CHAPTER V

DISCUSSION

The major sources of crude fat in the ration of laying hen come from various ingredients eg. vegetable oil, lard, tallow, palm stearin etc. There were a few previous researches in using crude palm oil as a source of fat in layer. Therefore, we examined the effect of supplementing 2% CPO in diet on hen performance and cholesterol concentration in experiment I compared to lard. This served as a preliminary trial to investigate, whether there was an adverse effect of crude palm oil in laying hens.

Experiment I

In experiment I, daily feed intake (DFI) and weight gain of control (lard 2%) and hens fed on CPO diet (2% CPO) were not significant different. Chickens regulate their feed consumption quite well in relation to dietary energy content. This can be varied within relatively wide limits. The amount of feed consumption depends on the energy level of the feed, egg production rate, body size and environmental temperature (Stadelman, 1995a). In this experiment, hen-day basis percentage (%HD) was not different in layers received CPO compared to control diets in each time interval. The hen-day basis percentage of CPO group was higher than control group in week 5 and week 6 but there were no statistically difference, since number of hens was small (5 hens/group).

The average egg weights of hens fed on CPO were significantly ($P<0.05$) greater than hens fed on lard at week 3 and week 6 of trial. Hens fed on 2% CPO had average of egg yolk weight higher ($P<0.05$) than hens fed on lard at week 5 and week 6. In this experiment, it is demonstrated that there were no difference in specific gravity of egg in CPO group compared to control. It is possible that CPO did not have any effect on shell thickness and shell quality, which was major factors affecting specific gravity.

Haugh unit (HU) of the control and CPO groups were not different. The Haugh unit is an expression in relation to egg weight and height of albumin. Haugh (1937) reported that the higher HU demonstrated the better albumin quality of the egg. Yolk color of CPO group had a trend toward an increase when compared with control group, but it was not significantly difference. In general, the majority of carotenoids in yolk are hydroxy compounds called xanthophylls with minor amounts of carotenes (Gunguly et al., 1953 and Smith and Perdue, 1966). The major xanthophylls in the yolk are derived from commonly used pigmented feed ingredients such as yellow corn (Brockman and Volker, 1934; Gillam and Heibron, 1935 and Smith and Perdue, 1966). It is possible that the level of CPO used in this preliminary trial was too low, to cause change on yolk color. At weeks 5 and 6, yolk weight of CPO group was significantly higher than control group. This increase yolk weight may explain the increased egg weight shown previously. The effect of CPO on egg weight may be due to the heavier yolk weight.

The cholesterol concentrations in egg yolk of control and CPO groups were not different. In previous literatures, it is shown that the cholesterol content of the yolk was influenced by the genotype of birds, weight of hen, DFI and dietary fat (Stadelman, 1995b). Since, the nutrient compositions of both control and CPO diets were similar, changes in cholesterol level in egg may result from levels of tocotrienol, which are abundant in CPO. Anyhow CPO at 2% supplementation may be too low to reduce cholesterol level in egg. Qureshi et al. (1986) and Qureshi et al. (1988) reported that palm vitamin E (α-, γ- and δ-tocotrienol) has been shown to suppress cholesterol biosynthesis and reduce total serum cholesterol and LDL cholesterol levels in hypercholesterolemic chickens.

It can be concluded from Experiment I that CPO at 2% did not have adverse effect on hen performance and its effect on reducing cholesterol in egg yolk was not different with control group, probably due to its low level and small number of hens studied. Therefore, we investigated the effect of crude palm oil inclusion at higher level (3% and 4%) in experiment II. The reason why we did not increased CPO more than 4% was that it was difficult to make all diets isocaloric (equal ME) and diets with large amount of CPO resulted in more rancidity and clumping of the feed ingredients.

Experiment II

Hen performance

Weight gain, average daily gain (ADG) and hen-day basis percentage (%HD) of hens fed on CPO diets were not different from control. Although, ME of CPO3 diet was slightly higher than other group but this did not affect feed intake. Moreover CPO supplementation seems to have no effect on feed intake of laying hens.

In layer, feed conversion ratio (FCR) shows amount of feed intake for 1 g egg weight (g diet/g egg). The lower FCR demonstrates the better efficiency of laying hen in producing eggs. The reason why FCR of hen received 4% CPO (CPO3) was better than other groups was the larger average egg weight in this group. With almost similar feed intake, the FCR of CPO3 group (1.91) was the best.

Egg quality

The specific gravity measurement of egg is used to determine shell quality. The procedure requires a series of sodium chloride solutions at varying level and a good hydrometer that can detect difference in specific gravity between adjacent paired of solutions of not more than 0.005 g per milliliter. The range of specific gravities found in most layer flock varied from 1.064 to 1.104 (Stadelman, 1995b). The higher specific gravity indicated more shell thickness and shell quality. The specific gravity of egg is depended on strain, temperature, time of measurement, shell thickness and egg weight (Stadelman, 1995b). Although, egg weight of CPO3 group was higher (approximately 2 g) than control group, its specific gravity was not different. In this study, the specific gravity of control group and CPO groups were not different, indicating that CPO did not affect shell quality and thickness.

Haugh unit express the albumin quality of the egg, which can be divided into 3 groups, AA-Grade (HU values over 72), A-Grade (HU value between 60-72) and B-Grade (HU values below 60) (Haugh, 1937). In experiment II, There was no significant difference in Haugh unit between hens received CPO and control diets. Haugh unit in this experiment were greater than 72 (AA-Grade). It is demonstrated that CPO did not have any effect on albumin quality. Haugh unit of eggs in all groups were measured at the same time, thus excluding the effect of time on albumin quality. Basically the albumin decrease as time increased. It is postulated that carbon dioxide gas escapes through the shell, resulting in an increase in the pH of the albumin and a reduction in its viscosity (Stadelman, 1995b).

Yolk color of hens in CPO3 group was markedly greater than hens fed on control diet. Our result was similar to Kang et al (1998) who reported that egg yolk form hens fed CPO had higher yolk color than control. The yellow-orange color of yolk has been attributed to the presence of fat-soluble carotenoids in the lipid portion of lipoproteins (Shenstone, 1968). The majority of carotenoids in yolk were hydroxy compounds called xanthophylls with minor amount of carotenes (Smith and Perdue, 1966). The type and amounts of carotenoids in yolk were diet dependent, since yolk pigmentation involved intestinal absorption and biotranslocation were affinity to feed carotenoids to the ovary (Brown, 1938; Nelson and Baptist, 1968; Scott et al., 1968). The crude palm oil contains high amounts of carotenoids at 500-700 mg/L, comprising of α- and β-carotenes at 36 and 54%, respectively and γ-carotene, lycopene and xanthophylls contributing to 10% (Goh et al., 1985). In this experiment, we did not supplement any commercial pigment in the basal diet. Our result was in agreement with the study of Cherian et al. (2000) who showed that egg yolks from hens fed on palm oil had significantly increased yolk color than hens fed control diet (2% fish oil + 1.5% tallow + tocopherol mix). Therefore, xanthophylls in β-carotenes in CPO diets increased yolk color dramatically than control diet.

In current study, egg weight of hens fed on CPO2 and CPO3 diets were higher than hens fed on control diet from week 4 to week 6 with a concurrent increase in yolk weight. It is demonstrated that increased average egg weight resulted from the increased yolk weight, similar the study of Roca et al (1984) who reported that the density of yolk and

albumin and the proportion of egg yolk were related to egg weight. Hens can form an egg in about two weeks. This is true except for the very small core of yolk in ovary. The yolk is formed during the final 10 to 12 days prior to the laying of the egg (Stadelman, 1995b). It is demonstrated that egg yolk can be changed in approximately 3-4 weeks if the diet of laying hens altered. Although, all experimental diets certain approximately equal crude protein. ME in diet of CPO3 (2,781.83 kcal/kg) group was slightly higher than other groups (2,750 kcal/kg). The increased ME in diet may increase weight of the yolk. Grobas et al. (1999) reported that an increase in the AMEn of the diets from 2,680 to 2,810 kcal/kg increased the weight of the yolk, but the AMEn of the diets did not modify albumin weight.

In this study, the percentage of crude fat was varied from 4.64% (control) to 6.50% (CPO3). The crude fat intake (percentage of crude fat x feed intake) in various groups increased from 4.96 (control) to 5.06 (CPO1), 6.00 (CPO2) and 7.02 (CPO3) g/d, respectively. Higher crude fat intake may affect the yolk weight and egg weight of the egg. The beneficial effect of dietary fat on improving egg weight was well documented (Merkel et al., 2002; Sohail et al., 2003). Bohnsack et al. (2002) reported that egg weight increased as the level of fat in the diet increased. Cherian et al. (2000) showed that the diets differently in vitamin E had no effect on egg weight on yolk weight. Yolk weight may increase from linoleic acid in CPO. Jensen et al. (1958) pointed out that linoleic acid increased egg weight by augmenting yolk weight. CPO had approximately 9% linoleic acid (Gottenbos and Vles, 1983), but lard very low amount slightly of linoleic acid. Since, linoleic acid content might enhance synthesis of lipoproteins in the liver that eventually would be secreted and taken up by the developing oocytes (March and McMillan, 1990). Dietary fat (i.e., long chain fatty acids) entering the portal blood would be in the form of chylomicrons, which were broken down by the liver. The lipids were then re-synthesized as components of very low density lipoprotein particles for direct transport and deposition into the yolk (Nimpf and Schneider, 1991).

Previous study (Moudgal, 1999) suggested that some unsaturated dietary fat (sunflower oil) improved egg weight when compared with lard, deserved further elucidation of the possible but still unknown mechanism. In vitro study across the follicular wall

indicated that this difference might be because of relatively higher transport rate of moisture in control group compared to sunflower oil fed group. In turns, it may be possible that the yolk of developing egg in oviduct of sunflower fed group may receive more water because of higher osmolarity difference compared to control group (Moudgal, 1999).

Egg yolk cholesterol concentration

Results showed that hens fed on CPO had significantly lower egg yolk cholesterol concentrations than hens fed on control diet (lard) on week 4 to week 6. Egg yolk cholesterol concentrations were gradually reduced when the increased percentage of CPO was supplemented. The cholesterol content of the yolk is influenced by the genotype of the birds. Harris and Wilcox (1963) reported that yolk cholesterol per gram of wet yolk differed between dam families within series in a random-bred White Leghorn strain. Diets or drugs could influence cholesterol synthesis by the laying hens and its concentration in the egg. Laying hens usually meet their needs for cholesterol by de novo synthesis, synthesizing 300 mg/day in the liver and ovary. However, ovarian-synthesized cholesterol is rarely transferred to developing oocyte, contributing minimally to egg cholesterol. Therefore, egg cholesterol was synthesized in the liver and secreted into the blood as very low-density lipoprotein particles, the main yolk cholesterol carrying macromolecules (Andrew et al., 1968). Plasma very low-density lipoprotein particles were then internalized by the oocyte vitellogenesis receptor in the rapidly growing follicles, and deposited to yolk (Hall and McKay, 1993).

In this experiment, hens fed 4% CPO in diet for 28 days produced egg with 36% reduction in cholesterol concentration (from 18.61 to 11.89 mg/g yolk). The lowest concentration of cholesterol reported in this trial was 202.68 mg/yolk weight in 4% CPO in diet at week 6. CPO contained the richest source of tocotrienol (palm TRF), which could suppress cholesterol biosynthesis. The hypocholesterolemic effect of tocotrienols resulted from the isoprenoid side-chain's ability to increase the concentration of cellular farnesol. Farnesol is derived from mevalonate, the product of the HMG-CoA reductase reaction. Farnesol, post-transcriptionally, suppressed HMG-CoA reductase synthesis and enhanced

the proteolytic catabolism of this enzyme. This mechanism was different from that of the statin hypocholesterolemic drugs (atorvastatin, cerivastatin, lovastatin and provastatin), which were competitive inhibitors of the enzyme HMG-CoA reductase. γ-Tocotrienol and δ-tocotrienol were significantly more active than α-tocotrienol in suppressing HMG-CoA reductase activity (Elson, 1995).

Elkin and Rogler (1989) reported that there was an 11% reduction in total cholesterol content of egg yolk (mg cholesterol/g yolk) after feeding lovastatin to hens for 35 days (5 weeks). Inhibition of cholesterol synthesis by lovastatin has been shown to have little effect on VLDL composition (Arad et al., 1990). Previous study reported that lovastatin reduce plasma cholesterol concentrations in some patients by stimulating hepatic uptake of LDL via the induction of LDL receptors, a response that in turn is due to the reduction in hepatic levels of cholesterol (Arad et a., 1990).

Approximately 20% of yolk cholesterol in eggs is in the form of cholesterol esters; a decrease in yolk cholesterol could have been due to a reduction in yolk cholesterol ester content. In another experiment, supplementing sitosterol and high fiber in diet may result in marginal decreases in cholesterol (Naber, 1991). Crude palm oil contains about 800 ppm vitamin E which, 70% is in the form of tocotrienol called palm tocotrienol-rich fraction (palm TRF) (Ab Gapor, 1990). Tocotrienols exhibited a hypocholesterolemic effect by reducing the activity β-hydroxy-β-methylglutaryl coenzyme A reductase, thus suppressing the synthesis of cholesterol in the liver (Qureshi et al., 1988; Quershi et al., 1989). The tocotrienols have a profound effect on the biosynthesis of cholesterol. Several studies indicated that tocotrienols were very effective in lowering blood cholesterol and LDL cholesterol levels by suppressing HMG-CoA reductase (Goldstein and Brown, 1990). β-Hydroxy-β-methylglutaryl coenzyme A (HMG-C0A) reductase catalyzes the rate-determining step in the biosynthesis of cholesterol (Siperstein and Fagan, 1966). Under certain conditions, HMG-CoA synthase controls the rate of cholesterogenesis (Ramachandran et al., 1978). It is demonstrated that cholesterol reduction in egg yolk using natural product like CPO is better than other cholesterol reducing drugs.

Tocopherol and tocotrienol concentrations in liver, plasma, egg yolk and adipose tissue

Our result demonstrated that hens fed on CPO had lower α- and γ-tocopherol concentrations in liver than hens fed on control diet. It is possible that control diet had higher α- and γ-tocopherol in diet than CPO diets. Since, α- and γ-tocopherol are riches in corn. γ-Tocopherol was a major form of vitamin E deposited in liver, but α- tocopherol was a major form of vitamin in plasma. There was close association of the vitamin E concentrations in liver, plasma, egg yolk and adipose tissue. Basically, vitamin E was absorbed in the intestine and entered the circulation via the lymphatic system. It is absorbed together with the chylomicrons and the remnants derived thereof (Reilly et al., 1996).

Lipids, including tocopherol, must be emulsified and solubilized before their absorption across the brush-border membrane of enterocyte. Emulsification begins in the stomach by predominantly mechanical forces that break up large emulsion particles into smaller particles. Within the small intestine, chyme mixes with pancreatic and biliary secretions, which are necessary for the efficient absorption of tocopherol. Pancreatic lipase is necessary for the hydrolysis of triglyceride in the small intestine to monoglycerides and fatty acids. These lipolytic products, together with bile salts (which are synthesized by the liver and secreted into the small intestine by gallbladder), form molecular aggregates known as mixed micelles, which are able to solubilize more hydrophobic molecules, such as tocopherol. These mixed micelles are able to transport tocopherol across the stirred water layer to the brush-border membrane of the enterocyte (Traber et al, 1993). Results of both in vitro (Hollander et al., 1975) and in vivo (Muvalidhara and Hollander, 1977) studies suggested that the uptake to tocopherol by the enterocyte takes place by passive diffusion. Thus, the process is non-saturable, non carrier-mediated, and unaffected by metabolic inhibitors and does not require energy. Within the enterocyte, tocopherol is incorporated into chylomicrons and secreted into portal vein, especially in fowl (Traber et al., 1988).

Total tocopherol of hens fed on 4% CPO was lowest in all tissues and plasma. α-Tocopherol was the major form of vitamin E deposited in liver and plasma when compared with other forms of tocotrienol. In 1999, Briglius-Flohe and Traber observed that α-tocopherol transfer protein in the liver specifically sorted out *RRR*-α from all incoming tocopherols for incoporation into plasma lipoprotein, and that α-tocopherol had signaling functions in vascular smooth muscle cell that cannot be exerted by other forms of tocopherol with similar antioxidative properties. The reason for the plasma preference for α-tocopherol was its specific selection by the hepatic α-tocopherol transfer protein (αTTP) (Hosomi et al., 1997).

The results demonstrated that hens fed on CPO had α-tocopherol deposited in egg higher than hens fed on lard, whereas the highest level γ-tocopherol was found in control group. In several literatures, it is shown that corn oil is the richest source of γ-tocopherol (412 mg/kg) (Gapor et al, 1983). The control and CPO1 diets had similar level corn of in (55.33% and 55.39%, respectively), which were higher than CPO2 and CPO3 diets (51.53% and 49.72%, respectively). This may explain more deposition of γ-tocopherol is control and CPO1 groups than others. The higher antioxidant vitamin, α-tocopherol, in egg yolk resulted from the addition of CPO in diet. The yellow corn is also a riches in β-carotene. It is well known that high intake of one fat-soluble vitamin can interfere with the utilization of other fat-soluble vitamins (Kang et al., 1998). High levels of β-carotene have been reported to reduce plasma and liver tocopherol (Bendich and Shapiro, 1986; Pellet et al., 1993). Kang et al. (1998) suggested that this might be explained by the competitive interaction between vitamins or their isomers for absorption.

The results showed that CPO significantly increased total tocotrienol concentrations in egg yolk with the highest concentration in hens fed on CPO2 and CPO3. The major form of tocotrienol in egg yolk was α-tocotrienol and γ-tocotrienol. Both forms increased markedly in egg yolk from hens fed on CPO diet compared to control diet. It is interested to note that α-tocotrienol deposited in egg in larger amount than γ-tocotrienol, which was contrasted to tocopherol. γ-Tocopherol deposited in the larger amount in egg yolk than

α-tocopherol. Our results were similar to Kang et al. (1998). The various levels of forms of vitamin deposited in egg yolk may result from the metabolism of vitamin E in liver on competitive absorption among forms of vitamin E.

Total tocotrienols, especially γ-tocotrienol was highest deposited in adipose tissue. α-Tocotrienol of hens fed on CPO, especially 4% CPO were highest deposited in egg yolk. Tocotrienol concentrations in plasma of hens fed on 4% CPO were associated with tocotrienol concentration in egg yolk and adipose tissue. It is demonstrated that tocotrienol in plasma could be transported and deposited in egg yolk and adipose tissue, although the mechanism was still unclear.

It is possible that the high tocotrienol in hens fed on CPO3 could compete with tocopherol for absorption and transportation into tissues. Kang et al. (1998) reported that egg yolk content of vitamin E (tocopherols + tocotrienols) increased by incorporating tocopherol mix or palm oil in diet. They suggested that the concentrations of vitamin E and carotenoids in the egg and liver were dependent upon their concentrations in the diet. This was expected because in hens, due to their rudimentary lymphatic system, liver is important in the metabolism of fat and fat-soluble vitamins. Apart from eggs, the adipose tissue incorporated the highest level of tocotrienols. The high levels when compared to liver suggested that tissue retention of tocotrienols might vary considerably. This difference in tissue incorporation could be due to differences in absorption, metabolic functions or oxidative status of the tissues (Kang et al., 1998). Adipose tissue may serve as the storage organ for regulating tocotrienol concentrations in plasma.

Tissue uptake occured by one of two ways: by lipase digesting the lipoprotein constituents or by " receptor mediated uptake " by binding of the lipoprotein to a specific tissue receptor site. This allowed for the vitamin to enter the tissue. Vitamin E enters a variety of different tissue types, with adipose tissue and adrenal gland having the highest levels. It is found primarily in mitochondria, vitamin can be stored in tissue for long periods of time (years) due to its exceedingly slow turnover rate.

In conclusion, the present study demonstrated that crude palm oil did not affect parameter on hen performance and egg performance. CPO supplementation at 4% is beneficial in improving FCR and increasing egg yolk color, egg weight and yolk weight compared to the control diet. Moreover, egg yolk cholesterol decreased markedly starting from week 4 to week 6 of the trial. Hens fed on 2% CPO had the highest tocopherol concentration in egg yolk and hens fed on 3% and 4% CPO had the highest α-tocotrienol concentration in egg yolk and the highest γ-tocotrienol concentrations in adipose tissue.