METABOLISM AND NUTRITION

Effects of supplementation with different rapeseed oil sources and levels on production performance, egg quality, and serum parameters in laying hens

N. Yuan,[*,2] J. P. Wang,[*,2] X. M. Ding,[*] S. P. Bai,[*] Q. F. Zeng,[*] Z. W. Su,[*] Y. Xuan,[*] H. W. Peng,[*] G. S. Fraley,[†] and K. Y. Zhang[*,1]

[*]*Animal Nutrition Institute, Sichuan Agricultural University, Key Laboratory for Animal Disease-Resistance Nutrition of China, Ministry of Education, Chengdu 611130, China; and* [†]*Biology Department and Neuroscience Program, Hope College, Holland, MI 49423, United States of America*

ABSTRACT This study was to determine the effects of rapeseed oil on production performance, egg quality, and serum parameters in laying hens. A total of 1,080 hens (33-wk-old) were randomly divided into a 1 plus 4 × 2 factorial design including four different rapeseed oil sources [high erucic acid of Mianyang city (MH); high erucic acid of Deyang city (DH); low erucic acid of Mianyang (ML); low erucic acid of Deyang (DL)] at two levels (2% and 4%) for 12 wk. The egg production and egg weight were decreased ($P < 0.05$) during 9 to 12 wk and 1 to 12 wk, while the average daily feed intake (ADFI) and feed conversion ratio were decreased ($P < 0.01$) in all phases compared to the control group. Adding ML as oil source had higher ($P < 0.05$) egg weight compared to DH in all periods in spite of levels. Meanwhile, layers fed 4% rapeseed oil decreased ($P < 0.01$) egg production compared with 2% in all phases except 1 to 4 wk. Regardless of rapeseed oil sources, hens fed 4% oil decreased ($P < 0.05$) egg weight in contrast to 2% during the whole experiment except 5 to 8 wk. The ADFI was lower ($P < 0.01$) in 4% oil inclusion groups compared with 2% during overall phase. Rapeseed oil decreased the yolk color ($P < 0.01$) and yolk ratio ($P = 0.02$) and increased ($P < 0.01$) the albumen height and Haugh unit at 12 wk. Dietary rapeseed oil supplementation resulted in a decreased total triglyceride (TG; $P < 0.01$) and increased high-density lipoprotein cholesterol ($P = 0.02$). Regardless of rapeseed oil levels, layers fed MH had higher TG ($P < 0.01$), TC ($P < 0.05$), low-density lipoprotein cholesterol ($P < 0.05$), alanine transaminase ($P < 0.01$) than those fed other sources. Taken together, the addition of rapeseed oil decreased laying performance, reduced TC and TG in the serum, and increased Haugh unit, with low erucic acid or 2% group showed more pronounced results among all treatments.

Key words: egg quality, erucic acid, laying hens, production performance, rapeseed oil

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INTRODUCTION

Rapeseed oil is a major domestic oil in China, accounting for 17.4% of the total consumption of domestic vegetable oil (National Bureau of Statistics of China, 2017). The Sichuan province is a major source of cabbage-type and mustard-type rapeseed oil (Feng, 2012). Rapeseed oil is the only vegetable oil that contains erucic acid (Muhammad et al., 2017). China National Standards suggest that the content of erucic acid is not more than 5% in a diet that includes no more than 45.0 μmol/g glucosinolate (GB 1536-2004). Levels of glucosinates in rapeseed meal can be defined at four levels: very low glucosinolate rapeseed meal (1 to 5 μmol/g glucosinolate), low glucosinolate rapeseed meal (10 to 30 μmol/g glucosinolate), moderate glucosinolate rapeseed meal (30 to 60 μmol/g glucosinolate), and high glucosinolate rapeseed meal (≥60 μmol/g glucosinolate), respectively (Mawson et al., 1993; Tripathi and Mishra, 2007). Different sources of rapeseed oils contain differning levels of the various fatty acids, for example, the content of erucic acid is 38% to 45% in Chinese cabbage, and 43% to 53% in Brassica napus (Muhammad et al., 2017). Compared to the other levels of glucosinolate, the low level of rapeseed oil has a high oleic acid content and low erucic acid content, which is easier to be digested and more beneficial to human health (Crowe et al., 2008). In recent years, there have been

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[1]Corresponding author: zkeying@sicau.edu.cn
[2]These two authors contributed this work equally.

Table 1. Composition and nutrient level of the basal diets (air dry basis).

Item	Control	2%	4%
Ingredients, %			
Corn	63.88	56.62	49.35
Soybean meal	21.84	23.95	26.07
Wheat bran	–	4.80	9.60
Corn gluten meal	3.30	1.65	–
Rapeseed oil[3]	–	2.00	4.00
Calcium carbonate	8.35	8.35	8.34
Dicalcium phosphate	1.20	1.16	1.11
NaCl	0.40	0.40	0.40
Vitamin premix[1]	0.03	0.03	0.03
Mineral premix[2]	0.50	0.05	0.50
L-Lysine HCl	0.11	0.05	–
DL-Methionine	0.08	0.10	0.12
L-Threonine	–	–	0.01
Choline chloride	0.10	0.10	0.10
Unite bran	0.21	0.29	0.38
Total	100	100	100
Calculated nutrients			
Metabolizable energy, kcal/kg	2700	2700	2700
Crude protein, %	16.5	16.5	16.5
Calcium, %	3.50	3.50	3.50
Available phosphorus, %	0.32	0.32	0.32
Digestible Lysine, %	0.75	0.78	0.78
Digestible Methionine, %	0.37	0.34	0.34
Digestible Threonine, %	0.55	0.55	0.55
Digestible Tryptophan, %	0.15	0.16	0.16

[1]Provided per kilogram of diet: vitamin A, 6,875 IU; vitamin D_3, 1,640 IU; vitamin E, 30.01 mg; thiamine, 1 mg; riboflavin, 3.9 mg; pyridoxine, 3.375 mg; vitamin B_{12}, 0.01 mg; calcium pantothenate, 8.85 mg; folate,0.5 mg; biotin, 0.1 mg; niacin, 49.25 mg.

[2]Provided per kilogram of diets: Fe ($FeSO_4 \cdot H_2O$) 60 mg, Cu ($CuSO_4 \cdot 5H_2O$) 10 mg, Mn ($MnSO_4 \cdot H_2O$)100 mg, Zn ($ZnSO_4 \cdot H_2O$) 80 mg, I (KI) 1 mg, Se (Na_2SeO_3) 0.35 mg.

[3]Four different sources: high erucic acid of Mianyang (MH), high erucic acid of Deyang (DH), low erucic acid of Mianyang (ML), low erucic acid of Deyang (DL).

Table 2. Quality indicators and fatty acid composition of four different oil sources.

Items[1]	MH	DH	ML	DL
Acid value (KOH mg/g)	0.996	0.592	0.867	0.813
Iodine value (g/100 g)	92.22	103.22	108.65	112.28
Refractive index (n^{40})	1.466	1.466	1.466	1.466
Specific gravity(d_{20}^{20})	0.912	0.915	0.918	0.919
Fatty acid composition (% total fatty acids)				
Palmitic acid C16:0	2.5	3.4	3.7	4.2
Palmitoleic acid C16:1	0.1	0.2	0.2	0.2
Stearic acid C18:0	0.7	1.1	1.7	1.6
Oleic acid C18:1	11.4	28.4	54.0	58.5
Linoleic acid C18:2	11.5	13.4	16.4	16.9
Linolenic acid C18:3	8.0	8.6	8.9	10.1
Arachidic acid C20:0	0.7	0.6	0.6	0.5
Eicosenoic acid C20:1	3.4	12.6	2.6	1.5
Eicosapentaenoic acid C20:5	–[2]	–	–	–
Erucic acid C22:1	54.0	36.0	7.3	2.1
Docosahexaenoic acid C22:6	–	–	–	–
Others	7.5	5.7	4.6	4.3
∑Saturated	3.9	5.1	6.0	6.3
∑Unsaturated	88.4	99.2	89.4	89.3
∑Polyunsaturated	19.5	22	25.3	27

[1]Abbreviation represented: MH = high erucic acid of Mianyang, DH = high erucic acid of Deyang, ML = low erucic acid of Mianyang, DL = low erucic acid of Deyang.

[2]"–"means below the detection limit.

several studies that investigated the nutritional value of rapeseed oil.

In recent years, many types of oils are used commercially in laying hens to provide lipids in the diet. The addition of lipids in layer diets have been shown by some to alter feed intake, energy efficiency, egg production, and egg weight (Shafey et al., 2003; Fouladi et al., 2008; Agah et al., 2012). However, others have shown that dietary lipids do not affect egg production, egg weight, egg yolk weight, and serum cholesterol levels (Ceylan et al., 2011; Garcia et al., 2013). These discrepancies may be attributed to different erucic acid content in the oil source. The erucic acid content of rapeseed oil is an important factor affecting its edible value and nutritional value (Lei et al., 2010). The rapeseed oil with more than 10% of erucic acid content can inhibit the growth performance and increased the oxidation rate of long-chain fatty acids in the liver. At the same time, it increase the accumulation of erucic acid in different tissues and lead to myocardial fat metabolism of the rats (Dewailly et al., 1978; Murphy, 2008). However, there is not known whether the different varieties of rapeseed oil with varient erucic acid content would affect laying performance and serum lipid profile.

Therefore, the aim of this study was to investigated the effect of dietary different sources of rapeseed oil supplementation on layer production performance, egg quality and serum biochemical indexes.

MATERIALS AND METHODS

Rapeseed Oil Preparation

Rapeseed of four typical different sources was selected and purchased from represented farms in city of Mianyang and Deyang (Sichuan, China). Rapeseed without peeling was processed by spiral hot extrusion to obtain rapeseed oil of four varieties (MH, high erucic acid of Mianyang; DH, high erucic acid of Deyang; ML, low erucic acid of Mianyang; DL, low erucic acid of Deyang).

Chemical Analysis of Rapeseed Oils

The chemical composition of these four rapeseed oil source was shown in Table 2. Acid value, iodine value, refractive index, and specific gravity of four different rapeseed oils were measured by the official methods of the AOCS (American Oil Chemists' Society 1998; cd 3d-63, cd 1d-92, Cc 7-25, and Cc 10b-25, respectively). Fatty acids were analyzed by gas chromatography at the Institute of Chengdu Grain and Oil Quality Supervision and Inspection Testing Center. Lipids (0.15 to 0.20 g) were extracted by ether from each sample (total of two) and saponified with 5 mL NaOH in methanol for 10 min. Five milliliter BF3-methanol was added and refluxed for 2 min. Then 5 mL of heptane was added to the mixture and boiled for 1 min. The final mixture was transferred into 25 mL volumetric flasks and the

Table 3. The fatty acid composition of the diets (% total fatty acids).

Items[1]	Control	MH		DH		ML		DL	
		2%	4%	2%	4%	2%	4%	2%	4%
Palmitic acid C16:0	16.85	9.51	7.43	10.04	7.73	10.27	8.37	11.01	8.44
Palmitoleic acid C16:1	–	–	0.19	–	0.22	0.2	–	0.2	0.22
Stearic acid C18:0	1.78	1.31	1.08	1.61	1.3	1.69	1.61	1.71	1.57
Oleic acid C18:1	22.13	17.9	16.24	28.86	31.12	40.53	45.47	41.3	49.12
Linoleic acid C18:2	56.4	33.98	27.07	34.77	27.06	36.33	31.7	38.25	30.48
Linolenic acid C18:3	2.84	6.16	7.1	6.33	7.42	6.36	7.28	6.7	8.31
Eicosaenoic acid C20:1	–[2]	2.5	3.26	6.96	10.21	1.45	1.6	0.84	1.11
Erucic acid C22:1	–	28.63	37.62	11.43	14.94	3.18	3.98	–	0.76
$\sum$Saturated	18.63	10.82	8.51	11.65	9.03	11.96	9.98	12.72	10.01
$\sum$Unsaturated	81.37	89.17	91.48	88.35	90.97	88.05	90.03	87.29	90
$\sum$Polyunsaturated	59.24	40.14	34.17	41.1	34.48	42.69	38.98	44.95	38.79

[1]Abbreviation represented: MH = high erucic acid of Mianyang, DH = high erucic acid of Deyang, ML = low erucic acid of Mianyang, DL = low erucic acid of Deyang.
[2]"–"means below the detection limit.

Table 4. Effect of different rapeseed oil sources and levels on production performance of laying hens.[1]

Item		Egg production, %				Egg weight, g				ADFI, g				FCR			
Source	Level	1 to 4 wk	5 to 8 wk	9 to 12 wk	1 to 12 wk	1 to 4 wk	5 to 8 wk	9 to 12 wk	1 to 12 wk	1 to 4 wk	5 to 8 wk	9 to 12 wk	1 to 12 wk	1 to 4 wk	5 to 8 wk	9 to 12 wk	1 to 12 wk
Control	0	89.37	84.76	81.24^{a}	85.13^{a}	59.0^{a,b}	60.4^{a}	61.3^{a}	60.2^{a,b}	113^{a}	113^{a}	112^{a}	113^{a}	2.16^{a}	2.22^{a}	2.27^{a}	2.21^{a}
MH	2%	87.54	82.33	81.97^{a}	83.42^{a-c}	59.1^{a,b}	59.4^{a,b}	60.4^{a-c}	59.6^{a-c}	107^{b}	100^{b}	101^{b,c}	103^{b}	2.09^{a,b}	2.06^{a,b}	2.07^{a,b}	2.07^{a,b}
MH	4%	88.32	79.20	71.27^{b}	79.75^{c}	57.9^{b}	58.9^{a,b}	59.7^{b,c}	58.8^{b,c}	103^{c}	95^{c}	93^{f}	97^{c}	2.02^{a,b}	2.06^{a,b}	2.20^{a,b}	2.09^{a,b}
DH	2%	88.33	82.95	76.22^{a,b}	82.27^{a-c}	58.6^{a,b}	59.6^{a,b}	60.7^{a-c}	59.7^{a-c}	107^{b}	102^{b}	101^{b}	103^{b}	2.07^{a,b}	2.06^{a,b}	2.21^{a,b}	2.11^{a,b}
DH	4%	87.74	78.36	73.27^{a,b}	79.61^{c}	59.7^{b}	58.5^{b}	59.5^{c}	58.6^{c}	103^{c}	95^{c}	94^{f}	98^{c}	2.04^{a,b}	2.08^{a,b}	2.17^{a,b}	2.11^{a,b}
ML	2%	86.55	83.23	79.68^{a,b}	82.83^{a-c}	58.7^{a}	60.4^{a}	61.4^{a}	60.5^{a}	108^{b}	101^{b}	99^{c,d}	103^{b}	2.09^{a,b}	2.02^{b}	2.03^{b}	2.04^{a,b}
ML	4%	87.20	79.79	75.76^{a,b}	80.75^{a-c}	58.9^{a,b}	59.8^{a,b}	61.1^{a,b}	59.9^{a-c}	102^{c}	98^{c}	97^{e}	99^{c}	1.99^{b}	2.05^{a,b}	2.09^{a,b}	2.06^{a,b}
DL	2%	91.31	84.17	79.78^{a,b}	84.88^{a,b}	58.8^{a,b}	59.7^{a,b}	60.4^{a-c}	59.6^{a-c}	107^{b}	101^{b}	99^{d}	103^{b}	1.99^{b}	2.03^{a,b}	2.06^{a,b}	2.02^{b}
DL	4%	87.89	80.43	72.85^{a,b}	80.32^{b,c}	59.1^{a,b}	60.0^{a,b}	60.7^{a-c}	59.9^{a-c}	101^{c}	97^{c}	94^{f}	98^{c}	1.95^{b}	2.01^{b}	2.12^{a,b}	2.02^{b}
SEM		1.31	1.68	1.77	1.44	0.33	0.35	0.28	0.29	0.51	0.42	0.28	0.25	0.03	0.04	0.05	0.03
P-value		0.36	0.08	<0.01	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.05	<0.01	<0.01
Main effect																	
Source	MH	87.93	80.77	76.62	81.58	58.5^{a,b}	59.1^{b}	60.1^{b}	59.2^{b}	105	98	97^{b,c}	100	2.06	2.06	2.13	2.08
	DH	88.04	80.66	74.75	80.94	58.2^{b}	59.1^{b}	60.1^{b}	59.1^{b}	105	99	98^{a,b}	100	2.05	2.07	2.19	2.11
	ML	86.88	81.51	77.72	81.79	59.2^{a}	60.1^{a}	61.2^{a}	60.2^{a}	105	99	98^{a}	101	2.04	2.03	2.06	2.05
	DL	89.60	82.30	76.32	82.60	58.9^{a,b}	59.9^{a,b}	60.5^{a,b}	59.8^{a,b}	104	99	97^{c}	100	1.97	2.02	2.09	2.02
Level	2%	88.43	83.17^{a}	79.42^{a}	83.35^{a}	59.1^{a}	59.8	60.7^{a}	59.9^{a}	107^{a}	101^{a}	100^{a}	103^{a}	2.06^{a}	2.04	2.09	2.06
	4%	87.79	79.45^{b}	73.29^{b}	80.11^{b}	58.4^{b}	59.3	60.2^{b}	59.3^{b}	102^{b}	96^{b}	94	98^{b}	2.00^{b}	2.05	2.15	2.07
SEM		0.73	0.81	0.91	0.75	0.17	0.18	0.14	0.15	0.23	0.27	0.13	0.15	0.02	0.02	0.03	0.02
P-value																	
Source		0.32	0.73	0.44	0.74	0.03	<0.01	<0.01	<0.01	0.14	0.06	<0.01	0.11	0.06	0.58	0.06	0.07
Level		0.53	<0.01	<0.01	<0.01	<0.01	0.07	0.02	0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.77	0.12	0.77
Source*level		0.45	0.97	0.15	0.85	0.13	0.24	0.09	0.12	0.11	0.12	<0.01	0.04	0.73	0.92	0.4	0.99
Contrast[2]		0.37	0.06	0.01	0.03	0.48	0.03	<0.01	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a–f]Means with different superscripts in the same column differ significantly ($P < 0.05$).
[1]Each mean represents five cages, with 3 layers/cage. Abbreviation represented: MH = high erucic acid of Mianyang, DH = high erucic acid of Deyang, ML = low erucic acid of Mianyang, DL = low erucic acid of Deyang, ADFI = Average Daily Feed Intake, FCR = Feed Conversion Ratio.
[2]Contrast: control vs other rapeseed oil groups.

volume was adjusted with saturated NaCl to 25 mL of which 1 mL of the heptane phase within the volumetric flasks was used to determine the fatty acids composition. Fatty acids were analyzed with gas chromatography (Agilent 6890 N, Hewlett Packard, Palo Alto, CA) using a capillary column (supel covax 10,60 m × 0.25 mm ID). The chromatographic conditions were: detector temperature 280°C; injector temperature 200°C; initial column temperature 100°C for 8 min and programmed to increase at a rate of 5°C per 5 min up to 200°C and then at 4°C per minute up to the final temperature of 250°C. The helium carrier gas flow was set at 1.2 mL/min, hydrogen at 30 mL/min, and air at 300 mL/min. Injection of the 1 μL samples was performed with a split ratio of 20:1. Identification of individual fatty acids was based on comparisons of retention times of unknown peaks to authentic fatty acid methyl ester standards.

Birds, Diets, and Management

The experimental procedures were approved by the Animal Care and Use Committee of Sichuan Agricultural University (No. SYXK2014-197). At 33 wk of age, a total of 1,080 Lohman pink shelled hens were randomly divided into nine treatment groups, including 1 plus 4 × 2 design experiment in which hens were fed a diet without rapeseed oil or diets containing four

Table 5. Effect of different rapeseed oil sources and levels on egg quality of laying hens at 12 wk.[1]

Item		Egg shell color			Eggshell strength, kg/cm^3	Eggshell thickness, mm	Albumen height, mm	Yolk color	HU	Yolk ratio, %	Shell ratio, %	Albumen ratio, %
		L*	a*	b*								
Source	Level											
Control	0	75.23	4.14	14.67	5.27	35.94	6.50^{b}	10.50^{a}	78.72^{b}	27.05^{a}	10.77	62.76^{b}
MH	2%	75.80	3.89	14.50	5.15	36.92	6.83^{a,b}	10.14^{a,b}	82.35^{a,b}	26.63^{a}	10.80	62.46^{b}
MH	4%	75.56	3.87	14.85	5.06	37.24	6.83^{a,b}	8.57^{d}	82.46^{a,b}	25.04^{b}	10.69	64.13^{a}
DH	2%	75.39	4.04	14.81	5.23	35.51	6.66^{a,b}	9.60^{b,c}	81.14^{a,b}	26.76^{a}	10.72	62.18^{b}
DH	4%	76.40	4.02	14.58	5.40	35.83	6.98^{a,b}	8.67^{d}	83.30^{a}	26.47^{a}	10.76	62.67^{b}
ML	2%	77.40	3.48	14.30	5.33	35.97	7.03^{a}	9.85^{b,c}	83.53^{a}	27.06^{a}	10.66	61.96^{b}
ML	4%	75.99	3.94	14.75	5.03	36.08	6.62^{a,b}	8.63^{d}	80.07^{a,b}	26.05^{a,b}	10.41	63.20^{a,b}
DL	2%	75.68	4.05	14.38	4.80	35.67	6.90^{a,b}	9.47^{c}	82.43^{a,b}	26.90^{a}	10.55	62.32^{b}
DL	4%	75.07	4.33	14.54	4.96	36.35	7.03^{a}	8.70^{d}	83.32^{a}	26.62^{a}	10.65	62.12^{b}
SEM		0.57	0.26	0.36	0.20	0.57	0.11	0.12	0.87	0.25	0.15	0.3
P-value		0.13	0.61	0.97	0.49	0.44	<0.01	<0.01	<0.01	<0.01	0.69	<0.01
Main effect												
Source	MH	75.68	3.88	14.68	5.10	37.08	6.83	9.36	82.4	25.83	10.74	63.30
	DH	75.89	4.03	14.69	5.31	35.67	6.82	9.14	82.22	26.61	10.74	62.42
	ML	76.70	3.71	14.52	5.18	36.03	6.82	9.24	81.8	26.56	10.53	62.58
	DL	75.37	4.19	14.46	4.88	36.01	6.96	9.09	82.88	26.76	10.6	62.22
SEM		0.39	0.18	0.26	0.15	0.42	0.08	0.09	0.61	0.18	0.11	0.22
Level	2%	76.06	3.87	14.50	5.13	36.02	6.85	9.77^{a}	82.36	26.84^{a}	10.68	62.23^{b}
	4%	75.76	4.04	14.68	5.11	36.37	6.86	8.64^{b}	82.29	26.04^{b}	10.62	63.03^{a}
SEM		0.28	0.13	0.19	0.11	0.29	0.10	0.06	0.43	0.13	0.08	0.18
P-value												
Source		0.11	0.29	0.90	0.22	0.10	0.57	0.12	0.66	<0.01	0.42	<0.01
Level		0.43	0.34	0.49	0.93	0.39	0.9	<0.01	0.9	<0.01	0.59	<0.01
Source∗level		0.18	0.74	0.81	0.63	0.97	0.02	0.01	<0.01	0.03	0.67	0.02
Contrast[2]		0.26	0.51	0.83	0.49	0.68	<0.01	<0.01	<0.01	0.02	0.47	0.68

[a-c]Means with different superscripts in the same column differ significantly ($P < 0.05$).

[1]Each mean represents 24 cages, with 1 layers/cage. Abbreviation represented: MH = high erucic acid of Mianyang, DH = high erucic acid of Deyang, ML = low erucic acid of Mianyang, DL = low erucic acid of Deyang L* = lightness; a* = redness; b* = yellowness.

[2]Contrast: control vs other rapeseed oil groups.

Table 6. Effect of different rapeseed oil sources and levels on storage egg quality of laying hens (stored for 7 d).[1]

Item		Eggshell strength, kg/cm^3	Eggshell thickness, mm	Albumen height, mm	Yolk color	HU	Yolk ratio, %	Shell ratio, %	Albumen ratio, %
Source	Level								
Control	0	4.199	31.57	6.45^{b}	9.92^{a}	79.74^{b}	27.23^{a}	10.53	62.43^{b}
MH	2%	4.301	30.18	6.83^{a,b}	9.53^{a,b}	82.35^{a,b}	26.54^{a,b}	10.72	62.46^{b}
MH	4%	4.222	30.48	6.83^{a,b}	8.32^{c}	82.46^{a,b}	25.32^{b}	10.71	64.12^{a}
DH	2%	4.057	30.51	6.66^{a,b}	9.63^{a,b}	80.75^{a,b}	26.77^{a,b}	10.92	62.60^{a,b}
DH	4%	4.483	30.33	7.04^{a}	8.09^{c}	84.01^{a}	26.30^{a,b}	11.03	62.68^{a,b}
ML	2%	4.350	30.98	7.03^{a,b}	9.33^{b}	83.13^{a,b}	26.70^{a,b}	10.72	62.59^{a,b}
ML	4%	4.061	30.47	6.61^{a,b}	8.05^{c}	80.37^{a,b}	26.16^{a,b}	10.52	63.33^{a,b}
DL	2%	4.027	30.29	6.83^{a,b}	9.20^{b}	82.43^{a,b}	26.90^{a,b}	10.84	62.45^{b}
DL	4%	4.091	30.33	6.95^{a,b}	8.10^{c}	83.32^{a,b}	26.86^{a,b}	10.8	62.32^{b}
SEM		0.170	0.40	0.13	0.11	0.84	0.36	0.18	0.36
P-value		0.58	0.31	0.03	<0.01	<0.01	0.02	0.57	<0.01
Main effect									
Source	MH	4.262	30.33	6.83	8.92^{a}	82.4	25.93	10.71	63.29
	DH	4.270	30.42	6.85	8.86^{a,b}	82.38	26.53	10.97	62.64
	ML	4.206	30.73	6.82	8.69^{a,b}	81.75	26.43	10.62	62.96
	DL	4.059	30.31	6.89	8.65^{b}	82.87	26.88	10.82	62.39
	2%	4.184	30.49	6.84	9.42^{a}	82.16	26.73^{a}	10.8	62.53^{b}
Level	4%	4.214	30.40	6.86	8.14^{b}	82.54	26.16^{b}	10.76	63.11^{a}
SEM		0.09	0.20	0.07	0.05	0.42	0.18	0.1	0.19
P-value									
Source		0.61	0.72	0.95	0.03	0.62	0.08	0.36	0.09
Level		0.81	0.76	0.84	<0.01	0.53	0.03	0.81	0.03
Source∗level		0.23	0.78	0.03	0.18	<0.01	0.46	0.91	0.07
Contrast[2]		0.99	0.33	<0.01	<0.01	<0.01	0.04	0.19	0.31

[a-c]Means with different superscripts in the same column differ significantly ($P < 0.05$).

[1]Each mean represents 24 cages, with 1 layers/cage. Abbreviation represented: MH = high erucic acid of Mianyang, DH = high erucic acid of Deyang, ML = low erucic acid of Mianyang, DL = low erucic acid of Deyang L* = lightness; a* = redness; b* = yellowness.

[2]Contrast: control vs other rapeseed oil groups.

Table 7. Effect of different rapeseed oil sources and levels on storage egg quality of laying hens (stored for 14 d)[1].

Item		Eggshell strength, kg/cm^3	Eggshell thickness, mm	Albumen height, mm	Yolk color	HU	Yolk ratio, %	Shell ratio, %	Albumen ratio, %
Source	Level								
Control	0	4.057	31.74	5.19^{a,b}	9.75^{a}	70.22^{b,c}	29.56	12.28	58.16
MH	2%	4.138	31.82	5.76^{a}	9.61^{a}	77.43^{a}	28.67	12.16	59.17
MH	4%	4.241	31.71	5.40^{a,b}	8.50^{b}	74.50^{a,b}	28.64	12.24	59.12
DH	2%	4.140	33.43	5.29^{a,b}	9.25^{a}	73.16^{a-c}	29.57	11.85	58.58
DH	4%	4.265	31.35	5.36^{a,b}	8.25^{b}	75.71^{a}	29.95	11.85	58.20
ML	2%	4.480	33.28	4.66^{b}	9.36^{a}	68.94^{c}	29.11	12.21	58.69
ML	4%	4.294	32.58	5.36^{a,b}	8.41^{b}	73.75^{a-c}	29.00	12.36	58.64
DL	2%	3.867	32.36	5.43^{a,b}	9.18^{a}	74.69^{a,b}	29.10	12.20	58.69
DL	4%	4.034	31.63	4.79^{b}	8.21^{b}	68.75^{c}	28.81	12.01	59.18
SEM		0.190	0.80	0.19	0.12	1.16	0.43	0.22	0.51
P-value		0.50	0.54	<0.01	<0.01	<0.01	0.39	0.71	0.81
Main effect									
Source	MH	4.189	31.76	5.58^{a}	9.05^{a}	75.97^{a}	28.66	12.20	59.14
	DH	4.202	32.39	5.33^{a,b}	8.75^{a,b}	74.43^{a,b}	29.76	11.85	58.39
	ML	4.387	32.93	5.01^{b}	8.89^{a,b}	71.34^{b}	29.06	12.28	58.66
	DL	3.951	31.99	5.11^{a,b}	8.69^{b}	71.72^{b}	28.96	12.11	58.94
SEM		0.130	0.58	0.14	0.09	0.85	0.36	0.16	0.42
	2%	4.156	32.72	5.29	9.35^{a}	73.55	29.11	12.11	58.78
Level	4%	4.208	31.82	5.23	8.34^{b}	73.18	29.10	12.11	58.79
SEM		0.09	0.41	0.10	0.06	0.60	0.26	0.11	0.30
P-value									
Source		0.14	0.51	0.02	0.02	<0.01	0.18	0.26	0.61
Level		0.70	0.12	0.68	<0.01	0.66	0.97	0.96	0.99
Source∗level		0.77	0.67	<0.01	0.91	<0.01	0.93	0.89	0.91
Contrast[2]		0.52	0.53	0.74	<0.01	<0.01	0.33	0.47	0.26

[a-c]Means with different superscripts in the same column differ significantly ($P < 0.05$).

[1]Each mean represents 24 cages, with 1 layers/cage. Abbreviation represented: MH = High erucic acid of Mianyang, DH = High erucic acid of Deyang, ML = Low erucic acid of Mianyang, DL = Low erucic acid of Deyang, L* = lightness; a* = redness; b* = yellowness.

[2]Contrast: control vs other rapeseed oil groups.

different rapeseed oil sources (MH, DH, ML, and DL). Basal diet containing no rapeseed oil was considered as a control group. Each group included eight replicates with 15 hens per replicate. The composition and calculated nutrient levels of all diets are presented in Table 1. The basal diet was formulated to meet or exceed nutrient requirements of laying hens according to the NRC recommendations (1994). All diets were isoenergetic and iso-nitrogenous and formulated on a digestible amino acid basis. The hens were randomly assigned to cages (38.1 cm-width × 50 cm-length × 40 cm-height) of three hens per cage. The hens were housed in stainless steel cages, and room environment was controlled at 22°C with a daily lighting schedule of 16 h light and 8 h dark, and were allowed ad libitum access to experimental diets and water. All diets were provided in mash form.

Egg Quality

Hen-day egg production, egg weight, and hen mortality were recorded daily. Feed consumption was recorded per week. The feed conversion ratio was expressed as the kilogram of feed consumed per kilogram of egg produced. Three eggs for each replicate (24 eggs per treatment) were collected to assess egg quality parameters at 4, 8, and 12wk. At the end of the experiment, 48 eggs from each treatment was collected and egg quality were also determined after 7 d and 14 d (24 egg for each time point) storage at room temperature (22∼24°C), respectively.

The eggshell color [L*(lightness), a*(redness), and b*(yellowness)] value was measured by a color meter (Minolta CR410 chroma meter, Konica Minolta Sensing Inc., Osaka, Japan). Eggshell strength was evaluated using an egg shell force gauge model II (Robotmation Co., Ltd., Tokyo, Japan). In addition, eggshell thickness was measured on the large end, equatorial region, and small end, respectively, using an eggshell thickness gauge (Robotmation Co., Ltd., Tokyo, Japan). The egg weight, egg yolk color, and Haugh unit (**HU**) were evaluated using an egg multi tester (EMT-7300, Robotmation Co., Ltd., Tokyo, Japan). Egg white and yolk were separate carefully, and the weight of yolk and albumen (calculated by total egg weight minus weight of yolk and eggshell) was also measured. The yolk ratio and albumen ratio were calculated by the weight of yolk and albumen to the egg weight, respectively.

Blood Collection and Analysis

At the end of 12 wk, a total of 72 blood samples (eight samples per group) were taken from the sub-wing vein and placed in non-additives blood collection tubes to produce serum after feed withdrawal for 12 h. After coagulation, the blood was centrifuged at 3,000 rpm for 10 min. Serum was collected and stored at –20°C until certain biochemical parameters were assayed. The serum parameters [TC∗(total cholesterol), TG∗(triglyceride),

Table 8. Effect of different rapeseed oil sources and levels on serum parameters of laying hens.

Item		TG	LDL-C	ALT	AST	TC	HDL-C
Source	Level						
Control	0	15.04^{a}	0.81^{a,b}	5.03^{a,b}	158.91^{a,b}	2.53	0.34^{b}
MH	2%	14.32^{a}	0.93^{a}	5.78^{a}	141.55^{b}	2.45	0.54^{a,b}
MH	4%	11.78^{a,b}	0.76^{a,b}	5.19^{a,b}	153.94^{a,b}	2.48	0.72^{a}
DH	2%	10.43^{b}	0.87^{a,b}	5.80^{a}	175.18^{a}	2.40	0.72^{a}
DH	4%	6.28^{c}	0.69^{a,b}	4.17^{a,b}	155.52^{a,b}	2.13	0.60^{a,b}
ML	2%	10.12^{b}	0.74^{a,b}	3.03^{b}	141.63^{b}	2.03	0.41^{a,b}
ML	4%	9.37^{b,c}	0.75^{a,b}	3.78^{a,b}	174.80^{a}	1.93	0.51^{a,b}
DL	2%	12.24^{a,b}	0.71^{a,b}	3.92^{a,b}	161.13^{a,b}	2.05	0.40^{a,b}
DL	4%	8.95^{b,c}	0.66^{b}	6.03^{a}	156.76^{a,b}	1.93	0.45^{a,b}
SEM		0.72	0.05	0.43	4.80	0.20	0.08
P-value		<0.01	<0.01	<0.01	<0.01	0.17	<0.01
Main effect							
Source							
	MH	13.05^{a}	0.85^{a}	5.49^{a}	147.75^{b}	2.46^{a}	0.63^{a,b}
	DH	8.36^{c}	0.78^{a,b}	4.98^{a}	165.35^{a}	2.27^{a,b}	0.66^{a}
	ML	9.75^{b,c}	0.75^{a,b}	3.41^{b}	158.22^{a,b}	1.98^{b}	0.46^{b,c}
	DL	10.59^{b}	0.68^{b}	4.97^{a}	158.94^{a,b}	1.99^{b}	0.42^{c}
SEM		0.54	0.04	0.32	3.34	0.13	0.05
Level							
	2%	11.78^{a}	0.81^{a}	4.63	154.87	2.23	0.52
	4%	9.10^{b}	0.72^{b}	4.79	160.26	2.12	0.57
SEM		0.38	0.02	0.22	2.36	0.10	0.04
P-value							
Source		<0.01	0.01	<0.01	<0.01	0.04	<0.01
Level		<0.01	0.01	0.62	0.11	0.40	0.34
Source∗level		0.16	0.19	<0.01	<0.01	0.89	0.26
Contrast[1]		<0.01	0.42	0.49	0.79	0.10	0.02

[a–c]Means with different superscripts in the same column differ significantly ($P < 0.05$).

[1]Contrast: control vs other rapeseed oil groups.

Abbreviation represented: MH = High erucic acid of Mianyang, DH = High erucic acid of Deyang, ML = Low erucic acid of Mianyang, DL = Low erucic acid of Deyang.

HDL-C∗ (high-density lipoprotein cholesterol), LDL-C∗(low-density lipoprotein cholesterol), ALT∗(alanine transaminase), AST∗(aspartate transaminase)], which were important indices to evaluate lipid metabolism and liver function, were analyzed using a commercial biochemistry analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA).

Statistical Analysis

All data were analyzed as one-way ANOVA using the GLM procedure in SAS software (SAS Institute Inc., Cary, NC). Data excluding the control were further analyzed as a 2 × 4 (levels × sources) factorial arrangement of treatments by two-way ANOVA with a model that included the main effects of rapeseed oil levels and sources, as well as their interaction. Data are expressed as means and standard error of the mean (SEM). When an effect was significant ($P < 0.05$), means were compared by Tukey's test to determine specific differences between means.

RESULTS

Chemical Analysis and Fatty Acid Composition

The MH had the highest acid value; however, the DH had the lowest. The DL had much higher iodine value compared to other sources (Table 2). The biggest difference between the four varieties of rapeseed oil is the content of erucic acid, followed by oleic acid; the other fatty acid profiles are roughly the same. The fatty acid profiles of dietary treatments were consistent with source and levels of four different rapeseed oil (Table 3). Particularly, the erucic acid content of MH (4%) diet is up to 37.62%, whereas the DL (2%) diet is below the limit detection.

Production Performance

The data showed that the egg production and egg weight were decreased ($P < 0.05$) during 9 to 12 wk and 1 to 12 wk, while the average daily feed intake (**ADFI**) and feed conversion ratio were decreased ($P < 0.01$) in all phases compared to the control treatment (Table 4). Layers fed 4% rapeseed oil decreased ($P < 0.01$) egg production compared with 2% during 5 to 8 wk, 9 to 12 wk, and 1 to 12 wk. Meanwhile, adding ML as source had higher ($P < 0.05$) egg weight compared to DH in all periods in spite of inclusion levels. Regardless of rapeseed oil sources, hens fed 4% oil decreased ($P < 0.05$) egg weight in contrast to 2% during the whole experiment except 5 to 8 wk. Layers fed ML had the higher ($P < 0.01$) ADFI than MH and DL during 9 to 12 wk in spite of levels. The ADFI was lower ($P < 0.01$) in 4% oil inclusion groups compared with supplementing oil at 2% overall phases. Otherwise, ADFI decreased (interaction, $P < 0.01$) more obviously in 4% MH oil added group during 9 to 12 wk.

Egg Quality

Supplementation of rapeseed oil decreased the yolk color ($P < 0.01$) and yolk ratio ($P = 0.02$) and increased ($P < 0.01$) the albumen height and HU at 12 wk (Table 5). A main effect of source was observed for b value, eggshell thickness, albumen height, HU ($P < 0.01$) at 4 wk and yolk ratio ($P < 0.05$), albumen ratio ($P < 0.01$) at 8 wk (Figure 1). The effect of oil supplementation level was observed to increase egg shell b value at 4 wk and yolk color at 8 wk (Figure 2). Regardless of oil sources, yolk color and yolk ratio were decreased ($P < 0.01$) but albumen ratio was increased ($P < 0.01$) as rapeseed oil supplementation levels increased. Supplementation of rapeseed oil decreased ($P < 0.01$) the yolk color and yolk ratio and increased ($P < 0.01$) the albumen ratio. After stored for 7 d, egg quality results showed a similar pattern to 12 wk except that there was a source effect for yolk color (Table 6). MH oil added group had the higher ($P < 0.01$) yolk color compared to DL. After stored for 14 d, there was an effect of source × level and a source effect on albumen height and HU. Meanwhile, when DL oil was added at 4%, the HU showed opposite result in contrast to 12 wk (Table 7).

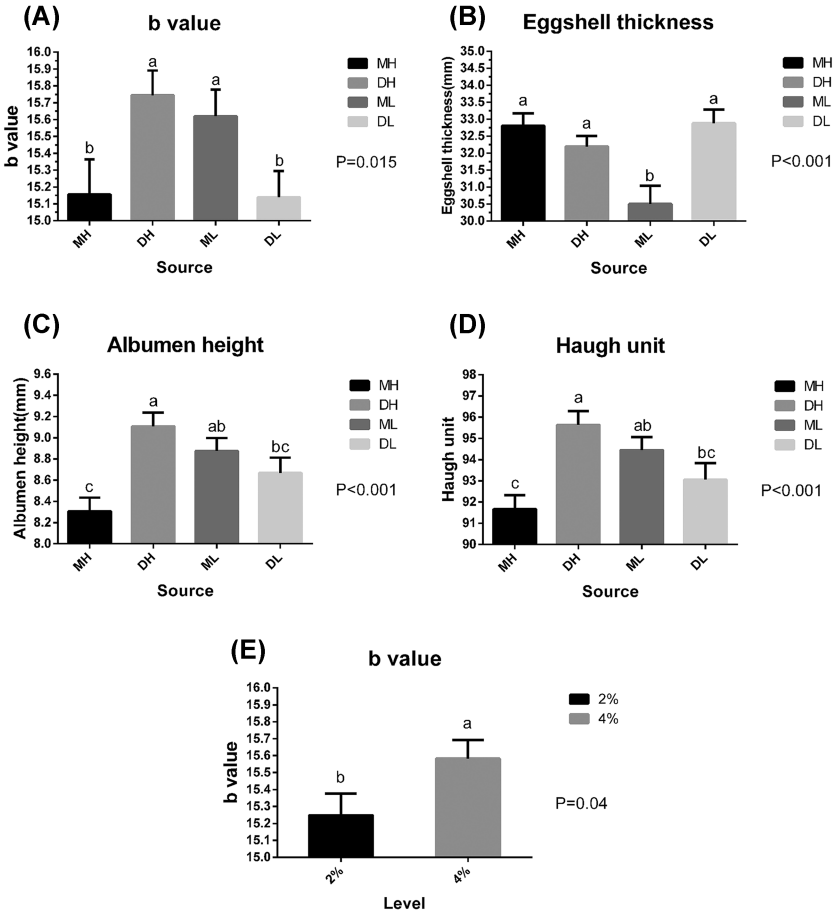

Figure 1. Effect of different rapeseed oil sources and levels on eggshell color b value, eggshell thickness, albumen height, and Haugh unit at 4 wk. **A**, The b value was higher ($P = 0.015$) in DH and ML treatments than MH and DL groups at 4 wk. **B**, The eggshell thickness was lower ($P < 0.01$) in ML group than other groups at 4 wk. **C** and **D**, Supplementation of MH decreased ($P < 0.01$) albumen height and Haugh unit than other sources at 4wk. **E**, The b value was higher ($P = 0.04$) in 4% level than 2% level groups at 4 wk. Values are means ± SEM (n = 8). The a, b, and c means every bars without same letter differ significantly ($P < 0.05$).

Serum Parameters

Dietary rapeseed oil supplementation resulted in a decreased TG ($P < 0.01$) and increased HDL-C (Table 8; $P = 0.02$). Regardless of rapeseed oil levels, layers fed MH had higher TG ($P < 0.01$), TC ($P < 0.05$), LDL-C ($P < 0.05$), and ALT ($P < 0.01$) than those fed other sources. A significant decrease ($P < 0.01$) in HDL-C was found in the serum from the hens receiving the diets containing DL as compared with other sources. The TG ($P < 0.01$) and LDL-C ($P = 0.01$) in the group containing 4% rapeseed oil was lower than 2% group.

DISCUSSION

As stated in National standards, the content of oleic acid is 8.0% to 60.0% in general rapeseed oil and 51% to 70% in low erucic acid rapeseed oil; the content of linoleic acid is 11% to 23% in general rapeseed oil and 15% to 30% in low erucic acid rapeseed oil; the content of eicosenoic acid is 3% to 15% in general rapeseed oil and 0.1% to 4.3% in low erucic acid rapeseed oil; the content of erucic acid is 3% to 60% in general rapeseed oil, and ≤3.0% in low erucic acid rapeseed oil (GB 1536-2004). In our study, the DL source belongs to the low erucic acid rapeseed oil sources, while ML, DH, and MH are of high eruci acid rapeseed oil sources. Acid value is a measurement of the amount of free fatty acids in fat. The presence of free fatty acids makes it vulnerable to lipid peroxidation, thus greatly reducing the quality of fat. So the MH was inferior to other sources of rapeseed oil. The DL had the highest iodine value, which indicated its higher unsaturated fatty acid content and may be also associated with its lower erucic acid content.

In the current study, we found that supplementation of rapeseed oil led to a reduction in feed intake, egg production, and egg weight. The data obtained from the present study were consistent with previous findings that reported a decrease in egg weight (Mazalli et al., 2004; Cherian, 2008; Nobakht et al., 2011), egg production (Agah et al., 2012), and feed intake (Shafey et al., 2003; Celebi and Utlu, 2006) when canola oil was supplemented into laying hens' diet. This may because the crude protein level of 4% MH and 4% DH groups was decreased by 3.04 g (9 to 12 wk), 2.54 g (1 to 12 wk), 2.90 g (9 to 12 wk), and 2.50 g (1–12 wk), respectively. Ceylan et al. (2011) reported that feeding 1.5% and 3.0% rapeseed oil had no effect on egg

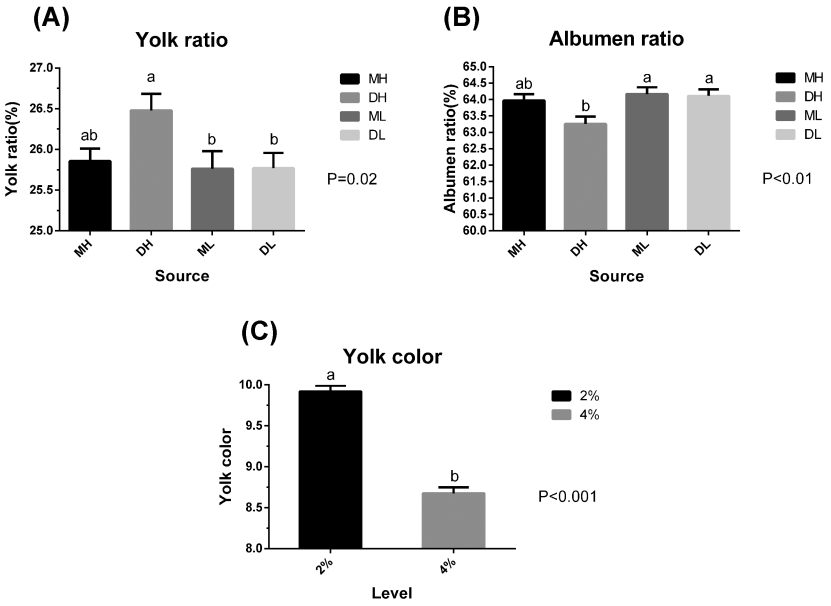

Figure 2. Effect of different rapeseed oil sources and levels on the yolk ratio, albumen ratio, and yolk color at 8 week. **A** and **B**, Supplementation of DH increased ($P < 0.05$) yolk ratio but decreased ($P < 0.01$) albumen ratio compared to other sources at 8 wk. **C**, The yolk color was higher ($P < 0.01$) in 2% rapeseed oil containing treatments than 4% level groups at 8 wk. Values are means ± SEM (n = 8). The a and b means every bars without same letter differ significantly ($P < 0.05$).

production, egg weight, feed intake, and feed conversion. Similarly, egg weight was not significantly different between 3% and 5% canola oil addition groups (Rowghani et al., 2007). However, Vogtmann et al. (1974) showed that feeding Leghorn hens with 15% high (26.2%) erucic acid rapeseed oil decreased feed intake and egg production in comparison with 15% low (4.1%) erucic acid soybean oil. In a similar study, the effects of different levels of canola oil (0, 2.0, 4.0 and 6.0%) on the performance of laying hens were investigated and it observed that the use of increasing levels of canola oil decreased egg production, egg weight, and daily feed intake but did not affect the feed conversion (Gul et al., 2012). It has been demonstrated that supplementation of different oils levels into diet decreased feed intake (Fouladi et al., 2008; Agah et al., 2012) and egg weight (Grobas et al., 2001), whereas the feed conversion was not affected (Shafey et al., 2003; Lelis et al., 2009). This can also be explained that the shortage of linoleic acid in the diet might be the limiting factor contributing to the decrease in egg weight (Nobakht et al., 2011; Rasoulpour et al., 2011). These results indicated that the content of erucic acid might influence the production of laying hens.

In a present study, supplementation of rapeseed oil led to a lower yolk color. Similarly, Gul et al. (2012) reported a similar decline in yolk color compared to the control when layers fed different levels of canola oil (2.0%, 4.0%, and 6.0%). The yellow color of the egg yolk depends on the dietary carotenoids that may varied according to the source and level of natural pigment precursor in the diet (An et al., 2010). The rapeseed oil groups contained less corn than the control treatment in this study, which may account for the different color we observed here. In our study, supplementation of rapeseed oil decreased yolk ratio and increased albumen ratio. However, in a previous study, Horniakova (1997) reported that yolk and albumen weight were not changed by adding 2% or 6% canola oil in the diets (Shaver Starcross 288). However, it was observed that the supplementation of 3% canola oil in the diets had no effect on yolk weight of Hy-line but 5% increased yolk weight (Rowghani et al., 2007).The discrepancy may be associated with the genetic background of different laying hens in above researches. The oil sources had no effect on egg quality, but the HU showed opposite result when DL oil was added at 4%. It may be related to the difference of fatty acid composition, especially the unsaturated fatty acids and its resistance to oxidation in experimental diets.

It also found that the high levels of serum TG, TC, and LDL-C were obtained from group that was fed MH containing low proportion n-3 PUFA (polyunsaturated fatty acid). Generally, saturated fatty acids increase plasma LDL (Grundy, 1987). Dietary n-3 PUFA can reduce TG synthesis and chylomicron secretion from intestinal cell and suppress hepatic fatty acid synthesis on TG production (Harris, 1989). Diet enrich in linoleic acid and oleic acid also suppress LDL concentration, but n-3 PUFA appear to be more effective (Nestel et al., 1984). Dietary PUFA may promote lipoprotein metabolism by altering the activity of certain lipolysis and transfer enzymes function in the plasma. Dietary PUFA of vegetable oils, containing mostly linoleic acid, are effective in counter-acting the effects of dietary saturated fatty acids (Grundy, 1987). In the present study, ML had the lower n-3 PUFA and DL had higher oleic acid; therefore, the results of our study were consistent

with above findings. As is shown current study, erucic acid and oleic acid have a certain inverse relationship, so the difference in erucic acid content may be one of the causes of changes in serum lipid composition.

CONCLUSION

Supplementation of rapeseed oil decreased laying performance, reduced TC and TG in the serum, and increased HU, with low erucic acid content rapeseed oil such as ML and DL or 2% group showed more pronounced results among all treatments.

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