Effects of Exogenous Amylase on In Vitro Nutrient Digestibility and Metabolizable Energy in Corn-Soybean Meal-Based Diets for Broilers

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Abstract

This experiment aimed to utilize the Single-gastric Animal Bionic Digestive System (SDS-II) to investigate the effects of exogenous amylase on the in vitro nutrient digestibility and metabolizable energy of corn-soybean meal diets for broiler chickens, providing a basis for accurate evaluation of feed enzyme efficacy. A 2×4 factorial completely randomized design was employed. Two basal corn-soybean meal diets were formulated for broiler chickens aged 1-21 days and 22-42 days according to the Chinese 'Feeding Standard of Chickens' (NY/T 33-2004) and NRC (1994) nutrient requirements for chickens. Three levels of exogenous amylase (1,840, 9,200, and 18,400 U/g) were added to each basal diet to prepare six amylase-supplemented diets, with the two basal diets without exogenous amylase serving as controls. The SDS-II was used to determine in vitro dry matter digestibility (IVDMD), in vitro apparent crude protein digestibility (IVACPD), in vitro standardized crude protein digestibility (IVSCPD), in vitro starch digestibility (IVSTD), in vitro gross energy digestibility (IVGED), and in vitro metabolizable energy (IVME) of the eight diets. Each diet had five replicates, with one digestion tube per replicate. The results showed: 1) Compared with the control group, the gastric phase IVDMD and IVGED of diets in the 1,840, 9,200, and 18,400 U/g amylase groups were significantly increased ($P<0.05$); the IVME of the 18,400 U/g amylase group was significantly higher than other groups ($P<0.05$); the whole-tract IVDMD, IVGED, and IVME of diets for 22-42 days were significantly higher than those for 1-21 days ($P<0.05$). 2) The whole-tract IVACPD and IVSCPD of the 1,840 and 9,200 U/g amylase groups were significantly higher than the control group ($P<0.05$), and the whole-tract IVACPD and IVSCPD of diets for 22-42 days were significantly higher than those for 1-21 days ($P<0.05$). 3) The whole-tract IVSTD of all eight diets was above 99.40%; the whole-tract IVSTD of the 9,200 and 18,400 U/g amylase groups was significantly lower than the control group ($P<0.05$);

the whole-tract IVSTD of diets for 1-21 days was significantly higher than that for 22-42 days (P<0.05). 4) Dietary nutrient level and amylase supplementation level exhibited interactive effects on IVDMD, IVGED, IVACPD, IVSCPD, IVSTD, and IVME of broiler diets (P<0.01). Under the conditions of this experiment, exogenous amylase supplementation improved gastric phase IVDMD and IVGED of broiler diets; 1,840 and 9,200 U/g exogenous amylase improved IVACPD and IVSCPD of broiler diets; 18,400 U/g exogenous amylase improved IVME of broiler diets; starch in corn-soybean meal diets was almost completely degraded, and the effect of exogenous amylase on IVSTD could be neglected; dietary nutrient level and exogenous amylase supplementation level exhibited interactive effects on in vitro nutrient digestibility and metabolizable energy of broiler diets, with in vitro nutrient digestibility and metabolizable energy of diets for 22-42 days being higher than those for 1-21 days.

Full Text

Effects of Exogenous Amylase on *in Vitro* Nutrient Digestibility and Metabolic Energy of Corn-Soybean Meal Diets for Broilers

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Abstract

This experiment investigated the effects of exogenous amylase on the *in vitro* nutrient digestibility and metabolic energy of corn-soybean meal diets for broilers using a Simulated Digestion System for monogastric animals (SDS-), aiming to provide a basis for accurately evaluating the efficacy of feed enzymes. A 2×4 factorial completely randomized design was employed. Two corn-soybean meal basal diets were formulated for broilers aged 1–21 days and 22–42 days according to the Chinese Feeding Standard of Chicken (NY/T 33-2004) and NRC (1994) requirements. Six experimental diets were created by supplementing the two basal diets with exogenous amylase at 1,840, 9,200, and 18,400 U/g, with the two unsupplemented basal diets serving as controls. The *in vitro* dry matter digestibility (IVDMD), *in vitro* apparent crude protein digestibility (IVACPD), *in vitro* standardized crude protein digestibility (IVSCPD), *in vitro* starch digestibility (IVSTD), *in vitro* gross energy digestibility (IVGED), and *in vitro* metabolic energy (IVME) of the eight diets were determined using SDS- . Each diet had five replicates with one digestion tube per replicate.

The results showed: (1) Compared with the control, diets supplemented with amylase at 1,840, 9,200, and 18,400 U/g significantly increased IVDMD and

IVGED in the gastric stage (P<0.05). The IVME of the 18,400 U/g amylase group was significantly higher than other groups (P<0.05). The total tract IVDMD, IVGED, and IVME of 22–42 day diets were significantly higher than those of 1–21 day diets (P<0.05). (2) The total tract IVACPD and IVSCPD of the 1,840 and 9,200 U/g amylase groups were significantly higher than the control (P<0.05), and these values for 22–42 day diets were significantly higher than for 1–21 day diets (P<0.05). (3) Total tract IVSTD of all eight diets exceeded 99.40%, with the 9,200 and 18,400 U/g amylase groups showing significantly lower IVSTD than the control (P<0.05). The total tract IVSTD of 1–21 day diets was significantly higher than that of 22–42 day diets (P<0.05). (4) Significant interactions between dietary nutrient level and amylase supplementation level were observed for IVDMD, IVGED, IVACPD, IVSCPD, IVSTD, and IVME (P<0.01). Under these experimental conditions, exogenous amylase supplementation improved gastric stage IVDMD and IVGED. Amylase at 1,840 and 9,200 U/g improved IVACPD and IVSCPD, while 18,400 U/g amylase improved IVME. Starch in corn-soybean meal diets was almost completely degraded, making the effect of exogenous amylase on IVSTD negligible. Interactions between dietary nutrient level and amylase dose affected *in vitro* nutrient digestibility and metabolic energy, with 22–42 day diets showing higher values than 1–21 day diets.

Keywords: exogenous amylase; SDS- ; nutrient; digestibility; metabolic energy

1. Introduction

Exogenous enzymes offer advantages in improving feed nutrient digestibility, enhancing animal growth performance, and maintaining animal health, making them a research hotspot in feed nutrition. However, their efficacy depends not only on enzymatic properties but also on the physiological status of target animals and basal diet composition. Therefore, accurate evaluation and application of feed enzymes remains an urgent challenge. Traditional animal testing methods consume substantial resources, suffer from uncontrollable conditions, show high result variability, and cannot provide rapid evaluation [1-2].

Studies show that supplemental -amylase alone can improve organic matter and starch digestibility and metabolic energy in diets, increasing daily weight gain and feed conversion ratio in broilers [3-6]. Conversely, other research indicates -amylase does not promote starch digestibility in young broiler diets (1–14 days) [7] or improve growth performance [8]. These inconsistent results suggest amylase efficacy is influenced by diet type, enzyme source, and supplementation level. Researchers have explored rapid, standardized *in vitro* methods for evaluating enzyme efficacy. Alabi et al. [9] and Malathi et al. [10] proposed pepsin-pancreatin *in vitro* methods for rapid assessment. Park et al. [11] demonstrated that enzyme complexes containing -amylase increased *in vitro* dry matter digestibility of corn and wheat. However, methodological variability across

laboratories limits stability in evaluation approaches.

Our research group has long studied rapid evaluation methods for feed enzymes, assessing protease effects on nutrient digestibility [12], establishing a rapid screening platform for non-starch polysaccharide enzymes in pig and poultry diets [13], and investigating non-starch polysaccharidase efficacy using biomimetic methods. However, *in vitro* evaluation of single exogenous amylase remains rarely reported. This study utilized SDS- to simulate digestive processes in broiler gastric and total gastrointestinal tracts, examining amylase effects on IVDMD, IVGED, IVACPD, IVSCPD, IVSTD, and IVME to provide a basis for accurate evaluation and application of feed enzymes.

2. Materials and Methods

This experiment was conducted from December 2016 to March 2017 at the State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences.

2.1. Experimental Design

A 2×4 factorial completely randomized design was used. Two basal corn-soybean meal diets were formulated for broilers aged 1–21 days and 22–42 days according to Chinese Feeding Standard of Chicken [14] (NY/T 33—2004) and NRC (1994) [15] requirements. The two basal diets served as controls, while six experimental diets were created by supplementing the basal diets with exogenous -amylase (provided by Beijing Yinong Feed Center, activity 24,500 U/g; one unit defined as enzyme activity releasing 1 μmol maltose per minute at 25°C, pH 6.90) at 1,840, 9,200, and 18,400 U/g. This yielded eight dietary treatment groups with five replicates each (one digestion tube per replicate). Diet samples were quarter-sampled, ground through a 60-mesh sieve, thoroughly mixed, and stored at -20°C. Basal diet composition is shown in Table 1 , and conventional nutrient contents in Table 2 .

2.2. Test Methods

The SDS- system simulated broiler gastric and total gastrointestinal digestion by preparing gastric buffer, small intestinal buffer, simulated gastric fluid, and simulated small intestinal fluid to evaluate amylase effects on IVDMD, IVGED, IVACPD, IVSCPD, IVSTD, and IVME of 1–21 day and 22–42 day corn-soybean meal diets. All procedures and parameters followed the *Simulated Digestion System for Monogastric Animals Operation Manual* [16].

2.2.1. Buffer and Simulated Digestive Fluid Preparation Buffer compositions are shown in Table 3 . All buffers were dissolved in deionized water, pH-adjusted at 41°C, and brought to 2,000 mL final volume. Gastric buffer was adjusted to pH 2.00 with 2 mol/L HCl, while anterior and posterior small

intestinal buffers were adjusted to pH 6.50 and 7.99, respectively, with 1 mol/L NaOH. Prepared buffers were preheated in the SDS- system.

Simulated gastric fluid (pepsin activity 1,550 U/mL): 387.5 kU pepsin (Sigma, P7000) was dissolved in 250 mL pH 2.0 HCl buffer (calibrated at 41°C) with gentle stirring (prepared fresh before use).

Simulated small intestinal fluid (amylase activity 401.46 U/mL, trypsin activity 49.28 U/mL, chymotrypsin activity 11.31 U/mL): 110.40 kU amylase (Sigma, A3306), 13.55 kU trypsin (Amresco, 0785), and 3.11 kU chymotrypsin (Amresco, 0164) were dissolved in 25 mL deionized water with gentle stirring (prepared fresh before use).

2.2.2. Operating Procedures A 2.0000 g diet sample (weighed to 0.0002 g precision) was mixed with 20 mL simulated gastric fluid, transferred without loss into a dialysis bag (molecular weight cutoff 14,000 Da) in a simulated digestion tube, sealed with a rubber stopper, and installed in the preheated SDS- . Gastric simulation parameters: 41°C for 4 h. After gastric digestion, 2 mL simulated small intestinal fluid was added to the small intestinal fluid reservoir, and small intestinal simulation continued at 41°C for 7.5 h each in anterior and posterior segments.

After digestion, undigested residues in dialysis bags were transferred without loss to pre-weighed (oven-dried) culture dishes, dried at 65°C for 8–10 h, then at 105°C to constant weight for 4 h, and weighed.

Residues were scraped from dishes: 0.3 g was taken for crude protein determination, 0.1 g for starch content, and the remainder was transferred to pre-weighed glass sand-core crucibles, defatted three times with anhydrous ethanol, dried at 105°C to constant weight after complete ethanol evaporation, and weighed. Simultaneously during biomimetic digestion, diet samples were analyzed for dry matter, crude protein, starch content, and gross energy.

2.2.3. Calculation Formulas Data were calculated as follows:

$$\begin{aligned}
\text{IVDMD}(\%) &= 100 \times (M_1 - M_2)/M_1 \\
\text{IVGED}(\%) &= 100 \times (GE_1 - GE_2)/GE_1 \\
\text{IVACPD}(\%) &= 100 \times (CP_1 - CP_2)/CP_1 \\
\text{IVSCPD}(\%) &= 100 \times (CP_1 - CP_2 + CP_0)/CP_1 \\
\text{IVSTD}(\%) &= 100 \times (ST_1 - ST_2)/ST_1 \\
\text{IVME}(\text{MJ/kg}) &= (GE_1 - GE_2)/(GE_1 \times 1000)
\end{aligned}$$

Where: M_1 = weight of diet dry matter (g); M_2 = weight of undigested residue dry matter (g); GE_1 = gross energy of diet (J); GE_2 = gross energy of residue (J); CP_0 = endogenous crude protein loss (g); CP_1 = weight of diet crude protein

(g); CP_2 = weight of residue crude protein (g); ST_1 = weight of diet starch (g); ST_2 = weight of residue starch (g).

2.3. Statistical Analysis

Data were analyzed using SAS 9.2 MEANS module for descriptive statistics and GLM module for two-way ANOVA. When interactions were significant (P<0.05), means were compared using Tukey' s method. Results are expressed as "mean ± standard deviation" with P<0.05 considered significant and 0.05<P<0.10 indicating a trend.

3. Results

3.1. Effects of Dietary Nutrient Level and Amylase Supplementation on IVDMD, IVGED, and IVME

As shown in Table 4 , significant interactions existed between dietary nutrient level and amylase supplementation for IVDMD, IVGED, and IVME (P<0.01). Amylase supplementation at all levels significantly increased gastric stage IVDMD and IVGED (P<0.05). The 18,400 U/g amylase group showed significantly higher IVME than other groups (P<0.05). Total tract IVDMD, IVGED, and IVME were significantly higher for 22–42 day diets than 1–21 day diets (P<0.05).

For 1–21 day diets, gastric and total tract IVDMD and gastric IVGED were significantly higher in the 9,200 and 18,400 U/g amylase groups compared to control and 1,840 U/g groups (P<0.05). IVME in the 9,200 and 18,400 U/g groups was significantly higher than control (P<0.05), increasing by 0.14 and 0.18 MJ/kg, respectively.

For 22–42 day diets, gastric IVDMD and IVGED were significantly higher in all amylase groups versus control (P<0.05). However, total tract IVDMD and IVGED were significantly lower in the 1,840 U/g group (P<0.05), and total tract IVDMD was significantly lower in the 9,200 U/g group (P<0.05). The 1,840 U/g group showed significantly lower IVME than control (P<0.05), while 9,200 and 18,400 U/g groups did not differ significantly from control (P>0.05).

3.2. Effects of Dietary Nutrient Level and Amylase Supplementation on IVACPD, IVSCPD, and IVSTD

As shown in Table 5 , significant interactions existed between dietary nutrient level and amylase supplementation for IVACPD, IVSCPD, and IVSTD (P<0.01). Total tract IVACPD and IVSCPD were significantly higher in the 1,840 and 9,200 U/g amylase groups than control (P<0.05), and significantly higher for 22–42 day diets than 1–21 day diets (P<0.05). Total tract IVSTD exceeded 99.40% for all diets, but was significantly lower in the 9,200 and 18,400 U/g groups versus control (P<0.05). Total tract IVSTD was significantly higher for 1–21 day diets than 22–42 day diets (P<0.05).

For 1–21 day diets, total tract IVACPD and IVSCPD were significantly higher in the 9,200 and 18,400 U/g groups than control ($P<0.05$), while total tract IVSTD was significantly lower in these groups ($P<0.01$).

For 22–42 day diets, total tract IVACPD and IVSCPD were significantly lower in the 18,400 U/g group than all other groups ($P<0.05$), with no significant differences between the 1,840 and 9,200 U/g groups and control ($P>0.05$). No significant differences in total tract IVSTD were observed among amylase supplementation groups ($P>0.05$).

4. Discussion

Exogenous amylase efficacy is influenced by enzyme source, supplementation level, and dietary nutrient level. Gracia et al. [3] found that supplemental -amylase improved organic matter and starch digestibility and metabolic energy in corn-soybean diets without affecting crude protein or fat digestibility, while also improving broiler weight gain and feed conversion [4-5]. Conversely, Mahagna et al. [7] reported no improvement in starch digestibility for young broilers (1–14 days), and Kaczmarek et al. [6] found no significant effects on starch and crude protein digestibility or performance. These inconsistencies highlight the need for standardized evaluation methods.

4.1. Effects of Dietary Nutrient Level and Amylase Supplementation on *in Vitro* Nutrient Digestibility

Exogenous enzyme effects are closely related to dietary nutrient level [17-18], though few studies examine single amylase effects. The 1–21 day diets were high-protein, low-energy, while 22–42 day diets were low-protein, high-energy. Significant interactions existed between nutrient level and amylase dose, with more pronounced effects in 1–21 day diets. Lower dietary energy levels showed greater amylase responses, consistent with animal studies where enzyme complexes containing -amylase improved digestibility more in 18–21 day diets than 42 day diets when metabolizable energy was reduced by 836 kJ/kg [17]. Huang [18] also reported greater enzyme effects on crude protein digestibility in ducks fed lower-energy diets.

4.2. Effects of Exogenous Amylase on IVCPD and IVSTD

Young chicks have immature digestive systems with insufficient endogenous enzyme secretion, necessitating exogenous enzyme supplementation. Gracia et al. [3] found amylase improved crude protein and starch digestibility at 7 days, but by 28 days, starch digestibility remained higher while crude protein digestibility decreased non-significantly. Our SDS- simulation showed that 9,200 and 18,400 U/g amylase significantly improved IVACPD and IVSCPD in 1–21 day diets but not 22–42 day diets, similar to animal trial results [17], possibly due to amylase-protease interactions. Jiang et al. [19] reported that microbial -amylase supplementation at 250–2,250 mg/kg increased total protease

and trypsin activities in 21-day-old broiler intestinal contents. Improved crude protein digestibility may result from enhanced endogenous enzyme activity or reduced starch molecular hindrance of protein digestion [20].

Although 9,200 and 18,400 U/g amylase statistically reduced total tract IVSTD in 1–21 day diets, the practical effect was negligible since corn-soybean diets showed nearly complete starch degradation (>99.40%). Starch digestibility correlates with endogenous amylase secretion, which is low at 4 days but increases with age before stabilizing [21].

4.3. Effects of Exogenous Amylase on IVDMD and IVGED

The acidic gastric environment can destroy exogenous enzyme activity. Ao et al. [22] demonstrated that avian digestive tract pH is a major limiting factor for exogenous enzymes. In our study, 9,200 and 18,400 U/g amylase improved gastric stage IVDMD in both diet types, indicating acid tolerance. Similar studies showed that enzyme complexes containing -amylase (8.5×10 U/t) increased corn IVDMD and IVGED by 2.07% and 2.82%, and soybean meal IVDMD and IVGED by 5.00% and 0.26% [23]. Our results demonstrate that amylase supplementation improved IVDMD, IVGED, and IVME, increasing digestible nutrients (e.g., crude protein) *in vitro*.

Research suggests low enzyme doses enhance endogenous enzyme activity, moderate doses degrade endogenous enzymes, and high doses show positive effects [24]. The interaction between exogenous and endogenous enzymes depends on enzyme source, substrate, and dosage, requiring further mechanistic study.

5. Conclusion

Under the conditions of this experiment:

1. Exogenous amylase supplementation improved gastric stage IVDMD and IVGED. Amylase at 1,840 and 9,200 U/g improved IVACPD and IVSCPD, while 18,400 U/g amylase improved IVME in broiler diets.
2. Starch in corn-soybean meal diets was almost completely degraded, making the effect of exogenous amylase on IVSTD negligible.
3. Significant interactions existed between dietary nutrient level and amylase supplementation level on *in vitro* nutrient digestibility and metabolic energy, with 22–42 day diets showing higher values than 1–21 day diets.

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