Effects of dietary oxidized oil on laying performance, lipid metabolism, and apolipoprotein gene expression in laying hens

H. Y. Yue, J. Wang, X. L. Qi, F. Ji, M. F. Liu, S. G. Wu, H. J. Zhang, and G. H. Qi[1]

Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

ABSTRACT We studied the effects of dietary oxidized oils on serum lipid metabolic indices, estradiol level, and the gene expression of apolipoprotein B-100 and apolipoprotein VLDL-II in laying hens. Hy-Line Grey hens (280 ± 10 d old; average egg production, 90.0 ± 2.5%) were allotted to 1 of 4 dietary treatments, which were supplemented with 0 (control group), 1% (low oxidized group), 2% (moderately oxidized group), or 4% (highly oxidized group) thermally oxidized soybean oil. Each treatment contained 6 replicates, with 12 birds each. The feeding trial lasted for 30 d. Laying performance data were recorded weekly. Other indices were measured on d 0, 2, 6, 14, and 30 of the feeding trial. Hens in the moderately and highly oxidized groups had significantly lowered feed conversion ratios ($P < 0.05$). Those in the highly oxidized group also had decreased concentrations of serum very low density lipoprotein cholesterol on d 30 ($P < 0.05$) compared with the very low density lipoprotein cholesterol of hens in the moderately oxidized group. Hens in the moderately oxidized group had significantly increased expression of apolipoprotein B-100 ($P < 0.05$) from d 6 to 30. Consequently, hepatic triglyceride increased in this group on d 30 ($P < 0.05$). Serum triglyceride decreased in the moderately oxidized group on d 30 ($P < 0.05$), which may have been caused by the activation of peroxisome proliferator-activating receptor α. Serum estradiol levels were not significantly affected by oxidized oils at any time of measurement, but were significantly different between d 0 and 30 within the moderately oxidized group. This fact indicated that the effect of oxidized oils on apolipoprotein B-100 might partially be a cumulative result of the increasing secretion of estradiol. The results suggested that oxidized oil may affect the performance of laying hens through the regulation of apolipoproteins and estradiol.

Key words: dietary oxidized oil, lipid metabolism, estradiol, apolipoprotein, laying hen

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INTRODUCTION

Oils are often used in poultry diets, especially in the summer, to protect the birds from heat stress by decreasing the heat. Vegetable oils, which contain high levels of unsaturated fatty acids, are commonly used in poultry diets. Storage and feed preparation of the vegetable oils inevitably lead to lipid peroxidation, which produces large numbers of reactive oxygen species and causes the feed to contain various levels of oxidants (Warnants et al., 1996). When ingestion of the excess reactive oxygen species surpasses the ability of the antioxidant system of an organism to remove them, oxidative stress occurs (Urso and Clarkson, 2003).

Dietary oxidized lipids have also been related to serum oxidized lipoproteins in human (Zalejska-Fiolka, 2001; Dhawan and Jain, 2004) and rodent studies (Eder and Kirchgessner, 1997; Eder, 1999). In multiple animal species, oxidized oils decrease feed intake, depress growth, and even cause disease (Alexander, 1981; Sanchez-Muniz et al., 1998). In addition, dietary oxidized oil in the diets of rodents can change the lipid composition of certain organs, such as the liver and brain (Ammouche et al., 2002; Kode et al., 2005); affect milk triglyceride concentrations (Brandsch et al., 2004); and even alter erythrocyte antioxidant indices (Zalejska-Fiolka et al., 2007). In humans, dietary oxidized lipids are probably absorbed directly by the small intestine and incorporated into chylomicrons, which contribute to the oxidative status of the body (Staprans et al., 1994).

The effects of oxidized oil on laying hens, which possess a unique lipid metabolic system, have not yet been thoroughly investigated. In sexually mature laying hens, triglycerides are synthesized in the liver, transported mainly in the form of very low density lipoprotein cholesterol (**VLDL-C**), and finally deposited in egg yolks in the form of small-sized yolk-targeted

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[1]Corresponding author: qiguanghai@mail.caas.net.cn or guanghai_qi@163.com

VLDL-C (**VLDLy**; Walzem et al., 1999). This pathway is extremely important for egg production and may be regulated by many related factors, such as hormones and apolipoproteins. Thus, a laying hen experiment was set up to study the effects of different levels of dietary oxidized oils on serum lipid and lipoprotein composition, estrogen secretion, and related hepatic gene expression.

MATERIALS AND METHODS

Preparation of Oil

A modified thermal heating method was used to prepare the dietary oil (Seppanen and Csallany, 2004, 2006). Specifically, fresh food-grade soybean oil was purchased from the supermarket and exposed to air for 3 mo at room temperature. The oil was then heated and kept at a temperature of 185°C for 6 h with constant blending. The heated oil was cooled to room temperature and stored unstirred for 24 h. The oil was then heated again to 185°C for 3 h with constant blending and air supplementation. The experimental diets were made within 48 h after the oxidized oil was prepared. Control oil was purchased when the heating was in process to avoid further oxidation during storage. The peroxide value (**PV**), acid value, and iodine value of the oil mixtures were analyzed using methods ISO 3960:2001 IDT (ISO, 2001), ISO 660:1996 IDT (ISO, 1996a), and ISO 3961:1996 MOD (ISO, 1996b), respectively. The malondialdehyde (**MDA**) content was measured using an MDA assay kit (Nanjing Jiancheng Corp., Nanjing, China; Tan et al., 2010). The fatty acid composition of the experimental oils was analyzed according to methods EN ISO 5509:2000 (ISO, 2000) and ISO 5508:1990 (ISO, 1990), in which the *trans*-fatty acid was analyzed according to method ISO 15304:2002 IDT (ISO, 2002). Data on the concentrations of total polar compounds (**TPC**) in the experimental oils were obtained using an automatic oil tester (Testo 270, Testo AG, Lenzkirch, Germany) at room temperature. The concentrations of vitamin E (**VE**) isomers of the oils were analyzed using the method of Surai (2000) with modifications. Briefly, the oil samples were saponified in ethanol by potassium hydroxide in the presence of ascorbic acid. The VE isomers were then extracted with hexane. The hexane extract was dried by nitrogen, redissolved in methanol, and injected into an HPLC system (Waters E2595, Waters Technologies Corporation, Milford, MA) fitted with a Zorbax SB-C_{18} reverse-phase HPLC column (5-μm particle diameter, 250 × 4.6 mm, Agilent Technologies, Palo Alto, CA). Standards of α-, β-, γ-, and δ-VE isomers were purchased from Sigma-Aldrich (St. Louis, MO).

Birds and Diets

Two hundred eighty-eight 280 ± 10-d-old Hy-Line Grey hens (initial egg production of approximately 90.0 ± 2.5%) were randomly allotted to 1 of the 4 dietary treatments, which were supplemented with 0 (control group, **NX**), 1% (low oxidized group, **LX**), 2% (moderately oxidized group, **MX**), or 4% (highly oxidized group, **HX**) thermally oxidized soybean oil preparations. All diets were supplemented with fresh soybean oil to a total dietary oil percentage of 4%. Each treatment had 6 replicates, and each replicate had 4 cages with 3 hens/cage. The cage size was 43 × 43 × 43 cm. All birds were housed under routine conditions of temperature, humidity, illumination (16L:8D), and ventilation. The experimental diets were formulated according to NRC (1994) recommendations (Table 1). The pretrial lasted for 7 d, during which all birds were fed the NX diet to acclimate them to the high-oil diet. The feeding trial lasted for 30 d. All the experimental procedures were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences.

Sampling Procedures

On d 0 (i.e., the last day of the pretrial), 2, 6, 14, and 30, one hen from each replicate was randomly selected for blood and liver sampling. Blood samples were collected via exsanguination of the left jugular vein. Serum was isolated by centrifugation at 3,300 × *g* for 10 min at 4°C and stored at −20°C until analysis.

Table 1. Composition and nutrient levels of the experimental diets

Item	Amount
Ingredient (%)	
Corn	50.1
Soybean meal	21.3
Calcium carbonate	8.8
Wheat bran	6.42
Soybean oil[1]	4
Rapeseed meal	3.0
Rice-hull powder	2.1
Fish meal	2.0
Calcium hydrophosphate	1.0
Premix[2]	1.0
Sodium chloride	0.28
Total	100.0
Nutrient level	
AME[3] (MJ/kg)	11.21
CP[4] (%)	16.50
Ca[4] (%)	3.51
Total P[3] (%)	0.58
Nonphytate P[3] (%)	0.35
Lysine[3] (%)	0.835
Methionine[3] (%)	0.382

[1]The oxidized oils were as follows: 100% fresh soybean oil for the nonoxidized (control) group; 25% oxidized soybean oil + 75% fresh oil for the low oxidized group; 50% oxidized soybean oil + 50% fresh soybean oil for the moderately oxidized group; and 100% oxidized soybean oil for the highly oxidized group.

[2]Premix provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3, 4,125 IU; vitamin E, 15 IU; vitamin K, 2 mg; thiamine, 1 mg; riboflavin, 8.5 mg; calcium pantothenate, 50 mg; niacin, 32.5 mg; pyridoxine, 8 mg; biotin, 2 mg; folic acid, 5 mg; vitamin B_{12}, 5 mg; choline, 500 mg; Mn, 65 mg; I, 1 mg; Fe, 60 mg; Cu, 8 mg; Zn, 66 mg; Se, 0.3 mg.

[3]Calculated values.

[4]Determined values.

After slaughter, the birds were hung for 10 min, and then the left hepatic lobe was sampled and quickly frozen in liquid nitrogen within 2 min. The liver samples were kept at −80°C until analysis.

Serum Lipid and Hormone Analysis

Serum total cholesterol (**TC**), triglyceride (**TG**), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured using an enzymatic kit (both made by Beijing BHKT Clinical Reagent Co. Ltd., Beijing, China; Yu et al., 2009). Serum estradiol was analyzed with an RIA kit (SINO-UK Biological Technology, Beijing, China; Yuan et al., 2007). The above indices were measured with an automatic biochemical analyzer (Hitachi 7600-020, Hitachi Co., Tokyo, Japan). The VLDL-C concentration was determined with an ELISA kit (Groundwork Biotechnology Diagnosticate Ltd., San Diego, CA; Han et al., 2011) and a Multiskan Spectrum spectrophotometer (Thermo Labsystems, Franklin, MA).

Quantification of apoB-100 and apoVLDL-II mRNA with Real-Time PCR

Isolation of Total RNA. Total RNA was extracted from approximately 80 mg of liver using TRIzol reagent (Invitrogen Gibco-BRL, Bethesda, MD). The quality of the isolated total RNA was estimated from the 260:280 nm absorbance spectra (values of 1.9 to 2.1 in 10 m*M* Tris-HCl, pH 7.5, were considered acceptable) and by examination of the 18S and 28S bands after electrophoresis in 1% agarose gels stained with ethidium bromide. The RNA concentration was determined by measuring absorbance at 260 nm.

Reverse Transcription and cDNA Synthesis. According to the instructions for the QuantScript Reverse-Transcription Kit (Tiangen Biotech Co. Ltd., Beijing, China), 2 μL of 10× reverse transcription mix (buffering solution with RNAase inhibitor and dithiothreitol), 2 μL of deoxynucleoside triphosphate mixture (each 2.5 mmol/L), 2 μL of oligo-dT_{15} (10 μmol/L), 2 μg of template RNA, and 1 μL of Quant Reverse Transcriptase were added to the PCR tube, and the volume was brought to 20 μL with ribonuclease-free water. The mixture was vortexed for 5 s, centrifuged briefly, and then incubated in a 37°C water bath for 1 h. The cDNA templates for PCR amplification were stored at −20°C.

Quantitative Real-Time Reverse Transcription. Expression of the genes encoding apoB-100 and apoVLDL-II was assessed by 2-step relative quantitative real-time reverse-transcription PCR following the RealMasterMix (SYBR Green) and reverse-transcription PCR protocol (Tiangen Biotech Co. Ltd.). Messenger RNA amounts were quantified using an iCycler iQ multicolor real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA). Briefly, a 20-μL PCR mixture was set up as follows: cDNA, 0.8 μL; each primer (10 pmol/mL), 0.8 μL; 2.5× RealMasterMix:20× SYBR solution mixture, 9 μL; double-distilled H_2O, 8.6 μL. The temperature cycles were as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 30 s, 60°C for 20 s, 68°C for 30 s, and 80°C for 10 s. The SYBR green fluorescence was measured at the end of each cycle. The temperature interval for melting curve recording began at 60°C and ended at 100°C. Oligonucleotide sequences of primers, annealing temperatures, and the sizes of the expected PCR products are shown in Table 2. All primer concentrations were optimized before actual runs. All measurements were carried out in triplicate, and the average values were obtained. The values were normalized to mRNA expression of avian β-actin and are expressed as the ratio of the β-actin mRNA values in arbitrary units. The relative expression levels of hepatic apoB-100 and apoVLDL-II mRNA were calculated with the $2^{-\Delta\Delta Ct}$ method (where Ct is cycle threshold; Livak and Schmittgen, 2001).

Statistical Analysis

All data were subjected to one-way ANOVA, and the means were compared for significance by Duncan's multiple-range tests. All statistical analyses were carried out using SAS version 8.02 (SAS Institute, 2001). A *P*-value of 0.05 or less was considered significant unless otherwise stated.

RESULTS

Characterization of the Experimental Oils

The appearance, PV, acid value, iodine value, and TPC, VE, and MDA concentrations of the experimen-

Table 2. Sequences of real-time PCR primers

Gene[1]	Primer sequence[2] (5′–3′)	Fragment size (bp)	Annealing temperature (°C)	GenBank accession number
apoB-100	S: CTGCAAATGCTGGGCTGTTT AS: CTGGTTGAGCCATCCAGCTT	106	60	NM_001044633.1
apoVLDL-II	S: AGGGCTGAACTGGTACCAACAAAC AS: GGATGACCAGCCAGTCACGA	140	60	NM_205483.1
β-Actin	S: ATCCGGACCCTCCATTGTC AS: AGCCATGCCAATCTCGTCTT	120	60	NM205518

[1]apoB-100 = apolipoprotein B-100; apoVLDL-II = apolipoprotein VLDL-II; β-actin = avian β-actin.

[2]S = sense primer; AS = antisense primer.

Table 3. Characteristics of the experimental oil mixtures[1]

Item	NX (fresh oil)	LX (1:3)[2]	MX (1:1)[2]	HX (oxidized oil)
Appearance	Bright yellow, clear, with flavor			Dark brown, clear, thick, unpleasant smell
PV[3] (mEq of O_2/kg)	3.8	21.2	56.0	88.0
Acid value (mg of KOH/g)	0.60	0.85	1.35	2.15
Iodine value	140.2	132.0	136.0	130.0
MDA[4] (nmol/mL)	13.5	119.6	204.2	342.2
Total polar compounds (%)	1.1	3.4	6.2	12.0
Total tocopherol (mg/kg)	586.4	530.0	502.2	440.5
α-Tocopherol (mg/kg)	94.5	70.3	52.1	16.5
Fatty acid composition (mg/g of total analyzed fatty acids)				
18:2n-6	514.9	494.5	474.1	450.3
18:1n-9	209.3	206.3	205.0	203.9
16:0	111.8	112.3	112.5	116.2
18:3n-3	69.8	57.2	54.2	50.8
18:0	42.8	44.8	45.1	47.2
18:1n-7	14.0	13.7	13.6	13.6
18:2 *cis*-9, *trans*-12	2.17	2.80	2.91	3.29
18:2 *cis*-12, *trans*-9	1.81	2.45	2.51	2.95
18:1 *trans*-9	0.20	0.34	0.72	0.76

[1]NX = nonoxidized (control) group; LX = low oxidized group (fed 1% oxidized oil); MX = moderately oxidized group (fed 2% oxidized oil); HX = highly oxidized group (fed 4% oxidized oil).

[2]The ratios in parentheses represent the ratio of oxidized oil to fresh oil in the mixtures, which were used in the corresponding diets.

[3]PV = peroxide value.

[4]MDA = malondialdehyde.

tal oils (before inclusion in the diets) are shown in Table 3. Increases in PV (23-fold), acid value (3.6-fold), and TPC and MDA concentrations (25-fold) were noted. However, no consistent change was observed in the concentrations of different VE isomers.

Performance

The weekly egg production, ADFI, and feed conversion ratio (**FCR**) are shown (Table 4), as well as those for the whole period. During wk 1, 2, 3, as well as for the whole period, dietary oxidized oil supplementation did not significantly affect egg production. However, as compared with the HX group, the low concentration of dietary oxidized oil significantly increased egg production at wk 4 ($P < 0.05$). The ADFI of the MX and HX groups increased slightly with time, but the differences among all groups were not significant. For the whole feeding period, the FCR of the HX and MX groups were significantly higher than that of the NX group ($P < 0.05$). In contrast, the low concentration of oxidized oil did not significantly influence the FCR during the whole period.

Serum and Hepatic Lipids

The concentrations of serum TG, TC, high-density lipoprotein cholesterol, low-density lipoprotein choles-

Table 4. Effect of dietary oxidized oil on egg production, ADFI, and feed conversion ratio (FCR) in laying hens[1]

		Dietary treatment[2]				
Item	Time (wk)	NX	LX	MX	HX	Pooled SEM
Egg production (%)	1	88.7	88.7	87.7	88.4	0.7
	2	91.0	90.9	90.7	90.0	0.6
	3	92.5	91.3	92.3	88.6	0.7
	4	91.7^{ab}	94.6^{a}	91.5^{ab}	89.7^{b}	0.7
	1 to 4	91.0	91.4	90.6	89.2	0.5
ADFI (g/hen)	1	109.9	116.7	115.5	122.2	3.50
	2	114.8	112.6	126.7	124.7	4.85
	3	125.8	117.2	127.4	125.6	3.62
	4	122.0	124.8	128.8	127.2	3.57
	1 to 4	118.5	118.0	124.9	125.0	2.99
FCR (g of feed/g of egg)	1	2.00^{b}	2.06^{ab}	2.21^{a}	2.24^{a}	0.04
	2	2.06^{b}	2.12^{b}	2.27^{a}	2.32^{a}	0.03
	3	2.10^{b}	2.20^{ab}	2.25^{a}	2.31^{a}	0.02
	4	2.10^{b}	2.09^{b}	2.28^{a}	2.32^{a}	0.03
	1 to 4	2.06^{b}	2.12^{b}	2.25^{a}	2.30^{a}	0.03

[a,b]Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

[1]n = 6. Each replicate had 4 pens of birds.

[2]NX = nonoxidized (control) group; LX = low oxidized group (fed 1% oxidized oil); MX = moderately oxidized group (fed 2% oxidized oil); HX = highly oxidized group (fed 4% oxidized oil).

Table 5. Effect of dietary oxidized oil on serum and liver triglyceride (TG) and total cholesterol (TC) concentrations in laying hens[1]

		Dietary treatment[2]				
Item	Time (d)	NX	LX	MX	HX	Pooled SEM
Serum						
TG (mmol/L)	0	14.06	14.06	13.95	13.98	0.12
	2	13.97	13.82	13.66	13.86	0.29
	6	13.86	13.80	13.57	13.64	0.30
	14	14.69	14.00	13.62	13.69	0.26
	30	14.99^{a}	14.01^{ab}	13.67^{b}	13.82^{ab}	0.21
TC (mmol/L)	0	2.84	2.95	3.05	2.89	0.05
	2	2.87	2.92	2.93	2.95	0.10
	6	2.81	2.72	2.66	2.66	0.06
	14	2.85	2.83	2.65	2.77	0.08
	30	2.90	2.86	2.68	2.78	0.08
Liver						
TG (mmol/L)	0	13.76	13.98	13.90	13.69	0.39
	2	13.90^{a}	10.59^{ab}	10.35^{ab}	9.36^{b}	0.71
	6	13.97^{ab}	12.06^{b}	16.83^{a}	12.30^{b}	0.68
	14	13.95^{ab}	9.98^{b}	13.20^{ab}	14.40^{a}	0.70
	30	15.25	13.53	15.32	14.46	0.44
TC (mmol/L)	0	2.78	2.69	2.68	2.69	0.05
	2	2.67	2.83	2.88	2.76	0.07
	6	2.76	2.86	2.90	2.77	0.05
	14	2.79	2.90	2.73	2.78	0.04
	30	2.76^{ab}	2.94^{a}	2.55^{b}	2.92^{a}	0.05

^{a,b}Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

[1]n = 6. Each replicate had 1 hen.

[2]NX = nonoxidized (control) group; LX = low oxidized group (fed 1% oxidized oil); MX = moderately oxidized group (fed 2% oxidized oil); HX = highly oxidized group (fed 4% oxidized oil).

terol, and VLDL-C are shown in Tables 5 and 6. The initial values of all these indices were not significantly different. The MX group showed a decrease in the TG level at d 30 compared with the control group ($P < 0.05$). Serum TC, however, showed no significant differences among all treatments throughout the experiment. Compared with serum VLDL-C levels in the control group, levels in the MX group were significantly higher on d 14 and 30 ($P < 0.05$). The significance of this index appeared between the LX and NX groups on d 30 ($P < 0.05$). The differences in serum low-density lipoprotein levels among all treatments were not significant at all sampling times. Serum high-density lipoprotein showed inconsistent changes on d 2, 6, and 14 ($P < 0.05$), but on d 30, no significant differences could be observed.

The concentrations of hepatic TG and TC are listed in Table 5. From d 2 to 14, significant differences appeared among all treatments ($P < 0.05$) on the index of TG. Overall, the LX group had the lowest hepatic TG

Table 6. Effect of dietary oxidized oil on serum lipoprotein concentrations in laying hens[1]

		Dietary treatment[2]				
Item	Time (d)	NX	LX	MX	HX	Pooled SEM
HDL-C (mmol/L)	0	0.47	0.50	0.50	0.47	0.01
	2	0.44^{ab}	0.54^{a}	0.53^{a}	0.48^{b}	0.02
	6	0.48^{a}	0.39^{b}	0.42^{ab}	0.41^{ab}	0.01
	14	0.44^{ab}	0.42^{ab}	0.36^{b}	0.49^{a}	0.02
	30	0.52	0.56	0.52	0.56	0.02
LDL-C (mmol/L)	0	0.21	0.21	0.21	0.22	0.01
	2	0.20	0.19	0.21	0.25	0.01
	6	0.19	0.22	0.20	0.24	0.01
	14	0.23	0.24	0.23	0.25	0.01
	30	0.23	0.25	0.23	0.25	0.01
VLDL-C (mg/mL)	0	3.22	3.17	3.23	3.23	0.01
	2	3.22	3.14	3.16	3.27	0.04
	6	3.11	3.17	3.33	3.38	0.05
	14	3.19^{b}	3.36^{ab}	3.48^{a}	3.33^{ab}	0.09
	30	3.14^{b}	3.64^{ab}	3.73^{a}	3.25^{ab}	0.12

^{a,b}Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

[1]n = 6. Each replicate had 1 hen. HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very low density lipoprotein cholesterol.

[2]NX = nonoxidized (control) group; LX = low oxidized group (fed 1% oxidized oil); MX = moderately oxidized group (fed 2% oxidized oil); HX = highly oxidized group (fed 4% oxidized oil).

Table 7. Effect of dietary oxidized oil on serum estradiol concentration in laying hens[1]

Estradiol (pg/mL)	Dietary treatment[2]				Pooled SEM
	NX	LX	MX	HX	
0 d	68.72	68.00	65.15	65.92	0.09
2 d	73.14[a]	69.88[ab]	65.22[ab]	64.64[b]	0.13
6 d	68.56	68.52	66.14	66.96	0.10
14 d	67.22	65.88	67.28	67.70	0.11
30 d	67.34	65.48	69.08	68.62	0.13

[a,b]Means within a row with no common superscripts differ significantly (P ≤ 0.05).

[1]n = 6. Each replicate had 1 hen.

[2]NX = nonoxidized (control) group; LX = low oxidized group (fed 1% oxidized oil); MX = moderately oxidized group (fed 2% oxidized oil); HX = highly oxidized group (fed 4% oxidized oil).

levels at most sampling times, whereas the NX, MX, and HX groups had significantly higher levels on d 2, 6, and 14 ($P < 0.05$), respectively. Hepatic TC did not show any significant differences among the groups at most sampling times. However, the sudden decrease in hepatic TC in the MX group resulted in a significant difference on d 30 compared with hepatic TC in the LX and HX groups ($P < 0.05$)

Serum Estradiol Levels

Serum concentrations of estradiol are shown in Table 7. On d 2, serum estradiol concentration of the control group was significantly higher than that of the HX group ($P < 0.05$), which was largely caused by the sudden increase in estradiol in the control group. However, serum estradiol levels at all the other time points were not significantly different among the treatments.

Hepatic apoB-100 and apoVLDL-II Gene Expression

The effects of dietary oxidized oils on gene expression of hepatic apB-100 and apoVLDL-II are shown in Table 8. In this study, the whole flock showed very consistent apoB-100 gene expression levels at the beginning of the feeding trial. On d 2, 6, 14, and 30, significant differences in apoB-100 gene expression were observed ($P < 0.05$) among different groups. The MX group had the highest expression of apoB-100 on d 6 ($P < 0.05$), compared with the LX group on d 14 ($P < 0.05$) and with the LX and HX groups on d 30 ($P < 0.05$).

The differences in apoVLDL-II gene expression among the 4 treatments were significant beginning at d 2 ($P < 0.05$). During this period, the expression levels of apoVLDL-II in the control and LX groups were the lowest and were almost unchanged, whereas the HX group consistently had the highest relative values ($P < 0.05$). In addition, in comparison with d 0, the HX group had a significantly greater expression level of apoVLDL-II on d 30 (data not shown in individual tables, $P < 0.05$).

DISCUSSION

Some of the previous studies reported a performance-reducing effect of dietary oxidized oil (Sanchez-Muniz et al., 1998), whereas more studies found no negative effects on performance from feeding dietary oxidized oil (Suomela et al., 2004; Bou et al., 2005; Lewis-McCrea

Table 8. Effect of dietary oxidized oil on hepatic apolipoprotein B-100 (apoB-100) and apolipoproteins VLDL-II (apoVLDL-II) mRNA expression in laying hens[1,2]

Item	Time (d)	Dietary treatment[3]				Pooled SEM
		NX	LX	MX	HX	
apoB-100	0	1.00	1.00	1.00	1.00	0.02
	2	0.99[a]	0.85[ab]	0.75[b]	0.71[b]	0.03
	6	0.98[b]	0.86[b]	1.47[a]	0.93[b]	0.07
	14	0.98[a]	0.80[b]	1.07[a]	0.98[a]	0.03
	30	1.06[ab]	0.89[bc]	1.21[a]	0.83[c]	0.04
apoVLDL-II	0	1.00	1.00	1.01	1.06	0.01
	2	1.01[c]	0.88[c]	1.28[b]	1.55[a]	0.07
	6	1.02[bc]	0.89[c]	1.14[ab]	1.26[a]	0.04
	14	1.04[ab]	0.84[c]	0.98[bc]	1.19[a]	0.04
	30	1.05[b]	0.98[b]	1.07[b]	1.32[a]	0.04

[a–c]Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

[1]n = 6. Each replicate had 1 hen.

[2]The values of the NX group were used as control values for each sampling time to compare the differences among various treatments at the same time (Livak and Schmittgen, 2001).

[3]NX = nonoxidized (control) group; LX = low oxidized group (fed 1% oxidized oil); MX = moderately oxidized group (fed 2% oxidized oil); HX = highly oxidized group (fed 4% oxidized oil).

and Lall, 2007). It was surprising that, in our study, dietary oxidized oil depressed the performance of laying hens, even though the oils were not as oxidized as those in previous studies. In the LX group, the low amount of oxidized oil had almost no effect on the measured performance indices; thus, no apparent reduction in laying performance was found. Laying hens at this experimental age are fully mature and have adequate resistance against this type of exogenous challenge. The MX group had a significantly depressed laying performance; however, this group showed advantages on almost all the measured lipid metabolism-related indices. The reason for this contradiction may be partially explained as follows: Staprans et al. (1994) reported that oxidized lipids in the diet are a source of oxidized chylomicrons in humans, and a study by the same group (Staprans et al., 1993) showed that dietary oxidized oils also correlated with the levels of oxidized lipids in serum VLDL-C in rats. Thus, laying hens in the MX group may have had an increased level of oxidized VLDL-C and VLDLy. Thus, as a hypothesis, the deposition of these lipids in yolks could increase the oxidation of the yolk, which might disturb egg formation to some extent. In another study (our unpublished data), we found a slight decrease in egg weight ($P > 0.05$). In addition, in the present study, the MX and HX groups had a noticeable increase in ADFI ($P > 0.05$) in the last 2 wk of the experiment. These observations indicated that egg formation in these 2 groups was somehow inhibited and that the birds needed more nutrient uptake to counteract this challenge. The HX group did not show significant adverse effects on most indices related to lipid metabolism measured in this study. However, the increased ADFI and the slight change in egg weight caused a significant depression in FCR during the experiment, as compared with the control. This phenomenon indicates that highly oxidized dietary oil may have a negative influence on physiological functions of hens other than lipid metabolism. This implication is in accordance with many other studies in humans and other mammals that have investigated the effects of dietary oxidized oil on the fatty acid composition of tissues, antioxidant enzymes (Ammouche et al., 2002), erythrocyte antioxidant status, thyroid physiology (Skufca et al., 2003), and growth performance. As a hypothesis, highly oxidized oils might first affect these physiological aspects and then show a negative influence on lipid metabolism, which could be supported by the gene expression of apoB-100 being lowest in the HX group on d 30. Nevertheless, the high level of oxidation may also cause redox disorders in the birds we studied, leading to excess energy consumption.

Our data suggest that dietary supplementation of oxidized oils decreased serum TG in the MX and HX groups. Similar results were obtained by Eder (1999) in a rat study. It is well recognized that the decrease in serum TG is caused by reduced de novo fatty acid synthesis in the liver and the activation of peroxisome proliferator-activating receptor α, which enhances β-oxidation of fatty acids (Chao et al., 2001; Sülzle et al., 2004). In our study, the experimental oxidized soybean oil was relatively less oxidized than the oxidized sunflower oils used previously (Eder et al., 2003; Skufca et al., 2003); therefore, the decrease in TG was less apparent.

Studies on the effects of dietary oxidized oil on serum TC showed various results. Serum TC was not significantly affected by oxidized sunflower oil in rats (Ammouche et al., 2002). In contrast, Koch et al. (2007) observed a reduction in plasma and liver TC concentrations in rats fed oxidized sunflower oil. In the current study, serum TC showed a slight trend to a decrease over time except in the NX group. In our study, no significant differences in serum TC were observed among groups at any sampling time. However, once we included the feeding duration as a factor and looked especially at the MX group, a significant reduction in serum TC could be seen between d 0 and 14 ($P < 0.05$; data not shown) and between d 0 and 30 ($P < 0.05$; data not shown). The reduction in serum or plasma TC is considered to be a result of reduced activation of sterol regulatory element-binding protein-2 in adult liver (Koch et al., 2007), and our result supports this view, given the premise of treatment time. In addition to serum TC, the hepatic TC showed a significant reduction in the MX group compared with the LX group ($P < 0.05$). However, during the whole period (except on d 30), the hepatic TC concentrations in the LX, MX, and HX groups showed no differences from the NX group or were even higher than in the NX group ($P < 0.05$). This result was not in accordance with a study by Eder (1999), which showed a significant decrease in the level of hepatic TC in rats fed oxidized safflower oil. This contradiction might reflect the species specificity that laying hens differ from rats in hepatic lipid metabolism.

During the period of peak production, the serum VLDL-C and VLDLy concentrations in laying hens are of great importance for egg production. The VLDLy is the dominant form through which lipids can be deposited in the egg yolk (Walzem, 1996). Griffin et al. (1982) reported that VLDL-C in laying hens is mostly in the form of VLDLy, which is an important difference between laying hens and immature hens. According to this view, the alteration in serum VLDL-C that we observed may reflect the same tendency in serum VLDLy. In this study, significant differences appeared between the MX and the NX groups on d 14 and 30 ($P < 0.05$), which may indicate a cumulative effect of this level of dietary oxidized oil. Previous studies that observed similar increases in serum VLDL-C attributed the mechanism to alterations in endocrine function. Luskey et al. (1974) reported that estrogen greatly increased hepatic synthesis of VLDL-C in roosters. Walzem et al. (1999) observed that estrogen strongly stimulated the biosynthesis of VLDLy in the liver of laying hens. These studies mostly used direct injection of hormones, which was a positive stimulation of VLDL-C synthesis, and the results could be very significant. In our study,

the serum concentration of estradiol, a main component of estrogen, stayed at a similar level in all treatments at every sampling time. However, significant differences appeared between the levels of estradiol on d 30 and 0 in both the MX and HX groups ($P < 0.05$, data not shown). This phenomenon indicated that dietary oxidized oil could enhance estradiol secretion slowly and cumulatively. Thus, we hypothesized that the increase in estradiol secretion might partially contribute to the increase in serum VLDL-C in these 2 groups, especially in the later period of the experiment.

Walzem et al. (1999) proposed a possible pathway for generic VLDL-C and VLDLy synthesis. In this pathway, apoB-100 (in the kidney and liver) and apoVLDL-II (in the liver) are key factors that regulate VLDL-C and VLDLy synthesis. Furthermore, the synthesis of both apolipoproteins is regulated by estrogen. Capony and Williams (1980) reported that exogenous estrogen increases hepatic apoB-100 synthesis by 4- to 6-fold in the rooster. Williams (1979) observed a proportional increase in both apolipoproteins in the liver of roosters injected with exogenous estrogen. In our study, the MX group had significantly higher mRNA expression of apoB-100 from d 6 to 30, which kept the hepatic TG concentration in this group at a relatively high level during this period. The HX group had the highest mRNA expression of apoVLDL-II on all sampling times except d 0. However, the serum estradiol significantly increased only in the last few days of the experiment, as mentioned above, so estradiol probably did not contribute to the increase in gene expression of the lipoproteins in the early stage. On the other hand, although these 2 groups had the same change in estradiol secretion, they showed totally different patterns of change in the expression of apoB-100 and apoVLDL-II mRNA. This observation requires further investigation. Nevertheless, because apoB-100 triggers hepatic VLDLy synthesis (Walzem et al., 1999), this decrease may cause a decrease in serum VLDLy. This hypothesis was supported by decreased serum VLDL-C and reduced laying performance in the HX group. Pal et al. (2003) found that red wine polyphenols, like many lipid-lowering drugs, decrease apoB-100 secretion by 50% in HepG2 cells, which may reflect the redox-responsible nature of apoB-100. Thus, it can be hypothesized that the overoxidative status of the HX group may affect the mRNA expression of this apolipoprotein. Apolipoprotein VLDL-II is a unique apolipoprotein that appears only in VLDLy and that provides resistance for the VLDLy particle against degradation by lipoprotein lipase (Schneider, et al., 1990). Expression of the apoVLDL-II gene was significantly higher in the HX group than in the other groups, which could be a positive factor affecting VLDL-C synthesis. However, the fluctuation of the upriver apoB-100 gene expression disturbed the synthesis of VLDL-C. Thus, in the HX group, the serum VLDL-C level remained steady throughout the experiment.

In summary, dietary oxidized oil could decrease the performance of laying hens. The reasons for these negative effects might result from an impairment in the mRNA expression of apolipoproteins, a disturbance of VLDL-C biosynthesis, and an abnormal upregulation of estradiol secretion. All these effects could eventually lower laying hen productivity and, possibly, bird welfare.

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