Determining Tocotrienol Rich Fraction Effects on Laying, Mortality and Egg Quality Parameters

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Abstract

The public interest and awareness in healthier lifestyle has increased the demand of functional food, nutraceuticals and designer foods, which has resulted in the creation of new foods in marketplace, for instance designer and specialty eggs. A feeding trial was carried out to evaluate the laying performance of H&N layer chickens fed diets supplemented with tocotrienol rich fraction (TRF), as well as to quantify tocopherol and tocotrienol accumulation in eggs and their effect on egg quality. A total of 300 H&N laying hens (17-wk old) were randomly allocated to 50 battery cages with 6 birds per cage. The birds were assigned equally to 6 different treatment diets; commercial diet (F1), corn-soy based diet (F2), corn-soy based diet added with 25ppm (F3), 50ppm (F4), 75ppm (F5) and 100ppm (F6) of tocotrienol rich fraction (TRF). Egg production and egg quality measurements were not significantly different (P>0.05) between treatments. Egg yolk from hens fed TRF supplemented diets contained more tocotrienol compared to those fed corn-soy based diet (P<0.05), however the accumulation of tocopherol in eggs between all the treatments was not significantly different (P>0.05). As feeding time progressed, the tocotrienol content tended to accumulate in the egg yolks (P<0.05).

Keywords: laying hens, tocotrienol, egg production

Introduction

Functional food can be described as food containing bioactive components that are beneficial to health and are able to reduce risk of chronic disease; however there is no official definition for functional foods used in Malaysia. The key words for functional food are fortified, enriched, altered and enhanced products, either with vitamins and/or minerals (Lau et al., 2013). The global health based food market, which includes functional food, has witnessed tremendous growth in recent times due to the increasing consumer health awareness, especially related to chronic diseases and ageing. These had encouraged studies to be carried out in order to create new food in the marketplace to meet the consumers' preference for healthy food that may also improve human health status. Consumers nowadays are willing to pay a premium for specialty food products that have enhanced nutritional value (Wong and Mardhati, 2015). Functional food are predominantly produced from animal products such as dairy and eggs as well as manufactured confectionery, soft drinks, bakery and baby food (Menrad, 2003).

Malaysia's egg production is projected to increase by 3% in 2014 (679,803 MT) from

2013 (659,664 MT). It is estimated that Malaysians consume 20 mill eggs every day (USDA, 2014) which are multifunctional food products. They are well known for their emulsification properties and also a good source of high quality protein, vitamin D, vitamin B and selenium. Eggs supply essential amino acids and minerals, including calcium and potassium. Several brands of eggs enriched with vitamins and/or minerals, or polyunsaturated fatty acids (PUFA) or organic eggs packaged with health-enhancing claims or eggs from hens raised in cage free, natural environments are available to the consumer (Cherian et al., 2002). The enrichment of some nutritive values in the eggs can be achieved by manipulating the layer diet, especially through feed supplementation. The most common way in enhancing the nutritive value of eggs is by enriching with vitamin E (Schiavone, 2011).

Vitamin E consists of two families of compounds which are tocopherols and tocotrienols. The members of each family are designated alpha (α), beta (β), gamma (γ) and delta (δ), according to the position of methyl groups attached to the chroman nucleus. The use of vitamin E as an antioxidant has been widely accepted as a preventive means to combat free radical formation, which reacts aggressively on tissues (Combs, 1992; Barby et al., 1999). Incorporating vitamin E into poultry diets tends to bring about oxidative stability of the eggs and reduce the development of off-flavors (Ajuyah et al., 1993). Vitamin E is also used in the poultry diet because of the reported benefits of its supplementation to laying hens during heat stress (Whitehead et al., 1998, Bollengier-Lee et al., 1998), and because vitamin E is reduced during heat stress (Feenster, 1985; Bollengier-Lee et al., 1999; Sahin et al., 2001). Poultry cannot synthesize vitamin E; therefore, vitamin E requirement must be met from dietary sources (Chan and Decker, 1994).

Palm oil derived from *Elaeis guineensis* represents the richest source of tocotrienol (Sen, 2010). Palm oil remains the undisputed world leader in oil and fat production, contributing 38.2 mill tonnes (25%) to the global output of 154 mill. tonnes. It also accounts for 51% of trade in oils and fats, or 29.5 mill. tonnes of 57.8 mill. tonnes of exports worldwide. Malaysian palm oil supplies 13.7 mill. tonnes (26%) of this volume, representing 47% of market share in palm oil (MPOC, 2008). The utilization of palm oil based products is to be expected to accelerate the local livestock industry. Therefore, this study was conducted to evaluate the laying performance of H&N layer chickens fed with diets formulated with addition of tocotrienol rich fraction and also to quantify the tocopherol and tocotrienol accumulation in eggs and their effect on egg quality characteristics.

Materials and Methods

House Management and Feeding Trial

A total of 432 pullets (17-wk old) were received from CP Chicken, Kulim for the study. These pullets were raised in 3-tier battery cages in Climatic Control Houses (CCHs) at Malaysian Palm Oil Board Research Station, Keratong, Pahang. The temperature in these houses was kept at 24-28°C, relative humidity was maintained at 70-85% and light was provided for 17 h per day. Pullets were fed with 6 different diets: commercial diet (F1), corn-soy based diet (F2), corn-soy based diet added with 25ppm (F3), 50ppm (F4), 75ppm (F5) and 100ppm (F6) of tocotrienol rich fraction (TRF). The minimal calculated nutrients in the typical corn-soybean meal diets were ME 11.60 MJ/kg, CP 16.70%, Calcium 3.90%, available Phosphorus 0.42%, Lysine 0.80%, Methionine 0.41%, Threonine 0.53%, Methionine+Cystine 0.75%, Sodium 0.15%, Chloride 0.18%, Linoleic acid 1.38% and Choline 1000 mg/kg. A mineral-vitamin premix was added at 0.3% to the ration. Each diet has 6 replicates with 12 birds per replicates. Water was supplied *ad-libitum*. Diets formulated were isocaloric and isonitrogenous. The birds were fed the same diets for the rearing period (17-23 wks) and laying period (24-56wks).

Laying Performance (% HDP)

Egg production was recorded daily for each treatment group throughout the trial. Egg production was expressed as hen-day production (%HDP). The mean was then calculated for every 4 wk of laying period. Each day, mortality was recorded. Mortality rate was recorded throughout the experimental period. Feed conversion ratio (FCR) was calculated as feed consumption (g) per hen per day divided by egg mass (g) per hen per day. The experiment lasted for 56 weeks.

Egg Quality

Eggs from every replicate (6 replicates per treatment) were collected from week 20 to week 73 at 4 week intervals to determine egg quality. Shell thickness was measured using a micrometer gauge and albumen height with a micrometer (AMES). Haugh unit (HU) was calculated using following formula (Haugh 1973):

$$HU = 100 \log_{10} (h - 1.7w^{0.37} + 7.6)$$

where,
h = observed height of the albumen in mm
w = weight of egg in g

Vitamin E and Fatty Acid Analysis

A total of 4 eggs sampled weekly from the 6 replicates for every treatment diet were subjected to further processing to extract tocotrienols from the yolk using technique described by Sundram and Rosnah (2000). Hexane extracted eggs yolk was subjected to detection and quantification of tocotrienols via HPLC (Waters Binary Pump 1525) with normal-phase 5µm silica column (4.6 x 250mm; Agilent Zorbax Rx-SIL). The mobile phase consisted of hexane:isopropanol with flow rate of 2ml/min. Tocotrienol content was determined using a fluorescent detector (Waters 2475) at 295 nm.

Statistical Analysis

Data collected were subjected to statistical analysis using ANOVA procedure of SAS and any difference between the treatments means was determined by Duncan Multiple Range Test using SAS 9.1 at P<0.05.

Results and Discussion

Hens' Performance and Egg Quality Characteristics

Egg production and egg quality measurements were not significantly different between treatment diets (Table 1). Mean % HDP of F2 was 79.96% followed by F1 (78.63%), F4 (78.30%), F3 (77.15%), F6 (76.48%) and F5 (74.80%). However the treatment diets supplemented with TRF produced lower number of eggs compared to commercial diet (80.96%). There was there was no significantly difference in FCR (Table 1) between the dietary treatments (P>0.05). These findings indicate that inclusion of TRF in the diets did not affect the ratio of feed consumption to egg production. F2 has the best FCR (2.21) among TRF supplemented treatments followed by F1 (2.22), F4 (2.24), F6 (2.28), F3 (2.28) and F5 (2.33).

There were no significant differences among all treatments in terms of shell thickness, yolk height and HU (Table 1). However, there were indications of improvement in shell thickness and HU value of birds fed experimental diets. F5 and F6 had thicker shells (0.40mm) compared to F1, F2, F3, F4 at 0.39, 0.39, 0.39, 0.38mm, respectively. Higher value of HU was shown in eggs produced by hens fed with F5 (81.31). The HU value for F1, F2, F3, F4, F5 and F6 were 79.52, 79.59, 81.03, 80.46, 81.31 and 80.56, respectively.

Table 1: Hen day production and egg quality of hens fed with different experimental diets

Parameter	Hen-day production (%) by diet						
	Commercial	F1	F2	F3	F4	F5	F6
Egg production (%)*	80.96 ± 13.41	78.63 ± 12.03	79.96 ± 11.77	77.15 ± 11.40	78.30 ± 12.77	74.80 ± 10.90	76.48 ± 11.12
FCR*	2.36 ± 0.91	2.22 ± 0.27	2.21 ± 0.15	2.28 ± 0.15	2.24 ± 0.23	2.33 ± 0.24	2.28 ± 0.21
Egg weight (g)*	64.01 ± 5.52	61.98 ± 5.54	62.33 ± 5.94	62.46 ± 5.64	62.64 ± 5.32	62.53 ± 5.42	62.68 ± 5.57
HU*	80.58 ± 9.67	79.52 ± 9.27	79.59 ± 9.05	81.03 ± 9.65	80.46 ± 9.36	81.31 ± 8.76	80.56 ± 8.84
Shell thickness (mm)*	0.38 ± 0.02	0.39 ± 0.02	0.39 ± 0.02	0.39 ± 0.02	0.38 ± 0.02	0.40 ± 0.02	0.40 ± 0.02

*Mean ± SD; No significant difference between treatments (P>0.05)

As shown in Figure 1, the egg production efficiency in hens decreased with time. Although there were some minor problems with the housing environmental control, the production of all of the treatments was within the H&N standard. At the later stage all diets were better in producing eggs than H&N standard but not in average value (Figure 1).

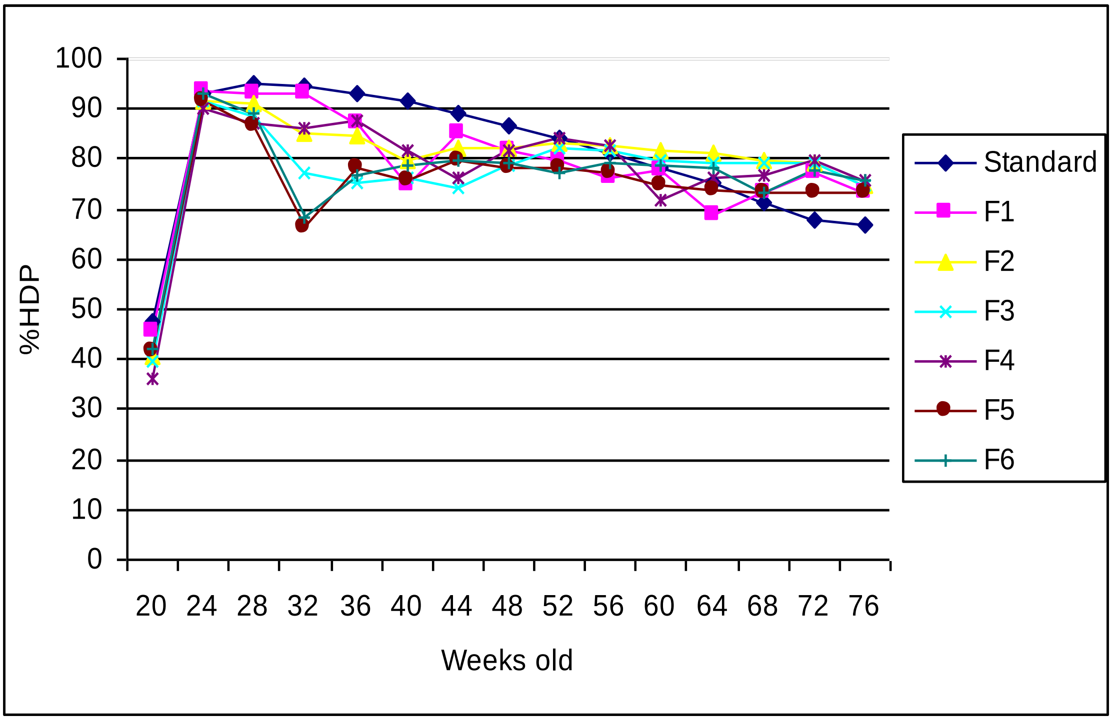

Figure 1: Laying performance of H&N layer chicken fed different experimental diets

The effect of treatments on mortality was significantly different (Table 2). The mortality rate was highest in F5 (1.06%) followed by F2 (0.92%), F3 (0.91%), F4 (0.82%), F1 (0.77) and F6 (0.75%). Even though supplementation of vitamin E (tocopherol and tocotrienol) is always associated with reducing birds stress, however, in this experiment, it can be clearly seen that the effect of that supplementation was not very effective during the early stage of feeding (Figure 1 and Table 2). The highest mortality rate in layer chickens rate can be related to the position of cages in the house as well.

According to Bozkurt et al. (2006), being located in the top row had adverse effects on pullet performance, and these effects became clearer when the pullets got older. One explanation is the higher light intensity at the top, which might be a stress on the birds housed there. It is much more difficult to provide uniformity of light intensity in cage operations, especially with multi-decked cages. This finding will be discussed further in the next experiment.

Table 2: Mortality rate for layer chickens

Feed	Mortality rate (%)
F1	0.77[c]
F2	0.92[ab]
F3	0.91[ab]
F4	0.82[b]
F5	1.06[a]
F6	0.75[c]

[abc] Means with different superscripts in the same column differ significantly(P<0.05)

Accumulation of Tocopherol and Tocotrienol

The content of tocopherol and tocotrienol in layer feed and eggs is shown in Table 3. TRF was incorporated into all feed formulation except F1 and F2. Table 3 shows that there was no significant difference in tocopherol content between all treatments (P>0.05). However the content increased with time (Figure 3). Tocotrienol content in eggs differed among the various treatments (Table 3). All data was collected starting at the age of hens at 20 wk-old equivalent to 2 wk of feeding period. The tocotrienol content in eggs increased with time although there was a declining trend at certain week (Figure 2). Addition of palm tocotrienol into diets gave an increasing effect on tocotrienol content in layer hen eggs. Laying-hens are very efficient at transferring nutrients from their feed to the resulting eggs. This is because the fat content in yolk can enhance absorption of lipid soluble vitamin (Lanari et al., 2004).

Results showed that without adding tocotrienol, layer feed still contained tocotrienol but in lower quantity. This is probably due to the inclusion of crude palm oil (CPO), which is rich in vitamin E (tocopherol and tocotreinol), in commercial feed formulations. According to Qureshi et al. (1996), α-tocopherol may interfere with tocotrienol absorption and bind preferentially to the α-tocopherol transfer protein, resulting in a more rapid clearance of tocotrienol. Hosomi et al. (1997) determined the affinity of different vitamin E analogs for the α-tocopherol transfer protein in vitro. They showed that the α-tocopherol’s binding capacity was 8–11 times higher than that of α-tocotrienol (Hosomi et al., 1997). Incorporation of TRF in layer diet elevated tocopherols content in eggs, but lower amount of tocotrienols was detected in eggs. These data suggested that the transfer efficiency, transport, tissue uptake or stability of tocopherols and tocotrienols are different. Alpha-tocopherol being the most bioavailable could dominate the absorption, causing poor absorption of the tocotrienols (Hansen 2015).

Table 3: Mean tocopherol and tocotrienol content in eggs of chickens fed various treatment diets throughout the experimental period

Treatment		Tocopherol content (ppm)	Tocotrienol content (ppm)
Feed	F1	9.80±0.91	9.81[b]±0.98
	F2	7.52±0.61	10.46[b]±0.55
	F3	6.22±0.30	15.05[a]±0.84
	F4	6.83±0.23	17.08[a]±0.61
	F5	8.13±0.21	18.29[a]±0.97
	F6	9.77±0.15	19.12[a]±0.94
Egg	F1	19.32±0.20	0.82[b]±0.97
	F2	20.31±0.16	0.93[ab]±0.10
	F3	20.12±0.31	1.07[ab]±0.03
	F4	20.87±0.42	1.24[ab]±0.09
	F5	21.34±0.60	1.45[a]±0.07
	F6	21.09±0.57	1.45[a]±0.17

[ab]Means with different superscripts in the same row are significantly different (P<0.05)

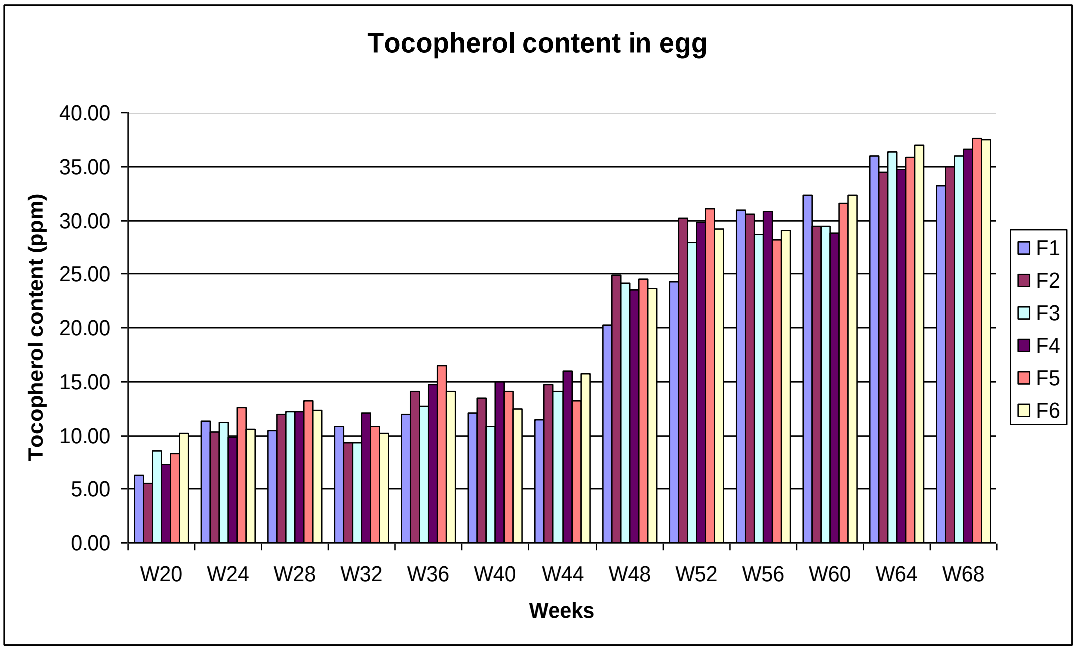

Figure 2: Tocopherol content in eggs

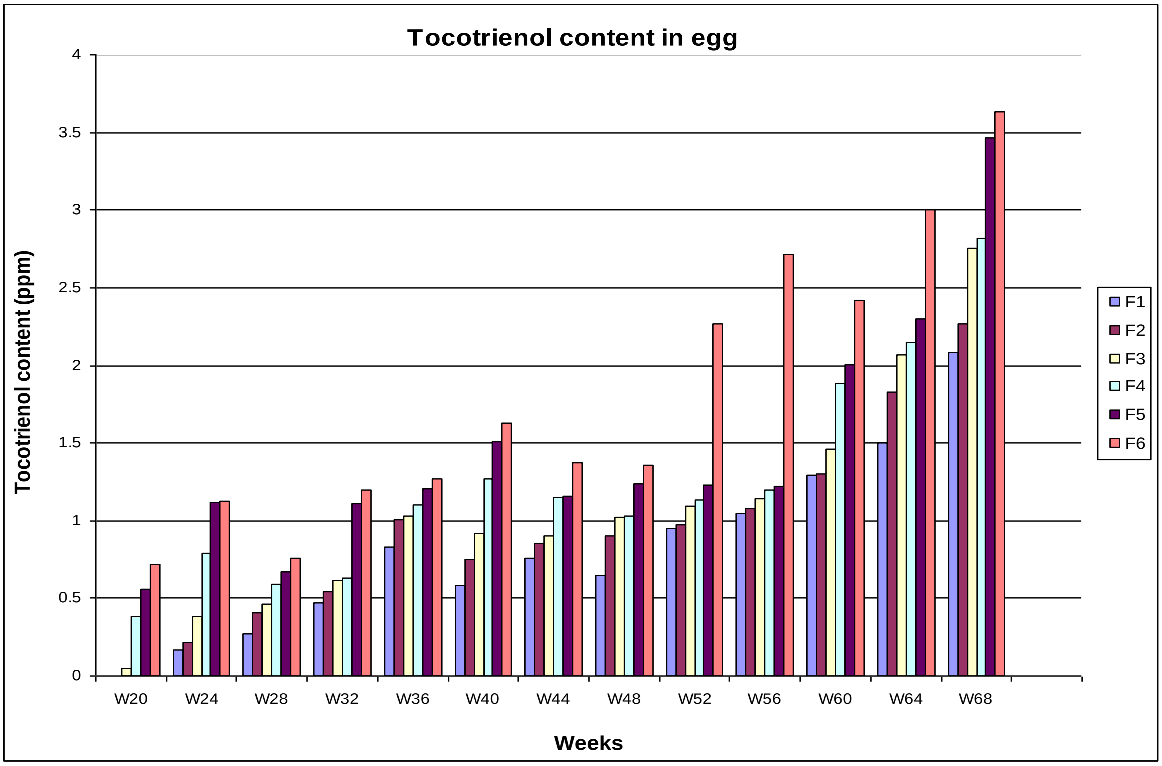

Figure 3: Tocotrienols content in eggs

The vitamin E (tocopherol and tocotrienol) content of eggs from hens fed the commercial diet was lower than eggs from hens fed the TRF diets. Result indicates a greater content of vitamin E in eggs when hens were fed 200ppm of tocotrienol in the diet. A number of investigators also found that the content of vitamin E in eggs increased linearly according to its level in the diet (Jiang et al., 1994; Melluzi et al., 2000; Griffin 1992). Qi and Sim (1995) observed that the efficiency of vitamin E transferred from diet to eggs ranged from 20 to 40%, reaching the maximum deposition about 1 week after vitamin E level was increased.

Conclusion

The result in this study clearly indicates that it was technically feasible to use TRF in the formulation of layer diets. MPOB-HIE can be used as an effective source of energy for laying hens. However improvements in HDP cannot be expected but birds fed TRF can produce tocotrienol enriched egg with improved egg quality.

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