Influence of corn conditioning temperature and enzyme supplementation on growth performance, nutrient utilisation and intestine morphology of broilers fed mash corn-soy diets

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ABSTRACT
Two trials were conducted to investigate the effects of different corn conditioning temperature and enzyme supplementation on nutrient utilisation, growth performance, and small intestine morphology in broiler chickens. In trial one, nutrient utilisation were determined in basal diets. These diets contained unconditioned or conditioned corn (temperatures 55, 70 and 85 °C) supplemented with enzyme (Rovabio®) at the levels of 0 and 0.5 g/kg. Total excreta collection was performed with 144 chicks in 8 treatments with 6 replicates and 3 birds each, during 7–9 d. In trial two, 576 day-old broilers were distributed in 8 treatments, 6 pens and 12 chicks each. Birds were fed with diets containing conditioned corn in aforementioned temperatures and enzyme levels. A completely randomised design with 4 × 2 factorial arrangement was used in both trials. Conditioning of corn and enzyme supplementation had no significant effects on apparent metabolisable energy (AME) of corn and apparent total tract retention (ATTR) of corn nitrogen (N). In second trial, no significant differences were observed in growth performance of broilers during 1–24 d. Feed conversion ratio (FCR) in birds fed diets containing conditioned corn at 85 °C was significantly higher than that of 55 and 70 °C during 1–10 d. Corn conditioning significantly increased villus width (VW) and crypt depth (CD) but decreased VW:CD ratio. Enzyme supplementation increased jejunal villus height (VH), VW and ileal lactobacillus count and decreased pancreas weight at 24 d (p < .05). Generally, corn conditioning and enzyme supplementation did not significantly affect growth performance and carcase traits of broilers during 1–24 d, but improved histology and microbial population of small intestine.

HIGHLIGHTS
- Conditioning of corn used in mash diets of broiler chickens increased the population of bifidobacteria.
- Enzyme supplementation to the diets of broilers increased the height and width of intestinal villi.
- Enzyme supplementation to the diets of broilers decreased relative weight of pancreas.

Introduction
Pelleting is the most common form of heat processing in broilers feed production (Massuquetto et al. 2020). Many benefits that can be attributed to feed pelleting include improving nutrient availability, destroying inhibitors and toxins, facilitating the use of a wide range of raw materials in diet formulations, producing hygienic diet, and reducing feed wastage. At the same time, this method is costly, and if the usefulness of corn processing is proven, it may be possible to process only corn in mash corn-soy diets (Kiarie and Mills 2019).

Corn is one of the most important cereals used for poultry feeding due to its high starch content, available energy and protein. Starch is an important source of energy in cereals and it is important to pay attention to its availability. In the cell wall of the endosperm, cereal grains, contain some of the structural carbohydrates (often arabinoxylans in corn) that are soluble in the small intestine and have a high molecular weight which may cause viscosity (Sylvanus et al. 2015). Nowadays, different feed processing techniques have resulted in improved levels of nutrient digestibility. Physical and chemical changes by
steam conditioning, may have positive effects on nutrient utilisation (Silversides and Bedford 1999).

Conditioning is an important processing method and the most important step in broilers' feed processing is the conditioning of corn, which is done by adding hot steam to the ground corn because soybean meal is processed during oil extraction (Skoch et al. 1981). Balanced conditioning of broiler diets may affect the nutritional value of feed. This improvement may be associated with starch gelatinisation, destruction of heat-sensitive nutrients, destruction of cell walls, denaturation of digestive enzyme inhibitor proteins, and improvement of nutrient availability through the breakdown of protein, starch and fat (Abdollahi et al. 2010). Compared to balanced conditioning, high temperatures may cause adverse effects such as the Millard reaction which impairs nutrient digestibility (Silversides and Bedford 1999).

Commercial exogenous enzymes have been used in poultry diets for more than 30 years and they are now commonly used (Amerah et al. 2011). Some authors reported that supplementing carbohydrate enzymes could improve the nutrient utilisation in a corn-soybean meal based diet (Meng and Slominski 2005). Similar results were also observed in diets which were supplemented with a cocktail enzyme (Coppedge et al. 2012). Results indicated that NSP-degrading enzymes had the potential to improve growth performance and carcass traits of broiler chickens.

Several physical and chemical factors of grains may affect metabolisable energy of feed and consequently growth performance of the broilers, including viscosity and hardness (González-Alvarado et al. 2007), quality and quantity of starch, protein, unsaturated polysaccharides, and fat (Loar et al. 2014). Corn conditioning in corn-soy diets is one of the major ways to modify and improve the nutritional value of nutrients, especially metabolisable energy, starch and protein for poultry (Omede and Iji 2018) considering that soybean meal undergoes some sort of heat processing during oil extraction. Therefore, single ingredient processing of corn in poultry soy-corn mash diets may be a method to reduce feed processing costs.

Non-starch polysaccharides (NSP) content of corn vary with genetics and the environment where it is cultivated (Bach Knudsen 2014). Regardless of NSP content, some amounts of nutrients pass through the birds’ gut without being digested in corn-soy diets. Supplementing broiler diets with exogenous enzymes to degrade NSP has been a useful tool to release energy and nutrients (Cowieson 2010), which can increase the value of low quality corn in poultry feeds and improve growth performance (Cowieson 2005).

There is not sufficient data on the effect of conditioned corn incorporated in corn-soy diets in mesh form. Also, as far as the authors know, there is no available data that compares the effect of exogenous enzymes with corn processing in mesh diets. Thus the aim of this study was to investigate the effect of different conditioning temperatures of corn and enzyme supplementation to diets containing conditioned and unconditioned corn on the AME of corn and its effect on broiler performance in starter and grower periods, jejunal morphology and ileum microbial population.

**Materials and methods**

**Diets and treatments**

A batch of corn grains was obtained from a commercial supplier and ground by a hammer mill (Manufactured by Iranian Industrial Machine and Mill Production Company, 2019; 100 HP (75 kW) and 1480 rpm with 64 hammers) to pass through screen sizes of 3.0 and 5.0 mm for starter (1–10 d) and grower (11–24 d) periods, respectively. The ground batch was divided to four parts; one part remained unconditioned and three others were conditioned separately in temperatures of 55, 70 and 85 °C for 150 s by adjusting the steam flow rate in a commercial conditioner (Dordaneh Livestock and Poultry Feed Co, Chenaran, Iran). The conditioner had a capability of 1400 kg feed/h with two shafts, each having 16 pedals and an angle of 45 degrees. The steam pressure used in this conditioner was 2 bars. The steam entered through three steam injection valves for better and more uniform distribution, i.e. in the beginning, middle and end section of the conditioner. After applying heat treatment to ground corn, it was dried and cooled at 45 and 25 °C for 8 min in the dryer and the cooler, respectively. The conditioned corn was then bagged, marked and sent to the farm for use in experimental diets. In this experiment, only the corn used in the diets was conditioned and it was ground before being conditioned. The diets were not pelleted and the experimental diets were provided in mash form. Four of the experimental diets were supplemented with 0.5 g/kg of a multiple enzyme complex composed of cellulases, 6400 units; β-glucanase, 2000 units and Xylanase 22000 units per gram (Rovabio®, Adisseo, France) and 4 other diets were not supplemented with the enzyme. Enzyme complex was added on top and its matrix value was not considered for the formulation of the diets.
**Birds and management**

In trial one, AME and ATTR of corn N and dry matter (DM) were determined in basal diets (Mutucumarana et al. 2014; Beheshti Moghadam et al. 2020), in which the corn was the sole source of ME and N (Imari et al. 2020). Basal diets contained the conditioned (at temperatures of 55, 70 and 85 °C) or unconditioned corn and was or was not supplemented with the enzyme (at the level of 0.5 g/kg diet). Total excreta collection was performed with 144 male broiler chicks from Ross 308 strain in 8 treatments with 6 replicates and 3 birds each during 3 to 9 days of age using battery cages (Jiménez-Moreno et al. 2009).

On day 3, three male birds were individually weighed for each replicate and randomly distributed in battery cages (1 × 0.5 × 0.5 m; L × W × H). The birds were subjected to four days of adaptation to the experimental diets. The period of excreta collection started on day 7. The birds were deprived of feed 5 h before collection and the collection lasted 5 h after removing the experimental feeds. Plastic trays were put under the cages to collect the excreta. Experimental diets were weighed, on the first and on the final day of the collection period (day 9) to detect the feed intake (FI). Excreta from each replicate cage were collected early in the morning and in the evening. After removing feathers, feed residues, and other contamination sources, excreta were dried in an oven at 55 °C for 72 h. Then the excreta were weighed and homogenised, then a sample of approximately 30% of the excreta was randomly separated and kept at –20 °C for further analysis.

Then, dried excreta samples were ground in a micro-mill and submitted to the Animal Nutrition Lab. Feed and excreta DM, gross energy, and nitrogen contents were determined. The DM was determined according to Silva and Queiroz (2002). Gross energy was determined by a calorimetric bomb (1281, PARR Instruments, USA). Nitrogen content was determined by the method of Kjeldahl according to the method described by Silva and Queiroz (2002). The AME values were determined based on analytical results, according to Sakomura and Rostagno (2007).

In the second trial, 576 day-old broilers from Ross 308 strain with initial body weight (BW) of 43 g, were fed with mash diets containing conditioned corn which was or was not supplemented with the enzyme, during starter (1–10 d) and grower (11–24 d) periods. The experiment was done based on a completely randomised design with 4 × 2 factorial arrangement of treatments evaluating four corn conditioning temperatures (unconditioned and conditioned at 55, 70 and 85 °C) and two enzyme levels (0 and 0.5 g/kg diet). Day-old chicks were assigned in eight treatments with 6 floor pen replicates. Each floor pen of 1.2 × 1 × 0.8 m (L × W × H) included 12 chicks (6 male and 6 female, equally for all replicates). The floor pens were covered with 1.5 kg/m² of wood shavings.

Com-soy-based diets were formulated to meet the Ross 308 strain recommendations for major nutrients for starter and grower phases (Aviagen 2014; Table 1). Feed and water were supplied for ad libitum consumption throughout the trial with a tube feeder and two nipple drinkers in each floor pen. House temperature was initially set at 32 °C on day one and was decreased linearly by 0.5 °C per day to a temperature of 21 °C. During the experiment, the lighting program consisted of 18L:6D.

**Growth performance**

The group weight of birds in every pen was measured at the beginning and end of each feeding phase. The

| Table 1. Ingredients and nutrient composition of experimental diets, as-fed basis. |
|----------------------------------|-----------------|-----------------|
| **Ingredients (%)**              | **Diet for determination of nutrient utilisation** | **Starters** (1–10 d) | **Growers** (11–24 d) |
| **(trial 1)**                    | **(1–10 d)**    | **(11–24 d)**   |
| Corn (8.4% CP)                   | 96.22a          | 49.39a          | 52.51a          |
| Soybean meal (44% CP)            | –               | 41.52          | 37.90          |
| Soy Oil                          | –               | 4.37           | 5.42           |
| Calcium carbonate                | 0.94            | 0.98           | 0.93           |
| Dicalcium phosphate              | 1.87            | 2.20           | 1.74           |
| Vitamin and mineral premixb      | 0.5             | 0.5            | 0.5            |
| DL-Methionine                    | 0.38            | 0.38           | 0.35           |
| L-Lysine HCl                    | –               | 0.26           | 0.17           |
| L-Threonine                     | –               | 0.11           | 0.06           |
| Sodium chloride                  | 0.27            | 0.29           | 0.29           |
| Sodium bicarbonate               | 0.12            | 0.12           | 0.12           |
| Choline chloride (600 g/kg)      | 0.07            | 0.07           | 0.05           |
| Metabolisable energy (kcal/kg)   | 3223            | 3000           | 3100           |
| Crude protein                    | 8.17            | 23.0           | 21.5           |
| Calcium                          | 0.80            | 0.96           | 0.87           |
| Available phosphorus             | 0.39            | 0.48           | 0.435          |
| Methionine                       | 0.17            | 0.72           | 0.65           |
| Methionine + Cystine             | 0.34            | 1.08           | 0.99           |
| Lysine                           | 0.25            | 1.44           | 1.29           |
| Threonine                        | 0.28            | 0.97           | 0.88           |
| Sodium                           | 0.16            | 0.16           | 0.16           |
| Chloride                         | 0.21            | 0.27           | 0.25           |
| DCAD (mEq/Kg)c                   | 89.9            | 249.5          | 235.8          |
| Analysed values                  | 90.6            | 89.5           | 90.8           |
| Crude protein (N × 6.25)         | 8.10            | 22.56          | 19.5           |
| Calcium                          | 0.77            | 0.94           | 0.89           |
| Total phosphorus                 | 0.51            | 0.60           | 0.57           |

| aUnprocessed and processed corn at temperatures 55, 70 and 85 °C for 150 s with or without Rovabio® enzyme supplement were used to prepare 8 experimental diets. |
| Supplied per kilogram of diet: Vitamin A, 8,800U (retinyl acetate); Vitamin D3, 2,500U; Vitamin E, 11U (DL-α-tocopherol acetate); Vitamin K3, 1.9 mg; B1, 1.5 mg; B2, 4 mg; B3, 34.6 mg; B5, 7.9 mg; B6, 2.2 mg; B9, 0.48 mg; B12, 0.01 mg; H2, 0.15 mg; Choline chloride, 140.6 mg; Antioxidant, 1 mg; Mn, 75.2 mg; Fe, 75.0 mg; Zn, 65.5 mg; Cu, 6.01 mg; I, 0.86 mg; Se, 0.20 mg; Choline chloride, 140.6 mg. |
| Dietary cation-anion difference. |
average daily weight gain (ADG) of a replicate group during a specific period was calculated by subtracting the initial average weight of the group from its final average weight. The FI was calculated by subtracting the remaining feed from the offered feed in each pen during the period. The FCR (g/g) was calculated as the amount of feed consumed per unit of BW and was corrected for mortality. Mortality was recorded daily (Imari et al. 2020).

**Carcass yield**

After 6-h fasting at the end of the experiment (24 d of age), one male bird from each replicate was randomly selected and was killed by cervical dislocation. After peeling, carcase weight, breast yield, thighs, back and neck, wings, as well as internal organs including heart, spleen, gizzard, liver, pancreas, bursa of Fabricius, abdominal fat, duodenum, jejenum and ileum were weighed. Length of duodenum, jejenum and ileum were also measured.

**Intestinal histomorphology**

At the age of 24 d, one male bird from each replicate was selected and killed by cervical dislocation. About 1 cm section from the midpoint of jejenum was separated, flushed with 0.9% saline and was fixed in 10% buffered formalin and kept for further processing. Tissue samples were taken out from the solution and dehydrated by a series of graded ethanol solutions, cleared in xylene, and were infiltrated with paraffin. The infiltrated tissue samples were embedded in paraffin blocks. Imbedded tissue samples were sectioned at 5–6 μm thickness using a rotating microtome. The sections were floated in 40 °C distilled water, such that they were easily placed on the lamina after straightening the wrinkles. The slides were placed on a warm plate (45 °C), and while drying, the additional paraffin was melted. Slides were stained with Haematoxylin and Eosin. All chemicals were prepared from Sigma-Aldrich Co. (Sigma-Aldrich Chemical Co, St. Louis, MO).

Micrographs were taken with an Olympus BX41 optical microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera. The images were analysed using image software. Morphometric measurements were done on 10 healthy villi of each sample. Morphometric indices included VH from the tip of the villus to the crypt, VW (an average of VW at one-third and two-thirds of the villus), CD from the base of the villus to the submucosa, and muscular thickness (MT) from the submucosa to the external layer of the jejenum. The villus surface area (VSA) was calculated and expressed as μm (Garcia et al. 2007).

**Microbiological analysis**

On day 24, one male bird from each pen was euthanized by cervical dislocation. About 3 g of the ileum contents was sampled. To determine the microbial population, one gram of ileum content was used to make serial 10-fold dilutions using buffered peptone water. Lactobacillus were quantified on de Man Rogosa and Sharpe (MRS; Merck, Germany) agar and Rogosa agar (Engberg et al. 2002). Salmonella and coliforms (Morey et al. 2012) were quantified on BPLS agar and violet red bile (VRB; Merck, Germany) agar, respectively. All plates were incubated in an anaerobic cabinet at 37 °C for 24 h.

**Statistical analysis**

In order to investigate the effect of experimental treatments, a completely randomised design with 4 × 2 factorial arrangement was used in both trials. The main factors were included corn conditioning temperatures (unconditioned and conditioned at 55, 70 and 85 °C) and two enzyme levels (0 and 0.5 g/kg diet). All data were analysed for normality and homoscedacity (Shapiro and Wilk 1965) prior to statistical analysis. Data were analysed using general linear model (GLM) procedure, SAS software (2012) and the differences between means were tested by Duncan’s test (p < .05). Additionally, orthogonal contrasts were performed between the groups fed with diets containing conditioned corn and groups fed with diets containing unprocessed corn.

**Results**

**Nutrient utilisation**

Table 2 shows the effect of corn conditioning and enzyme supplementation on AME value, ATTR of N and DM in broiler chicks on 7–9 d. Conditioning and enzyme supplementation had no significant effects on AME value and ATTR of corn N (p < .05). Processing at 55 and 70 °C had no significant effect on ATTR of DM, but processing at 80 °C decreased it in comparison with the control group (p < .05). Enzyme supplementation had no significant effect on corn ATTR of DM. There were no significant interactions between corn conditioning and enzyme supplementation on AME value and ATTR of corn N and DM (p < .05). Orthogonal contrast analyses showed that
Table 2. Effect of corn conditioning and enzyme supplementation on apparent metabolisable energy (AME), apparent total tract retention of nitrogen (N) and dry matter (DM) in broiler chicks during 3–9 d.

| Treatments | AME (Kcal x kg⁻¹) | N (%) | DM (%) |
|------------|------------------|-------|--------|
| Main effects |                 |       |        |
| Temperature¹ |                 |       |        |
| Unprocessed | 3052.8           | 56.8  | 79.3²  |
| 55 °C      | 3057.7           | 55.0  | 77.2²  |
| 70 °C      | 3133.7           | 58.7  | 78.1¹  |
| 85 °C      | 3061.9           | 55.9  | 75.4³  |
| SEM        | 28.4             | 0.02  | 0.01   |
| Enzyme² |                 |       |        |
| -          | 3093.8           | 57.8  | 77.7   |
| +          | 3059.3           | 55.5  | 77.3   |
| SEM        | 20.1             | 0.01  | 0.01   |
| Interaction effects |           |       |        |
| Enzyme × Processing |             |       |        |
| -          | 3048.8           | 56.5  | 79.3   |
| 55 °C      | 3073.6           | 56.6  | 77.5   |
| 70 °C      | 3176.2           | 60.4  | 78.3   |
| 85 °C      | 3076.4           | 57.6  | 75.8   |
| SEM        | 40.2             | 0.02  | 0.01   |
| p Value |                 |       |        |
| Processing | .150             | .380  | .300   |
| Enzyme     | .230             | .140  | .600   |
| Processing × Enzyme | .710   | .760  | .980   |
| Orthogonal contrast analysis | |       |        |
| P vs. Unp. | .013             | .220  | .050   |
| E-P vs. E-Unp | .038  | .316  | .235   |
| E + P vs. E + Unp | .045  | .493  | .124   |

¹Unprocessed and processed corn at temperatures of 55 °C, 70 °C and 85 °C for 150 s.
²Rovabio® enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g x kg⁻¹ diet.
³P: processed; Unp: unprocessed; E-: without enzyme supplementation; E+: with enzyme supplementation.
⁴Values within a column with different superscripts differ (p < .05).
⁵SEM: standard error of the mean.

Conditioning of corn significantly increased (p < .01) its AME value compared to unconditioned corn (3084.4 vs. 3052.8 kcal/kg). When the processed corn without enzyme supplementation was compared with the unprocessed corn without enzyme supplementation, the AME value of the processed corn was significantly (p < .05) higher than that of unprocessed corn (3108.7 vs. 3048.8 kcal/kg). Also, comparison of enzyme-supplemented processed corn with enzyme-supplemented unprocessed corn showed that the AME value of processed corn was significantly (p < .05) higher than that of unprocessed corn (3060.1 vs. 3056.7 kcal/kg) (Table 2). These comparisons were not statistically significant (p > .05) regarding ATTR of N. But, orthogonal contrast showed that ATTR of DM in birds fed with unconditioned corn was higher than groups fed with conditioned corn (79.3 vs. 76.9%). Comparison of birds fed with processed corn without enzyme supplementation with the groups fed with unprocessed corn and without enzyme supplementation as well as comparison of enzyme-supplemented processed corn with enzyme-supplemented unprocessed corn did not show a significant difference (p > .05) regarding ATTR of DM.

### Growth performance and carcass traits

The effect of corn conditioning on BW, WG and FI during 1–10 d was not significant (Table 3). The FCR of the birds fed with diets containing conditioned corn at 85 °C was significantly (p = .012) more than that of 55 °C and 70 °C diets; however, it was not significantly different with the control group. The effect of the enzyme supplementation on BW, WG, FI and FCR was not significant during starter phase (Table 3).

Interaction effects of corn conditioning and enzyme supplementation on BW at d 10 and WG on 1–10 d were significant (p < .05); so that BW and WG in non-enzyme supplemented diets were decreased as the processing temperature increased and vice versa in enzyme supplemented diets. There were no significant interactions between corn processing and enzyme supplementation regarding FI and FCR during 1–10 d (Table 3). The orthogonal comparison for growth performance of birds during 1–10 d showed that diets containing processed corn without enzyme supplementation caused lower BW on 10 d and ADG during 1–10 d (p < .05) compared with the groups fed with diets containing unprocessed corn and without enzyme supplementation (240.8 vs. 255.8 g and 20.1 vs. 21.7 g/b/d, respectively). In contrast, comparison of enzyme-supplemented diets containing processed corn showed that birds fed with these diets had higher BW and ADG (p < .05) compared with birds fed with diets supplemented with enzyme and containing unprocessed corn (253.8 vs. 240.8 g and 21.4 vs. 20.4 g/b/d, respectively). Orthogonal comparisons between different groups did not show significant differences (p > .05) regarding FI and FCR during 1–10 d and BW, WG, FI and FCR during 11–24 and 1–24 d.

As shown in Tables 4–7, neither corn processing nor enzyme supplementation and their interactions had a significant effect on 11–24 and 1–24 d growth performance, carcass characteristics (Table 6) and gut morphology (Table 7) on 24 d. Except that, the enzyme addition significantly reduced the relative weight of the pancreas on 24 d compared with birds fed with diets without enzyme supplementation (p < .05). Orthogonal comparisons between different groups did not show significant differences (p > .05)
regarding carcase traits and internal organs (Table 6) and also gut morphology on 24 d. Except for gizzard relative weight on 24 d (Table 7) that its weight in groups fed with enzyme-supplemented diets containing processed corn is higher than \((p < .01)\) that of birds fed with diets supplemented with enzyme and containing unprocessed corn (2.2 vs. 1.87%).

**Jejunum morphology**

Effect of corn conditioning and enzyme supplementation on jejunum histomorphology in broiler chickens (24 d) is presented in Table 8. Corn conditioning at different temperatures had no significant effect on VH, but enzyme supplementation significantly increased it \((p < .05)\). Interaction effect of corn conditioning and enzyme supplementation on VH was significant \((p < .05)\). Jejunal VH of the birds fed with non-enzyme supplemented diets was decreased as the processing temperature increased and vice versa in enzyme supplemented diets.

Corn conditioning significantly affected VW, CD and VH to CD ratio (VH:CD) in the jejunum of broiler chickens at the age of 24 d \((p < .05)\). Villus width in the birds fed with diet containing conditioned corn in \(70^\circ C\) was significantly more than other treatments. Crypt depth in the jejunum of the birds fed with diet containing conditioned corn in \(55^\circ C\) and \(85^\circ C\) were significantly higher than the control group; however, this parameter in birds fed with diet containing conditioned corn at \(70^\circ C\) was not significantly different with the control group (Table 8).

The main factor of dietary enzyme supplementation significantly affected VH and CD. The VH and CD were significantly increased by enzyme addition to the diets \((p < .05)\). Crypt depth and VH:CD ratio did not respond to enzyme supplementation (Table 8).

Interaction effects of corn conditioning and dietary enzyme supplementation on VH at the age of 24 d

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**Table 3. Effect of corn conditioning and enzyme supplementation on the growth performance of broilers (1–10 day).**

| Treatments | Body weight (g/bird) | Weight gain (g/bird/day) | Feed intake (g/bird/day) | Feed conversion ratio (g/g) |
|------------|----------------------|--------------------------|--------------------------|-----------------------------|
| **Main effects** |                       |                          |                          |                             |
| **Temperature** |                       |                          |                          |                             |
| Unprocessed | 249.8                | 21.0                     | 22.4                     | 1.08<sup>ab</sup>           |
| 55°C       | 249.8                | 21.0                     | 22.3                     | 1.05<sup>b</sup>           |
| 70°C       | 246.7                | 20.8                     | 22.1                     | 1.07<sup>b</sup>           |
| 85°C       | 245.5                | 20.6                     | 21.1                     | 1.11<sup>+</sup>           |
| **SEM**    | 3.45                 | 0.33                     | 0.60                     | 0.01                        |
| **Enzyme** |                       |                          |                          |                             |
| –          | 244.6                | 20.5                     | 21.6                     | 1.09                        |
| +          | 251.3                | 21.2                     | 22.3                     | 1.07                        |
| **SEM**    | 2.44                 | 0.23                     | 0.42                     | 0.01                        |
| **Interaction effects** |                       |                          |                          |                             |
| Enzyme × Processing |                       |                          |                          |                             |
| –          | 255.8<sup>a</sup> | 21.7<sup>a</sup>     | 22.0                     | 1.07                        |
| 55°C       | 246.8<sup>b</sup> | 20.7<sup>b</sup>     | 22.5                     | 1.06                        |
| 70°C       | 238.2<sup>b</sup> | 19.9<sup>b</sup>     | 21.3                     | 1.1<sup>b</sup>            |
| 85°C       | 237.5<sup>b</sup> | 19.8<sup>b</sup>     | 20.6                     | 1.14                        |
| **SEM**    | 4.88                 | 0.47                     | 0.85                     | 0.02                        |
| **p Value** |                       |                          |                          |                             |
| Processing | .754                 | .746                     | .449                     | .006                        |
| Enzyme     | .057                 | .058                     | .229                     | .070                        |
| Processing × Enzyme | .016                 | .013                     | .656                     | .131                        |
| Orthogonal contrast analysis |                       |                          |                          |                             |
| P vs. Unp. | .199                 | .192                     | .956                     | .398                        |
| E-P vs. E-Unp | .041                 | .039                     | .562                     | .214                        |
| E + P vs. E + Unp | .021                 | .018                     | .755                     | .273                        |

<sup>1</sup>Each value represents the mean of six replicates (12 birds per replicate).
<sup>2</sup>Unprocessed corn and processed corn at temperatures of 55°C, 70°C and 85°C for 150 s.
<sup>3</sup>Rovabio<sup>V</sup><sup>R</sup> enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g × kg<sup>−1</sup> diet.
<sup>P</sup>: processed; <sup>Unp</sup>: unprocessed; <sup>E</sup>-: without enzyme supplementation; <sup>E +</sup>: with enzyme supplementation.
<sup>a,b</sup>Values within a column with different superscripts differ \((p < .05)\).

SEM: standard error of the mean.
was significant \((p < .05)\), such that VH increasing in the jejunum of the birds fed with enzyme supplemented diets by increasing processing temperature was more prominent than non-enzyme supplemented fed birds. In other words, the enzyme was more efficient in diets containing conditioned corn in higher temperatures. Interaction effects of enzyme and corn conditioning were not significant regarding VW, CD and VH:CD ratio (Table 8).

Orthogonal comparisons between different experimental groups showed that jejunal VH in birds fed with diets containing processed corn was higher than \((p < .05)\) that of groups fed diets containing unprocessed corn \((179.3 \text{ vs. } 155.7 \mu m)\). Other comparisons did not show significant differences (Table 8).

**Ileum microbial population**

Bifidobacteria, E. coli and clostridium population in the ileal contents were not affected by processing, enzyme supplementation and their interaction. As shown in Table 9, salmonella was not detected in the samples. However, processing of corn and enzyme addition to the broilers diet significantly affected the ileum lactobacillus population. Lactobacillus population was increased in the ileum of the birds fed with diets containing heat-treated corn at temperature \(70^\circ C\) compared with the control group. The orthogonal comparisons did not show significant differences in microbial count of ileum contents in broiler chickens at 24 days of age (Table 9).

**Discussion**

**Nutrient utilisation**

In the current study, conditioning of ground corn did not increase its AME value and ATTR of N. Processing at 55 and \(70^\circ C\) had no significant effect on ATTR of corn DM; however, \(85^\circ C\) reduced it compared to the control treatment. Jiménez-Moreno et al. (2009) cooked the corn by steam for 15 min at \(90^\circ C\), 10 min

| Table 4. Effect of corn conditioning and enzyme supplementation on the growth performance of broilers (11–24 day)a. | Body weight (g/bird) | Weight gain (g/bird/day) | Feed intake (g/bird/day) | Feed conversion ratio (g/g) |
|---|---|---|---|---|
| **Main effects** | | | | |
| **Temperature**b | | | | |
| Unprocessed | 952.4 | 50.2 | 72.2 | 1.55 |
| \(55^\circ C\) | 945.9 | 49.7 | 76.6 | 1.70 |
| \(70^\circ C\) | 954.4 | 50.5 | 75.7 | 1.67 |
| \(85^\circ C\) | 954.3 | 50.6 | 73.1 | 1.66 |
| SEM | 19.7 | 1.37 | 1.92 | 0.05 |
| **Enzymec** | | | | |
| – | 943.8 | 50.0 | 73.1 | 1.64 |
| + | 959.7 | 50.6 | 75.7 | 1.64 |
| SEM | 14.0 | 0.97 | 1.35 | 0.04 |
| **Interaction effects** | | | | |
| Enzyme \(\times\) Processing | | | | |
| – | 943.6 | 49.1 | 70.6 | 1.57 |
| \(55^\circ C\) | 973.5 | 51.9 | 77.1 | 1.70 |
| \(70^\circ C\) | 933.5 | 49.7 | 72.8 | 1.62 |
| \(85^\circ C\) | 924.6 | 49.1 | 71.8 | 1.69 |
| + | 961.3 | 51.3 | 73.8 | 1.53 |
| \(55^\circ C\) | 918.2 | 47.5 | 76.0 | 1.70 |
| \(70^\circ C\) | 975.3 | 51.4 | 78.7 | 1.53 |
| \(85^\circ C\) | 984.1 | 52.2 | 74.4 | 1.72 |
| SEM | 27.9 | 1.94 | 2.71 | 1.07 |
| **p Value** | | | | |
| Processing | .988 | .965 | .335 | .220 |
| Enzyme | .420 | .648 | .176 | .991 |
| Processing \(\times\) Enzyme | .196 | .222 | .639 | .715 |
| Orthogonal contrast analysis | | | | |
| P vs. Unp. | .750 | .497 | .129 | .144 |
| E-P vs. E-Unp | .990 | .486 | .323 | .159 |
| E + P vs. E + Unp | .958 | .757 | .402 | .130 |

aEach value represents the mean of six replicates (12 birds per replicate).

bUnprocessed corn and processed corn at temperatures of \(55^\circ C, 70^\circ C\) and \(85^\circ C\) for 150 s.

cRovabio enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g × kg\(^{-1}\) diet.

P: processed; Unp: unprocessed; E-: without enzyme supplementation; E+: with enzyme supplementation.

SEM: standard error of the mean.
at 117 °C and fed to broilers. They reported that AME value of cooked corn was higher than raw corn. Also, these researchers suggested that thermal processing may dissolve some of the hemicelluloses in corn and that by releasing sugars, some of them may become available to the bird, increasing the AME of corn. But in another experiment, the ileal digestibility of starch was not affected by the thermal processing in corn-based diets. In the study of Abdollahi et al. (2010) with increasing processing temperature in a wheat-based diet, ileal digestibility was decreased. In the present study, AME value and ATTR of N were not affected which may be due to the high natural digestibility of nutrients in corn. As a result, enzyme supplementation and thermal processing could not have a significant effect on nutrient utilisation. Abdollahi et al. (2011) stated that thermal processing of feed dissolves hemicelluloses, increases the starch gelatinisation and increases protein digestibility for broilers. Due to the high temperature, disulphide bridges break in the protein structure, causing denaturation and increased efficiency of endogenous enzymes.

In contrast, Massuquetto et al. (2018) found that steam conditioning of corn-soybean meal diet for 80 and 120 s improves protein digestibility. They stated that heat treatment breaks down disulphide bridges and denatures proteins, making proteases easier to access nutrients. However, in the case of DM, due to the heat applied during processing, this heat treatment may have been able to affect the ATTR of DM. Enzyme supplementation had no significant effect on ATTR of DM, N and AME value of corn. The interaction effect of corn conditioning with enzyme supplementation on AME value and ATTR of N and DM of corn was not significant, which may be due to high digestibility values of nutrients in corn-soy diets. The usefulness of carbohydrase enzymes in corn-soy-based diets for broiler is still unclear (Rabello et al. 2021).

Massuquetto et al. (2020) applied 84 °C for 13 s for corn conditioning and 105 °C for corn expansion. They found that DM digestibility increased in both processing methods compared with non-processed corn, which contradicts the results of this experiment. The reason for the decrease in ATTR of DM in this

| Treatments | Body weight (g/bird) | Weight gain (g/bird/day) | Feed intake (g/bird/day) | Feed conversion ratio (g/g) |
|------------|---------------------|--------------------------|--------------------------|-----------------------------|
| **Main effects** |                      |                          |                          |                            |
| Temperature |                    |                          |                          |                            |
| Unprocessed | 952.4               | 38.0                     | 51.4                     | 1.45                        |
| 55 °C       | 945.9               | 37.8                     | 52.7                     | 1.56                        |
| 70 °C       | 944.4               | 38.1                     | 53.0                     | 1.49                        |
| 85 °C       | 954.4               | 38.1                     | 51.2                     | 1.52                        |
| SEM         | 19.7                | 0.82                     | 1.22                     | 0.04                        |
| Enzyme      |                    |                          |                          |                            |
| –           | 943.8               | 37.7                     | 51.5                     | 1.49                        |
| +           | 959.7               | 38.3                     | 53.1                     | 1.52                        |
| SEM         | 14.0                | 0.58                     | 0.86                     | 0.03                        |
| **Interaction effects** |                |                          |                          |                            |
| Enzyme × Processing |                      |                          |                          |                            |
| –           | 943.6               | 37.7                     | 50.2                     | 1.43                        |
| 55 °C       | 973.5               | 38.9                     | 54.4                     | 1.53                        |
| 70 °C       | 933.5               | 37.3                     | 51.3                     | 1.46                        |
| 85 °C       | 924.6               | 36.9                     | 49.3                     | 1.54                        |
| +           | 961.3               | 38.4                     | 52.6                     | 1.47                        |
| 55 °C       | 918.2               | 36.6                     | 52.1                     | 1.59                        |
| 70 °C       | 973.3               | 39.0                     | 54.7                     | 1.53                        |
| 85 °C       | 984.1               | 39.4                     | 52.0                     | 1.49                        |
| SEM         | 27.9                | 1.16                     | 1.73                     | 0.06                        |
| **p Value** |                      |                          |                          |                            |
| Processing  | .988                | .980                     | .390                     | .366                        |
| Enzyme      | .420                | .423                     | .207                     | .538                        |
| Processing × Enzyme | .196          | .199                     | .562                     | .730                        |
| Orthogonal contrast analysis |                  |                          |                          |                            |
| P vs. Unp.  | .750                | .752                     | .213                     | .149                        |
| E-P vs. E-Unp | .990          | .980                     | .440                     | .214                        |
| E + P vs. E = Unp | .958     | .935                     | .708                     | .382                        |

*aEach value represents the mean of six replicates (12 birds per replicate).*

*bUnprocessed corn and processed corn at temperatures of 55 °C, 70 °C and 85 °C for 150 s.*

*cRovabio® enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g × kg⁻¹ diet.*

P: processed; Unp: unprocessed; E-: without enzyme supplementation; E+: with enzyme supplementation.

SEM: standard error of the mean.
experiment is not clear to the authors. Some investigations have shown that pelleting has little effect on nutrients digestibility, but more severe processing as expansion and extrusion, with higher moisture and higher temperatures in a short time, resulted in larger improvements in the feed structures. The results obtained in this study suggested that the processing time or temperatures in this study may not be suitable for changing the structure of corn to increase the utilisation of the diet.

**Growth performance and carcase traits**

Corn processing and enzyme supplementation had no significant effect on the growth performance of broilers. Only the interaction of corn processing and enzyme supplementation significantly affected BW at 10 days of age and WG in the starter period. In the study of Loar et al. (2014) a corn-soy diet was conditioned at temperatures 74, 85 or 96°C; none of the processing temperatures had a significant effect on FCR, which is in agreement with the results of the current study. The results obtained by Ghobadi and Karimi (2012) showed that enzyme supplementation to broilers diet in the period of 0 to 20 days of age had a significant effect on BW and WG. This is somewhat consistent with the results of this study as the use of enzyme supplement increased BW on 10 d and WG during the starter period. The beneficial effects of xylanase and amylase supplementation on intestinal viscosity and nutrient availability in diets based on cooked corn at 145°C for 10 min were more pronounced than unprocessed one (Svihus 2014). The results reported by Cowieson et al. (2005) showed that enzyme supplementation at the age of 0 to 21 days of broiler chickens did not have a significant effect on FI, but it had a significant effect on ADG, which is consistent with the present study. Some researchers have stated that cooking corn or dietary enzyme supplementation affects the growth performance of broilers

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Table 6. Effect of corn conditioning and enzyme supplementation on carcase characteristics of broiler chickens (24 day)1.

| Treatments | Edible carcase | Thighs | Breast yield | Neck + back | Liver | Pancreas | Heart | Spleen | Bursa of Fabricius | Abdominal fat pad |
|------------|---------------|--------|--------------|-------------|------|----------|-------|--------|------------------|------------------|
| Main effects | % of live body weight | | | | | | | | | |
| Temperature2 | | | | | | | | | | |
| Unprocessed | 60.8 | 18.0 | 23.6 | 19.1 | 2.53 | 0.37 | 0.57 | 0.10 | 0.20 | 0.64 |
| 55°C | 60.1 | 17.8 | 23.5 | 18.8 | 2.59 | 0.35 | 0.59 | 0.12 | 0.17 | 0.54 |
| 70°C | 60.9 | 18.4 | 23.3 | 19.2 | 2.45 | 0.35 | 0.54 | 0.12 | 0.18 | 0.74 |
| 85°C | 60.4 | 17.8 | 24.0 | 18.7 | 2.67 | 0.36 | 0.58 | 0.12 | 0.19 | 0.54 |
| SEM | 0.62 | 0.25 | 0.48 | 0.18 | 0.09 | 0.02 | 0.03 | 0.01 | 0.02 | 0.06 |
| Enzyme3 | | | | | | | | | | |
| Unprocessed | 60.3 | 17.8 | 23.8 | 19.6 | 2.52 | 0.38 | 0.59 | 0.12 | 0.18 | 0.60 |
| + | 60.8 | 18.2 | 23.3 | 19.3 | 2.60 | 0.34 | 0.55 | 0.11 | 0.19 | 0.63 |
| SEM | 0.44 | 0.18 | 0.34 | 0.13 | 0.06 | 0.01 | 0.02 | 0.01 | 0.01 | 0.04 |
| Interaction effects | | | | | | | | | | |
| Enzyme × Processing | | | | | | | | | | |
| Unprocessed | 59.9 | 17.6 | 23.6 | 18.7 | 2.40 | 0.40 | 0.63 | 0.12abc | 0.20 | 0.54 |
| 55°C | 60.8 | 18.0 | 23.7 | 18.4 | 2.58 | 0.38 | 0.61 | 0.14a | 0.13 | 0.52 |
| 70°C | 61.2 | 18.3 | 24.1 | 18.8 | 2.47 | 0.36 | 0.55 | 0.11abc | 0.20 | 0.73 |
| 85°C | 59.7 | 17.4 | 23.9 | 18.4 | 2.62 | 0.36 | 0.58 | 0.11abc | 0.20 | 0.59 |
| SEM | 0.88 | 0.36 | 0.69 | 0.26 | 0.13 | 0.02 | 0.04 | 0.01 | 0.02 | 0.08 |
| p Value | | | | | | | | | | |
| Processing | .780 | .321 | .857 | .240 | .390 | .730 | .630 | .440 | .638 | .072 |
| Enzyme | .390 | .243 | .310 | .105 | .411 | .031 | .084 | .288 | .394 | .608 |
| Processing × Enzyme | .490 | .400 | .600 | .950 | .720 | .670 | .494 | .046 | .065 | .368 |
| Orthogonal contrast analysis | | | | | | | | | | |
| P vs. Unp. | .591 | .380 | .951 | .545 | .262 | .098 | .121 | .702 | .612 | .540 |
| E-P vs. E-Unp | .668 | .488 | .743 | .563 | .253 | .315 | .327 | .611 | .484 | .458 |
| E + P vs. E + Unp | .299 | .558 | .457 | .323 | .690 | .583 | .264 | .106 | .552 | .223 |

1Each value represents the mean of six replicates.
2Unprocessed corn and processed corn at temperatures of 55°C, 70°C and 85°C for 150 s.
3Rovabio® enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g × kg⁻¹ diet.
P: processed; Unp: unprocessed; E−: without enzyme supplementation; E+: with enzyme supplementation.
abcValues within a column with different superscripts differ (p < .05).
SEM: standard error of the mean.
only at the age of 0 to 4 days (Abdollahi 2010). Processing of feed at the lower 85 °C is suitable for obtaining suitable growth performance in broilers and processing temperature at 95 °C leads to an increase in BW but a poorer FCR (Abdollahi 2010). In the study conducted by Abdollahi et al. (2010), increasing processing feed temperature caused increased FCR in birds. Nitrogen digestibility in corn-soy based diets processed at 75 °C was lower than those processed ones at 60 and 90 °C. Also, ileal digestibility of DM, N or starch in pelleted diets was not affected by processing time. In addition, these researchers observed that processing temperature did not affect the AME valve.

In this study, carcase traits of broilers were not affected by experimental treatments and their interactions. Only the addition of enzyme significantly reduced the pancreatic weight of the chicks. Rabello et al. (2021) supplemented corn-soy based diets with a carbohydrase and observed that carcase traits of broilers were not affected by the enzyme addition, which is in agreement with our findings. Ghobadi and Karimi (2012) evaluated the effects of feed processing (pelleted vs. mash) and enzyme supplementation (Grindazym) on broiler chick performance from 1 to 36 days of age. They also reported that neither feed processing nor enzyme supplementation had significant effects on the pancreas weight. In the present study, the enzyme supplement containing carbohydrases, pectinases and proteases could relieve the pressure on the pancreas and this secretory organ did not secrete excessively; thus, its relative weight was decreased.

The orthogonal comparison showed that gizzard relative weight in birds fed with diets containing processed corn was higher than that of unprocessed corn. There are conflicting reports comparing the effects of pelleted feeds with unprocessed mash diets on gizzard weight of broilers. Results of these studies showed that pelleting either reduced the weight of the gizzard or had no effect (Kiarie and Mills 2019). While in this study, in which only corn of diet was processed, processing increased the weight of gizzard.

### Table 7. Effect of corn conditioning and enzyme supplementation on gut morphology of broiler chickens at 24 days of age.

| Treatments                  | Proventriculus | Gizzard | duodenum | Jejunum | ileum | Small intestine | Duodenum | Jejunum | Ileum |
|-----------------------------|----------------|---------|----------|---------|-------|----------------|----------|---------|-------|
| % of live body weight cm/kg body weight | 0.62           | 2.01    | 1.05     | 1.70    | 1.25  | 190.8          | 31.5     | 76.7    | 82.6  |
| SEM                         | 0.04           | 0.08    | 0.05     | 0.07    | 0.05  | 5.77           | 2.92     | 2.15    | 2.39  |
| Enzyme                      |                |         |          |         |       |                |          |         |       |
| -                           | 0.61           | 2.16    | 1.02     | 1.80    | 1.32  | 189.4          | 32.0     | 77.6    | 79.9  |
| +                           | 0.61           | 2.12    | 1.03     | 1.73    | 1.22  | 189.3          | 34.5     | 75.4    | 79.5  |
| SEM                         | 0.03           | 0.05    | 0.03     | 0.05    | 0.03  | 4.08           | 2.07     | 1.52    | 1.69  |
| Interaction effects         |                |         |          |         |       |                |          |         |       |
| Enzyme × Processing         |                |         |          |         |       |                |          |         |       |
| Unprocessed                 | 0.69           | 2.14    | 1.00     | 1.70    | 1.30  | 192.4          | 31.6     | 77.0    | 83.8  |
| 55 °C                       | 0.56           | 2.21    | 1.06     | 1.97    | 1.36  | 203.5          | 35.9     | 84.5    | 83.1  |
| 70 °C                       | 0.61           | 2.00    | 0.94     | 1.79    | 1.28  | 180.1          | 29.0     | 74.8    | 76.4  |
| 85 °C                       | 0.56           | 2.27    | 1.08     | 1.75    | 1.32  | 181.8          | 31.5     | 74.0    | 76.3  |
| SEM                         | 0.06           | 0.10    | 0.06     | 0.10    | 0.07  | 8.16           | 4.14     | 3.05    | 3.38  |

*p Value

| Processing | .960 | .143 | .830 | .516 | .939 | .566 | .686 | .214 | .311 |
| Combination with Enzyme |        |      |      |      |      |      |      |      |      |
| Processing × Enzyme | .980 | .621 | .801 | .373 | .070 | .987 | .401 | .320 | .866 |

Orthogonal contrast analysis

- P vs. Unp. | .196 | .766 | .846 | .405 | .742 | .705 | .654 | .863 | .164 |
- E-P vs. E-Unp | .196 | .912 | .774 | .321 | .735 | .652 | .810 | .865 | .163 |
- E-P vs. E-Unp | .166 | .006 | .267 | .654 | .788 | .991 | .544 | .668 | .524 |

*a Each value represents the mean of six replicates.

*Unprocessed corn and processed corn at temperatures of 55 °C, 70 °C and 85 °C for 150 s.

*Rovabio enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g × kg⁻¹ diet.

P: processed; Unp: unprocessed; E-: without enzyme supplementation; E+: with enzyme supplementation.

SEM: standard error of the mean.
Jejunum morphology

The gastrointestinal tract’s (GIT) growth and development is a very important parameter for broiler chickens because the utilization of nutrients depends on it. Researchers have reported that broilers fed with conditioned diets compared to those fed with mash diets have a lower relative length of the digestive tract segments (Abdollahi et al. 2011, 2013). Although several studies showed that the birds fed with conditioned feed exhibit lower GIT segments length and weight, Amerah et al. (2007) and Zang et al. (2009) reported that VH and CD were higher in the intestine of broilers fed processed diets than those fed unprocessed mash diets. The villi increase the surface area of the small intestine, which greatly enhance the absorption of nutrients. The increased VH increases the total villus absorptive area and result in higher digestive enzyme action and increased transport of nutrients at the villus surface (Naderinejad et al. 2016). These researchers indicated that N digestibility in wheat-based pellets is lower than mash diets.

Abdollahi et al. (2013) showed that conditioned diets had no remarkable effects on N, starch, fat, Ca and P ileal digestibility in corn-based diets, whereas digestibility of all nutrients was reduced in wheat-based diets.

In the current study, the relative weight of the gizzard, proventriculus and different segments of the small intestine as well as the relative length of the small intestine and its segments were not affected by experimental treatments, which is in contradiction with the findings of Abdollahi et al. (2011, 2013). In addition, the enzyme supplementation to the broilers diet improved intestinal histological parameters, which is in agreement with previous findings (Amerah et al. 2007; Zang et al. 2009).

Ileum microbial population

In the current experiment, the conditioning of corn and dietary supplementation of carbohydase enzyme increased the number of lactobacilli in ileum contents.

Table 8. Effect of corn conditioning and enzyme supplementation on jejunum histomorphology of broiler chickens at 24 days of age.

| Treatments | Villus height (μm) | Villus width (μm) | Crypt depth (μm) | Villus height to Crypt depth |
|------------|--------------------|------------------|-----------------|-----------------------------|
| Main effects |                   |                  |                 |                             |
| Temperature |                   |                  |                 |                             |
| Unprocessed | 733.5              | 155.8b           | 155.3c          | 4.78a                       |
| 55 °C     | 737.0              | 176.3b           | 174.5b          | 4.30b                       |
| 70 °C     | 745.5              | 200.3a           | 166.8bc         | 4.51ab                      |
| 85 °C     | 767.2              | 161.5b           | 194.6a          | 3.94bc                      |
| SEM       | 15.8               | 7.78             | 6.49            | 0.20                        |
| Enzyme     |                   |                  |                 |                             |
| –         | 728.6b             | 164.3b           | 174.1           | 4.25                        |
| +         | 763.0a             | 182.6a           | 171.4           | 4.51                        |
| SEM       | 11.2               | 5.50             | 4.59            | 0.14                        |
| Interaction effects |               |                  |                 |                             |
| Enzyme × Processing |             |                  |                 |                             |
| –         | Unprocessed        | 731.0bc          | 149.0           | 157.0           | 4.76 |
| +         | 696.0bc            | 157.0           | 181.5           | 3.85 |
| 55 °C     | 760.5abc           | 188.0           | 171.5           | 4.49 |
| 70 °C     | 727.0bc            | 163.0           | 186.5           | 3.90 |
| 85 °C     | 736.0bc            | 162.5           | 153.5           | 4.79 |
| SEM       | 778.0abc           | 195.5           | 167.5           | 4.79 |
| 70 °C     | 730.5bc            | 212.5           | 162.0           | 4.52 |
| 85 °C     | 807.3a             | 160.0           | 202.7           | 3.98 |
| SEM       | 22.4               | 11.0            | 9.18            | 0.28 |
| Orthogonal contrast analysis |             |                  |                 |                             |
| P vs. Unp. | .520               | .049            | .076            | .143 |
| E-P vs. E-Unp | .918               | .204            | .076            | .074 |
| E + P vs. E + Unp | .194               | .100            | .075            | .274 |

1Each value represents the mean of six replicates.
2Unprocessed corn and processed corn at temperatures of 55 °C, 70 °C and 85 °C for 150 s.
3Rovabio® enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g x kg−1 diet.
4P: processed; Unp: unprocessed; E-: without enzyme supplementation; E+: with enzyme supplementation.
5Values within a column with different superscripts differ (p < .05).
6SEM: standard error of the mean.
but had no remarkable effects on the bifidobacteria population, E. coli and clostridium. Salmonella was also not found in the samples. In accordance with these results, Goodarzi et al. (2014) stated that the expansion of broilers feed might reduce the size of the microscopic structure of the particles, which in turn reduced the feed density and increased the solubility of the fibre, which may make fermentable carbohydrates available for lactobacilli. They reported that increasing the processing temperature led to an increase in the number of lactobacilli in the crop and ileum contents, followed by an increased lactate concentration in the ileal contents of chickens. At the same time, the population of clostridium and Enterobacteriaceae were not affected (Goodarzi et al. 2014).

Increasing processing temperature increased lactobacilli count in the crop and ileum contents, whereas clostridia and enterobacteria remained unaffected by hydrothermal processing (HTP) of feed. The impact of different HTP treatments in the crop and small intestine was mostly confined to lactobacilli and lactic acid concentration (Goodarzi et al. 2014). This study concluded that typical HTP applied in feed does not have a remarkable effect on the gut microbial dynamics in poultry. However, the number of studies investigating the effects of HTP on the microbiology of poultry intestine is limited. A better understanding of the effects of steam conditioning time and temperature manipulation can help manufacturers maintain the hygienic, physical and nutritional quality of feed in antibiotic-free feeding programs.

**Conclusions**

Processing of ground corn and enzyme supplementation to mash diets of broiler chicks did not improve AME value and nutrient utilisation of corn. The growth performance and carcase traits of broilers were not affected by corn processing, enzyme addition and

### Table 9. Effect of corn conditioning and enzyme supplementation on microbial count (Log10CFU/g) of ileum contents in broiler chickens at 24 days of age.

| Treatments | Lactobacillus | Bifidobacterium | Clostridium | Escherichia Coli | Salmonella |
|------------|---------------|-----------------|-------------|------------------|------------|
| Main effects |               |                 |             |                  |            |
| Temperature |               |                 |             |                  |            |
| Unprocessed | 6.44<sup>a</sup> | 5.19            | 1.57        | 4.73             | NF<sup>d</sup> |
| 55 °C       | 6.93<sup>ab</sup> | 5.23            | 1.52        | 4.69             | NF         |
| 70 °C       | 7.07<sup>a</sup> | 5.59            | 1.76        | 4.73             | NF         |
| 85 °C       | 6.40<sup>a</sup> | 5.21            | 1.52        | 4.58             | NF         |
| SEM         | 0.19           | 0.25            | 0.10        | 0.28             |            |
| Enzyme<sup>3</sup> |               |                 |             |                  |            |
| +           | 6.48<sup>a</sup> | 5.20            | 1.69        | 4.71             | NF         |
| –           | 6.93<sup>a</sup> | 5.41            | 1.50        | 4.66             | NF         |
| SEM         | 0.13           | 0.18            | 0.07        | 0.20             |            |
| Interaction effects |               |                 |             |                  |            |
| Enzyme × Processing |               |                 |             |                  |            |
| –           |                |                 |             |                  |            |
| +           | 6.19           | 4.97            | 1.55        | 4.61             | NF         |
| 55 °C       | 6.54           | 4.93            | 1.60        | 4.58             | NF         |
| 70 °C       | 7.12           | 5.78            | 1.92        | 5.05             | NF         |
| 85 °C       | 6.09           | 5.12            | 1.70        | 4.59             | NF         |
| SEM         | 0.27           | 0.35            | 0.14        | 0.40             |            |

**p Value**

| Processing | 0.044 | .639 | .330 | .979 |
| Enzyme     | 0.027 | .420 | .072 | .867 |
| Processing × Enzyme | .401 | .527 | .501 | .666 |

**Orthogonal contrast analysis**

| P vs. Unp. | .114 | .319 | .752 | .893 |
| E-P vs. E-Unp | .919 | .413 | .847 | .843 |
| E + P vs. E = Unp | .456 | .972 | .448 | .561 |

<sup>1</sup>Each value represents the mean of six replicates.
<sup>2</sup>Unprocessed corn and processed corn at temperatures of 55 °C, 70 °C and 85 °C for 150 s.
<sup>3</sup>Rovabio<sup>®</sup> enzyme was provided from Adisseo company (France) and supplemented at the rate of 0.50 g × kg<sup>−1</sup> diet.
<sup>4</sup>P: processed; Unp: unprocessed; E-: without enzyme supplementation; E+: with enzyme supplementation.
<sup>a,b</sup>Values within a column with different superscripts differ (p < .05).
<sup>d</sup>SEM: standard error of the mean.
<sup>d</sup>Not found.
their interaction. Conditioning of ground corn and dietary supplementation of Rovabio® enzyme complex used in mash diets improved histology of small intestine. Ileum microbial population was improved by enzyme addition in compare with birds fed with diets without enzyme supplementation.

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Ethical approval

The present study was approved by the Animal Ethics Committee of the Ferdowsi University of Mashhad, Mashhad, Iran.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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