

Effects of Dietary Protein Level and Fiber Source on Cecal Fluid Characteristics in Growing Pigs

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Abstract

This study aimed to investigate the effects of dietary protein level and fiber source on the digestive enzyme activities and hydrolytic capacity of cecal fluid in growing pigs, providing a reference for simulating large intestinal digestive fluid in growing pigs. Experiment 1 examined the effect of dietary protein level (diets 1 and 2 contained 19.03% and 13.96% crude protein, respectively) on the composition of cecal fluid in growing pigs. A completely randomized design was employed, with 12 growing pigs fitted with cecal cannulas randomly assigned to 2 groups based on body weight, with 6 replicates per group and 1 pig per replicate, a 5-day preliminary period, and a 10-day experimental period. Experiment 2, conducted after a 10-day recovery period following Experiment 1, examined the effect of dietary fiber source (diet 3: corn-soybean hull diet; diet 4: corn-wheat bran diet) on the composition of cecal fluid in growing pigs, with the same experimental design as Experiment 1. Experiment 3 investigated the effects of cecal fluid source and substrate on reducing sugar release based on the porcine cecal digesta obtained from Experiments 1 and 2. A 4\$×8two – factorcompletelyrandomizeddesignwasadopted,withcecalfluidsourceas4treatments, namelycecalfluidcollectedfromdiets5and6, twocorn–soybeanmealdiets(diets5and6), andfreeze–drieddigestafromtheterminalileumofpigsfeddiets5and6. 1)Comparedwiththelow–proteindiet(diet2), thehigh–proteindiet(diet1)significantlydecreasedthedrymattercontentofporcinececaldigesta, while dietary protein level had no significant effects on cecal fluid pH and the activities of cellulase, xylanase, glucanase, amylase, neutral protease, and acid protease ($P > 0.05$); 2) Dietary fiber source had no significant effects on the dry matter content of porcine cecal digesta, pH, and the activities of cellulase, xylanase, glucanase, amylase, neutral protease, and acid protease ($P > 0.05$); 3) Both cecal fluid source and substrate had significant effects on reducing sugar release ($P < 0.05$), and the two factors had a significant interaction effect on reducing sugar release ($P < 0.05$). Higher hydrolytic enzyme activities in cecal fluid and lower dietary fiber content resulted in greater reducing sugar release after in vitro digestion of cecal fluid. The activities of xylanase, glucanase,

neutral protease, and amylase in cecal fluid affected the digestive capacity for diets, while xylanase and amylase activities affected the digestive capacity for terminal ileal digesta. These results indicate that although dietary nutrient levels did not reach statistical significance in their effects on the six hydrolytic enzyme activities in growing pig cecal fluid, small differences in multiple hydrolytic enzymes accumulated to cause differences in hydrolytic capacity.

Full Text

Effects of Dietary Protein Levels and Fiber Sources on Characteristics of Cecal Fluid of Growing Pigs

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Abstract: This study investigated the effects of dietary protein levels and fiber sources on digestive enzyme activities and hydrolytic capacity of cecal fluid in growing pigs to provide a reference for simulating large intestinal digestion.

Experiment 1 examined the effect of dietary protein levels (crude protein content of 19.03% in Diet 1 and 13.96% in Diet 2) on cecal fluid composition. Using a completely randomized design, 12 growing pigs fitted with cecal cannulas were randomly divided into 2 groups based on body weight, with 6 replicates per group and 1 pig per replicate. The adaptation period was 5 days, followed by a 10-day experimental period.

Experiment 2, conducted after a 10-day recovery period following Experiment 1, investigated the effect of dietary fiber sources (Diet 3: corn-soybean hull diet; Diet 4: corn-wheat bran diet) on cecal fluid composition, using the same experimental design as Experiment 1.

Experiment 3 examined the effects of cecal fluid source and substrate on reducing sugar release based on the cecal digesta obtained from Experiments 1 and 2. A 4×8 two-factor completely randomized design was employed, with 4 cecal fluid sources (collected from pigs fed Diets 1, 2, 3, and 4) and 8 substrates (Diets 1-4, two corn-soybean meal diets [Diets 5 and 6], and freeze-dried ileal digesta from pigs fed Diets 5 and 6). Each treatment had 5 replicates with 1 digestion tube per replicate.

The results showed: (1) Compared with the low-protein diet (Diet 2), the high-protein diet (Diet 1) significantly decreased dry matter content in cecal digesta ($P \leq 0.05$), while dietary protein level had no significant effect on pH or activities of cellulase, xylanase, glucanase, amylase, neutral protease, and acid protease in cecal fluid ($P > 0.05$). (2) Dietary fiber source had no significant effect on dry matter content, pH, or activities of cellulase, xylanase, glucanase, amylase,

neutral protease, and acid protease in cecal fluid ($P>0.05$). (3) Both cecal fluid source and substrate significantly affected reducing sugar release ($P<0.05$), with a significant interaction between the two factors ($P<0.05$). Higher hydrolytic enzyme activity in cecal fluid and lower dietary fiber content resulted in greater reducing sugar release after in vitro digestion. The activities of xylanase, glucanase, neutral protease, and amylase in cecal fluid affected digestive capacity for diets, while xylanase and amylase activities affected digestive capacity for ileal digesta. These results indicate that although dietary nutrient levels did not significantly affect the activities of six hydrolytic enzymes in cecal fluid of growing pigs, the combination of small differences in multiple hydrolytic enzymes caused differences in hydrolytic capacity.

Keywords: growing pig; cecal digesta; digestive enzyme activity; hydrolysis capacity

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Introduction

The large intestine of growing pigs is generally 3.5-6.0 m in length, with digestion retention time exceeding 18 hours. The large intestine makes significant contributions to nutrient digestion from high-fiber feed ingredients, making it crucial to investigate large intestinal digestive physiology in pig digestion simulation studies. The cecum, located at the anterior portion of the large intestine and accounting for 7-8% of total large intestine length, serves as an important site for microbial growth and structural carbohydrate digestion. Microbial concentration in the cecum of growing pigs is approximately 2.37×10^{10} cells/g digesta [1], and these microbes produce or secrete carbohydrate and proteolytic enzymes that degrade dietary components not digested and absorbed by the small intestine.

Research indicates that the cecal digestion rate of dietary organic matter in growing pigs exceeds 11.7%, representing 90% of total large intestine digestion [2]. Dietary nutrients can induce changes in cecal microbial populations [3], thereby affecting nutrient digestion. For example, low-protein diets can significantly increase the ratio of Firmicutes to Bacteroidetes in pig intestines, affecting concentrations of short-chain fatty acids and biogenic amines in feces [4]. In vitro digestion of high-protein diets by pig cecal microbes can significantly increase ammonia nitrogen, microbial protein, and short-chain fatty acid contents [5]. Dietary fiber sources affect microbial diversity including *Prevotella*, *Lactobacillus*, *Ruminococcus*, and *Bacteroides* in cecal digesta, thereby influencing activities of non-starch polysaccharide enzymes such as cellulase, pectinase, β -glucanase, and xylanase [6-7].

These findings demonstrate that dietary protein levels and fiber sources significantly affect characteristics of pig cecal fluid. Investigating differences in enzyme

activities and hydrolytic capacities of cecal fluid from growing pigs under different dietary nutrient levels provides a biological basis for preparing simulated cecal fluid. Therefore, this study compared differences in cecal fluid composition and hydrolytic characteristics of growing pigs fed two crude protein levels and two fiber sources through in vivo cecal digesta collection, providing basic parameters for simulating large intestinal digestion in growing pigs.

1.1 Experimental Animals and Management

Twelve Duroc×Landrace×Yorkshire crossbred barrows with similar genetic background and parity, with initial body weight of $(20.05\pm 1.87)kg$, were individually housed in metabolism cages. Type cannulas were installed at the terminal cecum (10 cm posterior to the ileocecal junction). Post-surgery, pigs were housed in metabolism cages with routine care for 3 weeks. Female experiments began after recovery, respectively.

1.2 Experimental Diets and Ileal Terminal Digesta

Basal and experimental diets were formulated according to NRC (2012) nutrient requirements for 20-60 kg growing pigs. Diets 1-4 were experimental diets for Experiments 1 and 2, representing high-protein diet, low-protein diet, corn-soybean hull diet, and corn-wheat bran diet, respectively (Table 1). Diets 5-6 were corn-soybean meal diets formulated in a previous experiment. Freeze-dried ileal digesta 1-2 were prepared from ileal digesta collected from pigs fed Diets 5 and 6, respectively.

1.3 Experimental Design

This study consisted of three parts. Experiment 1 investigated the effect of dietary protein levels (crude protein content of 19.03% and 13.96%) on cecal fluid composition. Using a two-sample completely randomized design, 12 cecum-cannulated growing pigs were randomly divided into 2 groups based on body weight, with 6 replicates per group and 1 pig per replicate, fed Diets 1 and 2, respectively.

Experiment 2, conducted after a 10-day recovery period following Experiment 1, investigated the effect of dietary fiber sources (soybean hull vs. wheat bran) on cecal fluid composition using the same design. Pigs with no significant difference in average body weight were fed Diets 3 and 4. Both Experiments 1 and 2 had 5-day adaptation periods and 10-day experimental periods. During the experimental periods, diets were fed wet at 08:00 and 16:00 daily at 4% of body weight to eliminate feed intake differences, with free access to water. Other management procedures followed routine protocols of the State Key Laboratory of Animal Nutrition. The first 5 days were for fecal collection, and the last 5 days for cecal digesta collection on alternate days.

Experiment 3 investigated the effects of cecal fluid source and dietary substrate

on reducing sugar release based on cecal digesta obtained from Experiments 1 and 2. A 4\$×\$8 two-factor completely randomized design was used, with 4 cecal fluid sources (A: from pigs fed high-protein Diet 1; B: from pigs fed low-protein Diet 2; C: from pigs fed corn-soybean hull Diet 3; D: from pigs fed corn-wheat bran Diet 4) and 8 substrates (Diets 1-6 and 2 types of freeze-dried ileal digesta). Each treatment had 5 replicates with 1 digestion tube per replicate for measuring reducing sugar release.

1.4 Sample Collection

In Experiments 1 and 2, total fecal collection was conducted from 09:00 on day 6 to 09:00 on day 11. Daily fecal samples were mixed uniformly, dried at 65°C, equilibrated for 24 hours, sampled by quartering method, and stored at -20°C. From days 11, 13, and 15, digesta samples were continuously collected from cannulas using self-made sampling bags with low-temperature cooling function during 09:30-10:30, 13:30-14:30, and 17:30-18:30. After each collection period, digesta samples from each pig were mixed proportionally by collection volume. A 40 mL sample was immediately centrifuged at 4°C and 1,250×g for 10 minutes. The supernatant was mixed uniformly, aliquoted into 1.5 mL tubes, and stored at -80°C for enzyme activity analysis. Remaining digesta samples from each pig were processed similarly to obtain cecal fluid, which was then pooled by volume within each dietary treatment group and aliquoted into 50 mL tubes for later use.

1.5 Measurements and Methods

Dry matter content of cecal digesta was determined by weighing fresh digesta, drying at 65°C until no water trace remained, then calculating according to GB/T 6435-2006 [9]. pH of cecal fluid was measured using a Sartorius PB-10 pH meter at 39°C in a constant temperature water bath. Activities of cellulase, xylanase, and β -glucanase were determined according to NY/T 912-2004, GB/T 23874-2009, and NY/T 911-2004, respectively. Amylase activity was determined using the method of Dahlqvist [10]. Acid protease and neutral protease activities were determined according to GB/T 28715-2012.

Reducing sugar release from diets and digesta by cecal fluid was determined using the “Operating Manual for Monogastric Animal Bionic Digestive System” established by our laboratory, measured in the vertical digestion module of the third-generation bionic digestive system. The sample loading amount was 0.5 g with 10 mL cecal fluid. After digestion, digested material was accurately transferred to a 100 mL volumetric flask, diluted to volume, filtered through a 0.22 μ m membrane, and reducing sugar release (as glucose) was measured using a double-beam UV spectrophotometer at 530 nm wavelength.

1.6 Data Processing and Statistical Analysis

The MEANS module of SAS 9.0 was used to calculate basic statistics for enzyme activities and nutrient digestibility. The t-test module was used to test significance differences in enzyme activities and nutrient digestibility between treatments in Experiments 1 and 2. For Experiment 3, the GLM module of SAS 9.0 was used for two-factor ANOVA. When interactions were significant, Duncan's multiple comparison test was used for mean separation. The CORR module was used to analyze correlations between digestive enzyme activities and reducing sugar release. Data were expressed as mean \pm standard deviation, with P \leq 0.05 indicating significant difference.

2.1 Effects of Diet Type on Cecal Digesta Characteristics and Nutrient Digestibility

As shown in Table 2, dry matter content in cecal digesta of pigs fed low-protein diet was significantly higher than that of pigs fed high-protein diet ($P=0.05$), while no significant difference was observed between corn-soybean hull diet and corn-wheat bran diet groups ($P>0.05$). Dietary treatments in Experiments 1 and 2 had no significant effects on pH or activities of cellulase, xylanase, glucanase, neutral protease, acid protease, and amylase in cecal fluid ($P>0.05$).

In Experiment 1, digestibility of dry matter, gross energy, crude protein, and crude fiber in the high-protein diet group was significantly higher than in the low-protein diet group ($P<0.05$). In Experiment 2, crude fiber digestibility in the corn-soybean hull diet group was significantly higher than in the corn-wheat bran diet group ($P<0.05$), while no significant differences were observed in dry matter, gross energy, or crude protein digestibility between the two groups ($P>0.05$).

2.2 Effects of Cecal Fluid Source and Substrate on Reducing Sugar Release

As shown in Table 3, both cecal fluid source and substrate significantly affected reducing sugar release ($P<0.05$), with a significant interaction between the two factors ($P<0.05$). Among the six dietary substrates, the ranking of reducing sugar release after cecal fluid digestion was: corn-soybean hull diet fluid > corn-wheat bran diet fluid > low-protein diet fluid > high-protein diet fluid. For ileal digesta 1 and 2, the rankings were: corn-soybean hull diet fluid > high-protein diet fluid > low-protein diet fluid > corn-wheat bran diet fluid, and corn-soybean hull diet fluid > high-protein diet fluid > corn-wheat bran diet fluid > low-protein diet fluid, respectively.

Among the eight substrates, Diet 6 showed the highest average reducing sugar release at 352 mg/g, significantly higher than other substrates ($P<0.05$). The

other five dietary substrates had reducing sugar release ranging from 250-296 mg/g with significant differences among groups ($P < 0.05$). The two ileal digesta samples had reducing sugar release of 51 mg/g, significantly lower than dietary substrates ($P < 0.05$).

2.3 Correlation Between Digestive Enzyme Activities and Reducing Sugar Release

Correlation analysis between digestive enzyme activities in cecal fluid and reducing sugar release (Table 4) revealed that reducing sugar release from the six diets was significantly correlated with xylanase, glucanase, neutral protease, and amylase activities ($r = 0.74$; $P < 0.05$), but not with cellulase and acid protease activities ($|r| = 0.49$; $P > 0.05$). Reducing sugar release from ileal digesta 1 was significantly positively correlated with xylanase and amylase activities ($r = 0.74$; $P < 0.05$), significantly negatively correlated with acid protease activity ($r = -0.79$; $P < 0.05$), but not correlated with cellulase, glucanase, or neutral protease activities ($r = 0.19$; $P > 0.05$). Reducing sugar release from ileal digesta 2 was significantly correlated with cellulase, xylanase, glucanase, and neutral protease activities ($r = 0.65$; $P < 0.05$), but not with acid protease activity ($r = -0.16$; $P > 0.05$).

3.1 Effects of Dietary Composition on Cecal Fluid Components

After digestion in the stomach and small intestine of growing pigs, starch, fat, and amino acids are digested at rates above 97%, 87%, and 83%, respectively [11-13], while crude fiber digestibility varies below 25% [14]. This indicates that substrates entering the large intestine consist primarily of plant cell walls from feed, along with small amounts of resistant starch and undigested dietary and sloughed intestinal proteins.

This study found that dry matter content in cecal digesta was 9.2-10.4% when pigs consumed high-protein and low-protein diets, and 9.9-10.4% when fed corn-soybean hull and corn-wheat bran diets. These values are slightly lower than the 13.68% dry matter content in pig cecal digesta obtained by Sun et al. [5] using slaughter methods, possibly due to different collection methods. However, pigs fed high-protein diets had higher moisture content in cecal digesta than those fed low-protein diets. This may be because high-protein diets increase concentrations of volatile fatty acids, ammonia, phenols, and indoles in the intestine, resulting in higher osmotic pressure and water-holding capacity [5,15].

The buffering capacity of pig intestine stabilizes with age, with cecal content pH stabilizing at 6.40-6.48 by 31 days of age [16]. This study found cecal fluid pH of 6.43-6.62, with neither dietary protein level nor fiber source significantly affecting pH, indicating strong buffering capacity of pig cecum for entering substrates. This result is consistent with Zhong [17].

Regarding the relationship between dietary substrates and cecal fluid enzyme activities, Morita et al. [18] and Liu et al. [19] found that increasing indigestible

protein levels in diets increased substrate flow into the cecum and enhanced total volatile fatty acid production. Rist et al. [20] also showed that dietary protein levels could affect butyrate production in pig hindgut, thereby altering cecal microbial composition. However, no reports have examined the effect of dietary protein levels on cecal fluid digestive enzyme activities.

Additionally, when fiber substrate concentrations are low, it is difficult to detect effects of dietary fiber levels on carbohydrate enzyme activities in growing pig cecal fluid. Under high fiber substrate concentrations, hindgut microbial populations increase significantly [21-22], and high fiber levels highlight the effect of fiber source on cellulase activity in pig cecal digesta [23]. In this study, neither dietary protein level nor fiber source affected hydrolytic enzyme activities in cecal fluid, mainly because under current nutrient requirements, differences in crude protein levels and fiber sources (with neutral detergent fiber [NDF] content below 15%) resulted in minimal differences in substrate concentrations in cecal digesta after gastric and small intestinal digestion, insufficient to cause statistically significant differences in microbial hydrolytic enzyme activities. This result is consistent with Gao [24], who reported no significant differences in cellulase, xylanase, and glucanase activities in cecal digesta of growing pigs fed four diets with NDF content below 20%.

3.2 Differences in Digestive Capacity of Cecal Fluid for Diets and Ileal Terminal Digesta

Pig cecal fluid contains various hydrolytic enzymes. Using current feed enzyme supplementation levels as reference for preparing simulated digestive fluid [25-26], the activities of non-starch polysaccharide enzymes and proteases in cecal fluid far exceed the activity concentrations of these exogenous feed enzymes when added to diets. Although this study found no statistically significant differences in hydrolytic enzyme activities in cecal fluid from pigs fed different diets, small differences in multiple hydrolytic enzymes combined may lead to differences in digestive capacity.

Knudsen et al. [11] found that when growing pigs were fed diets with different β -glucan contents, large intestinal digestibility of dietary fiber and carbohydrates increased linearly. Jørgensen et al. [27] showed that when growing pigs consumed low-fiber and high-fiber diets, large intestinal digestibility of entering dry matter was 45.9% and 64.7%, respectively, and non-starch polysaccharide digestibility was 55.9% and 75.0%, respectively. These findings indicate that large intestinal digestive capacity is affected by dietary composition. In vitro simulation studies also show that adding different non-starch polysaccharide enzymes during the large intestinal digestion stage significantly affects dietary dry matter and energy digestibility [28].

In this study, collected pig cecal fluid was only thawed and centrifuged, retaining over 95% of original enzyme activity. Reducing sugar release after substrate digestion expressed the carbohydrate hydrolytic capacity of cecal fluid. Different

cecal fluid sources significantly affected hydrolytic capacity for diets, which was correlated with xylanase, glucanase, amylase, and neutral protease activities. Among the six hydrolytic enzymes measured, pigs fed high-protein diets had the lowest activities for five enzymes, while pigs fed corn-soybean hull diets had the highest activities for five enzymes. The hydrolytic capacities of these two cecal fluids for six diets corresponded accordingly, indicating that small changes in multiple hydrolytic enzymes combined to amplify obvious differences in hydrolytic capacity.

The hydrolytic capacities of the two cecal fluids for ileal terminal digesta were correlated with xylanase and amylase activities, related to xylan and resistant starch contents in ileal terminal digesta of pigs fed corn-soybean meal diets. Furthermore, hydrolytic capacity of cecal fluid for six diets was much higher than for two ileal terminal digesta samples, because ileal terminal digesta had already undergone gastric and small intestinal digestion, with most nutrients digested and absorbed, reducing substrates for cecal fluid hydrolytic enzymes.

4 Conclusion

1. Dietary protein level significantly affected dry matter content in pig cecal digesta but had no significant effect on cecal fluid pH or hydrolytic enzyme activities. Dietary fiber source had no significant effect on dry matter content, pH, or hydrolytic enzyme activities in cecal fluid.
2. Cecal fluid from growing pigs fed different diets showed significant differences in digestive capacity. Activities of xylanase, glucanase, neutral protease, and amylase in cecal fluid affected digestive capacity for diets, while xylanase and amylase activities affected digestive capacity for ileal terminal digesta.

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