. The resulting supernatant is designated serum. Following centrifugation, it is important to immediately transfer the liquid component (serum) into ependorf tube using a pipette. If the serum is not analyzed immediately, the serum should be stored and transported at -20°C or lower.

Hormone concentration measurements: Concentrations of estriol and Progesteron were measured with medical diagnosis RIA kits. To minimize interference from steroid-binding proteins, 100 µL sample aliquots were mixed with an equal volume of 0.01 M PBS (pH7.4) and preheated in a 70°C water bath for 30 min to denature binding proteins and release bound hormone. The samples were analyzed according to the protocols supplied by the kit provider.

Observations hierarchical follicles: At the end of the experimental carried out observations of hierarchical follicles.

Hormones profile: The data were graphed to determine the profile of the hormones estriol and progesterone and compared between the control and laying hen treated with turmeric powder in an ovulatory cycle.

RESULTS AND DISCUSSION

Profile of progesterone: Progesterone profiles can be seen in Fig. 1.

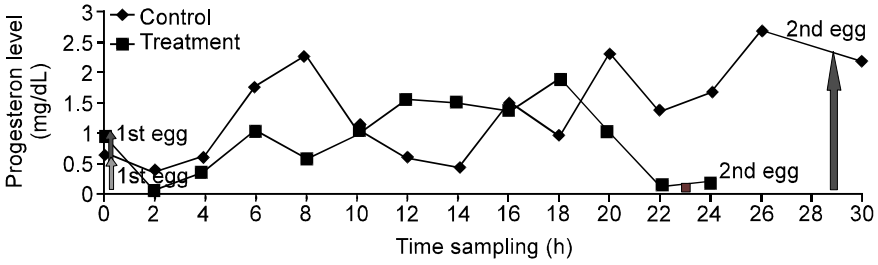

Fig. 1: Profile of hormone progesterone in control and laying hen treated with turmeric powder in an ovulatory cycle

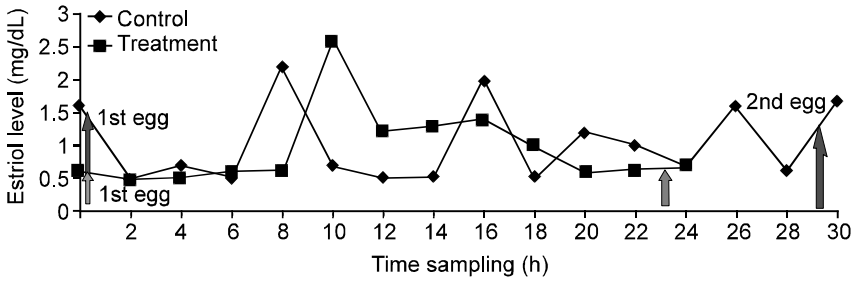

Fig. 2: Profile of hormone estriol in control and laying hens treated with turmeric powder in an ovulatory cycle

The results of measurements of the levels of plasma progesterone levels fluctuate for an ovulatory cycle. After oviposition decreased progesterone levels. Ovulation usually occurs in chickens 15-75 min following oviposition (Sturkie *et al*., 2000). After ovulation progesterone levels begin to increase again and peak progesterone occurs 4 times before the next ovulation with 4-8 h intervals. Progesterone peak occurred approximately 4 h before oviposition. This is in accordance with the opinion of Doi *et al*. (1980) that plasma progesterone levels peaked 4 h before ovulation, the research is also supported by Buchanan *et al*. (2002), that increased plasma progesterone concentrations prior to ovulation along with elevated levels of LH that occurs between 4-8 h before ovulation. The study of blood progesterone levels after treated with turmeric powder 405 mg/day for a month intervals obtained with the first egg the next 23 h and 25 min. Measurement of progesterone levels indicated that progesterone levels fluctuation pattern similar to the pattern of fluctuations in the control animals, which means that the addition of turmeric powder did not affect the secretion of progesterone. Average progesterone levels in animals treated lower than control animal's progesterone levels. When ovulation hormone progesterone decreased then increased and there is a fluctuation to form 4 peak progesterone levels in an ovulatory cycle, with intervals of 6 h. The highest progesterone levels peak about 5 h before oviposition.

Fluctuations related to the secretion of progesterone components of egg white and eggshell membrane, is also associated with the movement of the ovum as it passes through the reproductive tract (Sturkie, 2000). This is supported by research Shimada and Tanabe (1982) on the electrical activity of the infundibulum during the ovulatory cycle, that the frequency of electrical activity in the infundibulum tend to decline shortly after ovulation, but then increased again for several h.

After ovulation the egg will be captured by fimbrae then enter the infundibulum for 15-30 min. Ovum subsequently entered magnum approximately 2-3 h, proceed to the isthmus for 1-2 h, then enter the uterus for 18-26 h, then pass through the vagina and oviposition occurs. Highest plasma progesterone concentrations obtained 6-4 h before ovulation and in conjunction with LH peak. Primarily from an increase in progesterone secretion preovulasi the largest follicle (Etches, 1996). Wave progesterone pre ovulation stimulates the release of LH, which causes ovulation of the largest follicle (Biswas *et al*., 2010).

Progesterone has specific receptors on the hypothalamus, pituitary gland and a number of receptors in the oviduct, smooth muscle cells of the arterial wall, myometrium and stigma. Progesterone plays a role in the contraction of the myometrium. So that the direct effect of progesterone mediate ovulation (Kawashima *et al*., 1994). Corpus luteum in laying hens didn't form, so post ovulation low progesterone levels. Plasma progesterone increased shortly after 4 times decreased and reached a peak before ovulation.

Profile of estriol: Estriol hormone profiles during a cycle of ovulation in hens control and its treated with turmeric powder can be seen in Fig. 2.

There are fluctuations in the levels of estriol during the ovulation cycle of the control laying hens. Peak occurred four estriol levels. Estriol peak occurs at intervals of time between 4-8 h. In accordance with research by

Graber and Nalbandov (1976) there are four peaks in estrogen levels in an ovulatory cycle, which first occurred about an hour before ovulation and the other three at intervals of 5-6 h. The observation of estriol levels in hens treated with turmeric powder showed a different pattern of fluctuations with control animals, which means giving turmeric powder affect the secretion of estriol. Estriol peak occurs once per cycle ovulation occurs 14 h prior to oviposition. When oviposition occurs either when the first oviposition and oviposition latter was no increase in plasma levels of estriol, which means that for the occurrence of ovulation with estrogen should not be too high. By Sturkie (2000), estrogen was not directly involved in inducing the secretion of LH or ovulation. Ovulation can occur even if there is no increase in estrogen is high. Together estrogen progesterone needed to induce the release of LH (Sturkie, 2000).

Low levels of estriol in plasma at the time of oviposition allegedly caused phytoestrogens contained in turmeric powder capable of replacing estrogen plays a role in influencing the physiological function of the laying hens. Phytoestrogens give negative feedback to suppress GnRH secretion by the hypothalamus in rats (Zhao and Mu, 2011), thereby suppressing the secretion of follicle stimulating hormone and causing plasma estrogen levels decline. According to Zhao and Mu (2011) phytoestrogens are polyphenolic compounds that are structurally or functionally similar to estrogen. Phytoestrogens cause estrogenic effects. Mechanism of estrogen receptor bioactivity of phytoestrogens bonded because structural similarity with estradiol. The biological effects of estrogen are mediated through two distinct intracellular receptors, ERα and ERβ. Phytoestrogens exhibit higher affinity for ERβ binding (Jefferson, 2010). Although phytoestrogens have weak affinity than natural estrogen, phytoestrogens have a significant role to the regulation of reproduction. Axis of the hypothalamic-pituitary-gonadal endocrine system is to regulate reproductive prime. Regulation by hypothalamic control of reproduction for release pituitary gonadotropin hormone follicle is stimulating hormone (FSH) and luteinizing hormone (LH). Both FSH and LH are released into the general circulation to stimulate the production of sex steroids, including estrogen. Estrogen also has the role of positive and negative feedback on gonadotropin through direct interaction with ER.

The results showed shortening of time laying occurs for about 5 h 35 min in laying hens which is treated by turmeric powder compared with control laying hens. The distance between the first egg laying with the second egg in the control laying hens during 29 h, whereas in laying hens treated with turmeric powder, eggs distance between the first and second egg is 23 h 25 min. Shortening the time spawn after supplementation with turmeric powder suspected to be related to the role of

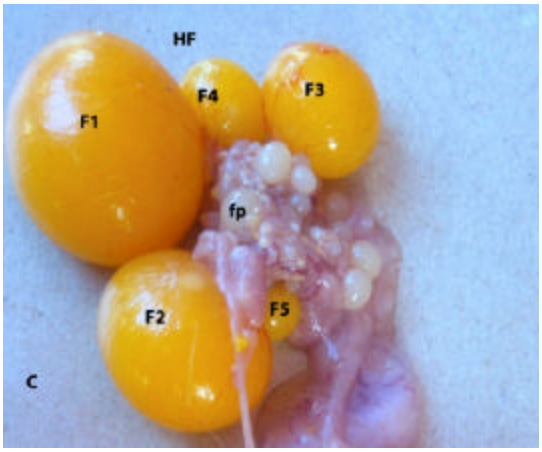

Fig. 3: Hierarchy of ovarian follicles in laying hens controls. HF: Hierarchy follicles, fp: white follicles

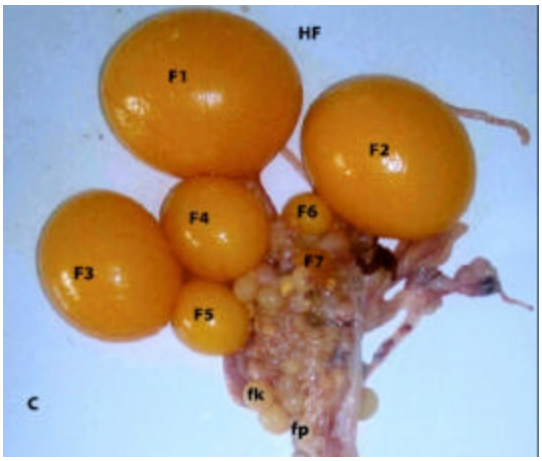

Fig. 4: Hierarchy of ovarian follicles in laying hens treated with turmeric powder for a month. Hierarchical follicles (F3, F4,F5, F6, F7, yellow follicles (fk); White follicles (fp), HF: Hierarchy follicles

turmeric powder in improving liver function to synthesize vitellogenin, which further vitellogenin transported to the ovaries to follicle growth hierarchy. Phytoestrogens have estrogen-like activity to induce the biosynthesis of vitellogenin. Phytoestrogens stimulates hepatocytes to synthesize vitellogenin that will be transported to the ovary through circulation as yolk precursors. More and more the follicles growing, the follicles ovulation becomes faster.

Hierarchy of ovarian follicles: Although the level of estriol in the treatment of laying hens is low, but the hierarchy of follicles in laying hens treated with turmeric powder (Fig. 4) more developed than the control laying hens (Fig. 3), it is proved that turmeric powder is able to

induce the synthesis vitellogenin in the liver and then carried by the blood stream to the ovarian follicle and is used as a constituent component of egg yolk. Phytoestrogens in turmeric powder capable of binding to estrogen receptors in the theca and granulosa cells to stimulate the growth of ovarian follicles. Phytoestrogens are also working on to prepare the reproductive tract cells of the reproductive tract to secrete other material constituent of egg components (Nadia *et al*., 2008).

The existence of an ovum in the reproductive tract cells stimulates the gland to secrete components of egg white (Saraswati *et al*., 2013b). Besides the presence of phytoestrogens in turmeric powder plays a role in inducing the development of the reproductive tract cells to synthesize components of egg white. Both of these factors will contribute to the acceleration of the process of laying eggs. The high one-time peak estriol administration on the treatment of turmeric powder which occurred 14 h before oviposition and then did not happen until late oviposition, estriol fluctuations allegedly associated with the growth of cells of the reproductive tract that are ready to secrete components of egg white and egg shell and can accelerate the journey formation of eggs in the reproductive tract, so that it can shorten the cycle of ovulation.

Conclusion: Provision of turmeric powder did not affect the pattern of hormonal fluctuations progesterone, but turmeric powder supplementation suppressed the hormone estriol so pattern estriol hormone fluctuations was affected. Provision of turmeric powder is shortening the cycle of ovulation 5 h 35 min. Provision of turmeric powder also increases follicular growth hierarchy. Supplemented with turmeric powder had higher follicular hierarchy as compared to control laying hens.

ACKNOWLEDGEMENTS

The authors thanks to the Directorate General of Higher Education, Ministry of National Education of Republic Indonesia for providing BPPS scholarship.

REFERENCES

Adelin, T., Frengki and O. Aliza, 2013. Curcumin and tethering molecule analogues on Cyclooxygenase-2., J. Medika Vet., 7: 30-34.

Amstrong, D.G., 1984. Ovarian aromatase activity in the domestic fowl (*Gallus domesticus*) J. Endocrinol., 100: 81-86.

Bedecarrats, G.Y., S.R. Maddineni and R. Ramachandran, 2009. Gonadotropin-inhibitory hormone receptor signaling and its impact on reproduction in chickens. General and Comparative Endocrinol., 163: 7-11.

Biswas, A., J. Mohan, K. Venkata and H. Sastry, 2010. Age-dependent variation in hormonal concentration and biochemical constituents in blood plasma of Indian native fowl. Vet. Med. Int., 2010: 737292.

Buchanan, S., G.W. Robertson and P.M. Hocking, 2002. Comparative changes in plasma concentrations of progesterone, oestradiol and LH during the ovulatory cycle in a multiple ovulating male line and a single ovulating traditional line of turkeys. Reprod., 123: 127-133.

Doi, O., T. Takai, T. Nakamura and Y. Tanabe, 1980. Changes in the pituitary and plasma LH, plasma and follicular progesterone and estradiol and plasma testosterone and estrone concentrations during the ovulatory cycle of the quail (*Coturnix coturnix japonica*). J. General and Comparative Endocrinol., 41: 156-163.

Elnagar, S.A. and A.M. Alhady, 2009. Exogenous Estradiol: productive and reproductive performance and physiological profile of japanese quail hens. Int. J. Poult. Sci., 8: 634-641.

Etches, R.J., 1996. Reproduction in Poult. Singapore. CAB. Int., 125-207.

Farghaly, H.S. and M.A. Hussein, 2010. Protective effect of curcumin against paracetamol-induced liver damage. Aust. J. Basic and Appl. Sci., 4: 4266-4274.

Fischer, M., C. Regitz, M. Kahl, M. Werthebach, M. Boll and U. Wenzel, 2012. Phytoestrogens genistein and daidzein affect immunity in the nematode Caenorhabditis elegans via alterations of vitellogenin expression. Mol. Nutr. Food Res., 56: 957-65.

Graber, J.W. and A.V. Nalbandov, 1976. Peripheral estrogen levels during the laying cycle of the hen (*Gallus domesticus*). Biol. Reprod., 14: 109-114.

Golzieher, J.W. and V.V. Castracane, 2008. Physiology and mechanism of action of steroids. Glob.Libr.Women's med.Doi 10.3843/GLOWM. 10387.

Han, D., N.H. Haunerland and T.D. Williams, 2009. Variation in yolk precursor receptor mRNA expression is a key determinant of reproductive phenotype in the zebra finch (*Taeniopygia guttata*). J. Exp. Biol., 212: 1277-1283.

Harris, D.M., E. Besselink, S.M. Henning, V.L. Go and D. Heber, 2005. Phytoestrogens induce differential estrogen receptor alpha- or Beta-mediated responses in transfected breast cancer cells. Exp. Biol. Med., 230: 558-68.

Jagetia, G.C. and B.B. Aggarwal, 2007. Spicing up of the immune system by curcumin J. Clin. Immunol., 27: 19-35.

Jefferson, W.N., 2010. Adult Ovarian Function Can Be Affected by High Levels of Soy. J. Nutr., 27: 2322-25.

Kawashima, M., M. Kamiyoshi and K. Tanaka, 1994. Changes in progesterone receptor binding of preoptic hypothalamus during an ovulatory cycle of the hen. Poult. Sci., 73: 855-863.

Koc, N.D. and C. Akbulut, 2012. Electron and light microscopic investigations of follicular epithelium in vitellogenic oocyte of zebrafish (Danio rerio). Pak. J. Zool., 44: 1581-1586.

Kumar, P. and S.F. Sait, 2011. Luteinizing hormone and its dilemma in ovulation induction. J. Hum. Reprod. Sci., 4: 2-7.

Labban, L., 2014. Medicinal and pharmacological properties of Turmeric (Curcuma longa): A Rev. Int. J. Pharm. Biomed. Sci., 5: 17-23.

Levi, L., I. Pekarski, E. Gutman, P. Fortina, T. Hyslop, J. Biran, B. Levavi and E. Lubzens, 2009. Licensee BioMed Central Ltd.http://www.biomedcentral. com/1471-2164/10/141.

Nadia, R.L., R.A. Hassan, M. Qota and H.M. Fayek, 2008. Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. Int. J. Poult. Sci., 7: 134-150.

Nasri, H., N. Shinfard, M. Rafieian, S. Rafieian, M. Shirzad and M.R. Kopaei, 2014. Turmeric: A spice with multifunctional medicinal properties. J. Herb. Med. Pharmacol., 3: 5-8.

Morito, K., T. Hirose, J. Kinjo, T. Hirakawa, M. Okawa T. Nohara, S. Ogawa, S. Inoue, M. Muramatsu and Y. Masamune, 2001. Interaction of phytoestrogens with estrogen receptors alpha and beta. Biol. Pharm. Bull., 24: 351-6.

Salama, S.M., M.A. Abdulla, A.S. Alrasdi, S. Ismail, S.A. Alkiyumi and S. Golbabapour, 2013. Hepatoprotective effect of ethanolic extract of Curcuma longa on thioacetamide induced liver cirrhosis in rats. BMC Complementary and Alternative Med., 13: 56.

Saraswati, T.R., W. Manalu, D.R. Ekastuti and N. Kusumorini, 2013a. The Role of Turmeric Powder in Lipid Metabolism and Its Effect on Quality of The First Quail's Egg. J. Indonesian Trop. Anim. Agric., 38: 123-129.

Saraswati, T.R., W. Manalu, D.R. Ekastuti and N. Kusumorini, 2013b. Increased egg production of japanese quail (cortunix japonica) by improving liver function through turmeric powder supplementation. Int. J. Poult. Sci., 12: 601-614.

Sengupta, M., G.D. Sharma and B. Chakraborty, 2011. Hepatoprotective and immunomodulatory properties of aqueous extract of Curcuma longa in carbon tetra chloride intoxicated Swiss albino mice. Asian Pac. J. Trop. Biomed., 1: 193-9.

Shimada, K. and Y. Tanabe, 1982. Electrical activity of the infundibulum in relation to ovulation in the chicken. J. Reprod. Fert., 65: 419-423.

Singh, H., P.S. Bedi and B. Singh, 2011. Hepatoprotective activity of turmeric and garlic against 7-12, dimethylbenzanthracene induced liver damage in wistar albino rats. Eur. J. Med. Plants., 1: 162-170.

Steffi, P.F. and M. Srinivasan, 2014. Curcumin, a potent anticarcinogenic polyphenol - A review. Asian J. Pharm. Clin. Res., 7: 1-8.

Sturkie, P.D., 2000. Avian Physiology. Academic Press., pp: 155.

Tan, X., E.M. Poulose, D.J. Stechschulte and K.N. Dileepan, 2009. Curcumin stimulates cyclooxygenase-2 (COX-2), prostaglandin I_2 synthase (PGI_2S) and PGE_2 synthase (PGE_2S) gene expression in human coronary artery endothelial cells. J. Immunol., 182: 93-19.

Turker, H., Bozcaarmutlu, 2009. Effect of total isoflavones found in soybean on vitellogenin production in common carp. Research article. Kafkas Univ. Vet. Fak Derg., 15: 561-568.

Wojtysiak, D. and E. Kapkowska, 2005. Steroid hormone concentration in the small ovarian follicles of the goose. Biotech. Anim. Husbandry, 21: 211-215.

Zhao, E. and Q. Mu, 2011. Phytoestrogen biological action on mammalian reproductive system and cancer growh. Sci. Pharm., 79: 1-20.