

## Feed form affects performance of weaned pigs

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**ABSTRACT.** This study was conducted to assess the effects of mash and pellet diets with the same starch gelatinisation degree on growth performance, nutrient digestibility, serum biochemical indicators, free amino acid concentrations, and digestive enzyme activities in piglets. A total of 96 crossbred (Duroc × Landrace × Yorkshire) piglets with an average initial body weight of  $8.91 \pm 0.15$  kg were randomly assigned to one of two dietary treatments based on body weight and sex (four barrows and four gilts per replicate). Each treatment consisted of six replicates, with eight pigs per replicate. The trial lasted 35 days, during which piglets were fed either a mash or pellet diet. Compared with the mash diet, piglets fed the pellet diet had higher average daily gain and average daily feed intake, while feed efficiency was not affected by the feed form. The pellet diet also improved the apparent total tract digestibility of ether extract and gross energy on day 14. Serum analysis demonstrated increased ghrelin and decreased leptin concentrations in pellet-fed piglets on day 35. Additionally, serum methionine and threonine levels were higher in the pellet diet group. Regarding digestive enzyme activity, piglets fed the pellet diet showed increased amylase activity in the posterior jejunum, while decreased lipase activity in the anterior jejunum, and sucrase activity in the posterior ileum. These findings indicate that, under identical starch gelatinisation conditions, the pellet diet increased growth performance by improving nutrient digestibility, gut hormone levels, and digestive enzyme activities.

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### Introduction

Weaning is a critical stage in pig production, marked by significant physiological, environmental, and dietary changes that disrupt intestinal and immune function, leading to reduced growth and feed intake (Wijtten et al., 2011; Campbell et al., 2013). To mitigate weaning stress, strategies such as optimising diet formulation and selecting high-quality ingredients are essential. Additionally, feed form plays a key role in nutrient utilisation and growth performance (Wondra et al., 1995; Medel et al., 2004; Lunedo et al., 2023). Mash and pellets are the two most common feed types in commercial pig production, which differ in physical properties, processing technology, and their effects on piglet

performance. Mash diets increase the contact area between feed and endogenous enzymes, thereby improving nutrient digestibility (Kim et al., 2002; Boroojeni et al., 2016). Meanwhile, pelleting, the most prevalent hydrothermal processing method, alters the physicochemical properties of feed ingredients through the combined effects of heat, moisture and pressure (Massuquetto et al., 2020; Lancheros et al., 2020). Lunedo et al. (2023) observed that feeding a pelleted diet during the last pig nursery phase improved average daily gain (ADG), feed conversion ratio (G:F), and final body weight (BW). Similarly, Medel et al. (2004) found that pelleting improved nutrient digestibility and feed conversion in piglets. In addition to reducing feed wastage, lowering energy consumption,

and increasing nutrient density, the major reason for these improvements is higher gelatinisation of starch in compound feeds (Solà-Oriol et al., 2009; Jo et al., 2021; Almeida et al., 2021). However, previous studies have often failed to consider the interactive effects between feed form and starch gelatinisation, making it difficult to accurately pinpoint the specific feed-related factor responsible for improved piglet growth performance. Therefore, the objective of this study was to determine the effects of feed form on piglet growth performance, serum biochemical parameters, free amino acid concentrations, nutrient digestibility, and digestive enzyme activities, while controlling for the confounding influence of starch gelatinisation. The present results contribute to a deeper understanding of the underlying mechanism by which feed form affects nutrient utilisation and overall performance in weaned piglets.

## Material and methods

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of China Agricultural University (Beijing, AW82803202-1-2) and this study was conducted at the Fengning Swine Research Base (Chengde, HE, China).

### Diets preparation

The experimental diets were formulated to meet or exceed the nutritional requirements for piglets as recommended by the National Research Council (NRC, 2012; Table 1). The pellet diet was produced using an MUZL-180 pellet mill (Muyang Co., Yangzhou, JS, China) fitted with a die measuring 2.5 mm in diameter and 20 mm in thickness. The feeds were steam-conditioned for approximately 10 s at a constant temperature of 65 °C. After pelleting, the diets were cooled using a cooler. Subsequently, half of the pellet feed was ground using an SFSP56 × 40C hammer mill (Muyang Co.) with a 2.0 mm screen to prepare the mash diet.

### Animal management and housing

A total of 96 crossbred piglets (Duroc × Landrace × Yorkshire), weaned at 28 days of age with an average initial body weight of  $8.91 \pm 0.15$  kg, were randomly assigned to one of two treatment groups based on BW and sex. Each treatment group included six replicates with eight pigs per pen (four barrows and four gilts). The pigs were fed either a mash or a pellet diet. Water and feed were available *ad libitum*, and the ambient tem-

**Table 1.** Composition and nutrient content of the experimental diet (% as-fed basis)

Ingredients, %	
Maize	62.72
Beet pulp	5.00
Soy protein concentrate	5.00
Soybean meal	6.50
Fish meal	5.00
Whey powder	5.00
Fermented soybean meal	5.00
Dicalcium phosphate	1.30
Limestone	0.60
Salt	0.50
Soybean oil	2.00
L-lysine HCL	0.48
DL-methionine	0.07
L-threonine	0.19
L-tryptophan	0.04
Acidifier	0.10
Vitamin-mineral premix <sup>1</sup>	0.50
Total	100
Calculated nutrients	
metabolisable energy, kcal/kg	3420.94
crude protein, %	18.68
calcium, %	0.78
phosphorus, %	0.63
lysine, %	1.47
methionine, %	0.40
tryptophan, %	0.24
threonine, %	0.87
standardised ileal digestible lysine	1.27
standardised ileal digestible methionine	0.36
standardised ileal digestible threonine	0.72
standardised ileal digestible tryptophan	0.18

<sup>1</sup> premix provided per kg of diet: IU: vit. A 12000, vit. D<sub>3</sub> 2500, vit. E 30; mg: vit. K<sub>3</sub> 3.0, vit. B<sub>2</sub> 4.0, vit. B<sub>5</sub> 15.0, vit. B<sub>3</sub> 40.0, choline chloride 400.0, vit. B<sub>9</sub> 0.7, vit. B<sub>1</sub> 1.5, vit. B<sub>6</sub> 3, manganese 40, iron 100, zinc 100, copper 100, iodine 0.3, selenium 0.3; µg vit. B<sub>12</sub> 12

perature was maintained at 26 °C. The trial lasted for 35 days. Piglets were weighed individually on days 1, 14, and 35, with feed consumption recorded to calculate ADG, average daily feed intake (ADFI) and the feed-to-gain ratio (F:G).

### Sample collection

Uncontaminated faecal samples were collected twice daily for three consecutive days on days 12–14 and 33–35. Samples were obtained from at least four different pigs per pen, with individual pigs marked after sampling to avoid repeated collection on the same day. All faecal samples were stored at –20 °C. Samples collected over the three day periods were pooled by pen and dried at 65 °C for 72 h. Diets and dried faeces were ground using a 1 mm sieve prior to analysis. Blood samples were collected from the jugular vein into vacutainer tubes after

a 12-hour fasting period on days 14 and 35 from piglets representing the average body weight in each pen. All samples were centrifuged at 3500 g for 15 min at 4 °C to separate serum, which was subsequently aliquoted and stored at –20 °C until biochemical analysis. On day 35, one piglet per pen with body weight closest to the pen average (six pigs per treatment group) was selected for slaughter. Segments (10 cm each) of the duodenum, anterior jejunum, posterior jejunum, anterior ileum and posterior ileum segments were collected, rinsed, and the mucosa samples were collected from each segment. All samples were flash-frozen in liquid nitrogen and stored at –80 °C for digestive enzyme activity analysis.

### Starch gelatinisation analysis

Starch gelatinisation in the feed was analysed using the methods of the Agricultural Industry Standard of the People's Republic of China (NY/T 4125-2022).

### Nutrient digestibility analysis

The dry matter (DM), ether extract (EE), and crude protein (CP) content in feed and faecal samples were analysed according to the methods of the Association of Official Agricultural Chemists (AOAC, 2006). Gross energy (GE) was determined using a Parr 1281 adiabatic bomb calorimeter (Automatic Energy Analyzer, Moline, IL, USA). Acid-insoluble ash (AIA) was determined using the methods described by McCarthy et al. (1974). The apparent total tract digestibility (ATTD) of nutrients was calculated using the following equation:

$$\text{ATTD of nutrients} = 100 - \left[ \left( \frac{\text{AIA}_{\text{diet}}}{\text{AIA}_{\text{faeces}}} \times \frac{\text{Nutrient}_{\text{faeces}}}{\text{Nutrient}_{\text{diet}}} \right) \right] \times 100$$

### Serum biochemical and amino acid concentration analysis

Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB) and urea were determined using commercial kits (BJ XinchuangYuan Biotech Co., Ltd., Beijing, China) with an automatic biochemical analyser (Toshiba, Tokyo, Japan). Serum ghrelin, leptin and lactate levels were determined by the enzyme-linked immunosorbent assay (ELISA) method using commercial kits (Beijing Lai-boTairui Technology Development Co., Ltd., Beijing, China).

The concentration of free amino acid in serum was determined according to the method of Yin et al. (2016), with minor modifications. Briefly, 200 µl of serum was transferred into a 1.5 ml

centrifuge tube, mixed with 8 µl of an internal standard (2.5 mM norleucine) and 800 µl of methanol. After vortexing, the samples were centrifuged at 14 000 rpm for 10 min at 4 °C, and the supernatants were collected. A 500 µl aliquot of the supernatant was evaporated to dryness in a vacuum concentrator, and then reconstituted in 100 µl of borate buffer. Subsequently, 10 µl of the sample was mixed with 50 µl of borate buffer and 20 µl of derivatisation reagent, vortexed immediately, and incubated at 55 °C for 10 min. After cooling to room temperature, the samples were filtered through a 0.1 µm filter and transferred to sampler vials for UHPLC-Q-Orbitrap HRMS analysis.

### Digestive enzyme activity analysis

Mucosal samples were accurately weighed and nine volumes of normal saline were added at a weight-to-volume ratio of 1:9 (g/ml). The samples were homogenised mechanically in an ice-water bath, followed by centrifugation at 2500 rpm for 10 min at 4 °C to obtain the supernatant. The activities of lipase, amylase, trypsin, maltase, sucrase and lactase were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, JS, China).

### Statistical analysis

All data were assessed for normality using the UNIVARIATE procedure in SAS v9.4 (SAS Institute, Cary, NC, USA). Statistical analysis was performed using the t-test in SAS, with pen as the experimental unit. Results are presented as mean ± standard error of the mean (SEM). Differences were considered statistically significant at  $P \leq 0.05$ , while  $0.05 < P < 0.1$  was considered a tendency.

## Results

### Starch gelatinisation

Both pellet and mash diets had identical starch gelatinisation levels of 29.23%.

### Growth performance

Feed form showed no significant effect on growth performance during days 1–14 (Table 2). However, from days 15–35, piglets fed pelleted diets showed higher ADG (372.77 vs 433.94;  $P = 0.009$ ) and ADFI (714.35 vs 830.26;  $P = 0.040$ ) compared to those fed the mash diet during days 15–35. Throughout the experimental period, ADG was significantly increased in the pellet group (389.19 vs 349.07;  $P = 0.030$ ), with a tendency towards higher ADFI (698.80 vs 627.11;  $P = 0.062$ ).

**Table 2.** Effect of feed form on growth performance in piglets

Items	Treatments		SEM	P-value
	mash	pellet		
BW, kg				
day 1	8.91	8.96	0.154	0.885
day 14	13.44	13.39	0.273	0.926
day 35	21.17	22.67	0.432	0.082
Days 1–14				
ADG, g	320.15	312.04	10.313	0.740
ADFI, g	496.52	498.22	28.496	0.935
F:G	1.58	1.60	0.040	0.828
Days 15–35				
ADG, g	372.77	433.94	11.767	0.009
ADFI, g	714.35	830.26	36.333	0.040
F:G	2.02	1.96	0.067	0.326
Days 1–35				
ADG, g	349.07	389.19	9.272	0.030
ADFI, g	627.11	698.80	30.768	0.062
F:G	1.85	1.82	0.041	0.616

ADG – average daily gain, ADFI – average daily feed intake, F:G – the ratio of average daily feed intake to average daily gain, SEM – standard error of the mean;  $P < 0.05$  indicates a statistically significant difference

### Serum biochemical indicators

Compared to the mash diet, the pellet diet increased serum ghrelin concentration on day 14 (104.00 vs. 115.45;  $P = 0.005$ ) and day 35 (118.04 vs. 140.66;  $P = 0.002$ ), while decreasing leptin level (2.05 vs. 1.79;  $P = 0.047$ ) on day 35 (Table 3).

**Table 3.** Effect of feed form on serum biochemical indicators in piglets

Items	Treatments		SEM	P-value
	mash	pellet		
Day 14				
ALT, U/l	36.83	37.67	2.270	0.864
AST, U/l	44.50	42.17	3.700	0.769
TP, g/l	47.53	48.22	1.451	0.827
ALB, g/l	27.75	28.77	0.728	0.511
GLB, g/l	19.78	19.45	1.507	0.918
urea, mmol/l	2.05	2.42	0.274	0.530
lactate, umol/l	5.80	5.69	0.195	0.785
leptin, ng/ml	1.63	1.76	0.063	0.312
ghrelin, pg/ml	104.00	115.45	2.373	0.005
Day 35				
ALT, U/l	35.83	42.83	2.333	0.140
AST, U/l	47.17	43.20	3.501	0.600
TP, g/l	55.78	54.32	1.841	0.710
ALB, g/l	35.43	32.32	1.279	0.240
GLB, g/l	20.35	22.00	1.428	0.588
urea, mmol/l	3.53	2.93	0.262	0.272
lactate, umol/l	6.23	6.90	0.205	0.104
leptin, ng/ml	2.05	1.79	0.069	0.047
ghrelin, pg/ml	118.04	140.66	4.242	0.002

ALT – alanine aminotransferase, AST – aspartate aminotransferase, TP – total protein, ALB – albumin, GLB – globulin, SEM – standard error of the mean;  $P < 0.05$  indicates a statistically significant difference

### Serum free amino acid concentrations

Piglets fed the pellet diet had significantly higher serum concentrations of methionine (2.67 vs 3.72;  $P = 0.038$ ), threonine (11.83 vs 15.76;  $P = 0.016$ ) and valine (10.66 vs 13.45;  $P = 0.035$ ) on day 14. Additionally, arginine concentration was decreased (0.72 vs 0.46;  $P = 0.023$ ), while glycine level was increased (16.47 vs 19.91;  $P = 0.039$ ) in the pellet group on day 35 (Table 4).

**Table 4.** Effect of feed form on serum free amino acid concentrations in piglets (ug/ml)

Items	Treatments		SEM	P-value
	mash	pellet		
Day 14				
Essential amino acids				
lysine	0.74	0.88	0.073	0.342
methionine	2.67	3.72	0.261	0.038
threonine	11.83	15.76	0.876	0.016
tryptophan	4.58	5.26	0.555	0.561
isoleucine	9.75	10.89	0.469	0.242
valine	10.66	13.45	0.690	0.035
arginine	0.47	0.55	0.034	0.284
histidine	0.30	0.37	0.030	0.232
leucine	16.79	17.31	0.684	0.725
phenylalanine	10.61	12.19	0.482	0.103
Non-essential amino acids				
aspartate	1.06	0.97	0.050	0.380
glutamate	2.33	2.37	0.143	0.884
alanine	19.78	17.74	0.861	0.253
cystine	0.02	0.02	0.004	0.965
proline	18.03	18.86	0.765	0.614
glycine	15.79	16.74	1.579	0.782
serine	3.59	3.15	0.289	0.468
tyrosine	9.39	11.53	0.644	0.097
Day 35				
Essential amino acids				
lysine	1.16	0.83	0.107	0.124
methionine	3.26	3.42	0.178	0.681
threonine	11.80	12.78	1.113	0.682
tryptophan	9.46	7.42	0.864	0.257
isoleucine	11.19	10.00	0.548	0.299
valine	15.70	14.20	0.676	0.285
arginine	0.72	0.46	0.060	0.023
histidine	0.45	0.32	0.035	0.062
leucine	21.18	18.15	0.944	0.110
phenylalanine	11.36	10.59	0.356	0.290
Non-essential amino acids				
aspartate	1.28	1.16	0.051	0.292
glutamate	2.69	2.88	0.091	0.323
alanine	23.71	20.16	1.015	0.078
cystine	0.01	0.01	0.003	0.959
proline	21.63	19.33	0.984	0.261
glycine	16.47	19.91	0.865	0.039
serine	3.44	3.10	0.167	0.344
tyrosine	12.30	10.51	0.688	0.208

SEM – standard error of the mean;  $P < 0.05$  indicates a statistically significant difference

### Apparent total tract digestibility of nutrients

Piglets fed the pellet diet showed improved ATTD of ether extract (EE) on day 14 (69.86 vs 73.99;  $P = 0.040$ ) and day 35 (74.03% vs 71.09%;  $P = 0.047$ ), as well as increased ATTD of gross energy (GE) on day 14 (83.86% vs 82.28%;  $P = 0.038$ ) compared to those fed the mash diet (Table 5).

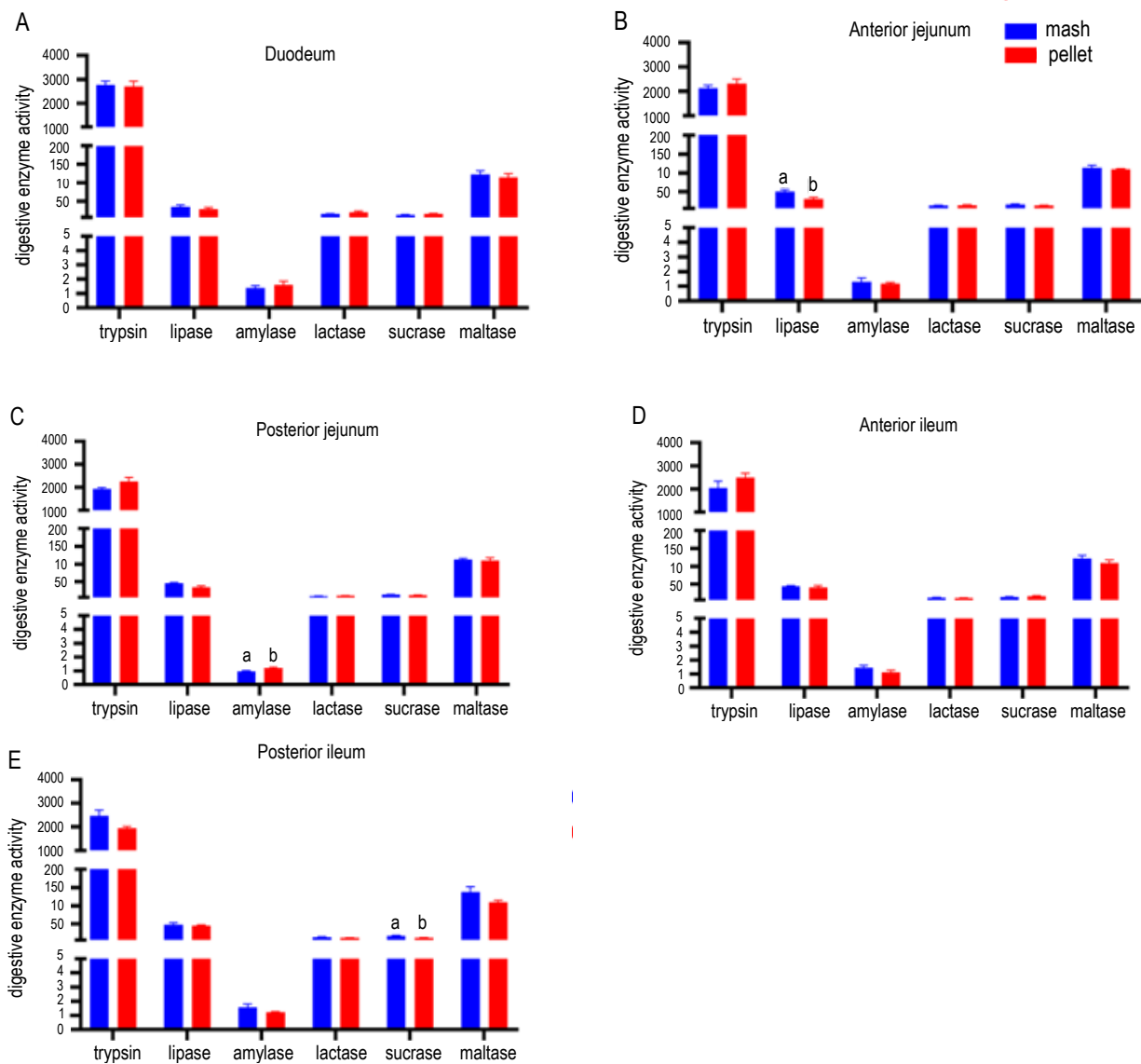
### Digestive enzyme activity

Higher lipase and sucrase activities were observed in the anterior jejunum and posterior ileum of piglets fed the mash diet ( $P < 0.05$ ). Amylase activity in the posterior jejunum was increased in piglets fed with pellet ( $P < 0.05$ ). However, no significant differences in digestive enzyme activities were observed in the duodenum or anterior ileum between feed forms (Figure 1).

**Table 5.** Effect of feed form on nutrient digestibility in piglets

Items	Treatments		SEM	P-value
	mash	pellet		
Day 14				
DM, %	82.08	83.62	0.388	0.048
EE, %	69.86	73.99	1.024	0.040
CP, %	72.53	73.44	0.807	0.591
GE, %	82.28	83.86	0.389	0.038
Day 35				
DM, %	85.25	85.70	0.332	0.509
EE, %	71.09	74.03	0.734	0.047
CP, %	77.51	78.21	0.712	0.635
GE, %	85.18	86.59	0.392	0.072

DM – dry matter, EE – ether extract, CP – crude protein, GE – gross energy, SEM – standard error of the mean;  $P < 0.05$  indicates a statistically significant difference.



**Figure 1.** Effect of feed form on the activity of digestive enzymes: lipase, amylase, trypsin, maltase, sucrase and lactase in the duodenum (A), anterior jejunum (B), posterior jejunum (C), anterior ileum (D) and posterior ileum (E) of piglets. Results are presented as means  $\pm$  SEM (standard error of the mean),  $n = 4-6$ ; <sup>ab</sup> – means with different superscripts in the columns are significantly different at  $P < 0.05$

## Discussion

Several studies have reported the beneficial effects of pelleted diets compared to mash feed forms. A maize-based pellet diet has been shown to improve ADG by 5.9% and FCR by 5.3% compared to a mash diet in finishing pigs (Potter et al., 2009). Similar improvements have been observed in piglets, where pelleted diets increased ADG by 11% and improved the G:F ratio by 7% in relation to mash diets (Traylor, 1997). In the present study, piglets fed the pellet diet reached higher ADG and ADFI even when starch gelatinisation was standardised between the two feed forms. Comparable findings have been reported in poultry by Abdollahi et al. (2014) and Selle et al. (2012), who found that broilers fed pellet diets had higher ADG and ADFI than those receiving re-ground pellets. These results suggest that even when starch gelatinisation is controlled, pelleted diets still provide growth performance benefits. This advantage may be partly attributed to intact physical integrity of pelleted diets, facilitating feed distribution and reducing feed wastage (9.15% vs 1.68% in mash vs pellet diets; Surek, 2012). However, feed form had no effect on FCR in the present study, suggesting that while the pellet diet increased feed intake and subsequent weight gain, they did not substantially alter energy metabolism or promote greater fat deposition or protein synthesis in piglets.

Blood biochemical indicators provide indirect information on the metabolic status of the body. In the present study, serum levels of leptin and ghrelin were significantly affected by feed form, as piglets fed the pellet diet showed higher ghrelin and lower leptin concentrations. Leptin plays an important role in regulating feed intake and energy balance, inducing weight loss by suppressing appetite (Klok et al., 2007). In contrast, ghrelin not only stimulates growth hormone secretion but also acts as an orexigenic hormone, enhancing appetite by activating hypothalamic neurocircuits (Poher et al., 2017). Serum urea nitrogen, an indicator of protein and amino acid metabolism, is negatively correlated with nitrogen retention and protein utilisation (Brown et al., 1974). The present results showed that urea levels were lower in the pellet diet group during the later stage of the experiment, suggesting greater amino acid oxidation in piglets fed the mash diet. This interpretation was further supported by the elevated concentrations of essential amino acids (methionine, threonine and arginine) in the serum of these animals. These changes in gastrointestinal hormone and

amino acid profiles were consistent with the observed improvements in ADFI and ADG, suggesting that feed form could influence growth performance through metabolic and endocrine regulation.

The improvements in ATTD, typically associated with pelleting (approximately 5–8% increases) are conventionally attributed to the hydrothermal processing effects, including starch gelatinisation and partial hydrolysis. These physicochemical changes stimulate lactic acid production in the gut, which suppresses pathogenic bacteria while improving nutrient absorption (Svihus et al., 2011; Röhe et al., 2014; Vukmirovic et al., 2017). Additionally, the mechanical action of pelleting disrupts plant cell walls, improving access to intracellular nutrients for digestive enzymes (O'Doherty et al., 2000). However, in the current study, both pelleted and mash diets underwent hydrothermal processing, and had the same degree of starch gelatinisation, yet the pelleted form still demonstrated higher ATTD for GE and EE. This improvement may be attributed to the increased bulk density of pelleted diets, which prolongs the mean retention time of digesta in the gastrointestinal tract, allowing for more complete digestion and absorption of nutrients. Previous studies have shown that feed form can influence feeding behaviour. Compared with pelleted diets, mash formulations have been associated with increased feeding time and feeder occupancy, along with a reduced feeding rate. These behavioural changes may lead to a higher heat increment and a lower net energy value of the diet (Laitat et al., 2004).

Digestive enzymes are one of the important factors affecting nutrient digestion in pigs. Dysfunction of digestive glands or insufficient secretion of endogenous enzymes can negatively affect both growth performance and nutrient digestibility (Liu et al., 2014). The present study showed that the physical form of feed influenced the intestinal activities of lipase, sucrase, and amylase in piglets. The lower amylase activity in the mash diet group may be associated with the hydrothermal process, which could cause dietary fibre to encapsulate starch, forming a barrier that prevent enzymatic hydrolysis and slows starch digestion. Grinding, on the other hand, can disrupt this fibre-starch matrix structure, thereby improving starch accessibility (Dhital et al., 2014; Wang et al., 2015). However, the observed lipase activity did not correspond with the ATTD results, suggesting a more complex relationship. The underlying mechanisms of how feed form affects

digestive enzyme activities are not fully elucidated and require further studies.

The effects of feed form on piglet growth performance were investigated here by controlling variables such as starch gelatinisation degree. Although differences were observed in weight gain, feed intake, and digestibility, the feed conversion ratio was not affected. The reasons for this finding remain unclear, and further research is required to clarify the mechanism by which feed form influences piglet growth performance, including factors such as feed wastage, feeding behaviours, energy metabolism, and processing conditions.

## Conclusions

This study has demonstrated that a pelleted diet improves piglet growth performance even when starch gelatinisation is standardised between feed forms. The observed growth improvements likely stem from multiple interacting factors, including reduced feed wastage, modified feeding behaviours and altered digesta retention times, rather than simply improved metabolic efficiency. The unchanged feed conversion ratio suggests that the additional energy intake from the pelleted diet was fully utilised for growth rather than being metabolised more efficiently.

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## Conflict of interest

The Authors declare that there is no conflict of interest.

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