Animal Nutrition 20 (2025) 311–317

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Effects of different rapeseed varieties on egg production performance, egg quality, hormone levels, follicle development, and thyroid function in hens

Liping Zhu [a, b], Jianping Wang [a], Xuemei Ding [a], Shiping Bai [a], Qiufeng Zeng [a], Yue Xuan [a], Keying Zhang [a, *]

[a] Animal Nutrition Institute, Key Laboratory for Animal Disease-Resistance Nutrition of China, Ministry of Education, Sichuan Agricultural University, Chengdu 611130, Sichuan Province, China

[b] Air Traffic Management College, Civil Aviation Flight University of China, Deyang 618300, Sichuan Province, China

ARTICLE INFO

Article history:
Received 13 January 2024
Received in revised form
29 May 2024
Accepted 15 October 2024
Available online 19 October 2024

Keywords:
Brassica napus rapeseed variety
Laying hens
Egg production performance
Hormone level
Follicle number
Organ index

ABSTRACT

This study was to evaluate the effects of rapeseed (*Brassica napus* L.) from China with different content of glucosinolate (Gls) and erucic acid (EA) on laying hens. A total of 600 laying hens at 33 wk of age were randomly divided into 5 treatments containing a control diet and 4 diets with 11.5% rapeseed. The 11.5% rapeseed diets varied in Gls and EA levels: 1) Deyou no. 6 (DY6) with Gls at 22.67 μmol/g and EA at 0.7%, 2) Mianbangyou no. 1 (MB1) with Gls at 43.23 μmol/g and EA at 3.5%, 3) Deyou no. 5 (DY5) with Gls at 74.66 μmol/g and EA at 16.20%, 4) Xiheyou no. 3 (XH3) with Gls at 132.83 μmol/g and EA at 44.60%. Each group had eight replicates and each replicate had 15 hens. The trial lasted for 12 wk with 4 wk withdrawal. From 1 to 8 wk of the trial, 11.5% rapeseed reduced average daily feed intake (ADFI) compared to the control group ($P = 0.002$), and egg-laying rate of XH3 rapeseed was lower than that of DY6 rapeseed ($P = 0.006$), and egg weight of MB1, DY5, and XH3 rapeseed were lower than that of the control group ($P = 0.007$). Egg mass was reduced by 11.5% rapeseed and egg mass of XH3 and DY5 rapeseed were lower than that of DY6 rapeseed ($P = 0.004$). Feed conversion ratio (FCR) of 11.5% rapeseed was higher than that the control group and FCR was higher in XH3 rapeseed than in DY6 rapeseed from 1 to 8 wk ($P = 0.008$). At wk 8, the lightness value of eggshell color of XH3 rapeseed was significantly lower than that of the control and DY5 rapeseed ($P = 0.012$). Xiheyou no. 3 rapeseed had a higher redness value of eggshell color than the control and MB1 and DY5 rapeseed ($P = 0.008$). Albumen height of DY5 rapeseed was lower than that of the control group at wk 8 ($P = 0.012$). Mianbangyou no. 1 and DY5 rapeseed decreased Haugh unit at wk 4 and 8, respectively ($P = 0.011$, $P = 0.024$). Serum estradiol (E2) content was decreased by 11.5% rapeseed ($P = 0.003$). Thyroid index increased as the Gls and EA content increased ($P = 0.008$). The smallest hierarchical follicle numbers of XH3 and MB1 rapeseed were lower than that of the control group ($P = 0.009$). After 4 wk withdrawal, the egg weight, egg mass, and FCR did not recover ($P = 0.011$, $P = 0.033$, $P = 0.024$, respectively). In conclusion, 11.5% rapeseed decreased egg production performance which might be caused by decreasing hormone levels, and high Gls and EA rapeseed had a lower performance than low Gls and EA rapeseed.

1. Introduction

Rapeseed or canola is an oil crop that is widely grown around the world. Full-fat rapeseed contains approximately 40% oil and 21% to 23% protein and is therefore a valuable source of energy and protein in poultry diets (Ajuyah et al., 1993). Rapeseed protein has a physiologically suitable amino acid composition, including a high content of essential amino acids like lysine, threonine, tryptophan

* Corresponding author.
E-mail address: zkeying@sicau.edu.cn (K. Zhang).

Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2024.10.001

and sulphur-containing amino acids (Chmielewska et al., 2021). Moreover, rapeseed has also been known to be a good source of α-linolenic acid (ALA, C18:3n-3), which can be readily converted to n-3 LC-PUFA in poultry (Li et al., 2017). However, rapeseed contains nutritionally unfavorable substances such as glucosinolates, sinapine, tannins, and phytates which may negatively influence many physiological processes and adversely impact health status (Chmielewska et al., 2021). There are relatively few reports in the literature on the nutritive value of unextracted rapeseed for laying birds. Olomu et al. (1975) observed that 15% rapeseed had no adverse effects on egg production performance and internal egg quality. Lichovnikova and Zeman (2004) also observed that 13.5% rapeseed had no effect on egg performance or internal egg quality. Canola was originally derived from rapeseed varieties; its components have been altered through genetic selection which has markedly reduced its detrimental components, erucic acid and the glucosinolates, to a negligible level and to less than 20 μg/g which is low enough to be of little or no harm to poultry (Najib and Al-Khateeb, 2004). Research has shown that 5% to 10% canola seed in hens' diets improved egg production, and 20% canola seed reduced egg performance including egg production, egg weight, and feed conversion (Najib and Al-Khateeb, 2004 had no effect on feed intake. Leslie and Summers (1972) showed that 5% rapeseed significantly reduced egg weight, 10% rapeseed had no effect on egg performance except egg weight, and for egg quality, 15% rapeseed could be used in laying hens' diets (Leslie and Summers, 1972). There are some inconsistencies in the results of the current study, and more data is needed to determine its dosage in layer diets.

China is not only the world's second largest producer of rapeseeds (FAO, 2019), but also breeds many rapeseed varieties with high or low glucosinolates (Gls) and erucic acid (EA) content. Given that rapeseed may affect the performance and egg quality in laying hens, and there are no studies to compare the difference between different rapeseed varieties used in feed, we conducted the following experiment. We chose 4 kinds of *Brassica napus* rapeseed with different Gls and EA content: Deyou no. 5 (DY5), Deyou no. 6 (DY6), Mianbangyou no. 1 (MB1), and Xiheyou no. 3 (XH3) (Gls and EA content: DY6 < MB1 < DY5 < XH3), which were produced from different geographic regions in Sichuan province of China (DY5 and DY6 rapeseed from Deyang City, MB1 and XH3 rapeseed from Mianyang City) to evaluate the effects of different kinds of raw unbroken rapeseed on production performance, egg quality, and organ index. At the same time, we also examined the production performance after the 4-wk rapeseed withdrawal period to provide some theoretical data for the use of rapeseed in industrial production.

2. Material and methods

2.1. Animal ethics statement

Animal procedures were performed according to the Guidelines for Care and Use of Laboratory Animals of Sichuan Agricultural University and approved by the Animal Care Advisory Committee of Sichuan Agricultural University (approval no. YYS160915).

2.2. Hens, diets, and management

At 33 wk of age, a total of 600 laying hens (Lohmann white, Sun Daily Inc., Chengdu, Sichuan, China) were randomly assigned to 5 dietary treatments with 8 replicates per group and 15 hens per replicate (3 hens/cage, cage size: 38.2 cm × 50.1 cm × 40.0 cm) including a control group (corn-soybean diet) and 4 groups with 11.5% raw unbroken rapeseed (DY5, DY6, MB1, and XH3). The experiment lasted for 12 wk, which comprised of an 8-wk experimental deposition period and a 4-wk withdrawal period. Hens were housed individually in stainless steel cages and the room environment was controlled at 22 °C by a daily lighting schedule of 16 h light and 8 h dark. Hens were allowed ad libitum access to experimental diets and water. The hens were fed diets in mash form (Table 1) during the experiment, 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 feeds (Table 2) in this study referred to the China Feed Database (2014).

2.3. Productive performance

Feed intake (FI) and feed conversion ratio (FCR) were recorded every week, and egg production, egg weight, and mortality rate were monitored daily to evaluate the performance outcomes. The average weight of the eggs was calculated weekly. The egg production rate was determined by dividing the number of daily eggs by the number of hens present on the same day. To determine the egg mass, the weight was multiplied by the rate of egg production. The FCR was also calculated by dividing the average FI by the egg mass.

Table 1
Ingredients of experimental diets (%, as-fed basis).

Item	Control	11.5% Rapeseed
Corn	61.04	49.63
Soybean meal (43% CP)	26.77	21.69
Wheat bran	0.94	6.00
Full-fat rapeseed	0.00	11.50
Corn gluten meal	0.02	0.01
Soybean oil	0.98	0.00
Calcium carbonate	3.65	3.57
Calcium carbonate	4.26	4.26
Calcium hydrophosphate	1.17	1.11
NaCl	0.40	0.40
Mineral premix [1]	0.50	0.50
Vitamin premix[2]	0.03	0.03
L-Lys·HCl	0.00	0.14
DL-Met	0.14	0.14
Thr	0.00	0.02
Trp	0.00	0.02
Chloride choline	0.10	0.10
Rice bran and hull	0.00	0.88
Total	100.00	100.00

CP= crude protein.
[1] Provided per kilogram of diet: 60 mg Fe ($FeSO_4 \cdot 7H_2O$), 8 mg Cu ($CuSO_4 \cdot 5H_2O$), 60 mg Mn (MnSO4·H_2O), 80 mg Zn ($ZnSO_4 \cdot 7H_2O$), 0.3 mg Se ($NaSeO_3$), and 0.35 mg I (KI).
[2] Provided per kilogram of diet: 8000 IU, vitamin A, 1600 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.

Table 2
Nutrient composition of experimental diets (%, as-fed basis).

Item	Control	DY6	MB1	DY5	XH3
Gross energy, Cal/g	3641.42	3654.93	3556.17	3557.62	3612.31
Dry matter	89.22	89.28	89.54	89.10	89.89
CP	16.43	16.85	16.68	16.87	17.00
EE	4.21	6.63	6.14	5.71	6.57
CF	2.99	10.50	10.47	9.37	11.12

CP= crude protein; EE= ether extract; CF= crude fiber; DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.

2.4. Sampling procedure

A daily record of egg production (2 wk) and a weekly record of feed consumption were maintained. Egg-laying rates were expressed as an average hen-day production. Every 2 wk, 3 eggs were chosen from each replicate for egg-quality measurement. At wk 8, one hen was chosen from each replicate randomly and blood samples were collected from wing veins for serum biochemical analysis, and then sacrificed by cervical dislocation. Organs including liver, thyroid, and kidneys were extracted and weighed. After the blood was collected, the tubes containing blood were centrifuged at 3000 × g at 4 °C for 10 min, and then serum was collected in new tubes and stored at −20 °C until further analysis. The organ index was calculated using the following equation: organ index (%) = (organ weight/body weight) × 100. Ovaries were removed and the hierarchical follicles were identified by size and counted. The follicles were classified by size, including small white follicles (SWF; 1 to 2 mm), large white follicles (LWF; 3 to 5 mm), small yellow follicles (SYF; 6 to 8 mm), and the smallest hierarchical follicle (F6; >8 mm).

2.5. Nutritional and antinutritional factor determination

For rapeseed, fatty acid composition was determined according to GB/T 21514-2008 (China National Standard, 2008). Glucosinolates were detected according to NY/T 1582-2007 (Ministry of Agriculture of the People's Republic of China, 2007). Isothiocyanates were detected according to NY/T 1596-2008 (Ministry of Agriculture of the People's Republic of China, 2008). Oxazolidinethione was detected according to NY/T 1799-2009 (Ministry of Agriculture of the People's Republic of China, 2009). Sinapine was detected as the following steps. A sample (1 g) was accurately weighed and placed in a 100-mL triangular bottle with 50 mL methanol for ultrasonic extraction for 20 min, then filtered, and a small amount of methanol washing filter was used and the lotion was incorporated into the filtrate. The residue was extracted twice, and then the extracted liquid was combined and evaporated to dry. The residue was dissolved with 0.08 mol/L potassium dihydrogen phosphate solution in a 50-mL volumetric flask and filtered with a microporous filter membrane (0.45 μm) for HPLC analysis. Dry matter, gross energy, ether extract, crude protein, and crude fiber content of diets were detected according to the methods of GB/T 6435-2018 (China National Standard, 2018), ISO 9831:1998 (ISO, 1998), GB/T 6433-2006 (China National Standard, 2006), GB/T 24318-2009 (China National Standard, 2009), and GB/T 6434-2022 (China National Standard, 2022), respectively.

2.6. Determination of egg quality

At the end of feeding experiment, 24 eggs (3 eggs per replicate) were chosen from each group for egg quality analysis. Eggshell color (lightness: L*, redness: a*, and yellowness: b*) was measured by a color meter (Minolta CR410 chroma meter, Konica Minolta Sensing Inc., Osaka, Japan). Eggshell strength was evaluated using an eggshell force gauge model II (Robotmation Co., Ltd., Tokyo, Japan). 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 were evaluated using an egg multi tester (EMT-7300, Robotmation Co., Ltd., Tokyo, Japan).

2.7. Serum biochemical analysis

The level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triiodothyronine (T3), tetraiodothyronine (T4), urea, creatinine (CREA), estradiol (E2), and progesterone (P4) in serum were measured using an automatic serum biochemical analyzer (HITACH 7020, Japan).

2.8. Statistical analysis

The data are presented as the mean and SEM. One-way ANOVA and post hoc multiple mean comparison (Tukey's HSD test) were performed in order to determine differences between the samples with a confidence interval of 95%. $P < 0.05$ was used to indicate a significant difference. The statistical method model is as follows:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + C_{(k)l} + T \times S_{ik} + e_{ijkl}$$

where Y_{ijkl} refers to the dependent variable; μ, the overall mean; T_i, the ith fixed treatment effect; P_j, the jth random period effect; S_k, the kth random square effect; $C_{(k)l}$, the random effect of the lth steer within the kth square; $T \times S_{ik}$, the interaction between the ith treatment and the kth square; and e_{ijkl}, the error residual.

3. Results

3.1. Antinutritional factor content of rapeseed

Antinutritional factor content is shown in Table 3. Total Gls and oxazolidinethione content increased along a gradient (DY6 < MB1 < DY5 < XH3). In rapeseed, about 25% to 50% Gls was 2-OH-3-butenyl and Gls 3-Butenyl Gls. DY5 rapeseed had the highest sinapine content. The erucic acid content increased along a gradient (DY6 < MB1 < DY5 < XH3). Sinapine content of DY5 rapeseed was higher than that of other three rapeseeds.

3.2. Egg production performance

The effects of rapeseed on egg production performance are shown in Tables 4 and 5. Compared to the control group, four kinds of 11.5% rapeseeds reduced ADFI from wk 1 to 4, wk 5 to 8, and wk 1 to 8 ($P = 0.003$, $P = 0.001$, $P = 0.002$). From wk 1 to 4, XH3 rapeseed had a significantly lower ADFI than DY6 rapeseed ($P = 0.003$). Egg production was reduced by 11.5% rapeseed from wk 1 to 4, wk 5 to 8, and wk 1 to 8 ($P = 0.0004$, $P = 0.003$, $P = 0.006$). From wk 1 to 4, there was no difference in egg-laying rate between the DY6 rapeseed and control group; as the time increased, the egg-laying rate of DY6 rapeseed was significantly lower than the control group ($P = 0.003$). Egg-laying rate of XH3 rapeseed was significantly lower than that of DY6 rapeseed from wk 1 to 4, wk 5 to 8, and wk 1 to 8. DY5 rapeseed had a lower egg weight than the control group from wk 1 to 4 ($P = 0.030$). Mianbangyou no. 1, DY5, and XH3 rapeseed had a lower

Table 3
Antinutritional factor content of four varieties rapeseed (as fed basis).

Item	DY6	MB1	DY5	XH3
Total Gls[1], μmol/g rapeseed cake	22.67	43.23	74.66	132.83
2-OH-3-butenyl Gls, μmol/g	4.04	8.93	17.68	42.96
3-Butenyl Gls, μmol/g	1.76	5.54	10.70	23.44
4-OH-3-indolylmethyl Gls, μmol/g	1.78	1.87	2.96	2.75
Phenethyl Gls, μmol/g	1.76	1.46	2.32	1.26
Isothiocyanate, mg/g	0.13	1.35	0.82	5.20
Oxazolidinethione, mg/g	0.06	0.60	0.82	2.24
Sinapine, g/kg	5.60	4.43	7.21	6.43
Erucic acid (relative to total fatty acids), %	0.50	8.50	22.90	54.00

Gls = glucosinolates; DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
[1] Total Gls only contains Gls with a relatively large proportion of content.

Table 4
Effects of different rapeseed varieties on egg production in laying hens.[1]

Item	Treatment					SEM	*P*-value
	Control	DY6	MB1	DY5	XH3		
ADFI, g/d per bird							
1–4 wk	110.50^{a}	105.76^{b}	105.97^{b}	103.34^{bc}	101.99^{c}	0.721	0.003
5–8 wk	105.30^{a}	99.83^{b}	99.07^{b}	99.07^{b}	99.79^{b}	0.571	0.001
1–8 wk	107.90^{a}	102.79^{b}	102.52^{b}	101.20^{b}	100.88^{b}	0.483	0.002
Egg-laying rate, %							
1–4 wk	91.67^{a}	88.21^{ab}	86.31^{bc}	83.57^{bc}	83.24^{c}	1.195	0.004
5–8 wk	88.48^{a}	78.09^{b}	74.06^{bc}	75.43^{bc}	70.77^{c}	1.642	0.003
1–8 wk	90.07^{a}	83.15^{b}	80.19^{bc}	79.50^{bc}	77.01^{c}	1.074	0.006
Egg weight, g/d per bird							
1–4 wk	60.10^{a}	59.05^{ab}	58.82^{ab}	58.63^{b}	58.91^{ab}	0.337	0.030
5–8 wk	60.94^{a}	59.73^{ab}	58.67^{b}	58.47^{b}	58.98^{b}	0.431	0.008
1–8 wk	60.52^{a}	59.39^{ab}	58.74^{b}	58.55^{b}	58.94^{b}	0.358	0.007
Egg mass, g/d per bird							
1–4 wk	55.11^{a}	52.10^{ab}	50.79^{b}	49.03^{b}	49.08^{b}	0.783	0.005
5–8 wk	53.94^{a}	46.63^{b}	43.47^{bc}	44.08^{bc}	41.74^{c}	0.910	0.006
1–8 wk	54.52^{a}	49.37^{b}	47.13^{bc}	46.55^{c}	45.41^{c}	0.639	0.004
FCR							
1–4 wk	2.01	2.04	2.09	2.12	2.09	0.032	0.100
5–8 wk	1.96^{c}	2.15^{b}	2.31^{ab}	2.26^{ab}	2.41^{a}	0.051	0.009
1–8 wk	1.99^{c}	2.09^{bc}	2.20^{ab}	2.19^{ab}	2.25^{a}	0.033	0.008

ADFI = average daily feed intake; FCR = feed conversion ratio; DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
Means with different superscripts in the same row differ significantly ($P < 0.05$).
[1] Data are expressed as mean and SEM.

egg weight than the control group from wk 5 to 8 ($P = 0.008$). For the wk 1 to 8 period, egg weight of MB1, DY5, and XH3 rapeseed were significantly lower than the control group ($P = 0.007$). For egg mass, MB1, DY5, and XH3 rapeseed were significantly lower than the control group from wk 1 to 4 ($P = 0.005$). From wk 5 to 8, four kinds of rapeseed had a lower egg mass than the control group ($P = 0.006$), and egg mass of XH3 rapeseed was lower than that of DY6 rapeseed. For the wk 1 to 8 period, 11.5% rapeseed reduced the egg mass, and egg mass in XH3 and DY5 rapeseed were lower than that in DY6 rapeseed ($P = 0.004$). From wk 1 to 4, there was no difference in FCR between each group ($P = 0.100$). From wk 5 to 8 and wk 1 to 8, 11.5% rapeseed had a higher FCR than the control group ($P = 0.009$), and FCR in XH3 rapeseed was higher than that in DY6 rapeseed ($P = 0.008$).

After 4 weeks withdrawal of rapeseed, the ADFI and egg-laying rate had recovered in the rapeseed groups, and there was no difference between each group ($P = 0.191$, $P = 0.152$, respectively). But the egg weight, egg mass, and FCR did not recover. DY5 and MB1 rapeseed had a lower egg weight than the control group ($P = 0.011$). XH3 and MB1 rapeseed had a lower egg mass than the control group ($P = 0.033$). XH3 and MB1 rapeseed had a higher FCR than the control group ($P = 0.024$).

3.3. Egg quality

The effects of rapeseed on eggshell and egg internal quality are shown in Tables 6–8. DY5 rapeseed had a lower L* than MB1 rapeseed at wk 2 ($P = 0.009$). At wk 4, The value of L* significantly decreased with the increase of antinutritional factors in rapeseed, and L* of DY6 rapeseed was significantly higher than that of XH3 rapeseed ($P = 0.023$). At wk 8, L* of XH3 rapeseed was significantly lower than the control and DY5 rapeseed ($P = 0.012$). XH3 rapeseed had a higher a* value than the control and MB1 and DY5 rapeseed ($P = 0.008$). There was no difference in b* between each group at wk 2, 4, 6, and 8 ($P = 0.524$, $P = 0.714$, $P = 0.592$, $P = 0.642$). For the eggshell strength, MB1 rapeseed tended to be higher than DY6 rapeseed at wk 8 ($P = 0.053$), at the same time, eggshell thickness of DY6 rapeseed was lower than other groups ($P = 0.009$). Albumen height of XH3 rapeseed was lower than the control group at wk 2 ($P = 0.022$). MB1, DY5, and XH3 rapeseed had a lower albumen height than the control group at wk 4 ($P = 0.009$). Albumen height of DY5 rapeseed was lower than the control group at wk 8 ($P = 0.012$). Yolk color was increased by 11.5% rapeseed before wk 6 ($P = 0.005$, $P = 0.003$, $P = 0.004$), and XH3 had a higher yolk color than other rapeseed groups. But there was no difference in yolk color between each treatment at wk 8 ($P = 0.122$). Mianbangyou no. 1 and DY5 rapeseed decreased Haugh unit at wk 4 and 8 ($P = 0.011$, $P = 0.024$).

3.4. Serum biochemical indices

The effects of different varieties on serum organ function parameters of laying hens are shown in Table 9. There was no significant difference in serum ALT, CREA, UREA, T3, T4, and T3/T4 ($P > 0.05$), but XH3 rapeseed tended to increase serum AST content ($P = 0.091$). Adding 11.5% rapeseed to diets significantly decreased serum E2 content ($P = 0.003$), and there was no difference between rapeseed variety ($P > 0.05$). Adding 11.5% rapeseed to diets tended to decrease serum P4 content ($P = 0.062$).

3.5. Organ index and follicle number

The effects of different variety on organ index and follicle number of laying hens are shown in Table 10. Thyroid index increased as the GIs and EA content increased, and DY5 and XH3 rapeseed had a higher thyroid index than the control group, and there were differences between rapeseed varieties (XH3 > DY6/MB1, $P = 0.008$). There was no difference in liver index between each group ($P = 0.412$). DY5 rapeseed had a higher kidney index than DY6 rapeseed ($P = 0.034$). Adding 11.5% rapeseed in diets decreased follicle number, although there was no difference in SYF, LWT, SWF, and total follicles ($P > 0.05$), but total follicle number of

Table 5
Effects of different rapeseed varieties on egg production during elimination phase (9–12 wk of the trial).[1]

Item	Treatment					SEM	*P*-value
	Control	DY6	MB1	DY5	XH3		
ADFI, g/d per bird	103.72	101.04	101.47	101.38	101.99	0.800	0.191
Egg-laying rate, %	87.37	82.63	82.57	86.07	82.15	1.661	0.152
Egg weight, g/d per bird	62.07^{a}	61.44^{ab}	59.81^{b}	59.75^{b}	60.62^{ab}	0.465	0.011
Egg mass, g/d per bird	54.21^{a}	50.74^{ab}	49.38^{b}	51.43^{ab}	49.78^{b}	0.963	0.033
FCR	1.91^{b}	1.99^{ab}	2.07^{a}	1.98^{ab}	2.06^{a}	0.032	0.024

ADFI = average daily feed intake; FCR = feed conversion ratio; DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
Means with different superscripts in the same row differ significantly ($P < 0.05$).
[1] Data are expressed as mean and SEM.

Table 6
Effects of different varieties of rapeseed on eggshell color of laying hens.[1]

Item	Treatment					SEM	*P*-value
	Control	DY6	MB1	DY5	XH3		
L*							
2 wk	72.55^{ab}	72.55^{ab}	75.19^{a}	71.86^{b}	74.25^{ab}	0.721	0.009
4 wk	76.58^{a}	76.20^{ab}	74.62^{bc}	74.61^{bc}	74.42^{c}	0.593	0.023
6 wk	73.9	75.04	74.08	73.9	74.24	0.572	0.601
8 wk	74.95^{a}	74.75^{ab}	74.55^{ab}	75.12^{a}	72.92^{b}	0.474	0.012
a*							
2 wk	4.93	5.04	4.98	5.49	5.12	0.200	0.271
4 wk	4.56	4.96	5.34	5.1	5.41	0.261	0.153
6 wk	5.28	4.68	4.93	5.03	5.23	0.252	0.441
8 wk	4.67^{b}	5.05^{ab}	4.88^{b}	4.68^{b}	5.80^{a}	0.221	0.008
b*							
2 wk	15.44	15.53	15.28	15.93	15.24	0.302	0.524
4 wk	15.68	15.78	16.31	15.94	15.86	0.334	0.714
6 wk	15.95	15.57	15.24	15.61	15.86	0.331	0.592
8 wk	15.19	14.86	15.10	14.74	15.53	0.391	0.642

L* = lightness; a* = redness; b* = yellowness; DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
Means with different superscripts in the same row differ significantly ($P < 0.05$).
[1] Data are expressed as mean and SEM.

Table 8
Effects of different varieties of rapeseed on egg internal quality of laying hens.[1]

Item	Treatment					SEM	*P*-value
	Control	DY6	MB1	DY5	XH3		
Albumen height, mm							
2 wk	8.63^{a}	8.17^{ab}	8.22^{ab}	8.27^{ab}	7.92^{b}	0.142	0.022
4 wk	9.11^{a}	8.46^{ab}	8.12^{b}	8.26^{b}	8.32^{b}	0.171	0.009
6 wk	8.40^{ab}	8.76^{a}	8.09^{ab}	8.43^{ab}	7.84^{b}	0.193	0.011
8 wk	8.39^{a}	8.11^{ab}	8.09^{ab}	7.51^{b}	7.87^{ab}	0.186	0.012
Yolk color							
2 wk	7.61^{b}	8.65^{a}	8.77^{a}	8.83^{a}	8.93^{a}	0.201	0.005
4 wk	7.65^{b}	8.50^{a}	8.68^{a}	8.93^{a}	8.69^{a}	0.142	0.003
6 wk	7.53^{b}	7.68^{b}	7.72^{b}	7.68^{b}	8.38^{a}	0.144	0.004
8 wk	7.30	7.19	7.45	7.40	7.79	0.163	0.122
Haugh unit							
2 wk	91.58	90.24	90.01	90.49	89.26	0.791	0.343
4 wk	95.02^{a}	91.93^{ab}	90.40^{b}	91.35^{ab}	91.65^{ab}	0.962	0.011
6 wk	91.69^{ab}	93.51^{a}	90.98^{ab}	91.95^{ab}	88.46^{b}	1.076	0.023
8 wk	90.74^{a}	89.40^{ab}	90.23^{a}	86.30^{b}	89.43^{ab}	0.997	0.024

DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
Means with different superscripts in the same row differ significantly ($P < 0.05$).
[1] Data are expressed as mean and SEM.

the XH3 rapeseed group was 6% lower than that of the control group, meanwhile, the number of F6 that could develop into egg yolk of XH3 and MB1 rapeseed was lower than that of the control group ($P = 0.009$).

4. Discussion

The nutrient profile of rapeseed makes it an ideal ingredient for high nutrient density diets, and it is also a good source of unsaturated fatty acids for poultry products (Drażbo et al., 2019; Du et al., 2017). Research on the use of rapeseed in poultry was mostly published in the 1970s, and very little research has been done in laying hens and the results are inconsistent. Egg production, particularly at the highest level of inclusion of 15% rapeseed was adversely affected as reported by Leslie and Summers (1972), but according to Olomu et al. (1975), 15% span rapeseed did not affect production performance in hens. In our present study, 11.5% rapeseed significantly decreased feed intake, egg mass, feed conversion efficiency, and F6 follicle number, and rapeseed with high Gls and EA content had a lower production performance than rapeseed with low Gls and EA content. As all we know, antinutritional factors such as Gls, sinapine, and their derivatives, tannins, phytic acid, and crude fiber have an adverse effect on animals (Yadav et al., 2022). Because the egg production performance was different between different kinds of rapeseed in this study, we postulated that the inconsistencies between the various studies might be due to the different antinutritional factor content in rapeseed varieties.

In the current study, during the last 4 wk (9–12 wk) for recovery, there was no difference in ADFI and egg laying-rate, but egg weight, egg mass, and feed conversion efficiency of rapeseed were still lower than that of the control group. Some studies have found that the level of antinutritional factors in the blood return to normal following withdrawal of rapeseed supplementation, facilitating a recovery in production performance (Mena et al., 2004; Zeng et al., 2014). Our previous study also showed that the egg production performance, some serum parameters, and organ weight recovered after 4 wk withdrawal of 29.4% rapeseed meal (Zhu et al., 2018). In this study, 4 wk withdrawal of 11.5% rapeseed did not help to recover egg weight and FCR. This might indicate that the organs of laying hens in this study were irreversibly damaged and need additional nutrients to for repair, or that it will take a longer time for egg performance to recover.

Egg quality ss a crucial factor affecting the economic benefits of the poultry industry. Eggshell color affects consumer preference (Samiullah et al., 2015), and research shows that preferences for eggshell color and yolk color vary by geographic region, with darker colors preferred in Northeast China (Chen et al., 2023). In present study, the 11.5% rapeseed diet increased a* value of eggshell and decreased L* value. This result showed that the eggshell color was darker and redder after feeding rapeseed. Research has indicated that feed source affects eggshell color (Mori et al., 2020) and that the pigmentation derived from rapeseed might induce darker and redder eggshell color for eggs. Although the eggshell thickness of DY6 was a little lower than the control and other rapeseed variety groups, there was no difference in eggshell strength. Interestingly, all rapeseed groups had a higher yolk color before six weeks, but there was no difference in wk 8 among groups. The darker color of egg yolk might be caused by pigments in rapeseed itself, but as the experimental time increased, the digestion and absorption of nutrients might have been affected by high EA content in the diet, so the egg yolk color decreased. This was in line with a previous study, in which Yuan et al. (2019) indicated that high EA rapeseed oil decreased yolk color. The lower albumen height and Haugh unit in the rapeseed groups might be caused by the reduced dry matter digestibility induced by the high content of Gls, EA, or crude fiber in rapeseed (Zhu et al., 2019).

Table 7
Effects of different varieties of rapeseed on eggshell quality of laying hens.[1]

Item	Treatment					SEM	*P*-value
	Control	DY6	MB1	DY5	XH3		
Eggshell strength, kg/cm^2							
2 wk	3.59	3.34	3.36	3.59	3.61	0.112	0.230
4 wk	5.44	5.19	5.12	5.58	5.41	0.214	0.501
6 wk	5.26	5.14	5.35	5.47	5.05	0.221	0.682
8 wk	4.84	4.54	5.36	5.11	5.15	0.203	0.053
Eggshell thickness, mm							
2 wk	0.39	0.38	0.38	0.39	0.38	0.006	0.414
4 wk	0.35	0.35	0.33	0.35	0.35	0.006	0.192
6 wk	0.35	0.33	0.34	0.34	0.34	0.006	0.231
8 wk	0.34^{a}	0.32^{b}	0.34^{a}	0.34^{a}	0.34^{a}	0.004	0.009

DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
Means with different superscripts in the same row differ significantly ($P < 0.05$).
[1] Data are expressed as mean and SEM.

Table 9
Effects of different varieties of rapeseed on serum organ function parameters of laying hens (wk 8 of the trial).[1]

Item	Treatment					SEM	*P*-value
	Control	DY6	MB1	DY5	XH3		
ALT, U/L	5.96	5.83	5.87	3.93	5.19	0.633	0.141
AST, U/L	161.87	165.47	161.03	169.68	196.91	10.061	0.091
CREA, μmol/L	48.17	62.06	57.62	70.72	73.20	9.423	0.352
Urea, μmol/L	0.46	0.57	0.51	0.48	0.48	0.043	0.253
T3, ng/dL	52.85	61.12	70.93	64.64	68.58	6.182	0.284
T4, ng/L	1013.72	810.66	1196.71	774.63	939.60	133.671	0.192
T3/T4	0.062	0.088	0.069	0.093	0.081	0.0144	0.301
E2, ng/L	61.15^{a}	30.59^{b}	34.00^{b}	31.34^{b}	17.84^{b}	4.286	0.003
P4, μg/L	1685.25	1145.11	1728.8	1416.58	1827.75	171.336	0.062

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CREA = creatinine; T3 = triiodothyronine; T4 = tetraiodothyronine; E2 = estradiol; P4 = progesterone; DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
Means with different superscripts in the same row differ significantly ($P < 0.05$).
[1] Data are expressed as mean and SEM.

Table 10
Effects of different varieties of rapeseed on organ index and follicle number of laying hens (wk 8 of the trial).[1]

Item	Treatment					SEM	*P*-value
	Control	DY6	MB1	DY5	XH3		
Thyroid index, $\times 10^{-5}$	12.50^{c}	13.00^{bc}	15.00^{bc}	19.90^{ab}	23.30^{a}	1.703	0.008
Liver index, %	2.04	1.93	1.83	2.00	1.86	0.094	0.412
Kidney index, %	0.77^{ab}	0.72^{b}	0.75^{ab}	0.86^{a}	0.83^{ab}	0.032	0.034
F6	5^{a}	4^{ab}	3^{b}	4^{ab}	3^{b}	0.4	0.009
SYF	5	5	3	4	2	1.2	0.343
LWF	15	11	14	15	11	2.2	0.612
SWF	25	30	29	31	29	3.1	0.543
Total follicles	50	50	52	54	47	4.2	0.812

F6 = the smallest hierarchical follicle; SYF = small white follicles; LWF = large white follicles; SWF = small yellow follicles; DY5 = Deyou no. 5 rapeseed; DY6 = Deyou no. 6 rapeseed; MB1 = Mianbangyou no. 1 rapeseed; XH3 = Xiheyou no. 3 rapeseed.
Means with different superscripts in the same row differ significantly ($P < 0.05$).
[1] Data are expressed as mean and SEM.

Blood biochemical indices are often used to reflect changes in metabolism and organ function (Zhang et al., 2021). Thyroid hormone regulates metabolic processes essential for normal growth and development (Mullur et al., 2014). It is well established that thyroid hormone status correlates with growth and energy expenditure (Mullur et al., 2014). Gls metabolites impair the thyroid uptake of iodide, its oxidation, iodine binding to thyroglobulin, synthesis, and the release of hormones, which then cause thyroid hyperplasia and goiter (Schöne et al., 1993). In this study, T4 content in serum of high Gls and EA rapeseed was a little lower than the control group, and the thyroid index increased gradually with the increase in Gls content. This was in line with a previous study in which Gls linearly increased thyroid index (Zhu et al., 2018). The rapeseed diets decreased serum E2 content, and this was in line with previous research (Mandiki et al., 2002). Gls metabolites have been proven to act as antiestrogens, blocking estradiol-induced cell proliferation (Ju et al., 2000). This might affect ovarian function, which in turn affects hormone production. Because estrogen regulates follicle development (Fortune 1994), this was the reason why the F6 number and egg laying rate decreased.

5. Conclusion

In summary, regardless of the level of Gls and EA content in rapeseed in the present study, Gls and EA were found to have an adverse effect on egg production performance. There was a difference in egg production performance between rapeseed varieties, and egg laying rate and feed conversion efficiency of high Gls and EA content rapeseed were lower than that of low Gls and EA content rapeseed. Although a rapeseed diet resulted in darker eggshell color, it could decrease internal egg quality as indicated by albumen height and Haugh unit. The negative effects of rapeseed in the diet might be due to its impact on hormone levels, follicle development, and thyroid function. A 4-wk withdrawal period of 11.5% rapeseed was not effective in recovering the egg weight and FCR. It is therefore recommended that when raw and whole rapeseed is used as a feed material for laying hens, the addition amount should not be too high, and rapeseed with a low content of antinutritional factors should be used.

CRediT authorship contribution statement

Liping Zhu: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. **Jianping Wang:** Conceptualization, Formal analysis, Writing – original draft. **Xuemei Ding:** Conceptualization, Data curation, Methodology. **Shiping Bai:** Conceptualization, Data curation, Methodology. **Qiufeng Zeng:** Conceptualization, Data curation. **Yue Xuan:** Investigation. **Keying Zhang:** Conceptualization, Data curation, Investigation, Formal analysis, Resources, Project administration, Writing – review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This project was supported by National Science and Technology Project (2014BAD13B04), the earmarked fund for Modern Agro-Industry Technology Research System and Sichuan Provincial Science and Technology Project (2014NZ0043, 2014NZ0002).

References

Ajuyah AO, Hardin RT, Sim JS. Studies on canola seed in Turkey grower diet: effects on ω-3 fatty acid composition of breast meat, breast skin and selected organs. Can J Anim Sci 1993;73(1):177–81.

China Feed Database. Tables of feed composition and nutritive values in China (in Chinese). 2014. https://www.chinafeeddata.org.cn/admin/Login/slcfb. [Accessed 18 March 2016].

China National Standard. Determination of crude fat in feeds (GB/T 6433-2006). Beijing, China: Standards Press of China; 2006.

China National Standard. Determination of moisture in feedstuffs (GB/T 6435-2018). Beijing, China: Standards Press of China; 2018.

China National Standard. Determination of the content of fatty acids in feeds (GB/T 21514-2008). Beijing, China: Standards Press of China; 2008.

China National Standard. Determination of total nitrogen content in animal feeding stuffs by combustion according to the Dumas principle and calculation of the crude protein content (GB/T 24318-2009). Beijing, China: Standards Press of China; 2009.

China National Standard. Feeding stuffs-Determination of crude fiber content (GB/T 6434-2022). Beijing, China: Standards Press of China; 2022.

Chen R, Jiang C, Li X, Shi X, Zhuang L, Zhou W, et al. Research on Chinese consumers' shell egg consumption preferences and the egg quality of functional eggs. Poultry Sci 2023;102(10):103007.

Chmielewska A, Kozowska M, Rachwa D, Wnukowski P, Rosicka-Kaczmarek J. Canola/rapeseed protein–nutritional value, functionality and food application: a review. Crit Rev Food Sci 2021;61(22):3836–56.

Drażbo A, Kozłowski K, Ognik K, Zaworska A, Jankowski J. The effect of raw and fermented rapeseed cake on growth performance, carcass traits, and breast meat quality in Turkey. Poultry Sci 2019;98(11):6161–9.

Du X, Liu Y, Lu L, Wang W, Zeng T, Tian Y, et al. Effects of dietary fats on egg quality and lipid parameters in serum and yolks of Shan Partridge Duck. Poultry Sci 2017;96(5):1184–90.

FAO (Food and Agriculture Organization). FAOSTAT data: rape or colza seed. https://www.fao.org/faostat/zh/#data/QCL. [Accessed 15 May 2021].

Fortune J. Ovarian follicular growth and development in mammals. Biol Reprod 1994;50(2):225–32.

ISO (International Organization for Standardization). Animal feeding stuffs, animal products, and Faeces or urine-determination of gross calorific value: bomb calorimeter method, standard 9831. Geneva, Switzerland: International Organization for Standardization; 1998.

Ju YH, Carlson KE, Sun J, Pathak D, Katzenellenbogen BS, Katzenellenbogen JA, et al. Estrogenic effects of extracts from cabbage, fermented cabbage, and acidified brussels sprouts on growth and gene expression of estrogen-dependent human breast cancer (MCF-7) cells. J Agric Food Chem 2000;48(10):4628–34.

Leslie AJ, Summers JD. Therapy. Feeding value of rapeseed for laying hens. Pharmacotherapy 1972;52(3):563–6.

Li S, Vestergren AS, Wall H, Trattner S, Pickova J, Ivarsson E. Feeding steam-pelleted rapeseed affects expression of genes involved in hepatic lipid metabolism and fatty acid composition of chicken meat. Poultry Sci 2017;96(8):2965–74.

Lichovnikova M, Zeman L. The effects of a higher amount of iodine supplement on the efficiency of laying hens fed extruded rapeseed and on eggshell quality. Czech J Anim Sci 2004;49(5):199–203.

Mandiki SNM, Derycke G, Bister JA, Mabon N, Wathelet JP, Marlier M, et al. Chemical changes and influences of rapeseed antinutritional factors on gestating and lactating ewes: 1. Animal performances and plasma hormones and glucose. Anim Feed Sci Technol 2002;98(1–2):25–35.

Mena H, Santos JEP, Huber JT, Tarazon M, Calhoun MC. The effects of varying gossypol intake from whole cottonseed and cottonseed meal on lactation and blood parameters in lactating dairy cows. J Dairy Sci 2004;87(8):2506–18.

Ministry of Agriculture of the People's Republic of China. Rapeseed. Determination of glucosinolates content. Method using high-performance liquid chromatography (NY/T 1582-2007). Beijing, China: China Agriculture Press; 2007.

Ministry of Agriculture of the People's Republic of China. Determination of isothiocyanates in rapeseed meal. Thiourea colorimetry method (NY/T 1596-2008). Beijing, China: China Agriculture Press; 2008.

Ministry of Agriculture of the People's Republic of China. Determination of oxazolidinethione in rapeseed meal and feed Spectrometric method (NY/T 1799-2009). Beijing, China: China Agriculture Press; 2009.

Mori H, Takaya M, Nishimura K, Goto T. Breed and feed affect amino acid contents of egg yolk and eggshell color in chickens. Poultry Sci 2020;99(1):172–8.

Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev 2014;2.

Najib H, Al-Khateeb SA. The effect of incorporating different levels of locally produced canola seeds (Brassica napus, L.) in the diet of laying hen. Int J Poultry Sci 2004;3(7):490–6.

NRC. Nutrition requirements of poultry. 9th ed. Washington (DC): National Academy Press; 1994.

Olomu JM, Robblee AR, Clandinin DR, Hardin RT. Evaluation of full-fat Span rapeseed as an energy and protein source for laying hens. Can J Anim Sci 1975;55(2):219–22.

Samiullah S, Roberts JR, Chousalkar K. Eggshell color in brown-egg laying hens-a review. Poultry Sci 2015;94(10):2566–75.

Schöne F, Jahreis G, Richter G, Lange R. Evaluation of rapeseed meals in broiler chicks: effect of iodine supply and glucosinolate degradation by myrosinase or copper. J Sci Food Agric 1993;61(2):245–52.

Yadav S, Teng PY, Choi J, Singh AK, Vaddu S, Thippareddi H, et al. Influence of rapeseed, canola meal and glucosinolate metabolite (AITC) as potential antimicrobials: effects on growth performance, and gut health in Salmonella Typhimurium challenged broiler chickens. Poultry Sci 2022;101(1):101551.

Yuan N, Wang JP, Ding XM, Bai SP, Zeng QF, Su ZW, et al. Effects of supplementation with different rapeseed oil sources and levels on production performance, egg quality, and serum parameters in laying hens. Poultry Sci 2019;98(4):1697–705.

Zeng QF, Yang GL, Liu GN, Wang JP, Bai SP, Ding XM, et al. Effects of dietary gossypol concentration on growth performance, blood profiles, and hepatic histopathology in meat ducks. Poultry Sci 2014;93(8):2000–9.

Zhang Q, Zhang S, Cong G, Zhang Y, Madsen MH, Tan B, et al. Effects of soy protein concentrate in starter phase diet on growth performance, blood biochemical indices, carcass traits, immune organ indices and meat quality of broilers. Animals 2021;11(2):281.

Zhu LP, Wang JP, Ding XM, Bai SP, Zeng QF, Su ZW, et al. The effects of varieties and levels of rapeseed expeller cake on egg production performance, egg quality, nutrient digestibility, and duodenum morphology in laying hens. Poultry Sci 2019;98(10):4942–53.

Zhu LP, Wang JP, Ding XM, Bai SP, Zeng QF, Su ZW, et al. The deposition and elimination of glucosinolate metabolites derived from rapeseed meal in eggs of laying hens. J Agric Food Chem 2018;66(6):1560–8.