

Olive Cake and Barley Malt Rootlets in Hen Diets to Improve Egg Lipids and Fatty Acids

Research Article

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ABSTRACT

The aim of the present study was to investigate the possibility of improving egg lipids and fatty acids composition of laying hens through inclusion of olive cake plus barley malt rootlets in the diets. Seventy, 54-week-old, Lohman laying hens were fed for a 12 weeks laying period on 3 iso-caloric and iso-nitrogenous diets contained 0 (control), 28.5 g olive cake (OC) + 25.5 g barley malt rootlets (BMR), 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR or 57.0 g OC + 51.1 g BMR per kg. All the tested diets decreased plasma cholesterol ($P < 0.05$) and triglycerides (TG) ($P < 0.001$) and yolk concentrations ($P < 0.0001$) of total lipids, TG, cholesterol, low-density lipoproteins and phospholipids. Inclusion of OC plus BMR in the diets at 28.5 g OC + 25.5 g BMR, 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR or 57.0 g OC + 51.1 g BMR per kg decreased concentration of total saturated fatty acids (SFA) of egg yolk by 22.4, 50.1, 58.5 and 55.4%, respectively and increased total polyunsaturated fatty acids (PUFA) (2.4, 1.4, 2.1 and 1.2 fold, respectively) and total n-6 PUFA (2.5, 1.9, 2.7 and 1.9 fold, respectively). Total monounsaturated fatty acids (MUFA) were decreased by 8.1% with dietary 28.5 g OC + 25.5 g BMR per kg diet, while it was increased by 105, 93.4 and 128.5% with dietary 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR and 57.0 g OC + 51.1 g BMR per kg, respectively. To feed laying hens with rations that contained 28.5 g OC + 25.5 g BMR, 28.5 g OC + 51.1 g BMR and 57.0 g OC + 25.5 g BMR per kg diet increased yolk concentrations of total n-3 PUFA (217, 4.9 and 57.5%, respectively), while ration with 57 g OC + 51.1 g BMR per kg on the diet decreased it by 56.5% compared to the control. It is concluded that inclusion of OC at rate of 28.5 or 57.0 g/kg on the diet in combination with BMR at rate of 25.5 or 51.1 g BMR/g/kg on the diet of laying hens decreased cholesterol and SFA with great increases in MUFA and moderate increases in n-3 PUFA in egg yolk lipids.

KEY WORDS barley malt rootlets, egg yolk, fatty acids, laying hen, lipids, olive cake.

INTRODUCTION

Monounsaturated and polyunsaturated fats may lower blood cholesterol levels when they replace saturated fat in human diet. Saturated fat in human diet, not dietary cholesterol, is what influences blood cholesterol levels the most (Howell *et al.* 1997). In addition, Lesson *et al.* (1998) and Baucells *et al.* (2000) reported a strong positive correlation between concentrations of fatty acids (FAs) in hen diet and their

concentrations in the egg yolk. The dietary fiber means that fraction of the edible part of plants are resistant to the digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine and a reduction in blood cholesterol (Truswell, 1999). Dietary fiber can be classified as either soluble or insoluble, based on whether it forms a dispersion when mixed with water (soluble fibre) or not (insoluble fibre) (Jiménez-Escrig and Sánchez-Muñiz, 2000). Olive cake a by-product of olive oil

industry contains a high content of residual unextracted olive oil (the predominant fatty acid in it is oleic acid with important amount of linoleic acid) along with high content of crude fiber (CF) (300-400 g/kg) according to results reported by Francisco *et al.* (1989).

Barley malt rootlets, a by-product of the brewing industry, consist of the plumule and radicle of barley and also may include some of the malt hulls

Barley malt rootlets contain (on a dry matter basis) 156 g/kg of CF and 22 g/kg of ether extract (McDonald *et al.* 1995). Fedak and De La Roche (1977) reported that the lipid (as fatty acid) content did not exceed 3.1% in barley. Linoleic acid was present in the highest proportions (50.7-57.9%), palmitic (18.3-27.0%), oleic (12.2-21.2%) and linolenic acid (4.3-7.1%).

Therefore, the objective of this study was to investigate the effects of inclusion of olive cake plus barley malt rootlets in four combinations in laying hen diets on plasma concentrations of cholesterol and triglycerides (TG) and on lipids and FAs profile of egg yolk.

MATERIALS AND METHODS

Seventy, 54-week-old, Lohman laying hens were randomly allotted into five dietary treatment groups each of 14 hens (7 replicates, each consisted of 2 hens housed in one cage). Diets were: diet 1 (control), while diets 2, 3, 4 and 5 contained 28.5 g olive cake (OC) + 25.5 g barley malt rootlets (BMR) or 28.5 g OC + 51.1 g BMR or 57.0 g OC + 25.5 g BMR or 57.0 g OC + 51.1 g BMR/kg, respectively. Formulated to contain 0.0 g olive oil/kg (diet 1- control), 28.5 g olive oil/kg (diets 2 and 3) and 57.0 g olive oil/kg (diets 4 and 5), respectively (Table 1). All diets were iso-caloric and iso-nitrogenous, covering the nutritional requirements of laying hens (NRC, 1994). Diets, in mash form, and fresh water were supplied *ad libitum*. Birds were reared at room temperature and were exposed to 18 hours light/day. Hens were fed with the experimental diets for a 12 weeks laying period. The diets were analyzed for proximate composition according to AOAC (1996) methods. FAs composition of OC were also analyzed (Table 2).

Table 1 Feed ingredients and chemical composition (g/kg) of the experimental diets

Ingredient g/kg diet	Control	28.5 g OC		57 g OC	
		25.5 g BMR	51.1 g BMR	25.5 g BMR	51.1 g BMR
Yellow corn	570.0	541.5	541.5	513.0	513.0
Olive cake ¹	0.0	28.5	28.5	57.0	57.0
Soybean meal (44%)	180.0	167.8	155.5	167.8	155.5
Barley malt rootlets ²	0.0	25.5	51.1	25.5	51.1
Rice polish	100.0	86.7	73.4	86.7	73.4
Herring fish meal	50.0	50.0	50.0	50.0	50.0
Bone meal	21.5	21.5	21.5	21.5	21.5
Limes tone	70.0	70.0	70.0	70.0	70.0
Vit. and Min. Premix ³	2.5	2.5	2.5	2.5	2.5
Sodium chloride	5.0	5.0	5.0	5.0	5.0
Methionin	1.0	1.0	1.0	1.0	1.0
<i>Composition g/kg</i>					
Crude protein	176.1	176.2	177.1	175.5	176.4
Crude fiber	30.0	49.6	50.2	60.3	61.0
Ether extract	43.1	51.5	54.8	67.8	68.3
Metabolizable energy, kcal/kg	2779	2768	2761	2761	2755

¹ 482 g/kg crude fiber and 132.3 oil g/kg, on DM basis, analytical values.

² 162 g/kg crude fiber and 21g/kg oil, on DM basis, analytical values.

³ Vitamin and mineral premix supplied per kg of diet: retinyl acetate 3.4 mg; cholecalciferol 0.075 mg; dl-alpha-tocopheryl acetate 10 mg; Vitamin K 2 mg; Vitamin B₁ 1 mg; Vitamin B₂ 4 mg; Vitamin B₆ 1.5 mg; Vitamin B₁₂ 0.001 mg; Pantothenic acid 10 mg; Niacin 20 mg; Folic acid 1 mg; Biotin 0.05 mg; Choline Chloride 500 mg; Fe 30 mg; Mn 40 mg; Cu 3 mg; I 3 mg; Co 0.2 mg; Zn 45 mg and Se 0.1 mg.

OC: olive cake; BMR: barley malt rootlets.

At the end of the experiment, heparinized blood samples were collected at random, via wing vein, from 4 hens/treatment. In these samples, plasma was separated by centrifugation and kept frozen at -20 °C until analyzed for cholesterol and TG.

Plasma cholesterol concentration was determined according to a quantitative-enzymatic-colorimetric method for determination of total cholesterol in serum or plasma (Stein, 1986). Cholesterol esterase hydrolyzes cholesterol esters to free cholesterol and FAs.

The free cholesterol so produced plus the preformed cholesterol are then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. A quinoneimine chromogen, with absorption maxima at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase (POD) with hydrogen peroxide. The intensity of the final red color is proportional to total cholesterol concentration. Plasma TG concentration was determined according to a quantitative-enzymatic-colorimetric method for determination of TG in

serum or plasma (Scheletter and Nussel, 1975). Glycerol and FAs are first formed by lipase action on the TG.

Table 2 Analyzed fatty acids of olive cake (g/kg of total fatty acids)

Fatty acids	Olive cake
16:0	94.1
16:1cis/tr9	8.2
17:1	0.4
18:0	26.7
18:1cis9	643.3
18:1 isomere ^a	26.3
18:2cis9,12	124.0
18:3cis9,12,15	5.8
20:0	4.5
20:1n-9	3.7
22:0	2.4
22:1 n-9	1.3
22:2 n-6	1.2
22:3n-3	4.5
24:0	3.5
24:1	7.4
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TSFA ^b	131.2
TMUFA ^c	690.6
TPUFA ^d	135.5
n-9	648.3
n-6	125.2
n-3	10.3
n-6:n-3	12.2

^atrans-C18:1.

^b Total saturated fatty acids.

^c Total monounsaturated fatty acids.

^d Total polyunsaturated fatty acids.

Glycerol is then phosphorylated by adenosine-5¹-triphosphate (ATP) to produce glycerol-3-phosphate and adenosine-5¹- diphosphate in a reaction catalyzed by glycerol kinase. The glycerol-3-phosphate is oxidized by glycerylphosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Peroxide reacts with α -4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase to form quinoneimine. The intensity of the color, which is proportional to TG concentration, is read at 500 nm. Eggs were collected for chemical analysis during the last 3 days of the experimental period. Twelve eggs per each treatment were taken at random, and then were weighed, cracked and their yolks were separated. Then each 4 yolks were pooled and homogenized and considered as one sample, i.e. each treatment had 3 samples of these pooled egg yolks for chemical analysis. These samples of the pooled yolks were freezed and stored at -20 °C until the chemical analysis was performed. Egg yolk samples and olive cake were analyzed for total lipids, TG, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and phospholipids. Total lipid content of egg yolk was determined gravimetrically after extraction with chloroform:methanol (2:1) according to Folch *et al.* (1957). TG content of egg yolk was

determined colorimetrically according to the method of Lowell *et al.* (1973). The developed colour was read at 410 nm.

Cholesterol concentration in egg yolk was determined according to an enzymatic-colorimetric method for the determination of cholesterol in egg (Shen *et al.* 1982). Cholesterol is oxidized by cholesterol oxidase to cholestenone. In the presence of catalase, the hydrogen peroxide produced in this reaction oxidizes methanol to formaldehyde. The latter reacts with acetylacetone forming a yellow lutidine-dye in the presence of NH_4^+ -ions. The concentration of the lutidine-dye (3,5-diacetyl-1,4-dihydrolutidine) formed is stoichiometric to the amount of cholesterol and it is measured by the increase of light absorbance in the visible range at 405 nm. Accurately weight approx. 0.5 g yolk into a 50 ml volumetric flask and add 1 g sea-sand (the volume displacement of 0.400 mL must be taken into account in the calculation formula); heat under a reflux condenser for 30 min with 20 mL freshly prepared methanolic potassium hydroxide solution (1.0 mol/L) and 10 mL isopropanol while stirring (magnetic stirrer). Allow the turbid solution to cool, and fill up to the mark with isopropanol at room temperature after removal of the magnetic rod (rinse with iso-propanol); mix, filter through a fluted filter and use the clear solution for the assay. LDL cholesterol was determined colorimetrically according to Wieland and Seidel (1983) method, while HDL cholesterol was determined colorimetrically according to Eckel (1977).

Phospholipids concentration in egg yolk was determined after precipitation of the phospholipids in egg yolk according to the method of Kates (1972), which depends on the fact that precipitation of phosphatides from neutral lipid depends on the general insolubility in cold acetone of most phosphatides in salt form. An aliquot of neutral lipids phospholipids mixture was placed in a 15 mL centrifuge tube and the solvent was evaporated in nitrogen stream at 30 °C to 0.2-0.3. Acetone (7.5 mL) plus MgCl_2 and $6\text{H}_2\text{O}$ in methanol (0.15 mL, 10% w/v) were added. Then, they were mixed on a Vortex mixer and cooled on an ice bath for 1 hour. The precipitated phospholipids were separated by centrifugation at 5000 r.p.m. for 5 min. The acetone supernatant was removed by Pasteur pipette. The precipitate was then washed twice by suspending it in 2 mL of cold acetone, cooling on ice and centrifuged as above. The precipitated phospholipids were freed of excess solvent in a stream of nitrogen and the dry residue was dissolved to a known concentration in redistilled chloroform (2 mL). Then, the colorimetric method of Kaur *et al.* (1973) was employed for the quantitative determination of the phospholipids.

The lipid extract of pooled yolk samples were pooled in one sample/treatment. Samples of pooled lipid extract and olive cake were methylated (Vogel, 1975), FAs were sepa-

rated and identified using a Pye: Unicam gas chromatography (PU 4550) equipped with dual flame ionization detectors and dual channel recorder. The fractionation of FAs methyl esters was conducted using a coiled glass column (1.5 m×4 mm) packed with polyethelene glycol adipate (PEGA) 10%. The fractionation condition for FAs was as described by Farag *et al.* (1990). Peak identification for FAs was conducted by comparing the retention time with that of a standard of known composition. Peak areas were measured by normalization method and the relative proportions of the individual FAs were computed using ATI Unicam 4880 data station.

The effects of dietary treatments were examined using analysis of variance for completely randomized design experiments using SAS (1996), while differences among means were evaluated using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Plasma lipids

Feed laying hens on diet contain 28.5 g OC + 25.5 g BMR, 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR or 57.0 g OC + 51.1 g BMR/kg diet decreased ($P<0.05$) concentrations of plasma cholesterol by 29.0, 40.0, 23.4 and 35.6%, respectively. Concentrations of plasma TG ($P<0.001$) increased by 33.2, 47.1, 49.4 and 44.4%, respectively compared to the control (Table 3).

Such effects of OC plus BMR feeding could be attributed to their contents of CF and oil (Table 1). Decreases in blood cholesterol levels were reported for hens fed a standard layer diet with added 15% cellulose (Menge *et al.* 1974). Moreover, a great decrease in plasma cholesterol of laying hens occurred when vegetable oils were included in their diets (Fisher and Leveille, 1975). Shafey *et al.* (2003) observed that feeding laying hens a diet containing olive oil at 20 g/kg reduced plasma TG concentration in the low-density lipoproteins (LDL) plus HDL fraction. Moreover, barley is an excellent source of β -glucan (Sharma and Gural, 2010). It has been hypothesized that upon ingestion, β -glucans increase small intestinal viscosity resulting in reduced bile acid and cholesterol or triglyceride absorption thus lowering plasma cholesterol of chicks (Wang *et al.* 1992). Also, Jonker *et al.* (2010) showed that barley β -glucan lowers plasma cholesterol in rats. Wang *et al.* (1993) showed that total plasma cholesterol concentration of the chicks fed 10% barley oil was 34% lower ($P<0.05$) than that of the chicks fed margarine. Suggesting that polyunsaturated FAs of barley oil are hypocholesterolemic components in barley oil. El-Husseiny *et al.* (1997) found that dietary barley malt rootlets lowered plasma cholesterol concentration of rabbit.

Egg yolk lipids

Feeding laying hens on diets containing 28.5 g OC + 25.5 g BMR, 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR and 57.0 g OC + 51.1 g BMR/kg diet decreased ($P<0.0001$) yolk concentrations of total lipids (11.6, 25.5, 25.3 and 34.9%, respectively), TG (11.6, 25.4, 25.1 and 34.9%, respectively), cholesterol (11.9, 25.5, 25.2 and 34.6%, respectively), LDL cholesterol (12.8, 26.1, 25.6 and 35.0%, respectively) and phospholipids (12.8, 26.6, 26.1 and 35.6 %, respectively) (Table 4).

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).

Cholesterol is largely synthesized in the liver and like lipids, transported to the growing follicles primarily in the VLDL (McDonald and Shafey, 1989). Moreover, Gallaher *et al.* (1993) reported that the cholesterol-lowering effect of soluble fiber in hamsters was due to the reduction of VLDL cholesterol. Accordingly to results obtained in the present study, the lowering effect of olive cake plus barley malt rootlets on cholesterol and lipids concentrations of egg yolk are more likely a secondary consequence arising from their lowering effects on cholesterol and TG in plasma (Table 3).

Fatty acid composition of yolk lipids

Feeding laying hens on diets containing 28.5 g OC + 25.5 g BMR, 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR and 57.0 g OC + 51.1 g BMR/kg diet decreased concentration of total saturated fatty acids (TSFA) in egg yolk by 22.4, 50.1, 58.5 and 55.4%, respectively (Table 5). Total monounsaturated fatty acids (TMUFA) was decreased by 8.1% with dietary 28.5 g OC + 25.5 g BMR/kg diet, while it was increased by 105, 93.4 and 128.5% with dietary 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR and 57.0 g OC + 51.1 g BMR/kg, respectively.

The inhibition of FAs synthesis in the liver is greater during digestion of unsaturated fats, rather than saturated fats (Sim and Qi, 1995). The main FA in olive oil is the mono-unsaturated oleic acid (643.3 g/kg of total FAs, Table 2). Fedak and De La Roche (1977) reported that barley oil contain palmitic (18.3-27.0%) and oleic (12.2-21.2%). Therefore, the decrease in TSFA and the increase in TMUFA could be attributed to the FAs profile of oil due to OC and BMR and to their high CF content. In accordance with the present results, Aydin *et al.* (2001) found that olive oil inclusion in hens diets decreased SFA and increased MUFA of egg yolk.

Also, Shafey *et al.* (2003) observed that feeding laying hens a diet containing olive oil at 20 g/kg increased yolk oleic acid.

Table 3 Cholesterol and triglycerides concentrations in blood plasma of hens fed diets containing olive cake (OC) plus barley malt rootlets (BMR)

Item	Control	28.5 g OC		57 g OC	
		25.5 g BMR	51.1 g BMR	25.5 g BMR	51.1 g BMR
Cholesterol*, mg/dL	129 ^a ±12	92 ^b ±6.7	78 ^b ±15	99 ^b ±13	83 ^b ±5.5
Triglycerides**, mg/dL	762 ^a ±12	509 ^b ±27	403 ^b ±61	386 ^b ±66	424 ^b ±34

* Means within a row with different superscript are significantly different (P<0.05).

** Means within a row with different superscript are significantly different (P<0.001).

Table 4 Total lipids, triglycerides, cholesterol, low density lipoprotein (LDL) and phospholipids in egg yolk of hens fed diets containing olive cake (OC) plus barley malt rootlets (BMR)

Item	Control	28.5 g OC		57 g OC	
		25.5 g BMR	51.1 g BMR	25.5 g BMR	51.1 g BMR
Total lipid, g	15 ^a ±0.58	13 ^b ±0.26	11 ^c ±0.38	11 ^c ±0.13	9.8 ^c ±0.63
Triglycerides, g	9.8 ^a ±0.38	8.6 ^b ±0.17	7.3 ^c ±0.25	7.3 ^c ±0.06	6.3 ^d ±0.41
Cholesterol, g	1.4 ^a ±0.05	1.2 ^b ±0.03	1.0 ^c ±0.03	1.0 ^c ±0.01	0.88 ^c ±0.06
LDL, mg	0.18 ^a ±0.006	0.16 ^b ±0.004	0.13 ^c ±0.005	0.13 ^c ±0.002	0.12 ^d ±0.007
HDL, mg	0.000	0.000	0.000	0.000	0.000
Phospholipids, g	3.0 ^a ±0.10	2.7 ^b ±0.06	2.2 ^c ±0.07	2.2 ^c ±0.02	1.9 ^d ±0.12

** Means within a row with different superscript are significantly different (P<0.001).

LDL: low density lipoprotein; HDL; high density lipoprotein; OC: olive cake; BMR: barley malt rootlets.

Table 5 Fatty acid composition of egg yolk (g/kg of total fatty acids) of hens fed diets containing olive cake (OC) plus barley malt rootlets (BMR)

	Control	28.5 g OC		57 g OC	
		25.5 g BMR	51.1 g BMR	25.5 g BMR	51.1 g BMR
14:0	13	2.8	2.6	3.9	6.5
16:0	323	335	233	196	217
16:1	81	57	36	44	36
18:0	294	151	79	62	58
18:1 n-9	145	150	426	393	480
18:2 n-6	34	197	183	236	186
18:3 n-3	24	39	14	22	11
20:4 n-6	30	28	41	2.7	0.0
22:6 n-3	0.0	39	12	17	0.0
TSFA ^a	630	489	315	262	282
TMUFA ^b	226	207	462	437	515
TPUFA ^c	88	303	250	278	197
n-6	64	225	224	238	186
n-3	24	78	26	39	11
n-6:n-3	2.7	2.9	8.6	6.1	18

^a Total saturated fatty acids.

^b Total monounsaturated fatty acids.

^c Total polyunsaturated fatty acids.

OC: olive cake; BMR: barley malt rootlets.

Moreover, Silversides and Lefrançois (2005) found that increasing the amount of hemp seed meal that had an important amount of oleic acid, with high fiber content (414 g/kg), in the diet of laying hens lowered the percentage of palmitic acid in yolk and it increased the percentages of linoleic and linolenic acid.

Diets containing 28.5 g OC + 25.5 g BMR, 28.5 g OC + 51.1 g BMR, 57.0 g OC + 25.5 g BMR or 57 g OC + 51.1g BMR/kg in hens increased yolk concentrations of total polyunsaturated fatty acids (TPUFA) reaching the following values 2.4, 1.8, 2.1 and 1.2 fold, respectively.

Total n-6 PUFA (2.5, 2.5, 2.7 and 1.9 fold, respectively) and the ratio of n-6:n-3 PUFA in egg yolk with 11.3, 219, 138 and 573% reaching the previous values compared to the control. Feeding laying hens on diets containing 28.5 g OC + 25.5 g BMR, 28.5 g OC + 51.1 g BMR and 57.0 g OC + 25.5 g BMR/kg diet increased yolk concentrations of total n-3 PUFA (217, 4.9 and 57.5%, respectively), while 57.0 g OC + 51.1g BMR/kg diet decreased it by 56.5% compared to the control. Olive oil contained linoleic acid (124.0 g/kg) and α -linolenic acid (LNA, 5.8 g/kg) of total FAs (Table 2).

Fedak and De La Roche (1977) reported that barley oil contained linoleic acid (507-579 g/kg) and linolenic acid (43-71 g/kg) of total FAs.

Diets containing OC plus BMR in the present study had higher amounts of α -linolenic acid (α -LNA) and this can be the cause of the increased concentration of docosahexanoic acid (DHA) in egg yolk. Laying hens can convert α -LNA to its elongated metabolite DHA and then deposit it in the yolk but this efficiency is limited. Enrichment of hen's diets with sources rich in LNA has resulted in the production of eggs with significantly increased levels of yolk LNA and small but significantly higher increases in the long chain (LC) n-3 PUFA, mainly as eicosapentanoic acid (EPA) and DHA.

However, the increase in this n-3 LC-PUFA FAs in yolk has not been in a similar proportion to that of LNA. Moreover, higher dietary fat levels in the form of flax and canola (rich in LNA) seed resulted in significantly smaller increases in the n-3 LC-PUFA compared to the increased levels of the same FAs reported by Hargis *et al.* (1991) in response to relatively low amounts (3%) of dietary FAs.

CONCLUSION

It is concluded that inclusion of OC at 28.5 or 57.0 g/kg diet in combination with BMR at 25.5 or 51.1 g BMR/g/kg diet of laying hens decreased plasma cholesterol and TG and yolk concentrations of total lipids, cholesterol, LDL, TG, phospholipids with greater decrease in the concentration of SFA, greater increase in the concentrations of MUFA and moderate increases in the concentrations of PUFA n-3 in egg yolk lipids.

REFERENCES

- AOAC. (1996). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA.
- Aydin R., Pariza M.W. and Cook M.E. (2001). Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. *J. Nutr.* **131**, 800-806.
- Baucells M.D., Crespo N., Barroeta A.C., López-Ferrer S. and Grashorn M.A. (2000). Incorporation of different polyunsaturated fatty acids into eggs. *Poult. Sci.* **79**, 51-59
- Chapman M.J. (1980). Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* **21**, 789-853.
- Duncan D.B. (1955). Multiple range and multiple F-test. *Biom.* **11**, 1-42.
- Eckel W. (1977). A fully enzymatic colorimetric method for determination of HDL-cholesterol in the serum. *Ärztl Laborgemeinschaft.* **23**, 101-108.
- El-Husseiny O.M., Hanafy M.A., Radwan M.A.H. and Azouz H.M.M. (1997). Evaluation of traditional and untraditional protein sources in rabbit diets. *Egypt J. Anim. Prod.* **34**, 57-66.
- Farag R.S., Ali M.N. and Taha S.H. (1990). Use of some essential oils as natural preservation for butter. *J. Am. Oil Chem. Soc.* **68**, 188-191.
- Fedak G. and De La Roche I. (1977). Lipid and fatty acid composition of barley kernels. *Can. J. Plant Sci.* **57**, 257-260.
- Fisher H. and Leveille G.A. (1975). Observations on the cholesterol, linoleic and linolenic acid content of eggs as influence by dietary fats. *J. Nutr.* **44**, 120-129.
- Folch O.J., Lees M. and Sloan-Stanely G.H. (1957). A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.
- Francisco T., Rioperez J. and Luisa R.M. (1989). Nutritional value for rabbits of olive pulp and the effect of visceral organs. *Anim. Feed Sci. Technol.* **25**, 79-85.
- Gallaher D.D., Hassel C.A. Lee K.J. and Gallaher C.M. (1993). Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect in hamsters. *J. Nutr.* **123**, 240-252.
- Hargis P.S., Van Elswyk M.E. and Hargis B.M. (1991). Dietary modification of yolk lipid with menhaden oil. *Poult. Sci.* **70**, 87-88.
- Howell W.H., McNamara D.J., Tosca M.A., Smith B.T. and Gaines J.A. (1997). Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. *Am. J. Clin. Nutr.* **65**, 1747-1764.
- Jiménez-Escrig A. and Sánchez-Muñiz F.J. (2000). Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res.* **20**, 585-598.
- Jonker D., Hasselwander O., Tervilä-Wilo A. and Tenning P.P. (2010). 28-Day oral toxicity study in rats with high purity beta-glucan (Glucagel™). *Food Chem. Toxicol.* **48**, 422-428.
- Kates M. (1972). Techniques of lipidology: isolation analysis and identification of lipids. Amsterdam: North Holland Publishing Co. Pp. 558-564.
- Kaur C., Raheja R., Singh A. and Bhatia I.S. (1973). New colorimetric method for the quantitative estimation of phospholipids without acid digestion. *J. Lipid Res.* **41**, 50-56.
- Lesson S., Caston L. and Maclaurin T. (1998). Evaluation of eggs produced by laying hens fed diets containing graded levels of flaxseed and vitamin E. *Poult. Sci.* **77**, 1436-1440.
- Lowell P., Foester A. and Galph T.D. (1973). Determination of triglyceride in serum. *Clin. Chem.* **19**, 338-340.
- McDonald M.W. and Shafey T.M. (1989). Nutrition of the hen and egg cholesterol. Pp. 33-39 in Proc. Eggs Seminar. Egg Industries Research Council, Sydney, Australia.
- Menge H., Littlefield L.H., Frobish L.T. and Weinland B.T. (1974). Effect of cellulose and cholesterol on blood and yolk lipids and reproductive efficiency of the hen. *J. Nutr.* **104**, 1554-1566.
- NRC. (1994). Nutrient Requirements of Poultry. 9th Rev. Ed. National Research Council, National Academy Press, Washington, DC., USA.
- SAS Institute. (1996). SAS®/STAT Software, Release 6.11. SAS Institute, Inc., Cary, NC.
- Scheletter G. and Nussel E. (1975). Quantitative enzymatic Colorimetric determination of triglycerides in serum or plasma.

- Arb. Sozial. Prac.* **10**, 25-31.
- Shafey T.M., Dingle J.G., McDonald M.W. and Kostner K. (2003). Effect of type of grain and oil supplement on the performance, blood lipoproteins, egg cholesterol and fatty acids of laying hens. *Int. J. Poult. Sci.* **2**, 200-206
- Sharma P. and Gujral S. (2010). Antioxidant and polyphenol oxidase activity of germinated barley and its milling fractions. *Food Chem.* **120**, 673-678
- Shen Ch.S.J., Chen I.S. and Sheppard A.J. (1982). Enzymatic determination of cholesterol in egg yolk. *J. Assoc. Offic. Anal. Chem.* **65**, 1222-1224.
- Silversides F.G. and Lefrançois M.R. (2005). The effect of feeding hemp seed meal to laying hens. *Brit. Poult. Sci.* **46**, 231-235.
- Sim J.S. and Qi G.H. (1995). Designing poultry products using flaxseed. Pp. 315-333 in *Flaxseed in Human Nutrition*, S.C. Cunnane and L.U. Thompson (Eds.). AOCS Press, Champaign, IL.
- Stein E.A. (1986). Quantitative enzymatic colorimetric determination of total cholesterol in serum or plasma. Pp. 879-886 and Pp. 1818-1829 in *Textbook of Clinical Chemistry*. W.B. Saunders. Ed. Philadelphia.
- Truswell A.S. (1999). Cereal grains and coronary heart disease. A review of the literature commissioned by "Go Grains". Accessed 01.11.2004.
<http://www.gograins.com.au>
- Vogel A.I. (1975). Methylation with diazomethane. A Textbook of Partial Organic Chemistry. 3rd Ed. Longman. Group Limited. London.
- Wang L., Newman R.K., Newman C.W. and Hofer P.J. (1992). Barley β -glucans alter intestinal viscosity and reduce plasma cholesterol concentrations in chicks. *J. Nutr.* **122**, 2292-2297.
- Wang L., Newman R.K., Newman C.W., Jackson L.L. and Hofer P.J. (1993). Tocotrienol and fatty acid composition of barley oil and their effects on lipid metabolism. *Plant. Foods Hum. Nutr.* **43**, 9-17.
- Wieland H. and Seidel D. (1983). A fully enzymatic colorimetric determination of LDL-cholesterol in serum. *J. Lipid Res.* **42**, 9-17.
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