



## Processed Canola Meal Effects on the Traits of Egg, Fertility, Cecal Microbial Population and Carcass of Broiler Breeder Hens

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### ■ Keywords

Caecum, Fermentation, Fertility, Processing.



### ABSTRACT

The aim of the current research was to study the effect of canola meal processing methods on the traits of egg, fertility, cecal microbial population, and the carcass of broiler breeder hens. Canola meal was processed by fermentation and enzymatic hydrolysis methods, then was fed to the hens. Four hundred and fifty broiler breeder hens of Ross strain weighing  $3300 \pm 150$  g (40 weeks) were used for 12 weeks. A completely randomized design was used with 6 treatments (unprocessed, processed by *Lactobacillus Plantarum*, *Bacillus Subtilis*, *Aspergillus Oryzae*, *Neurospora Sitophila*, and Alcalase enzyme) and 5 replications. Fifteen hens were included in each pen. The data collected were analyzed using the LSmeans procedure of SAS software. The treatment effect was significant on the fertility hatchability ( $p < 0.05$ ). The highest fertility hatchability was observed in the processing method by *Neurospora Sitophila* fungi (85%). Experimental treatments had significant effects on the germs and pH of the caecum ( $p < 0.05$ ). The use of processing methods reduced the population of salmonella and coliforms and increased the population of lactobacillus in the caecum. Most of the population of salmonella, lactobacillus, and coliforms of the caecum was observed in the treatments of unprocessed, *Bacillus Subtilis* bacteria, and unprocessed, respectively. The effect of the experimental treatment had significant effect on abdominal fat ( $p < 0.05$ ). Processing methods of canola caused the reduction of abdominal fat. The highest decrease in abdominal fat was observed in the treatment that processed *Aspergillus Oryzae* fungi (1.89%). Processing of canola meal improved its digestibility and reduced its anti-nutritional factors.

### INTRODUCTION

Canola is scientifically named *Brassica Napus* from the family Brassicaceae Cruciferae. Canola meal is the most important plant protein product after soybean meal. In poultry rations, soybean can be replaced by canola. Canola is derived from the rapeseed breeding by 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  $30 \mu\text{mol} / \text{g}$  (Zeb, 1998). The major anti-nutrient components of canola are phytate, glucosinolate, and tannin. These compounds reduced the ration palatability. Glucosinolates are not normally toxic. But secondary products from their decomposition can have adverse effects on bird performance (Tripathi & 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, and also changes liver activity, and increases mortality (McNeill *et al.*, 2004; Kermanshahi & Abbasi Pour, 2006).



Nowadays, the production of new feeds has been considered. These feeds were made with the fermentation technology by fungal species (such as *Rhizopus Oligosporus*, *Aspergillus Oryza*, *Neurospora Cytophila* and *Aspergillus Niger*) and bacterial species (such as *Enterococcus Faecium* and *Bacillus Subtilis*) (Ravindra, 2000; Singhanian *et al.*, 2009). 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 methods to produce high-quality protein and free from anti-nutritional compounds has been considered (Khalil, 2006).

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. (Brij *et al.*, 2014). In the enzymatic hydrolysis of proteins, the hydrolysis process is completely controlled, resulting in the production of peptides with biologically active properties (Pasupuleti & Demain, 2010). The enzymatic hydrolysis of plant proteins, such as canola meal, 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 (Brij *et al.*, 2014).

The purpose of the present research was to study the effect of canola meal processing by bacteria, fungi, and enzyme on the traits of egg, fertility, cecal microbial population and carcass of broiler breeder hens.

## **MATERIALS AND METHODS**

### **Canola meal and processing methods**

The canola meal was processed by fermentation and enzymatic hydrolysis. Fermentation processing was done by 2 bacteria strains named *Lactobacillus Plantarum* and *Bacillus Subtilis*, and 2 fungi strains named *Aspergillus Oryzae* and *Neurospora Sitophila*. Enzymatic hydrolysis was done by the Alcalase enzyme. Then, the canola meal processed was fed to the hens.

### **Hens and experimental treatments**

In this study, four hundred and fifty broiler breeder hens of Ross strain weighing  $3300 \pm 150$  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 by *Lactobacillus Plantarum*, 3) canola meal processed by *Bacillus Subtilis*, 4) canola meal processed by *Aspergillus Oryzae*, 5) canola meal processed by *Neurospora Sitophila*, and 6) canola meal processed by Alcalase enzyme.

### **Studied traits**

#### **Egg and fertility traits**

Eggs were collected 3 times per day. The egg weight and egg production were measured. The egg storage room had good conditions. Its temperature and relative humidities were 15-16 °C and 70%, respectively. The eggs were weighed before the incubation. The eggs were incubated. After incubation, healthy and salable chickens and unhatched eggs were recorded. Total hatchability was calculated through the number of healthy hatched chickens divided by egg number multiples 100. The fertility rate and fertility hatchability (the number of healthy hatched chickens divided by fertile eggs number multiples 100) were calculated.

#### **Total population of bacteria, Salmonella and Lactobacillus**

At the end of the experiment, after the slaughter of the hens, the contents of the caecum were drained with a syringe (3 ml) and the syringe was kept in the refrigerator. Samples were transferred to the laboratory of Islamic Azad University, Qaemshahr Branch. MacConkey medium was used for Salmonella count and MRS agar for lactobacillus count. Salmonella was cultured in aerobic conditions and lactobacillus under anaerobic conditions. To prepare the sample, one gram of feces with 9 ml of physiological serum solution was homogenized by Vertex (tube shaker). Then, one ml of the sample was mixed with 9 ml of physiological serum and the diluted solution was obtained. Dilution with physiological serum continued until one-eighth. Samples were cultured in MRS agar and McConkey medium. The culture media were incubated for 2 h at 4 °C. Then, the colonies were counted using a colony counting machine.

#### **Carcass traits**

At the end of the experiment, two hens were randomly selected from each pen and slaughtered after 12 hours of starvation. After slaughter, live weight, chest, thighs, wings, back and neck muscles, liver, spleen and pancreas were measured.



### Experimental ration formulation

The ration was formulated based on the nutritional requirements for the broiler breeder hens 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                        |
| 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: 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.

### Statistical analysis

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

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

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

## RESULTS

### Eggs and fertility traits

Effects of canola meal process methods on Hen-day egg production, Egg weight, and Egg mass production are presented in Table 2. No significant effect was observed ( $p > 0.05$ ). As shown in Table 3, the effect of the treatments was significant on fertility hatchability ( $p < 0.05$ ). The highest fertility hatchability was observed in the processing method with *Neurospora Sitophila* fungi (85%).

**Table 2** – Effect of treatments on egg traits of Ross broiler breeder hens.

| Treatments   | Hen-day egg production (%) | Egg weight (g) | Egg mass production (g/hen/day) |
|--|----------------------------|----------------|---------------------------------|
| Unprocessed  | 61.86                      | 52.23          | 34.12                           |
| Processed by <i>Lactobacillus Plantarum</i> (Bacteria) | 62.90                      | 52.90          | 34.98                           |
| Processed by <i>Bacillus Subtilis</i> (Bacteria)       | 62.57                      | 53.01          | 34.26                           |
| Processed by <i>Aspergillus Oryzae</i> (Fungi)         | 61.97                      | 52.84          | 35.13                           |
| Processed by <i>Neurospora Sitophila</i> (Fungi)       | 62.13                      | 53.10          | 34.84                           |
| Processed by Alcalase enzyme                           | 62.30                      | 52.92          | 34.69                           |
| SEM  | 1.29                       | 1.63           | 1.01                            |
| <i>p</i> value   | 0.09                       | 0.19           | 0.24                            |

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

**Table 3** – Effect of treatments on fertility traits (%) of Ross broiler breeder hens.

| Treatments   | Fertility rate | Fertility hatchability | Total hatchability |
|--|----------------|------------------------|--------------------|
| Unprocessed  | 87             | 79 <sup>a</sup>        | 70                 |
| Processed by <i>Lactobacillus Plantarum</i> (Bacteria) | 89             | 83 <sup>b</sup>        | 73                 |
| Processed by <i>Bacillus Subtilis</i> (Bacteria)       | 88             | 83 <sup>b</sup>        | 72                 |
| Processed by <i>Aspergillus Oryzae</i> (Fungi)         | 90             | 84 <sup>b</sup>        | 73                 |
| Processed by <i>Neurospora Sitophila</i> (Fungi)       | 90             | 85 <sup>b</sup>        | 72                 |
| Processed by Alcalase enzyme                           | 90             | 84 <sup>b</sup>        | 72                 |
| SEM  | 0.52           | 0.61                   | 0.48               |
| <i>p</i> value   | 0.12           | 0.00                   | 0.10               |

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

### Caecum germs and pH

Experimental treatments had significant effects on germs and pH of the caecum ( $p < 0.05$ ). The use of processing methods reduced the population of salmonella and coliforms and increased the population of lactobacillus in the caecum. Most of the population of salmonella, lactobacillus, and coliforms of the caecum was observed in treatments of unprocessed, *Bacillus Subtilis* bacteria, and unprocessed, respectively (Table 4). The highest and lowest pH of caecum were related to unprocessed (6.82) and *Aspergillus Oryzae* fungi (6.21) treatments, respectively.


**Table 4** – Effect of treatments on microbial population of caecum ( $\log_{10}$  CFU/g).

| Treatments   | Salmonella        | Lactobacillus     | Coliforms         | pH                |
|--|-------------------|-------------------|-------------------|-------------------|
| Unprocessed  | 4.90 <sup>a</sup> | 3.85 <sup>b</sup> | 3.91 <sup>a</sup> | 6.82 <sup>a</sup> |
| Processed by <i>Lactobacillus Plantarum</i> (Bacteria) | 3.91 <sup>b</sup> | 4.51 <sup>a</sup> | 3.23 <sup>b</sup> | 6.27 <sup>b</sup> |
| Processed by <i>Bacillus Subtilis</i> (Bacteria)       | 3.85 <sup>b</sup> | 4.64 <sup>a</sup> | 3.20 <sup>b</sup> | 6.25 <sup>b</sup> |
| Processed by <i>Aspergillus Oryzae</i> (Fungi)         | 3.70 <sup>b</sup> | 4.59 <sup>a</sup> | 3.27 <sup>b</sup> | 6.21 <sup>b</sup> |
| Processed by <i>Neurospora Sitophila</i> (Fungi)       | 3.76 <sup>b</sup> | 4.63 <sup>a</sup> | 3.29 <sup>b</sup> | 6.24 <sup>b</sup> |
| Processed by Alcalase enzyme                           | 3.81 <sup>b</sup> | 4.49 <sup>a</sup> | 3.32 <sup>b</sup> | 6.25 <sup>b</sup> |
| SEM  | 0.06              | 0.10              | 0.09              | 0.13              |
| <i>p</i> value   | 0.00              | 0.00              | 0.00              | 0.00              |

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

### Carcass traits

The effects of canola meal process methods on carcass traits are presented in Table 5. The effect of experimental treatment had significant effect on abdominal fat ( $p < 0.05$ ). Processing methods of canola meal caused the reduction of abdominal fat. The highest decrease in abdominal fat was observed in the treatment that process with *Aspergillus Oryzae* fungi (1.89%).

## DISCUSSION

Canola contains anti-nutritional substances such as glucosinolate. Therefore, if canola is not processed before consumption, its nutritional value will be low and will have adverse effects on poultry health. In the present study, the processed canola by bacteria, fungi, and enzymes did not affect the egg traits of broiler breeder hens, which is in accordance with other reports (Goldberg *et al.*, 2016; Zhu *et al.*, 2019; Ziyad *et al.*, 2019).

Canola processing improved fertility traits. But there was no difference between the methods used with bacteria, fungi, and enzymes. The processed canola has increased the palatability of the ration by reducing

its antioxidants. On the other hand, phytate and available phosphorus have decreased and increased, respectively. Phosphorus has a positive effect on fertility traits. These results are consistent with other studies (Van Emous *et al.*, 2015; AL-Dhanki *et al.*, 2019; Ziyad *et al.*, 2019).

In a study, the decreasing effect of enzymatic hydrolysis of canola with Alcalase was reported on the microbial population of broiler ceca (Karimzadeh *et al.*, 2016b). In another study, the use of high levels of unprocessed canola had no effect on the microbial population of broiler ceca (Toghyani *et al.*, 2017). These results are consistent with the results of the present study. In the present study, canola processing reduced the cecal pH. Subsequently, conditions for the growth of beneficial bacteria and the destruction of harmful microbes in the caecum were provided and the microbial balance of the gastrointestinal tract improved.

In a study, rapeseed meal was processed by *Lactobacillus Acidophilus* and *Bacillus Subtilis*, and *Aspergillus Niger*. It was reported that processed meal increased carcass traits weight and reduced abdominal fat (Ashayerizadeh *et al.*, 2018). In another study, the use of high levels of unprocessed canola improved the carcass traits of broilers (Toghyani *et al.*, 2017). These findings are consistent with the results of the present study. Canola processing improves the protein quality and fatty acid profile and improves bird performance.

Canola processing improved fertility hatchability, which caused an increase in the number of chickens. On the other hand, by reducing the population of harmful microbes and increasing the population of beneficial intestinal microbes, it improves the ability to absorb nutrients. All of the above is due to the reduction in the anti-nutritional substances of canola, which is caused by processing. Thus, canola processing improved its digestibility. In other words, protein quality and fatty acid profiles 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.

**Table 5** – Effect of treatments on carcass traits (%).

| Treatments   | Breast | Thigh | Abdominal Fat     | Spleen | Liver | Heart |
|--|--------|-------|-------------------|--------|-------|-------|
| Unprocessed  | 33.21  | 28.76 | 2.73 <sup>a</sup> | 0.14   | 2.85  | 0.82  |
| Processed by <i>Lactobacillus Plantarum</i> (Bacteria) | 34.61  | 29.21 | 1.95 <sup>b</sup> | 0.14   | 2.80  | 0.85  |
| Processed by <i>Bacillus Subtilis</i> (Bacteria)       | 35.12  | 28.65 | 1.93 <sup>b</sup> | 0.13   | 2.79  | 0.85  |
| Processed by <i>Aspergillus Oryzae</i> (Fungi)         | 34.10  | 28.76 | 1.89 <sup>b</sup> | 0.14   | 2.78  | 0.87  |
| Processed by <i>Neurospora Sitophila</i> (Fungi)       | 35.31  | 29.12 | 1.94 <sup>b</sup> | 0.13   | 2.78  | 0.86  |
| Processed by Alcalase enzyme                           | 33.19  | 29.93 | 1.96 <sup>b</sup> | 0.14   | 2.81  | 0.87  |
| SEM  | 1.13   | 1.09  | 0.06              | 0.03   | 0.05  | 0.04  |
| <i>p</i> value   | 0.34   | 0.26  | 0.00              | 0.40   | 0.35  | 0.37  |

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



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