The effects of varieties and levels of rapeseed expeller cake on egg production performance, egg quality, nutrient digestibility, and duodenum morphology in laying hens

L. P. Zhu,* J. P. Wang,* X. M. Ding,* S. P. Bai,* Q. F. Zeng,* Z. W. Su,* Y. Xuan,* T. J. Applegate,† 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, Sichuan, China; and †Department of Poultry Science, University of Georgia, Athens 30602, GA, USA

ABSTRACT The objective of this study was to evaluate the effects of rapeseed expeller cake (REC) derived from *Brassica napus* rapeseed with different concentrations of glucosinolate (Gls) and erucic acid (EA) on the egg-production performance, egg quality, apparent nutrient digestibility, and intestinal morphology in laying hens. At 33 wk of age, a total of 1,080 laying hens were randomly divided into 9 treatment groups in a completely randomized design involving a control treatment without REC (a corn–soybean diet) and a 2 × 4 factorial arrangement with 2 concentrations of REC (at 7 and 14%) from 4varieties of rapeseed varying in Gls and EA concentrations [DY6 REC: 22.67 μmol/g (Gls, relative to rapeseed meal), 0.7% (EA, relative to total fatty acids); MB1 REC: 43.23 μmol/g, 3.5%; DY5 REC: 74.66 μmol/g, 16.20%; XH3 REC: 132.83 μmol/g, 44.60%]. The trial lasted for 8 wk. Compared with the control group, REC addition decreased the ADFI, egg production, egg weight, and egg mass of laying hens during wk1 to wk4, wk5 to wk8, and wk1 to wk8 ($P < 0.05$), and REC did not affect FCR, mortality during wk1 to wk4, wk5 to wk8, and wk1 to wk8 ($P > 0.05$). The XH3 REC group had a trend to lower egg weight when compared with the DY6 REC group during wk1 to wk8 ($P = 0.07$).REC decreased AME and DM digestibility at wk8 ($P < 0.01$), and REC addition in diet did not affect apparent nitrogen digestibility ($P = 0.6$). REC decreased villi height ($P < 0.01$) and increased crypt depth ($P < 0.01$). The XH3 REC group had a lower crude fat digestibility than the DY6 REC group, and the crude fat digestibility of the DY5 and MB1 REC groups was lower than the XH3 REC group ($P < 0.01$). The DY6 REC group had a higher villi height than the DY5, MB1, and XH3 REC groups ($P < 0.01$). The XH3 REC group had a higher crypt depth than the DY6, DY5, and MB1 REC groups ($P < 0.01$). The DY6 REC group had a higher value of the ratio of villi height to crypt depth than the DY5 and MB1 REC groups, and the DY5 and MB1 REC groups had a higher value of the ratio of villi height to crypt depth than the XH3 REC group ($P < 0.01$).REC decreased albumen height and Haugh unit during wk1 to wk8 ($P < 0.01$ and $P = 0.004$), and increased yolk color during wk1 to wk8 ($P < 0.01$).The XH3, MB1, and DY5 REC groups had a lower albumen height than the DY6 REC group during wk1 to wk8 ($P < 0.01$), and the XH3 and DY5 REC groups had a lower Haugh unit than the DY6 REC group during wk1 to wk8 ($P < 0.01$). The DY6 REC group had the highest value of yolk color than other three varieties of REC (DY5, MB1, XH3) at wk6 and wk8 ($P < 0.01$ and $P < 0.01$). It can be concluded that the exposure of laying hens to REC with higher Gls and EA (DY5, MB1, XH3) led to a lower egg weight, nutrient digestibility, intestinal absorptive area, and egg internal quality than those with lower Gls and EA (DY6).

Key words: *Brassica napus* rapeseed, egg production performance, egg quality, nutrient digestibility, duodenum morphology

2019 Poultry Science 98:4942–4953
http://dx.doi.org/10.3382/ps/pez254

INTRODUCTION

Rapeseed is an important oilseed crop with 7.41 million tons of rapeseed oil and 11.3 million tons of rapeseed meal (**RSM**) produced in 2018 (https://www.indexmundi.com/agriculture). There are

Received December 29, 2018.
Accepted April 15, 2019.

[1]Corresponding author: zkeying@sicau.edu.cn

different ways to produce rapeseed oil and its by-products, including heat-screw pressing, cold-pressing, and prepressing extraction (Unger, 1990; Bell, 1984; Hobson-Frohock, Fenwick and Heaney, 1977; Vig and Walia, 2001). First of all, rapeseed is preheated at 30 to 40°C, and then cooked at a high temperature (Unger, 1990). Then, the cooked rapeseed is screw-pressed (Unger, 1990). Heat-processing the rapeseed could not only reduce glucosinolates (**Gls**) content, but also inactivate myrosinase activity, which could hydrolyze Gls to toxic metabolites containing OZT, ITC, and nitriles (Huang et al., 1995), whereas heat-pressing could decrease the amino acid content or availability of amino acids like Lys and Thr, which play key roles in biological reactions (Huang et al., 1995; Liu et al., 2019). Rapeseed has lots of antinutritional factors, including Gls, erucic acid (**EA**), tannins, phytic acid, or certain fiber components, which could limit the use of RSM or cake (Zhu et al., 2018). Biologically inactive Gls and EA are the 2 main antinutritional factors in RSM, and adverse effects of Gls metabolites containing thiocyanate ions (SCN^-) and 5-vinyl-1,3-oxazolidine-2-thione (5-VOT) are thought to be goitrogenic (Tripathi and Mishra, 2007), hepatotoxic, or nephrotoxic (Choi et al., 2014). A combination of 20% rapeseed oil with 22% EA can cause cardiac toxicity and impaired mitochondrial function in male rats, and therefore it is recommended that EA contents in infant formulas should not exceed 1% of the total fatcontent (Hulan et al., 1976; Koletzko, 2005). Fiber components are poorly utilized by poultry and are inversely related to metabolic energy and digestible protein content of RSM (Slominski, 1997). Several research works compared the chemical and nutritive composition of meals derived from prepress solvent extracted seeds of the black-seeded *Brassica napus* canola and the canola-quality yellow-seeded *B. juncea* in broilers and turkeys (Slominski et al., 1999; Radfar et al., 2017; Kozlowski et al., 2018; Rad-Spice et al., 2018). The black-seeded *B. napus* canola had a higher content of crude fiber and metabolic energy than yellow-seeded *B. juncea*, and broilers fed the yellow-seeded *B. napus* canola showed the lowest feed-to-gain ratio. The RSM/rapeseed expeller cake (**REC**) are sorted into 4 categories by the Gls content, including very low Gls RSM/REC (1 to 5 μmolGls/g), low-Gls RSM/REC (10 to 30 μmolGls/g), moderate-Gls RSM/REC (30 to 60 μmolGls/g), and high-Gls RSM/REC ($\geq$ 60 μmolGls/g) (Tripathi and Mishra, 2007). According to the EA content in oil, RSM/REC are sorted into 2 categories, containing high-EA RSM/REC ($\geq$ 43%, NY/T 1990–2011) and low-EA RSM/REC (< 3%, GB/T 1536–2004). Some reports have compared the effects of high- and low-Gls RSM on laying hens (Grandhi et al., 1977; Campbell, 1979; Ibrahim and Hill, 1980). The results showed that hens receiving 20% lower Gls RSM maintained egg production well when compared with hens receiving soybean meal, but hens receiving 20% higher Gls RSM had the lowest egg production. Laying hens fed diet containing 10 or 15% higher Gls RSM had a lower egg production and Haugh unit than those fed diet containing 15% Tower RSM (low-Gls RSM) (Thomas et al., 1978).

In China, there are many varieties of rapeseed with different levels of Gls and EA (http://www.cgris.net/#), but little is known about the effects of expeller cake from different varieties of rapeseed on the production performance and intestinal physiology in poultry. Therefore, we chose 4 kinds of *B. napus* rapeseed with different Gls and EA contents containing DY5, DY6, MB1, and XH3 (Gls and EA content: DY6<MB1<DY5<XH3) to evaluate the effects of REC on the egg production performance, egg quality, apparent nutrient digestibility, and intestinal morphology in laying hens.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Sichuan Agricultural University approved all procedures used in this study (Chengdu, Sichuan, China).

Hens, Diets, and Management

At 30 wk of age, a total of 1,080 laying hens (Lohmann white, Sun Daily Inc., Chengdu, Sichuan, China) were randomly divided into 9 treatment groups in a completely randomized design involving a 2 × 4 factorial arrangement (REC levels ×rapeseed varieties) and a control treatment without REC (a corn–soybean diet). The 4 kinds of REC were made from 4 varieties of Chinese *B. napus* rapeseeds containing DY5, DY6, MB1, and XH3 by a hot expeller [processing temperatures: 70 to 87°C (10 min), 87 to 130°C (10 min), and 130°C (2 to 3 min)]. The REC were supplemented in diets at 7 and 14%. The trial lasted for 8 wk. The analyzed nutrients and antinutritional factor concentrations of 4 varieties of rapeseed and REC are given in Table 1. There were 8 replicates per treatment with 15 hens per replicate. Hens were housed individually in stainless-steel cages (38.2 × 50.1 × 40.0 cm), and the room environment was controlled at 22°C by a daily lighting schedule of 16 h light and 8 h dark. They were allowed ad libitum access to experimental diets and water and were offered mash-form diets (Table 2), which were formulated to meet or exceed energy and nutrient requirements of hens according to NRC (1994) and a published management guide (Lohmann Tierzucht GmbH, Cuxhaven, Lower Saxony, Germany). The nutrient composition of feedstuff in this study refers to the China feed-database information network centre (CFD, 2014). The analyzed nutrients and antinutritional factors of diets are given in Table 3.

Sampling Procedure

A daily record of egg 2-wk production and a weekly record of feed consumption were maintained.

Table 1. Analyzed nutrient and antinutritional factor content of rapeseed or rapeseed expeller cake (as-fed basis).[1]

Item	DY5	DY6	XH3	MB1
Dry matter (%)	94.84	94.30	94.84	94.21
Gross energy (kcal/kg)	4,631	4,701	4,773	4,713
Crude protein (%)	36.59	36.98	38.30	36.69
Crude fat (%)	8.76	8.32	11.10	8.47
Sinapine (mg/kg)	9.98×10^3	8.77×10^3	7.61×10^3	6.89×10^3
Crude fiber (%)	19.02	18.65	20.79	19.69
Erucic acid (%)	16.20	0.70	44.6	3.50
Isothiocyanates[2] (mg/g)	2.09	0.13	2.63	0.49
Oxazolidinethione[2] (mg/g)	1.11	0.13	1.24	0.61
2-OH-3-butenyl Gls[2] (μmol/g)	17.68	4.04	42.96	8.93
3-Butenyl Gls[2] (μmol/g)	10.7	1.76	23.44	5.54
4-OH-3-indolylmethyl Gls[2] (μmol/g)	2.96	1.78	2.75	1.87
Phenethyl Gls[2] (μmol/g)	2.32	1.76	1.26	1.46
Total Gls[2] (μmol/g)	74.66	22.67	132.83	43.23

[1]Gl = glucosinolate. DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 = Mianbangyou No.1, XH3 = Xiheyou No.3. Erucic acid content is relative to total fatty acid content.

[2]Those analyzed values were measured from rapeseed, and the Gls content was calculated on the basis of rapeseed meal.

Egg-laying rates were expressed as an average hen-day production. At intervals, 3 eggs were chosen from each replicate for egg-quality measure. At wk 8, 2 hens were chosen from each replicate randomly. One hen was sacrificed by cervical dislocation. A 2cm segment of mid-duodenum was removed from the duodenum and flushed with physiological saline, followed by fixation in 10% formalin. Furthermore, the other hen was used for a 72-h balance experiment using a total fecal collection method. Hens were allocated to 72 metabolic cages. Excreta samples from each hen were collected every 3 h and immediately stored at −20°C (Yan et al., 2019). Care was taken during collection to avoid contaminations from feathers, feed, and foreign materials.

Table 2. Ingredient composition and energy and nutrient content of experimental diets (as-fed basis).[1]

Item	REC (%) 0	7	14
Ingredients (%)			
Corn	61.04	59.52	58.00
Soybean meal (CP, 43%)	26.77	20.84	14.91
Wheat bran	0.94	0.50	0.05
REC (CP, 35.7%)	0.0	7.00	14.00
Corn gluten meal	0.02	0.19	0.35
Soybean oil	0.98	1.62	2.26
Calcium carbonate	7.91	7.87	7.82
Calcium phosphate	1.17	1.14	1.10
NaCl	0.40	0.40	0.40
Mineral premix[2]	0.50	0.50	0.50
Vitamin premix[3]	0.03	0.03	0.03
L-Lys.HCl	0.0	0.12	0.23
DL-Met	0.14	0.14	0.13
L-Thr	0.0	0.03	0.06
L-Trp	0.0	0.01	0.01
Chloride choline	0.10	0.10	0.10
Rice hull powder	0.0	0.03	0.05
Total	100	100	100
Calculated energy and nutrient contents (%)			
AMEn (kcal/kg)	2700.0	2700.0	2700.0
CP	16.50	16.50	16.50
Ca	3.50	3.50	3.50
Total *P*	0.53	0.55	0.57
Available *P*	0.32	0.32	0.32
Digestible Lys	0.78	0.78	0.78
Digestible Met	0.37	0.37	0.37
Digestible Thr	0.55	0.55	0.55
Digestible Trp	0.17	0.17	0.17

[1]AMEn = nitrogen-corrected apparent metabolizable energy.

[2]Provided per kilogram of diet: 60 mg Fe ($FeSO_4 \cdot 7H_2O$), 8 mg Cu ($CuSO_4 \cdot 5H_2O$), 60 mg Mn ($MnSO_4 \cdot H_2O$), 80 mg Zn ($ZnSO_4 \cdot 7H_2O$), 0.3 mg Se ($NaSeO_3$), and 0.35 mg I (KI).

[3]Provided per kilogram of diet: 8,000 IU vitamin A, 1,600 IU vitamin D_3, 5 IU vitamin E, 0.8 mg vitamin B_1, 2.5 mg vitamin B_2, 1.5 mg vitamin B_6, 0.004 mg vitamin B_{12}, 2.2 mg D-pantothenic acid, 0.25 mg folic acid, 20 mg nicotinic acid, and 0.1 mg biotin.

Chemical Analysis

The concentrations of antinutritional factors of REC were analyzed by a laboratory (Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan, China) using HPLC (Agilent 1200, Santa Clara, CA) and GC (Agilent 7890A, Agilent Technologies Inc., Santa Clara, CA). The 5-VOT content of diets was analyzed by HPLC (Agilent 1200, Santa Clara, CA) according to Matthäus and Fiebig (1996). The content of free SCN^- was quantified by ion chromatography (Dionex ICS1100; Thermo Fisher Scientific, Waltham, MA) proposed by Michigami et al. (1992). Eggshell strength was evaluated using an egg shell force gauge model II (Robotmation Co., Ltd., Tokyo, Japan). In addition, the eggshell thickness was measured at the large end, equatorial region, and small end by an eggshell thickness gauge (Robotmation Co., Ltd., Tokyo, Japan). The egg weight, yolk color, and Haugh unit were evaluated by an egg multitester (EMT-7300; Robotmation Co., Ltd.). The fixed duodenum tissues were trimmed and embedded in paraffin. Thin sections (5 μm) were sliced and mounted on a slide, stained with hematoxylin eosin for histopathological examination by a pathologist unaware of the treatment groups. Sections were photographed using MoticMicroscope BA200 at 40 × magnification (Motic®, Japan). The gross energy of feed and excreta were analyzed

Table 3. Analyzed nutrient and antinutritional factor content of experiment diets (as-fed basis).[1]

	0% REC	7% REC				14% REC			
Item	Control	DY6	MB1	DY5	XH3	DY6	MB1	DY5	XH3
Gross energy (kcal/kg)	3,641	3,631	3,569	3,589	3,618	3,699	3,695	3,694	3,681
Dry matter (%)	89.22	89.35	89.44	90.25	90.53	89.95	89.61	89.40	89.74
Crude protein (%)	16.43	16.87	16.89	16.96	16.93	16.90	16.86	16.41	17.02
Crude fat (%)	4.21	4.54	4.41	4.86	5.00	6.01	6.24	5.87	6.21
Crude fiber (%)	2.99	8.62	6.11	7.20	9.65	8.03	9.79	7.04	10.70
5-VOT (ma/kg)	N.D	0.81	22.00	39.31	76.13	4.46	33.69	93.04	117.48
SCN^- (mg/kg)	N.D	12.80	16.30	20.40	15.40	30.20	29.80	28.60	33.20
Gls (μmol/g)	N.D	0.57	1.68	2.79	5.20	2.98	3.53	6.13	7.98
Erucic acid (%)	N.D	N.D	N.D	1.66	6.20	0.20	1.34	3.70	11.23

[1]N.D = not detected; erucic acid is relative to total fatty acids; 5-VOT = 5-vinyl-1,3-oxazolidine-2-thione, SCN^- = thiocyanate (free), Gls = glucosinolates. DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 = Mianbangyou No.1, and XH3 = Xiheyou No.3.

by an adiabatic oxygen bomb calorimeter (Model 1281; Parr Instrument Company, Moline, IL). The dry matter and crude protein of the experimental diets and excreta were analyzed as described by AOAC Int. (2012). Crude fiber was analyzed by an automatic fiber analyzer (ANKOM 2000i; ANKOM Technology, Fairport, NY).

Statistical Analysis

A transformation selector was used to determine the appropriate transformation for total mortality (Kirk, 1968). All data were analyzed as one-way ANOVA using the GLM procedure in SAS software (SAS Institute, 2003). To test the effect of REC in diets, data were analyzed using single *df* contrast to compare all REC diet treatments with the control (Jonker et al., 2011). Data excluding the control were further analyzed as a 2 × 4(REC levels × REC varieties) factorial arrangement of treatments by 2-way ANOVA with a model that included the main effects of REC levels and varieties, as well as their interaction. When an effect was significant ($P < 0.05$), means were compared by Turkey's HSD test to determine specific differences. Data were expressed as means and standard error of the mean.

RESULTS

Antinutritional Factors Contents of RSM

Antinutritional factor contents of 4 varieties of rapeseed and its by-product used in this study are presented in Table 1. Glucosinolates are secondary plant metabolites that occur in all Brassica-originated feeds (Tripathi et al., 2007). The rapeseed used in the present study primarily contains 3-butenyl and 2-OH-3-butenyl Gls. The total Gls contents of DY6, MB1, DY5, and XH3 rapeseed are 22.67, 43.23, 74.66, and 132.83 μmol/g (relative to RSM), respectively, and the EA contents are0.7, 3.5, 16.2, and 44.6% (relative to total fatty acids), respectively. The crude fiber contents of DY6, MB1, DY5, and XH3 REC are18.65, 19.69, 19.02, and 20.79%, respectively. The crude protein content of XH3 REC is about 2% higher than DY5, DY6, and MB1 REC, and the crude fat content of XH3 REC is about 3% higher than the other 3 varieties of REC.

Egg Production Performance

The effects of different varieties and levels of REC on egg-production performance are presented in Tables 4 and 5. In the present study, some laying hens died due to liver hemorrhage after ingesting REC diet (Figure 1). Compared with the control group, supplementation of REC decreased the ADFI, egg production, egg weight, and egg mass during wk1 to wk4, wk5 to wk8, and wk1 to wk8 ($P < 0.05$), but did not affect FCR, and mortality ($P > 0.05$). The source of REC had no effect on the ADFI, FCR, and mortality ($P > 0.05$), while XH3 REC had a trend to lower egg weight when compared with DY6 REC during wk1 to wk8 ($P = 0.07$). XH3 REC with high EA and Gls was 2.01% and 1.73 g/D per hen lower than DY6 RSM with low EA and Gls in egg production and egg mass during wk1 to wk8, respectively. Feeding 14% REC had a higher mortality than 7% REC during wk1 to wk4 ($P = 0.04$), and 14% REC had a trend to decrease ADFI during wk5 to wk8 and wk1 to wk8 ($P = 0.08$, $P = 0.06$). There was a trend of interaction effect of REC source and concentration on ADFI during wk1 to wk4 ($P = 0.09$). The REC concentration and interaction between REC source and concentration had no effect on egg production, egg weight, and egg mass ($P > 0.05$). There was no interaction effect of REC source and level on FCR and mortality ($P > 0.05$).

Nutrient Digestibility and Duodenum Morphology

The effects of different varieties and levels of REC on nutrient digestibility and AME are presented in Table 6. Compared with control treatment, supplementation of REC decreased AME and DM digestibility at wk8 ($P < 0.001$ and $P < 0.001$), and REC did not affect apparent nitrogen digestibility ($P = 0.6$). XH3 REC had a lower crude fat digestibility than DY6 REC, and

Table 4. Effects of rapeseed expeller cake varieties and levels on egg production performance in laying hens during wk1 to wk8.[1]

		Egg-laying rate (%)			Egg weight (g)			Egg mass[2] (g/D per hen)		
Variety	Level (%)	wk1 – wk4	wk5 – wk8	wk1 – wk8	wk1 – wk4	wk5 – wk8	wk1 – wk8	wk1 – wk4	wk5 – wk8	wk1 – wk8
Control	0	91.68	88.48	90.07	60.11[a]	60.94[a]	60.52[a]	55.11	53.94[a]	54.52[a]
DY6	7	90.83	84.42	87.63	59.04[a,b]	59.37[b]	59.21[b]	53.63	50.12[a,b]	51.87[a,b]
MB1	7	89.44	86.06	87.75	59.02[a,b]	59.07[b]	59.04[b]	52.79	50.84[a,b]	51.81[a,b]
DY5	7	89.91	85.51	87.71	58.97[a,b]	59.05[b]	59.01[b]	53.02	50.49[a,b]	51.75[a,b]
XH3	7	90.12	85.55	87.84	58.38[b]	58.67[b]	58.52[b]	52.62	50.21[a,b]	51.41[a,b]
DY6	14	90.98	86.36	88.67	59.12[a,b]	59.19[b]	59.15[b]	53.771	51.10[a,b]	52.43[a,b]
MB1	14	87.28	81.67	84.48	59.09[a,b]	59.07[b]	59.07[b]	51.60	48.22[b]	49.91[b]
DY5	14	88.87	85.94	87.41	59.13[a,b]	58.99[b]	59.06[b]	52.55	50.66[a,b]	51.61[a,b]
XH3	14	87.12	81.77	84.44	58.49[b]	58.46[b]	58.47[b]	51.09	47.79[b]	49.44[b]
SEM		1.52	1.62	1.38	0.32	0.29	0.26	0.96	0.97	0.86
P ANOVA		0.39	0.09	0.11	0.04	<0.001	<0.001	0.16	0.004	0.009
P-value[3]		0.02	<0.01	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Variety										
DY6		90.91	85.39	88.15	59.08	59.28	59.18	53.70	50.61	52.16
DY5		89.39	85.72	87.56	59.05	59.02	59.03	52.78	50.58	51.68
MB1		88.36	83.86	86.11	59.06	59.07	59.06	52.19	49.53	50.86
XH3		88.62	83.66	86.14	58.44	58.57	58.50	51.86	49.00	50.43
SEM		1.09	1.10	0.95	0.23	0.22	0.19	0.66	0.66	0.58
Level (%)										
14		88.56	83.94	86.25	58.96	58.93	58.94	52.25	49.45	50.85
7		90.07	85.38	87.73	58.85	59.04	58.95	53.02	50.42	51.72
SEM		0.77	0.78	0.67	0.16	0.16	0.14	0.47	0.47	0.41
P-value	Variety	0.35	0.44	0.33	0.14	0.16	0.07	0.23	0.24	0.16
	Level	0.17	0.19	0.13	0.65	0.62	0.98	0.25	0.15	0.14
	Variety × Level	0.76	0.12	0.27	1.00	0.98	0.99	0.81	0.14	0.32

[1]Data are expressed as mean and SEM (n = 8). SEM = standard error of the mean. DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 = Mianbangyou No.1, and XH3 = Xiheyou No.3.

[2]Egg mass = (egg production × egg weight)/100.

[3]This is a contrast of all REC versus the control group.

[a,b]"*P-value*" is a contrast comparison between all REC groups and control group, and "*P* ANOVA" is one-way ANOVA comparison between the 9 groups, and there was no difference in egg-laying rate between the 9 groups.

Table 5. Effects of rapeseed expeller cake varieties and levels on egg production performance in laying hens during wk1 to wk 8.[1]

		ADFI (g/D/bird)			FCR			Morality		
Variety	Level (%)	wk1 – wk4	wk5 – wk8	wk1 – wk8	wk1 – wk4	wk5 – wk8	wk1 – wk8	wk1 – wk4	wk5 – wk8	wk1 – wk8
Control	0	110.50[a]	105.30[a]	107.90[a]	2.01	1.96	1.99	0	0	0
DY6	7	107.78[b]	99.01[b]	103.39[b]	2.01	1.98	2.00	0	0	0
MB1	7	107.95[b]	99.25[b]	103.59[b]	2.06	1.96	2.01	0	0.82	0.57
DY5	7	106.63[b]	99.91[b]	103.26[b]	2.02	1.99	2.00	0	0	0
XH3	7	107.14[b]	99.86[b]	103.50[b]	2.04	2.00	2.02	0	0	0
DY6	14	107.09[b]	99.24[b]	103.16[b]	1.99	1.95	1.97	0.82	0	0.57
MB1	14	106.10[b]	98.41[b]	102.25[b]	2.07	2.06	2.06	0.82	0	0.57
DY5	14	107.82[b]	99.57[b]	103.69[b]	2.06	1.97	2.01	0	0	0
XH3	14	105.89[b]	98.16[b]	102.02[b]	2.09	2.06	2.07	1.6	0	1.1
SEM		0.54	0.54	0.47	0.04	0.04	0.03	0.54	0.27	0.42
P ANOVA		<0.001	<0.001	<0.001	0.63	0.37	0.41	0.25	0.45	0.45
P-value[2]		<0.01	<0.0001	<0.0001	0.32	0.24	0.22	0.48	0.72	0.43
Variety										
DY6		107.44	99.13	103.28	2.00	1.97	1.98	0.41	0	0.29
DY5		107.22	99.74	103.48	2.04	1.98	2.01	0	0	0
MB1		107.03	98.83	102.93	2.06	2.01	2.04	0.41	0.41	0.57
XH3		106.51	99.01	102.76	2.06	2.03	2.05	0.82	0	0.57
SEM		0.43	0.37	0.35	0.03	0.03	0.02	0.4	0.2	0.3
Level (%)										
14		106.72	98.85	102.79	2.05	2.01	2.03	0.82[a]	0	0.57
7		107.37	99.51	103.44	2.03	1.98	2.01	0[b]	0.2	0.14
SEM		0.31	0.26	0.25	0.02	0.02	0.016	0.3	0.1	0.2
P-value	Variety	0.48	0.34	0.45	0.28	0.36	0.22	0.56	0.39	0.53
	Level	0.14	0.08	0.06	0.38	0.34	0.29	0.04	0.32	0.18
	Variety × Level	0.09	0.31	0.17	0.81	0.24	0.54	0.56	0.39	0.53

[1]Data are expressed as mean and SEM (n = 8). DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 =Mianbangyou No.1, XH3 = Xiheyou No.3. ADFI = average daily feed intake, FCR = feed conversion ratio, and SEM = standard error of the mean.

[2]This is a contrast of all REC versus the control group.

[a,b]"*P-value*" is a contrast comparison between all REC groups and control group, and "*P* ANOVA" is one-way ANOVA comparison between the 9 groups, and there was no difference in egg-laying rate between the 9 groups.

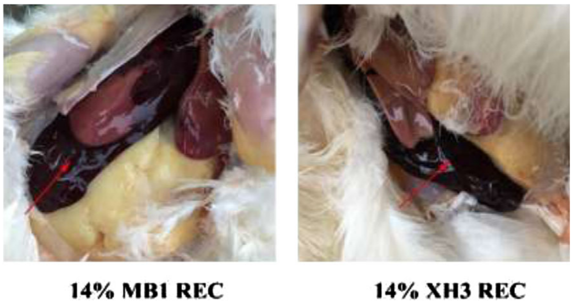

Figure 1. Hen livers died from liver hemorrhage during wk 1 to wk 8. MB1 REC = Mianbangyou No.1 rapeseed expeller cake, XH3 REC = Xiheyou No.3 rapeseed expeller cake.

the crude fat digestibility of DY5 and MB1 REC treatment was lower than that of XH3 REC treatment ($P = 0.01$). Although the REC source had no significant effect on AME, DM digestibility, and apparent nitrogen digestibility ($P = 0.12$, $P = 0.34$, $P = 0.54$), the high EA and Gls REC (XH3) was 3.03% and 58.01 kcal/kg lower than the low EA and Gls REC (DY6) in apparent nitrogen digestibility and AME, respectively. In terms of AME, 14% RSM had a lower AME than 7% REC ($P = 0.01$), and REC content did not affect DM, apparent nitrogen, and crude fat digestibility ($P > 0.05$). There were no interaction effect of REC source and content on apparent AME, DM, nitrogen, and crude fat digestibility ($P > 0.05$).

The effects of different varieties and levels of REC on duodenum morphology in laying hens at wk8 are presented in Table 7 and Figure 2. Compared with the control group, supplementation of REC decreased villi height ($P < 0.001$) and increased crypt depth ($P < 0.001$). REC addition had no effect on the ratio of villi height to crypt depth ($P = 0.12$). Feeding DY6 REC resulted in a higher villi height than DY5, MB1, and XH3 REC ($P < 0.01$). Feeding XH3 REC had a higher crypt depth than DY6, DY5, and MB1 REC ($P < 0.01$). Feeding DY6 REC resulted in a higher ratio of villi height to crypt depth than DY5 and MB1 REC, and DY5 and MB1 REC had a higher ratio of villi height to crypt depth than XH3 REC ($P < 0.01$). Feeding 14% REC resulted in a lower villi height and ratio of villi height to crypt depth than feeding 7% REC ($P < 0.01$ and $P < 0.01$). There was an interaction effect of REC source and content on villi height and ratio of villi height to crypt depth ($P < 0.01$ and $P = 0.002$). There was no difference in villi height among the birds fed the 4 varieties of 7% REC, but 14% XH3 and DY5 REC had a lower villi height than 14% DY6 and MB1 REC. In terms of ratio of villi height to crypt depth,7% XH3 REC had a lower ratio of villi height to crypt depth than 7% DY6 REC, but 14% MB1 REC had a lower

Table 6. Effects of rapeseed expeller cake varieties and levels on apparent metabolic energy and nutrient availability in laying hens at wk 8.[1]

Variety	Level (%)	AME (kcal/kg)	Dry matter (%)	Apparent nitrogen (%)	Crude fat (%)
Control	0	3,323^{a}	78.35^{a}	47.64	80.08^{c}
DY6	7	3,208^{a-c}	75.24^{a,b}	46.95	86.96^{a,b}
MB1	7	3,141^{b,c}	75.24^{a,b}	46.58	80.88^{b,c}
DY5	7	3,105^{c}	74.26^{b}	45.04	81.61^{b,c}
XH3	7	3,151^{b,c}	75.58^{a,b}	43.51	85.56^{a-c}
DY6	14	3,263^{a,b}	74.72^{a,b}	50.03	90.17^{a}
MB1	14	3,187^{a-c}	73.42^{b}	45.46	81.61^{b,c}
DY5	14	3,213^{a-c}	73.57^{b}	45.25	80.71^{b,c}
XH3	14	3,205^{a-c}	75.10^{a,b}	47.39	84.42^{a-c}
SEM		34	0.82	2.41	1.40
*P*ANOVA		<0.01	<0.01	0.76	<0.01
P-value[2]		<0.001	<0.001	0.60	0.01
Variety					
DY6		3,236	74.98	48.49	88.56^{a}
DY5		3,159	73.92	45.15	81.16^{c}
MB1		3,164	74.33	46.02	81.25^{c}
XH3		3,178	75.34	45.46	84.99^{b}
SEM		25	0.60	1.78	1.00
Level (%)					
14		3,217^{a}	74.20	47.03	84.23
7		3,151^{b}	75.08	45.53	83.75
SEM		18	0.42	1.26	0.71
P-value	Variety	0.12	0.34	0.54	<0.01
	Level	0.01	0.15	0.40	0.64
	Variety × Level	0.80	0.84	0.73	0.40

[1]Data are expressed as mean and SEM (n = 8). DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 = Mianbangyou No.1, XH3 = Xiheyou No.3, and SEM = standard error of the mean.

[2]This is a contrast of all REC versus the control group.

[a–c]"*P-value*" is a contrast comparison between all REC groups and control group, and "*P* ANOVA" is one-way ANOVA comparison between the 9 groups, and there was no difference in egg-laying rate between the 9 groups.

Table 7. Effects of rapeseed expeller cake varieties and levels on duodenum morphology in laying hens at wk8.[1]

Variety	Level (%)	Villi height (μm)	Crypt depth (μm)	Villi height/ crypt depth
Control	0	1484.09[a,b]	234.73[a,b]	6.38[c,d]
DY6	7	1464.06[a,b]	189.78[c,d]	7.87[a]
MB1	7	1354.25[b]	184.24[d]	7.40[a-c]
DY5	7	1472.82[a,b]	198.57[c,d]	7.54[a,b]
XH3	7	1432.09[a,b]	218.71[b,c]	6.55[b,c]
DY6	14	1534.93[a]	183.23[d]	8.43[a]
MB1	14	1359.49[b]	208.81[b-d]	6.66[b,c]
DY5	14	1147.74[c]	213.01[b-d]	5.43[d,e]
XH3	14	1172.61[c]	261.06[a]	4.65[e]
SEM		59	11.81	0.49
P ANOVA		<0.01	<0.01	<0.01
P-value[2]		<0.001	<0.001	0.12
Variety				
DY6		1491.32[a]	187.39[b]	8.09[a]
DY5		1333.50[b]	204.76[b]	6.64[b]
MB1		1357.16[b]	197.89[b]	6.99[b]
XH3		1318.57[b]	237.24[a]	5.72[c]
SEM		23.21	5.12	0.19
Level (%)				
7		1438.18[a]	197.62[b]	7.39[a]
14		1299.32[b]	215.61[a]	6.27[b]
SEM		16.41	3.62	0.13
P-value	Variety	<0.01	<0.01	<0.01
	Level	<0.01	0.009	<0.01
	Variety × level	<0.01	0.117	0.002

[1]Data are expressed as mean and SEM (n = 8). DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 = Mianbangyou No.1, XH3 = Xiheyou No.3, and SEM = standard error of the mean.

[2]This is a contrast of all REC versus the control group.

[a–e]"*P-value*" is a contrast comparison between all REC groups and control group, and "*P* ANOVA" is one-way ANOVA comparison between the 9 groups, and there was no difference in egg-laying rate between the 9 groups.

ratio of villi height to crypt depth than 14% DY6 REC, and 14% XH3 REC had the lowest ratio of villi height to crypt depth.

Egg Quality

Eggshell Quality The effects of different varieties and levels of REC on eggshell quality are given in Table 8. Compared with the control group, REC addition did not affect eggshell strength and thickness in the whole experiment period ($P > 0.05$). The REC source had no effect on eggshell strength and thickness in the whole experiment period ($P > 0.05$), but feeding 14% REC resulted in a higher eggshell strength than 7% REC at wk6 ($P = 0.002$). In terms of eggshell thickness, 14% REC had a lower eggshell thickness than 7% REC at wk8 ($P = 0.01$), and 14% REC was 0.04 kg/m^3 lower than 7% REC at wk8. There was no interaction effect of REC source and content on eggshell strength or thickness at wk8 ($P > 0.05$).

Egg Internal Quality The effects of different varieties and levels of REC on egg internal quality in laying hens are given in Table 9. Compared to the control group, supplementation of REC decreased albumen height and Haugh unit during wk1 to wk8 ($P < 0.001$ and $P = 0.004$), and increased yolk color during wk1 to wk8 ($P < 0.001$). XH3, MB1, and DY5 REC reduced albumen height compared to DY6 REC during wk1 to wk8 ($P < 0.001$), and XH3 and DY5 REC had a lower Haugh unit than eggs from hens fed DY6 REC during the entire experiment period ($P < 0.01$). Feeding DY6 REC resulted in the highest value of yolk color compared to other 3 varieties of REC (DY5, MB1, XH3) at wk6 and wk8 ($P < 0.001$ and $P < 0.001$). Furthermore, feeding 14% REC resulted in a lower albumen height and Haugh unit than 7% REC at wk6 ($P < 0.01$ and $P < 0.01$), and eggs from hens fed 14% REC had a higher yolk-color value than when fed 7% REC at wk 2, wk 4, wk 6, wk 8, and the whole phase ($P < 0.01$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). There was an interaction effect of REC source and content on albumen height and Haugh units during wk1 to wk8 ($P = 0.02$ and $P = 0.02$), and yolk color at wk8 ($P < 0.001$).

DISCUSSION

Mortality attributable to hemorrhagic liver was evident only among hens receiving the high-Gls RSM (Target), which significantly related to reticulolysis (Campbell, 1979; Martland et al., 1984). In the present study, large hematomas covering a major portion of the liver was observed in hens fed 14% MB1 REC and 14% XH3 REC after 8 wk of feeding. Yamashiro et al. (1975) observed a high incidence of "hemorrhagic liver syndrome" in hens receiving 20% rapeseed oil. Erucic acid could inhibit fatty acid oxidation (Christophersen and Bremer, 1972) and increase liver lipids (Kienle et al., 1976), and high fat in the liver could induce vascular friability and breakdown and eventually inducing liver hemorrhage (Savary, 2013). High Gls and EA content of MB1 RSM and XH3 RSM might be a cause of hepatorrhagia. Factors that affected the nutrient value of RSM contained Gls, EA, sinapine, phytic acid, tannins, dietary fiber, and electrolyte balance (Khajali and Slominski, 2012). Our results were consistent with the previous study showing that ADFI, egg production, egg weight, and egg mass of laying hens increased linearly with dietary RSM level (Leslie and Summers, 1972; Zhu et al., 2018). Diet palatability could be adversely affected by RSM due to the bitter taste of the Gls metabolites (Fenwick et al., 2010), and the high crude fiber content of REC used in this study could decrease feed intake by increasing satiety (Razdan et al., 1997). Reduced feed intake could decrease egg production performance (Tripathi et al., 2001). In the present study, the REC variety used had no effect on ADFI, egg production, and egg mass. Those observations may indicate that the Gls and EA content in REC is not the main factor affecting ADFI, egg production, and egg mass.

Supplementation of REC led to a reduction in AME and dry matter digestibility when compared to the

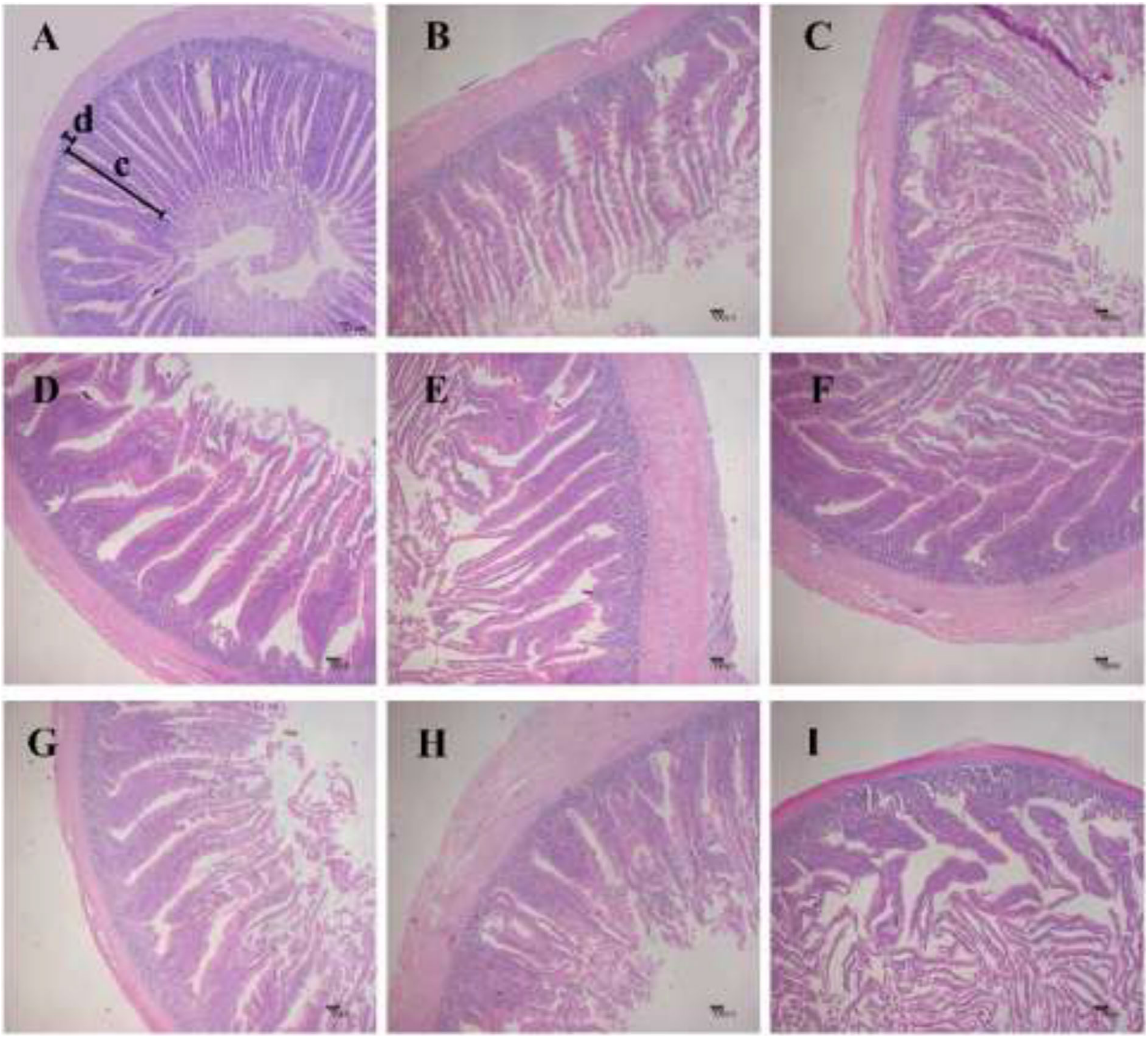

Figure 2. Effects of rapeseed expeller cake varieties and levels on duodenum morphology in laying hens at wk 8. (A): Control, (B): 7% DY6 REC, (C): 7% MB1 REC, (D): 7% DY5 REC, (E): 7% XH3 REC, (F): 14% DY6 REC, (G): 14% MB1 REC, (H): 14% DY5 REC, and (I): 14% XH3 REC. c: villi height, d: crypt depth.

control group. Isothiocyanates (Gl metabolites) were pungent to the intestinal tract, and they could decrease the intestinal villus height, which could drive the adverse effects of RSM on nutrient digestibility (Xu et al., 2012). High dietary fiber, tannins, and phytic acid content in RSM could also reduce nutrient digestibility due to its physiochemical properties that limit nutrient breakdown (Thacker and Petri, 2011). Supplementation of REC did not affect apparent nutrient digestibility in the present study; however, 17.64% RSM decreased nutrient digestibility in our previous study (Zhu et al., 2018). This difference may be explained by lower inclusion in the present study. Carroll and Richards (1958) reported that EA had a lower coefficient of digestibility than other unsaturated fatty acids; therefore, the high EA content in REC may induce a lower crude fat digestibility and AME than REC with lower EA (XH3, MB1, and DY5 vs. DY6 REC). This might also be the reason why there was an effect of different varieties of REC on egg weight.

Changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the presence of toxins (Yason et al., 1987). A shortening of the villus and a large crypt can lead to poor nutrient absorption and lower performance (Xu et al., 2003). In the present study, REC decreased villi height and villi height-to-crypt depth ratio and increased the crypt depth of duodenum compared with the control group, which was in line with Gopinger et al. (2014), who reported that 20% canola meal decreased villi height and increased crypt depth. The REC source had an effect on villi height, crypt depth, and villi height-to-crypt depth ratio, and the different duodenum morphologies of 4 varieties of REC might be the reason why there was a difference in egg weight among hens fed the 4 varieties of REC.

Table 8. Effects of rapeseed expeller cake varieties and levels on eggshell quality in laying hens during wk1 to wk8.[1]

Variety	Level (%)	Eggshell strength (kg/cm^3)					Eggshell thickness (mm)				
		wk 2	wk 4	wk 6	wk 8	wk1 to wk8	wk2	wk 4	wk 6	wk 8	wk1 to wk8
Control	0	3.59	5.44	5.26^{a,b}	4.84	4.78	0.39	0.35^{a,b}	0.34^{a,b}	0.34	0.36
DY6	7	3.55	5.29	5.19^{a,b}	5.54	4.90	0.39	0.34^{a,b}	0.33^{a,b}	0.34	0.35
MB1	7	3.57	5.16	5.05^{a,b}	5.15	4.73	0.38	0.33^{a,b}	0.33^{a,b}	0.34	0.35
DY5	7	3.42	5.30	4.63^{b}	5.08	4.61	0.39	0.32^{b}	0.32^{b}	0.33	0.34
XH3	7	3.56	5.53	5.33^{a,b}	5.04	4.87	0.38	0.35^{a,b}	0.35^{a,b}	0.34	0.35
DY6	14	3.63	5.38	5.48^{a,b}	5.25	4.94	0.39	0.35^{a,b}	0.34^{a,b}	0.33	0.35
MB1	14	3.65	5.33	5.91^{a}	5.13	5.01	0.40	0.35^{a,b}	0.36^{a}	0.33	0.36
DY5	14	3.53	5.40	5.44^{a,b}	5.24	4.90	0.39	0.36^{a}	0.34^{a,b}	0.32	0.35
XH3	14	3.46	5.18	5.29^{a,b}	5.04	4.74	0.39	0.33^{a,b}	0.33^{a,b}	0.32	0.34
SEM		0.10	0.21	0.21	0.21	0.17	0.01	0.01	0.01	0.005	0.01
P ANOVA		0.84	0.96	0.009	0.56	0.84	0.82	0.003	0.003	0.06	0.41
P-value[2]		0.69	0.58	0.87	0.13	0.77	0.71	0.13	0.30	0.27	0.27
Variety											
DY6		3.59	5.34	5.34	5.39	4.92	0.39	0.35	0.34	0.34	0.35
DY5		3.47	5.35	5.04	5.16	4.76	0.39	0.34	0.33	0.33	0.35
MB1		3.61	5.25	5.48	5.14	4.87	0.39	0.34	0.35	0.33	0.35
XH3		3.51	5.36	5.31	5.04	4.81	0.39	0.34	0.34	0.33	0.35
SEM		0.07	0.15	0.15	0.15	0.12	0.0043	0.0041	0.0043	0.0038	0.0038
Level (%)											
14		3.57	5.32	5.53^{a}	5.16	4.90	0.39	0.35^{a}	0.34	0.33^{b}	0.35
7		3.53	5.32	5.05^{b}	5.20	4.78	0.39	0.34^{b}	0.33	0.34^{a}	0.35
SEM		0.05	0.10	0.11	0.11	0.08	0.004	0.004	0.004	0.004	0.004
P-value	Variety	0.45	0.95	0.21	0.41	0.79	0.87	0.59	0.14	0.29	0.75
	Level	0.57	0.97	0.002	0.80	0.31	0.22	0.05	0.14	0.01	0.53
	Variety × Level	0.70	0.59	0.11	0.76	0.56	0.64	<0.01	<0.01	0.89	0.15

[1]Data are expressed as mean and SEM (n = 24). DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 = Mianbangyou No.1, XH3 = Xiheyou No.3, and SEM = standard error of the mean.
[2]This is a contrast of all REC versus the control group.
[a,b]"*P-value*" is a contrast comparison between all REC groups and control group, and "*P* ANOVA" is one-way ANOVA comparison between the 9 groups, and there was no difference in egg-laying rate between the 9 groups.

Eggshell and internal egg quality are of major importance to the egg industry worldwide (Roberts, 2006). Supplementation of REC had no effect on eggshell strength and eggshell thickness compared with the control group during wk1 to wk8, which was in agreement with previous studies, which reported 15% RSM or 29.4% RSM had no effect on eggshell strength and thickness (Riyazi et al., 2009; Zhu et al., 2018). Supplementation of REC decreased albumen height and Haugh units, which was in line with the previous study, which reported that RSM linearly decreased albumen height and Haugh units (Zhu et al., 2018). Similarly, Thomas et al. (1978) reported that 10% RSM had a high Gls had a lower Haugh unit than feeding 15% RSM with low Gls. The RSM source had an effect on albumen height and Haugh units in the present study. Metabolites of Gls could affect the secretion of estrogen, which can activate the expression of ovalbumin gene by binding with the estrogen receptor (Gaub et al., 1990; Mawson et al., 1994), and, thus, Gls metabolites might inhibit ovalbumin synthesis and decrease albumen height and Haugh units. Alternatively, decreased ADFI and apparent nitrogen digestibility also might be the reason for the decreasing albumen height in the present study too (Roberts, 2006). REC increased yolk color compared with the control group in the present study, which was in accordance with our previous study, which reported 100% RSM replacement of soybean meal 29.4% RSM increased yolk color (Zhu et al., 2018). Interestingly, high-Gls and high-EA REC had a lower yolk color than low-Gls and low-EA REC in the present study. Some pigments, including chlorophyll, phaeophytin, carotene, and xanthophyll, are fat soluble (Davidson, 1954). Xanthophyll, cryptoxanthin, and β-carotene were the main pigments in egg-yolk (Grimbleby and Black, 1952), and, thus, the low fat digestibility of REC with high Gls and EA might be the reason for the low pigmentation of yolk and the low yolk color of REC with high Gls and EA.

In summary, Gls and EA from REC induced low apparent nutrient digestibility, and intestinal morphology damage could decrease laying performance and egg internal quality. Antinutritional factors in REC had an interaction effect of enhancing its negative impact on the intestinal absorption area and egg internal quality. Feeding the REC with higher Gls and EA REC had (DY5, MB1, XH3) a lower laying performance and egg quality than with feeding lower Gls and EA REC (DY6). Therefore, the content of Gls and EA in REC should be taken into consideration when formulating feeds for hens.

Table 9. Effects of rapeseed expeller cake varieties and levels on egg internal quality in laying hens during wk1 to wk8.[1]

		Albumen height (mm)					Haugh unit					Yolk color				
Variety	Level (%)	wk 2	wk 4	wk 6	wk 8	wk1 to wk 8	wk 2	wk 4	wk 6	wk 8	wk1 to wk8	wk 2	wk 4	wk 6	wk 8	wk1 to wk8
Control	0	8.63^{a}	9.11^{a}	8.40^{a-c}	8.39^{a}	8.63^{a}	91.58^{a}	95.02^{a}	91.69^{a-c}	90.74	92.26^{a}	7.61^{c}	7.65^{c}	7.53^{c}	7.30^{c}	7.52^{d}
DY6	7	8.06^{a-c}	8.56^{a-c}	8.36^{a-c}	8.33^{a,b}	8.33^{a-c}	88.96^{a,b}	92.61^{a,b}	91.55^{a-c}	91.16	91.07^{a}	8.86^{a,b}	8.78^{a,b}	8.09^{b,c}	7.55^{b,c}	8.32^{b,c}
MB1	7	7.63^{b,c}	8.63^{a-c}	8.55^{a,b}	8.05^{a,b}	8.21^{a-c}	87.21^{a,b}	93.34^{a,b}	91.85^{a-c}	89.79	90.55^{a,b}	8.55^{b}	8.47^{b}	7.77^{b,c}	7.62^{b,c}	8.10^{c}
DY5	7	7.56^{b,c}	8.38^{b,c}	8.51^{a,b}	8.03^{a,b}	8.12^{b-d}	87.42^{a,b}	92.425^{a,b}	92.34a^{b}	89.60	90.45^{a}	8.72^{a,b}	8.75^{a,b}	7.90^{b,c}	7.40^{b,c}	8.19^{b,c}
XH3	7	8.07^{a-c}	8.67^{a-c}	8.13^{a-c}	7.78^{a,b}	8.16^{a-d}	89.47^{a,b}	93.77^{a,b}	90.43^{a-c}	88.03	90.43^{a}	8.89^{a,b}	8.65^{a,b}	7.71^{c}	7.30^{c}	8.14^{b,c}
DY6	14	8.39^{a,b}	8.94^{a,b}	8.84^{a}	8.21^{a,b}	8.59^{a,b}	91.27^{a}	94.75^{a}	93.12^{a}	90.33	92.36^{a}	9.06^{a,b}	9.16^{a}	8.96^{a}	8.87^{a}	9.01^{a}
MB1	14	8.21^{a,b}	8.30^{b,c}	7.66^{c}	8.08^{a,b}	8.06^{c,d}	90.92^{a}	91.46^{a,b}	88.88^{a-c}	90.22	90.37^{a,b}	9.32^{a}	8.84^{a,b}	8.06^{b,c}	7.58^{b,c}	8.45^{b,c}
DY5	14	8.07^{a-c}	8.28^{b,c}	7.74^{b,c}	7.99^{a,b}	8.02^{c,d}	90.36^{a}	90.88^{b}	87.56^{c}	89.49	89.58^{a,b}	9.14^{a,b}	9.09^{a}	8.10^{b,c}	7.74^{b,c}	8.52^{a-c}
XH3	14	7.30^{c}	8.09^{c}	7.82^{b,c}	7.55^{b}	7.69^{d}	85.15^{b}	90.32^{b}	87.85^{b,c}	86.89	87.55^{b}	9.03^{a,b}	9.20^{a}	8.35^{b}	7.93^{b}	8.63^{a,b}
SEM		0.19	0.16	0.19	0.18	0.11	1.11	0.86	1.05	0.99	0.64	0.17	0.14	0.14	0.13	0.12
P ANOVA		<0.001	<0.001	<0.001	0.033	<0.001	<0.001	0.001	<0.001	0.067	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P-value[2]		<0.001	<0.01	0.34	0.04	<0.001	0.02	0.005	0.27	0.22	0.004	<0.01	<0.01	<0.01	0.002	<0.01
Variety																
DY6		8.23^{a}	8.75^{a}	8.60^{a}	8.27^{a}	8.46^{a}	90.11	93.68	92.33^{a}	90.74^{a}	91.72^{a}	8.96	8.97	8.53^{a}	8.21^{a}	8.67^{a}
DY5		7.82^{a,b}	8.33^{b}	8.12^{a,b}	8.01^{a,b}	8.07^{b}	88.89	91.66	89.95^{a,b}	89.55^{a,b}	90.01^{b}	8.93	8.92	8.00^{b}	7.57b	8.36^{a,b}
MB1		7.92^{a,b}	8.47^{a,b}	8.11^{b}	8.06^{a,b}	8.14^{b}	89.06	92.40	90.37^{a,b}	90.00^{a,b}	90.46^{a,b}	8.93	8.65	7.92^{b}	7.61^{b}	8.28^{b}
XH3		7.69^{b}	8.38^{a,b}	7.98^{b}	7.67^{b}	7.93^{b}	87.31	92.05	89.14^{b}	87.46^{b}	88.98^{b}	8.96	8.93	8.03^{b}	7.62^{b}	8.38^{a,b}
SEM		0.14	0.11	0.13	0.13	0.08	0.81	0.61	0.75	0.72	0.46	0.11	0.10	0.10	0.09	0.09
Level (%)																
14		7.99	8.40	8.01^{b}	7.96	8.0	89.42	91.86	89.35^{b}	89.23	89.97	9.14^{a}	9.07^{a}	8.37^{a}	8.03^{a}	8.65^{a}
7		7.83	8.56	8.39^{a}	8.05	8.21	88.26	93.04	91.55^{a}	89.64	90.62	8.75^{b}	8.67^{b}	7.87^{b}	7.48^{b}	8.19^{b}
SEM		0.10	0.08	0.09	0.09	0.06	0.57	0.43	0.53	0.51	0.33	0.08	0.07	0.07	0.06	0.06
P-value	Variety	0.05	0.04	<0.01	0.012	<0.01	0.11	0.11	0.02	0.01	<0.01	1.00	0.11	<0.01	<0.01	0.01
	Level	0.24	0.17	<0.01	0.49	0.16	0.15	0.054	<0.01	0.57	0.15	<0.01	<0.01	<0.01	<0.01	<0.01
	Variety × level	<0.01	0.02	0.001	0.91	0.02	<0.01	0.01	0.02	0.87	0.02	0.21	0.89	0.06	<0.01	0.41

[1]Data are expressed as mean and SEM (n = 24). DY5 = Deyou No.5, DY6 = Deyou No.6, MB1 = Mianbangyou No.1, XH3 = Xiheyou No.3, and SEM = standard error of the mean.
[2]This is a contrast of all REC versus the control group.
$^{a-c}$"*P-value*" is a contrast comparison between all REC groups and control group, and "*P* ANOVA" is one-way ANOVA comparison between the 9 groups, and there was no difference in egg-laying rate between the 9 groups.

ACKNOWLEDGMENTS

This project was fanatically supported by the National Science and Technology Project (2014BAD13B04), the Sichuan Provincial Science and Technology project (2018NZ20009, 2014NZ0043), and the 111 Project. We also want to thank Gregory S. Fraley (Professor Biology, Hope College, Holland, MI) for giving us lots of suggestions on this manuscript.

REFERENCES

AOAC Int., 2012. Official Methods of Analysis of AOAC Int. 19th ed. Assoc. Off. Anal. Chem. Int., Gaithersburg, MD.

Bell, J M 1984. Nutrients and toxicants in rapeseed meal: a review. J. Anim. Sci. 58:996–1010.

Campbell, L. D. 1979. Incidence of liver haemorrhage among white leghorn strains fed on diets containing different types of rapeseed meals. Br. Poult. Sci. 20:239–246.

Carroll, K. K., and J. F. Richards. 1958. Factors affecting digestibility of fatty acids in the rat. J. Nutr. 64:411–424.

Chinese Feed Database (CFD). 2014. Tables of feed composition and nutritive values in China. China feed-database information network centre, Beijing. Accessed Sept. 2016. (in Chinese). http://www.chinafeeddata.org.cn/

Choi, E. J., P. Zhang, and H. Kwon. 2014. Determination of goitrogenic metabolites in the serum of male wistar rat fed structurally different glucosinolates. Toxicol. Res. 30:109–116.

Christophersen, B. O., and J. Bremer. 1972. Erucic acid–an inhibitor of fatty acid oxidation in the heart. BBA – Lipids Lipid Metabol. 280:506–514.

Davidson, J. 1954. Procedures for the extraction, separation and estimation of the major fat-soluble pigments of hay. J. Sci. Food Agric. 5:1–7.

Fenwick, G. R., N. M. Griffiths, and R. K. Heaney. 1983. Bitterness in brussels sprouts (*Brassica oleracea* L. var.*gemmifera*): therole of glucosinolates and their breakdown products. J. Sci. Food Agric. 34:73–80.

Gaub, M. P., M. Bellard, I. Scheuer, P. Chambon, and P. Sassonecorsi. 1990. Activation of the ovalbumin gene by the estrogen receptor involves the Fos-Jun complex. Cell. 63:1267–1276.

Gopinger, E., E. G. Xavier, M. C. Elias, A. A. Catalan, M. L. Castro, A. P. Nunes, and V. F. Roll2014. The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens. Poult. Sci. 93:1130–1136.

Grandhi, R. R., S. J. Slinger, and J. D. Summers. 1977. Productive performance and liver lesions in two strains of laying hens receiving two rapeseed meals. Poult. Sci. 56:1904–1908.

Grimbleby, F. H., and D. J. Black 1952. Variations in the composition of egg-yolk pigment. Br. J. Nutr. 6:393–397.

Hobson-Frohock, A., G. R. Fenwick, and R. K. Heaney et al. 1977. Rapeseed meal and egg taint: association with sinapine. Br. Poult. Sci. 18:539–541.

Huang, S., M. Liang, G. Lardy, H. E. Huff, M. S. Kerley, and F. Hsieh 1995. Extrusion processing of rapeseed meal for reducing glucosinolates. Anim. Feed Sci. Technol., 56:1–9.

Hulan, H. W., J. K. G. Kramer, S. Mahadevan, F. D. Sauer, and A. H. Corner1976. Relationship between erucic acid and myocardial changes in male rats. Lipids. 11:9–15.

Ibrahim, I. K., and R. Hill. 1980. The effects of rapeseed meals from Brassica napus varieties and the variety Tower on the production and health of laying fowl. Br. Poult. Sci. 21:423–430.

Jonker, A., M. Y. Gruber, Y. Wang, B. Coulman, A. Azarfar, J. J. McKinnon, D. A. Christensen, and P. Q. Yu 2011. Modeling degradation ratios and nutrient availability of anthocyanidin-accumulating *Lc*-alfalfa populations in dairy cows. J. Dairy Sci. 94:1430–1444.

Khajali, F., and B. A. Slominski2012. Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. 91: 2564–2575.

Kienle, M. G., G. Cighetti, C. Spagnuolo, and C. Galli1976. Effects of rapeseed oil on fatty acid oxidation and lipid levels in rat heart and liver. Lipids. 11:670–675.

Kirk, R. E. 1968. Experimental Design: Procedures for the Behavioral Sciences. Pages 276–283, Wadsworth Publishing House Company, Belmont, California.

Koletzko, B. 2005. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. J. Pediatr. Gastroenterol. Nutr. 41:584–599.

Kozlowski, K., D. Mikulski, A. Rogiewicz, Z. Zdunczyk, M. Rad-Spice, H. Jeroch, J. Jankowski, and B. A. Slominski. 2018. Yellow seeded *B. napus* and *B. juncea* canola. Part 2. Nutritive value of the meal for turkeys. Anim. Feed Sci. Technol. 240:102–116.

Leslie, A. J., and J. D. Summers. 1972. Feeding value of rapeseed for laying hens. Pharmacotherapy. 52:102–116.

Liu, J. B., H. L. Yan, and S. C. Cao et al. 2019. The response of performance in grower and finisher pigs to diets formulated to different tryptophan to lysine ratios. Livest. Sci. 222:25–30.

Martland, M. F., E. J. Butler, and G. R. Fenwick. 1984. Rapeseed induced liver haemorrhage, reticulolysis and biochemical changes in laying hens: the effects of feeding high and low glucosinolate meals. Res. Vet. Sci. 36:298–309.

Matthäus, B., and H. J. Fiebig. 1996. Simultaneous determination of isothiocyanates, indoles, and oxazolidinethiones in myrosinase digests of rapeseeds and rapeseed meal by HPLC. J. Agric. Food Chem. 44:3894–3899.

Mawson, R., R. Heaney, Z. Zdunczyk, and H. Kozlowska1994. Rapeseed meal-glucosinolates and their antinutritional effects Part 5. Animal reproduction. Nahrung. 38:588–598.

Michigami, Y., K. Fujii, K. Ueda, and Y. Yamamoto. 1992. Determination of thiocyanate in human saliva and urine by ion chromatography. Analyst. 117:1855–1858.

Ministry of Agriculture of the People's Republic of China, 2011. High Erucic Acid Rapeseed (NY/T 1990–2011).Ministry of Agriculture of the People's Republic of China, Beijing, China.

Ministry of Health of the People's Republic of China and Standardization Administration of China, 2004. Rapeseed Oil (GB /T 1536–2004).Ministry of Health of the People's Republic of China and Standardization Administration of China, Beijing, China.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Rad-Spice, M., A. Rogiewicz, J. Jankowski, and B. A. Slominski. 2018. Yellow-seeded *B. napus* and *B. juncea* canola. Part 1. Nutritive value of the meal for broiler chickens. Anim. Feed Sci. Technol. 240:66–77.

Radfar, M., A. Rogiewicz, and B. A. Slominski. 2017. Chemical composition and nutritive value of canola-quality *Brassica juncea* meal for poultry and the effect of enzyme supplementation. Anim. Feed Sci. Technol. 225:97–108.

Razdan, A., D. Pettersson, and J. Pettersson. 1997. Broiler chicken body weights, feed intakes, plasma lipid and small-intestinal bile acid concentrations in response to feeding of chitosan and pectin. Br. J. Nutr. 78:283–291.

Riyazi, S. R., Y. Ebrahimnezhad, K. Nazeradl, N. Maherisis, R. Salamatdust, and T. Vahdatpour. 2009. The effects of replacing soybean meal with different levels of rapeseed meal on egg quality characteristics of commercial laying hens. Asian J. Anim. Vet. Adv. 4:337–341.

Roberts, J. R. 2004. Factors affecting egg internal quality and egg shell quality in laying hens. Jpn. Poult. Sci. 41:161–177.

SAS, 2003. SAS User's Guide: Statistics, Version 9.1. SAS Inst. Inc., Cary, NC.

Savary, R. K. 2013. Evaluating canola (*brassica napus*) meal and juncea (*brassica juncea*) meal with or without supplemental enzymes for two commercial strains of laying hens. MSc Diss. Univ. Canada, Halifax, Nova Scotia.

Slominski, B. A. 1997. Developments in the breeding of low fibre rapeseed/canola. J. Anim. Feed Sci. 6:303–318.

Slominski, B. A., J. Simbaya, L. D. Campbell, G. Rakow, and W. Guenter. 1999. Nutritive value for broilers of meals derived from newly developed varieties of yellow-seeded canola. Anim. Feed Sci. Technol. 78:249–262.

Thacker, P. A., and D. Petri. 2011. Nutritional evaluation of canola protein concentrate for broiler chickens. Asian Australas. J. Anim. Sci. 24:1607–1614.

Thomas, D., A. R. Robblee, and D. R. Clandinin. 1978. Effects of low and high glucosinolate rapeseed meals on productive performance, egg quality, composition of liver and incidence of haemorrhagic liver syndrome in laying birds. Br. Poult. Sci. 19:449–454.

Tripathi, M. K., and A. S. Mishra. 2007. Glucosinolates in animal nutrition: a review. Anim. Feed Sci. Technol. 132:1–27.

Tripathi, M. K., A. S. Mishra, A. K. Misra, D. Mondal, and S. A. Karim 2001. Effect of substitution of groundnut with high glucosinolate mustard (*Brassica juncea*) meal on nutrient utilization, growth, vital organ weight and blood composition of lambs. Small Rumin. Res. 39:261–267.

Unger, E. H. 1990. Commercial processing of canola and rapeseed: crushing and oil extraction. Pages 235–249 in Canola and Rapeseed. F. Shahidi, ed. Springer, Boston, MA.

Vig, A. P., and A. Walia 2001. Beneficial effects of Rhizopus oligosporus fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (Brassica napus) meal. Bioresour. Technol. 78:309–312.

Xu, F. Z., X. G. Zeng, and X. L. Ding. 2012. Effects of replacing soybean meal with fermented rapeseed meal on performance, serum biochemical variables and intestinal morphology of broilers. Asian Australas. J. Anim. Sci. 25:1734–1741.

Xu, Z. R., C. H. Hu, M. S. Xia, X. A. Zhan, and M. Q. Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82:1030–1036.

Yamashiro, S., M. K. Bhatnagar, J. R. Scott, and S. J. Slinger. 1975. Fatty haemorrhagic liver syndrome in laying hens on diets supplemented with rapeseed products. Res. Vet. Sci. 19:312–321.

Yan, H. L., L. Zhang, Z. D. Guo, H. F. Zhang, and J. B. Liu 2019. Production phase affects the bioaerosol microbial composition and functional potential in swine confinement buildings. Animals. 9:90.

Yason, C. V., B. A. Summers, and K. A. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. Am. J. Vet. Res. 48:977.

Zhu, L. P., J. P. Wang, X. M. Ding, S. P. Bai, Q. F. Zeng, Z. W. Su, Y. Xuan, and K. Y. Zhang. 2018. Effects of dietary rapeseed meal on laying performance, egg quality, apparent metabolic energy, and nutrient digestibility in laying hens. Livest. Sci. 214:265–271.