Effects of Exogenous Amylase on In Vitro Nutrient Digestibility and Metabolizable Energy of Corn-Soybean Meal Diets for Broiler Chickens

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Abstract

This study aimed to investigate the effects of exogenous amylase on in vitro nutrient digestibility and metabolizable energy of corn-soybean meal diets for broiler chickens using the Simulated Digestive System for monogastric animals (SDS-), providing a basis for accurately evaluating the efficacy of feed enzyme preparations. A 2$×$4 factorial completely randomized design was employed. Basal corn-soybean meal diets for broilers aged 1 to 21 days and 22 to 42 days were formulated according to China' s "Feeding Standard of Chickens" (NY/T 33-2004) and NRC (1994) nutrient requirements for chickens. Six amylase-supplemented diets were prepared by adding exogenous amylase at 1,840, 9,200, and 18,400 U/g to the two basal diets, with the two basal diets without exogenous amylase serving as controls. The SDS- system was used to determine in vitro dry matter digestibility (IVDMD), in vitro apparent crude protein digestibility (IVACPD), in vitro standardized crude protein digestibility (IVSCPD), in vitro starch digestibility (IVSTD), in vitro gross energy digestibility (IVGED), and in vitro metabolizable energy (IVME) of the eight diets. Each diet was allocated five replicates, with one digestion tube per replicate. The results showed: 1) Compared with the control group, gastric phase IVDMD and IVGED of diets in the 1,840, 9,200, and 18,400 U/g amylase groups were significantly increased ($P<0.05$); IVME of diets in the 18,400 U/g amylase group was significantly higher than that of other groups ($P<0.05$); whole-tract IVDMD, IVGED, and IVME of diets for 22 to 42 days of age were significantly higher than those for 1 to 21 days of age ($P<0.05$). 2) Whole-tract IVACPD and IVSCPD of the 1,840 and 9,200 U/g amylase groups were significantly higher than those of the control group ($P<0.05$); whole-tract IVACPD and IVSCPD of diets for 22 to 42 days of age were significantly higher than those for 1 to 21 days of age ($P<0.05$). 3) Whole-tract IVSTD of all eight diets exceeded 99.40%; whole-tract IVSTD of the 9,200 and 18,400 U/g amylase groups was significantly lower than that

of the control group (P<0.05); whole-tract IVSTD of diets for 1 to 21 days of age was significantly higher than that for 22 to 42 days of age (P<0.05). 4) Dietary nutrient level and amylase supplementation dose exhibited interactive effects on IVDMD, IVDGE, IVACPD, IVSCPD, IVSTD, and IVME of broiler diets (P<0.01). Under the experimental conditions, exogenous amylase supplementation improved gastric phase IVDMD and IVDGE 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 dose had interactive effects on in vitro nutrient digestibility and metabolizable energy of broiler diets, and in vitro nutrient digestibility and metabolizable energy of diets for 22 to 42 days of age were higher than those for 1 to 21 days of age.

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 in vitro nutrient digestibility and metabolic energy of corn-soybean meal diets for broilers using the Simulated Digestion System for Monogastric Animals (SDS-), providing a basis for accurately evaluating feed enzyme efficacy. 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 for Chickens (NY/T 33-2004) and NRC (1994) requirements. Six enzyme-supplemented diets were created by adding 1,840, 9,200, and 18,400 U/g of exogenous amylase to each basal diet, 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 measured using SDS- . Each diet had five replicates with one digestion tube per replicate. The results showed: (1) Compared with the control, gastric phase IVDMD and IVGED were significantly increased in diets supplemented with 1,840, 9,200, and 18,400 U/g amylase (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 those of 22–42 day diets were significantly higher than 1–21 day diets (P<0.05). (3) The total tract IVSTD of all eight diets exceeded 99.40%. The total tract IVSTD of the 9,200 and 18,400 U/g amylase groups was significantly lower than the control (P<0.05), while that of 1–21 day diets was significantly higher than 22–42 day diets (P<0.05). (4) Significant interactions between dietary nutrient level and amylase dosage were observed for IVDMD, IVGED, IVACPD, IVSCPD, IVSTD, and IVME (P<0.01). Under these experimental conditions, exogenous amylase supplementation improved gastric phase IVDMD and IVGED. Amylase at 1,840 and 9,200 U/g increased IVACPD and IVSCPD, while 18,400 U/g amylase increased IVME. Starch in corn-soybean meal diets was almost completely degraded, making the effect of amylase on IVSTD negligible. Interactions between dietary nutrient level and amylase dosage 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- ; nutrients; digestibility; metabolic energy

Introduction

Exogenous enzyme supplementation improves feed nutrient digestibility, animal growth performance, and health maintenance, making it a research hotspot in feed nutrition. However, enzyme efficacy depends not only on enzymatic properties but also on animal physiological status and basal diet composition, necessitating accurate evaluation methods. Traditional animal trials for feed enzyme evaluation consume substantial resources, suffer from uncontrollable conditions, show high result variability, and cannot provide rapid assessment [1-2]. Studies show that α-amylase supplementation improves organic matter and starch digestibility, increases metabolic energy, and enhances daily gain and feed conversion in broilers [3-6]. Conversely, other research indicates α-amylase does not promote starch digestibility in young broilers (1–14 days) [7] or improve growth performance [8]. These inconsistent results reflect influences from diet type, enzyme source, and dosage. 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 enzyme evaluation. Park et al. [11] demonstrated that α-amylase-containing enzyme complexes increased in vitro dry matter digestibility of corn and wheat. Despite progress, variability in methods, materials, and conditions across laboratories limits standardization. Our research group has developed rapid evaluation methods for feed enzymes, assessing protease effects on nutrient digestibility [12] and establishing non-starch polysaccharide enzyme screening platforms for pigs

and poultry [13]. However, few studies have evaluated single exogenous amylase efficacy using in vitro methods. This experiment used SDS- to simulate gastric and whole digestive tract processes in chickens, examining amylase effects on IVDMD, IVGED, IVACPD, IVSCPD, IVSTD, and IVME to provide a basis for accurate enzyme evaluation and application.

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1 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.

1.1 Experimental Design

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 for Chickens [14] (NY/T 33—2004) and NRC (1994) [15] requirements. The two basal diets served as controls. Three amylase supplementation levels (1,840, 9,200, and 18,400 U/g) were added to each basal diet using exogenous α-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 and pH 6.90). This created eight dietary treatment groups with five replicates each (one digestion tube per replicate). Diet samples were quartered, ground through a 60-mesh sieve, mixed thoroughly, and stored at -20°C. Basal diet composition is shown in Table 1 , and conventional nutrient contents of experimental diets are shown in Table 2 .

1.2 Experimental Methods

The SDS- system simulated gastric and whole digestive tract processes in chickens 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 broiler diets. All procedures and parameters followed the Simulated Digestion System for Monogastric Animals Operation Manual [16].

1.2.1 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 diluted to 2,000 mL. Gastric buffer pH was adjusted to 2.00 with 2 mol/L HCl, while anterior and posterior intestinal buffers were adjusted to 6.50 and 7.99 with 1 mol/L NaOH, respectively. Prepared buffers were preheated in the SDS- system.

Simulated gastric fluid (pepsin activity 1,550 U/mL) was prepared by dissolving 387.5 kU pepsin (Sigma, P7000) 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) was prepared by dissolving 110.40 kU amylase (Sigma, A3306), 13.55 kU trypsin (Amresco, 0785), and 3.11 kU chymotrypsin (Amresco, 0164) in 25 mL deionized water with gentle stirring (prepared fresh before use).

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

After digestion, undigested residues in the dialysis bag 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.

Digestion residues were scraped from dishes. Approximately 0.3 g was taken for crude protein determination, 0.1 g for starch content, and the remainder was weighed and transferred to pre-weighed glass sand-core crucibles. After three defatting steps with anhydrous ethanol and complete ethanol evaporation, samples were dried at 105°C to constant weight and weighed.

During simulated digestion, diet samples were simultaneously analyzed for dry matter, crude protein, starch content, and gross energy.

1.2.3 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 = dry matter weight of diet sample (g); M_2 = dry matter weight of undigested residue (g); GE_1 = gross energy of diet sample (J); GE_2 = gross energy of undigested residue (J); CP_0 = endogenous crude protein loss (g); CP_1 = crude protein weight of diet sample (g); CP_2 = crude protein weight of undigested residue (g); ST_1 = starch weight of diet sample (g); ST_2 = starch weight of undigested residue (g).

1.3 Statistical Analysis

Data were analyzed using SAS 9.2. The MEANS procedure calculated descriptive statistics, and the GLM procedure performed two-way ANOVA. When interactions were significant ($P<0.05$), means were compared using Tukey' s test. Results are expressed as "mean ± standard deviation." Significance was declared at $P<0.05$, with $0.05<P<0.10$ indicating a trend.

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2 Results

2.1 Effects of Dietary Nutrient Level and Amylase Dosage on IVDMD, IVGED, and IVME

As shown in Table 4 , significant interactions between dietary nutrient level and amylase dosage were observed for IVDMD, IVGED, and IVME ($P<0.01$). Following amylase supplementation, gastric phase IVDMD and IVGED increased significantly ($P<0.05$). The IVDMD and IVGED of 1–21 day diets were significantly higher than those of 22–42 day diets in the gastric phase ($P<0.01$). The total tract IVDMD and IVGED of the 1,840 U/g amylase group were significantly lower than other groups ($P<0.05$), while those of 22–42 day diets were significantly higher than 1–21 day diets ($P<0.05$). The IVME of the 18,400 U/g amylase group was significantly higher than other groups ($P<0.05$), and IVME of 22–42 day diets was significantly higher than 1–21 day diets ($P<0.05$).

In 1–21 day diets, gastric and total tract IVDMD and gastric phase IVGED were significantly higher in the 9,200 and 18,400 U/g amylase groups compared with the control and 1,840 U/g group ($P<0.05$). The IVME of these two groups was also significantly higher than the control ($P<0.05$), increasing by 0.14 and 0.18 MJ/kg, respectively.

In 22–42 day diets, gastric phase IVDMD and IVGED were significantly higher in all amylase groups compared with the 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 IVME of the 1,840 U/g group was significantly lower than the control ($P<0.05$), while the 9,200 and 18,400 U/g groups did not differ significantly from the control ($P>0.05$).

2.2 Effects of Dietary Nutrient Level and Amylase Dosage on IVACPD, IVSCPD, and IVSTD

As shown in Table 5 , significant interactions between dietary nutrient level and amylase dosage were observed for IVACPD, IVSCPD, and IVSTD ($P<0.01$). 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 those of 22–42 day diets were significantly higher than 1–21 day diets ($P<0.05$). The total

tract IVSTD of all eight diets exceeded 99.40%, but was significantly lower in the 9,200 and 18,400 U/g amylase groups compared with the control ($P<0.05$). The total tract IVSTD of 1–21 day diets was significantly higher than 22–42 day diets ($P<0.05$).

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

In 22–42 day diets, total tract IVACPD and IVSCPD were significantly lower in the 18,400 U/g group compared with all other groups ($P<0.05$), while the 1,840 and 9,200 U/g groups did not differ significantly from the control ($P>0.05$). No significant differences in total tract IVSTD were observed among amylase groups ($P>0.05$).

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3 Discussion

Exogenous amylase efficacy is influenced by enzyme source, dosage, and dietary nutrient level. Gracia et al. [3] found that α-amylase supplementation improved organic matter and starch digestibility and metabolic energy in corn-soybean meal diets without affecting crude protein and fat digestibility. Single α-amylase also improved broiler weight gain and feed conversion [4-5]. However, 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, metabolic energy, or performance. These inconsistent results highlight the need for standardized evaluation methods. This study used SDS- to examine interactions between dietary nutrient level and amylase dosage, providing a reference for rapid enzyme evaluation.

3.1 Effects of Dietary Nutrient Level and Amylase Dosage on In Vitro Nutrient Digestibility

Exogenous enzyme effects are closely related to dietary nutrient level [17-18], but few studies have examined single amylase interactions with diet composition. The 1–21 day diets were high-protein, low-energy formulations, while 22–42 day diets were low-protein, high-energy. Significant interactions indicated that amylase effects were more pronounced in 1–21 day diets, with greater improvements observed in lower-energy diets. Similar animal trials showed that enzyme complexes containing α-amylase improved total tract digestibility of dry matter, crude protein, and energy more effectively in 18–21 day diets than in 42 day diets when dietary metabolizable energy was reduced by 836 kJ/kg [17]. Huang [18] also reported that enzyme supplementation improved crude protein utilization in ducks, with greater effects in lower-energy diets.

3.2 Effects of Exogenous Amylase on IVCPD and IVSTD

Young chicks have immature digestive systems with insufficient endogenous enzyme secretion, necessitating exogenous supplementation to support early development. Gracia et al. [3] found that amylase improved crude protein and starch digestibility at 7 days, but by 28 days, crude protein digestibility decreased non-significantly while starch digestibility remained elevated. Using SDS- to simulate digestion in 1–21 day and 22–42 day broilers, we found that 9,200 and 18,400 U/g amylase significantly improved IVACPD and IVSCPD in 1–21 day diets but not in 22–42 day diets, consistent with animal trial results [17]. This may reflect interactions between exogenous amylase and endogenous proteases. Jiang et al. [19] reported that microbial α-amylase supplementation at 250, 750, and 2,250 mg/kg increased total protease and trypsin activities in 21-day broiler intestinal contents. The improved crude protein digestibility may result from enhanced endogenous enzyme activity or reduced steric hindrance from starch molecules, facilitating protein digestion [20].

Although 9,200 and 18,400 U/g amylase statistically reduced total tract IVSTD in 1–21 day diets, the practical improvement was negligible because corn-soybean meal starch was almost completely degraded (IVSTD >99.40% for all diets). Starch digestibility correlates with endogenous amylase secretion, which is low at 4 days of age but increases and stabilizes with age [21].

3.3 Effects of Exogenous Amylase on IVDMD and IVGED

The acidic gastric environment can denature exogenous enzymes. Ao et al. [22] evaluated enzyme activity across avian digestive tract pH levels, identifying pH as a major limiting factor. In this study, 9,200 and 18,400 U/g amylase improved gastric phase IVDMD in both age groups, indicating acid tolerance and enhanced gastric nutrient digestibility. Similar research showed that α-amylase-containing complexes (activity $ 8.5×10^{6}$ 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%, respectively [23]. Our results demonstrate that amylase supplementation improved IVDMD, IVGED, and IVME, increasing digestible nutrients (e.g., crude protein) in vitro.

Research suggests that low enzyme doses enhance endogenous enzyme activity, moderate doses may degrade endogenous enzymes, and high doses exhibit positive effects [24]. The mechanisms depend on enzyme source, substrate, and dosage, requiring further investigation.

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4 Conclusion

Under the experimental conditions:

1. Exogenous amylase supplementation improved gastric phase IVDMD and

IVGED. Amylase at 1,840 and 9,200 U/g increased IVACPD and IVSCPD, while 18,400 U/g amylase increased IVME of broiler diets.

2. Starch in corn-soybean meal diets was almost completely degraded, making the effect of amylase on IVSTD negligible.
3. Significant interactions existed between dietary nutrient level and amylase dosage 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.