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Effect of canola processing by bacteria, fungi, and enzyme on the intestine traits and blood metabolites of broiler breeder hens

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ABSTRACT

Anti-nutritional factors of canola meal are reduced by processing. The purpose of the current research was to study the canola processing effect on the intestinal traits and blood metabolites of broiler breeder hens. Broiler breeder hens (450) were reared for 12 weeks. A completely randomized design was used with 6 treatments (unprocessed, processed by *Lactobacillus plantarum*, *Bacillus subtilis*, *Aspergillus oryzae*, *Neurospora cytophilla*, and Alkalase enzyme) and 5 replications. The LS-means procedure of SAS statistical software was used for the analysis of the data. The treatments' effect was significant on all blood metabolites. Process methods were increased glucose concentration and HDL. The effect of processing methods was significant on caecum, jejunum, and duodenum. The use of experimental treatments increased the weight of the small intestine (duodenum and jejunum) and decreased caecum weight. The processing of canola meal increased the length of the villi and decreased the depth of the crypt of jejunum. The canola process improved its digestibility. Protein quality, fatty acid profile, and antimicrobial properties were improved. The processed canola used by the bird improves its traits and can be recommended to use in the ration.

Keywords: Canola, Intestine, Processing, Triglyceride, Villi

Canola meal is the second most important plant protein product after soybean meal. Canola is derived from breeding the rapeseed for reducing the glucosinolate amount (Recoules *et al.* 2019). The amount of erucic acid in canola oil is less than 2% and the amount of glucosinolate in its meal is less than 30 μmol/g (Popova and Mihaylova 2019). The major anti-nutrient components of canola are phytate, glucosinolate and tannin (Tripathi and Mishra, 2007). High consumption of glucosinolates in broiler chickens reduces feed intake, decreases growth rate, hyperthyroidism, decreases thyroid hormone levels, enlarges liver, kidney and thyroid gland, changes liver activity, and increases mortality (Kermanshahi and Abbasi pour 2006, Toghyani *et al.* 2017).

Now a days, the production of new feeds through fermentation technology has been favored by fungal and bacterial species (Van Emous *et al.* 2015, Singhania *et al.* 2009). The use of microbial fermentation method to produce high quality protein and free of anti-nutritional compounds has been considered. Fermentation technology is better at processing feed than chemical methods (Khalil 2006). Probiotics and prebiotics are another group of additives introduced as alternatives to antibiotics. Probiotics are living microbial food additives that have a beneficial effect on the host by improving the gut microbial balance and enhancing the immune system (Alkhalf *et al.* 2010). Prebiotics are contained non-digestible feed components and increase the growth or activity of beneficial bacterial species in the intestine and reduce the population of harmful host bacteria (Zhu *et al.* 2019). In the enzymatic hydrolysis of proteins, the hydrolysis process is completely controlled, resulting in peptides are produced with biologically active properties (Goldberg *et al.* 2016). Enzymatic hydrolysis of plant proteins, such as canola meal, produce peptides that are used as natural ingredients in the production of useful feeds and can be used in animal nutrition because of their high absorption capacity from the small intestine (Singh *et al.* 2014).

The purpose of the present research was to study the effect of canola processing by bacteria, fungi, and enzyme on the traits and intestine morphology, blood metabolites and kidney enzymes of broiler breeders.

MATERIALS AND METHODS

Time and place of the research: The study was done in 2018, using the chicken farm, facilities and laboratory of the agriculture faculty of Islamic Azad University-Qaemshahr branch of Iran. Broiler breeder hens was rear for 12 weeks (weeks 40 to 52). Experimental procedures of the present research were done based on the laws of the

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national committee for ethics in biomedical research of Iran (2018).

Birds and treatments: In this study, 450 hens of broiler breeder Ross strain weighing 3,300±150 g (40 weeks) were used for 12 weeks. A completely randomized design was used with 6 treatments and 5 replications. Thirty pens were designed and prepared to study the effect of treatments. Fifteen broiler breeder hens were included in each pen. Treatments included: 1) unprocessed canola meal, 2) canola meal processed with *Lactobacillus plantarum*, 3) canola meal processed with *Bacillus subtilis*, 4) canola meal processed with *Aspergillus oryzae*, 5) canola meal processed with *Neurospora cytophilla*, and 6) canola meal processed with Alkalase enzyme.

Processing of canola meal: After preparation of canola meal, three samples (500 gm) were supplied and sent to the laboratory for chemical analysis. Five 25 kg samples were supplied and fermentation processing (fermentation with *Lactobacillus plantarum*, *Bacillus subtilis*, *Aspergillus Oryzae*, and *Neurospora cytophilla*) and enzymatic hydrolysis (Alkalase) was performed on them. Then, 3 sub-samples were prepared from each sample and sent to a specialized laboratory for evaluation of quality, and traits measurement.

Studied traits

Blood metabolites: At the end of the experiment, two birds were randomly selected from each pen and about two ml of blood was collected through a jugular vein of the wing. Concentrations of glucose, triglyceride, cholesterol, HDL (high density lipoprotein) and LDL (low density lipoprotein) of blood samples were determined using the laboratory kits (Iran Pars-Azmoon) and spectrophotometer device (UK Jenway Genova MK3). The data was recorded for analysis.

(*Small*) *intestine traits:* At the end of the experiment, after slaughtering of hens and emptying the contents of the intestine; weight and lengths of the three small intestine sections were measured. Then, 2 cm of the jejunum was washed with cold PBS solution (4ÚC) and placed in 10% neutral formalin buffer and transferred to the histological laboratory (for supply transverse sections and determination of villi length and crypt depth). Histological studies were performed according to an accuracy recommended method (Bird *et al.* 1994b). In this study, the villi length and crypt depth were measured by Graticule.

Ration: The ration was formulated based on the nutritional requirements for the broiler breeder hen of Ross 308 (weeks 40 onwards) by corn, and soybean meal (Table 1).

Statistical analysis: The data collected from the present study were analyzed using the GLM procedure of SAS statistical software (9.1). The statistical model used was as follows:

$$y_{ij}=\mu+A_i+_{eij}$$

where, y_{ij}, value of each observation; μ is the effect of the mean; A_i, effect of the treatment and e_{ij}, residual effect.

Table 1. Feed ingredients and chemical compositions of the experimental ration

Ingredient	Amount in the ration (%)
Corn	56.80
Soybean meal (43% CP)	24.70
Wheat bran	6.00
Soybean oil	1.20
Di-calcium phosphate	1.50
Oyster powder	8.00
Salt	0.30
Mineral supplement	0.25
Vitamin supplement	0.25
DL-methionine	1.00
Chemical composition	
Metabolizable energy (Kilocalories per kilogram)	2740
Crude protein (%)	15.50
Methionine + Cysteine (%)	0.62
Lysine (%)	0.77
Calcium (%)	3.30
Available Phosphorus (%)	0.38
Sodium (%)	0.18

Mineral supplement provides the following items: 50 mg of manganese, 50 mg of iron, 24 mg of Zinc, 10 mg of copper, 2 mg of iodine, 200 µg of selenium, 500 µg of cobalt. Vitamin supplement provides the following items: 12,000 international units (IU) of vitamin A, 3,000 IU of vitamin D3, 100 IU of Vitamin E, 5 mg of vitamin K3, 3 mg of vitamin B1, 12 mg of vitamin B2, 55 mg of vitamin B3, 15 mg of vitamin B5, 4 mg of pyridoxine, 2 mg of vitamin B9, 40 µg of vitamin B12, 1,000 mg of vitamin choline and 250 µg of vitamin biotin.

RESULTS AND DISCUSSION

Blood metabolites: The effect of experimental treatments was significant (P<0.05) on all blood metabolites traits (Table 2). Process methods were increased glucose concentration and HDL. The highest concentration of glucose and HDL were observed in the processing method with *Aspergillus oryzae* fungi (184 mg/dl) and *Neurospora cytophilla* fungi (36 mg/dl), respectively. Processing methods of canola meal reduced the concentration of triglyceride, cholesterol, and LDL. The lowest concentration of triglyceride (60 mg/dl), cholesterol (144 mg/dl), and LDL (41 mg/dl) was observed in the processing method with *Bacillus subtilis* bacteria.

By studying the results of the effects of canola processing on blood metabolites in the present study, it can be observed that the processing reduces triglyceride, cholesterol and LDL levels in the broiler breeder blood. The decrease in cholesterol may be due to the inhibition of the activity of the 3-hydroxy-3-methyl-glutaryl-coA enzyme, which subsequently decreases cholesterol production. It can also be due to the production of short-chain fatty acids such as propionic acid, which subsequently restricts the production of cholesterol (Ashayerizadeh *et al.* 2018). The results of the present study are consistent with other studies (Hu *et*

Table 2. Effect of treatments on blood metabolites of Ross broiler breeder hens (mg/dl)

Treatment	Glucose	Triglyceride	Cholesterol	HDL	LDL
Unprocessed	173[a]	70[b]	160[a]	27[b]	50[a]
Processed by *Lactobacillus plantarum* (Bacteria)	179[b]	63[a]	146[b]	35[a]	43[b]
Processed by *Bacillus subtilis* (Bacteria)	183[b]	60[a]	144[b]	34[a]	41[b]
Processed by *Aspergillus oryzae* (Fungi)	184[b]	62[a]	147[b]	33[a]	42[b]
Processed by *Neurospora cytophilla* (Fungi)	182[b]	61[a]	149[b]	36[a]	43[b]
Processed by Alkalase enzyme	183[b]	62[a]	146[b]	34[a]	43[b]
SEM	7.81	3.93	10.02	0.80	0.91
P Value	0.00	0.00	0.00	0.00	0.00

SEM, Standard error of means; HDL, High density lipoprotein; LDL, Low density lipoprotein. Means with different letters are significant (P<0.05).

Table 3. Effect of treatments on (small) intestine traits

Treatment	Caecum (%)	Ileum (%)	Jejunum (%)	Duodenum (%)	Villi height (µm)	Crypt) depth (µm	Villi height/ Crypt depth
Unprocessed	0.27[a]	0.95	2.09[b]	0.69[b]	1732.21[b]	197.65[a]	8.76[b]
Processed by *Lactobacillus plantarum* (Bacteria)	0.23[b]	0.97	2.18[a]	0.75[a]	1750.56[a]	186.11[b]	9.41[a]
Processed by *Bacillus subtilis* (Bacteria)	0.23[b]	0.97	2.19[a]	0.76[a]	1752.43[a]	185.34[b]	9.46[a]
Processed by *Aspergillus oryzae* (Fungi)	0.21[b]	0.96	2.21[a]	0.77[a]	1754.91[a]	185.11[b]	9.48[a]
Processed by *Neurospora cytophilla* (Fungi)	0.23[b]	0.96	2.19[a]	0.75[a]	1753.98[a]	185.20[b]	9.47[a]
Processed by Alkalase enzyme	0.22[b]	0.97	2.20[a]	0.76[a]	1754.21[a]	185.32[b]	9.47[a]
SEM	0.00	0.02	0.09	0.02	24.21	9.65	0.31
P Value	0.00	0.13	0.02	0.03	0.00	0.00	0.00

SEM, Standard error of means. Means with different letters are significant, P<0.05.

al. 2016, Karimzadeh *et al.* 2017, Ashayerizadeh *et al.* 2018). Uric acid is a marker of protein catabolism and is the most important excreted nitrogen product in birds. Changes in blood uric acid levels indicate changes that occur in protein catabolism and also depend on the protein content and quality of the poultry ration (Ziyad *et al.* 2019).

Intestinal traits and Jejunum morphology: It can be seen from the results of Table 3 that, the effect of processing methods was significant on caecum, jejunum, and duodenum (P<0.05). The use of experimental treatments increased the weight of the small intestine (duodenum and jejunum) and decreased caecum weight. The highest weight gains in the small intestine (duodenum and jejunum) was observed in treatments processed with *Aspergillus oryzae* fungi. The lowest caecum weight was observed in the treatment processed with *Aspergillus oryzae* fungi.

The processing of canola meal increased the length of the villi and decreased the depth of the crypt of jejunum (P<0.05). The highest villi length and the lowest depth of crypt were observed in the treatment processed with *Aspergillus oryzae* fungi (Table 3). In a canola study with Alkalase enzymatically hydrolyzed, the processed canola was reported to increase villus height, decrease crypt depth, and increase the villus height to crypt depth ratio in the broiler jejunum (Karimzadeh *et al.* 2016a). In another study, canola was processed by *Bacillus subtilis*, *Candida utilis*, and *Enterococcus faecalis*. The use of processed canola reduced the crypt depth and increased the villus height to crypt depth ratio in the broiler jejunum (Hu *et al.* 2016). These results are consistent with the results of the present study.

The canola process improved its digestibility. In other words, protein quality, fatty acid profile and antimicrobial properties were improved. Therefore, the processed canola used by the bird improves its traits. It can be recommended to use the processed meal instead of the raw canola meal.

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