

Effects of feed with different protein digestion kinetic profiles on intestinal health of growing pigs

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Abstract

This study evaluated the effects of feed ingredients with different protein digestion kinetic profiles on the intestinal health of growing pigs. Two protein sources were selected, namely casein (CAS) as a rapid release source of amino acids (AAs), and corn gluten meal (CGM) as a slow-release source. Twenty-four crossbred barrows (Duroc x Landrace x Yorkshire) with similar bodyweight (43.27 ± 3.51 kg) were selected and randomly assigned to four treatments with six barrows. These consisted of T1: 13.2% digestible crude protein (CP) with supplemental CAS; T2: 13.2% digestible CP with supplemental CGM; T3: 11.2% digestible CP with supplemental CAS (T3); and T4: 11.2% digestible CP with supplemental CGM. Diets with CGM had increased crypt depth in the duodenum, jejunum, and ileum and reduced villi height in the jejunum in comparison with CAS. They also had increased intestinal permeability, as seen by the high level of serum diamine oxidase (DAO) compared with CAS. The diets with CAS increased health-promoting *Lactobacillus* and decreased health-threatening *Treponema* compared with those fed CGM diets. The CAS diets had a positive effect on gut functions with increased villi height, decreased crypt depth and high villi height/crypt depth. Thus, use of CAS in diets for pigs is favoured over CGM.

Keywords: casein, corn gluten meal, microorganism, morphology, permeability

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Introduction

Several properties of protein sources can affect the release kinetics of free amino acids (AAs) and dipeptides and tripeptides. This may influence the timing of the postprandial appearance of AAs and peptides (Chen *et al.*, 2018). Protein sources can be regarded as fast and slow, depending on the timing and the level of postprandial increase of plasma AA and peptide concentration (Boirie *et al.*, 1997; Bos *et al.*, 2003; Tang *et al.*, 2009). Dietary proteins need to be hydrolysed into free AA or small peptides (Ganapathy *et al.*, 2000; Webb *et al.*, 1992) before they can be absorbed by enterocytes in the small intestinal mucosa. In pigs, the absorption of AAs, and di- and tripeptides by intestinal enterocytes takes place in the jejunum and ileum, for which the proximal jejunum is the major site (Bröer, 2008; Chen, 2017). Pigs have a longer retention time of digesta in the stomach and the small intestine than poultry (Liu *et al.*, 2013; Weurding *et al.*, 2001; Wilfart *et al.*, 2007).

The absorption rate for proteins is highly dependent on the protein source, since animal proteins (e.g. casein) are more completely digested than plant proteins (e.g. corn gluten meal, soybean) (López-Pedrouso *et al.*, 2019). Casein is a highly digestible protein source with a rapid AA release rate (Hambræus & Lönnerdal, 2003; Rist *et al.*, 2014; Wellock *et al.*, 2006). In addition, the true digestibility of the AAs in CAS is essentially 100% in pigs (Chung & Baker, 1992). Zhong *et al.* (2017) found that the total AA content in chyme proved that CAS feed digested rapidly in the duodenum of piglets. Corn gluten meal has a lower AA release rate (Abdallah *et al.*, 2019). This could be because of the high amount of prolamin in corn, which is composed of high contents of hydrophobic AAs and thus has poor solubility (Torres-Giner *et al.*, 2008). Additionally, humans show earlier postprandial appearance of AA and di- and tripeptides in blood when they consume fast digestible dietary proteins, such as whey, compared with more slowly digestible sources such as CAS (Boirie *et al.*, 1997). Boirie *et al.* (1997) noted that CAS induced a lower but more prolonged

postprandial increase of AAs and peptides in plasma. The intestinal barrier plays a critical role in maintaining the health and growth of animals (Che *et al.*, 2019). Therefore an ideal dietary pattern could increase nutrient absorption efficiency and maximize the economic performance of pigs (Kim *et al.*, 2009; Van Milgen & Dourmad, 2015).

Most published studies focused on the effect of different concentrations of AAs on growth performance and carcass characteristics. However, few investigated the effect of the release rate of dietary protein AAs on intestinal health. In the present study, CAS was selected as a rapid AA release rate protein source and CGM as a slow one. The use of these two protein sources was designed to obtain a broad range in protein supply in feed ingredients with different protein digestion kinetics on grower pigs. The aim of the study was to explore the effects of dietary protein level and sources with different AA release rate on the intestinal morphology, permeability and microorganism of growing pigs, and the expression of AA and glucose transporters.

Materials and Methods

The experiment was conducted at the Swine Metabolism Laboratory of Jilin Agricultural University (Changchun, Jilin). Permission to feed the animals and to use samples was acquired from the Ethical Clearance Committee of Jilin Agricultural University, permission number KT2019012 for swine (Changchun, Jilin).

A 2*2 factorial arrangement of treatments was used in this experiment. Twenty-four hybrid barrows (Duroc x Landrace x Yorkshire) weighing about 30 kg were selected and randomly divided into four groups of six barrows. T1 contained 13.2% digestible CP with supplemental CAS; T2 contained 13.2% digestible CP with supplemental CGM; T3 contained 11.2% digestible CP with supplemental CAS; and T4 contained 11.2% digestible CP with supplemental CGM. The pigs were fed these diets for four weeks. The diets (Table 1) were based on the guidelines of the National Research Council (2012).

Table 1 Formulation of experimental diets for 25 kg growing pigs with differing levels of digestible protein and supplemented with either casein or corn gluten meal

Ingredients, %	13.2% digestible protein diets		11.2% digestible protein diets	
	Casein supplemented	Corn gluten meal supplemented	Casein supplemented	Corn gluten meal supplemented
Corn	68.01	62.65	71.80	67.11
Wheat bran	5.00	5.00	5.00	5.00
Soybean meal	14.23	15.09	9.55	12.59
Casein	3.13	-	2.66	-
Corn protein meal	-	6.52	-	3.60
Sucrose	3.00	3.00	3.00	3.00
Soybean oil	1.54	2.53	1.59	2.36
Supplemental amino acids	0.45	0.52	0.96	1.04
Calcium phosphate	1.39	1.38	1.38	1.18
Salt	0.85	0.84	0.85	0.84
Premix ¹	1.00	1.00	1.00	1.00

¹Per kg diet: vitamin A: 28 500 IU, vitamin D: 36 000 IU, vitamin E: 67.5 IU, vitamin K: 37.5 mg, vitamin B: 17.5 mg, vitamin B₂: 215 mg, vitamin B₆: 69 mg, vitamin B₁₂: 0.075 mg, nicotinic acid 70 mg, folic acid: 3 mg, calcium pantothenate: 37.5 mg, biotin: 0.375 mg, antioxidant 0.15 mg, choline chloride: 105 mg, cobalt: 1 mg, copper: 155 mg, iron: 145 mg, manganese: 75 mg, zinc: 125 mg, iodine: 0.3 mg, selenium: 0.3 mg

The calculated nutritional composition of the diets is given in Table 2.

Table 2 Nutritional composition of experimental diets for 25 kg growing pigs with differing levels of digestible protein and supplemented with either casein or corn gluten meal

Nutrient	13.2% digestible protein diets		11.2% digestible protein diets	
	Casein supplemented	Corn gluten meal supplemented	Casein supplemented	Corn gluten meal supplemented
Net energy, MJ/kg	10.35	10.35	10.35	10.35
Digestible crude protein, %	13.18	13.18	11.21	11.21
Crude fibre, %	1.88	1.89	1.79	1.85
Arginine, %	0.85	0.85	0.85	0.85
Histidine, %	0.36	0.36	0.36	0.36
Isoleucine, %	0.59	0.59	0.59	0.59
Leucine, %	1.62	1.62	1.62	1.62
Lysine, %	0.98	0.98	0.98	0.98
Methionine + cysteine, %	0.98	0.98	0.98	0.98
Phenylalanine + tyrosine, %	2.42	2.42	2.42	2.42
Threonine, %	0.59	0.59	0.59	0.59
Tryptophan, %	0.17	0.17	0.17	0.17
Valine, %	0.68	0.68	0.68	0.68
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.40	0.40	0.40	0.41
		0.42	0.41	0.43

An intestinal morphological study was conducted on the duodenum, jejunum, and ileum of grower pigs. An approximately 1 cm piece was cut from the middle of each section. These intestinal tissue samples were rinsed with precooled saline solution, and stored in 10% paraformaldehyde (pH 7.4). When the study was conducted, the samples were cut into blocks of about 5 × 5 × 3 mm and put into the embedding box for water flushing. The samples were embedded with paraffin and sections of each intestinal segment were selected. Three pieces of villi were selected from each segment without missing structure, and the vertical field of vision was observed. The villi height and crypt depth of each segment was measured under a 40x objective lens by ToupView (x86) software, and the ratio of the two was calculated.

Levels of serum DAO and D-lactic acid (D-LA) were detected with an enzyme-linked immunosorbent assay (ELISA) kit (E05227, R&D Systems, Minneapolis, MN, USA). Blood samples were collected in tubes containing EDTA and mixed immediately to avoid coagulation. Serum was obtained after centrifugation at 4 °C for 15 minutes at 3000 rev/min and stored at -80 °C until analysis.

The contents of the jejunum anterior, middle, and posterior segments and ileum and cecum were amplified, and the extracted DNA was determined with 0.8% agarose gel electrophoresis. The DNA was quantified by ultraviolet spectrophotometer and tested to see whether the subsequent PCR could amplify an effective target band. The 16S rDNA v3-v4 region was used for PCR amplification. The amplification results were performed by 2% agarose gel electrophoresis. The target fragments were cut and recovered with an Axygen® gel recovery kit. The QUant-It™ PicoGreen™ dsDNA kit (Thermo Fisher Scientific) was used to quantify the PCR products, then the samples were mixed according to the amount of data required for each sample, and finally, the sequencing was carried out. QIIME was used to classify and analyse the composition and abundance of phylum, class, order, family, and genus. The alpha diversity index was determined with Mothur, including Shannon, ACE, Chao1 and Simpson indices.

The data were analysed with SPSS 23.0 software (IBM Corp., Armonk, New York, USA) for multivariate analysis of variance, and Duncan's test was used for multiple comparisons among treatments. The linear model was:

$$y_{ijk} = \mu + P_i + S_j + PS_{ij} + e_{ijk}$$

where: y_{ijk} = an observation of a dependent variable from the kth pig fed a diet with the ith protein level that contained supplemental protein from the kth source,
 μ = overall mean,

P = the effect of protein level,
 S = effects of the supplemental protein source,
 PS = the interaction of P and S, and
 e_{ijk} = the random error.

The threshold $P = 0.05$ was used for tests of significance. The distributions of the data were tested for normality using the Kolmogorov-Smirnov test.

Results and Discussion

No significant level of CP by dietary source interaction effects were observed ($P > 0.10$). Pigs fed diets containing CAS had increased villi height and villi height/crypt depth in the duodenum, jejunum and ileum compared with those fed CGM. Villi height and crypt depth in the duodenum decreased when pigs were fed a diet supplemented with CAS at low protein level (T3). Pigs fed T4 had increased crypt depth in the duodenum compared with T3. CGM had increased crypt depth in the duodenum, jejunum, and ileum compared with CAS. Different levels of protein did not affect villi height/crypt depth in the duodenum and villi height in the jejunum and ileum. Table 3 shows the effects of these feed ingredients.

Table 3 Effects of feed ingredients with different protein digestion kinetic profiles on intestinal morphogenesis of growing pigs

Morphological measurement	13.2% digestible protein diets		11.2% digestible protein diets	
	Casein supplemented	Corn gluten meal supplemented	Casein supplemented	Corn gluten meal supplemented
Duodenum				
Villi height μm	372.45 ^a	304.10 ^c	347.45 ^{ab}	322.05 ^{bc}
Crypt depth μm	291.94 ^{ab}	264.93 ^a	236.45 ^b	280.46 ^a
Villi height/ crypt depth	1.421 ^a	1.088 ^b	1.485 ^a	1.190 ^b
Jejunum				
Villi height μm	401.12 ^{ab}	355.26 ^a	412.16 ^b	357.09 ^a
Crypt depth μm	247.60	270.01	252.58	284.81
Villi height/ crypt depth	1.642 ^{ab}	1.341 ^b	1.756 ^a	1.284 ^b
Ileum				
Villi height μm	356.41 ^a	288.88 ^c	340.49 ^{ab}	311.94 ^{bc}
Crypt depth μm	223.12	242.82	252.91	248.73
Villi height/ crypt depth	1.638 ^a	1.214 ^b	1.403 ^{ab}	1.316 ^{ab}

^{a,b,c} Within a row, means with a common superscript were not different with probability $P < 0.05$

Serum DAO and D-LA activity can be used as markers for monitoring the extent of intestinal permeability and mucosal damage (Wu *et al.*, 2013). Both molecules are released directly into the blood circulation when the intestinal mucosa is destroyed (Quiros & Nusrat, 2014). D-LA is the final product of bacterial fermentation in the gut (Nieto *et al.*, 2000; Tossou *et al.*, 2016). Feeding T2 resulted in increased DAO concentration compared with T1. Among protein sources, CGM meal had increased DAO level compared with CAS. No differences were observed in serum D-LA concentration among the treatments. The interaction effect of protein level and source of AAs was not significant for either DAO or D-LA. The effects of different protein sources on DAO and D-LA in growing pigs are shown in Table 4.

Table 4 Effects of feed ingredients with different protein digestion kinetics profile on diamine oxidase and D-lactic acid in growing pigs

	13.2% digestible protein diets		11.2% digestible protein diets	
	Casein supplemented	Corn gluten meal supplemented	Casein supplemented	Corn gluten meal supplemented
DAO, ng/mL	153.37 ^b	293.99 ^a	186.76 ^{ab}	232.21 ^{ab}
D-LA, μ mol/mL	508.18 ^a	513.87 ^a	531.70 ^a	535.57 ^a

^{a,b} Within a row, means with a common superscript were not different with probability $P < 0.05$
 DAO: diamine oxidase; D-LA: D-lactic acid

Bacterial richness (Chao1) was decreased when pigs were fed T3 and T4. No differences among treatments were detected in the Simpson and Shannon diversity indices for the intestinal microbial populations of the pigs. (See Table 5.)

Table 5 Effects of feed ingredients with different protein digestion kinetic profiles on microbial diversity in growing pigs

	13.2% digestible protein diets		11.2% digestible protein diets	
	Casein supplemented	Corn gluten meal supplemented	Casein supplemented	Corn gluten meal supplemented
Chao1	2708.26	3182.52	2282.94	2870.28
Simpson	0.96224	0.96563	0.96137	0.96293
Shannon	7.92827	8.18041	7.49596	7.45459

The four major bacterial phyla that accounted for more than 90% of the total bacterial community were *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Spirochaetes*, with *Firmicutes* being most abundant in the intestines of pigs that were fed all diets. However, the interaction of protein level and source of AAs was significant only for *Firmicutes*, because *Firmicutes* was much more prevalent in pigs fed T4, reaching a peak of 70% of the bacteria. *Proteobacteria* were most abundant in the T1 and T3 diets, which were not different from each other. The response in *Proteobacteria* to T2 and T4 was similar. Neither the dietary protein level nor the source of supplemental AAs affected the concentrations of *Actinobacteria* and *Spirochaetes*. Feeding supplemental CGM increased the prevalence of *Bacteroidetes* (See Table 6.)

Table 6 Effects of feed ingredients with different protein digestion kinetic profiles on the relative abundance of the most common phyla found in the intestine of growing pigs

Phylum	13.2% digestible protein diets		11.2% digestible protein diets	
	Casein supplemented	Corn gluten meal supplemented	Casein supplemented	Corn gluten meal supplemented
Proteobacteria, %	35.0945 ^a	22.8971 ^{ab}	30.9498 ^a	14.6574 ^b
Actinobacteria, %	0.5837	0.3357	0.6100	0.3638
Firmicutes, %	53.2479 ^b	52.3187 ^b	55.9498 ^b	65.2509 ^a
Spirochaetes, %	3.6881	2.8171	0.4159	0.9239
Bacteroidetes, %	4.3117 ^b	13.1343 ^a	6.5846 ^b	16.9458 ^a

^{a,b} Means within the row with a common superscript did not differ with probability $P < 0.05$

The four major genera were *Acinetobacter*, *Streptococcus*, *Treponema*, and *Lactobacillus*. *Lactobacillus* was more prevalent in pigs that were fed T1 and T3 than T2 or T4. Despite more than fourfold differences in abundance, the incidences of *Acinetobacter*, *Treponema*, and *Bifidobacteria* were not detectably different across treatments. *Streptococcus* was more abundant when pigs were fed T2 compared with the other diets. The interaction between level of dietary protein and the source of supplemental amino acids was not significant for any of these genera.

Table 7 Effects of feed ingredients with different protein digestion kinetic profiles on taxonomic statistics of the most common genera of intestinal bacteria found in growing pigs

Genera	13.2% digestible protein diets		11.2% digestible protein diets	
	Casein supplemented	Corn gluten meal supplemented	Casein supplemented	Corn gluten meal supplemented
Lactobacillus, %	8.4723 ^a	3.9822 ^b	7.8729 ^a	3.1270 ^b
Acinetobacter, %	25.4121	18.1788	21.3803	7.0250
Streptococcus, %	0.8136 ^b	5.4852 ^a	0.1215 ^b	2.1339 ^b
Treponema, %	2.9780	14.5092	1.5731	8.1521
Bifidobacterium, %	0.1468	0.0267	0.0046	0.0201

The formation of crypts and villi enlarges the surface of the mucus membrane in the small intestine (Wiese *et al.*, 2003). An enhanced villus to crypt ratio improved nutrient digestibility (Shen *et al.*, 2009). Increased crypt depth and reduced villus height has been observed in piglets fed excess protein (Boudry *et al.*, 2013). In the present study, pigs fed CGM showed damage in the duodenum and ileum, resulting in lower villi height and higher crypt depth, irrespective of the level of digestible CP. Pigs fed T4 diets also showed increased crypt depth in the jejunum. Chen *et al.* (2018) found increased crypt depth in the ileum of pigs fed a 15% CP diet relative to those fed either 12% or 18% CP. Additionally, as a result of the various sources of AAs, CGM was found to increase crypt depth in the duodenum, jejunum, and ileum compared with CAS. Corn gluten meal reduced the height of the villi in the jejunum in comparison with CAS. In the present study, pigs fed diets containing CAS (T1 and T3) had increased villi height and villi height/crypt depth ratio in the duodenum, jejunum and ileum compared with those fed CGM. Reduction of dietary protein concentration from 16% to 10% damaged ileal mucosa dramatically with a lower ratio of intestinal villus height to crypt depth, which influenced the absorptive capacity for nutrients (Fan *et al.*, 2017).

The various levels of protein did not affect villi height/crypt depth ratio in the duodenum and villi height in the jejunum and ileum of grower pigs. Leonard *et al.* (2011) found no effect on the ratio of villus height to crypt depth in pigs weaned from sows supplemented with seaweed extract. Although Chen *et al.* (2018) found a decrease in the ratio of villi height to crypt depth in the ileum of pigs fed a diet with 15% CP, in the current study villi height and crypt depth in the duodenum decreased when pigs were fed the T4 diet, which was lower in protein. Decreasing the ratio of villi height to crypt depth in the ileum reduced nutrient absorption (Chen *et al.*, 2018). In the present study, no differences were observed in serum D-LA among treatments. Serum D-LA concentration may increase if the mucosa of the small intestine is injured as a result of dysfunction in the intestinal barrier (Vella & Farrugia, 1998).

Diamine oxidase is a highly active intracellular enzyme produced by the intestinal epithelial. It exists only in the intestinal mucosa and ciliated cells and may play a role in the control of mucosal proliferation (Chen *et al.*, 2017; Tossou *et al.*, 2016). In this study, T2 resulted in an increase in DAO concentration compared with T1. Among protein sources, CGM supplementation produced higher serum DAO compared with CAS, suggesting that diets containing CGM affected intestinal permeability in grower pigs. An increase in both D-LA and DAO levels reflected changes in the intestinal permeability (Liu *et al.*, 2008) and damage to the intestinal mucosa (Luk *et al.*, 1980; Vella & Farrugia, 1998). The permeability of the gastrointestinal tract allows the selective barrier to absorb needed nutrients and prevent the penetration of harmful entities from the external environment, including pathogens and antigens (Li *et al.*, 2016). Gut microbiota and their fermented metabolites have a probable effect on the health of the pig (Zhao *et al.*, 2020). Dietary habits modify the structure of the gut microbiota and have a significant effect on gut microbiota (De Filippo *et al.*, 2010). In the current experiment, all of the treatments altered the structural composition of the microbiota.

In the present study, bacterial richness and diversity were indicated by the Chao 1 statistic and by the Simpson and Shannon indices, respectively. When grower pigs were fed the T2 diet, bacterial richness increased compared with T1. However, bacterial richness (Chao 1) decreased when fed T3 and T4. No differences were observed in the microbial diversity (Simpson and Shannon indices) in the intestine of pigs in both protein sources and levels. Similarly, Yu *et al.* (2019) found no significant difference in bacterial diversity when pigs were fed a protein-restricted diet. Weaning pigs fed a diet 14% CP were found to have reduced bacterial diversity relative to those fed a 20% CP diet despite the diets having similar levels of lysine, methionine, threonine, and tryptophan (Luo *et al.*, 2015). On the contrary, Peng *et al.* (2017) found an increase in bacterial diversity when pigs were fed a diet with a moderate reduction of CP level from 20.00% to 15.30%. The microbiota goes through an extreme change when cereal-based diets are introduced at weaning (Mach *et al.*, 2015). Moderate protein restriction can enhance the microbiota structure (Chen *et al.*, 2018), increasing microbes such as *Lactobacillus*, therefore enhancing mucin production, which then enriches the gut barrier (Che *et al.*, 2014).

Lactobacillus can ferment carbohydrates into lactic acid and improve the intestinal environment (Fukada *et al.*, 2011; Kleerebezem *et al.*, 2003). It is one of the predominant genera in the ileum of growing pigs (Zhao *et al.*, 2015) and is seen as a beneficial microbe that prevents disease in piglets during the preweaning phase (Konstantinov *et al.*, 2006; Petri *et al.*, 2010). In the current study, *Lactobacillus* was more abundant in pigs fed the CAS diets than those fed the CGM diets. In addition, when pigs were fed the T3 diet *Lactobacillus* concentration remained on the same level as that of the T1 diet. Thus, *Lactobacillus* increased when grower pigs were fed T3 compared with T4. Yu *et al.* (2019), who found a decrease in *Lactobacillus*, stated that this could be because pig diets were short of protein and were composed predominately of corn and oil. Zhao *et al.* (2020) found that *Lactobacillus* was sensitive to dietary alterations. However, in studies in which highly digestible animal protein sources such as CAS (Wellock *et al.*, 2006) were fed to weaned piglets, no effect could be found on the composition of the intestinal microbiota, in comparison with plant proteins with lower protein digestibility such as soybean meal (SBM) (Rist *et al.*, 2014).

Similar to *Lactobacillus*, *Bifidobacteria* benefit intestinal health as a result of their fermenting carbohydrates into lactic acid (Gibson & Macfarlane, 1995; Loh *et al.*, 2006). However, *Bifidobacterium* was previously found to be a more rare microbe in the intestinal tract of pigs (Fouhse *et al.*, 2016; Rist *et al.*, 2013). Similarly, *Bifidobacteria* was the least abundant genus in pigs in the present study. In a study by Peng *et al.* (2017), a reduction of CP level to 13.90% decreased *Bifidobacterium* concentration.

In the current study, abundance of *Acinetobacter* was found in the pigs fed the CAS diets. Bacteria belonging to *Streptococcus* play a major role in AA utilization in animals (Boudry *et al.*, 2013; Mao *et al.*, 2016; Neis *et al.*, 2015; Niu *et al.*, 2015). CGM had a high *Streptococcus* concentration compared with CAS diets. Pigs fed a low protein diet had decreased *Streptococcus* in the colon, suggesting a shortage of protein substrate for fermentation (Chen *et al.*, 2018; Fan *et al.*, 2016; Zhou *et al.*, 2016).

The disease-causing bacteria *Treponema* was more abundant in pigs fed T4 compared with T2. Thus, the decreased level of *Treponema* in pigs fed CAS could be beneficial (Bunker *et al.*, 2015; Palm *et al.*, 2014).

In the present study, *Firmicutes* was the most dominant phylum in pigs that were fed all diets. When dietary protein concentration decreased, the proportion of *Firmicutes* increased, reaching a peak of 70% in T4. Contrary to the present findings, Luo *et al.* (2015) found a decrease in *Firmicutes* when feeding a low protein diet to pigs. *Proteobacteria* reached its peak in pigs fed CAS diets T1 and T3).

Neither dietary protein level nor source affected the concentrations of *Actinobacteria* and *Spirochaetes*, which had the lowest concentrations in all diets. *Bacteroidetes* are potentially beneficial bacteria and are capable of fermenting complex carbohydrates (Flint *et al.*, 2008; McCormack *et al.*, 2017; Umu *et al.*, 2015). In the current study, the CGM protein source had increased *Bacteroidetes* compared with CAS. Similarly, Cervantes-Pahm and Stein (2008) reported that higher dietary CP stimulated the growth of *Bacteroides* in the faeces of piglets fed SBM-based diets, but not in CAS-based diets, possibly because of less digestible protein from SBM compared with CAS. The unpredictable effects of dietary protein levels on the intestinal microbial diversity might be owing to the reduced levels of dietary protein and different sources of protein.

Conclusions

Grower pigs fed diets supplemented with CAS had a better outcome than those supplemented with CGM, as seen by a lower concentration of DAO, increased *Lactobacillus* and decreased *Treponema* in the intestinal tract. This study provided added insight into the theoretical basis for full and efficient utilization of protein sources and improved economic benefits.

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Authors' Contributions

MRT conducted animal feeding, sample and data collection, statistical analysis and writing up of the manuscript. GQ developed the idea, gave input in the writing of the manuscript, and was the main supervisor of the study. YZ assisted in developing the study, edited the manuscript, and was a co-supervisor. BW helped with animal feeding, sample and data collection, and statistical analysis. ZWS collected samples and data. QZ contributed to animal feeding and sample collection.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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