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THE EFFECT OF VITAMIN E SUPPLEMENTATION IN RATION CONTAINING MANHADDEN FISH AND KERNEL PALM OILS TO NATIVE CHICKEN'S SPERM QUALITY AND HISTOLOGICAL PROFILE OF TESTES

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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 sperm quality (mortality, abnormality, motility, konsentration), and histological profile of testes. 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, and was carried out from April 2004 to November 2005. Chickens used in this research consist of 20 cockerels of 22 weeks old. The rations at the first step were: R_0 = 0% manhadden fish oil and 0% kernel palm oil; R_1 = 10% manhadden fish oil and 0% kernel palm oil; R_2 = 5% manhadden fish oil and 5% kernel palm oil; R_3 = 0% manhadden fish oil and 10% kernel palm oil. The second step: 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. Research was done use Completely Randomized Design (CRD), each treatment was repeated 6 times. Data then were analyzed using analysis of varian continued with Duncan Test. The result showed that the first step rationof affeccted sperm motility and concentration, but very significantly ($P<0.01$) not significantly ($P>0.05$) affected mortality and abnormality of sperm. The second step of study showed that the treatment affected abnormality, motility and concentration of sperm very significantly ($P<0.01$), and significantly ($P<0.05$) affected sperm mortality. It was concluded that vitamin E supplementation in feed containing manhadden fish and kernel palm oils improved the sperm quality (improved motility and concentration, reduced mortality and abnormality) and improve the testicular development.

Keywords: Vitamin E, Sperm Quality, Histological Profile Of Testes

INTRODUCTION

Animal reproduction in animal develop especially in avian, was influenced by nutrient intake. In sufficient of nutrient intake could fatally affect to the animals such as embryonic fatality, reproduction organ disorder, sex maturity disorder, less number and vitality of sperm or may stop the spermatogenesis. Thus, the availability of food needed by animal is necessity in order to keep the reproduction and production process run well.

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Good quality of food should contain certain ingredient such as essential fatty acids especially long chain fatty acids which contain omega 3 (linolenic acid) omega 6 (linoleic acid) and omega 9 (oleic acid). Linoleic acid (18:3ω3) and oleic acid (18:1ω3) were found in plant oils such as from soybean, rapeseed and peanut. However, linolenic acids such as eicosa penthanoic acid, *EPA* (20:5ω3) and docosa hexanoic acid, DHA (22:6ω3) could be found such as in fish oil.

Increasing the need of fatty acid resulted in demand on vitamin E which needed as antioxidant as well as male and female fertilities factor. Unsaturated fatty acids were easily oxidized to peroxides. By the availability of vitamin E, the fatty acids were protected from oxidation. It was due to the role of vitamin E as antioxidant. Broodstock food composition was a main factor in developing antioxidant system in embryo during embryogenesis and initial was days of newly hatched chicks (DOC) (Surai *et al.*, 1999).

MATERIALS AND METHODS

The experiment was carried out from April 2004 to November 2005. 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

Cages and Utensils

20 cockerels of native chickens were reared for 5 months, in indovodual cages (pen cages) with 50 cm length, 33 cm width and 25 cm height, were completed with drinking water cups made from plastic and bamboo feeding container. The barn were accompany by utensils such as wall thermometer, wall hygrometer, balance, disinfectant sprayer, spuit, plastic boxes, cleaning utensil and microscop.

The research was *in vivo* experiment using Completely Randomized Design (Steel and Torrie, 1981). The treatment consisted of 4 ration and 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.

Experiment 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

Experiment 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

Data collection procedures

Semen kolections has been done when the age of cockerels reach their puberty (5-6 month old). Semen collecting activities done 3 times as long asa the research proceeds, and each semen collection repeated 3 times with internal of one week.

1. Mortality

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2. Motility and abnormality by semen determination under microscope with magnitude of 40 x 10. Motility percentage based on mass movement / progress movement.
3. Abnormality, based on percentage of normal sperm cells number devided by total sperm cell count
4. Sperm cell concentration, based on Toelihere (1985) that is :
   $Y = X \times \frac{400}{80} \times 200 \times 10 = 10.000 \text{ x/mm}^3$ or $10 X \times 10^6$ sperm cell/ml
5. Parafin and hematoxylin methods used to make histological preparation to examine histological profile of testis.

Table 1. Nutrient composition of native chicken ration

	Step I				Step II			
Food material (%)	R_0	R_1	R_2	R_3	R_{31}	R_{32}	R_{33}	R_{34}
Vitamin E (mg/kg feed)	0,00	0,00	0,00	0,00	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	65,00	11,00	11,00	11,00	36,00	36,00	36,00	36,00
Ricebran	7,00	52,00	52,00	52,00	36,00	36,00	36,00	36,00
Soy flake	15,00	14,00	14,00	14,00	6,00	6,00	6,00	6,00
Fishmeal	6,00	6,00	6,00	6,00	4,00	4,00	4,00	4,00
Calcium powder	2,50	2,50	2,50	2,50	2,00	2,00	2,00	2,00
Salt	1,50	1,50	1,50	1,50	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	0,00	0,00	0,00	0,00	2,50	2,50	2,50	2,50
Soy hull	0,00	0,00	0,00	0,00	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 (%)	15,07	15,08	15,08	15,08	12,60	12,24	12,93	12,64
Metabolic energy (Kcal/Kg)	2900,00	2900,00	2900,00	2900,00	3034	3034	3034	3034
Lipid (%)	3,92	6,57	6,57	6,57	6,88	6,88	6,88	6,88
Raw fibre (%)	2,59	3,69	3,69	3,69	6,39	6,39	6,39	6,39
Ca (%)	1,25	1,26	1,26	1,26	1,20	1,20	1,20	1,20
P *available* (%)	0,50	0,90	0,90	0,90	0,81	0,81	0,81	0,81
Lysin (%)	1,03	1,01	1,01	1,01	0,40	0,40	0,40	0,40
Methionine (%)	1,59	1,63	1,63	1,63	0,75	0,75	0,75	0,75

RESULTS AND DISCUSSION

The measurement of sperm quality i.e. mortality, abnormality, motility, and konsentration of sperm can be seen in Table 2. abnormality, motility and concentration of sperm, and significantly ($P<0.05$) affected sperm mortality.

The result of first step showed that treatment effect on cockerel at the age of 22 months is not significantly different on mortility and abnormality of sperm cell, however was higly significant effect ($P<0.01$) on motility and concentration of sperm cells effect of R_3 treatment resulted in lowest rate of mortility and abnormality, and highest rate of motility and concentration of sperm cell. The effect of feeding combination of menhaden fish and kernel palm oils show increasing in sperm quality as showed by Surai *et al.* (2000) was stated that supply of phospholipids decrease than the

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result also decreased spermatozogenesis rate and semen quality (Kelso *et al.*, 1997; and Cerolini *et al.*, 1997), and it was followed by formation of Sertoli cells and increasing number of Leydig cells, due to hyperplasia activity (Lamming, 1990).

Analysis of variance on second step of experiment showed that the effect of treatment is significantly ($P<0.05$) higher in mortality, and highly significanty ($P<0.01$) effect on abnormality and sperm cell concentration. The average result showed that mortality was about 12,99% (R_{31}) to 16,63% (R_{32}) and abnormality 6,74%(R_{33}) to 9,49% (R_{32}). The result of this experiment was better than the result of Haryati (2001), that the mortality of Kedu chicken range from 16.45 to 20.98 %, and the abnormality range between 21.1 to 24.37 %. Result of this experiment shows that concentration of sperm cells range between $3,88x10^9$/ml to $4,58x10^9$/ml (R_{34}). The result was almost the same with resukt of Utomo (1997) experience, that concentration of sperm cells range between 3,09 x 10^9/ml to 3,82 x 10^9 /ml. Nasroedin *et al.*, (1993) stated that sperm cells concentration of native chicken range between 3,08 x 10^9 /ml to 3,9 x 10^9 /ml. Lake (1983) stated that sperm cell concentration is affected by nutrition and the age of chicken. Cooper *et al* (1987) stated that deficiency of Vitamin E in feed cause decreate of proliferation and diferrerentiation of germ cells, however did not affect on gonadotropin function and serum tetstosterone concentration, asa well as the seminal vesicle weight and citrate concentration Vitamin E deficiency will inhibit on LH-Testoterone feedback, and it is clear that Vitamin E will decrease member of germ cell, and testicular degeneration.

Histological profile of testis could be examined in fifuge 1. It showed that the treatment effected on histological profile, manhadden fish oil addition caused increasing number *tubuli seminiferus* compared to without manhadden fish oil. The role of manhadden fish oil that contained abundant of long chain fatty acids improve testis development as stated by Surai *et al.*, (2000), and the addition of long chain fatty acid containing omega 3 and 6 could improve testis and liver development of chicken. The role of long chain fatty acid on sperm cell function were to increasing in sperm phospholipids content. Rettersol *et al.*, (1998) stated that the role of long chain fatty acid on testis was to join together among phospholipids cell membrane. Long chain fatty acids especially C20:4n-6 would be increasing spermatogenesis, and resulted in increasing testis mass as well as increasing sperm cell are count, too.

The result of experiment II on histological profile testis were showen in Figure 2. It is identified that R_{34} treatment that composed of basal ration with manhadden fish and kernel palm oils addition, then added with vitamin E in the amount of 30 mg/kg ration would cause increasing number were *tubuli seminiferus* And germ cells, compared to the other treatments.

The role Vitamin E were as antioxcidant agent and protected *epitelium germination* degeneration. Djawadun (2001) stated, that vitamin E feed increased tubuli seminiferus development, *epitelium germinativum*, and *Leydig cells* in duck. Tocopherol antioxcidant in vitamin E was fat diluted antioxcidant, so that it was easily enter the cell and reacted on free radicals (Miller dan Slebodzinska, 1993; Wijaya, 1996). Lipid peroxcidation could be protected by tocopherol antioxcidant, decreasing the rate of unsaturated fatty acid oxidation of the membrane and prolecting the intact of plasma membrane. Alpha tocopherol has the ability as effective inhibitor on sate of lipid peroxcidation, due to the fact that each tocopherol molecule could react with 2 peroxcyl radicals (Krinsky, 1992).

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Tabel 2. Effect of treatments on sperm cell quality

	Step I				Step II				
	R_0	R_1	R_2	R_3	R_{31}	R_{32}	R_{33}	R_{34}	Sig
Mortality (%)^{ns}	15,93	11,35	14,09	11,23	12,99^{a}	16,63^{b}	14,79^{ab}	13,39^{a}	*
Abnormality (%)^{ns}	14,47	12,71	16,48	12,23	7,14^{a}	9,49^{ab}	6,74^{b}	7,71^{b}	**
Motility (%) **	80,83^{a}	89,17^{b}	85,83^{b}	85,83^{b}	80,00^{a}	81,67^{b}	81,67^{b}	83,33^{b}	**
Concentration 10^7 **	2,78^{a}	2,88^{a}	4,17^{b}	5,38^{c}	4,01^{a}	3,64^{a}	4,05^{a}	4,58^{b}	**

Note : ns = non significant ^{ns} (P>0.05) Different superscript in the same line indicated significant differences;

** = very significant differences ($P \leq 0.01$), * = significant differences ($P \leq 0.05$)

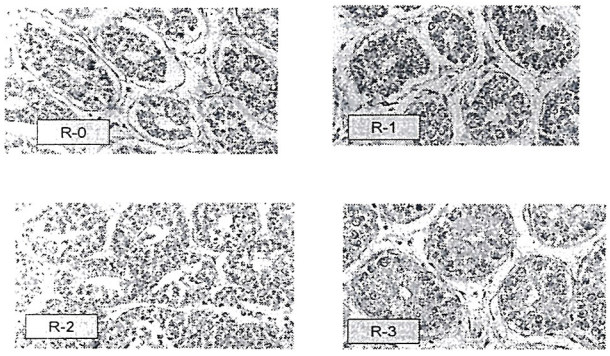

Figure 1. Histological profile of testis, as observed from experiment I (magnitude of 400 x)

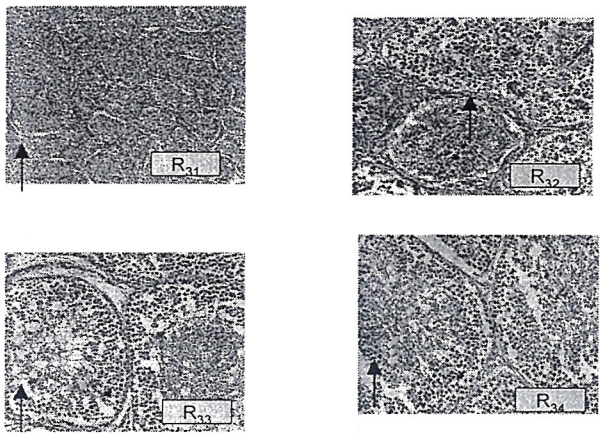

Figure 1. Histological profile of testis, as observed from experiment II (magnitude of 400 x)

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CONCLUSION

The use of 5 % manhadden fish and 5 % kernel palm oils combination and vitamen E suplementation at 30 mg/kg of ration for native chicken had been approved in native chicken sperm quality dan testes develop.

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Table 1. Nutrien composition in ration of native chicken

Bahan pakan (%)	R_0	R_1	R_2	R_3	R_{31}	R_{32}	R_{33}	R_{34}
Vitamin E (mg/kg pakan)	-	-	-	-	0,00	10,00	20,00	30,00
Minyak ikan lemuru	0,00	0,00	10,00	5,00	5,00	5,00	5,00	5,00
Minyak kelapa sawit	0,00	**10,00**	**0,00**	**5,00**	5,00	5,00	5,00	5,00
Jagung kuning	65,00	11,00	11,00	11,00	36,00	36,00	36,00	36,00
Dedak padi	7,00	52,00	52,00	52,00	36,00	36,00	36,00	36,00
Bungkil kedele	15,00	14,00	14,00	14,00	6,00	6,00	6,00	6,00
Tepung ikan	6,00	6,00	6,00	6,00	4,00	4,00	4,00	4,00
Tepung batu kapur	2,50	2,50	2,50	2,50	2,00	2,00	2,00	2,00
Garam	1,50	1,50	1,50	1,50	0,40	0,40	0,40	0,40
DL-Metionin	0,75	0,75	0,75	0,75	0,10	0,10	0,10	0,10
L-Lisin 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
Onggok	100,00	100,00	100,00	100,00	2,50	2,50	2,50	2,50
Kulit kedele	R_0	R_1	R_2	R_3	2,50	2,50	2,50	2,50
Total		15,08	15,08	15,08	**100,00**	**100,00**	**100,00**	**100,00**
Komposisi Nutrien :		2900,00	2900,00	2900,00	**R_{31}**	**R_{32}**	**R_{33}**	**R_{34}**
Protein (%)	15,07	6,57	6,57	6,57	12,60	12,24	12,93	12,64
Energi Metabolis (Kkal/Kg)	2900,00	3,69	3,69	3,69	3034	3034	3034	3034
Lemak (%)	3,92				6,88	6,88	6,88	6,88
Serat Kasar (%)	2,59				6,39	6,39	6,39	6,39
Ca (%)	1,25	1,26	1,26	1,26	1,20	1,20	1,20	1,20
P *Available* (%)	0,50	0,90	0,90	0,90	0,81	0,81	0,81	0,81
Lisin (%)	1,03	1,01	1,01	1,01	0,40	0,40	0,40	0,40
Metionin (%)	1,59	1,63	1,63	1,63	0,75	0,75	0,75	0,75
Total n = 3	0,77	13,22	14,07	14,92				
Total n = 6	1,12	13,21	12,07	10,93				
Total n = 9	49,14	21,70	19,16	16,62				

Ket : Hasil analisis Lab. Nutrisi dan Makanan Ternak UNSOED (2005)
*) Hasil perhitungan dengan Tabel NRC (1984)