Growth performance and amino acid digestibility responses of broiler chickens fed diets containing purified soybean trypsin inhibitor and supplemented with a monocomponent protease

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ABSTRACT  Trypsin inhibitors (TI) resident in soybeans affects protein utilization. While heat treatment influences residual TI, it simultaneously affects the structure and solubility of the soybean proteins and confounds any response to exogenous proteases. Using purified TI, the effect of exogenous protease to TI can be dissociated from changes in the soybean protein. Thus, the current study was designed to evaluate the growth performance and protein utilization responses of broiler chickens to purified TI and exogenous protease. Soybean meal (SBM) was preanalyzed for basal TI (2,996 TIU/g SBM), formulated into nutritionally adequate experimental diets to contain 1,033 TIU/g diet, and purified TI was added at 9,000 TIU/g diet. A total of 320 Cobb-500 broiler chicks were allocated to 4 diets, with 8 replicate cages and 10 birds per replicate. The experimental diets were arranged as a 2 × 2 factorial with factors being dietary TI (1,033 or 10,033 TIU/g) and exogenous protease (0 or 15,000 PROT/kg). On day 7, 14, and 21 posthatching, protease supplementation improved the BW gain (P < 0.01) and gain to feed ratio (P < 0.05) of birds. On day 14 and 21 posthatching, the relative weight of pancreas increased (P < 0.05) with added TI but was reduced (P < 0.001) with protease supplementation. Apparent ileal digestibility of all amino acids, except methionine, decreased (P < 0.001) with added TI but increased (P < 0.05) with protease supplementation. Jejunal MUC-2 was downregulated (P < 0.01) and SCL7A-2 was upregulated (P < 0.05) by protease supplementation. Duodenal trypsin and chymotrypsin activities reduced (P < 0.05) with added TI but increased (P < 0.01) with protease supplementation. Exogenous protease produced longer villi (P < 0.05) and deeper crypts (P < 0.01) in the jejunal tissue. In conclusion, dietary addition of purified TI negatively affects nutrient utilization by broiler chickens. Furthermore, the study showed that the efficacy of the exogenous protease might be independent of dietary TI concentration.

Key words: broiler, gene expression, protease, soybean meal, trypsin inhibitor

INTRODUCTION  Although soybean meal (SBM) is an excellent protein feed ingredient for nonruminants, it contains several antinutritional factors, which may contribute to variance in its nutritional value. Among these are the protease inhibitors, specifically of the Bowman-Birk and Kunitz types. Both inhibitors have considerable antinutritive effects which impedes the activation of gastrointestinal proteolytic enzymes, thereby affecting dietary protein digestion. In soybeans, the Kunitz-type inhibitor is bigger (>20 kDA), found in much larger concentrations, and acts by forming stable stoichiometric complexes with the digestive enzyme trypsin and chymotrypsin (Liener, 1994). The resulting noncovalent complex renders the proteases inactive and significantly reduces the digestibility and utilization of proteins and amino acids (AA) by nonruminants (Rawlings et al., 2004). Although the inhibitors are heat labile and can be deactivated by heat treatment, excessive processing can negatively influence the nutritional quality of SBM, necessitating care in the management of processing conditions (Newkirk, 2010).

Supplementing poultry diets with exogenous proteases may be a complementary strategy to improve the digestibility of SBM for nonruminant animals (Ghazi et al., 2002, 2003; Clarke and Wiseman, 2005; Costa
et al., 2008; Erdaw et al. 2017). One potential mode of action of microbial protease is by outcompeting the trypsin inhibitor (TI) for active sites, thereby improving the overall protein and AA utilization. Alternatively, the microbial protease could also destroy or inactive the TI. Huo et al. (1993) found that fungal and bacterial protease enzymes could inactivate TI in raw soybean and low-temperature extruded soybean in vivo. Rooke et al. (1998) incubated soybean meal using 0.1% acid protease for 3 h at 50°C and pH 4.5 and reported fewer antigenic proteins compared with the nonprotease treatments. In another study, dietary addition of monocomponent protease reduced the SBM-specific antibodies in serum of broiler chicks fed a corn–SBM–based diet (Ghazi et al., 2002).

Previous work to explore the potential for microbial protease to reduce the antinutritional effect of TI in SBM have been conducted using variably heat-treated SBM. Thus, the effect of the exogenous protease on utilization of the soybean protein may be confounded by the level and or method of processing. Hence, by the dietary addition of a purified source of soybean TI, it becomes possible to clearly delineate any direct effects of the exogenous protease on growth performance and nutrient utilization of the birds.

To our knowledge, there has not been any previous reports in literature that assess the efficacy of exogenous protease on nutrient utilization of birds fed diets containing purified TI. Therefore, the hypothesis of the current study was that purified soybean TI and exogenous protease will not affect the nutrient utilization and performance of broiler chickens. To test this hypothesis, specific objectives were set out to 1) determine the effect of dietary addition of purified soybean TI on growth performance, nutrient utilization, intestinal morphology, and enzyme secretion of broiler chickens from day 0 to 21 posthatching and 2) evaluate the effect of exogenous protease administration in diets containing purified TI on growth performance, nutrient utilization, intestinal morphology, and enzyme secretion of broiler chickens from day 0 to 21 posthatching.

A total of 320 male 0-day-old broiler chicks (Cobb 500, Siloam Springs, AR) were purchased from a commercial hatchery. Birds were individually tagged, weighed, and raised in heated battery brooders (model SB 4 T; Alternative Design Manufacturing, Siloam Springs, AR) with temperature and lighting maintained as previously described by Park et al. (2017). Using a 2 × 2 factorial arrangement, with 2 concentrations of dietary TI (1.033 or 10.033 TIU/g) and exogenous protease (0 or 15,000 PROT/kg; Ronozyme ProAct, DSM Nutritional Products, Kaiseraugst, Switzerland), the chicks were alloted to 4 experimental diets (Table 2) in a randomized complete block design, each diet with 8 replicates cages and 10 birds per replicate cage. All diets were corn–SBM–based, formulated to meet breeder nutrient specifications, and fed as mash. All diets were formulated to be nutritionally equivalent in terms of energy, protein, calcium, phosphorus, and dietary electrolyte balance. All diets contained phytase (RONOZYME HiPhos, DSM Nutritional Products) at 1,000 FYT/kg, and titanium dioxide was included as an indigestible marker.

**Sampling Procedures**

For growth performance assessment, feed and water was available ad libitum during the 21-D experimental period. Body weights and feed intake was recorded weekly on day 7, 14, and 21 posthatching, and mortality records was taken daily. Gain to feed ratio was calculated and corrected for the body weight of any bird that died or was culled during the experimental period. On day 7 and 14 posthatching, 2 birds (heaviest and lightest) from each cage were selected and euthanized by CO₂ asphyxiation. The pancreas, liver, and duodenal loop were excised for respective weight and length measurements. On day 21 posthatching, the 6 birds remaining in each cage were euthanized by CO₂ asphyxiation. Ileal digesta was collected from the distal two-thirds of the ileum (i.e., from the Meckel’s diverticulum to approximately 2 cm cranial to the ileocecal junction), by flushing with distilled water into plastic containers and stored at −20°C before nutrient analyses.

**Intestinal Morphological Analysis**

On day 21 posthatching, mid-jejunal segments were collected from 1 bird per replicate with median BW, flushed with ice-cold 10% phosphate-buffered saline (VWR International, Radnor, PA) and fixed in 10% neutral buffered formalin (VWR International) for approximately 30 D. Samples were subsequently dehydrated with ethanol (VWR International), cleared with Sub-X (Polysciences, Inc., Warrington, PA) and placed in paraffin (Polyfin paraffin, Sigma Polysciences, St. Louis, MO). The segments (5 μm) were stained with hematoxylin and eosin at the Purdue Histology and Phenotyping Laboratory (Purdue University, West Lafayette, IN). Villus height and crypt depth were measured from 4 complete, vertically oriented villi per slide and villus height to crypt depth ratio was calculated. Villus...
length is defined as the length from the villus tip to the valley between each villus, whereas crypt depth is defined as the length between the crypt opening and base. All measurements were performed under a binocular light microscope (National Optical and Scientific Instruments, Inc., Schertz, TX).

**Digestive Enzyme Assay**

Duodenal digesta and the pancreas was collected from 1 bird per replicate with median BW day 21 posthatching, frozen in liquid nitrogen, and stored at −80°C until required for assay. Enzymes activities were determined using a commercially available assay kit (Sigma Chemical Co., St. Louis, MO). The absorbance of the colorimetric final product was measured in a UV/visible spectrophotometer, and the concentration of the respective enzymes was calculated accordingly. For duodenal digesta, the samples were centrifuged at 13,000 rpm at 4°C for 10 min, and aliquots of the supernatant was used for enzyme assay. The activity of the pancreatic enzymes was determined after the whole organ was homogenized in appropriate buffers and centrifuged at 13,000 rpm at 4°C for 10 min to get a clear supernatant. Trypsin activity (EC 3.4.21.4) was determined with benzoyl-DL-arginine p-nitroanilide as substrate (Sigma Aldrich, CN MAK290). The product of p-nitroaniline was measured at an absorbance of 405 nm. One activity unit of trypsin was expressed as nanomoles of p-nitroaniline released per minute per milligram of protein.

**Total RNA Extraction and Reverse Transcription**

On day 21 posthatching, a section of the jejunum from 1 bird per replicate with median BW was removed and flushed with ice-cold PBS (VWR International), cut longitudinally in half exposing the lumen, and mucosal contents were scraped with a metal spatula. Mucosal contents were immediately placed in 2 mL of Trizol reagent (Invitrogen, Grand Island, NY) and stored at −80°C until RNA isolation. Total RNA was extracted from the tissues using Trizol reagent (Invitrogen) following the manufacturer’s protocol. RNA concentrations were determined by NanoDrop 1000 (Thermo Scientific, Waltham, MA), and RNA integrity was verified by 1% agarose gel electrophoresis. To prevent the contamination of the DNA, extracted RNA was purified with DNase-free DNase Treatment and Removal Kit (Ambion, Austin, TX). Afterwards, 2 μg of total RNA from each sample were reverse transcribed into cDNA using the MMLV reverse transcription system (Promega, Madison, WI). The cDNA was then diluted 1:10 with nuclease-free water (Ambion) and stored at −20°C until use.

**Quantitative Real-time PCR Analysis**

Real-time PCR was performed with Bio-Rad iCycler with the Faststart SYBR green-based mix (Life Technologies, Carlsbad, CA). PCR programs for all genes were designed as follows: 10 min at 95°C; 40 cycles of 95°C for 30 s, primer-specific annealing temperature for 30 s, and 72°C for 30 s, followed by melting curve analysis. The primer sequences used in the current study are listed in Table 3. Primer specificity and efficiency were verified, subsequently the samples were analyzed in duplicate, and a difference lesser than or equal to 5% was acceptable. Relative gene expression was calculated using the 2−ΔΔCt method (Livak and Schmittgen, 2001) with normalization against glyceraldehyde 3-phosphate dehydrogenase as the housekeeping gene (Tan et al., 2014).

**Chemical Analyses**

Ileal digesta samples were freeze-dried for 96 h and subsequently ground to pass through a 0.5-mm screen (Retsch ZM 100, GmbH, Haan, Germany). For DM analysis, diets and ileal digesta samples were analyzed by drying overnight at 105°C (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006), and the nitrogen (N) content of the samples was subsequently determined by combustion using a LECO FP-428 nitrogen analyzer (LECO Corp., St. Joseph, MI) with EDTA.
as a calibration standard. Samples for AA analysis were prepared using a 24-h hydrolysis in 6 N hydrochloric acid at 110°C under an atmosphere of N. Samples were oxidized in performic acid before acid hydrolysis for methionine and cysteine analyses. Samples for tryptophan analysis were hydrolyzed using barium hydroxide.

Table 2. Ingredient and calculated nutrient composition of experimental diets, as-fed basis.

| Ingredients, g/kg | 0          | 15,000    | 0          | 15,000    |
|------------------|------------|-----------|------------|-----------|
| Purified trypsin inhibitor, TIU/g: | 0          | 9,000     | 0          | 9,000     |
| Protease, PROT/kg: | 0          | 15,000    | 0          | 15,000    |
| Corn              | 562.6      | 552.6     | 512.6      | 502.6     |
| Soybean meal      | 345.0      | 345.0     | 345.0      | 345.0     |
| Soybean oil       | 18.0       | 18.0      | 18.0       | 18.0      |
| Monocalcium phosphate | 11.0   | 11.0      | 11.0       | 11.0      |
| Lime                | 13.5       | 13.5      | 13.5       | 13.5      |
| Salt                | 2.8        | 2.8       | 2.8        | 2.8       |
| Vitamin-mineral premix | 3.0    | 3.0       | 3.0        | 3.0       |
| α-Methionine       | 2.1        | 2.1       | 2.1        | 2.1       |
| L-Lysine HCl       | 2.2        | 2.2       | 2.2        | 2.2       |
| Threonine          | 1.5        | 1.5       | 1.5        | 1.5       |
| Tryptophan         | 0.3        | 0.3       | 0.3        | 0.3       |
| NaHCO₃             | 3.0        | 3.0       | 3.0        | 3.0       |
| Trypsin inhibitor premix | 0.0    | 0.0       | 50.0       | 50.0      |
| Ronozyme ProAct premix | 0.0    | 10.0      | 0.0        | 10.0      |
| Ronozyme HiPhos premix | 10.0  | 10.0      | 10.0       | 10.0      |
| Titanium dioxide premix | 25.0  | 25.0      | 25.0       | 25.0      |
| Total              | 1,000      | 1,000     | 1,000      | 1,000     |

Calculated composition

| Crude protein, g/kg | 222.34 | 222.34 | 222.34 | 222.34 |
| ME, kcal/kg         | 3,000  | 3,000  | 3,000  | 3,000  |
| Ca, g/kg             | 7.95   | 7.95   | 7.95   | 7.95   |
| P, g/kg              | 6.11   | 6.11   | 6.11   | 6.11   |
| Nonphytate P, g/kg   | 3.54   | 3.54   | 3.54   | 3.54   |
| Ca:total P           | 1.30   | 1.30   | 1.30   | 1.30   |
| Na, g/kg             | 2.34   | 2.34   | 2.34   | 2.34   |
| K, g/kg              | 9.66   | 9.66   | 9.66   | 9.66   |
| Cl, g/kg             | 3.72   | 3.72   | 3.72   | 3.72   |
| Dietary electrolyte balance, mEq/kg | 244.49 | 244.49 | 244.49 | 244.49 |

Digestible amino acids, g/kg

| Arg                | 13.23  | 13.23  | 13.23  | 13.23  |
| His                | 5.20   | 5.20   | 5.20   | 5.20   |
| Ile                | 8.34   | 8.34   | 8.34   | 8.34   |
| Leu                | 17.38  | 17.38  | 17.38  | 17.38  |
| Lys                | 12.24  | 12.24  | 12.24  | 12.24  |
| Met                | 5.19   | 5.19   | 5.19   | 5.19   |
| Cys                | 3.81   | 3.81   | 3.81   | 3.81   |
| Phe                | 9.49   | 9.49   | 9.49   | 9.49   |
| Tyr                | 7.36   | 7.36   | 7.36   | 7.36   |
| Thr                | 8.61   | 8.61   | 8.61   | 8.61   |
| Thr                | 2.89   | 2.89   | 2.89   | 2.89   |
| Val                | 9.04   | 9.04   | 9.04   | 9.04   |
| Total sulfur amino acids | 9.00   | 9.00   | 9.00   | 9.00   |
| Phe + Tyr          | 16.85  | 16.85  | 16.85  | 16.85  |

Analyzed composition

| Crude protein, g/kg | 232.1  | 233.0  | 237.8  | 238.6  |
| Crude fiber, g/kg   | 24.7   | 25.2   | 25.2   | 25.0   |
| Ether extract, g/kg  | 36.5   | 35.7   | 36.2   | 34.1   |
| Protease, PROT/kg*  | LOD    | 16,760 | LOD    | 17,010 |
| Trypsin inhibitor, TIU/g | 1,181 | 1,218  | 8,882  | 8,833  |

*16% Ca, 21% P.
*38% Ca.
*Supplied the following per kg diet: vitamin A, 5,484 IU; vitamin D3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulphite, 4.38 mg; riboflavin, 5.49 mg; pantothenic acid, 11 mg; nicin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 μg; biotin, 55.2 μg; thiamine mononitrate, 2.2 mg; folic acid, 990 μg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 44.4 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 μg.
*1Purified trypsin inhibitor (PTI) from soybeans product contains 9,000,000 TIU/g. 1 g of PTI added to 49 g of corn supplied 180,000 TIU/g of premix. 50 g premix delivered 9,000,000 TIU/kg feed. Product contained 75,000 PROT/g. 1 g Protease added to 49 g ground corn supplied 15,000 PROT/kg feed. 10 g premix delivered 15,000 PROT/kg feed.
*2Phytase product contained 5,000 units/g. 1 g of phytase added to 49 g of ground corn supplied 100 units/g of premix. 10 g premix delivered 9,000 units/kg feed. 1,000 units/kg supplied 1.5 g P/kg and 1.7 g of Ca/kg.
*31 g of Titanium dioxide added to 4 g of corn.
*LOD = limit of detection.
Amino acids in hydrolysates were determined by cation-exchange chromatography coupled with postcolumn derivatization (AOAC, 2000; method 982.30 E [a, b, c]). Titanium concentration was measured on a UV spectrophotometer following the method of Short et al. (1996).

The index method was used to calculate the apparent ileal digestibility (AID) of N and AA, according to the following equation:

\[
\text{AID} \%, \% = 100 - [(\text{Ti}_i / \text{Ti}_o) \times (\text{P}_o / \text{P}_1) \times 100]
\]

where \(\text{Ti}_i\) is Titanium concentration in diets; \(\text{Ti}_o\) is Titanium concentration in output (ileal digesta); \(\text{P}_1\) is N or AA concentration in diets; and \(\text{P}_o\) is N or AA output in ileal digesta.

### Statistical Analyses

Data were analyzed as a randomized complete block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Initial body weight was used as the blocking criterion. The main effects of dietary TI and protease concentrations and the interaction were tested accordingly, and an \(\alpha\) level of 0.05 was considered significant. Where interactions exist, Tukey’s mean separation test was used to make pairwise comparisons.

#### Table 3. Primers used in real-time quantitative PCR.

| Genes              | Primer sequence (5’to 3’) | Gene Bank ID     | Reference          |
|--------------------|---------------------------|------------------|--------------------|
| Housekeeping gene  |                           |                  |                    |
| GAPDH F: TCCTAGGATACACAGGAGCCA R: CGGTGTCATATGCAAATCA | ENSGALG0000001442 | Grenier et al., 2015 |
| Markers of inflammation |                       |                  |                    |
| IL-1β F: GCATCAAGGGCTCAAGCTC R: CAGGGGGTAGGAAGATGAGC | NM_204524 | Adedokun et al., 2012 |
| IL-8 F: GGGGCCCCCCTGCAAGAAT R: TCACAGTGGGTGCATCAAGAATGAGC | ENSGALG00000001670 | Grenier et al., 2015 |
| IL-10 F: GCTGAAGGGTGAAGTTGAGG R: AGACTGGCAGCACAAGATTGTC | ENSGALG00000000892 | Grenier et al., 2015 |
| Marker of gut integrity |                       |                  |                    |
| MUC-2 F: GCTACAGGATCTGCTTGTCC R: AATGGGCGCTTCGAGTTTTT | XM_421035 | Adedokun et al., 2012 |
| Markers of nutrient transport |                     |                  |                    |
| ASCT-1 F: TTGGCCCCGGAAGGAGAAG R: AGACCATATGTTGGCTATCTGGAGT | XM_001232899.4 | Paris and Wong (2013) |
| SLC7A-2 F: TGCTGCAGTCTCCCAAGA R: GGCCCAAGTCCACCAACAG | NM_001199102.1 | Gilbert et al. (2007). |

Abbreviations: ASCT-1, neutral amino acid transporter-1; F, forward primer; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; MUC2, mucin 2; R, reverse primer; SLC7A-2, cationic amino acid transporter-2.

1Sequence obtained from Ensembl chicken genome data resources.

#### Table 4. Growth performance of broiler chickens fed diets supplemented with protease (PROT/kg) and purified trypsin inhibitor (TIU/g), from day 0 to 21 posthatching.

| Item         | TI     | 0       | 9,000   | Protease | Protease | SEM  | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 | 0     | 15,000 |
|--------------|--------|---------|---------|----------|----------|------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|
| BW, kg       |        |         |         |          |          |      |       |        |       |        |       |        |       |        |       |        |       |        |       |        |       |        |       |        |       |        |       |        |       |        |       |
| Day 0        |        | 36.3    | 36.3    | 36.3     | 36.3     | 0.01 | 36.3  | 36.3  | 36.3  | 36.3  | 0.01  | 36.3  | 36.3  | 36.3  | 36.3  | 0.238 | 0.728  | 0.728  |
| Day 7        |        | 141     | 148     | 132     | 145      | 2.29 | 137   | 146   | 144   | 138   | 0.001 | 0.017 | 0.159 |
| Day 14       |        | 415     | 432     | 394     | 429      | 6.36 | 405   | 430   | 423   | 411   | 0.001 | 0.074 | 0.172 |
| Day 21       |        | 899     | 943     | 870     | 936      | 11.63| 884   | 940   | 921   | 903   | <0.001| 0.144 | 0.356 |
| Day 0 to 7   |        | 105     | 111     | 96      | 108      | 2.29 | 100   | 110   | 108   | 102   | <0.001| 0.017 | 0.159 |
| BW gain, g   |        | 154     | 137     | 132     | 132      | 2.35 | 133   | 134   | 136   | 132   | 0.634 | 0.126 | 0.594 |
| Feed intake, g |       | 783     | 813     | 727     | 824      | 20.20| 755   | 819   | 798   | 776   | 0.005 | 0.255 | 0.114 |
| Day 0 to 14  |        | 379     | 395     | 358     | 392      | 6.36 | 368   | 394   | 384   | 375   | 0.001 | 0.044 | 0.169 |
| BW gain, g   |        | 466     | 465     | 465     | 463      | 10.66| 466   | 464   | 466   | 464   | 0.873 | 0.879 | 0.957 |
| Feed intake, g |       | 813     | 851     | 773     | 854      | 23.74| 793   | 853   | 832   | 813   | 0.019 | 0.429 | 0.379 |
| Day 0 to 21  |        | 862     | 906     | 834     | 876      | 15.32| 848   | 891   | 884   | 855   | 0.011 | 0.046 | 0.946 |
| BW gain, g   |        | 967     | 983     | 970     | 971      | 10.29| 969   | 977   | 975   | 971   | 0.452 | 0.692 | 0.431 |
| Feed intake, g |       | 892     | 922     | 860     | 902      | 14.46| 876   | 912   | 907   | 881   | 0.019 | 0.087 | 0.656 |
| G:F, g/kg    |        | 8       | 8       | 8       | 8        | 16   | 16    | 16    | 16    | 16    | 16    | 16    | 16    | 16    | 16    | 16    | 16    |

Abbreviations: G:F, gain to feed ratio; P, protease; TI, trypsin inhibitor.
Table 5. Organ response of broiler chickens at day 21, fed diets supplemented with protease (PROT/kg) and purified trypsin inhibitor (TIU/g).

| Item                        | 0         | 15,000     | 0         | 15,000     | SEM       | 0         | 15,000     | 0         | 9,000      | Protease TI | P-value     | P × TI     |
|-----------------------------|-----------|------------|-----------|------------|-----------|-----------|------------|-----------|------------|-------------|-------------|------------|
| Day 7, Absolute             |           |            |           |            |           |           |            |            |            |             |             |            |
| Pancreas, g                 | 0.64      | 0.59       | 0.68      | 0.62       | 0.029     | 0.66      | 0.61       | 0.62      | 0.65       | 0.074       | 0.266       | 0.838      |
| Liver, g                    | 6.22      | 6.11       | 5.63      | 5.45       | 0.200     | 5.92      | 5.78       | 6.17      | 5.54       | 0.477       | 0.005       | 0.854      |
| Duodenal loop, cm/kg BW     | 14.37     | 14.31      | 14.29     | 14.99      | 0.389     | 14.33     | 14.65      | 14.34     | 14.64      | 0.415       | 0.448       | 0.338      |
| Day 7, Relative             |           |            |           |            |           |           |            |            |            |             |             |            |
| Pancreas, g/kg BW           | 4.75      | 4.24       | 4.99      | 4.63       | 0.233     | 4.88      | 4.44       | 4.50      | 4.81       | 0.072       | 0.189       | 0.736      |
| Liver, g/kg BW              | 45.39     | 42.36      | 41.62     | 41.35      | 1.872     | 43.50     | 41.85      | 43.87     | 41.48      | 0.388       | 0.216       | 0.469      |
| Duodenal loop, cm/kg BW     | 110.1     | 103.50     | 109.1     | 114.9      | 3.89      | 109.6     | 109.2      | 106.8     | 112.0      | 0.919       | 0.195       | 0.127      |
| Day 14, Absolute            |           |            |           |            |           |           |            |            |            |             |             |            |
| Pancreas, g                 | 1.58      | 1.55       | 1.92      | 1.58       | 0.063     | 1.75      | 1.57       | 1.56      | 1.75       | 0.009       | 0.008       | 0.027      |
| Liver, g                    | 13.67     | 14.94      | 13.70     | 13.70      | 0.455     | 14.40     | 14.32      | 14.30     | 14.42      | 0.861       | 0.799       | 0.007      |
| Duodenal loop, cm/kg BW     | 4.20      | 3.73       | 4.75      | 3.82       | 0.149     | 4.47      | 3.78       | 3.97      | 4.28       | <0.001      | 0.046       | 0.137      |
| Day 14, Relative            |           |            |           |            |           |           |            |            |            |             |             |            |
| Pancreas, g/kg BW           | 36.67     | 35.80      | 37.38     | 33.59      | 1.583     | 37.03     | 34.70      | 36.23     | 35.49      | 0.156       | 0.642       | 0.367      |
| Liver, g/kg BW              | 52.44     | 50.64      | 49.59     | 46.68      | 1.796     | 51.02     | 48.66      | 51.54     | 48.14      | 0.204       | 0.072       | 0.759      |
| Day 21, Absolute            |           |            |           |            |           |           |            |            |            |             |             |            |
| Pancreas, g                 | 2.74      | 2.78       | 4.31      | 2.86       | 0.113     | 3.53      | 2.82       | 2.76      | 3.59       | <0.001      | <0.001      | <0.001     |
| Liver, g                    | 27.00     | 25.62      | 28.10     | 25.86      | 0.094     | 27.55     | 25.74      | 26.31     | 26.98      | 0.016       | 0.346       | 0.545      |
| Duodenal loop, cm/kg BW     | 21.56     | 21.31      | 21.57     | 21.41      | 0.451     | 21.56     | 21.36      | 21.43     | 21.49      | 0.602       | 0.902       | 0.924      |
| Day 21, Relative            |           |            |           |            |           |           |            |            |            |             |             |            |
| Pancreas, g/kg BW           | 3.10      | 3.01       | 4.78      | 3.17       | 0.128     | 3.94      | 3.11       | 3.07      | 3.97       | <0.001      | <0.001      | <0.001     |
| Liver, g/kg BW              | 30.44     | 27.96      | 31.06     | 28.46      | 0.781     | 30.75     | 28.21      | 29.20     | 29.76      | 0.004       | 0.485       | 0.942      |
| Duodenal loop, cm/kg BW     | 24.37     | 23.49      | 23.93     | 23.91      | 0.099     | 24.15     | 23.70      | 23.93     | 23.92      | 0.530       | 0.990       | 0.521      |
| N                           | 8         | 8          | 8         | 8          |           | 16        | 16         | 16        | 16         |             |             |            |

*a,b*Means within the same row with different superscripts are significantly different (*P* < 0.05).

Abbreviations: P, protease; TI, trypsin inhibitor.

RESULTS

The analyzed nutrient composition and quality characteristics of the soybean meal are presented in Table 1, and the ingredient composition and nutrient provision of the experimental diets are presented in Table 2. The effect of exogenous protease and dietary TI concentration on growth performance of the birds are presented in Table 4. There were no interactions between protease and TI on growth performance. Supplementation of the diets with exogenous protease resulted in an increase in BW gain (*P* < 0.01) and gain to feed ratio (*P* < 0.05) in all experimental phases. Increased dietary TI resulted in a reduction in BW gain (*P* < 0.05) in all experimental phases but had no effect on the gain to feed ratio. There was no effect of any of the experimental treatments on feed intake.

Table 5 shows the effect of exogenous protease and TI supplementation of diets on pancreas weight, liver weight, and length of the duodenal loop. On an absolute basis, addition of TI to diets reduced (*P* < 0.01) the liver weight of birds only on day 7 posthatching. Notably, exogenous protease supplementation reduced (*P* < 0.01) the relative weight of the liver on day 21 posthatching. On the other hand, absolute pancreas weight on day 14 and 21 increased (*P* < 0.01) in response to dietary TI increase. Relative to BW, the increase in dietary TI led to 8 and 29% increases in pancreas weight on day 14 (*P* < 0.05) and day 21 (*P* < 0.01) posthatching, respectively. Addition of exogenous protease to the diet resulted in a reduction (*P* < 0.01) in both absolute and relative pancreas weight on day 21 to a greater extent in the diet that contained supplemental TI compared with the diet with low TI, resulting in a protease by TI interaction (*P* < 0.01).

Added dietary TI reduced (*P* < 0.01) the AID of all AA (Table 6). There were no interactions between dietary TI concentration and exogenous protease on AA digestibility. Protease supplementation increased the AID of N (*P* < 0.01) and all AA (*P* < 0.05), with the exception of Met. The increase in Cys digestibility by exogenous protease supplementation was only marginal (*P* = 0.057). Overall, the magnitude of the relative protease effect was numerically greater in the low TI diet for most of the AA than the high TI diet (Figure 1). For example, the protease effect on Lys digestibility was +1.6% in the low TI diet and +1.2% in the high TI diet.

The effects of exogenous protease and dietary TI concentration on histology of jejunal tissue and mRNA expression of markers of inflammation, integrity of the intestinal wall, and nutrient transport in the mucosa of the chicken jejunum are presented in Table 7. There were no interactions between dietary TI concentration and exogenous protease. The expression of interleukin (IL)-1β, IL-8, IL-10, and neutral amino acid transporter-1 were not affected by dietary TI or exogenous protease supplementation. However, increased dietary TI downregulated (*P* < 0.05) the expression of
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Table 6. Effect of exogenous protease (PROT/kg) and purified trypsin inhibitor (TIU/g) concentration on apparent ileal digestibility (%) of nitrogen and amino acids in broiler chickens, at day 21 posthatching.

| Item     | 0 Protease | 9,000 Protease | SEM | 0 Protease | 9,000 Protease | SEM | P-value          |
|----------|------------|----------------|-----|------------|----------------|-----|------------------|
| Nitrogen | 84.8       | 86.3           | 0.43| 82.8       | 84.9           | 0.38| <0.001          |
| Arg      | 89.6       | 90.7           | 0.34| 88.7       | 90.1           | 0.29| <0.001          |
| His      | 86.6       | 87.9           | 0.34| 85.2       | 86.3           | 0.29| <0.001          |
| Ile      | 84.5       | 86.0           | 0.34| 82.8       | 84.0           | 0.29| <0.001          |
| Leu      | 85.9       | 87.2           | 0.34| 84.3       | 85.6           | 0.29| <0.001          |
| Lys      | 87.7       | 89.1           | 0.34| 86.3       | 87.7           | 0.29| <0.001          |
| Met      | 92.9       | 93.3           | 0.34| 91.8       | 92.0           | 0.29| <0.001          |
| Phe      | 85.2       | 86.6           | 0.34| 83.8       | 85.1           | 0.29| <0.001          |
| Thr      | 80.3       | 82.3           | 0.34| 78.2       | 79.8           | 0.29| <0.001          |
| Trp      | 86.9       | 88.3           | 0.34| 84.8       | 86.3           | 0.29| <0.001          |
| Val      | 82.8       | 84.4           | 0.34| 80.8       | 82.3           | 0.29| <0.001          |

Dispensable AA

| Item     | 0 Protease | 9,000 Protease | SEM | 0 Protease | 9,000 Protease | SEM | P-value          |
|----------|------------|----------------|-----|------------|----------------|-----|------------------|
| Ala      | 84.8       | 86.2           | 0.45| 83.1       | 84.3           | 0.38| <0.001          |
| Asp      | 82.6       | 84.2           | 0.45| 80.6       | 81.9           | 0.38| <0.001          |
| Cys      | 73.7       | 75.7           | 0.45| 69.6       | 71.3           | 0.38| <0.001          |
| Glu      | 89.0       | 90.0           | 0.45| 87.9       | 88.8           | 0.38| <0.001          |
| Gly      | 80.1       | 82.2           | 0.45| 78.0       | 79.5           | 0.38| <0.001          |
| Pro      | 85.0       | 86.5           | 0.45| 83.5       | 84.9           | 0.38| <0.001          |
| Ser      | 82.6       | 84.3           | 0.45| 80.6       | 82.1           | 0.38| <0.001          |
| Tyr      | 84.8       | 86.3           | 0.45| 82.8       | 84.9           | 0.38| <0.001          |

N: 8 8 8 8 16 16 16 16

Abbreviations: AA, amino acid; P, protease; TI, trypsin inhibitor.

cationic amino acid transporter-2 (SLC7A-2). Protease supplementation downregulated ($P < 0.01$) the expression of mucin-2 and upregulated ($P < 0.05$) the expression of SLC7A-2 in the jejunum. Exogenous protease increased the villus height ($P < 0.05$) and crypt depth ($P < 0.05$) in the jejunal tissue of birds.

Table 8 shows the effect of exogenous protease and dietary TI concentration on digestive enzyme activities. In the pancreas, there were no effects (or interactions) of exogenous protease and dietary TI concentration on digestive enzyme activities. Despite this, there was a reduction in trypsin activity ($P < 0.05$) in the duodenal digesta in response to increased dietary TI. However, duodenal trypsin activity increased ($P < 0.01$) with exogenous protease supplementation. Similarly, chymotrypsin activity was reduced ($P < 0.01$) by increased dietary TI concentration but increased ($P < 0.01$) with exogenous protease supplementation.

**DISCUSSION**

There is a substantial body of work on the nutritional implications of TI in broiler chickens. To the authors’ knowledge, the data obtained from the current study are the first to test the efficacy of a monocomponent protease on a purified source of TI in broiler chickens. This approach becomes valuable for optimization and indeed validation of the protease enzyme as a useful additive for improvements in the nutrient utilization of broiler chickens.

In the current study, the effect of increased dietary TI on the final BW of birds diminished with age, although there were improvements because of protease supplementation. This was evident with marked improvements in BW gain and feed efficiency over the entire feeding period and are similar to previous reports (Wang et al., 2008; Barekatain et al., 2013). However, there have also been reports of a lack of positive effect of exogenous protease on BW gain, even though the feed conversion significantly improved (Ghazi et al., 2002; Freitas et al., 2011). The diminishing effect of increased dietary TI on live BW agrees with previous authors (Erdaw et al., 2017) at 24 D or 35 D posthatching. However, Ruiz and De Belalcázar (2005) previously reported

Figure 1. Correlation between inherent amino acid digestibility in the control diet (%) and response to exogenous protease (% change relative to the control diet without added protease). Solid blue circles represent data points from the low TI diet group, and solid orange triangles represent data points from the high TI diet group. Solid and dashed linear lines indicate the respective best fit model for the low and high TI groups. Abbreviation: TI, trypsin inhibitor.
that the impact of dietary TI may simply be intake related, and as age approaches 21 D and older, excess TI intake could cause moderate to severe rapid feed passage syndrome and a consequent drop in overall growth performance of the birds. In the current study, there were no differences in feed intake between birds fed the low or high TI diets at 21 D posthatching which could open up an avenue for exogenous protease in complementing the functions of the endogenous proteases (Noy and Sklan, 1995).

Addition of purified TI to the diets increased both absolute and relative weight of the pancreas. This was especially true for birds at day 14 and 21 posthatching and is indicative of pancreatic hypertrophy or hyperplasia (Embaby, 2010). Owing to an intrinsic negative feedback control, enzyme secretion by the pancreas is inversely related to the enzyme activity in the gut. The ingested TI acts by forming an irreversible complex with trypsin in the small intestine, which limits the concentration and function of trypsin and leads to overproduction of digestive enzymes and thus, enlargement of the pancreas (Cabrera-Orozco et al., 2013). It is surprising that increased dietary TI had no effect on the relative pancreas weight at day 7 posthatching, even though the growth performance data suggest that birds were more susceptible to TI at this age. One possible explanation is that although dietary TI was high, low feed intake in the first week of the bird’s life could limit total TI intake, which might not be enough to elicit major morphological changes in the pancreas. This is corroborated by a previous report by Clarke and Wiseman (2007) who showed a strong dose-dependent response of relative pancreas weight to dietary TI intake. However, Erdaw et al. (2017) reported that the pancreas of younger birds was more sensitive to dietary TI concentrations, but it is pertinent to state that responses in that study was for 10-day-old birds. Interestingly, the magnitude of change in the relative weight of the pancreas to dietary TI tends to increase with age, and this might be partly because of increased dietary intake of TI.

Table 7. Relative gene expression1 of cytokines, mucosa, amino acid transporter proteins in jejunal mucosa, and histology of jejunal tissue of broiler chickens fed diets containing exogenous protease (PROT/kg) and purified trypsin inhibitor (TIU/g), at day 21 posthatching.

| Item          | TI 0 9,000 | Protease | SEM | Protease | TI 0 9,000 | Protease | TI 0 9,000 | Protease | TI 0 9,000 | Protease | TI 0 9,000 |
|---------------|------------|----------|-----|----------|------------|----------|------------|----------|------------|----------|------------|
| Genes         |            |          |     |          |            |          |            |          |            |          |            |
| IL-1β         | 1.18       | 0.96     | 0.96| 0.95     | 0.246      | 1.07     | 0.95       | 1.07     | 0.95       | 0.638    | 0.651      | 0.683     |
| IL-8          | 1.23       | 1.51     | 1.02| 1.14     | 0.258      | 1.12     | 1.33       | 1.37     | 1.08       | 0.435    | 0.278      | 0.763     |
| IL-10         | 1.14       | 0.95     | 0.92| 0.69     | 0.164      | 1.03     | 0.82       | 1.05     | 0.81       | 0.217    | 0.154      | 0.913     |
| MUC-2         | 1.31       | 1.02     | 1.25| 0.57     | 0.130      | 1.28     | 0.79       | 1.16     | 0.91       | 0.001    | 0.062      | 0.155     |
| ASCT-1        | 1.11       | 1.18     | 0.79| 1.06     | 0.206      | 0.95     | 1.12       | 1.14     | 0.92       | 0.407    | 0.301      | 0.629     |
| SLC7A2        | 1.01       | 1.41     | 0.52| 0.93     | 0.177      | 0.77     | 1.17       | 1.21     | 0.73       | 0.055    | 0.014      | 0.945     |
| Histology     |            |          |     |          |            |          |            |          |            |          |            |
| Villus height | 1,057.5    | 1,273.8  | 849.4| 1,084.0  | 88.88      | 953.5    | 1,178.9    | 1,165.7  | 966.7      | 0.019    | 0.036      | 0.919     |
| Crypt depth   | 100.6      | 130.5    | 96.7| 112.8    | 6.75       | 98.7     | 121.7      | 115.6    | 104.8      | 0.003    | 0.125      | 0.322     |
| VH:CD ratio   | 10.7       | 9.8      | 8.8 | 9.7      | 0.78       | 9.8      | 9.7        | 10.2     | 9.3        | 0.968    | 0.216      | 0.244     |

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Abbreviations: ASCT-1, neutral amino acid transporter-1; CD, crypt depth; IL, interleukin; MUC2, mucin 2; P, protease; SLC7A-2, cationic amino acid transporter-2; TI, trypsin inhibitor; VH, villus height.

1Relative gene expression (2^−ΔΔCt) was calculated with GAPDH as the endogenous control.

Table 8. Effect of exogenous protease (PROT/kg) and purified trypsin inhibitor (TIU/g) concentration on enzyme activity in the duodenal digesta (units/mL) and Pancreas (units/mg), at day 21 posthatching.

| Item          | 0 9,000 | Protease | SEM | Protease | TI 0 9,000 | Protease | TI 0 9,000 |
|---------------|---------|----------|-----|----------|------------|----------|------------|
| Duodenal digesta |         |          |     |          |            |          |            |
| Trypsin       | 10.13   | 11.69    | 7.76| 10.40    | 0.699      | 8.95     | 11.04      | 10.91     | 9.08       | 0.007    | 0.016      | 0.446     |
| Chymotrypsin  | 5.49    | 6.77     | 3.79| 5.41     | 0.123      | 4.64     | 6.09       | 6.13      | 4.60       | <0.001   | <0.001     | 0.178     |
| Amylase       | 121.49  | 115.10   | 117.9| 119.44   | 3.284      | 119.74   | 117.27     | 118.30    | 118.71     | 0.460    | 0.900      | 0.247     |
| Lipase        | 1.30    | 1.32     | 1.41| 1.35     | 0.047      | 1.35     | 1.33       | 1.31      | 1.38       | 0.682    | 0.140      | 0.393     |
| Pancreas      |         |          |     |          |            |          |            |
| Trypsin       | 5.81    | 5.67     | 6.02| 5.40     | 0.333      | 5.92     | 5.53       | 5.74      | 5.71       | 0.263    | 0.915      | 0.473     |
| Chymotrypsin  | 4.28    | 4.31     | 4.12| 4.21     | 0.076      | 4.20     | 4.26       | 4.29      | 4.16       | 0.433    | 0.102      | 0.708     |
| Amylase       | 34.91   | 33.59    | 35.66| 35.13    | 1.331      | 35.28   | 34.36      | 34.27     | 35.39      | 0.495    | 0.399      | 0.770     |
| Lipase        | 0.95    | 0.98     | 1.07| 1.00     | 0.045      | 1.01     | 0.99       | 0.97      | 1.03       | 0.670    | 0.143      | 0.325     |

N 8 8 8 8 16 16 16 16

Abbreviations: P, protease; TI, trypsin inhibitor.
These results contradict findings by Erdaw et al. (2017), that the relative weight of the chicken pancreas fed extruded full fat SBM compared with the control, decreased with age. This suggests that purified TI exerts a more critical effect on the pancreas than the native TI in full fat SBM. In the current trial, birds were fed diets containing high-quality SBM with highly digestible AA, and this might increase the negative effects of the added purified TI. On the other hand, previous experiments that fed raw SBM, full-fat SBM, or underprocessed SBM used SBM with inherently low AA digestibility with a lot of residual TI. Therefore, it is possible that the TI as an antinutrient tends to exert different influences depending on the inherent quality of the diet that it is associated with. Although both lectin and TI contained in raw or full fat soybean may have complementary effects on pancreatic function (Grant, 1989), TI seems a more relevant antinutritional factor in young broilers (Douglas et al., 1999). Although lectin can increase pancreas weight (Liener, 1994) by stimulating the accumulation of polyamines (Pusztai et al., 1995), Fasina et al. (2004) reported no trophic changes in pancreas weights of turkey pouls fed diets containing increasing concentrations of purified soybean lectin. Nevertheless, the current study showed that the exogenous protease was effective in ameliorating the negative effect of increased dietary TI on pancreas weight.

In the current study, increased dietary TI as a result of the addition of purified TI decreased the AID of N and all of the reported AA. This was at least a 4% drop in AID of Thr and Trp and up to 8% decrease for Cys. This result is similar to reports by Schulze (1994), who observed a significant decrease in N digestibility and increase in ileal endogenous N flow when growing pigs were fed diets containing increasing concentrations of purified TI. However, relative to the control, protease supplementation improved the overall N digestibility by 2.5%. This is similar to observations of Cowieson and Ravindran (2008) when a multi-enzyme complex, containing protease, was used. Angel et al. (2011) also reported an increased N digestibility with increasing dietary protease concentration. Bertechini et al. (2009) reported increased true AA digestibility for soybean meal, and Carvalho et al. (2009) reported increased true AA digestibility for corn, in the presence of a monocomponent protease. This indicates that variable quantities of N that could escape digestion and absorption were partially captured by the protease and made available to the birds. The relative effect of protease on control AA digestibility were consistent with those in the review of 25 independent experiments published by Cowieson and Roos (2014).

These improvements in growth performance responses can also be attributed to the observed improvements in the digestibility of AA. This is because birds that more readily reach their target intake of digestible AA have improved feed efficiency compared with the corresponding controls. In the current study, we observed a lack of effect of exogenous protease on methionine digestibility, which is also consistent with previous reports (Ravindran et al., 2005; Angel et al., 2011; Cowieson and Roos, 2014). This may be related to the typically high AID of methionine (90–95%) in a corn–SBM–based diet. However, there are some reports of significant improvements in AID of methionine when protease was supplemented at dietary concentrations of 400 mg/kg or higher (Angel et al., 2011). Although relatively low, but similar to a previous report (Cowieson, 2010), the AID of Cys in birds was improved by protease supplementation. These data on AA digestibility indicate a reliance by the exogenous protease on the inherent digestibility of dietary AA or “starting material” (Cowieson and Roos, 2014). However, in diets with high TI concentration, the protease effect was not large enough to counteract the drastic drop in digestibility of AA in the control diet. This suggests that the exogenous protease may not directly act on the TI, and any improvements in AA digestibility, attributable to the exogenous protease, is largely independent of dietary TI concentration. The reason for this observation is not clear but might be ascribed, in part, to a lack of an effective enzyme-substrate specificity, interference with other dietary components, or interference of endogenous proteolytic activity by the exogenous protease effect (Yuan et al., 2015).

Exogenous protease downregulated the expression of mucin-2 in the jejunal of birds fed diets with increased dietary TI concentration. This agrees with reports (Cowieson and Roos, 2014; Cowieson et al., 2017) that noted a beneficial effect of exogenous protease on mucin secretion in the intestine of the chicken. This is usually associated with a reduction in mucin secretion or an increase in the autolytic recovery of mucin or both. It is possible that the protease acts by reducing the metabolic demand for mucoprotein by reducing the erosion of the mucosal layer by the incoming feed matrix. Hence, there is usually a marked reduction in the mucin layer thickness, goblet cells, and endogenous protein losses. For example, birds fed diets supplemented with protease show significant reductions in the digesta sialic acid concentration (a component of mucin) and goblet cell numbers (Peek et al., 2009; Cowieson et al., 2017). It is also possible that a portion of the beneficial effect of protease on AA digestibility is conferred through a reduction in the loss of mucoprotein from the intestine, with possible implications for gut health.

In addition, mucin being a family of mucus glycoproteins have some essential AA in the core structure (e.g., Trp and Thr), and it is possible that an increase in the digestibility of these AA, conferred by the exogenous protease, would correlate with improvements in the integrity of mucus layer. Protease supplementation also upregulated (almost doubling) the expression of the Na-independent cationic AA transporter, SLC7A-2. Consistent with the reports of Cowieson et al. (2017), this suggests an effect of the exogenous protease in modulating protein absorption, which may also be because of the availability of more AA released in the gut. However, there was no protease effect on the expression of Na-dependent neutral AA transporter, amino acid transporter-1, and the reason for this observation is not totally clear. The exogenous protease also increased the villus height and crypt depth in the jejunal
tissue of the birds. This suggests a better absorptive capacity of dietary nutrients and may corroborate the observed improvements in growth performance and nutrient utilization responses previously noted. This observation is similar to reports by Wang et al. (2008) in broilers and Zuo et al. (2015) in piglets.

In the current study, increased dietary TI and exogenous protease supplementation had no effect on pancreatic enzyme activities. Although, birds fed high TI diet showed signs of pancreatic hypertrophy, it is unclear why the pancreatic enzyme activity was not different from birds fed the low TI diet. This is in dissonance with reports by Erdaw et al. (2017), who observed reduced activities of pancreatic trypsin and chymotrypsin of 24-day-old birds fed diets with increasing levels of raw soybean meal. However, in the duodenum, the activities of trypsin and chymotrypsin decreased as a result of increased dietary TI but increased with exogenous protease administration. The reduction in enzymatic activity might be because TI not only has inhibitory specificity to trypsin but will also affect the activity of chymotrypsin, which is activated by trypsin. On the other hand, the improvements in intestinal trypsin and chymotrypsin activity because of protease supplementation suggest a complementarity or additivity between the exogenous and endogenous proteases. This is because the enzyme activity in the small intestine is composed of both exogenous and endogenous components.

Given the foregoing, the current study shows that increased dietary TI affected the growth performance and digestibility of AA and that exogenous protease administration improves these responses in the birds. Furthermore, responses to protease administration seem more likely to have arisen from a general improvement in protein digestion. Pronounced pancreatic hypertrophy was identified in birds fed diets with added purified TI, which was ameliorated by exogenous protease administration. The data from the current study suggest that the efficacy of dietary exogenous protease might be independent of dietary TI concentration.

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