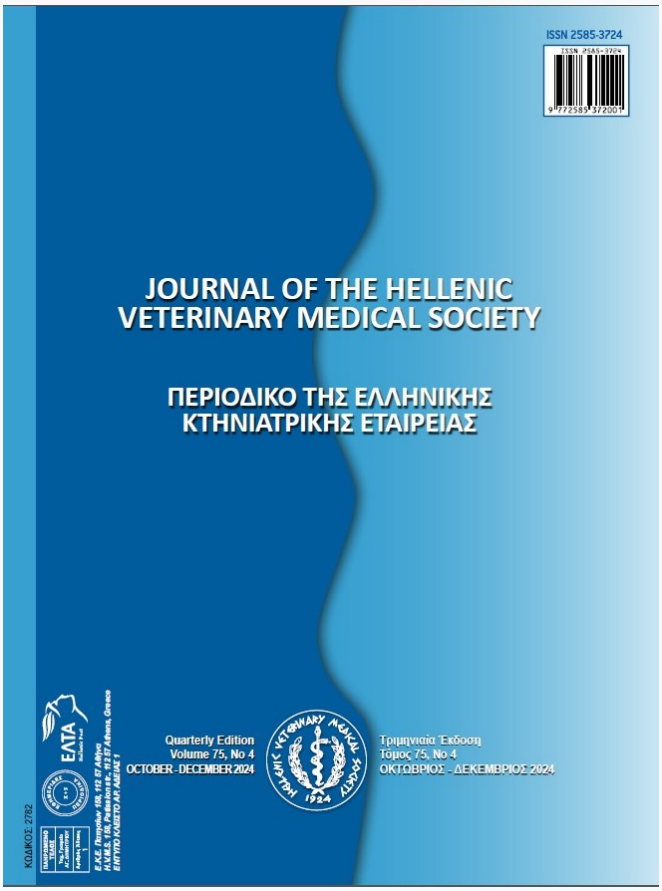
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 4 (2024)



The effect of processing methods of canola seed on growth performance, blood parameters, liver enzymes, and immune status of broiler chickens

H Keyhani, KJ Khorshidi, MA Jafari

doi: 10.12681/jhvms.36756

To cite this article:

Keyhani, H., Khorshidi, K., & Jafari, M. (2025). The effect of processing methods of canola seed on growth performance, blood parameters, liver enzymes, and immune status of broiler chickens. *Journal of the Hellenic Veterinary Medical Society*, *75*(4), 8313–8324. https://doi.org/10.12681/jhvms.36756

J HELLENIC VET MED SOC 2024, 75 (4): 8313-8324
ΠΕΚΕ 2024, 75 (4): 8313-8324

Research article
Ερευνητικό άρθρο

The effect of processing methods of canola seed on growth performance, blood parameters, liver enzymes, and immune status of broiler chickens

H. Keyhani, K.J. Khorshidi, M.A. Jafari

Department of Animal Science, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

ABSTRACT: Canola meal is a protein source for poultry feeding. Canola has anti-nutritional compounds whose effects can be reduced during processing. The research aim is to investigate the processing methods of canola seed by irradiation, roasting, and enzyme methods on the broiler chicken traits. To experiment, 450 day-old broiler chickens of the Ross 308 strain were placed in 30 pens. Treatments included the control (without canola), the raw canola, and 3 treatments of the processed canola (by enzymes, gamma rays, and roasting). The repeat number was 6 for each treatment. The traits were growth performance, blood and immune parameters, liver enzymes, and antibody titers against Bronchitis and Gumboro disease. GLM procedure and Duncan's test (α=0.05) by SAS software were used for data analysis and to compare treatment' means, respectively. The processing methods effect was significant ($P<0.01$) on the protein solubility percentage in KOH, the peroxide number, and the urease activity. Also, the processing methods effect was significant ($P<0.05$) on the feed consumption in different rearing phases, body weight gains in the starter and grower phases, FCR in all rearing phases, the total protein concentration, uric acid, kidney enzymes, and antibody titers. The highest increase in total protein and uric acid was observed in canola seeds processed by gamma ray and enzyme. The enzyme processing method produced the highest antibody titers. In general, the gamma radiation and roasting improved the chicken's traits. The canola seed processing increases the absorption of its nutrients by reducing anti-nutrient compounds. Improving antibody titers against Bronchitis and Gumboro diseases is very effective in health management and herd vaccination, and will reduce related costs. Feed processing can change nutrient requirements by influencing activities related to feed consumption.

Keywords: Broiler; Canola; Enzyme; Gamma ray; Roasting;Processing.

Corresponding Author:
Kaveh Jafari Khorshidi, Qaemshahr Branch, Islamic Azad University- Tabarsi Road- Qaemshahr- Mazandaran- Iran
E-mail address: K.J.Khorshidi@Gmail.com

Date of initial submission: 2-2-2024
Date of acceptance: 19-9-2024

INTRODUCTION

Given the limitations on accessing vegetable protein sources in poultry nutrition, alternative sources like canola meal are necessary. Canola, scientifically known as *Brassica napus*, is a variety of *Eruca sativa*. The high level of unsaturated fats and omega 3 and 6 fatty acids in canola seed is remarkable (Nelda *et al*. 2007). Canola seed contains 25-55% crude fat and 18-24% crude protein (Gharahveysi and Jafari 2021). The use of canola meal in poultry ration is restricted because it contains high levels of fiber and anti-nutritional factors like non-starch polysaccharides (16.5-17.5%), sinapine (6-8%), tannin (0.5-1.5%), phytic acid (2-4%), phenolic compounds (4.5-10%), and glucosinolates (20-27%) (Gharahveysi and Jafari 2021, Khalil *et al.* 2023). Most glucosinolate compounds found in canola include gluconapin, glucobrassicanapine, progoitrin, and glucobrassicin. Tannins are polyphenolic compounds ranging in molecular weight from 500 to 3000 daltons and are categorized into two main groups: hydrolyzable and non-hydrolyzable (dense). The major part of tannin in the canola seed shell is the dense type (70-96% of total tannin) known as cyaniding (Yadav *et al*. 2022). In addition to darkening the feed color, tannin forms an indigestible complex with proteins and protein-digesting enzymes in the digestive system and severely disrupts protein digestion and absorption (Yasothai 2016, Yadav *et al*. 2022). Phytic acid or inositol polyphosphate is the main form of phosphorus storage in most seeds. The formation of this insoluble complex makes the phosphorous unavailable. About 35-70% of canola phosphorus exists as phytic acid (Agyekum and Woyengo, 2022). Sinapine is the choline ester of sinapic acid, constituting around 1% of canola meal. It leads to a fishy odor, particularly noticeable in brown-shelled eggs, attributed to the presence of a sinapine metabolite in egg yolk known as tri-methylamine (Niu et al. 2022).

To decrease anti-nutritional compounds, feed compounds may undergo different processing techniques before being used in feed. Various methods are available to minimize or remove the impact of anti-nutritional components in canola. Feed processing encompasses all procedures conducted before feeding the animals (Gharahveysi and Jafari 2021). Some of these methods include roasting, irradiation, micronizing, expansion, and extrusion. The two main effects of processing are 1) changes in the micro and macro structure of the feed, and 2) changes in some of the feed compounds. These changes can significantly impact the function of the bird's digestive system (Gharahveysi and Jafari 2021, Inglis *et al.* 2021). The use of enzymes in the ration containing canola meal improves the digestibility of non-starch polysaccharides, soluble polysaccharides, and oligosaccharides. About 60-70% of the phosphorus in canola meal is bound to phytic acid. So, the combination of phytic acid with proteins, solutes, and starch reduces phosphorus absorbability. Phytase enzymes facilitate the gradual release of phosphate from phytic acid, making phosphorus available for utilization (Niu *et al.* 2022).

Researchers have confirmed the positive impacts of microwave radiation (Sadeghi and Shawrang 2006). It is understood that the chemical composition of canola seeds is altered by microwave radiation, impacting their digestibility as well (Sadeghi and Shawrang 2006). Processing by gamma rays and microwaves results in the reduction of phytic acid and glucosinolates, a constant rate of decomposition, and the effective degradability of dry matter and raw protein of native *Eruca sativa* seeds (Yadav *et al.* 2022). In a study, canola seed processing by gamma radiation at doses of 10, 20, and 30 kGy led to the reduction of the anti-nutritional substances such as phytic acid and glucosinolate (about 10%), weight gain (about 5%), and FCR improvement (about 0.10) (Siddhuraju *et al.* 2002).

Due to the limited information and scientific references available, the study aims to investigate the processing methods of canola seed by irradiation, roasting, and enzyme methods on the broiler chicken traits.

MATERIALS AND METHODS

The research conditions

The research was conducted between July and September 2022. The poultry research farm of the agriculture faculty at Qaemshahr Islamic Azad University was used for the study. The current study was conducted taking into account the procedures of sample collection, management, and the ethical, scientific, and administrative standards for animal research outlined in the laws of the National Committee for Ethics in Biomedical Research of Iran (2018).

Rearing management

Before rearing, the farm was carefully disinfected by the flamethrower and formalin gas. The dimensions of each pen were 1.5×1.5 m^2. The farm had thermometers and ventilation regulators to manage temperature. Thermometers were placed in the rearing area to

control temperature, which started at 32°C, gradually dropping to 23°C. Each pen had its own feed and water trough. Lighting was provided for 23 hours during the rearing phase

Vaccination schedule

The vaccination program was developed based on guidelines for rearing Ross 308 chickens and local farm conditions (such as existing microbes, chicken health, and feed quality). The vaccines administered included: bronchitis vaccine (eye drops) at 1-day old, influenza and Newcastle Lasota vaccines (via drinking) at 8 days old, bronchitis vaccine (eye drops) at 13 days old, Gambro vaccine (via drinking) at 14 days old, Newcastle Lasota vaccine (via drinking) at 17 days old, bronchitis vaccine (via drinking) at 24 days old, and Newcastle Lasota vaccine (via drinking) at 28 days old

Canola seed processing

Canola seed processing with electron (gamma) rays at a 100 kGy dose was carried out using a Rhodotron system (Model TT-2200, IBA Co., Belgium). Gamma radiation was conducted by the gamma cell radiation system at the Yazd radiation center, utilizing cobalt 60 gamma rays affiliated with the Iranian Atomic Energy Organization (Gharaghani *et al*. 2008). Canola seed processing was done by a roasting technique using direct dry heat inside a cylinder. This exposed the seeds to hot air (190 to 200 °C) for 18 to 20 minutes. By the end of this process, the seed temperature reached 144-146 °C. Subsequently, the seeds were cooled by transferring them to the ventilation section (Maclsaac et al. 2005). The enzyme utilized in this treatment was Avizyme® 1502, which was used to process the canola seeds (Bampidis *et al.* 2020). Avizyme® 1502 contained protease, amylase, and xylanase in effective amounts. This enzyme product by Biochem is imported and distributed in Iran by its exclusive representative, Arya Dalman Company. Avizyme® is coated and can withstand temperatures up to 90°C. Specifically designed for feed rations with corn and soy. Its inclusion level was 500 grams per ton of feed (Bampidis *et al.* 2020)

Chickens, and experimental treatments

For the experiment, 450 Ross 308 strain broiler chickens aged one day were allocated into 30 pens. The study treatments included 1) a control group (no canola seeds), 2) raw canola seeds, 3) enzyme-processed canola seeds, 4) gamma-ray-processed canola seeds, and 5) roasted canola seeds. The repeat number was 6 for each treatment. Treatments were randomly assigned to the pens, with 15 birds in each pen

Experimental rations: The experimental rations were formulated using UFFDA software (Pesti *et al.* 1992) based on the nutritional requirements catalog of chicken broilers of the Ross 308 strain. The same metabolic energy and crude protein were considered for all experimental rations (Table 1). Rations were divided into three phases including the starter (1-11 days old), grower (12-24 days old), and finisher (25-42 days old)

Chemical composition determination of canola seed: Canola seed samples were placed in 65×35 cm^2 polyethylene bags, with two kg in each. These samples were then analyzed for chemical compounds at the specialized laboratory of Qaemshahr Islamic Azad University

In the laboratory, the chemical composition of canola seeds was measured. They included the dry matter, ether extract, crude protein, crude fiber and ash (AOAC 1997), urease activity (AOCS 1998), protein solubility index in potassium hydroxide (AOCS 1998), and peroxide index (AOCS 1998)

Traits measured: From the beginning of the experiment's first week, experimental rations for each pen were poured into numbered buckets. At the end of each phase, the remaining feed of each pen was weighed. Then, the feed consumed in each phase was measured for each of the pens by deducting the feed remaining amount from the total amount of feed. So, the daily feed consumption was calculated for the starter, grower, finisher, and the whole phase (1 to 42 days old). At the end of each phase, the chickens of each pen were weighed. To weigh the chickens, feeding was stopped two hours before weighing. The dead chicks were collected and recorded daily. Daily weight gain was calculated for the starter, grower, finisher, and whole phase (1 to 42 days old). The FCR was calculated by dividing the amount of feed consumed of each pen by the weight gain of the same pen. Therefore, FCR was calculated for the starter, grower, finisher, and whole phase (1 to 42 days). Blood sampling was done at the end of the experiment (day 42)

Two chickens were randomly selected from each pen, and blood was taken from their wing veins. Blood samples were immediately collected with test tubes containing anticoagulants. Then, blood samples

Table 1 Experimental rations in different phases and treatments of the research

Rearing phases						
Starter phase		**Grower phase**		**Finisher phase**		
Treatments contain the raw and processed canola	Control (without canola)	Treatments contain the raw and processed canola	Control (without canola)	Treatments contain the raw and processed canola	Control (without canola)	**Ingredients**(%)
52.95	52	54	52.20	53.60	53.50	Corn (8% CP[1])
2.40	2	1.90	2	0	0	Corn gluten meal (60% CP)
34	27	31	27	28	19.30	Soybean meal (43% CP)
0	10	0	10	0	10	Canola seed (19% CP)
3	3	5	3	10	10	Wheat (12% CP)
1.60	0	2.35	0	3	1.60	Oil
1.90	1.90	1.90	1.90	1.80	1.90	Di-calcium phosphate[2]
1.40	1.40	1.40	1.40	1.40	1.40	Calcium carbonate
0.25	0.25	0.20	0.23	0.10	0.10	Salt
1	1	1	1	1	1	Bentonite
0.50	0.50	0.40	0.40	0.30	0.30	Vitamin premix[3]
0.50	0.50	0.40	0.40	0.30	0.30	Mineral premix[4]
0.20	0.17	0.15	0.17	0.17	0.20	DL Methionine
0.25	0.23	0.25	0.25	0.28	0.30	Lysine sulfate
0.05	0.05	0.05	0.05	0.05	0.10	L Threonine
chemical	*Composition*					*Calculated*
2860	2860	2920	2940	3000	3000	Metabolizable energy (kcal/kg[5])
20.40	20.60	19	19	17	17	Crude protein (%)
2.53	4.27	2.52	4.27	2.48	4.29	Crude fiber (%)
3.70	3.20	3.50	3	3.5	2.8	Crude fat (%)
1.06	1.04	1.06	1.04	1.06	1.02	Calcium (%)
0.46	0.44	0.45	0.44	0.43	0.42	Available phosphorus (%)
0.18	0.16	0.16	0.17	0.16	0.16	Sodium (%)
1.37	2.19	1.37	2.20	1.34	2.20	Linoleic acid (%)
1.35	1.38	1.26	1.34	1.18	1.17	Arginine[6] (%)
1.26	1.26	1.18	1.23	1.21	1.11	Lysine (%)
0.56	0.52	0.48	0.48	0.46	0.45	DL Methionine (%)
0.89	0.86	0.80	0.80	0.75	0.75	DL Methionine + Cysteine (%)
0.86	0.87	0.81	0.80	0.72	0.70	Threonine (%)
0.25	0.26	0.24	0.24	0.20	0.21	Tryptophan (%)

1. Crude protein- 2. Di-calcium phosphate contained: 16% phosphorous and 23% calcium- 3. Vitamin premix (per kg of ration); Vitamin A: 14000 IU, Vitamin D3: 5000 IU, Vitamin E: 50 IU, Vitamin k3: 4 mg, Thiamine: 3 mg, Riboflavin: 8 mg, Pantothenic acid: 20 mg, Pyridoxine: 4 mg, Cyanocobalamin: 0.016 mg, Niacin: 730 mg, Biotin: 0.02 mg, Folic acid: 1.75 mg and Choline chloride: 1.60 g- 4. Mineral premix (per kg of ration); Fe: 50.00 mg, Mn: 100.00 mg, Zn: 100.00 mg, Cu: 10.00 mg, I: 1.00 mg and Se: 0.20 mg- 5. Kilocalorie per kilogram- 6. On a digestible basis

were sent to the specialized laboratory. In the laboratory, blood parameters, immune parameters, and liver enzymes were measured using the Iranian Pars Azmoun kits and autoanalyzer (RA1000). The measured parameters were uric acid, glucose, albumin, cholesterol, triglyceride, total protein, lymphocyte, heterophile, alkaline phosphatase (ALP), alanine transferase (ALT), and aspartate aminotransferase (AST). Antibody titers against bronchitis and Gumboro diseases were determined by ELISA serological test (using

IDEXX kit)

Statistical analysis: This research was conducted in the form of a completely randomized design using 5 treatments, and 6 repeats. The statistical model used was as follows

$$yij = \mu + Ai + eij$$

which: yij, the value of each observation; μ, the mean effect; Ai, the effect of each treatment and eij, the random effect of the experimental error (residual). GLM procedure and Duncan's test (α= 0.05) by SAS software (2004) were used to study the effect of experimental treatments on different traits and compare the treatment means, respectively

RESULTS

Chemical composition determination of canola seed: The results of the chemical composition of canola seed and its quality indicators are presented in Table 2. The effect of canola seed processing methods was significant on KOH solubility percentage, peroxide value, and urease activity (P<0.01). The highest and lowest percentages of solubility in KOH were in the control treatment (91.55%) and canola seed treatment processed by roasting (82.35%), respectively. The highest and lowest peroxide values were in the canola seed treatment processed by roasting (4.45 kg/mE) and the control treatment (2.94 kg/mE), respectively. The highest and lowest urease activity was observed in raw canola seed treatment (1.88) and canola seed treatment processed by roasting (1.05), respectively. The solubility percentage in KOH was significantly higher (p<0.01) in the control treatment compared to the treatments with enzyme-processed canola and gamma-ray-processed canola. Peroxide levels were significantly higher (p<0.01) in the gamma-ray processed canola compared to treatments with raw canola and the control treatment. Additionally, Urease activity was significantly higher (p<0.01) in the raw canola compared to treatments with raw canola, enzyme-processed canola, and gamma-ray-processed canola

Feed consumption: According to Table 3, it can be seen that the effect of different canola seed processing methods on feed consumption in different rearing phases is significant (P<0.05). In the starter (1-11 days old), the highest and lowest feed consumption was observed in the control treatment (279.28 g) and the treatment containing raw canola seeds (272.78 g), respectively. In the grower (12-24 days old), the highest and lowest feed consumption is related to the processed treatment by gamma radiation (931.20 g) and the control treatment (837.16 g), respectively. In the finisher (25-42 days old), the highest and lowest feed consumption is related to the control treatment (2902.80 g) and the treatment containing raw canola seeds (2643.60 g), respectively. In the whole phase, the highest and lowest feed consumption was related to the control treatment (4019.24 g) and the treatment containing raw canola seeds (3760.65 g), respectively. Feed consumption was significantly higher (p<0.05) in the control treatment compared to treatments with raw, enzyme-processed, and roasted canola during the starter phase. In the grower phase, gamma-ray processed canola showed significantly higher (p<0.05) feed consumption compared to other treatments. For

Table 2 The effect of canola seed processing methods on the chemical composition, and quality indicators of canola seed

Indicators Quality				(%) **Compositions**	**Chemical**			
Urease activity (pH difference)	Peroxide (kg/mE)	Solubility in KOH (%)	Crude fat	Ash	Crude fiber	Crude protein	Dry matter	**Treatments\Traits**
1.85^{a}	2.94^{c}	91.55^{a}	48.35	2.47	9.10	19.42	91.82	Control
1.88^{a}	3.39^{b}	90.63^{ab}	48.27	2.36	9.22	19.43	91.72	Containing the raw canola
1.58^{b}	3.91^{ab}	88.81^{b}	48.46	2.50	9.37	19.54	91.40	Containing the enzyme processed canola
1.07^{c}	4.26^{a}	84.51^{bc}	48.48	2.35	9.32	19.46	91.46	Containing the gamma ray processed canola
1.05^{c}	4.45^{a}	82.35^{c}	48.37	2.45	9.28	19.50	91.52	Containing the roasted canola
0.04	0.08	0.337	0.09	0.05	0.06	0.07	0.14	SEM
0.01	0.01	0.01	0.82	0.55	0.62	0.94	0.58	P.value

SEM: Standard error of the mean. Non common superscripts in each column indicate the significant statistical difference (p<0.05).

Table 3 The effect of canola seed processing methods on the feed consumption of broiler chickens in rearing phases (g)

Whole phase	Finisher	Grower	Starter	Treatments\Traits
4019.24^{a}	2902.80^{a}	837.16^{b}	279.28^{a}	Control
3760.65^{b}	2643.60^{b}	844.27^{b}	272.78^{b}	Containing the raw canola
3889.60^{ab}	2738.00^{ab}	877.40^{b}	274.20^{b}	Containing the enzyme processed canola
3930.82^{ab}	2721.60^{ab}	931.20^{a}	278.02^{ab}	Containing the gamma ray processed canola
3778.90^{b}	2659.00^{ab}	846.54^{b}	273.36^{b}	Containing the roasted canola
22.67	23.63	11.69	0.86	SEM
0.03	0.02	0.04	0.04	P.value

SEM: Standard error of the mean. Non common superscripts in each column indicate the significant statistical difference (p<0.05).

Table 4 The effect of canola seed processing methods on the weight gain of broiler chickens in rearing phases(g)

Whole phase	Finisher	Grower	Starter	Treatments\Traits
2047.88	1345.80	539.88^{b}	217.32^{b}	Control
2069.06	1334.67	572.54^{a}	216.60^{b}	Containing the raw canola
2094.52	1365.60	572.73^{a}	211.86^{b}	Containing the enzyme processed canola
2057.22	1323.00	565.62^{a}	224.38^{a}	Containing the gamma ray processed canola
2057.64	1341.60	551.86^{a}	219.74^{a}	Containing the roasted canola
16.24	18.59	8.22	2.07	SEM
0.90	0.96	0.03	0.04	P.value

SEM: Standard error of the mean. Non common superscripts in each column indicate the significant statistical difference (p<0.05).

the finisher phase, feed consumption was significantly higher (p<0.05) in the control treatment compared to treatment with raw canola. Throughout all phases, feed consumption was significantly higher (p<0.05) in the control treatment compared to treatments with raw and roasted canola.

Weight Gain: The results of investigating the effects of different canola seed processing methods on broiler body weight gain are presented in Table 4. In the starter and grower phases, the effect of different canola seed processing methods on body weight gain was significant (P<0.05). In the starter (1-11 days old), the highest and lowest weight gain was related to the treatment containing canola seed processed by gamma rays (224.38 g) and the treatment containing canola seed processed by the enzyme (211.86 g), respectively. The highest and lowest weight gain during the grower phase was observed in the treatment containing canola seeds processed by the enzyme (572.73 g) and the control treatment (539.88 g), respectively. Weight gain was significantly higher (p<0.05) in gamma-ray-processed canola compared to treatments with raw and enzyme-processed canola during the starter phase. In the grower phase, weight gain was significantly higher (p<0.05) in all treatments containing canola compared to the control treatment. The canola seed processing by gamma radiation in the starter compared to other processing methods caused an improvement in weight gain by reducing the negative effects of raw canola seed consumption. During the grower phase, canola seed processing, especially the enzyme method, improved the weight gain of broilers (Table 4)

FCR: As can be seen in Table 5, the effect of different canola seed processing methods on the FCR of broilers was significant in all rearing phases (P<0.05). In the starter (1-11 days), the lowest FCR was observed in treatments containing canola seeds processed by gamma rays and roasting methods (1.24), and the highest FCR was observed in the treatment with enzyme processing (1.29). In the grower (12-24 days), the lowest and highest FCR was observed in treatments containing raw canola seeds (1.48) and treatments processed by gamma rays (1.64), respectively. In the finisher (25-42 days), the lowest and highest FCR was observed in the treatments containing raw canola seeds (1.98) and the control treatment (2.15), respectively. In the whole phase, the lowest and highest FCR was observed in the treatment containing raw canola seeds (1.81) and the control treatment (1.96), respectively. FCR was significantly lowest (p<0.05) in all treatments compared to those with enzyme-processed canola during the starter phase. In the grower phase, FCR was significantly lowest (p<0.05) in raw and enzyme-processed canola compared to treatment with gamma-ray-processed canola.

In the finisher phase, FCR was significantly lowest ($p<0.05$) in all treatments compared to those with the control treatment. Additionally, FCR was significantly lowest ($p<0.05$) in raw and enzyme-processed canola compared to the control treatment during the whole phase. By studying the results of the research, it can be seen that in the starter, the presence of canola seeds processed by gamma-ray and roasting methods improved the FCR in broiler chickens ($P<0.05$)

Blood parameters: The results of investigating the effects of canola seed processing methods on the blood parameters of broiler chickens are presented in Table 6. The effect of processing methods on the concentration of total protein, uric acid, AST, ALT and ALP was significant ($P<0.05$). The highest and lowest concentrations of total protein were observed in the treatment containing canola seed processed by gamma rays (4.22 mg/dl) and the treatment containing roasted canola seed (3.70 mg/dl), respectively. The highest and lowest concentrations of uric acid were observed in the treatment containing canola seeds processed by enzyme (5.62 mg/dl) and control treatment (3.78 mg/dl), respectively. Also, the highest and lowest concentrations of AST were observed in the treatment containing canola seed processed by enzyme (219.00 U/L) and the treatment containing canola seed processed by gamma radiation (181.80 U/L), respectively. On the other hand, the highest and lowest ALT concentrations were observed in the treatment containing raw canola seeds (17.90 U/L) and the control treatment (11.01 U/L), respectively. The highest and lowest concentrations of ALP were found in the treatment containing roasted canola seeds (71.80 U/L) and the treatment containing raw canola seeds (49.40 U/L), respectively. Total protein was significantly higher ($p<0.05$) in gamma-ray-processed canola compared to the others. Uric acid was significantly

Table 5 The effect of canola seed processing methods on the feed conversion ratio of broiler chickens in rearing phases

Whole phase	Finisher	Grower	Starter	Treatments\Traits
1.96^{a}	2.15^{a}	1.55^{ab}	1.28^{b}	Control
1.81^{b}	1.98^{b}	1.48^{b}	1.26^{b}	Containing the raw canola
1.85^{b}	2.00^{b}	1.53^{b}	1.29^{a}	Containing the enzyme processed canola
1.91^{ab}	2.06^{b}	1.64^{a}	1.24^{b}	Containing the gamma ray processed canola
1.84^{ab}	1.99^{b}	1.54^{ab}	1.24^{b}	Containing the roasted canola
0.02	0.02	0.02	0.01	SEM
0.02	0.02	0.03	0.04	P.value

SEM: Standard error of the mean. Non common superscripts in each column indicate the significant statistical difference ($p<0.05$).

Table 6 The effect of canola seed processing methods on blood parameters, and liver enzymes

ALP (U/L)	ALT (U/L)	AST (U/L)	Uric acid (mg/dl)	Albumin (mg/dl)	Total protein (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	Treatments\ Traits
52.60^{b}	11.01^{b}	195.20^{ab}	3.78^{b}	1.25	3.83^{b}	81.62	105.88	198.60	Control
49.40^{b}	17.90^{a}	194.00^{ab}	4.87^{ab}	1.25	3.75^{b}	79.95	104.76	187.20	Containing the raw canola
63.40^{ab}	14.89^{ab}	219.00^{a}	5.62^{a}	1.33	3.72^{b}	79.22	105.96	190.40	Containing the enzyme processed canola
59.80^{ab}	15.20^{ab}	181.80^{b}	5.02^{ab}	1.30	4.22^{a}	80.78	105.92	197.20	Containing the gamma ray processed canola
71.80^{a}	14.80^{ab}	181.90^{b}	5.16^{ab}	1.20	3.70^{b}	82.70	106.14	194.20	Containing the roasted canola
3.32	0.51	4.67	0.17	0.04	0.11	4.71	2.70	4.58	SEM
0.03	0.01	0.04	0.04	0.85	0.04	0.90	0.72	0.83	P.value

SEM: Standard error of the mean. Non common superscripts in each column indicate the significant statistical difference ($p<0.05$).
AST: Aspartate aminotransferase. ALT: Alanine transferase. ALP: Alkaline phosphatase.

higher ($p<0.05$) in enzyme-processed canola compared to the control treatment. AST was significantly higher ($p<0.05$) in enzyme-processed canola compared to treatments with gamma-ray processed and roasted canola. ALT was significantly higher ($p<0.01$) in raw canola compared to treatments with the control treatment. Additionally, ALP was significantly higher ($p<0.05$) in roasted canola compared to raw canola and control treatment. By studying the results of blood parameters in the present research, it can be seen that the treatment of canola seeds processed by the gamma-ray method increased the total protein (as an immune index). Also, the use of gamma-ray processing caused a significant decrease in the level of liver enzymes in broiler chickens

Antibody titers, and Immune parameters: As can be seen in Table 7, the effect of processing methods on the antibody titer against bronchitis and Gumboro diseases and Immune parameters were significant ($P<0.05$). The highest and lowest heterophile percentages were observed in the treatment containing canola seeds processed by gamma rays (20.20) and the control treatment (15.30), respectively. The highest and lowest percentages of lymphocytes were observed in the treatment containing roasted canola seeds (44.80) and the control treatment (40.00), respectively. Also, the highest and lowest ratio of heterophil to lymphocyte was found in the treatment containing canola seed processed by gamma radiation (0.50) and the control treatment (0.38), respectively. The highest and lowest antibody titers against Bronchitis were in the treatment containing canola seeds processed by enzyme (1739.40) and the treatment containing roasted canola seeds (1385.40), respectively. The highest and lowest antibody titers against Gumboro were observed in the treatment containing canola seed processed by enzyme (2676.00) and canola seed processed by gamma radiation (2286.40), respectively. The heterophile percentage was significantly higher ($p<0.05$) in gamma-ray-processed canola compared to the control treatment. The lymphocyte percentage was significantly higher ($p<0.05$) in roasted canola compared to gamma-ray processed canola and control treatment. The heterophile to lymphocytes ratio (H/L) was significantly higher ($p<0.05$) in gamma-ray processed canola compared to the control treatment and roasted canola. The antibody titer for Bronchitis was significantly higher ($p<0.05$) in raw and enzyme-processed canola compared to others. Also, the antibody titer for Gumboro was significantly higher ($p<0.05$) in enzyme-processed canola compared to others

DISCUSSION

Chemical composition determination of canola seed: Biological methods for determining the quality of processed canola are time-consuming and expensive. Therefore, various practical and reliable methods have been proposed to determine the quality of processed canola in laboratory conditions, among them tests of urease activity index, protein solubility in potassium hydroxide, and protein differentiation index. They are more reliable and common than other methods (Monari 1996). As seen in Table 2, roasting reduced the KOH solubility percentage index and improved the urease activity. To determine whether canola seeds have been overheated or not, there are suitable methods, one of which is protein solubility in potassium hydroxide. The amount of protein solubility in potassium hydroxide is used to identify canola

Table 7 The effect of canola seed processing methods on blood parameters, and the antibody titers

titer	Antibody		parameters	Blood	
Gumboro	Bronchitis	H/L	Lymphocytes (%)	Heterophile (%)	**Treatments\Traits**
2421.20^{b}	1408.40^{b}	0.38^{b}	40.00^{b}	15.30^{b}	Control
2290.80^{b}	1690.20^{a}	0.45^{ab}	41.90^{ab}	18.80^{ab}	Containing the raw canola
2676.00^{a}	1739.40^{a}	0.42^{ab}	42.20^{ab}	17.80^{ab}	Containing the enzyme processed canola
2286.40^{b}	1398.80^{b}	0.50^{a}	40.80^{b}	20.20^{a}	Containing the gamma ray processed canola
2552.20^{b}	1385.40^{b}	0.39^{b}	44.80^{a}	17.20^{ab}	Containing the roasted canola
83.68	93.02	0.01	0.38	0.38	SEM
0.04	0.03	0.04	0.04	0.03	P.value

SEM: Standard error of the mean. Non common superscripts in each column indicate the significant statistical difference ($p<0.05$).
H/L: The ratio of heterophil to lymphocyte.

seeds that have been overheated. This test is based on the solubility of canola proteins in a diluted solution of potassium hydroxide, which decreases as the temperature increases. The amount of protein solubility in potassium hydroxide is 70 to 85% indicating good seed processing, and an index lower than 70% indicates excessive heat of the seed, which reduces the growth of chickens (AOCS 1998). As can be seen in Table 2, the amount of protein solubility in the present research is more than 80% in all treatments. The results of a study showed that roasting decreased urease activity (Karr-Lilienthal *et al.* 2006), which was consistent with the results of the present experiment. Urease activity is an indicator of trypsin inhibitory activity. If the amount of trypsin inhibitors in the diet is very high, it can increase the size of the pancreas in birds (McNaughton *et al*. 1981). The urease activity test is one of the reliable methods to determine the quality. The inhibitory activity of trypsin is similar to the urease enzyme and is sensitive to heat and can be deactivated at high temperatures. The inhibitory activity of trypsin is deactivated in canola seeds processed by heat-based methods. Therefore, the use of urease is not suitable for canola seeds that have been processed by heat-based methods. The peroxide index, taste, and smell of the oil are highly related to the initial amount of Linolenate. By measuring the amount of Linolenate in the oilseed, it is possible to understand to some extent the stability of the taste and the stability of the oil against oxidation (when the oil is heated). Heat increases oxidation and increases peroxide value. The rate of production and breakdown of peroxides in canola oil is lower and slower than in soybean oil due to less linoleic acid and more oleic acid (Ifeoma *et al.* 2011). In the present research, the application of heat treatment during the roasting of canola seed increased its peroxide value compared to other processing methods

In a study, by extruding soybeans at the temperature of 140 °C for 20 seconds, it was observed that the urease activity index increased from 2.03 in raw soybeans to 0.05 in roasted soybeans (Perilla *et al.* 1997). In another experiment, researchers extruded soybean samples at different temperatures and reported a different urease activity index, which is slightly different from the urease activity in the present study (Palic *et al.* 2011). These partial differences are due to differences in the duration of extrusion, seed variety, or laboratory conditions and errors (Leeson *et al.* 1987)

Feed consumption: It has been reported that increasing the level of raw canola seed in the broilers' ration leads to a gradual decrease in feed consumption (Szymeczko *et al.* 2010, Talebali and Farzinpour 2005). Also, in another study, it was reported that the consumption of raw canola seed reduces feed consumption (Rakita *et al.* 2023, Katuk *et al.* 2003). In a study, the feed consumption of broilers increased under the influence of treatment containing processed canola seeds (Rezaeipour *et al.* 2015). In another study, it was reported that irradiation with gamma rays in soybean seeds reduced anti-nutrient compounds such as phytic acid and glucosinolates and improved the digestibility of seed nutrients (Maclsaac *et al.* 2005). The mentioned results are in accordance with the results of the present research. The decrease in feed consumption in the treatment containing raw canola seeds in the present study may be due to the presence of phenolic compounds and high levels of sulfur in canola glucosinolates. Of course, researchers consider the variety of canola used to be effective (Yadav *et al.* 2022). The processing methods improve the availability of fats and proteins and increase their nutritional value. Processing by reducing the speed of movement of materials in the digestive system causes the feed to stay longer in the digestive tract and digestive enzymes spend more time affecting nutrients. The processing increases the ability to digest and absorb nutrients (Fathi Lehmali and Jafari 2020)

Weight Gain: It has been reported that the canola seeds processing by enzymes improves the weight gain of broiler chickens (Rezaeipour *et al.* 2015). On the other hand, it has been reported that gamma radiation is effective in reducing glucosinolates, and phytic acid and improving chemical composition, degradability of dry matter, crude protein, and digestibility of the Eruca Sativa seed protein (Siddhuraju *et al.* 2002). Researchers reported that the use of multi-enzymes in a ration containing high-fat canola improved the absorption of nutrients and ultimately improved the body weight of broiler chickens (Jozefiak *et al.* 2010, Meng *et al.* 2006). The results of the aforementioned research are consistent with the results of the present research. One of the reasons for weight loss in the treatment containing raw canola seeds in the present study could be the high raw fibers of canola seeds, which prevents the absorption of nutrients in the two parts of soluble and insoluble non-starch polysaccharides. On the other hand, not being palatable (due to bitterness) reduces food consumption

FCR: The canola seed processing reduces the

FCR by reducing anti-nutritional factors, increasing feed consumption, and improving body weight. Several reports have confirmed the positive effect of canola seed processing by irradiation and enzyme on improving the FCR in broiler chickens (Rezaeipour et al. 2015, Meng *et al.* 2006). All these reports are consistent with the results of this research. Feed processing can change nutrient requirements by affecting activities related to feed consumption. Birds will use less energy to feed, so they will have more energy available for growth

Blood parameters: In the present research, the percentage of crude fiber and fat of all rations was similar, so the effect of processing temperature and its rate could not significantly affect triglycerides and lipoproteins in blood serum. In a study, canola seed processing by microwaves reduced the blood cholesterol concentration of broiler chickens (Abbasi Rad and Irani 2014), which is inconsistent with the results of the current research. One possible reason for this inconsistency could be a different processing method (microwave). An increase in the concentration of ALT or AST that enters the bird's bloodstream from the liver indicates liver damage (Sirtori et al. 1998, Gharahveysi and Jafari 2021)

Antibody titers, and Immune parameters: In the present research, the treatment containing canola seed processed by the enzyme improved the antibody titer against Bronchitis and Gumboro disease in broilers. Also, roasting and gamma-ray methods had an improving effect in increasing the percentage of lymphocytes and heterophils. By breaking large molecules of soluble non-starch polysaccharides into small polymers, enzymes reduce the viscosity of digestive materials and improve the immune status of birds by improving the absorption of micronutrients such as zinc (Scott *et al.* 1997). Some nutrients directly affect the immune system by changing the actions of immune cells. Some also affect the immune system indirectly through hormonal or nervous pathways. The most important nutrients that indirectly affect the immune system are energy and protein, which are provided through dietary sources (Szymeczko *et al.* 2010). Improving antibody titers against Bronchitis and Gumboro diseases will be very effective in health management, and herd vaccination. It will reduce related costs

CONCLUSIONS

The research results indicated that canola seed and its processing are beneficial for improving broiler chicken traits. Processing techniques involving gamma rays and roasting were found to decrease liver enzyme secretion, thus reducing liver damage. These methods also enhance immune parameters, and antibody titers for Bronchitis and Gumboro diseases, ultimately improving chicken health. Based on the findings of the present research, it can be hoped that the use of processed canola seeds in the ration will have beneficial and positive effects on the performance of broiler chickens. The present results can inspire future research

ACKNOWLEDGMENT

The authors acknowledge the support of the Islamic Azad University- Qaemshahr branch and the assistance of all individuals who contributed to this research

CONFLICT OF INTEREST

The authors do not have any potential conflicts of interest to declare

REFERENCES

Abbasi Rad A, Irani M (2014) The effect of whole canola seed processing with 800-watt microwave on blood parameters of broiler chickens. Proceedings of the first national conference on new achievements in biological and agricultural sciences, pp. 74-79, 10 May 2014, Zabul University, Zabol, Iran.

Agyekum AK, Woyengo TA (2022) Nutritive value of expeller/cold-pressed canola meal and pre-pressed solvent-extracted carinata meal for broiler chicken. Poultry Science 101(1):101528.

AOAC (1997) In W Horwitz (Ed), Official methods of analysis (17th ed.). Washinton, DC, USA, Association of official analytical chemists.

AOCS (1998) Official and recommended practices of the American oil chemists' society (5th ed.). Champaign Illinois, USA, AOCS press.

Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Durjava MK, Kouba M, López-Alonso M, Puente SL, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Maradona MP, Saarela M, Galobart J, Holczknecht O, Manini P, Pizzo F, Tarrés-Call J, Anguita M, Pettenati E (2020) Safety and efficacy of Avizyme® (endo-1,4-beta-xylanase, subtilisin and alpha-amylase) for all poultry species. European Food Safety Authority Journal 18(2):6027.

Fathi Lehmali I, Jafari MA (2020) Soybean processing effects on the performance, carcass traits, and blood metabolites of broiler chickens. Indian Journal of Animal Sciences 90(8):53-58.

Gharaghani H, Zaghari M, Shahhosseini G, Moravej H (2008) Effect of gamma irradiation on anti-nutritional factors and nutritional value of canola meal for broiler chickens. Asian-Australian Journal of Animal Science 21:1479-1485.

Gharahveysi S, Jafari MA (2021) Canola processing effects on the intestine, blood, and kidney of broiler breeder hens. Journal MVZ Cordoba 26(1):e1878.

Ifeoma I, Okechukwu W, Nwokohuru W, Chukwunonso E (2011) Oxidative stability of red palm oil from two oil palm varieties Elaeis guineensis and Elaeis oleifera: comparative effects of storage tempratura and duration. Journal of Food Science and Technology 5(2):18-22.

Jozefiak D, Ptak A, Kaczmarek S, Mackowiak P, Sassek M, Slominski BA (2010) Multi-carbohydrase and phytase supplementation improves growth performance and liver insulin receptor sensitivity in broiler chickens fed diets containing full-fat rapeseed. Poultry Science 89:1939-1946.

Karr-Lilienthal LK, Bauer LL, Utterback PL, Zinn KE, Frazier RL, Parsons CM, Fahey GC (2006) Chemical composition and nutritional quality of soybean meals prepared by extruder/expeller processing for use in poultry diets. Journal of agricultural and food chemistry 54(21):8108-8114.

Katuk Sh, Amanlou H, Kamyab A (2003) The effect of different levels of canola meal and calcium supplementation on the performance of broiler chickens. Iranian Journal of Chakavak 12(2):15-30.

Khalil MM, Abdollahi MR, Zaefarian F, Chrystal PV, Ravindran V (2023) Broiler Age Influences the Apparent Metabolizable Energy of Soybean Meal and Canola Meal. Animals 13:219.

Leeson S, Atteh JO, Summers JD (1987) The replacement value of canola meal for soybean meal in poultry diets. Canadian Journal of Animal Science 67:151-158.

Maclsaac JL, Burgoyne KL, Anderson DM, Rathgeber BR (2005) Roasted full fat soybeans in starter, grower and finisher diets for female broiler turkeys. Journal of Poultry Science 14:116-121.

McNaughton JL, Reece FN, Deaton JW (1981) Relationships between color, trypsin inhibitor contents, and urease index of soybean meal and effects on broiler performance. Poultry Science 60(2):393-400.

Meng X, Slominski BA, Campbell LD, Guenter W, Jones O (2006) The use of enzyme technology for improved energy utilization from full-fat oilseeds, Part 1: canola seed. Poultry Science 85:1025-1030.

Monari S (1996) Full fat soya handbook, American Soybean Association. Brussels, Belgium.

Nelda R, Paz R, Masson L, Ortiz J, Gonzalez K, Tapia K, Dobaganes C (2007) Effect of a-tocopherol, a-tocotrienol and Rosa Mosqueta Shell Extract on the Performance of Antioxidant-Stripped Canola Oil (Brassica Sp.) at High Temperature. Food Chemistery 104:383-389.

Niu Y, Rogiewicz A, Shi L, Patterson R, Slominski BA (2022) The effect of multi-carbohydrase preparations on non-starch polysaccharides degradation and growth performance of broiler chickens fed diets containing high inclusion level of canola meal. Animal Feed Science and Technology 293:115450.

Palic D, Modika KY, Oelofse A, Morey L, Coetzee SE (2011) The protein dispersibility index in the quality control of heat-treated full-fat soybeans: an inter-laboratory study. South African Journal of Animal Science 41(4):413-419.

Perilla N, Cruz MP, De Belalcazar F, Diaz G (1997) Effect of temprature of wet extrution on the nutritinal value of full fat soybeans for broiler chickens. British Poultry Science 38:412-416.

Pesti GM, Miller BR, Hargrave J (1992) User-Friendly Feed Formulation, Done Again (UFFDA), Programed by J. Hargrave. University of Georgia, USA.

Rezaeipour V, Agharajabi A, Ghareveisi S, Norozi M (2015) Effects of full-fat canola seed with an exogenous enxzyme supplementation on performance, carcass characteristics and thyroid hormones of broiler chickens. The Journal of Animal & Plant Sciences 25(5):1233-123.

Rakita S, Kokic B, Manoni M, Mazzoleni S, Lin P, Luciano A, Ottoboni M, Cheli F, Pinotti L (2023) Cold-Pressed Oilseed Cakes as Alternative and Sustainable Feed Ingredients: A Review. Foods 12:432-451.

Sadeghi AA, Shawrang P (2006) Effects of microwave irradiation on ruminal degradability and in vitro digestibility of canola meal. Animal Feed Science and Technology 127:45-54.

SAS Institute (2004) SAS/STAT User's Guide, Statistics. Release 9.1. SAS Institute Inc., Cary, NC.

Scott TA, Swift ML, Bedford MR (1997(Influence of feed milling, enzyme supplementation and nutrient regimen on broiler chick performance. Journal of Applied Poultry Research 6(4):391-398.

Siddhuraju P, Makker HPS, Becker K (2002) The effect of ionising radiation on anti-nutritional factors and nutritional value of plant material with reference to human and animal food. Food Chemistry 78(2):187-

205.
Sirtori CR, Lovati MR, Manzoni C, Gianazza E, Bondioli A, Staels B, Auwerx J (1998) Reduction of serum cholesterol by soy proteins: clinical experience and potential molecular mechanisms. Nutrition, Metabolism and Cardiovascular Diseases 8(5):334-340.
Szymeczko R, Topolinski T, Burlikowska K, Piotrowska A, Boguslawska-Tryk M, Blaszyk J (2010) Effects of different levels of rapeseeds in the diet on performance, blood and bone parameters of broiler chickens. Journal of Central European Agriculture 11(4):393-400.
Talebali H, Farzinpour A (2005) Effect of different levels of full-fat canola seed as a replacement for soybean meal on the performance of broiler chickens. International Journal of Poultry Science 4(12):982-985.
Yadav S, Teng PY, Choi J, Singh AK, Vaddu S, Thippareddi H, Kim WK (2022) 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 Science 101(1):101551.
Yasothai R (2016) Antinutritional factors in soybean meal and its deactivation. International Journal of Science, Environment and Technology 5(6):3793-3797.