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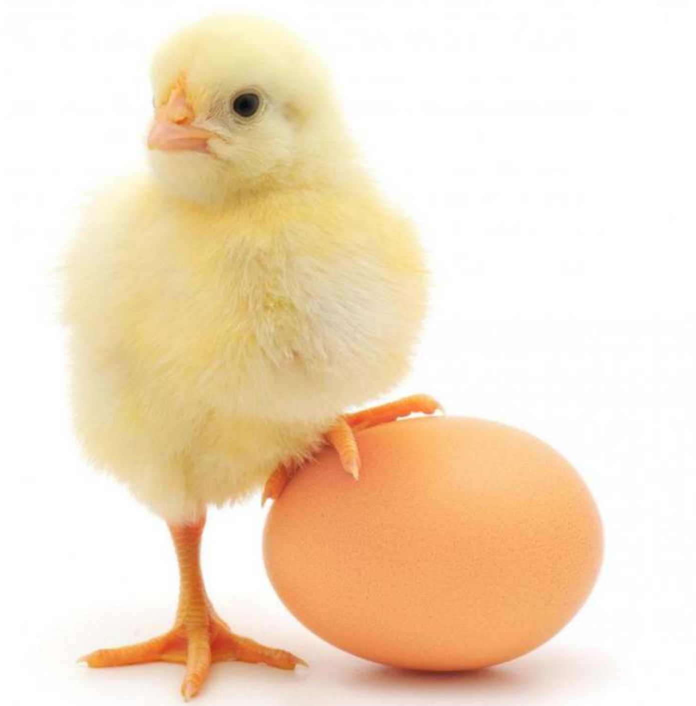

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Effect of Type of Grain and Oil Supplement on the Performance, Blood Lipoproteins, Egg Cholesterol and Fatty Acids of Laying Hens

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Abstract: The effects of type of grain (wheat *vs.* sorghum) and oil supplement/kg diet [0, 20g olive oil (OL), 20g safflower oil (SO), 10 g OL plus 10 g SO (OLSO)], over a 12-week period on the performance, plasma and lipoproteins lipids [cholesterol (C), triglycerides (TG), phospholipid (P)], and yolk C and fatty acids concentrations of laying hens were studied. Hens fed on the sorghum diet had significantly ($P<0.05$) higher yolk oleic acid concentration and oleic:linoleic acid ratio and plasma TG concentration in the low density plus high density lipoproteins (LDL plus HDL) fraction when compared with hens fed on the wheat diet. Birds fed on the OL diet had significantly ($P<0.05$) higher yolk oleic acid concentration and oleic:linoleic acid ratio, and plasma TG concentration in the VLDL fraction and lower plasma TG concentration in the LDL plus HDL fraction ($P<0.01$) when compared with those fed on the control diet. Hens fed on the SO diet had significantly ($P<0.05$) higher concentration of yolk linoleic acid and plasma lipids in the very low density lipoproteins (VLDL) fraction and lower yolk oleic:linoleic acid ratio and plasma TG concentration in the LDL plus HDL fraction ($P<0.01$) than hens fed on the control diet. Whilst, birds fed on the OLSO diet produced significantly ($P<0.05$) higher yolk unsaturated:saturated fatty acids ratio and lower palmitic and stearic acids concentrations when compared with those fed on the control diet. Dietary treatments did not significantly affect the weight gain, feed intake, rate of lay, egg and yolk weights, yolk lipid and C concentrations, daily C output, plasma total lipid concentration, plasma C and P concentrations in the LDL plus HDL fraction of hens. It was concluded that hens fed a sorghum based diet or a diet supplemented with olive oil produced eggs with high concentration of oleic and oleic:linoleic acid ratio and that hens fed a diet supplemented with safflower oil produced eggs with high concentration of linoleic and lower oleic:linoleic acid ratio and that hens fed a diet supplemented with olive plus safflower oils produced eggs with high concentration of unsaturated:saturated fatty acid ratio and lower palmitic and stearic acids.

Key words: Egg production, olive oil, safflower oil, yolk cholesterol, yolk fatty acids, plasma lipoproteins

Introduction

There is growing evidence that diet and health relationships are a function of both what is in the diet and what is missing from it. Consumers limit their intake of eggs because of adverse publicity about saturated fats and cholesterol (C). Health professionals suggest decreasing saturated fat intake is important. Consumption of polyunsaturated fatty acid has been reported to reduce the risk of atherosclerosis and stroke (Iso *et al.*, 2002; Lada and Rudel, 2003). Monounsaturated and polyunsaturated fats may lower blood C levels when they replace saturated fat in the diet. Howell *et al.* (1997) investigated the relationship between diet and blood C levels and found that saturated fat in the diet, not dietary C, is what influences blood C levels the most. Modification of egg yolk C and fatty acids contents requires better understanding of factors that influence the deposition of C and fatty acids in egg yolk.

Type of grain (Shafey *et al.*, 1992) and composition of fatty acids in the diet of laying hens (Marion and Edwards, 1964; McDonald and Shafey, 1989; Shafey and Dingle, 1992, Shafey *et al.*, 1999) are known to influence fatty acid profile of the egg yolk. Yolk lipid is not difficult to manipulate and to enrich the egg with mono and polyunsaturated fatty acids through dietary change. However, dietary fatty acids have a marked effect on fat and C metabolism. The presence of unsaturated fatty acids increases C and phospholipid (P) synthesis, while saturated fatty acids had little effect (Weiss *et al.*, 1967; Hargis, 1988). Lipids are synthesized in the liver of a laying hen and transported to the ovary by lipoproteins. Lipoproteins serve as precursors of egg yolk lipid, and plasma very low-density lipoproteins (VLDL) are the major components of egg yolk (Chapman, 1980). C is largely synthesized in the liver and like lipids, transported to the growing follicles primarily in the VLDL (McDonald and Shafey, 1989). Despite extensive research, little progress has been made in reducing C content of eggs (Hargis, 1988; McDonald and Shafey, 1989,1990; Shafey *et al*., 1999).

Wheat and sorghum are excellent feed ingredients and used as major grains in poultry diets. However, the effect of wheat and sorghum on plasma lipoproteins composition and egg yolk C and fatty acids has not been investigated. Therefore, this study was designed to investigate the effects of dietary type of grain (wheat vs. sorghum) and oil supplement [olive (OL) vs. safflower (SO)] on the performance, plasma lipids and lipoproteins, and egg yolk C and fatty acids of laying hens. Olive and safflower oils were added to the diet of laying hen to alter dietary content of oleic and linoleic acids.

Materials and Methods

A total of 480, Tegal strain birds (White Leghorn x New Hampshire), 36 weeks of age, were weighed and randomly housed in 40 blocks of 6 cages each with 2 birds per cage and each block was treated as a replicate. The experiment was a 2 x 4 factorial, the variables being type of grain (wheat, sorghum) and oil supplement (0, 20g OL/kg, 20g SO/kg, 10 g OL plus 10 g SO/kg (OLSO). The composition of the wheat and sorghum basal diets is shown in Table 1. The oil experimental diets were achieved by diluting the sorghum and wheat basal diets with oils. Dietary content of oleic and linoleic acids is shown in Table 1. Each experimental diet was randomly assigned to five replicates. Feed and water were available *ad libitum*. Diets were supplied as mash. A photoperiod of 14 h commenced when the birds were caged at 22 weeks of age and continued throughout the trial in a gabled roofed, naturally ventilated shed.

Egg production and feed intake were recorded for the entire replicate. Egg production was recorded daily for each replicate. Egg weight was based on the weight of a complete collection of eggs for two days each week. Calculations for egg production and egg weight were based on 2-week periods starting 4 weeks from the beginning of the experiment. Total feed consumption per diet was measured over the 12-week experimental period. The birds were re-weighed when the experiment concluded and body weight gain was calculated. Egg yolk C and fatty acids were determined every 2 weeks starting 4 weeks from the beginning of the experiment. Six eggs were randomly sampled from each replicate, their yolks separated and weighed. Each three yolks were pooled, homogenized, and two samples placed in airtight containers prior to analysis.

At the end of experiment, 3 birds were randomly selected from each replicate and 5 ml of blood were withdrawn from the wing-vein of each bird. Blood was collected into glass tubes with a blood-collecting cocktail (Edelstein and Scanu, 1986), centrifuged at 2300 x g for 20 minutes at 4 C and plasma was collected for analysis. Plasma samples from birds of each replicate were pooled into

Table 1: The composition of the basal diets (g/kg)

Ingredient	Wheat[1]	Sorghum[2]
Wheat	390.00	--------
Sorghum	--------	390.00
Cottonseed (36% protein)	50.00	50.00
Meatmeal (50% protein)	85.00	104.00
Soybean (48 %protein)	50.00	63.00
Maize	233.80	206.80
Mung beans (% protein)	100.00	100.00
Methionine	2.00	2.00
Salt	1.20	1.20
Dicalcium phosphate	5.00	--------
Limestone	80.00	80.00
Vitamin /mineral Premix[3]	3.00	3.00
Analysis:		
Crude protein (N% x 6.25)	18.1	17.9
Oleic acid	9.2	11.0
Linoleic acid	13.5	12.2
Oleic: linoleic acid ratio	0.7	0.9
Calculated metabolizable energy (MJ/kg)	11.7	11.8
Calculated calcium (g/kg)	37.3	37.6
Calculated available phosphorus (g/kg)	5.3	5.5

1: The olive, safflower and 50% olive plus 50% safflower oils were added at 20g/kg. Oleic and linoleic acids contents were 24.1 and 15.1, and 11.4 and 28.4 and 17.8 and 21.9 g/kg for OL, SO and OLSO diets.

2: The olive, safflower and 50% olive plus 50% safflower oils were added at 20g/kg. Oleic and linoleic acids contents were 25.6 and 13.9, 13.1 and 27.1, 19.4 and 20.5 g/kg for OL, SO and OLSO diets.

3: The composition of vitamins and minerals in the premix per kg diet were:
Retinol, 2.4 mg; cholecalciferol, 75 ug; DL-α-tocopheryl acetate, 5 mg; riboflavin, 3 mg; menadione sodium bisulphate, 300 ug; niacin, 15 mg; cyanocobalamin, 10 ug; biotin, 5 ug; choline, 100 mg; ethoxyquin, 20 mg; Co, 200 ug; I, 500 ug; Cu, 5 mg; Fe, 20 mg; Mn, 80 mg; Zn, 50 mg; Se, 100 ug; Mo, 200 ug; apocarotenoic ester, 150 mg ; canthaxanthin, 50 mg.

one sample prior to analysis. VLDL were separated at density 1.006 by ultracentrifugation using a Beckman (Beckman Inc., Palo Alto, CA) TL 100 centrifuge with a TL 41.2 rotor at 75,000 rpm for 3 hours. The substrate contained low density and high-density lipoproteins (LDL plus HDL). The concentrations of lipid and C in the total plasma and lipoprotein fractions were determined by kits provided by Boehringer Mannheim (cat. no. 124303 and 123087, respectively); triglycerides (TG) by a kit from Sigma (cat. no. 405-b); and P by using the method of Zilversmit and Davis (1950) after extraction of lipids according to Folch *et al*. (1957). Yolk lipid was extracted by the method of Folch *et al*. (1957). Fatty acid and C concentrations in the yolk lipid were determined

Table 2: Weight gain, feed consumption, egg weight, rate of lay and egg mass of laying hen fed diets with different types of grain and oil supplement

Treatment	Weight gain (g)	Feed consumption (g)	Egg weight (g)	Rate of lay (egg/hen/day)	Egg mass (g)
Type grain					
Wheat	237.5	108.2	57.5	0.91	52.0
Sorghum	224.2	107.2	57.2	0.89	51.5
SE[1] (P<0.05)	12.7	1.3	0.3	0.01	0.5
Type of oil supplement[2]					
Control	204.2	109.2	57.1	0.89	52.3
OL	250.0	105.8	57.2	0.89	51.2
SO	235.0	108.6	57.5	0.90	51.5
OLSO	234.2	107.2	57.5	0.90	52.1
LSD[3] (P<0.05)	51.4	5.2	1.0	0.03	2.2

[1]Standard error of means. [2]Control = Without oil; OL = With 20g olive oil/kg diet; SO = With 20g safflower oil/kg diet; OLSO = With 10 g olive oil plus 10 g safflower oil/kg diet. [3]Least significant differences (P< 0.05). There was no significant interaction between dietary types of grain and oil supplement on parameters measured.

by gas-liquid chromatography (Nugara and Edwards, 1970; Ishikawa *et al.*, 1974). Data collected were subjected to analysis of variance (SAS Institute, 1985). When significant variance ratios were detected, differences between treatment means were tested using the least significant difference procedure.

Results

The effect of type of grain and oil supplement of layer diets on the performance (weight gain, feed intake, rate of lay, egg weight and mass), yolk weight and mass, egg yolk C and fatty acids concentrations and on plasma lipids and lipoproteins are shown in Table 2, 3 and 4, respectively. Birds fed on the sorghum diet had significantly (P<0.05) higher oleic acid and oleic:linoleic acid ratio in their yolks (P<0.01) and plasma TG in the LDL plus HDL fraction when compared with those fed on the wheat diet. There were no significant differences between the two dietary grains in the performance of laying hens, yolk weight and mass, yolk lipid, C and palmitic, stearic and linoleic acids concentrations, yolk unsaturated:saturated (oleic plus linoleic : palmitic plus stearic acids) ratio, lipid composition (C, TG and P) in plasma and its VLDL fraction, and C and P concentrations in the LDL plus HDL fraction of plasma. Feeding the OL diet significantly (P<0.05) increased yolk oleic acid and oleic:linoleic acid ratio, plasma TG in the VLDL fraction and reduced plasma TG concentration in the LDL plus HDL fraction (P<0.01) when compared with those fed the control diet. Pullets fed the SO diet had significantly (P<0.05) higher concentration of linoleic acid in their egg yolk, plasma lipid concentration (C, TG and P) in the VLDL fraction and lower yolk oleic:linoleic acid ratio and plasma TG concentration in the LDL plus HDL fraction (P<0.01) than those fed on the control diet. Pullets fed the OLSO diet produced significantly (P<0.05) higher unsaturated:saturated ratio and lower palmitic and stearic acids concentrations of egg yolk when compared with those fed on the control diet. There was no significant difference between the OLSO and control birds in plasma lipid concentration of lipoproteins fractions. Dietary supplementation of oil did not affect the performance of laying hens, yolk weight and mass, and yolk lipid and C concentrations, daily C output, plasma total concentration of C, TG and P, plasma C and P concentrations in the LDL+HDL fraction (Table 2, 3 and 4).

Discussion

Results of this experiment showed that dietary type of grain and oil supplement altered fatty acid profile of egg yolk and composition of plasma lipoproteins without having a significant effect on the overall performance or egg yolk content of lipid and C. Several investigators have emphasized the role of dietary factors in modifying egg yolk lipid (Fisher and Leveille, 1957; Sim *et al.*, 1973; Hargis, 1988; Stadelman and Pratt, 1989; Burley and Vadehra, 1989; McDonald and Shafey, 1989, 1990; Shafey *et al.*, 1992). It is expected that diets containing oils from different origins will influence egg yolk fatty acid composition, reflecting their predominant fatty acids. The sorghum diets had a higher oleic acid and a lower linoleic acid contents than those found across the wheat diets (Table 1). Birds fed on the sorghum diet had higher egg yolk oleic and oleic:linoleic acid ratio when compared with birds fed on the wheat diet. Birds fed on the OL diet increased oleic acid and oleic:linoleic acid ratio in their egg yolk when compared with the control and SO diets. Whilst, the SO diet increased linoleic acid concentration and reduced oleic:linoleic acid ratio in the egg yolks when compared with the control and OL diets. The oleic and linoleic acids contents of the OL and SO diets were higher than those found in the control diets (Table 1). The deposition of oleic and linoleic acids in

Table 3: Yolk weight and mass and concentration of lipid, cholesterol and fatty acid in egg yolk of laying hen fed diets with different types of grain and oil supplement

Treatment	Yolk weight (g)	Yolk mass (g)	Lipid (%)	Cholesterol (CHL) concentration (mg CHL/g yolk)	Cholesterol (CHL) content (mg CHL/ yolk)	Cholesterol (CHL) daily output (mg CHL/day)	Fatty acids (% of total methyl esters of yolk lipid) 16:0	18:0	18:1	18:2	UNS/SAT ratio[1]	18:1/18:2 ratio[2]
Type of grain												
Wheat	16.2	14.6	35.3	14.78	246.9	225.2	24.39	9.21	39.87	12.83	1.57	3.11
Sorghum	16.5	14.7	36.1	14.73	246.9	225.2	24.58	8.76	41.31*	11.78	1.59	3.50**
SE[3] (P<0.05)	0.1	0.2	0.4	0.50	3.5	4.4	0.33	0.26	0.52	0.41	0.03	0.11
Type of oil supplement[4]												
Control	16.2	14.8	34.9	15.28	246.9	225.2	25.29^{a}	9.56^{a}	39.47^{b}	12.03^{bc}	1.48^{b}	3.28^{b}
OL	16.2	14.5	35.9	14.56	235.9	210.7	24.26^{ab}	9.09^{ab}	42.33^{a}	10.64^{c}	1.59^{ab}	3.98^{a}
SO	16.1	14.4	36.1	14.86	239.2	214.1	24.46^{ab}	8.72^{ab}	39.26^{b}	13.97^{a}	1.60^{ab}	2.81^{c}
OLSO	16.3	14.8	35.9	14.35	233.4	211.6	23.93^{b}	8.37^{b}	41.30^{ab}	12.58^{ab}	1.67^{a}	3.28^{b}
LSD[5] (P<0.05)	0.3	0.6	1.5	1.87	13.8	16.2	1.32	1.03	2.12	1.68	0.13	0.44

[1]The proportion unsaturated [oleic (18:1) plus linoleic (18:2)] to saturated [palmitic (16:0) plus stearic (18:0)] fatty acid in egg yolk lipid. [2]The proportion of monounsaturated (oleic) to polyunsaturated (linoleic) fatty acid in egg yolk lipid. [3]Standard error of means. [4] Control = Without oil; OLO = With 20 g olive oil/kg diet; SFO = With 20 g safflower oil/kg diet; OLSFO = With 10 g olive oil plus 10 g safflower oil/kg diet. [5]Least significant differences (P< 0.05). *Significantly different (P<0.05); **Significantly different (P<0.01). [abc]Treatment means within columns followed by different letters are significantly different at (P< 0.05). There was no significant interaction between dietary types of grain and oil supplement on parameters measured.

Table 4: The effects of type of grain and oil supplement on cholesterol (C), triglycerides (TG), and phospholipid (P) in plasma and lipoprotein fractions

Treatment	Plasma C (mmol/l)	Plasma TG (mmol/l)	Plasma P (g/l)	LDL +HDL[1] C (mmol/l)	LDL +HDL[1] TG (mmol/l)	LDL +HDL[1] P (g/l)	VLDL[2] C (mmol/l)	VLDL[2] TG (mmol/l)	VLDL[2] P (g/l)
Type of grain									
Wheat	3.6	25.9	7.7	0.4	0.9	0.6	3.2	25.0	7.1
Sorghum	3.8	29.1	6.2	0.5	1.2**	0.6	3.2	27.9	5.7
SE[3] (P<0.05)	0.4	2.4	0.8	0.05	0.03	0.1	0.3	1.6	0.6
Type of oil supplement[4]									
Control	3.1	23.8	6.4	0.4	1.2^{a}	0.5	2.7^{b}	22.6^{b}	5.9^{b}
OL	3.9	32.1	6.3	0.5	1.0^{b}	0.7	3.4^{ab}	31.1^{a}	5.5^{b}
SO	4.3	30.1	8.9	0.4	0.8^{c}	0.6	3.9^{a}	29.2^{a}	8.3^{a}
OLSO	3.5	24.1	6.3	0.5	1.1^{ab}	0.6	3.0^{ab}	20.3^{b}	5.8^{b}
LSD[5] (P<0.05)	1.5	9.9	2.8	0.2	0.1	0.3	1.2	6.6	2.4

[1]LDL+HDL = Low density plus high density lipoproteins fractions. [2]VLDL = Very low density lipoproteins. [3]Standard error of means. [4]Control = Without oil; OL = With 20 g olive oil/kg diet; SO = With 20 g safflower oil/kg diet; OLSO = With 10 g olive oil plus 10 g safflower oil/kg diet. [5]Least significant differences (P<0.05); **Significantly different (P < 0.01). [abc]Treatments means within columns followed by different letters are significantly different (P<0.05). There was no significant interaction between dietary types of grain and oil supplement on parameters measured.

egg yolks were positively related to the range of dietary oleic and linoleic acids contents. Similar findings with linoleic acid deposition in egg yolk were reported by Shafey *et al*. (1992).

However, birds fed on the OLSO reduced palmitic and stearic acids and increased unsaturated:saturated fatty acid ratio of egg yolk when compared with those of the control diet. It appears that the reduction in egg yolk saturated fatty acids (palmitic and stearic) was compensated for by an increase in the unsaturated fatty acids, albeit non significantly (oleic plus linoleic acids). Whilst, the addition of either OL or SO alone to the control diet did not significantly affect yolk palmitic and stearic acids concentrations (Table 3). Similar results were obtained from feeding other vegetable oils to layers (Fisher and Leveille, 1957; Sim *et al*., 1973; Shafey *et al*., 1992). The composition of egg yolk fatty acids is a reflection of the fatty acids synthesized by the liver of a laying hen on a standard diet since the amount of egg yolk fatty acids provided by adipose tissue is about 20% (Leclercq, 1973). The findings that the supplementation of oil to the hen's diet can influence the profile of fatty acid in egg yolk without a significant effect on total lipid content of egg yolk were in agreement with other investigators (Marion and Edwards, 1962; Balnave, 1970; Vogtmann and Clandinin; 1975; Ohtake and Hoshino, 1976; Cherian and Sim, 1991; Hargis *et al*., 1991; Ahn *et al*., 1995). These results suggest that it is possible to increase the ratio of unsaturated:saturaed fatty acids in egg yolk by feeding sorghum and OL plus SO to the laying hens.

Dietary type of grain and oil supplement did not alter egg yolk C (mg C/g yolk or mg C/yolk), and plasma total concentration of C. C is largely synthesized in the liver and transported to the ovum via VLDL. Plasma VLDL is the yolk lipid precursor of the laying hens (Bacon, 1981). However, the finding that C concentration in the VLDL fraction of plasma of birds fed the SO diet was significantly higher than that of the control group, whilst there was no significant difference in egg yolk C between the two groups of birds would suggest that egg yolk C is not influenced by C concentration in the VLDL fraction. It seems that the uptake of C is not determined by plasma VLDL concentration. These findings are in agreement with Hargis (1988) who concluded that the inability to reduce markedly egg yolk C is possibly due to natural selection pressures to maintain a certain level of C in the egg for use by the developing embryo. Similar finding on the effect of dietary fat on egg yolk C was reported by Chung *et al*. (1965); Grimes *et al*. (1996); Shafey *et al*. (1999) who found that dietary fat had little effects on cholesterol deposition in the egg yolk. Feeding laying hens diets containing corn oil, lard or hydrogenated coconut oil did not affect egg yolk cholesterol.

Dietary type of grain and oil supplement did not alter plasma total concentration of TG and P. Type of grain altered TG concentration in plasma LDL plus HDL fraction, and non-significantly in the VLDL fraction. However, the supplementation of OL and SO to the diet altered TG concentration in the plasma lipoprotein fractions (LDL plus HDL and VLDL, Table 4). These results suggest that dietary oleic and linoleic acids contents affected lipids composition of lipoprotein fractions through altering the synthesis of lipids in the liver and their transport in the blood to the developing follicles. Dietary fat that enters the portal blood (Noyan *et al*., 1964; Freeman, 1984) may supply a readily available source of lipid for direct transport and deposition in egg yolk (Griffin *et al*., 1984). Dietary fat is known to increase estrogen level in the blood of laying hens (Whitehead *et al*., 1993) and avian liver increases VLDL synthesis under the influence of estrogen (Nimpf and Schneider, 1991). These findings are in agreement with Weiss *et al*. (1967) and Hargis (1988) who reported that unsaturated fatty acids increased C and P synthesis. These results suggest that dietary oleic and linoleic acid concentrations are involved in the synthesis and secretion of VLDL into the hen plasma.

Dietary supplementation of oil did not affect the performance of laying hens and yolk weight and mass (Table 2 and 3). It seems that the rate of hepatic synthesis of fats is sufficient to supply the amount of lipid needed to achieve optimum performance, yolk and egg weight and exogenous fat might not needed to meet these requirements. Similar observations were made by different authors (Sell *et al*., 1979; Summers and Lesson, 1983; Douglas *et al*., 1989; Baucells *et al*., 2000). The linoleic acid concentration in the basal diet exceeded that recommended by NRC (1%, 1994). It appears that hens had an adequate capacity for hepatic VLDL synthesis to fulfill the requirements for egg production. This study was performed on birds during the period of 36 to 48 weeks of age. This suggestion is supported by the observation of Balnave and Weatherup (1973, 1974) that the favorable effects of supplemental fat in nutritionally adequate diets depend on age of the hens. Sell *et al*. (1987) reported that supplemental fat had greatest effects on yolk and egg weights when hens were 30 to 34 weeks of age, but these effects disappeared by 38 weeks of age. It was concluded that hens fed a sorghum based diet or a diet supplemented with olive oil produced eggs with high concentration of oleic and oleic:linoleic acid ratio and that hens fed a diet supplemented with safflower oil produced eggs with high concentration of linoleic and lower oleic:linoleic acid ratio and that hens fed a diet supplemented with olive plus safflower oils produced eggs with high concentration of unsaturated:saturated fatty acid ratio and lower palmitic and stearic acids. The composition of the plasma lipoproteins was influenced by dietary factors. Neither daily cholesterol output nor the egg yolk cholesterol was affected by dietary type of grain and oil supplements.

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