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Research Article
Roles of Curcumin and Monochromatic Light in Optimizing Liver Function to Support Egg Yolk Biosynthesis in Magelang Ducks

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Abstract

Backgroud: The biosynthesis of vitellogenin as an egg-yolk precursor in oviparous animals is occurred in the liver cells. The vitellogenin synthetic capacity of hepatocyte is under the control of estrogen that its secretion increase when the oviparous animals reach sexual maturity. This experiment was designed to study the uses of curcumin and artificial monochromatic light on liver performance to improve vitellogenin synthesis to support egg production during laying period. **Materials and Methods:** One hundred and ninety two female magelang ducks were assigned into a completely randomized design with a 4×4 factorial arrangement. The first factor was dose of curcumin supplementation consisted of 4 levels i.e., 0, 9, 18 and 36 mg. The second factor was the color of monochromatic light consisted of 4 levels i.e., white, red, green and blue. **Results:** The results showed the interaction effects between curcumin and monochromatic light on the liver weight, hepatocytes diameter, DNA and RNA concentrations in the liver cells ($p<0.05$). Serum estradiol and vitellogenin concentrations in female magelang ducks during sexual maturity prior to laying period ranged from 0.06-0.19 and 0.36-3.46 mg mL^{-1}, respectively. Curcumin supplementation at doses of 18 and 36 mg $duck^{-1}$ day^{-1} increased the diameters of F1 follicles by 21.62 and 17.91%, respectively. **Conclusion:** It is concluded that curcumin supplementation at a dose of 18 mg $duck^{-1}$ day^{-1} combined with the use of red or greenlight can improve the biosynthesis capacity of egg yolk protein precursors by liver cells without increasing liver cells proliferation. Moreover, the increased estradiol during vitellogenesis is also necessary in the recruitment of ovarian follicles in establishing the F1 hierarchical follicles.

Key words: Curcumin, monochromatic light, hepatocyte, vitellogenin, egg-yolk biosynthesis, diameter follicle, estradiol, sexually mature of magelang duck

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Follicle growth and development until ovulation is determined by the deposition of yolk precursors in the developing oocytes. The main component of egg yolk is vitellogenin. In oviparous animals, including birds, liver cells play critical and significant roles in synthesizing vitellogenin. The capacity of liver cells to synthesize vitellogenin is under the control and stimulation of estrogen. The vitellogenin synthesized by the liver cells is then secreted into the circulation and transported and absorbed by the ovaries for the development and maturation of oocytes. The process of synthesis, secretion, transport and absorption of vitellogenin by the oocyte and the deposition into the growing and developing oocytes is a biomarker that is highly crucial in oogenesis[1,2]. The concentration of vitellogenin in *Sturnus vulgaris* was reported to be low before entering the breeding period and increasesto the high level when the F1-F4 hierarchical follicles have been established[3].

The livers of female birds entering laying period have 2 times higher lipid concentrations compared with those of sexually immature birds. The increased lipid concentration in the liver is marked by the increased liver weight at the time of laying period that was started by the increased plasma lipid concentration[4]. During the life cycle of birds, liver experiences various changes in color and consistency. The liver color of newly hatched chickens is pale red and contains large amounts of fat. Furthermore, the pale red color turns into brownish red at the age of 5-7 days old when the yolk sac has been fully absorbed. Once again, the fat will be accumulated in the liver when female birds enter nesting period. These physiological changes are controlled by estrogen, as fat infiltration takes place in hepatocytes of nesting female birds as a basic material for the synthesis of yolks, hence the liver color becomes yellowish brown or pale brown with a crumbly consistency compared to that of male fowls at the same age[5].

The liver of laying bird is able to synthesize 1.0-1.5 g of yolk protein per day at the peak of laying period[6]. The increased biosynthesis of vitellogenin with the increased egg production triggers morphological changes in the liver cells, especially the proliferation of the endoplasmic reticulum and the golgi apparatus[7]. Various morphological and physiological changes in the liver cells during egg production period trigger deterioration in the liver functions or liver disorders as characterized by oxidative stress, especially lipid oxidation.

The increase in lipid oxidation is caused by the high level of free radicals production. Free radicals are unstable molecules that are capable of damaging cell structures and membranes through lipid oxidation reactions occurring abnormally. In turn, lipid oxidation will increase the malondyaldehid (MDA) levels in the liver which is the end product of lipid peroxidation. The SGPT enzyme concentration (ALT: Alanine aminotransferase) and SGOT (AST: Aspartate aminotrasnferase) in the plasma will also increase with the decreased level of antioxidant. Both of these enzymes are biomarkers of oxidative damage in liver tissues[8].

Our previous studies showed that blue light could be used to increase estrogen synthesis and production that increased shell gland development, hen day production and the weights of the egg in quails[9-11]. Turmeric has a hepatoprotector activity and this material has been used to prevent and cure liver damage. Curcumin contained in turmeric has been shown to have antioxidant activities and has significant preventive and curative effects in a number of diseases, including arteriosclerosis, cancer, diabetes, respiratory, hepatic, pancreatic, intestinal and gastric diseases, neurodegenerative and eye diseases[12,13]. This experiment was designed to study the combination effects of curcumin supplementation and the use of artificial monochromatic light on liver functions and performances to support the synthesis and production of vitellogenin as yolk precursor to increase egg production in local ducks.

MATERIALS AND METHODS

Local ducks, breeding management and research design: One hundred and ninety two female magelang ducks, aged 16 weeks old with body weight of 1500±0.09 g were used in the experiment. The experimental ducks were obtained from Breeding Center for non-ruminant Livestock Farming, Banyubiru, Ambarawa, Central Java, Indonesia. The experimental ducks were randomly selected and placed into 48 cage plots and acclimatized for a week before treatment. Each cage plot contained four ducks. Each duck was marked at the ankles using colored cable ties (white, red, black and blue). The research cages were in the form of litter system with a base made of rice husk mixed with calcite. Each cage plot has a size of 100×170×75 cm^3, equipped with feeding container and two nipple drinkers. The divider between cages used wooden slats coated with calcirata (GRC board).

The source of monochromatic light was Light Emitting Diode (LED) with the color of white, red, green and blue, G45 type Koss, 220 V. The lamps were arranged in series, hung on the top center of each cage plot. The light source was 70 cm away from the cage floor in order to obtain intensity of 10 1x for each cage. The light intensity was measured using luxmeter (Lutron Taiwan, LX-100) every week in 5 points for each cage plot. The lamp circuit was equipped with an

Table 1: Feed composition and nutrient content for each phase of local duck breeding

Feed composition (%)	Grower (16-20 weeks)	Production (21-25 weeks)
Yellow corn	57.0	58.00
Rice barn	15.0	11.05
Corn gluten meal	6.0	3.5
Soybean meal	11.0	13.0
Fishmeal	5.15	3.5
Palm oil	4.0	2.7
Dicalcium phosphate	0.1	0.1
$CaCO_3$	1.25	7.5
Salt	0.2	0.1
Premix*	0.3	0.3
L-lysine	0.0	0.1
DL-methionine	0.0	0.15
Total	100.0	100.0
Nutrient content from calculation result		
ME (kcal kg^{-1})	3133.13	2898.95
Protein (%)	18.01	16.02
Fat (%)	7.22	5.08
Crude fibers (%)	3.76	3.29
Lysine (%)	0.87	0.91
Methionine (%)	0.43	0.51
Methionine+cysteine (%)	0.70	0.76
Linoleic acid (%)	2.02	1.72
Ca (%)	0.88	3.14
P (%)	0.47	0.38
Na (%)	0.16	0.11
Cl (%)	0.21	0.14

*Each kg of premix contains: Vit A, 1.200.000 IU, Vit D3: 200.000 IU, Vit E: 800 mg, Vit K: 200 mg, Vit B1: 200 mg, Vit B2: 500 mg, Vit B6: 50 mg, Vit C: 2500 mg, DL-methionine: 8000 mg, L-lysine: 3000 mg, Ca: 280.000 mg, P: 150.000 mg, Mn: 12.000 mg, Fe: 2000 mg, I: 20 mg, Co: 20 mg, Zn: 10.000 mg, dan Cu: 400 mg and ME: Metabolic energy

adapter for managing voltage, stabilizer to stabilize the input and output electrical current, as well as timer to maintain the duration of the lighting period. The addition of monochromatic light was provided for 5 h every single day, i.e., at 06:00-11:00 pm, thereby resulted in 17 h light and 7 h dark each day (17L:7D).

The ducks were raised at ambient temperature of 28-32°C. The feed and water were provided *ad libitum*. The feed used during the experiment was in the form of wet mash formulated with curcumin, adapted to the nutritional needs of laying ducks development in the period of 16-20 weeks old (grower) and a production period of 21-25 weeks old (breeder) with different protein contents and metabolizable energy. The composition of the materials and nutrient content of the feed was the result of the calculation using Excel program (Table 1). The formula for feed requirements at each stage of duck breeding was prepared in accordance with recommendations of Leeson and Summers[14]. The curcumin extract (78.94%) in the form of flour was obtained from curcuminoid 95% (Plamed Green Science Ltd., China). The curcumin doses used in this research were 0, 9, 18 and 36 mg $duck^{-1}$ day^{-1}. Curcumin was added and mixed previously with the micronutrients. After homogenously, the mixture was then added to the feed materials with larger composition. The feed supplemented with curcumin was given twice daily in the morning at 07.00 am and in the afternoon at 03.00 pm. The duck handling during the experiment used the protocols approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Bogor Agricultural University.

The experimental ducks were assigned into a Completely Randomized Design (CRD) with a 4×4 factorial arrangement with 3 replications and each replication used 4 ducks. The first factor was the dose of curcumin supplementation consisted of 4 levels i.e., 0, 9, 18 and 36 mg $duck^{-1}$ day^{-1}. The second factor was light color consisted of 4 levels i.e., white, red, green and blue. The curcumin supplementation and use of monochromatic light in the experimental ducks were conducted for 8 weeks, starting from age of 17-25 weeks.

Sample collection and parameter measurement: The blood serum and liver collections were conducted at the end of treatment, when the ducks reached 25 weeks of age or undergone sexual maturity, as characterized by the ability of nesting. Each treatment was represented by three ducks to be sacrificed by cutting the vein and jugular artery, esophagus and trachea. The blood was collected in 10 mL test tube for each individual of experimental duck. The test tubes containing blood were allowed to stand for 24 h at a temperature of 4°C and were later centrifuged at 3000 rpm for 10 min. The individual serum formed was transferred into microtube (Eppendorf tubes), stored at -20°C for further analyses of vitellogenin and estradiol concentrations. The liver was isolated and was later weighed in order to determine its weight and subsequently cut by 1×1 cm and was stored in 10% BNF solution for making histological preparation. Analysis of DNA and RNA concentrations of liver tissues was conducted by isolating 2-3 g liver dried in an oven at 60°C for 3 days and then the dried liver was crushed into a mash by using mortar and then 10 mg of the liver tissue mash was weighed each for analysis of liver DNA and RNA.

The concentration of vitellogenin in the serum was measured by using polyacrylamide gel electrophoresis method (SDS-PAGE)[15,16]. The serum estradiol concentrations were measured by using ELISA[17] (kit DGR EIA 2693 International Inc.). The liver weight was obtained by weighing the fresh liver organs by using digital scale with accuracy of 0.1 g (HWH Osaka series). The qualitative observation of liver was determined by description of liver histology using paraffin method and hematoxylin-eosin staining[18], followed by the quantitative measurement in the form of liver cells diameter

obtained by the use of Olympus CX-52 microscope with Olympus DP2-BSW software. From each field of observation was chosen 5 liver cells and observations were conducted in 5 fields of observation. The concentrations of DNA and RNA in the liver were used to estimate the population of liver cells and synthetic activities of vitellogenin in liver cells, respectively. The DNA concentration was determined by DNA extraction method by using genomic DNA mini kit for the tissues (Geneaid GT050) and RNA concentration was determined by orcinol staining based on recommendations of Manalu and Sumaryadi[19]. The F1 follicle diameter was determined from the isolated follicle hierarchy and each follicle was measured by using a digital caliper (accuracy 0.01 mm), the greatest follicle categorized as F1.

Statistical analysis: The data obtained were analyzed by using analysis of variance (ANOVA) to test the effect of curcumin doses, light colors and the interaction between curcumin doses and light colors. The whole data analysis was done by general linear model procedure on SAS version 9.0 program[20]. If there was a significant effect of main factors, thus it would be continued with Duncan's multiple range tests. And if the interaction effect was also significant, it would be continued with interaction tests using Least Significantly Different (LSD). All results of significantly different are expressed with $p<0.05$.

RESULTS

Weight of the liver, the diameter of hepatocytes, the DNA and RNA concentrations of liver: Curcumin supplementation and the use of monochromatic light significantly affected the liver weight ($p<0.05$). However, there was an interaction effect of dose of curcumin supplementation and monochromatic light ($p<0.05$) (Table 2).

Regardless of the monochromatic light used, the increased dose of curcumin supplementation decreased the liver weight of the experimental ducks ($p<0.05$). Supplementation of 9 mg curcumin day^{-1} decreased liver weight by 8.61% ($p<0.05$) as compared to control ducks. The increased doses of curcumin supplementation to 18 mg decreased liver weight by 27.30 and 20.44% as compared to control ducks and ducks supplemented with 9 mg, respectively ($p<0.05$).However, the increased dose of curcumin supplementation from 18-36 mg did not significantly decrease the liver weight ($p>0.05$). Ducks supplemented with 36 mg curcumin day^{-1} had 31.40 and 24.94% lower liver weights as compared to those supplemented with 0 and 9 mg duck^{-1} day^{-1}, respectively.

Regardless of dose of curcumin supplementation, the use of monochromatic light significantly affected liver weight ($p<0.05$). Experimental ducks given blue light had 11.38% lower liver weight as compared to ducks given red light ($p<0.05$). However, the experimental ducks given white and green lights had similar liver weight as compared to those given red and blue lights ($p>0.05$). The highest liver weights from 16 combinations of curcumin and monochromatic light was found in ducks without curcumin supplementation given red and white lights and ducks supplemented with 9 mg curcumin and given white and green lights (Table 2).

The dose of curcumin supplementation and the use of monochromatic light significantly affected the hepatocyte diameters ($p<0.05$). However, there was an interaction effect of dose of curcumin supplementation and monochromatic light on the diamater of hepatocytes ($p<0.05$) (Table 2).

Regardless of monochromatic light used, the increased dose of curcumin supplementation significantly increased hepatocyte diameters ($p<0.05$). The experimental ducks supplemented with curcumin at doses of 9, 18 and 36 mg duck^{-1} day^{-1} had higher hepatocyte diameters by 2.89, 25.29 and 49.96%, respectively, compared to controlled ducks without curcumin supplementation (Table 2). The diameters of hepatocytes in experimental ducks supplemented with curcumin at doses of 18 and 36 mg duck^{-1} day^{-1} increased by 21.76 and 45.74%, respectively, compared to those supplemented with curumin at a dose of 9 mg duck^{-1} day^{-1}. The diameter of hepatocyte in ducks supplemented with curcumin at a dose of 36 mg duck^{-1} day^{-1} increased by 19.70% as compared to those supplemented with curcumin at a dose of 18 mg duck^{-1} day^{-1}.

Regardless of dose of curcumin supplementation, the use of monochromatic light significantly affected hepatocyte diameter ($p<0.05$). The experimental ducks given red and green lights had similar hepatocyte diameters that 13.78 and 14.68% higher, respectively as compared to those given white light ($p<0.05$). However, the experimental ducks given blue light had similar hepatocyte diameter as compared to those given white, red and green lights ($p>0.05$).

The highest hepatocyte diameters from 16 combinations of curcumin and monochromatic light were found in the experimental ducks supplemented with curcumin at a dose of 36 mg duck^{-1} day^{-1} given red, blue, white and green lights and experimental ducks supplemented with curcumin at a dose of 18 mg duck^{-1}day^{-1} given red and blue lights (Table 2).

Curcumin supplementation and the use of monochromatic light had significant effects on DNA concentrations of liver tissue ($p<0.05$). However, there was an

Table 2: Effect of curcumin supplementation and the used of monochromatic light on the liver weight and the hepatocytes diameter in sexually mature of magelang ducks

	Curcumin doses (mg duck^{-1} day^{-1})				
Light colors	0	9	18	36	Average
Liver weight (g)					
White	79.25±2.33^{b}	76.48±1.58^{b}	44.28±5.69^{i}	47.80±3.25^{hi}	61.95^{ab}
Red	91.10±1.74^{a}	61.38±2.03^{def}	57.45±1.63^{ef}	53.26±2.20^{fg}	65.79^{a}
Green	67.35±1.56^{c}	75.53±2.88^{bc}	52.41±2.69^{ghi}	55.51±0.45^{efg}	62.69^{ab}
Blue	61.39±5.54^{def}	59.92±0.31^{def}	63.30±3.96^{d}	48.60±2.97^{hi}	58.30^{b}
Average	74.77^{a}	68.33^{b}	54.36^{c}	51.29^{c}	
Hepatocytes diameter (µm)					
White	11.22±0.92^{g}	12.88±1.63^{fg}	14.52±2.85^{e}	19.14±2.65^{bc}	14.44^{b}
Red	12.68±1.97^{fg}	12.98±1.43^{fg}	17.60±2.33^{bcd}	22.44±3.15^{a}	16.43^{a}
Green	16.50±3.30^{de}	15.62±0.92^{de}	16.00±1.91^{de}	17.60±0.78^{bcd}	16.56^{a}
Blue	12.10±0.78^{fg}	12.54±1.25^{fg}	17.16±1.99^{bcd}	19.58±1.43^{b}	15.35^{ab}
Average	13.13^{d}	13.51^{c}	16.45^{b}	19.69^{a}	

$^{a-d}$Different superscripts within row shows significantly different ($p<0.05$), $^{a-b}$Different superscripts within colomn shows significantly different ($p<0.05$), $^{a-g}$Different superscripts within row and column shows significantly different ($p<0.05$), the data are shown as average±SD

Table 3: Effect of curcumin supplementation and the used of monochromatic light on the liver DNA and RNA concentrations in mature sexually of magelang ducks

	Curcumin doses (mg duck^{-1} day^{-1})				
Light colors	0	9	18	36	Average
DNA of liver tissues (µg mg^{-1} sample)					
White	18.49±0.04^{b}	15.79±0.08^{d}	10.42±0.01^{h}	8.49±0.01^{l}	13.29^{a}
Red	20.61±0.04^{a}	11.40±0.02^{f}	10.18±0.01^{i}	8.50±0.01^{l}	12.67^{b}
Green	16.43±0.01^{c}	10.69±0.01^{g}	9.88±0.03^{j}	8.67±0.02^{k}	11.41^{c}
Blue	14.15±0.01^{e}	10.44±0.02^{h}	8.72±0.01^{k}	8.41±0.03^{m}	10.43^{d}
Average	17.42^{a}	12.08^{b}	9.80^{c}	8.52^{d}	
RNA of liver tissues (µg mg^{-1} sample)					
White	78.82±1.41^{d}	71.20±0.71^{gh}	71.50±0.06^{g}	71.05±0.71^{ghi}	73.14^{b}
Red	67.06±0.01^{j}	70.05±0.01^{hi}	59.45±0.71^{k}	81.27±0.64^{c}	69.46^{c}
Green	74.36±0.69^{f}	83.96±0.70^{b}	78.48±0.72^{d}	70.37±0.12^{ghi}	76.79^{a}
Blue	69.07±0.72^{i}	76.01±0.69^{e}	77.01±0.01^{e}	85.67±0.74^{a}	76.94^{a}
Average	72.33^{c}	75.31^{b}	71.61^{d}	77.08^{a}	

$^{a-d}$Different superscripts within row shows significantly different ($p<0.05$), $^{a-d}$Different superscripts within colomn shows significantly different ($p<0.05$), $^{a-m}$Different superscripts within row and column shows significantly different ($p<0.05$), the data are shown as average±SD

interaction effect of dose of curcumin and monochromatic light on DNA concentrations of liver tissue ($p<0.05$) (Table 3). Regardless of monochromatic light used, the increased dose of curcumin supplementation decreased DNA concentrations of liver tissue ($p<0.05$). Supplementation of curcumin at doses of 9, 16 and 36 mg duck^{-1} day^{-1} decreased liver DNA concentrations by 30.65, 43.74 and 51.09%, respectively, as compared to control ducks. The increased dose of curcumin supplementation to 18 and 36 mg duck^{-1} day^{-1} decreased liver DNA concentrations by 18.87 and 29.47%, respectively as compared to ducks supplemented with curcumin at a dose of 9 mg duck^{-1} day^{-1}. The increased dose of curcumin supplementation to 36 mg duck^{-1} day^{-1} decreased liver DNA concentration by 13.06% compared to those supplemented with curcumin at a dose of 18 mg duck^{-1}day^{-1}.

Regardless of dose of curcumin supplementation, the use of monochromatic light significantly affected liver DNA concentrations ($p<0.05$). The experimental ducks given red, green and blue lights had lower liver DNA concentrations by 4.02, 14.15 and 21.52%, respectively, as compared to the experimental ducks given white light ($p<0.05$). Further, the experimental ducks given green and blue lights had lower liver DNA concentrations by 9.94 and 17.68%, as compared to those given red light and the experimental ducks given blue light had 8.59% lower liver DNA concentrations as compared to those given green light.

The highest liver DNA concentrations from 16 combinations of curcumin and monochromatic light were found in the experimental ducks without curcumin supplementation given red, white, green and blue lights and those supplemented with 9 mg duck^{-1} day^{-1} curcumin given white light (Table 3).

Dose of curcumin supplementation and the use of monochromatic light significantly affected the concentration of RNA of liver tissue (Table 3). However, there was an interaction effect of dose of curcumin and monochromatic light on RNA concentrations of liver tissue ($p<0.05$) (Table 3). Regardless of monochromatic light use, the increased

dose of curcumin supplementation significantly increased RNA concentration in the liver tissue ($p<0.05$), except dose of 18 mg duck^{-1} day^{-1}. Supplementation of curcumin at a dose of 9 mg duck^{-1} day^{-1} increased RNA concentration of liver tissue by 4.12% as compared to control ducks. However, the experimental ducks supplemented with curcumin at a dose of 18.0 mg duck^{-1} day^{-1} had lower RNA concentrations of liver tissue by 1.0% as compared to control ducks without curcumin supplementation ($p<0.05$). Experimental ducks supplemented with curcumin at a dose of 36.0 mg duck^{-1} day^{-1} had 6.57% higher RNA concentration of liver tissue as compared to control ducks ($p<0.05$). The increased dose of curcumin supplementation from 9.0-18.0 mg duck^{-1} day^{-1} decreased RNA concentrations of liver tissue by 4.91% ($p<0.05$). However, the increased dose of curcumin supplementation from 18-36.0 mg duck^{-1} day^{-1} increased RNA concentration of liver tissue by 7.64% ($p<0.05$). The increased dose of curcumin supplementation from 9.0-36.0 mg duck^{-1} day^{-1} only increased RNA concentration of liver tissue by 2.35% ($p<0.05$).

Regardless of curcumin supplementation, monochromatic light had significant effect on RNA concentrations of liver tissue ($p<0.05$). The experimental ducks given green and blue lights had 4.99 and 5.20% higher RNA concentrations of liver tissue, respectively as compared to those given white light ($p<0.05$). Ducks given green and blue lights had 10.55 and 10.77% higher RNA concentrations of liver tissue, respectively, as compared to ducks given red light ($p<0.05$). However, the experimental ducks given red light had 5.03% lower RNA concentration of liver tissue as compared to those given white light ($p<0.05$). The RNA concentrations of liver tissue in ducks given green and blue lights were similar ($p>0.05$).

The highest liver tissue RNA concentrations from 16 combinations of curcumin and monochromatic light were found in the experimental ducks supplemented with curcumin at a dose of 36 mg duck^{-1} day^{-1} given blue light, ducks supplemented with curcumin at a dose of 9.0 mg duck^{-1} day^{-1} given green light and ducks supplemented with curcumin at a dose of 36 mg duck^{-1} day^{-1} given red light (Table 3).

17-β-Estradiol (E2), serum vitelogenin (VTG) and first follicle diameter (F1): The doses of curcumin supplementation and the colors of monochromatic light had significant effects on serum estradiol concentrations of the experimental ducks ($p<0.05$). However, there was an interaction effect of dose of curcumin supplementation and monochromatic light on serum estradiol concentration of the experimental ducks ($p<0.05$).

Regardless of the monochromatic light use, the increased dose of curcumin supplementation decreased serum estradiol concentrations of the experimental ducks ($p<0.05$). The experimental ducks supplemented with curcumin at doses of 9 and 18 mg duck^{-1} day^{-1} had 7.69% lower serum estradiol concentrations as compared to control ducks ($p<0.05$). However, the increased dose of curcumin supplementation from 9.0-18.0 mg duck^{-1} day^{-1} did not affect serum estradiol concentrations ($p>0.05$). The experimental ducks supplemented with curcumin at a dose of 38.0 mg duck^{-1} day^{-1} had 15.38% lower serum estradiol concentrations as compared to control ($p<0.05$) (Fig. 1a). Serum estradiol concentrations decreased upto 8.33% in the experimental ducks supplemented with curcumin at a dose of 36 mg duck^{-1} day^{-1} compared to those supplemented with curcumin at doses of 9 and 18 mg duck^{-1} day^{-1}.

Furthermore, the colors of monochromatic light have the potentials to improve serum estradiol concentration. The experimental ducks given red and green lights had higher serum estradiol concentrations by 70.0 and 50.0%, respectively, compared to those given white light ($p<0.05$). However, the experimental ducks given blue light had lower serum estradiol concentrations by 10% as compared to those given white light ($p<0.05$). Ducks given green and blue lights had 11.76 and 47.6% lower serum estradiol concentrations, respectively, as compared to those given red light ($p<0.05$). The experimental ducks given blue light had 40.0% lower serum estradiol concentrations as compared to those given green light ($p<0.05$) (Fig. 1b).

The highest serum estradiol concentrations from 16 combinations of curcumin and monochromatic light were found in the control ducks without curcumin supplementation given green light, the experimental ducks supplemented with curcumin at a dose of 9.0 and given green light and the experimental ducks supplemented with curcumin at doses of 18.0, 36.0 and 9.0 mg duck^{-1} day^{-1} given red light (Fig. 1c).

Doses of curcumin supplementation and the colors of monochromatic light had significant effects on serum vitellogenin concentrations ($p<0.05$). However, there was an interaction effect of dose of curcumin supplementation and monochromatic light use on serum vitellogenin concentrations ($p<0.05$). Regardless of monochromatic light used, the increased dose of curcumin supplementation did not linearly increase serum vitellogenin concentrations. Supplementation of experimental ducks with curcumin at a dose of 9 mg duck^{-1} day^{-1} decreased serum vitellogenin concentrations by 33.73% ($p<0.05$). However, the increased dose of curcumin supplementation to 18 mg duck^{-1} day^{-1} increased serum vitellogenin concentrations by 69.88 and

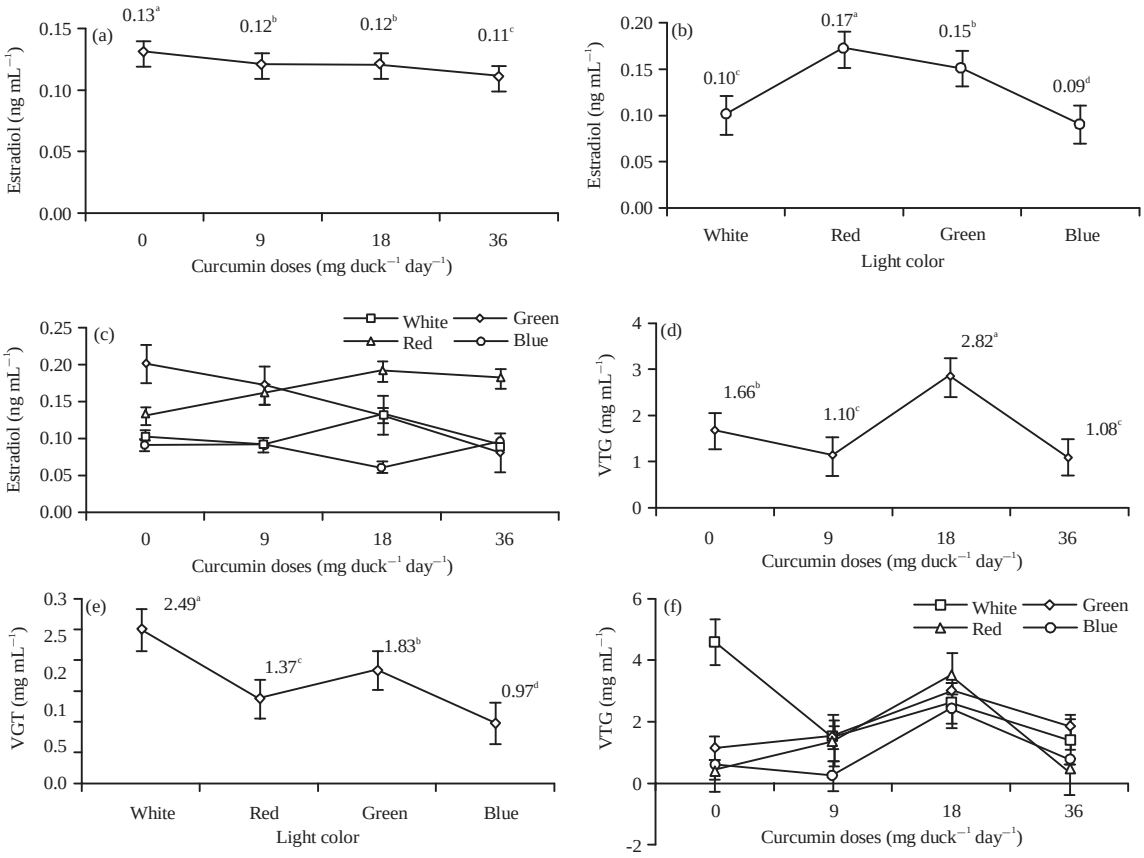

Fig. 1(a-f): Effects of curcumin supplementation and the used of monochromatic light on sexually mature magelang ducks, (a) Effect of curcuminsupplementation on estradiol, (b) Effect of monochromatic light on estradiol, (c) Interaction effect between the curcumin doses and light color on estradiol concentration, (d) Effect of supplemented with curcumin on serum VTG ($p<0.05$), (e) Effect of monochromatic light on serum VTG ($p<0.05$) and (f) Interaction effect between the curcumin doses and light colors ($p<0.05$) on serum VTG concentrations. The results are shown as an average±SE, VTG: Vitellogenin

156.36% as compared to control experimental ducks and the experimental ducks supplemented with curcumin at doses of 9 mg duck^{-1} day^{-1} ($p<0.05$). Further increase in dose of curcumin supplementation to 36.0 mg duck^{-1} day^{-1} decreased serum vitellogenin concentrations by 34.94 and 61.70% as compared to those supplemented with 0 and 18 mg duck^{-1} day^{-1} ($p<0.05$) (Fig. 1d). There was no significant difference in serum vitellogenin concentrations in the experimental ducks supplemented with curcumin at doses of 9.0 and 36.0 mg duck^{-1} day^{-1} ($p>0.05$).

Regardless of dose of curcumin supplementation, the use of white monochromatic light gave the highest serum vitellogenin concentrations and the experimental ducks given the blue light had the lowest serum vitellogenin concentrations. The experimental ducks given red, green and blue lights had 44.98, 26.51 and 61.04% lower serum vitellogenin concentrations, respectively, as compared to those given white light. The experimental ducks given green light had 33.58% increased serum vitellogenin concentrations as compared to those given red light ($p<0.05$). However, the experimental ducks given blue light had 29.20 and 46.99% lower serum vitellogenin concentrations as compared to those given red and green lights ($p<0.05$) (Fig. 1e).

The highest serum VTG concentrations from 16 combinations of curcumin and monochromatic light were found in the control ducks without curcumin supplementation given white light, the experimental ducks supplemented with curcumin at a dose of 18 mg duck^{-1} day^{-1} given red, green, white and blue lights (Fig. 1f).

Dose of curcumin supplementation significantly affected the diameter of follicle (F1) ($p<0.05$). Monochromatic light use did not significantly affect the diameter of follicle (F1) ($p>0.05$). However, there was an interaction effect of doses of curcumin supplementations and the color of monochromatic light on the size of F1 diameter ($p<0.05$) (Table 4).

Table 4: Effect of curcumin supplementation and the used of monochromatic light on the F1 follicle diameter (mm) in sexually mature of magelang ducks

	Curcumin doses (mg duck^{-1} day^{-1})				
Light colors	0	9	18	36	Average
White	28.64±1.82^{bc}	24.03±1.20^{d}	31.21±2.66^{bc}	32.68±0.76^{abc}	29.14
Red	27.62±5.39^{cd}	23.29±0.87^{d}	37.18±3.48^{a}	33.12±0.93^{abc}	30.30
Green	28.63±1.23^{bc}	30.71±4.61^{bc}	37.11±1.89^{a}	30.35±0.62^{bc}	31.02
Blue	30.07±1.38^{bc}	32.05±3.59^{abc}	28.37±1.01^{bcd}	33.61±4.47^{ab}	31.70
Average	28.74^{b}	27.52^{b}	33.47^{a}	32.45^{a}	

$^{a-d}$Different superscripts within row shows significantly different ($p<0.05$), $^{a-b}$Different superscripts within row and column shows significantly different ($p<0.05$), the data are shown as an Average±SD, F1: First follicle on hierarchical follicle

Regardless of the color of monochromatic light used, the increased dose of curcumin supplementation increased the diameter of F1 follicle ($p<0.05$). Supplementation of curcumin at a dose of 9.0 mg duck^{-1}day^{-1} did not significantly affect the diameter of F1 follicle ($p>0.05$). However, the increased dose of curcumin supplementation to 18.0 and 36.0 mg duck^{-1} day^{-1} increased diameters of F1 follicles by 16.46 and 12.91%, respectively, as compared to control ducks without curcumin supplementation ($p<0.05$). The increased doses of curcumin supplementations from 9.0-18 and 36 mg duck^{-1} day^{-1} increased the diameters of F1 follicles by 21.62 and 17.91%, respectively ($p<0.05$). However, the increased dose of curcumin supplementation from 18.0-36.0 mg duck^{-1} day^{-1} did not significantly increase the diameter of F1 follicle, even decreased by 3.05% ($p>0.05$).

Regardless of dose of curcumin supplementation, the color of monochromatic light used did not significantly affect the diameters of F1 follicles. However, there was a tendency of increased diameters of F1 follicles in the experimental ducks given red, green and blue lights as compared to those given white light ($p>0.05$). The experimental ducks given red, green and blue lights had 3.98, 6.45 and 8.79% higher diameters of F1 follicles, respectively as compared to those given white light ($p>0.05$). The experimental ducks given green light had 2.38% higher diameters of F1 follicles as compared to those given red light ($p>0.05$). Furthermore, the experimental ducks given blue light had 4.62 and 2.19% higher diameters of F1 follicle as compared to those given red and green lights, respectively ($p>0.05$).

The highest diameters of F1 follicle from 16 combinations of dose of curcumin supplementation and the color of monochromatic light were found in the experimental ducks supplemented with curcumin at a dose of 18.0 mg duck^{-1} day^{-1} given red and green lights (Table 4).

DISCUSSION

The results of the present experiment clearly showed that curcumin supplementation and the used of monochromatic light increased vitellogenin synthesis and its deposition in the developing oocytes as was indicated by the increased diameter of F1 follicle without increasing liver weight, the number of hepatocytes population and serum estradiol concentrations. It was found that curcumin supplementation did not stimulate the proliferation of liver cells of magelang ducks. Therefore, the increased biosynthesis of egg-yolk precursor in the experimental ducks is associated with the increased functionality of the liver cells as indicated by the increased RNA concentrations of the liver tissue. In the curcumin-supplemented ducks, the liver cells work optimally to synthesize egg yolk precursors under the stimulation of estradiol. In addition, the bioactive components of curcumin are very important as the hepatoprotector in protecting the hepatocytes from cell destruction by free radicals as was indicated by the decreased SGPT and SGOT concentrations in the experimental ducks supplemented with curcumin[21].

Previous observation showed that quails supplemented with turmeric powder had higher hepatocytes diameters[22]. The capacity of the hepatocyte cells to synthesize VTG in vertebrata is under the control estradiol[23]. In this present study, the observation in liver histomorphology showed that curcumin-supplemented ducks had large hepatocytes vacuoles containing fat droplets. Vacuole is a place for metabolic deposition of the components of egg-yolk precursors before being transported and distributed to the ovarian follicles for oocytes development at the time of sexual maturity. The increased size of the vacuoles reflects the improved storage capacity of the liver cells to store organic materials, such as vitellogenin as egg-yolk precursors before being secreted into the bloodstream for further deposition in the developing oocytes in the ovary.

The increased RNA concentration of liver tissue found in the present experiment is an indication of the increased synthetic capacity of the liver cells to produce VTG. It was reported that estradiol increased vitellogenin mRNA concentration and total RNA content of the liver tissue[24,25] without increasing the number of hepatocytes. However, the increased RNA concentrations of the experimental ducks

supplemented with curcumin was not associated with the increased serum estradiol concentrations. The lowered serum estradiol concentrations in the experimental ducks supplemented with curcumin could probably related to the increased use of circulating estradiol to stimulate RNA required for synthesizing of vitellogenin.

Estradiol plays an important role in the synthesis of vitellogenin by the hepatocytes. In general, the experimental ducks supplemented with curcumin had lower serum estradiol concentrations. This fact indicates that serum estradiol concentration had low correlation with the serum vitellogenin concentration ($r = 0.31$). In catfish it was found the same phenomenon that the increased vitellogenin synthesis is not related to the high serum estradiol concentration but related to the capacity and the functionality of the hepatocytes to synthesize vitellogenin[26].

In regulating vitellogenin synthesis, estradiol diffuses into the cell and nuclear membrane to binds to its specific receptors in the nucleus. Ovipar vertebrate liver has a high-level of estradiol's receptor concentration and its presence increases during the vitellogenin synthesis[27]. The synthesis of vitellogenin in the liver of bird is a long-term effect of estradiol on the expression of VTG genes in the liver. The genes that encode these egg-yolk protein are expressed at various levels and show the different levels of dependence on estradiol[28]. The vitellogenin gene only appears in the liver cells and shows exponential response to estradiol[29].

Estradiol is synthesized and secreted by the growing theca cells of ovarian follicles. Estradiol is released into the bloodstream bind to its receptor in the liver cells to initiate the biosynthesis of vitellogenin during the sexual maturity. Therefore, the concentration of estradiol in the serum is the result of the rate of estradiol synthesis and secretion into the circulation and the rate of estradiol binding to its receptor in the hepatocytes to synthesize vitellogenin. The lowered serum estradiol concentrations in the experimental ducks supplemented with curcumin could probably related to the increased use of circulating estradiol to stimulate RNA required for synthesizing of vitellogenin. The decreased serum estradiol concentration could be probably caused by the decreased endogenous estradiol synthesis and secretion since curcumin used in this study contain phytoestrogens at 0.14-0.21 ng mL^{-1}. However, vitellogenin synthesis and deposition in the oocytes increased in the experimental ducks supplemented with curcumin. The increased vitellogenin synthesis in the experimental ducks supplemented with curcumin could be related to the phytoestrogen content of curcumin even though it was reported that phytoestrogen in curcumin has a very little effect on gene expression that is normally regulated by endogenous estradiol[30].

In the present study, serum vitellogenin concentrations increased in the experimental ducks supplemented with curcumin at a dose of 18 mg $duck^{-1}$ day^{-1}. Vitellogenin is not permanently stored in the liver cells, but are secreted into the bloodstream immediately after the synthesis[31] and are further deposited into the growing oocytes in the ovary. Therefore, the concentration of vitellogenin in the circulation is the result of the rate of vitellogenin synthesis and secretion into the circulation and the rate of vitellogenin transport and deposition into the growing oocytes in the ovary. In the curcumin-supplemented ducks, the transport and deposition of vitellogenin into the growing ovarian follicles was increasethat eventually improved the recruitment of ovarian follicles with better hierarchical follicles. The increased diameter of F1 hierarchical follicle in the experimental ducks supplemented with curcumin at doses of 18 and 36 mg $duck^{-1}$ day^{-1} supported this hypothesis.

The results found in this study clearly showed that the use of monochromatic light could stimulate the synthesis of estradiol. The experimental ducks reared with red and green lights significantly showed the increase in serum estradiol concentrations. It is assumed that the monochromatic light used in the evening could stimulate the experimental ducks that will affect neuroendocrine synthesis and secretion that eventually affect gonadal development and estradiol synthesis. In addition, the results of daily observation showed that experimental ducks reared with the green light in the evening had less social activities and motions. These conditions allow the use of energy for sexual activity such as follicle recruitment and maturation of gonads that eventually increased follicles recruitment and development. In contrast, ducks reared with the red light showed more social activities are more aggressive, often spread their wings have increased locomotion and photo-sexual responses. The increased photo-sexual responses is assumed to be associated with the path of light penetration. The red light belongs to the longer wave lengths, more efficient penetration through the cranial and brain tissue so that the light signal can be received directly by the photoreceptors in the hypothalamus. The signal received by the hypothalamus stimulates the secretion of gonadotropins. Gonadotropin play a role in regulating normal reproductive function of ovaries mainly through the regulation of the hypothalamus-pituitary-gonad which in turn triggers the growth, development and maturation of ovarian follicles. The growing theca cells of ovarian follicles actively synthesize and secrete estradiol. The presence of estradiol is necessary to maintain the sustainability of VTG synthesis in liver cells.

Baxter *et al.*[32] suggests that the light exposure for the reproductive process that does not rely on eye photoreceptors but hypothalamus photoreceptors is a biological transformer that can convert photons (light energy) into a neural signal. The neural signal affects the endocrine system so that it can control the activity of the ovary, reproductive function, behavior and secondary avian sex characteristics. It was reported that light with a greater wavelength of 600 nm can penetrate directly into the enchepalon that activate the photo-sexual responses[33].

The effects of interaction between the curcumin doses and light colors in increasing estradiol secretion and VTG synthesis was found in the experimental ducks supplemented with curcumin at a dose of 18 mg duck^{-1} day^{-1} and given the red and green lights. Curcumin supplementation on this group of ducks is very effective in playing the hepatoprotective role in protecting hepatocytes from oxidative damage due to the increased VTG synthesis. The increased VTG synthesis is induced by the increased estradiol triggered by the light signals. The deposition of vitellogenin in the developing oocytes in the ovary of experimental ducks supplemented with curcumin at a dose of 18 mg duck^{-1} day^{-1} and given red and green lights were better than the other groups as were indicated by the highest diameters of F1 hierarchical follicles in both groups. The increased estradiol in both groups significantly increase vitellogenin synthesis by the increased capacity of hepatocytes and further deposition into the growing oocytes in the ovary.

CONCLUSION

It could be concluded that the curcumin supplementation of 18 mg duck^{-1} day^{-1} combined with the use of monochromatic light of red or green can improve the biosynthesis of egg yolk protein precursors in Magelang ducks indicated by the optimal work of the existing liver cells without being followed by the liver cells proliferation. Moreover, the increased estradiol during vitellogenesis is also necessary in the recruitment of ovarian follicles in establishing the F1 hierarchical follicles.

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REFERENCES

1. Arukwe, A. and A. Goksoyr, 2003. Eggshell and egg yolk proteins in fish: Hepatic proteins for the next generation: Oogenetic, population and evolutionary implications of endocrine disruption. Comp. Hepatol., Vol. 2. 10.1186/1476-5926-2-4
2. Turker, H. and A. Bozcaarmutlu, 2009. Effect of total isoflavones found in soybean on vitellogenin production in common carp. Kafkas Universitesi Veteriner Fakultesi Dergisi, 15: 561-568.
3. Challenger, W.O., T.D. Williams, J.K. Christians and F. Vezina, 2001. Follicular development and plasma yolk precursor dynamics through the laying cycle in the european starling (*Sturnus vulgaris*). Physiol. Biochem. Zool., 74: 356-365.
4. Hermier, D., G. Guy, S. Guillaumin, S. Davail, J.M. Andre and R. Hoo-Paris, 2003. Differential channelling of liver lipids in relation to susceptibility to hepatic steatosis in two species of ducks. Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol., 135: 663-675.
5. Tarek, K., M. Mohamed, B. Hassina, B. Omar and I. Messaouda, 2013. Histological changes in liver and pectoral muscles of broiler chickens slaughtered with and without naming of Allah. Int. J. Poult. Sci., 12: 550-552.
6. Stevens, L., 1991. Egg white proteins. Comp. Biochem. Physiol. Part B: Comp. Biochem., 100: 1-9.
7. Levi, L., I. Pekarski, E. Gutman, P. Fortina and T. Hyslop *et al.*, 2009. Revealing genes associated with vitellogenesis in the liver of the zebrafish (*Danio rerio*) by transcriptome profiling. BMC Genomics, Vol. 10. 10.1186/1471-2164-10-141
8. Rajput, N., M. Naeem, S. Ali, J.F. Zhang, L. Zhang and T. Wang, 2013. The effect of dietary supplementation with the natural carotenoids curcumin and lutein on broiler pigmentation and immunity. Poult. Sci., 92: 1177-1185.
9. Kasiyati, N. Kusumorini, H. Maheshwari and W. Manalu, 2009. Kadar estrogen dan profil oviduk puyuh (*Coturnix coturnix* Japonica) setelah pemberian cahaya monokromatik. Anatomi Fisiologi, 17: 1-11.
10. Kasiyati, N. Kusumorini, H. Maheshwari and W. Manalu, 2010. Fotostimulasi cahaya monokromatik untuk optimasi reproduksi dan karakteristik karkas puyuh (*Coturnix coturnix* Japonica) masak kelamin. Proceedings of the Prosiding Seminar Nasional: Peranan Teknologi Reproduksi Hewan Dalam Rangka Swasembada Pangan Nasional, October 6-7, 2010, Bogor, pp: 118-122.
11. Kasiyati, N. Kusumorini, H. Maheshwari and W. Manalu, 2011. Penerapan cahaya monokromatik untuk perbaikan kuantitas telur puyuh. Buletin Anatomi Fisiologi, 19: 1-7.

12. Bengmark, S., 2006. Curcumin, an atoxic antioxidant and natural NFκB, cyclooxygenase-2, lipooxygenase and inducible nitric oxide synthase inhibitor: A shield against acute and chronic diseases. J. Parenter. Entral Nutr., 30: 45-51.
13. Prasad, S., A.K. Tyagi and B.B. Aggarwal, 2014. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. Cancer Res. Treatment, 46: 2-18.
14. Leeson, S. and J.D. Summers, 2005. Commercial Poultry Nutrition. 3rd Edn., Nottingham University Press, England.
15. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
16. Gibbins, A.M.V., F.R. van de Voort and R. Braham, 1981. Comparative study of three different forms of both native and subunit vitellogenin identified in quail plasma. Compar. Biochem. Physiol. Part B: Compar. Biochem., 70: 731-738.
17. Nash, J.P., B. Davail-Cuisset, S. Bhattacharyya, H.C. Suter, F. Le Menn and D.E. Kirne, 2000. An Enzyme Linked Immunosorbant Assay (ELISA) for testosterone, estradiol and 17,20β-dihydroxy-4-pregenen-3-one using acetylcholinesterase as tracer: Application to measurement of diel patterns in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem., 22: 355-363.
18. Bancroft, J.D. and M. Gamble, 2008. Theory and Practice of Histological Techniques. 6th Edn., Churchill Livingstone Elsevier, China.
19. Manalu, W. and M.Y. Sumaryadi, 1998. Maternal serum progesterone concentration during gestation and mammary gland growth and development at parturition in Javanese thin-tail ewes carrying a single or multiple fetuses. Small Rumin. Res., 27: 131-136.
20. SAS., 2002. The SAS System for Windows. Release 9.0, SAS Institute Inc., Cary, NC., USA.
21. Kasiyati, W. Manalu, Sumiati and D.R. Ekastuti, 2016. Efficacy of curcumin and monochromatic light in improving liver functions of sexually mature magelang ducks. J. Indonesian Trop. Anim. Agric., 41: 153-160.
22. Saraswati, T.R., W. Manalu, D.R. Ekastuti and N. Kusumorini, 2013. Increased egg production of Japanese quail (*Cortunix japonica*) by improving liver function through turmeric powder supplementation. Int. J. Poult. Sci., 12: 601-614.
23. Tata, J.R., 1979. Control by oestrogen of reversible gene expression: The vitellogenin model. J. Steroid Biochem., 11: 361-371.
24. Skipper, J.K. and T.H. Hamilton, 1977. Regulation by estrogen of the vitellogenin gene. Proc. Nat. Acad. Sci., 74: 2384-2388.
25. Shapiro, D. and J.M. Taylor, 1982. Steroid hormone regulation of vitellogenin gene expressio. CRC Critical Rev. Biochem., 12: 187-203.
26. Dewi, C.D., D.R. Ekastuti, A.O. Sudrajat and W. Manalu, 2016. Improved vitellogenesis, gonad development and egg diameter in catfish (*Pangasianodon hypophthalmus*) supplemented with turmeric (*Curcuma longa*) powder. Aquaculture Research, (In Press).
27. Sumpter, J.P. and S. Jobling, 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ. Health Perspect., 103: 173-178.
28. Hache, R.J., S.P. Tam, A. Cochrane, M. Nesheim and R.G. Deeley, 1987. Long-term effects of estrogen on avian liver: Estrogen-inducible switch in expression of nuclear, hormone-binding proteins. Mol. Cell. Biol., 7: 3538-3547.
29. Wiskocil, R., P. Bensky, W. Dower, R.F. Goldberger, J.I. Gordon and R.G. Deeley, 1980. Coordinate regulation of two estrogen-dependent genes in avian liver. Proc. Nat. Acad. Sci., 77: 4474-4478.
30. Bachmeier, B.E., V. Mirisola, F. Romeo, L. Generoso and A. Esposito *et al*., 2010. Reference profile correlation reveals estrogen-like trancriptional activity of curcumin. Cell Physiol. Biochem., 26: 471-482.
31. Nath, P. and B.I. Sundararaj, 1979. Role of protein and steroid hormones in vitellogenesis in the catfish, *Heteropneustes fossilis*. Proc. Indian. Natl. Sci. Acad., 45: 491-496.
32. Baxter, M., N. Joseph, V.R. Osborne and G.Y. Bedecarrats, 2014. Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. Poult. Sci., 93: 1289-1297.
33. Hartwig, H.G. and T. van Veen, 1979. Spectral characteristics of visible radiation penetrating into the brain and stimulating extraretinal photoreceptors. J. Compa. Physiol., 130: 277-282.