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THE EGG QUALITY OF NATIVE CHICKEN'S WITH VITAMIN E SUPPLEMENTATION IN RATION CONTAINING MANHADDEN FISH OIL AND KERNEL PALM OIL

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ABSTRACT

The experiment was conducted to determine the effect of manhadden fish and kernel palm oils and their combination with different levels of vitamin E on the egg quality (egg weights, egg production/Hen Day Production, Haught Unit/HU, egg index, yolk colour, egg strength, cholesterol, High Density Lipoprotein, Low Density Lipoprotein, and egg yolk triglyceride). This research was done in Laboratory of Animal Feed Department of Animal Feed and Nutrition GMU, Yogyakarta. Laboratory of Animal Feed and Nutrition and Experimental Sub-station Faculty of Animal Sciences UNSOED, Purwokerto. The Research consisted of two steps of experiment which carried out from April 2004 to November 2005. fourty five hens and 20 cockerels of 22 weeks old native chicken were used in the experiment and they were kept for five months. Experiment design used was Completely Randomized Design (CRD), each treatment was repeated 6 times. Data then were analyzed using analysis of variance continued with Duncan Test. The rations of the study at first step were: R_0 = 0% manhadden oil and 0% kernel palm oil; R_1 = 10% manhaden oil and 0% kernel palm oil; R_2 = 5% manhadden oil and 5% kernel palm oil; R_3 = 0% manhadden oil and 10% kernel palm oil, where the second experiment used : R_{31} = basal feed + 0 mg vitamin E /kg of feed ; R_{32} = basal feed + 10 mg vitamin E /kg of feed; R_{33} = basal feed + 20 mg vitamin E /kg of feed; R_{34} = basal feed + 30 mg vitamin E /kg of feed. The result showed that the first step of treatment did was not significantly ($P>0.05$) affect egg index, yolk colour, cholesterol, HDL, and egg yolk triglyceride), but was very significantly ($P<0.01$) affected egg weights, HU, egg strength, and egg yolk LDL. The second step showed that the treatment was not significantly ($P>0.05$) affect HU, yolk colour, egg strength, cholesterol, HDL, LDL, and egg yolk triglyceride, but was very significantly ($P<0.01$) affect egg weights, HDA, and egg index. It can be concluded that vitamin E addition in ration containing manhadden fish and kernel palm oils improve the egg weights, egg production/HDA, and Haught Unit. In the other hand vitamin E addition reduced cholesterol, triglyceride and LDL of yolk.

Keywords : Native Chickens, Vitamin E, Egg Quality

INTRODUCTION

The success of chicken breeding can be measured by the high rate of fertility, hatchability and quality of chicks. Good quality egg produce better hatchability and hatching quality. There is co-relation between egg quality and chick quality. Furthermore, hatchability depends on the nutrient value of food for the broodstocks (Yuwanta, 1997) which is based on the amount and variety of food sources. Such kind

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of food is from material containing essential fatty acids, especially long chain fatty acids i.e omega 3 (linolenic acid), omega 6 (linoleic acid) and omega 9 (oleic acid). Linoleic acid (18:3ω3) and oleic acid (18:1ω3) can be found in plant such as soybean, rapeseed, and peanut. However, linolenic acids such as eicosa penthanoic acid, *EPA* (20:5ω3) and docosa hexanoic acid, DHA (22:6ω3) can be found in fish oil.

The need of fatty acid increases demand on vitamin E needed as antioxidant and fertilities factor. Unsaturated fatty acids are easily oxidized to peroxides. By the availability of vitamin E, the fatty acids are protected from oxidation. Broodstock food composition is a main factor in developing antioxidant system in embryo during embryogenesis and initial days of newly hatched chicks (DOC) (Surai *et al.*, 1999).

MATERIALS AND METHODS

Cages and Utensils

Fourty five native hens (pullets) and 20 native cockerel of 22 weeks old respectively, which were reared and kept up to 50 weeks old. Pullet cages are arranged individually (pen cages) with 50 cm length, 33 cm width and 25 cm height, which were completed with drinking water cups made from plastic and food container made from bamboo which placed in barn. The barn were accompaned with utensils such as wall thermometer, wall hygrometer, balance, disinfectant sprayer, syringe, plastic boxes, cleaning utensil, and hatching machine "Cemani"(trade mark).

Data collection procedures

Blood serum was taken from left wing blood vesel (vena), for the determination of hormon concentration, cholesterol, HDL, LDL and triglyceride analysis. Blood was taken three times at second, third and fourth months. Blood was taken using 3 ml syringe and placed in eppendorf tube, then kept over night in refrigerator. The tubes then centrifuged at 5000 rpm for 10 minutes, the clear aliquote is blood serum. Blood plasm was collected using similar method but EDTA was pre-added into the tubes. Hematological profile were analysed using smeared of fresh blood on glass slides.
Egg weight data were collected by weighing fresh eggs everyday
Number of egg (% HDA) was found by devided the number of eggs with the number of days of reared and number of chikens.
Egg yolk weight (g) was measured by weighing the egg yolk. The result was from average weight from a set number of eggs.

Haugh Unit (HU). HU was based on calculation of the height of gelatinous white egg using depth micrometer and weighing whole egg.

HU value = 100 log (H + 7.57 x W 0.37)
which : H = height of gelatinous white egg (mm)
W = weight of whole egg (g)

Shell strength (mm/pascal) was found by measured the egg using instrument "Egg Strength"

HDL and LDL concentrations of blood serum are measured using KIT from Diasys (*Diagnostic System)* using CHOD-PAP method, that was by filling three cuvets with 10 μl serum and 1 ml reagent, 10 μl cholesterol standard and *Blanc Reagent* (contains 1 ml colour reagent, 1 ml serum) respectively. The tubes then were incubated for 20 minutes

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at 20-25 °C, then the first and second tubes were measured their absorbance against blanc reagent (third tube) whitin 60 minute respectively. The measurement used spectrophotometer at 500 nm wave length, the calculation was:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Sample absorbance value}}{\text{Standard absorbance value}} \times 200$$

$$\text{HDL (mg/dl)} = \frac{\text{Sample absorbance value}}{\text{Standard absorbance value}} \times 150$$

$$\text{LDL (mg/dl)} = \text{Total cholesterol - Triglyceride/5}$$

Data Analysis

The research was *in vivo* experiment using Completely Randomized Design (Steel and Torrie, 1981). The treatment consisted of 4 kind of ration, each treatment was repeated 6 times. Data were analysed using analysis of varian and Duncan test (Gill, 1978). Complete ration composition is shown in Table 1.

Experimant I :

R_0 = 0% manhadden fish oil and 0% kernel palm oil

R_1 = 10% manhadden fish oil and 0% kernel palm oil

R_2 = 0% manhadden fish oil and 10% kernel palm oil

R_3 = 5% manhadden fish oil and 5% kernel palm oil

Experimant II

R_{31} = basal ration (5% manhadden fish oil and 5% kernel palm oil)

R_{32} = basal ration + 10 mg vitamin E/kg feed

R_{33} = basal ration + 20 mg vitamin E/kg feed

R_{34} = basal ration + 30 mg vitamin E/kg feed

RESULT AND DISCUSSION

The egg weight, egg index, HU, yolk colour, egg strength, cholesterol, HDL, LDL and triglycerides of egg yolk were presented in Table 2.

The result of variance analysis of experiment I indicated that the treatment was not significant to egg index, yolk colour and cholesterol; HDL and triglyceride contents in egg yolk. Therefore, the treatment significantly affected ($P<0.01$) egg weight, HU value, egg strength, and yellow egg LDL. However, step II indicated that feed treatment did not significantly affecte to HU value, yolk colour, egg strength and cholesterol; LDL; and triglycerides contents in yolk egg but significantly affected the egg index ($P<0.05$).

The best HU value, therefore, was found in R_3 but with higher cholesterol and lower HDL values. Such figure was caused by the fact that egg development require certain nutrient mainly lipoprotein and cholesterol as several reproduction hormons precursor which related to egg development processes. Such figure is in concordance with Bair *et al.* (1980) who stated that cholesterol content in egg has a negative correllation with egg production and the size of egg yolk, and in line with the age of chicken.

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Table 1. Nutrient composition of native chicken ration

Feed Ingridient (%)	R_0	R_1	R_2	R_3	R_{31}	R_{32}	R_{33}	R_{34}
Vitamin E (mg/kg feed)	-	-	-	-	0.00	10.00	20.00	30.00
Manhadden fish oil	0.00	0.00	10.00	5.00	5.00	5.00	5.00	5.00
Kernel palm oil	0.00	10.00	0.00	5.00	5.00	5.00	5.00	5.00
Yellow corn	62.00	27.00	27.00	27.00	41.00	41.00	41.00	41.00
Ricebran	7.00	31.00	31.00	31.00	25.00	25.00	25.00	25.00
Soy flake	13.00	15.00	15.00	15.00	10.00	10.00	10.00	10.00
Fishmeal	7.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Calcium powder	7.00	7.00	7.00	7.00	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00	0.40	0.40	0.40	0.40
DL-Methionine	0.75	0.75	0.75	0.75	0.10	0.10	0.10	0.10
L-Lysin HCl	0.75	0.75	0.75	0.75	0.10	0.10	0.10	0.10
Topmix	1.50	1.50	1.50	1.50	0.40	0.40	0.40	0.40
Cassava solid waste	-	-	-	-	2.50	2.50	2.50	2.50
Soy hull	-	-	-	-	2.50	2.50	2.50	2.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition :	R_0	R_1	R_2	R_3	R_{31}	R_{32}	R_{33}	R_{34}
Protein (%)	15.21	15.31	15.07	15.44	15.16	15.84	15.65	15.75
Metabolic energy (Kcal/Kg)	2900.00	3000.00	3000.00	3000.00	3044.63	3093.28	3011.17	3086.02
Lipid (%)	3.85	4.97	4.97	4.97	5.94	5.63	5.84	5.65
Raw fibre (%)	2.45	2.99	2.99	2.99	2.40	3.37	3.62	3.50
Ca (%)	3.00	3.00	3.00	3.00	1.31	1.31	1.31	1.31
P *available* (%)	0.53	0.72	0.72	0.72	0.75	0.75	0.75	0.75
Lysin (%)	1.03	1.01	1.01	1.01	0.89	0.89	0.89	0.89
Methionine (%)	1.57	1.60	1.60	1.60	1.40	1.40	1.40	1.40
Total n = 3	0.77	13.22	14.07	14.92				
Total n = 6	1.20	13.21	12.07	10.93				
Total n = 9	49.14	21.70	19.16	16.62				

Note : Analysis result from Nutrition and Animal feed Laboratory. UNSOED (2005)
*) Calculation result using NRC table(1984)

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Tabel 2.

Egg quality	Step I					Step II				
	R_0	R_1	R_2	R_3	Sig	R_{31}	R_{32}	R_{33}	R_{34}	Sig
Egg weight (g)	38.77^{a}	36.40^{b}	32.91^{c}	36.63^{b}	**	35.38^{a}	38.50^{a}	39.55^{b}	40.79^{b}	**
HDA (%)	10.91^{a}	18.15^{ab}	8.83^{a}	18.35^{b}	**	34.67^{a}	34.06^{ab}	39.92^{b}	35.26^{b}	**
Egg index	70.58	72.62	72.58	71.55	ns	79.96^{a}	76.91^{a}	76.04^{b}	73.45^{b}	**
HU	82.07^{a}	84.2^{a}	88.65^{a}	92.01^{b}	**	76.43	72.88	65.71	72.17	ns
Yolk Colour	6.30	5.5	6.67	6.60	ns	6.75	6.75	7.50	7.4167	ns
Egg strength /(mm/g)	0.279	0.227	0.325	0.28	**	0.26	0.34	0.28	0.29	ns
Egg yolk cholesterol (mg/dl)	158.80	137.43	121.19	106.66	ns	333.33	395.23	533.33	414.28	ns
Egg yolk HDL (mg/dl)	2.22	2.22	3.33	3.33	ns	2.60	2.22	1.85	2.96	ns
Egg yolk triglyceride (mg/dl)	272.72	181.81	281.81	109.09	ns	126.67	173.33	73.33	60.00	ns
Egg yolk LDL (mg/dl)	81.78^{a}	65.18^{b}	54.22^{c}	21.10^{d}	**	308.00	360.56	518.66	402.28	ns

Note : ns = non significant ($P>0.05$)
Different superscript in the same line indicated significant differences;
** = very significant differences ($P \leq 0.01$), * = significant differences ($P \leq 0.05$)

The highest HU value was found in R_{31} (79.96) with lowest cholesterol (333.33 mg/dl) and LDL (308.00 mg/dl) contents in yolk. However, the highest cholesterol content was found in R_{33} (533.33 mg/dl) but had the lowest triglyceride (73.33 mg/dl). These figures indicated that vitamin E did not act in cholesterol content reduction but maintenaning cell affinity through mechanisms which is not using cell apoprotein as mentioned by Mardones and Rigotti (2004).

Increasing weight of egg was related apparenmtly due to the increasing use of kernel palm oil rather than manhadden fish oil. The size and weight of egg are influenced by linoleic acid and methionine. Manhadden fish oil is source of linolenic acid (omega-3) and kernel palm oil is source of linoleic acid (omega-6) (Farrell. 1996). Linoleic acid is needed for lipoprotein complex synthesis which is conducted in liver stimulated by estrogen then it is transfered to follicle development and directly controls egg weight (March and Mc Millan. 1990).

According to van Elswyk (1997[b]), linolenic acid in fish oil can reduce estradiol circulation which is needed for lipogenesis in liver and egg development. Thus, low concentration of linolenic acid will reduce the weight and size of eggs. High linolenic acid in ration will result in acceleration of follicles maturity and reduction of VLDL secretion to follicles due to estradiol activity reduction (Sulistiawati. 1998). The increasing use of manhadden oil in ration resulted the reduction of egg weight. However, the increasing use of linoleic acid such as the used of kernel palm oil had been reported produce higher yolk as reported in quails (Saerang, 1997). Linoleic acid (omega-6) is a factor which control protein and lipid needed by follicle development to produce yolk egg (March and Mac Millan, 1990). According to Park *et al.* (1994). Saturated fatty acid such as omega-6 is rapidly absorbed by intestine and keeping the cholesterol excretion low. However, unsaturated fatty acid (omega-3) such as found in fish oil, inhibits cholesterol biosynthesis and reduces triglyceride and VLDL cholesterol

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as found in mice blood plasm. The use of manhadden fish oil (R_2) could increase HDL cholesterol and triglyceride, therefore decrease LDL cholesterol. However, the use of ideal mixture concentration of manhadden fish and kernel palm oils (R_3) could reduce cholesterol concentration due to synergistic absorption of saturated and unsaturated fatty acids, thus inhibits egg cholesterol synthesis.

This research found that yolk among the treatment animals was not significantly different ($P<0.05$), but the best result was found in R_2 (10 % menhaden fish oil). This result is due the fact that yolk influenced by carotene from yellow corn which contains cryptoxanthin and from manhadden fish oil which contains xanthophyl, astaxanthin and taraxantin (Kanoni. 1991). According to van Elswyk (1997[a]), fish oil can reduce *very low density lipoprotein* (VLDL) cholesterol and triglyceride in cockerel blood. Another benefit of fish oil is the fact that omega-3 fatty acid will be metabolized to produce eicosanoic such as prostaglandin which functionally reduce the inflammation, platelet aggregation and heart problem (Marshall *et al.*, 1994). According to Parakkasi (1983), combination of fatty acids produces different energy value compared to the used of single fatty acid. This figure due to the synergistic interaction with micelle development which facilitated the fatty acids absorption (Leeson and Atteh, 1995).

Cholesterol ($C_{27}H_{45}OH$) is lipid dissolved substance and freely found in ester-high molecule unsaturated fatty acid binding (Anggorodi. 1995) and act as precursor all steroid substances in animal body such as corticosteroid, sex hormons, bile and vitamin D (Murray *et al.*, 1995). Cholesterol precursor (*vitellogenin*) and triglyceride-lipoprotein (VLDL) products of liver synthesis, which is directly brought to ovarium (Griffin. 1992). Cholesterol deposition in egg is caused by lipoprotein deposition from synthesis process in the egg yolk. According to Bacon (1985), egg yolk development is started from VLDL synthesis in liver the secreted to ovarium wall for folliles development through filtration and special transport mechanism facilitated by certain receptor.

Griffin (1992) stated that egg yolk cholesterol is depended on VLDL rich triglyceride. A study in mammal indicated a cholesterol synthesis inhibition can reduce synthesis rate and secretion of lipoprotein in liver, but the effect to secreted lipoprotein was small so the role of cholesterol in steroid synthesis apparently inhibits cholesterol reduction in yolk. In addition, inhibition of cholesterol synthesis in initial phase conducted by HMG-CoA (*3-hidroxy-3-methylglutaril Coenzyme A*) which is also intermediate substance in ketogenesis in liver (Murray *et al.*, 1995).

Saerang (1997) reported that the use of oil in quail ration can reduce the cholesterol content in egg. About 95% of yolk cholesterol is found in triglyceride rich lipoproteins which shape 33% of yolk and 66% solid substance of yolk (Griffin. 1992). Furthermore, the rest of 5% was bound in other main component of yolk, lipovitellin. Research by Vilchez *et al.* (1990) reported that fatty acid composition of plasm and egg were influenced by food lipid composition.

The results of experiment II were shown in Table 2. Variance analysis indicated that the research was non-significant to HU value, yolk colour, egg strength and the values of egg yolk cholesterol; HDL; LDL and triglyceride. However, the result was significantly affect the egg index ($P<0.05$). The highest HU value was found in treatment with R_{31} (79.96), but with low cholesterol (333.33 mg/dl) and LDL (308.00 mg/dl) in egg yolk. The animals of R_{33} had high cholesterol content (533.33 mg/dl) but low triglyceride (73.33 mg/dl). This is indicated that vitamin E does not have any role in cholesterol reduction but in cell affinity protection (Mardones andn Rigotti, 2004). Vitamin E addition significantly increases the egg strength and yolk colour, as seen in R_4. Yolk colour is influenced by carotene as mentioned by BASF (2000) which stated that vitamin E addition significantly increase the egg weight, vitamin E less than 25 IU will result the yolk saturated with α tocoferol. However, when vitamin E is given in low level, the vitamin will be used for other purposes. R_{33} has cholesterol content 533.33 mg/dl and high LDL (518.66 mg/dl), but with low HDL (1.85 mg/dl) and triglyceride (73.33 mg/dl). R_{34} has better average values of yolk cholesterol, HDL, LDL, and triglyceride. Flohe *et al.* (2005) stated that vitamin E supplementation will reduce excessive oxidation of lipid, be able reduce 8-iso $PGF_{2\alpha}$ excretion and reduce the LDL sensitivity *in vitro*.

CONCLUSION

The use of 5 % manhadden fish and 5 % kernel palm oils combination and vitamin E suplementation at 30 mg/kg of ration for native chicken has been approved in native chicken egg quality.

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