

Postprint: Effects of Exogenous Protease on In Vitro Degradation Efficiency of Broiler Diets

Authors: Wang Xiong, Tang Xiaoyi, Chen Fengming, Chen Qinghua

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of exogenous protease on the in vitro degradation efficiency of broiler diets and to determine the optimal supplementation level of protease, providing a theoretical reference for rapid evaluation of protease efficacy. A single-factor completely randomized design was adopted, with 0, 100, 200, 400, and 800 mg/kg exogenous protease added to the basal diets of broilers during the starter phase (1-21 days of age) and grower phase (22-42 days of age), respectively. An in vitro simulated digestion model for broilers was established using the Simulated Digestive System for monogastric animals (SDS-III) to determine in vitro degradation efficiency. Each treatment had 5 replicates, with each replicate consisting of one simulated digestion tube. The results showed that: Adding protease to starter-phase diets significantly improved diet gross energy digestibility (GED) and enzymatic hydrolysate gross energy (EHGE) ($P < 0.05$), the dry matter digestibility (DMD) of the 200 mg/kg protease group was significantly higher than that of the control group ($P < 0.05$), and DMD, GED, and EHGE of starter-phase diets showed a significant quadratic relationship with exogenous protease supplementation level, first increasing and then decreasing ($P < 0.05$); adding protease to grower-phase diets significantly improved diet DMD, GED, and EHGE ($P < 0.05$), and these three parameters showed a significant quadratic relationship with exogenous protease supplementation level, first increasing and then decreasing ($P < 0.05$). It can be concluded that adding an appropriate amount of exogenous protease to corn-soybean meal basal diets can significantly improve the in vitro degradation efficiency of broiler diets; in this experiment, the 200 mg/kg protease group exhibited the highest in vitro degradation efficiency.

Full Text

Effects of Exogenous Protease on in Vitro Degradation Efficiency of Broiler Diets

WANG Xiong¹, TANG Xiaoyi¹, CHEN Fengming^{1,2}, CHEN Qinghua^{1*}

¹College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China

²Hunan Ling Dao Agriculture Environmental Protection Technology Co., Ltd, Changsha 410128, China

Abstract

This experiment was conducted to investigate the effects of exogenous protease on the in vitro degradation efficiency of broiler diets and to determine the optimal protease supplementation level, thereby providing a theoretical reference for rapid evaluation of protease effectiveness. A single-factor completely randomized design was employed, with exogenous protease added at levels of 0, 100, 200, 400, and 800 mg/kg to basal diets for both the starter phase (1-21 days of age) and finisher phase (22-42 days of age) of broilers. An in vitro simulated digestion model for broilers was established using the Simulated Digestion System for monogastric animals (SDS-III) to determine in vitro degradation efficiency. Each treatment consisted of 5 replicates, with one simulated digestion tube per replicate. The results demonstrated that protease supplementation significantly improved the gross energy digestibility (GED) and enzymatic hydrolysate gross energy (EHGE) of starter phase diets ($P < 0.05$). The dry matter digestibility (DMD) in the 200 mg/kg protease group was significantly higher than that in the control group ($P < 0.05$), and DMD, GED, and EHGE of starter phase diets exhibited a significant quadratic relationship with protease supplementation level ($P < 0.05$), increasing initially and then decreasing. For finisher phase diets, protease supplementation significantly enhanced DMD, GED, and EHGE ($P < 0.05$), with all three parameters showing a significant quadratic relationship with protease addition level ($P < 0.05$). These findings indicate that appropriate supplementation of exogenous protease in corn-soybean meal basal diets can significantly improve the in vitro degradation efficiency of broiler diets, with the 200 mg/kg protease group achieving the highest efficiency in this experiment.

Keywords: exogenous protease; dry matter digestibility; gross energy digestibility; enzymatic hydrolysate gross energy

Introduction

Exogenous protease can improve growth performance and enhance protein digestion and utilization efficiency in livestock and poultry [1]. However, its efficacy

depends not only on its enzymatic properties but also on the target animal and basal diet composition. Animal testing methods for evaluating exogenous protease effectiveness are complex and time-consuming, creating an urgent need in feed production and animal husbandry for a simple and rapid in vitro evaluation method. In vitro methods are categorized into three types: exogenous enzyme methods, endogenous enzyme methods, and bionic digestion enzyme methods [2]. The exogenous enzyme method, represented by the pepsin-pancreatin method established by Boisen et al. [3], has been shown to rapidly evaluate exogenous enzyme effectiveness and serve as a preliminary test for animal trials, though it suffers from poor accuracy and unstable results [4-6]. Endogenous enzyme methods utilize digestive enzymes with poor stability, yielding results with low comparability, and have largely been abandoned from in vitro evaluation systems [2].

In contrast, the bionic digestion enzyme method offers higher simulation fidelity of animal digestive physiology and demonstrates good biological validity for livestock diets, enabling the screening of optimal enzyme combinations and supplementation levels [7-8]. However, few studies have evaluated exogenous protease effectiveness using the bionic method. Therefore, this experiment employed the Simulated Digestion System for monogastric animals to establish an in vitro digestion model for broilers, investigating the effects of exogenous protease on in vitro degradation efficiency of broiler diets, determining the optimal protease supplementation level, and providing both a methodological reference for rapid protease evaluation and a scientific basis for its application in broiler production.

Materials and Methods

1.1 Experimental Materials

The exogenous protease was provided by Shandong Longkete Enzyme Preparation Co., Ltd., with measured activities of 11,464 U/g acid protease, 25,635 U/g neutral protease, and 21,655 U/g alkaline protease. Enzyme activity was defined as the amount of enzyme required to hydrolyze casein to produce 1 μ g of tyrosine per minute under specific pH conditions (pH=3.0, pH=7.5, and pH=10.5) at 40°C.

1.2 Experimental Diets and Design

Experimental diets were formulated according to the “Feeding Standard of Yellow-Feathered Broilers” (NY/T 33–2004) [9] and the NRC (1994) nutrient requirements for poultry [10]. The composition and nutrient levels of the basal diets are presented in . A single-factor completely randomized design was adopted, with a control group receiving basal diets for both starter (1-21 days) and finisher (22-42 days) phases, and treatment groups receiving the same basal diets

supplemented with 100, 200, 400, or 800 mg/kg exogenous protease. A total of 10 dietary samples were evaluated, with 5 replicates per sample and one simulated digestion tube per replicate. Diet samples were obtained using the quartering method, ground to pass through a 60-mesh sieve using a small feed grinder, thoroughly mixed, and stored at -20°C until use.

1.3 Experimental Procedures

This experiment utilized the Simulated Digestion System for monogastric animals (SDS-III) to simulate the digestion process of diets in the gastrointestinal tract of chickens. The dialysis bag specifications and pretreatment, preparation of gastric and small intestinal buffer solutions, and instrument operating parameters were performed according to reference [11].

The operational procedure was as follows: Approximately (2 ± 0.0002) g of diet sample was weighed and placed into a simulated digestion tube containing a dialysis bag, followed by the addition of 20 mL of prepared gastric fluid. The digestion tube was fixed onto the preheated SDS-III system and connected to pre-formulated gastric and small intestinal buffer solutions. After the gastric phase simulation (digestion at 41°C for 4 h), 2 mL of small intestinal fluid was accurately transferred into the small intestinal digestion reservoir of the system to continue digestion for 7.5 h in the anterior small intestine segment and 7.5 h in the posterior small intestine segment. Following digestion, the residual digesta was transferred without loss to pre-weighed constant-weight culture dishes, dried in a 65°C oven until no water marks remained, and then dried at 105°C to constant weight. The dried residues were completely scraped from the dishes and transferred to pre-weighed constant-weight glass sand-core crucibles, defatted with anhydrous ethanol (rinsed 3 times with anhydrous ethanol), and dried at 105°C to constant weight. The *in vitro* dry matter digestibility, gross energy digestibility, and enzymatic hydrolysate gross energy of samples were calculated using the following formulas [12]:

$$\begin{aligned} \text{DMD}(\%) &= (M - M')/M \times 100 \\ \text{GED}(\%) &= (\text{GE} - \text{GE}')/\text{GE} \times 100 \\ \text{EHGE} &= (\text{GE} - \text{GE}')/(M \times 1000) \end{aligned}$$

Where: DMD is the *in vitro* dry matter digestibility of the diet (%); GED is the *in vitro* gross energy digestibility of the diet (%); EHGE is the *in vitro* enzymatic hydrolysate gross energy of the diet (MJ/kg); M is the dry matter weight of the diet sample (g); M' is the dry matter weight of the undigested residue (g); GE is the gross energy of the diet sample (MJ); and GE' is the gross energy of the undigested residue (MJ).

1.4 Statistical Analysis

Experimental data were organized using Excel and analyzed using one-way ANOVA procedure in SPSS 19.0 software. Duncan's multiple range test was used for post-hoc comparisons, and linear and quadratic regression analyses were

performed to evaluate the effects of different protease supplementation levels. Results are expressed as mean \pm standard deviation, with $P < 0.05$ considered statistically significant.

Results

2.1 Effects of Exogenous Protease on In Vitro DMD, GED, and EHGE of Broiler Starter Diets

As shown in , the 200 mg/kg protease group exhibited significantly higher dry matter digestibility compared with the control group ($P < 0.05$), while other protease-supplemented groups showed an improving trend ($P > 0.05$). Dry matter digestibility displayed a quadratic relationship with protease supplementation level ($P < 0.05$). The 100, 200, and 400 mg/kg protease groups showed significantly higher gross energy digestibility and enzymatic hydrolysate gross energy compared with the control group ($P < 0.05$), with both GED and EHGE demonstrating a quadratic relationship with protease supplementation level ($P < 0.05$).

2.2 Effects of Exogenous Protease on In Vitro DMD, GED, and EHGE of Broiler Finisher Diets

As shown in , treatment groups exhibited significantly higher gross energy digestibility and enzymatic hydrolysate gross energy compared with the control group ($P < 0.05$), with both parameters showing a quadratic relationship with protease supplementation level ($P < 0.05$). Supplementation with 100, 200, and 400 mg/kg protease significantly improved gastrointestinal dry matter digestibility of finisher diets ($P < 0.05$), and DMD also displayed a quadratic relationship with protease supplementation level ($P < 0.05$).

Discussion

Compared with other in vitro methods, the bionic digestion method based on the Simulated Digestion System for monogastric animals offers advantages of standardization, instrumentation, and automation, ensuring accuracy and precision of experimental results [13-15]. Zhang et al. [12] demonstrated that the Simulated Digestion System can predict the effectiveness of exogenous protease, with whole-tract dry matter digestibility and enzymatic hydrolysate gross energy showing good linear regression relationships with exogenous protease supplementation levels. The present study found that exogenous protease improved in vitro dry matter digestibility, gross energy digestibility, and enzymatic hydrolysate gross energy of corn-soybean meal basal diets for both starter and finisher phases, with all three parameters showing quadratic relationships with protease supplementation levels.

These findings align with previous research. Fru-Nji et al. [16] reported that protease supplementation in corn-soybean meal basal diets significantly improved energy utilization in broilers. Stefanello et al. [17] observed significant improvements in ileal and total tract digestibility of dry matter and energy when protease was added to both soybean meal-based and corn-soybean meal semi-purified diets. Angel et al. [18] found that protease supplementation in diets for 7- to 22-day-old broilers improved apparent nutrient digestibility, consistent with our results. Exogenous protease can reduce anti-nutritional factors in diets and enhance hydrolysis efficiency, thereby increasing digestibility. For instance, Huang [19] found that protease rapidly degraded anti-nutritional factors in soybean meal and effectively improved its digestibility, while Lahaye et al. [20] reported that protease could eliminate trypsin inhibitors in soybeans. Yu et al. [21] demonstrated using the pepsin-pancreatin in vitro method that protease promoted degradation of soy protein.

Beyond degrading dietary protein and anti-nutritional factors, exogenous protease may also affect the activity of endogenous enzymes during simulated intestinal digestion. Li [22] found that 327 mg/kg protease supplementation in broiler diets tended to increase endogenous enzyme secretion, while 2,619 mg/kg protease reduced intestinal chyme amylase activity by 8.75% and 28.14% in 1-21 day and 22-42 day old broilers, respectively. Zhou [23] reported that 200 mg/kg protease significantly increased trypsin and chymotrypsin activities in jejunal chyme of broiler chickens, while 800 mg/kg protease significantly reduced pancreatic trypsin and chymotrypsin activities. In the present study, the quadratic response of in vitro dry matter digestibility, gross energy digestibility, and enzymatic hydrolysate gross energy to increasing protease supplementation may be explained by appropriate protease levels enhancing endogenous enzyme activity, while excessive supplementation inhibited endogenous enzyme activity. The results indicate that supplementation of 200 mg/kg exogenous protease in both starter and finisher basal diets achieved the best degradation effect, demonstrating that the Simulated Digestion System can rapidly evaluate protease effectiveness and provide a scientific basis for protease application in broiler diets.

Conclusion

Appropriate supplementation of exogenous protease can significantly improve the in vitro degradation efficiency of broiler basal diets. In this experiment, the 200 mg/kg protease group achieved the highest in vitro degradation efficiency.

References

- [1] WANG Xiong, CHEN Qinghua. Research progress on feed protease and its

- application in broiler diets[J]. *Feed Research*, 2016(21): 10-14.
- [2] AI Qin, ZUO Jianjun, ZHAO Jiangtao, et al. Discussion on evaluation methods of exogenous enzyme preparations[J]. *Feed Industry*, 2015, 36(10): 16-20.
- [3] BOISEN S, FERNÁNDEZ J A. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses[J]. *Animal Feed Science and Technology*, 1995, 51(1/2): 29-43.
- [4] ALABI O O, ATTEH J O, ADEJUMO I O. Comparative evaluation of in vitro and in vivo nutrient digestibility of dietary levels of rice husk supplemented with or without commercial enzyme[J]. *International Journal of Research in Agriculture and Forestry*, 2015, 2(6): 15-19.
- [5] MALATHI V, DEVEGOWDA G. In vitro evaluation of nonstarch polysaccharide digestibility of feed ingredients by enzymes[J]. *Poultry Science*, 2001, 80(3): 302-305.
- [6] KONG C S, CHAN S P, KIM B G. Effects of an enzyme complex on in vitro dry matter digestibility of feed ingredients for pigs[J]. *SpringerPlus*, 2015, 4: 261.
- [7] HOU Xiaofeng. Regulation of non-starch polysaccharide enzyme preparations on energy metabolism rate of broiler diets and study on its in vitro evaluation method[D]. Master's Thesis. Taigu: Shanxi Agricultural University, 2005.
- [8] HE Kelin, SA Renna, GAO Jie, et al. Optimization of non-starch polysaccharide enzymes for broiler diets by in vitro method[J]. *Scientia Agricultura Sinica*, 2012, 45(21): 4457-4464.
- [9] Ministry of Agriculture of the People's Republic of China. NY/T 33-2004 Feeding standard of chickens[S]. Beijing: China Agriculture Press, 2004.
- [10] NRC. Nutrient requirements of poultry[S]. 9th ed. Washington, D.C.: National Academy Press, 1994.
- [11] ZHAO Feng, DENG Yaohui, ZHANG Hongfu, et al. Operation manual of Simulated Digestion System for monogastric animals[M]. 3rd ed. Beijing: Institute of Animal Science, Chinese Academy of Agricultural Sciences, 2016.
- [12] ZHANG Lilan, CHEN Liang, ZHONG Ruqing, et al. Effects of exogenous protease on in vitro dry matter digestibility and enzymatic hydrolysate gross energy of broiler diets[J]. *Scientia Agricultura Sinica*, 2017, 50(7): 1326-1333.
- [13] ZHAO F, REN L Q, MI B M, et al. Developing a computer-controlled simulated digestion system to predict concentration of metabolizable energy of feedstuffs for rooster[J]. *Journal of Animal Science*, 2014, 92(4): 1537-1547.
- [14] ZHAO F, ZHANG L, MI B M, et al. Using a computer-controlled simulated digestion system to predict the energetic value of corn for ducks[J]. *Poultry Science*, 2014, 93(6): 1410-1420.

- [15] CHEN L, GAO L X, HUANG Q H, et al. Prediction of digestible energy of feed ingredients for growing pigs using a computer-controlled simulated digestion system[J]. *Journal of Animal Science*, 2014, 92(9): 3887-3894.
- [16] FRU-NJI F, KLUENTER A M, FISCHER M, et al. A feed serine protease improves broiler performance and increases protein and energy digestibility[J]. *The Journal of Poultry Science*, 2011, 48(4): 239-246.
- [17] STEFANELLO C, VIEIRA S L, RIOS H V, et al. Energy and nutrient utilisation of broilers fed soybean meal from two different Brazilian production areas with an exogenous protease[J]. *Animal Feed Science and Technology*, 2016, 221: 267-273.
- [18] ANGEL C R, SAYLOR W, VIEIRA S L, et al. Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens[J]. *Poultry Science*, 2011, 90(10): 2281-2286.
- [19] HUANG Mingyuan. Study on enzymatic hydrolysis of soybean meal, rapeseed meal and cottonseed meal by protease[J]. *Feed Research*, 2014(11): 5-7.
- [20] LAHAYE L, GAUTHIER L, XIONG Lingling. Application of protease in animal production[J]. *Feed and Husbandry*, 2011(7): 41-45.
- [21] YU B, WU S T, LIU C C, et al. Effects of enzyme inclusion in a maize-soybean diet on broiler performance[J]. *Animal Feed Science and Technology*, 2007, 134(3/4): 283-294.
- [22] LI Hui. Effects of protease and xylanase on growth performance, digestive function and blood indices of broilers[D]. Master's Thesis. Yangling: Northwest A&F University, 2010.
- [23] ZHOU Liang. Study on effects of exogenous protease (ProAct) on broiler performance and amino acid digestibility[D]. Master's Thesis. Beijing: Chinese Academy of Agricultural Sciences, 2014.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv – Machine translation. Verify with original.