Addition of a protease to low crude protein density diets of broiler chickens

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ABSTRACT
A total of 864 1-day-old Ross 308 broiler chickens with an average body weight of 40 ± 1.1 g were used to determine the effect of adding protease to broiler chickens diets with different crude protein (CP) density on growth performance, carcase characteristics, excreta microbiota, blood constituents, and nutrient digestibility. The chicks were allotted in pens with 18 birds/pen and 12 pen/treatment. Treatments were T1: basal diet, T2: T1 − 1.0% CP, T3: T2 + 0.05% protease, T4: T2 + 0.1% protease. Results showed that supplementing low CP diets with protease alleviated the negative effects of lowering dietary CP on BWG and FCR (P < .05) on days 1–35. Addition of protease to low CP diets improved BWG on days 1–35 (P < .05). Feeding the chickens with low CP diet reduced (P < .05) digestibility of dry matter (DM), but the addition of protease improved digestibility of DM. The results showed that lowering dietary CP had detrimental influence (P < .05) on digestibility of total essential amino acids (TEAA) and total non-essential amino acids (TNEAA). It is concluded that reduction of dietary CP had a negative effect on broiler chickens and the addition of protease alleviated the negative effects and improved chickens performance.

Introduction
Economic and environmental issues are two major factors affecting commercial animal agriculture. Increasing the price of feedstuffs and environmental pollution caused by excretion of excessive nutrients and the production of greenhouse gasses has exacerbated the need to reduce dietary nutrients. The use of synthetic amino acids and exogenous enzymes has played positive roles in reducing nutrients level of animal diets, especially the crude protein (CP) level, consequently resulting in lower cost of production without excessive excretion of nutrients. However, it is well-documented that lowering dietary CP level reduced the performance of poultry (Alleman and Leclercq 1997; Mohammadi Gheisar et al. 2011). Proteases are enzymes that can be synthesized in the gastrointestinal tract (GIT). Proteases can be classified into six groups based on their catalytic mechanisms: aspartic, glutamic, metalloproteases, cysteine, serine, and threonine proteases (Rawlings et al. 2004). Serine proteases isolated from Bacillus spp. are mostly used to produce commercial proteases. Lopez-Otin and Bond (2008) have stated that proteases have multiple functions, including regulating the fate, localization, and activity of many proteins, modulating protein–protein interactions, creating new bioactive molecules, contributing to the processing of cellular information, and generating, transducing, and amplifying molecular signals. Proteases can also influence DNA replication and transcription, cell proliferation and differentiation, tissue morphogenesis and remodeling, heat shock and unfolded protein responses, angiogenesis, neurogenesis, ovulation, fertilization, wound repair, stem cell mobilization, hemostasis, blood coagulation, inflammation, immunity, autophagy, senescence, necrosis, and apoptosis (Lopez-Otin and Bond 2008). Synthesized amount of proteases in GIT is generally considered to be sufficient for optimized feed protein utilization (Le Heurou-Luron et al. 1993; Nir et al. 1993). On the other hand, considerable amounts of crude protein might pass through the GIT without being completely digested (Parsons et al. 1997; Wang and Parsons 1998; Lemme et al. 2004, Angel et al. 2011). Many published studies have investigated the effect of multi-enzymes containing protease. However, the effect of each individual enzymes in multi-enzyme has rarely reported. A few published data have investigated individual proteases in poultry diet. It has been reported that the addition of protease into the diet can significantly improve the digestibility of amino acids (Angel et al. 2011; Liu et al. 2013). Kocher et al. (2003) suggested that the efficacy of proteases might be affected by the ingredients used in the diet. Some researchers have reported that benefit of protease may also be influenced by the presence of other enzymes such as xylanase and/or phytase (Sultan et al. 2011; Kalmendal and Tauson 2012). Reviewing the literature shows that the results of protease published to date are not consistent. Kocher et al. (2003) have stated that various functions of proteases may also depend on dietary formulation and ingredients used in the diet. Therefore, the objective of this study was to determine the influence of addition of a protease to low CP diets on the growth performance, nutrient digestibility, carcase characteristics, and excreta microbiota in broiler chickens.
Materials and methods

**Animals, housing, and diets**

Experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University, South Korea. A total of 864 1-day-old Ross 308 (as hatched) broiler chickens with an average initial BW of 40 ± 1.1 g were used in a 35-day trial period. Chicks were randomly assigned to pens with 18 birds/pen and 12 pen/treatment. Treatments were T1: basal diet, T2: T1 −1.0% dietary CP, T3: T2 + 0.05% protease, and T4: T2 + 0.1% protease. The birds were housed in 3-floor battery cages (1.55 × 0.75 × 0.55 m/cage), in an environmentally controlled room (temperature was started at 32°C and reduced by 2°C every week up to 24°C, and 65% relative humidity). Each cage was equipped with 2 feeders (one feeder in each side) and 2 nipple drinkers to provide feed and water ad libitum to birds. Diets were formulated to meet or exceed the nutrient requirements recommended by the National Research Council recommendations (Table 1; NRC 1994). The enzyme preparation used in this study was a commercial product (Ronozyme ProAct®, Novozymes A/S, Bagvaerd, Denmark) produced through submerged fermentation of Bacillus licheniformis.

| Item                              | Basal Low CP | Basal Low CP |
|-----------------------------------|--------------|--------------|
| **Ingredients (%)**               |              |              |
| Corn                              | 54.4         | 53.7         | 59.9         |
| Soybean, full fat                 | 0            | 10.8         | 6            |
| Soybean meal, 45%                 | 29           | 25.4         | 14.2         | 17.5         |
| Rapeseed meal, 38%                | 3            | 3            | 4            | 4            |
| Corn gluten                       | 3            | 2            | 2            |
| DDGS, corn                        | 0            | 4.24         | 5            |
| Tallow                            | 5            | 5            | 5            |
| Soybean oil                       | 0            | 0            | 0            |
| Limestone                         | 0.1          | 0.1          | 0.1          |
| Dicalcium phosphate               | 1.8          | 1.8          | 1.7          |
| Sodium chloride                   | 0.28         | 0.23         | 0.22         | 0.29         |
| NaCl                              | 0.1          | 0.1          | 0.1          |
| Methionine, 99%                   | 0.35         | 0.38         | 0.4          | 0.41         |
| L-Lysine, 24%                     | 1.61         | 1.85         | 1.35         | 1.42         |
| L-Threonine, 98.5%                | 0.19         | 0.22         | 0.19         | 0.21         |
| L-Tryptophan, 10%                 | 0.03         | 0.14         | 0            | 0.03         |
| Vitamin premixa                   | 0.1          | 0.1          | 0.1          |
| Mineral premix                    | 0.1          | 0.1          | 0.1          |
| Choline, 50%                      | 0.1          | 0.1          | 0.1          |
| CuSO4                             | 0.04         | 0.04         | 0.04         |
| **Calculated composition**        |              |              |
| DM (%)                            | 87.83        | 87.69        | 88.07        | 86.02        |
| ME (Mcal/kg)                      | 3.2          | 3.2          | 3.3          | 3.3          |
| CP (%)                            | 20.4         | 19.4         | 18.5         | 17.5         |
| EE (%)                            | 7.23         | 7.49         | 9.16         | 8.29         |
| Ca (%)                            | 0.90         | 0.91         | 0.90         | 0.90         |
| Total P (%)                       | 0.65         | 0.66         | 0.61         | 0.60         |
| Available P (%)                   | 0.44         | 0.45         | 0.41         | 0.41         |
| Lys (%)                           | 1.37         | 1.37         | 1.20         | 1.18         |
| Digestible Lys (%)                | 1.22         | 1.21         | 1.04         | 1.02         |
| Met + Cys (%)                     | 1.02         | 1.01         | 1.01         | 1.01         |
| Digestible Met + Cys (%)          | 0.90         | 0.89         | 0.90         | 0.88         |

*Provided per kg of premix: retinyl palmitate, 4.95 g; cholecalciferol, 0.09 mg; dl-a-tocopheroyl acetate, 37.5 mg; menadione sodium bisulphite, 2.55 mg; thiamine mononitrate, 3 mg; riboflavin, 7.5 mg; cyanocobalamin, 24 mg; niacin, 51 mg; folic acid, 1.5 mg; biotin, 126 mg; pantothenic acid, 13.5 mg.

Enzyme activity for this protease was measured in PROT units. One unit of protease was defined as the amount of enzyme that can release 1 µmol of p-nitroaniline from 1 µM of substrate per minute at pH 9.0 and 37°C. This protease was added to low CP diets at the concentration of 500 and 1000 mg/kg, providing 37,500 and 75,000 PROT units/kg, respectively. Diet samples were collected and sent to the enzyme manufacturer for protease analysis. Protease activity was determined using the method of DelMar et al. (1979).

**Sampling and measurements**

On days 0, 21, and 35, chickens and their remained feed were weighed to calculate body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). At the end of the experimental period, 12 birds were randomly selected from each treatment. They were weighed and slaughtered. Breast meat, abdominal fat, gizzard, liver, spleen, and Bursa of Fabricius were removed. Excess moisture from all samples was blotted and weighed. Hunter L* (lightness), a* (redness), and b* (yellowness) values of breast meat were measured using a Minolta CR410 chromometer (Konica Minolta Sensing Inc., Osaka, Japan). Drip loss percentage was determined at 1, 3, 5, and 7 days post-slaughter using procedures described by Honikel (1998). Duplicate pH values for each sample were measured using a pH meter (Fisher Scientific, Pittsburgh, PA). Fresh faecal samples were collected from cloacae into micro-tubes. The numbers of *Lactobacillus* and *Escherichia coli* colonies were counted. At the end of the experiment, faecal samples were collected and pooled. They were placed on ice and transported to the lab where analysis was immediately carried out. One gram of faecal sample from each bird was diluted in 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and homogenized. Viable counts of bacteria in the faecal samples were determined by plating serial 10-fold dilutions (in 1% peptone solution) onto McConkey agar plates (Difco Laboratories, Detroit, MI) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *E. coli* and *Lactobacillus*, respectively. The lactobacilli medium III agar plates were incubated at 39°C for 48 h under anaerobic conditions. The McConkey agar plates were incubated at 37°C for 24 h. The colonies of *E. coli* and *Lactobacillus* colonies were counted immediately after removing the plates from the incubator.

On day 28, 0.2% chromium oxide was added to all diets as an inert marker to determine apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and amino acids (AA). Birds were fed with diets mixed with chromium oxide during days 28–35. On day 35, faecal samples were collected from each pen and stored in a freezer at −20°C until analysis. For chemical analysis, faecal samples were thawed and dried at 60°C for 72 h. They were finely ground to a size smaller than 1 mm in diameter. All feed and faecal samples were analysed in duplicates for DM and N using a combustion N analyser (FOSS, DK-3400 Hilleroed, Denmark; methods 982.30a, 990.03, and 992.23; AOAC 2000). AA was specified in the ingredients. Chromium was analysed using UV absorption spectrophotometry (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan) based on the method of Williams et al. (1962). ATTD of nutrients was
calculated using the following formula:

\[
\text{Digestibility} (\%) = 1 - \left( \frac{Nf \times Cd}{Nd \times Cf} \right) \times 100,
\]

where \( Nf \) was the concentration of nutrient in faeces (%DM), \( Nd \) was the concentration of nutrient in the diet, \( Cd \) was the concentration of chromium in the diet, and \( Cf \) was the concentration of chromium in the faeces (Mohammadi Gheisar et al. 2015). The weights of breast meat, abdominal fat, and organs were expressed as percentages of live body weight.

### Statistical analysis

Data were analysed using GLM procedures of SAS (SAS Inst. 1996) as a completely randomized design. Orthogonal contrasts were used to test the overall effect of different levels of dietary CP (T1 vs. T2), effect of supplementing the basal diet and low CP diet with protease (T1 vs. T3&T4 and T2 vs. T3&T4, respectively), and effect of different concentrations of protease in diets (T3 vs. T4). Probability values of less than 0.05 were considered as statistically significant.

### Results

#### Growth performance

The results presented in Table 2 show that lowering dietary CP level had a detrimental effect on BWG and FCR \((P < .05)\). Addition of protease to low CP diets (T3 and T4 groups) significantly alleviated the negative effects of feeding the chickens with low CP diet (T2) and improved \((P < .05)\) BWG and FCR on days 1–21. During the overall experimental period feeding the chickens with low CP diets resulted in negative impacts on BWG and FCR \((P < .05)\). The results showed that the addition of protease to low CP diets improved BWG significantly \((P < .05)\) and a trend was observed in improving FCR \((P < .06)\).

Comparison of the chickens fed the diets containing 0.05% of protease (T3) and 0.1% protease (T4) showed that there was not any significant difference.

### Table 2. Effect of adding protease to low CP diet on growth performance and mortality rate in broilers.

| Items       | T1  | T2  | T3  | T4  | SE  | \( P \)-value |
|-------------|-----|-----|-----|-----|-----|--------------|
| Days 1–21   |
| BWG (g/bird) | 752.9 | 723.1 | 753.8 | 756.0 | 9.37 | 0.03 .86 .01 .87 |
| FI (g/bird)  | 1046.1 | 1048.8 | 1055.1 | 1046.7 | 16.41 | 0.91 .81 .92 .72 |
| FCR         | 1.39 | 1.45 | 1.40 | 1.39 | 0.02 | 0.05 .88 .03 .59 |
| Days 21–35  |
| BWG (g/bird) | 844.8 | 808.6 | 825.9 | 825.2 | 18.91 | 0.19 .41 .47 .98 |
| FI (g/bird)  | 1390.9 | 1408.4 | 1425.7 | 1408.1 | 20.26 | 0.55 .30 .73 .54 |
| FCR         | 1.65 | 1.75 | 1.73 | 1.72 | 0.04 | 0.09 .12 .66 .84 |
| Overall     |
| BWG (g/bird) | 1597.7 | 1531.8 | 1579.7 | 1581.2 | 18.15 | 0.02 .44 .04 .95 |
| FI (g/bird)  | 2437.0 | 2457.2 | 2480.8 | 2454.7 | 26.25 | 0.59 .35 .74 .49 |
| FCR         | 1.53 | 1.61 | 1.57 | 1.55 | 0.02 | 0.003 .09 .06 .49 |

Note: \# of observations: 12. T1: basal diet; T2: T1 \(-1.0\% \text{dietary CP; T3: T2 + 0.05\% protease; T4: T2 + 0.1\% protease; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; SE: Standard error of means.}

### Table 3. Effect of adding protease to low CP diet on nutrient digestibility in broilers.

| Items (%) | T1  | T2  | T3  | T4  | SE  | \( P \)-value |
|-----------|-----|-----|-----|-----|-----|--------------|
| Dry matter | 75.9 | 74.7 | 75.8 | 75.7 | 0.33 | 0.02 .72 .02 .85 |
| Nitrogen  | 64.3 | 63.1 | 64.0 | 63.9 | 0.71 | 0.24 .68 .34 .91 |
| Essential amino acid |
| Val       | 79.3 | 77.4 | 78.6 | 78.6 | 0.37 | 0.002 .18 .01 .99 |
| Met       | 79.2 | 77.5 | 78.9 | 78.7 | 0.83 | 0.18 .72 .22 .84 |
| Ile       | 79.6 | 75.5 | 77.9 | 77.7 | 0.75 | 0.001 .07 .82 .01 |
| Leu       | 82.1 | 80.1 | 81.7 | 81.8 | 0.36 | 0.02 .39 .06 .81 |
| Thr       | 79.4 | 79.9 | 80.2 | 80.7 | 0.78 | 0.67 .29 .57 .67 |
| Phe       | 80.1 | 79.0 | 79.7 | 79.7 | 0.43 | 0.09 .31 .32 .59 |
| His       | 82.2 | 82.1 | 82.6 | 81.5 | 1.01 | 0.96 .92 .97 .47 |
| Lys       | 79.4 | 77.7 | 78.5 | 78.7 | 0.60 | 0.06 .26 .26 .82 |
| Arg       | 80.1 | 79.4 | 79.7 | 79.7 | 0.44 | 0.25 .42 .59 .96 |
| Trp       | 80.2 | 78.6 | 78.5 | 78.5 | 0.95 | 0.25 .16 .91 .98 |
| TEAA      | 80.2 | 78.9 | 79.8 | 79.7 | 0.35 | 0.01 .28 .06 .95 |
| Non-essential amino acid |
| Tyr       | 81.4 | 80.7 | 81.8 | 81.8 | 0.49 | 0.30 .60 .09 .94 |
| Ser       | 79.5 | 78.2 | 79.2 | 79.0 | 0.63 | 0.16 .65 .24 .79 |
| Gln       | 78.7 | 77.9 | 78.1 | 78.0 | 0.38 | 0.15 .19 .73 .83 |
| Pro       | 82.0 | 80.6 | 82.6 | 82.1 | 1.19 | 0.42 .83 .25 .81 |
| Gly       | 79.4 | 78.6 | 78.7 | 78. | 1.07 | 0.62 .62 .94 .98 |
| Ala       | 81.1 | 80.6 | 82.0 | 81.8 | 0.64 | 0.54 .36 .11 .82 |
| Cys       | 81.4 | 79.5 | 80.4 | 80.5 | 0.46 | 0.01 .11 .11 .92 |
| Asp       | 80.8 | 79.0 | 79.2 | 79.2 | 0.59 | 0.05 .04 .83 .95 |
| TNEAA     | 80.1 | 79.0 | 79.7 | 79.6 | 0.37 | 0.05 .30 .18 .89 |

Note: \# of observations: 12. T1: basal diet; T2: T1 \(-1.0\% \text{dietary CP; T3: T2 + 0.05\% protease; T4: T2 + 0.1\% protease; SE: standard error of means.}
Our results showed that the BWG and FCR of chickens fed with low CP diet supplemented with either 0.05% or 0.1% of protease were significantly improved. In addition, during the 2nd phase of the experiment, feeding chickens with diets supplemented with different concentrations of protease failed to affect their growth performance. Yan et al. (2012) have reported that the effect of adding protease to the diet of broiler chickens during the starter phase is greater compared to that during other growth phases, suggesting that young animals might be more sensitive to supplementary protease, in agreement with the findings of this current study. It has been reported that adding protease to diet has a positive influence on the digestibility of amino acids (Angel et al. 2011; Liu et al. 2013). They have suggested that the beneficial effects of exogenous protease on AA digestibility might have derived from the ability to target protease inhibitors and/or targeting the cereal portion of the diet (Liu et al. 2013), Angel et al. (2011) have suggested that improvements in BWG and FCR are the results of improved digestibility of AA caused by the addition of protease were significantly improved. In addition, during the 2nd phase of the experiment, feeding chickens with diets supplemented with different concentrations of protease failed to affect their growth performance. Yan et al. (2012) have reported that the effect of adding protease to the diet of broiler chickens during the starter phase is greater compared to that during other growth phases, suggesting that young animals might be more sensitive to supplementary protease, in agreement with the findings of this current study. It has been reported that adding protease to diet has a positive influence on the digestibility of amino acids (Angel et al. 2011; Liu et al. 2013). They have suggested that the beneficial effects of exogenous protease on AA digestibility might have derived from the ability to target protease inhibitors and/or targeting the cereal portion of the diet (Liu et al. 2013), Angel et al. (2011) have suggested that improvements in BWG and FCR are the results of improved digestibility of AA caused by the addition of protease to the diet. It has been reported that feeding broiler chickens with diets supplemented with protease can lead to the improvement in digestibility for the majority of essential amino acids (Angel et al. 2011).

Nutrient digestibility

Comparison of dietary treatments (T1 vs. T2) showed that digestibility of DM was significantly reduced by lowering dietary CP level (P<.05), but the addition of protease to low CP diets (T3 and T4) improved DM digestibility (P<.05). Digestibility of nitrogen was not affected by dietary treatments. The results also showed that reduction in dietary CP level significantly reduced the digestibility of AA and the addition of protease led to a trend in improving AA digestibility (Table 3).

Carcase characteristics and excreta microbiota

Carcase characteristics assay (Table 4) demonstrated that dietary treatments did not affect meat quality parameters. The results of excreta microbiota presented in Table 5 showed that the counts of Lactobacillus and E. coli in excreta were not affected by dietary CP levels and supplementation of protease to the diet of broiler chickens.

Discussion

The findings of this current study were in agreement with the results of previous studies that feeding broiler chickens with low CP diets resulted in a detrimental impact on growth performance (Alleman and Leclerq 1997; Mohammadi Gheisar et al. 2011). Addition of a protease to the diet of broiler chickens has been helpful in optimizing the digestion of undigested protein passing through GIT (Parsons et al. 1997; Wang and Parsons 1998; Lemme et al. 2004; Angel et al. 2011). Some researchers have reported that the addition of protease to swine diet can improve their growth performance (Wang et al. 2011; Guggenbuhl et al. 2012; McAlpine et al. 2012). Our findings showed that the BWG and FCR of chickens fed with low CP diet supplemented with either 0.05% or 0.1% of protease were significantly improved. In addition, during the 2nd phase of the experiment, feeding chickens with diets supplemented with different concentrations of protease failed to affect their growth performance. Yan et al. (2012) have reported that the effect of adding protease to the diet of broiler chickens during the starter phase is greater compared to that during other growth phases, suggesting that young animals might be more sensitive to supplementary protease, in agreement with the findings of this current study. It has been reported that adding protease to diet has a positive influence on the digestibility of amino acids (Angel et al. 2011; Liu et al. 2013). They have suggested that the beneficial effects of exogenous protease on AA digestibility might have derived from the ability to target protease inhibitors and/or targeting the cereal portion of the diet (Liu et al. 2013), Angel et al. (2011) have suggested that improvements in BWG and FCR are the results of improved digestibility of AA caused by the addition of protease to the diet. It has been reported that feeding broiler chickens with diets supplemented with protease can lead to the improvement in digestibility for the majority of essential amino acids (Angel et al. 2011).

Conclusion

In conclusion, our results showed that it was possible to reduce dietary CP level without any negative effect on the growth performance of chickens by adding protease to the diet of broiler chickens. Adding exogenous protease was beneficial in improving the digestibility of AA, consequently improving the BWG and FCR. Our results also demonstrated that carcase characteristics and GIT microflora were not affected by the addition of exogenous protease to the diet of broiler chickens.

Table 4. Effect of adding protease to low CP diet on carcase characteristics in broilers.

| Items                           | T1          | T2          | T3          | T4          | SE       | T1 vs. T2 | T1 vs. T3&T4 | T2 vs. T3&T4 | T3 vs. T4 |
|--------------------------------|-------------|-------------|-------------|-------------|----------|-----------|-------------|-------------|-----------|
| pH value                       | 5.47        | 5.44        | 5.46        | 5.39        | 0.04     | 0.67      | .39         | .71         | .29       |
| Breast muscle colour           |             |             |             |             |          |           |             |             |           |
| Lightness (L*)                 | 49.11       | 49.09       | 49.32       | 48.97       | 0.53     | .99       | .95         | .93         | .64       |
| Redness (a*)                   | 11.73       | 11.92       | 12.00       | 11.64       | 0.19     | .50       | .71         | .68         | .20       |
| Yellowness (b*)                | 9.39        | 9.35        | 9.26        | 9.37        | 0.16     | .85       | .69         | .86         | .61       |
| Drip loss (%)                  |             |             |             |             |          |           |             |             |           |
| Day 1                          | 1.50        | 1.54        | 1.51        | 1.53        | 0.16     | .86       | .91         | .93         | .96       |
| Day 3                          | 4.32        | 4.30        | 4.28        | 4.33        | 0.23     | .96       | .95         | .99         | .89       |
| Day 5                          | 7.14        | 7.48        | 7.15        | 7.17        | 0.24     | .32       | .93         | .29         | .95       |
| Day 7                          | 9.40        | 9.94        | 9.47        | 9.42        | 0.30     | .21       | .90         | .19         | .92       |
| Relative organ weight (%)      |             |             |             |             |          |           |             |             |           |
| Breast muscle                  | 16.78       | 16.99       | 16.85       | 16.38       | 0.49     | .77       | .78         | .54         | .51       |
| Liver                          | 3.20        | 3.04        | 2.94        | 2.99        | 0.15     | .46       | .21         | .68         | .82       |
| Abdominal fat                  | 1.72        | 1.74        | 1.52        | 1.60        | 0.10     | .86       | .19         | .14         | .57       |
| Gizzard                        | 1.50        | 1.48        | 1.37        | 1.39        | 0.07     | .80       | .13         | .22         | .77       |

Note: # of observations: 12. T1: basal diet; T2: T1 – 1.0% dietary CP; T3: T2 + 0.05% protease; T4: T2 + 0.1% protease; SE: standard error of means.

Table 5. Effect of adding protease to low CP diet on excreta microflora in broilers.

| Items (log10 cfu/g) | T1    | T2    | T3    | T4    | SE    | T1 vs. T2 | T1 vs. T3&T4 | T2 vs. T3&T4 | T3 vs. T4 |
|---------------------|-------|-------|-------|-------|-------|-----------|-------------|-------------|-----------|
| Lactobacillus       | 7.57  | 7.51  | 7.54  | 7.55  | 0.05  | .43       | .73         | .57         | .97       |
| E. coli             | 6.52  | 6.57  | 6.56  | 6.55  | 0.03  | .20       | .36         | .56         | .88       |

Note: # of observations: 12. T1: basal diet; T2: T1 – 1.0% dietary CP; T3: T2 + 0.05% protease; T4: T2 + 0.1% protease; SE: standard error of means.
Disclosure statement

No potential conflict of interest was reported by the authors.

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