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Original

Canola processing effects on the intestine, blood, and kidney of broiler breeder hens

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ABSTRACT

Objective. Canola meal is one of the most important vegetable protein that contained the anti-nutritional factors. The aim of the study was to study the effect of canola processing on the intestine traits, blood metabolites, and kidney enzymes of broiler breeders. **Material and methods.** Four hundred fifty broiler breeder hens were used for 3 months. The 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 collected data were analyzed by the LSmeans procedure of SAS statistical software. **Results.** The effects of treatments were significant on blood metabolites ($p<0.05$). Glucose concentration and High-density lipoprotein (HDL) were increased by processing methods. Also, the caecum, jejunum and duodenum weight were influenced($p<0.05$). The weight of the duodenum and jejunum was increased and caecum weight was decreased. The processing of canola meal increased the length of the villi and decreased the depth of the crypt of the jejunum ($p<0.05$). Treatments effect was significant on the Alanine transaminase (ALT) and Alkaline phosphatase (ALP) ($p<0.05$). The effect of treatments was significant on the digestive amylase, lipase and protease activity ($p<0.05$). The canola process improved its digestibility. In other words, its protein quality, fatty acid profile and antimicrobial properties were improved. **Conclusions.** The different processing methods of canola improved the hen's traits. It can be recommended to use the processed meal instead of the raw canola meal.

Keywords: Blood; canola; enzyme; kidney; processing (Source: CAB).

RESUMEN

Objetivo. La harina de canola es una de las proteínas vegetales más importantes que contiene factores antinutricionales. El objetivo del estudio fue estudiar el efecto del procesamiento de la canola en los rasgos intestinales, los metabolitos sanguíneos y las enzimas renales de las reproductoras de pollos de engorde. **Material y Métodos.** Se utilizaron cuatrocientas cincuenta gallinas reproductoras de engorde durante 3 meses. Se utilizó el diseño completamente al azar con 6 tratamientos (sin procesar, procesados por *Lactobacillus Plantarum*, *Bacillus Subtilis*, *Aspergillus Oryzae*, *Neurospora*

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Cytophilla y Alkalasa enzima) y 5 repeticiones. Los datos recopilados se analizaron mediante el procedimiento LSmeans del software estadístico SAS. **Resultados.** Los efectos de los tratamientos fueron significativos sobre los metabolitos sanguíneos ($p<0.05$). La concentración de glucosa y las lipoproteínas de alta densidad (HDL) se incrementaron mediante métodos de procesamiento. Asimismo, se influyó en el peso del ciego, yeyuno y duodeno ($p<0.05$). Se aumentó el peso del duodeno y yeyuno y se redujo el peso del ciego. El procesamiento de la pasta de canola aumentó la longitud de las vellosidades y disminuyó la profundidad de la cripta del yeyuno ($p<0.05$). El efecto de los tratamientos fue significativo sobre la alanina transaminasa (ALT) y la fosfatasa alcalina (ALP) ($p<0.05$). El efecto de los tratamientos fue significativo sobre la actividad digestiva de amilasa, lipasa y proteasa ($p<0.05$). El proceso de canola mejoró su digestibilidad. En otras palabras, se mejoraron su calidad proteica, perfil de ácidos grasos y propiedades antimicrobianas. **Conclusiones.** Los diferentes métodos de procesamiento de la canola mejoraron los rasgos de la gallina. Se puede recomendar utilizar la comida procesada en lugar de la pasta de canola cruda.

Palabras clave: Sangre; canola; enzima; riñón; procesamiento (*Fuente: CAB*).

INTRODUCTION

The scientific name of canola is the *Brassica Napus* from the family Brassicaceae Cruciferea. In poultry nutrition, canola meal is the second most important vegetable protein after soybean meal. Canola was derived by breeding the rapeseed for reducing the glucosinolate amount (1). The erucic acid of canola oil is less than 2%, and the amount of glucosinolate in its meal is less than 30 µmol/g (2). The major anti-nutrient components of canola are phytate, glucosinolate, and tannin. These compounds reduce the palatability of the ration. Glucosinolates are not normally toxic. But, secondary products from their decomposition can have adverse effects on bird performance (3). 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 (4,5).

In modern nutrition science, the production of new feeds through fermentation technology has been favored by fungal species (such as *Rizopus Oligosporus, Aspergillus Oryza, Neurospora Cytophila* and *Aspergillus Niger*) and bacterial species (such as *Enterococcus Faecium* and *Bacillus Subtilis*) (6,7). Fermentation technology eliminates anti-nutritional compounds and improves the structure and taste of the feed. Fermentation technology is better at processing feed than chemical methods. The use of microbial fermentation method to produce high quality protein and free of anti-nutritional compounds has been considered (8).

The inadequate use of antibiotics in the chicken farms has increased the antibiotic resistance of the bacteria. For this, the use of growth-promoting antibiotics was banned in Denmark in 1995, and then in the EU in January 2006. With this prohibition, choosing the right alternative has become a challenge for the poultry nutrition (9). The alternative ways such as fermentation technology, as a potential alternative to antibiotics, has attracted the attention of poultry nutritionists. Probiotics and prebiotics are other groups 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 (9). 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 (10). The results of studies indicate that the addition of prebiotics to the diet not only stimulates the growth and activity of beneficial intestinal bacteria but can also increase the growth and activity of the host intestinal bacteria (10). Therefore, research has focused on other additives to address the weaknesses of probiotics and prebiotics. One of the most important and newest of these feed additives are peptides. Peptides are products obtained after incomplete hydrolysis of proteins by enzymes, acids, alkali, or fermented hydrolysis (11). Incomplete hydrolysis of proteins from plant or animal sources produces peptides of different molecular weights and high solubility in water (12). Research results show that in the process of hydrolyzing proteins by chemical methods (acid and alkaline solutions), enzymatic and fermentative, peptides are

produced with beneficial feed properties such as antioxidant properties, immune system stimuli, antimicrobial, blood pressure modulation, anti-cancer, and anti-obesity production. (13). In the enzymatic hydrolysis of proteins, the hydrolysis process is completely controlled, resulting in peptides are produced with biologically active properties (12). Enzymatic hydrolysis of plant proteins, such as canola meal, has produced 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 (13).

The aim of the current experiment was to study the effects of different canola processing methods by bacteria, fungi, and enzyme on the intestine traits, blood metabolites, and kidney enzymes of broiler breeder hens.

MATERIALS AND METHODS

The research conditions. The research 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 were rearing for 12 weeks (weeks 40 to 52). Experimental procedures of the current study were done base on the laws of the national committee for ethics in biomedical research of Iran (2018).

Hens and treatments. In the current research, 450 hens of broiler breeder Ross strain weighing 3300±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. Fifteen broiler breeder hens were included in each pen. The experimental treatments included: 1) unprocessed canola meal; 2) processed canola meal by *Lactobacillus Plantarum*; 3) processed canola meal by *Bacillus Subtilis*; 4) processed canola meal by *Aspergillus Oryzae*; 5) processed canola meal by *Neurospora Cytophilla*; and 6) processed canola meal by Alkalase enzyme

Canola meal processing method. After the preparation of the canola meal, three samples (500 gr) 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.

The ration formulation. 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).

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

Ingredient	Amount in the ration (%)
Corn	56.8
Soybean meal (43% CP)	24.7
Wheat bran	6
Soybean oil	1.2
Di-calcium phosphate	1.5
Oyster powder	8
Salt	0.3
Mineral supplement	0.25
Vitamin supplement	0.25
DL-methionine	1
Calculated chemical composition	
Metabolizable energy (Kilocalories per kilogram)	2740
Crude protein (%)	15.50
Methionine + Cysteine (%)(digestible)	0.62
Lysine (%)(digestible)	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: 12000 international units (IU) of vitamin A, 3000 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.

The studied traits.

The small intestine traits. At the end of the experiment, after the slaughtering of hens and emptying the contents of the intestine; weights and lengths of the three small intestine sections (duodenum, jejunum, and ileum) were measured. Then, 2 cm of the jejunum was washed with cold PBS solution (4°C) 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 accuracy recommended protocols (14). In this study, the villi length and crypt depth were measured by Graticule.

Blood metabolites and enzymes. At the end of the experiment, two hens were randomly selected from each pen and about two ml of blood was collected through a jugular vein of the wing. Concentrations of alanine aminotransferase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), total protein, albumin, uric acid, cholesterol, Low-density lipoprotein (LDL), High-density lipoprotein (HDL), calcium and phosphorus 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.

Statistical analysis. The data collected were analyzed by the generalized linear model (GLM) procedure of SAS statistical software (15). The statistical model used was as follows:

$$y_{ijk}=\mu+A_i+e_{ijk}$$

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

RESULTS

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.

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.

Table 2. Canola processing effects on blood metabolites of Ross broiler breeder hens (mg/dl).

Treatments	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 in each column are significant ($p<0.05$).

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

Treatments	Caecum	Ileum	Jejunum	Duodenum
Unprocessed	0.27[a]	0.95	2.09[b]	0.69[b]
Processed by *Lactobacillus Plantarum* (Bacteria)	0.23[b]	0.97	2.18[a]	0.75[a]
Processed by *Bacillus Subtilis* (Bacteria)	0.23[b]	0.97	2.19[a]	0.76[a]
Processed by *Aspergillus Oryzae* (Fungi)	0.21[b]	0.96	2.21[a]	0.77[a]
Processed by *Neurospora Cytophilla* (Fungi)	0.23[b]	0.96	2.19[a]	0.75[a]
Processed by Alkalase enzyme	0.22[b]	0.97	2.20[a]	0.76[a]
SEM	0.00	0.02	0.09	0.02
P.Value	0.00	0.13	0.02	0.03

SEM: Standard error of means. Means with different letters in each column are significant ($p<0.05$).

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 4).

Table 4. Effect of treatments on the villi height and crypt depth of small intestine.

Treatments	Villi height (µm)	Crypt depth (µm)	Villi height/ Crypt depth
Unprocessed	1732.21[b]	197.65[a]	8.76[b]
Processed by *Lactobacillus Plantarum* (Bacteria)	1750.56[a]	186.11[b]	9.41[a]
Processed by *Bacillus Subtilis* (Bacteria)	1752.43[a]	185.34[b]	9.46[a]
Processed by *Aspergillus Oryzae* (Fungi)	1754.91[a]	185.11[b]	9.48[a]
Processed by *Neurospora Cytophilla* (Fungi)	1753.98[a]	185.20[b]	9.47[a]
Processed by Alkalase enzyme	1754.21[a]	185.32[b]	9.47[a]
SEM	24.21	9.65	0.31
P.Value	0.00	0.00	0.00

SEM: Standard error of means. Means with different letters in each column are significant ($p<0.05$).

Kidney enzymes, and other blood metabolites. As shown in Table 5, treatments effect was significant on the ALT and ALP ($p<0.05$). The lowest concentration of ALT and ALP was observed in treatments processed with *Lactobacillus Plantarum* bacteria (6.08) and *Bacillus Subtilis* bacteria (2504.11), respectively.

Table 5. Effect of treatments on ALT, ALP, total protein, albumin, and uric acid.

Treatments	ALT (u/l)	ALP (u/l)	Total protein (mg/dl)	Albumin (mg/dl)	Uric acid (mg/dl)
Unprocessed	6.97[a]	2589.90[a]	3.54	1.54	4.50
Processed by *Lactobacillus Plantarum* (Bacteria)	6.08[b]	2510.04[b]	3.52	1.59	4.48
Processed by *Bacillus Subtilis* (Bacteria)	6.10[b]	2504.11[b]	3.50	1.58	4.45
Processed by *Aspergillus Oryzae* (Fungi)	6.12[b]	2512.61[b]	3.51	1.58	4.46
Processed by *Neurospora Cytophilla* (Fungi)	6.15[b]	2521.70[b]	3.52	1.57	4.47
Processed by Alkalase enzyme	6.16[b]	2518.34[b]	3.50	1.58	4.46
SEM	0.09	45.70	0.09	0.04	0.08
P.Value	0.02	0.00	0.14	0.23	0.19

SEM: Standard error of means. Means with different letters in each column are significant ($p<0.05$). ALT: Alanine transaminase. ALP: Alkaline phosphatase.

The effect of experimental treatments was significant ($p<0.05$) on the digestive amylase, lipase and protease activity (Table 6). The highest amylase, lipase and protease activity was observed in treatments processed with *Lactobacillus Plantarum* bacteria (8.98), *Bacillus subtilis* bacteria (21.43) and Alkalase enzyme (85.40), respectively.

Table 6. Effect of treatments on digestive amylase, lipase and protease activity (u/mg of digestible protein).

Treatments	ASU	LSTU	PU
Unprocessed	8.09[b]	19.18[b]	77.30[b]
Processed by *Lactobacillus Plantarum* (Bacteria)	8.98[a]	21.10[a]	83.87[a]
Processed by *Bacillus Subtilis* (Bacteria)	8.85[a]	21.43[a]	84.91[a]
Processed by *Aspergillus Oryzae* (Fungi)	8.89[a]	21.39[a]	83.09[a]
Processed by *Neurospora Cytophilla* (Fungi)	8.92[a]	21.28[a]	84.12[a]
Processed by Alkalase enzyme	8.87[a]	21.35[a]	85.40[a]
SEM	0.08	0.17	0.92
P.Value	0.02	0.00	0.00

SEM: Standard error of means. Means with different letters in each column are significant ($p<0.05$).
ASU = Amylase activity unit (1 Somogyi unit) was defined as the amount of amylase that would cause the formation of reducing power equivalent to 1 mg of glucose in 30 min at 40°C/mg of intestinal digesta protein (Somogyi, 1960).
LSTU = Lipase activity unit (Sigma-Tietz unit) was equal to the volume (mL) of 0.05 M NaOH required neutralizing the fatty acid liberated during 6 hrs incubation with 3 mL of lipase substrate at 37°C/mg of intestinal digesta protein (Tietz & Fiereck, 1966).
PU = Protease activity unit was defined as mg of azocasein degraded during 2 hrs incubation at 38°C/mg of intestinal digesta protein (Lynn & Clevette-Radford, 1984).

DISCUSSION

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 (16). These changes mean improved canola quality. Improvement of canola quality was achieved by different processing methods. The results of the present study are consistent with other studies (16,17,18). 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 depend on the protein content and quality of the poultry ration (19).

Alkalase is an endo-protease of the serine-type. It has a very broad substrate specificity. On another hand, it can hydrolyze most peptide bonds within a protein molecule. Peptides and amino acids are formed which are either dissolved or dispersed in the washing water. The processed canola (by alkalase enzyme) was reported to increase villus height, decrease crypt depth, and increase the villus height to crypt depth ratio in the broiler jejunum (11). 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. The higher this ratio, the greater the ability to digest and absorb nutrients (18). These results are consistent with the results of the present study.

The different canola processing methods increased the activity of amylase, lipase and protease enzymes. Increasing the activity of these enzymes results in the breakdown of long-chain sugars, large fat and protein molecules into smaller, more digestible molecules. This way the nutritional value of canola will increase (11). These results are consistent with a similar report in this field (11).

Increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (ALP) that enter the bloodstream of broiler chickens indicate liver damage (20). In the present study, the soybean processing by bacteria, fungi, and enzymes decreased the concentration of liver enzymes. Canola processing reduced the secretion of liver enzymes. It shows that the processing methods have reduced the anti-nutritional factors. As a result, the pressure on the liver was reduced. These results may be due to the favorable and protective effect of canola processing methods on the liver. The results of the present study are consistent with similar results in this field (20, 21).

The results of the present study showed that the processing of canola meals by various methods (such as bacteria, fungi, and enzyme) was reduced blood undesirable metabolites of hens (such as triglycerides). On the other hand, the characteristics of the small intestine of hens that are effective in absorbing nutrients (such as crypt and willi) were improved. Also, the concentration of secreted liver enzymes decreased. All of this means improving the quality of canola meal and reducing their anti-nutritional factors through the use of various processing methods. in other words, the processed methods improved canola meal digestibility. The protein quality, fatty acid profile, and anti-nutritional properties of canola meal were improved. Therefore, can be recommended to use the processed canola instead of the raw canola meal.

Conflict of interest

The authors declare that there is no conflict of interest associated with the paper.

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