



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF CAROTENES AND ALPHA-TOCOPHEROL IN CRUDE
PALM OIL ON LAYER AND BROILER CHICKEN PERFORMANCE***

YEASMIN AKTER

FP 2015 94



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By

YEASMIN AKTER

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

January 2015

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DEDICATION

***TO MY BELOVED MOTHER, FATHER,
DAUGHTER AND HUSBAND***



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the Degree of Doctor of Philosophy

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January 2015

Chairman: Associate Professor Azhar Kasim, PhD
Faculty: Agriculture

Commercial poultry producers use vegetable oils in poultry diets to supply a higher amount of dietary energy at an economically justifiable cost. Vegetable oils such as corn oil, canola oil and soybean oil are rich in unsaturated fatty acids (UFAs) which are more prone to lipid peroxidation. Oxidation of lipid causes serious damage to the poultry and its products. For this reason, poultry diets with an increased amount of vegetable oils should be supplemented with antioxidants, so that they may provide protection to the poultry and its products from oxidative damage. Crude palm oil (CPO) contains a vast amount of natural antioxidants such as carotenoids and vitamin E. The combination of high antioxidants and saturated fatty acids gives palm oil a higher oxidative stability than other vegetable oils. In this context, the objectives of this study were to investigate the antioxidant potency of CPO when fed to chicken and its effects on laying performance, egg quality, hatchability, and meat quality. To achieve the objectives, five experiments were conducted at the Poultry Unit, Department of Animal Science, Universiti Putra Malaysia.

In the first experiment, CPO was supplemented in layer diets at 0, 1.5, 3 and 5% levels, and the diet with 3% CPO significantly increased ($P<0.05$) egg production (88.89%) and improved feed conversion ratio (1.92). Yolk color score and carotene concentrations were significantly increased ($P<0.05$) with increasing levels of CPO in the diet, whereas lipid peroxidation (TBARS) values in yolk followed the opposite ($P<0.05$) trend. Diet containing 3% CPO showed maximum deposition of α -tocopherol (96.99 $\mu\text{g/g}$) in the yolk, while yolk cholesterol and serum lipid profiles were not significantly influenced ($P>0.05$) by dietary CPO. In the second experiment, experimental birds were assigned to three dietary treatments namely, control, 3 and 5% CPO. The inclusion of 3% CPO in the maternal diet significantly increased ($P<0.05$) fertility (87.22%) and hatchability (77.31%). Liver and muscle carotene concentrations (5.29 and 3.12 $\mu\text{g/g}$) of day old chicks from the 5% CPO fed hens were highest ($P<0.05$), while the maximum ($P<0.05$) deposition of α -tocopherol (25.41 $\mu\text{g/g}$) was found in the muscle of day old chicks obtained from the 3% CPO fed group. The lipid peroxidation (TBARS) values in the liver of day old chicks were significantly decreased ($P<0.05$) by the CPO supplemented maternal diets. In the third experiment, the hatching eggs produced during the second experiment were stored at 18 °C for 7, 10 and 14 days to observe the effect of storage

duration and carotenes and α -tocopherol in CPO on egg quality, hatchability and chick quality. The concentration of TBARS in stored eggs was significantly higher ($P<0.05$) in the control group compared to the CPO treated groups. Hatchability rate decreased with increased storage time, while the highest ($P<0.05$) hatchability rate was noticed in the 3% CPO followed by the 5% CPO and control groups. The fourth and fifth experiments were conducted to evaluate the influence of carotenes and α -tocopherol in CPO on carcass characteristics and meat quality of slow and fast growing broiler chicken. The oxidative stability, water holding capacity and tenderness value of meat from both slow and fast growing chickens were improved ($P<0.05$) by the dietary CPO. In slow growing chicken, meat produced in the 5% CPO fed group deposited maximum ($P<0.05$) amount of carotene (1.48 and 1.41 $\mu\text{g/g}$), whereas α -tocopherol content (15.34 and 23.93 $\mu\text{g/g}$) was highest in the meat of chicken fed 3% dietary CPO compared to other diets. In contrast, feeding fast growing broiler chicken with 4% CPO showed highest carotene (1.67 and 1.75 $\mu\text{g/g}$) and α -tocopherol concentrations (10.05 and 11.20 $\mu\text{g/g}$) in the meat than those fed other diets. However, the cholesterol and saturated fatty acids (SFAs) contents in meat from both slow and fast growing chickens did not show any remarkable alteration ($P>0.05$) due to CPO in chicken diets.

The results of this study indicate that supplementation of 3-4% CPO in the chicken diets could be effective for increasing egg production, feed efficiency and for improving fresh and stored egg quality, hatchability and meat quality of chicken. Therefore, dietary CPO could be an alternative, effective and natural way in reducing the chance of lipid peroxidation of poultry and its products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN KAROTIN DAN ALFA-TOKOFEROL DALAM MINYAK SAWIT MENTAH KE ATAS PRESTASI AYAM PENELUR DAN AYAM DAGING

Oleh

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Januari 2015

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Pengeluar ayam komersial menggunakan minyak sayuran dalam diet ayam untuk membekalkan tenaga pemakanan dalam jumlah yang lebih tinggi pada kos yang wajar dari segi ekonomi. Minyak sayuran yang lazim digunakan seperti minyak jagung, canola dan kacang soya kaya dengan asid lemak tak tepu (UFAs) yang lebih cenderung kepada peroksidaan lipid. Lipid teroksida menyebabkan kerosakan serius kepada ayam dan produk-produknya. Atas sebab ini, diet ayam yang mengandungi jumlah minyak sayuran yang meningkat hendaklah ditambah dengan antioksidan, supaya ia dapat memberi perlindungan kepada kedua-dua ayam dan produk-produknya daripada kerosakan oksidatif. Minyak sawit mentah (MSM) telah dikenalpasti mengandungi sejumlah besar antioksidan semula jadi seperti karotenoid dan vitamin E. Gabungan antioksidan yang tinggi serta asid lemak tepu memberikan MSM kestabilan oksidatif yang lebih tinggi berbanding minyak sayuran lain. Dalam konteks ini, objektif kajian ini adalah untuk mengkaji potensi antioksidan MSM apabila diberi makan kepada ayam dan kesannya terhadap prestasi peneluran, kualiti telur, penetasan, dan kualiti daging. Bagi mencapai objektif, lima eksperimen telah dijalankan di Unit Ternakan, Jabatan Sains Haiwan, Universiti Putra Malaysia.

Dalam eksperimen pertama, MSM telah ditambah dalam diet pada tahap 0, 1.5, 3 dan 5%. Diet dengan 3% MSM meningkat pengeluaran telur (88.89%) dengan ketara ($P < 0.05$) dan nisbah penukaran makanan (1.92). Warna yolka dan kepekatan karotena dalam yolka meningkat dengan ketara ($P < 0.05$) mengikut peningkatan aras MSM dalam diet. Peroksidaan lipid (TBARS) dalam yolka menunjukkan aliran bertentangan ($P < 0.05$). Diet yang mengandungi 3% MSM menunjukkan kandungan maksimum α -tokoferol (96.99 $\mu\text{g/g}$) dalam yolka. Kajian mendapati paras kolesterol yolka dan profil lipid serum tidak dipengaruhi dengan ketara ($P > 0.05$) dengan diet MSM. Dalam eksperimen kedua, induk ayam telah diberikan tiga rawatan pemakanan iaitu, kawalan, 3 dan 5% minyak sawit mentah. Kemasukan 3% MSM dalam diet induk meningkat kesuburan dengan ketara ($P < 0.05$) (87.22%) dan penetasan (77.31%). Kepekatan karotena dalam hati dan otot anak ayam satu hari dari induk betina yang menerima rawatan 5% MSM adalah paling tinggi ($P < 0.05$) manakala kandungan α -tokoferol didapati paling maksimum ($P < 0.05$) dalam otot (25.41 $\mu\text{g/g}$) dalam anak ayam satu hari dari induk yang menerima rawatan 3% MSM. Nilai peroksidaan lipid (TBARS) dalam hati anak ayam berusia sehari telah

menurun dengan ketara ($P < 0.05$) dari induk yang menerima diet yang ditambah MSM. Dalam eksperimen ketiga, ujian penetasan telur yang disimpan pada 18°C selama 7, 10 dan 14 hari untuk melihat kesan jangka masa penyimpanan dan karotin dan α -tokoferol dalam MSM kepada kualiti telur, penetasan dan kualiti anak ayam. Kepekatan TBARS dalam telur yang disimpan adalah jauh lebih tinggi ($P < 0.05$) dalam kumpulan kawalan berbanding kumpulan MSM dirawat. Kadar penetasan menurun apabila masa penyimpanan meningkat. Peratusan penetasan paling tinggi ($P < 0.05$) diperolehi dalam telur dari induk yang menerima 3% MSM diikuti dengan 5% MSM dan kawalan. Eksperimen keempat dan kelima telah dijalankan untuk menilai pengaruh karotin dan α -tokoferol dalam MSM kepada ciri dan kualiti badan ayam dari ayam yang membesar dengan perlahan dan cepat. Kestabilan oksidatif, keupayaan pegangan air dan kelembutan nilai daging ayam dari ayam yang membesar dengan perlahan dan cepat bertambah baik ($P < 0.05$) dari kumpulan yang menerima diet yang diperkaya dengan MSM. Dalam ayam yang lambat membesar, daging yang dihasilkan dalam kumpulan 5% MSM mendepositkan jumlah karotena (1.48 and 1.41 $\mu\text{g/g}$) maksimum ($P < 0.05$). Kumpulan yang menerima 3% MSM mempunyai kandungan α -tokoferol (15.34 and 23.93 $\mu\text{g/g}$) adalah paling tinggi dalam daging dengan diet yang lain. Sebaliknya, bagi ayam yang cepat membesar rawatan 4% MSM menunjukkan kepekatan karotena (1.67 and 1.75 $\mu\text{g/g}$) dan α -tokoferol (10.05 and 11.20 $\mu\text{g/g}$) tertinggi dalam daging berbanding dengan rawatan diet yang lain. Walaubagaimanapun, kolesterol dan kandungan asid lemak tepu dalam daging ayam yang lambat membesar dan cepat membesar tidak menunjukkan apa-apa perubahan ketara ($P > 0.05$) disebabkan oleh tambahan MSM dalam diet ayam.

Keputusan kajian ini menunjukkan bahawa suplementasi 3-4% MSM dalam diet ayam boleh berkesan untuk meningkatkan pengeluaran telur, kecekapan makanan dan untuk meningkatkan segar dan disimpan kualiti telur, penetasan dan kualiti daging ayam. Oleh itu, pemakanan MSM boleh menjadi satu alternatif, cara yang berkesan dan semulajadi dalam mengurangkan peluang pengoksidaan lipid dan untuk menstabilkan ayam dan produk-produknya.

ACKNOWLEDGEMENTS

First of all, praise to the almighty Allah for giving me the health, spirit and patience to complete this work successfully.

I would like to express my sincere gratitude to my highly respected supervisor, Associate Professor Dr. Azhar Kasim, Chairman of the Supervisory Committee, for his valuable advice, patience, enthusiastic guidance, willingness to help, encouragement, kindness and constructive comments during the years of my study and research. I thank him for his endless support during the preparation of the thesis.

I wish to express my thanks to the members of my supervisory committee, Dr. Awis Qurni Sazili, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM) and Dr. Hishamuddin Omar, Department of Biology, Faculty of Science, UPM for their encouragement, constructive discussion, excellent advice, comments, and suggestions throughout the project.

I would like to extend my deepest and sincere appreciation to the Organization for Women in Science for the Developing World (OWSD), Trieste, Italy and Swedish International Development Cooperation Agency (SIDA) for providing me the Ph.D Fellowship without which I could not complete my study.

Many thanks are extended to the Head of the Department, all lecturers and staff members of the Department of Animal Science, Faculty of Agriculture, UPM. I would like to acknowledge all staff of the Nutrition Analytical Laboratory, Meat Science Laboratory and Poultry Unit for their technical assistance, kind co-operation and help during the field study. My sincere appreciation goes to Dr. Mahdi Ebrahimi, Physiology Department, Faculty of Veterinary Medicine for his helpful efforts and valuable advice during my laboratory analysis.

I would like to express my appreciation to my loving, caring mother, Anjira Begum and father, Sheikh Md. Ahsanur Rashid who advised and supported me in my pursuit for higher education and expressed understanding and consideration towards me. Words cannot express my gratitude for their love, support and patience that have sustained me during my life and study. My deepest gratitude is extended to my husband, Dr. Md. Abul Kalam Azad for his patience, encouragement and support throughout the course of my study. My heartfelt thanks and appreciation goes to my understanding daughter Adiba Azad, whom I am indebted for her sacrifice, patience and understanding, throughout the course of my study. I would like to extend my special sincere thanks to my younger sister, Dr. Mahzabin Akter and brother, Eng. Sheikh Mizanur Rashid for their encouragement and support.

Many thanks are due to all of my friends for their co-operation and mental and physical support during the study, among them Dr. Zeiad Amjad Aghwan, Kamal Abu Alfath, Dr. Rakibul Islam, Farhana Yasmin and others.

I certify that a Thesis Examination Committee has met on 12 January 2015 to conduct the final examination of Yeasmin Akter on her thesis entitled " Effects of Carotenes and Alpha-Tocopherol in Crude Palm Oil on Layer and Broiler Chicken Performance" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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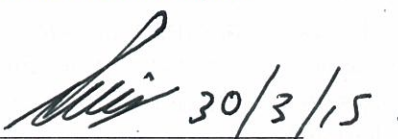
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LIST OF ABBREVIATIONS

a*	Redness
b*	Yellowness
BHT	Butyated hydroxyl toluene
Ca	Calcium
CHD	Coronary heart disease
cm	Centimetre
CP	Crude protein
CPO	Crude palm oil
d	Day
DFD	Dry, firm and dark
DHA	Docosahexaenic acid
DM	Dry matter
EE	Ether extract
FAME	Fatty Acid Methylation Esters
FCR	Feed conversion ratio
FFB	Fresh fruits bunch
g	Gram
GC	Gas chromatography
h	Hour
H ₂ SO ₄	Sulfuric acid
HCL	Hydrochloric acid
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
HU	Haugh unit
kcal	Kilocalorie
kg	Kilogram
KOH	Potassium hydroxide
L	Litre
L*	Lightness
LA	Linoleic acid
LDL	Low density lipoprotein
MDA	Malonaldehyde
ME	Metabolizable energy
mg	Milligram
ml	Millilitre
mm	Millimetre
mmol	milimole
MUFA	Monounsaturated fatty acid
N	Nitrogen
P	Phosphorus
PO	Palm oil
ppm	Parts per million
PSE	Pale Soft Executive
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
Se	Selenium
SE	Standard error
SFA	Saturated fatty acids

TBA	Thiobarbituric Acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
UFA	Unsaturated fatty acid
α -LNA	α -Linolenic acid
μ g	Microgram



CHAPTER 1

INTRODUCTION

Poultry eggs and meat play an important role in the human diet because they are healthy, nutritious and protein rich food for all categories of people. For this reason, in recent years, poultry production has not been considered as conventional farming, but considered as an industry to produce poultry products for the ever growing human population. Therefore, each producer has to be judicial in order to survive in the business. From that point of view, poultry producers are trying to improve the productivity of birds to ensure a sufficient supply to consumers at reasonable prices. Globally, corn and wheat are the main sources of dietary energy for poultry, but the cost and availability of these grains have made them expensive feed components in poultry diets, and as a result, commercial poultry producers are trying to incorporate vegetable oils or fats in poultry diets to supply higher amounts of metabolizable energy (ME) at a lower cost. Poultry can utilize high fat containing diets and deposit more energy compared to the same diet with low fat content (Hurwitz *et al.*, 1980). However, the composition and the stability of the dietary oils or lipid fractions seriously affect the quality and sensory characteristics of eggs and meat. Vegetable oils contain higher amounts of unsaturated fatty acids (UFAs) such as linoleic acid, oleic acid, and α -linolenic acid (Haggag *et al.*, 2014), and some of these fatty acids are essential for both humans and animals. It is well reported that oils rich in UFAs are more prone to oxidation by free radical chain mechanism (Heidar *et al.*, 2010, Yasin *et al.*, 2012). Oxidation causes serious damage to cells and cellular components which may affect the growth, development and reproduction of chicken and reduce the overall quality of poultry products (Jiang *et al.*, 1992; Hayat *et al.*, 2010). Oxidative products create cardiovascular and atherogenesis diseases in the human body. It also increases liver and kidney weights, and modify fatty acid composition in tissue lipids, cardiac fibrotic and hepatic bile duct lesions. Oxidative products are very reactive in cross-linking reactions with DNA and proteins. For this reason, poultry diets containing vegetable oils should be supplemented with antioxidants to protect the birds and its products from free radicals and oxidative damages (Barroeta, 2007; Surai and Fisinin, 2012). In nature there are thousands of antioxidants which can neutralize free radicals and prevent oxidation effectively (Panda and Cherian, 2014). Antioxidants react with free radicals and produce less reactive molecules and prevent oxidation of biological molecules (Surai, 2007). Reactive oxygen species (ROS) are highly reactive oxidants which can be converted to free radicals and initiate lipid peroxidation and damage polyunsaturated fatty acids (PUFAs).

Carotenoids and vitamin E are potential antioxidants that can minimize oxidative damage and contribute significant defenses within the body against free radical attack (El-Agamey *et al.*, 2004; Fotina, *et al.*, 2013). In poultry, the carotenoids and vitamin E are widely distributed in the eggs and have a strong correlation with the quality of eggs (Blount *et al.*, 2000). They could be transferred from the feed to the egg yolk and improve oxidative stability, egg quality, and reduce the development of undesirable flavours while increased egg production (Cherian *et al.*, 1996; Surai and Fisinin, 2012). Recently, carotenoids and vitamin E have been widely used in poultry rations for improving productivity and reproductive performance of birds (Fisinin and Surai, 2011; Panda and Cherian, 2014).

The hatching process is a critical period for developing chicks because during this process, considerable deposition of PUFAs occurs within the embryonic tissue and the rate of oxidation increases drastically specially at the 19th day of embryonic development and the first day of post hatched when the risk of oxidative damage is very high (Khan, 2011). During this time a portion of the yolk carotenoids and vitamin E may be used by the embryo, presumably in antioxidant reactions (Surai and Fisinin, 2012). Increased carotenoids and vitamin E concentrations in the chicken embryo increase the oxidative stability of embryonic tissues (Fotina *et al.*, 2013). Carotenoids and vitamin E play an important role in signaling and patterning of cells during embryonic development (Vermot and Pourqui'e, 2005). Eggs enriched with maternal carotenoids and vitamins E are also strongly linked to hatching success, disease resistance and oxidative stress (Tyndale *et al.*, 2008). Hence, hatching eggs should contain maximum amount of antioxidants as accumulated antioxidants in eggs are very critical factors in determining the reproductive fitness and hatching successes (Fisinin *et al.*, 2008).

Storing of hatching eggs is a common practice in commercial hatcheries. During storage of eggs, the albumen pH raises which is closely related to the degradation of albumen quality (Stadelman, 1995) or Haugh unit (HU). A decrease in albumin height and weight of eggs during storage results in decreased egg weight (Jones and Musgroove, 2005). Another important change during storage of eggs is the weakening of the vitelline membrane (Fromm and Matrone, 1962). Storage of eggs before incubation is often associated with lipid peroxidation within the egg membrane, particularly due to unsaturated oils or fats or fatty acids (Fisinin and Surai, 2011). Aging of hatching eggs for 7 days does not affect hatchability, but hatchability decreases remarkably after storage for 8 days or more (Fasenko *et al.*, 1992, 2001). Chicken eggs stored for 14 days increases embryonic mortality at first and last weeks of incubation (Fasenko *et al.*, 2001). Therefore, in order to retain egg quality and fatty acid stability during aging, it is important to protect or minimize lipid peroxidation in stored eggs (Fisinin and Surai, 2011). Inclusion of increased amounts of antioxidants can be effective to inhibit damaging effects of free radicals produced within the stored eggs, but low antioxidants with high temperatures, humidity and PUFAs increase the chance of lipid peroxidation in eggs (Surai, 2007).

Poultry meat is more susceptible to oxidation than red meat due to its higher content of phospholipids (Ali and Zahran, 2010). Unsaturated fatty acids of meat phospholipids oxidize easily and affect meat quality and consumer health (Kemin Europa, 2009; Mapiye *et al.*, 2012). However, the oxidation of lipids and the production of free radicals occur naturally in meat and changes the colour, flavour, taste, texture and nutritional value of meat (Karami *et al.*, 2011), which consequently determines meat quality to a wide extend (Augustini, *et al.*, 1998). The volatile lipid oxidation products strongly reduce the consumers' acceptability of the product (Ladikos and Lougovois, 1990; Sample, 2013). Dietary antioxidants are very effective for controlling lipid peroxidation in meat (Ao *et al.*, 2011). There are many scientific evidences that the quality characteristics of animal originated foods (such as colour, oxidation, tenderness, and storage properties) can be improved by dietary antioxidants (Flachowsky *et al.*, 2003). Carotenoids such as β -carotene with vitamin E or α -tocopherol significantly inhibit oxidation of lipids and their joint influence is synergic (Palozza and Krinsky, 1992), but some of these synthetic antioxidants have some adverse effects on health and they may also increase the cost of production

(Moyo *et al.*, 2011; Karre *et al.*, 2013). So, cheaper alternate natural antioxidant sources are needed for stabilizing prices of poultry products.

Crude palm oil (CPO) can be effectively used as a source of dietary energy and natural antioxidants in poultry feeding. It contains about 600-1000 ppm carotenoids and vitamin E (Goh *et al.*, 1985), which are very cheap and are potential natural free radical scavengers (King *et al.*, 1995). They can inhibit the formation of oxidative by-products and subsequently prolong the shelf life of poultry products. In addition, CPO contains about 50 percent saturated fatty acids which gives some stability against oxidation to the oil as compared to other vegetable oils (Basiron, 2005). However, in recent years a large number of studies have been conducted on dietary CPO in fish species (Bell *et al.*, 2002; Ng *et al.*, 2003, 2004), but very few reports are available on the effect of carotenes and α -tocopherol in CPO in poultry nutrition. Therefore, the objectives of this research were to examine the antioxidant potency of CPO fed to chickens and its effects on laying performance, egg quality, hatchability and meat quality.

Hypothesis statements

1. Supplementing CPO in the layer diet will increase laying performance, feed efficiency, yolk colour score as well as carotene and α -tocopherol contents in egg.
2. Incorporation of CPO in the maternal diet will have positive effect on fertility, hatchability and post-hatched mortality of chicks.
3. Inclusion of carotenes and α -tocopherol rich CPO in the layer diet will affect the egg weight loss, oxidative stability and hatchability of stored hatching eggs.
4. Dietary CPO will have positive effect on colour, water holding capacity, tenderness value, oxidative stability of chicken meat.

General objectives

The general objectives of this research were to determine the effect of carotene and α -tocopherol, on bird's productive and reproductive performance, egg and meat quality by incorporating CPO with the layer and broiler diets.

The specific objectives of the research were as follows:

1. To evaluate the influence of carotenes and α -tocopherol in CPO on laying performance, egg quality and blood lipid profile of layer chicken.
2. To observe the attribute of CPO as a source of carotenes and α -tocopherol on fertility, hatchability, chick quality, post hatched tissues carotenes and α -tocopherol status and post hatched performance.
3. To examine the effect of storage duration and carotenes and α -tocopherol in CPO on hatching eggs quality, hatchability and chick quality.

4. To evaluate the attributes of carotenes and α -tocopherol in CPO on carcass characteristics and meat quality of slow and fast growing broiler chicken.



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