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Overall assessment of fermented feed for pigs: a series of meta-analyses

Bocheng Xu, Luoyi Zhu, Jie Fu, Zhi Li, Yizhen Wang, and Mingliang Jin¹

National Engineering Laboratory of Biological Feed Safety and Pollution Prevention and Control, Key Laboratory of Animal Nutrition and Feed of Ministry of Agriculture, Key Laboratory of Animal Nutrition and Feed Science of Zhejiang Province, Institute of Feed Science, Zhejiang University, 866 Yuhangtang Road, Hangzhou, Zhejiang Province, 310058, P.R. China

¹Corresponding author: mljin@zju.edu.cn

Abstract

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As an alternative to antimicrobial growth promoters, fermented feed (FF) has been continuously developed for two decades; however, its effects on feed, performance, digestibility, and meat quality of pigs have yet to be systematically and comprehensively evaluated. This study aimed to (i) quantitatively evaluate the effects of fermentation on nutritional components of feed stuffs; (ii) quantitatively evaluate the effects of FF on pig growth performance, digestibility, and meat quality; and (iii) explore the dose–effect relationship. From PubMed and Web of Science (searched range from January 1, 2000 to April 4, 2019), we collected 3,271 articles, of which 30 articles (3,562 pigs) were included in our meta-analysis. Our analysis revealed that fermentation significantly increased the CP content in feed (P < 0.05). For weaned piglets and growing pigs, FF significantly improved ADG, G:F, DM digestibility, N digestibility, and energy digestibility in finishing pigs (P > 0.05). In the subgroup analyses, fermented ingredients increased the growth performance of weaned piglets and growing pigs, and fermented additives promoted the growth of pigs at all stages. The dose–effect analysis confirmed that the optimal doses of fermented ingredients and additives were 8% and 0.15%, respectively. Furthermore, FF had beneficial impacts on meat quality through increased lightness, redness, marbling and flavor and reduced drip loss (P < 0.05). In

Key words: dose-effect relationship, fermented feed, growth performance, meat quality, meta-analysis, pigs

Introduction

Antimicrobial growth promoters (AGPs) are widely used in animal husbandry due to their outstanding effects, such as bacterial pathogen inhibition and growth promotion (Cromwell, 2002; Van Boeckel et al., 2015). However, subtherapeutic AGP-treated animals have become a major contributor to antimicrobial-resistant bacterial strains, which are seriously endangering the public health globally (Marshall and Levy, 2011). Aimed at reversing or at least reducing the rising trend of antimicrobial resistance, governments, and world organizations have initiated a series of countermeasures and encouraged the research and development of AGP alternatives (Jensen and Hayes, 2014). Considering the beneficial impacts on feed safety, nutrient bioavailability, pig growth performance, and meat quality, fermented feed (FF) has been regarded as a novel alternative to AGPs in pigs. However, variability in the fermented products, bacterial strains, and experimental designs has restricted animal nutritionists from comprehensively evaluating the effects of FF on pigs. Thus, we performed a series of metaanalyses to (i) explore the effects of the fermentation process on

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feed nutrition components; (ii) quantitatively measure different FF factors on growth performance, digestibility, and meat quality in pigs at each growth stage; and (iii) investigate the dose–effect relationship between FF and growth performance to provide strategies for FF application in the pig industry. According to our knowledge, this is the first comprehensive and systematic overall assessment of FF in pigs.

Materials and Methods

This meta-analysis is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (Moher et al., 2009).

Search Strategy

We searched for relevant studies published during the last 20 years (from January 1, 2000 to April 8, 2019) on PubMed (https:// www.ncbi.nlm.nih.gov/pubmed; accessed April 8, 2019) and Web of Science (http://webofknowledge.com; accessed April 8, 2019). The search strategy involved a combination of the following free terms and keywords: "fermented" and "pig*". The complete search method is shown in Supplementary Table S1. The search was restricted to articles published in English. In addition, a manual search was performed to obtain more studies.

Inclusion and Exclusion Criteria

Studies were considered eligible if they met the following inclusion criteria: (i) the breeding background was commercial pigs; (ii) the studies investigated changes in the composition of ingredients before and after fermentation; (iii) the studies investigated the effects of FF on pig growth performance; and (iv) the studies investigating the effects of FF on meat quality. The exclusion criteria were as follows: (i) the studies used a Latin Square design; (ii) the pigs were given a probiotic supplementation without a fermentation period for the feedstuff; (iii) the studies investigated the inhibitory effects of FF on pathogenic bacteria and infective viruses; (iv) the studies investigated distillers dried grains with soluble supplements; (v) the studies investigated fermented liquid feed and liquid feed; (vi) the studies lacked a control group; (vii) the studies did not assess pig growth in stages; and (viii) the studies investigated sows and litters. The summarized information of included studies was showed in Table 1.

The Methodology of FF Preparation

The fermentation condition of FF from primary studies was summarized (Table 2). All methodologies of fermentation were solid-state fermentation. Fermentation temperature ranges from 25 to 40 °C. In general, the fermentation time is longer than 2 d. The main strain starters included Aspergillus, Bacillus, and multiple strain starters.

Information Extraction

The following crucial information was retrieved from each included study: treatment supplement, amount of additive, growth stage, and outcome data. The outcome data included changes in the composition of ingredients before and after fermentation (DM, DE, CP, crude fat, crude ash, and crude fiber); growth performance (ADG, ADFI, and G:F); digestibility (DM, N, energy, essential amino acids [EAAs], nonessential amino acids [NEAAs]); meat quality (lightness, redness, yellowness, drip loss, marbling, and flavor).

Study Quality Assessment

We utilized a novel approach called the Study Quality Assessment on Nonruminants (SQANR) to assess the studies of nonruminants. The potential risk of bias derived from whether within-group differences were reported, whether experimental data were reported in multiple articles, whether the experimental information was complete, whether the sample size was appropriate, and whether the experimental design was rational. Two investigators (B.X. and L.Z.) performed independent study quality assessments.

Statistical Analyses

For outcomes with the same units, such as ADG, ADFI, and G:F, pooled estimates were expressed as weighted mean differences (WMDs). For outcomes that were measured with different units, the reported pooled estimate was presented as the standardized mean difference (SMD), and mean changes were calculated from the available data if the change means were not reported (Wan et al., 2014). Furthermore, pooled estimates for each outcome were presented as the SMD with 95% CI for visualization purposes (Noronha et al., 2019). The pooled estimates of the SMD with 95% CI for each outcome were evaluated by using a random-effects model. If the 95% CI contained a zero value, there was no difference. For all the analyses, heterogeneity was assessed with the I² statistic (Higgins and Thompson, 2002) and Cochran's Q test (Higgins et al., 2003); $I^2 > 50\%$ and $P_{heterogeneity} < 0.1$ was regarded as a substantial heterogeneity. We used sensitivity analyses to exclude individual data with large deviations from the overall value. If 10 or more trials were available, we conducted subgroup analyses and meta-regression to explore potential sources of heterogeneity. The studies included in the metaanalysis were stratified into type of FF (fermented ingredients and fermented additives), growth stage (weaned piglets, growing pigs, and finishing pigs), dose (0.1–0.5%, 1–5%, 5–10%, and >10%), and strain starters (Aspergillus, Bacillus, multiple strain starters, and others). Publication bias was evaluated using Egger's tests, for which the significance level was defined at P < 0.1 (Eng et al., 2014). To help establish an optimal amount of FF, we considered dose-effect curves for FF supplementation and the outcomes of pig growth performance and meat quality.

The statistical analyses were performed with Stata 14.0 (Stata Corp., USA).

Results

We identified 3,271 studies, of which 30 (with data for 3,562 pigs) were included in the meta-analyses (Kim et al., 2006a, 2006b, 2006c; Cho et al., 2007, 2013; Feng et al., 2007; Wang et al., 2007, 2014; Hung et al., 2008; Lee et al., 2009; Ao et al., 2010, 2011; Jones et al., 2010; Li et al., 2011; Rafai et al., 2011; Yan et al., 2011, 2012a, 2012b; Yan and Kim, 2013; Jeong and Kim, 2015; Kraler et al., 2015; Zhou et al., 2015; Shi et al., 2016; Zhao et al., 2016; Chamorro-Ramirez et al., 2017; Liu et al., 2017; Tian et al., 2017; Yuan et al., 2017; Park and Kim, 2018; Zhang et al., 2018). The selection process is shown in Fig. 1. The characteristics of the studies included in the meta-analyses are shown in Table 1. The data indicating health status are shown in Supplementary Table S6. The mean initial BWs of weaned piglets, growing pigs, and finishing pigs were 7.3, 30.8, 70.4 kg, respectively. FF was divided into 13 fermented ingredients (soy protein, apple pomace, oat, corn, wheat, soybean meal, persimmon shell, wheat, apple diet, potato pulp, rapeseed meal, biogas residue, and Ginkgo

Study	Country	Treatment group	Bacteria	Add amount	Growth stage	Sample size	Crossbreed	Initial BW, kg	Trial duration or final BW at trial end
Ao et al. (2010)	Korea	Fermented soy protein	Aspergillus oryzae GB-107	5.00%	Weaned piglets	60	L×Y×D L×Y×D	5.68	4 weeks
Ao et al. (2011)	Korea	Fermented red ginseng	Bijtaobacterium H-1	0.10%, 0.20%, 0.40%	Finishing pigs	48	L×Y×U	/1.64	8 WK
Chamorro- Ramirez et	México	Solid-state fermented apple pomace	Yeast	5.00%, 10.00%, 5.00%, 10.00%	Growing pigs	∞	L×Y	47.1	4 wk
Cho et al.	Korea	Fermented soy protein	A. oryzae GB-107	5.00%, 10.00%,	Weaned piglets	72	NA	8.09	4 wk
(2007) Cho et al.	Korea	Fermented oat	Bacillus subtilis 2-20cx	15.00% 5.00%, 10.00%,	Growing pigs	50	L×Υ×D	20.5	2 wk
(2013)		Fermented corn	B. subtilis 2-25cx	15.00%, 20.00% 5.00%, 10.00%,	Growing pigs	48	L×Y×D	24.4	6 wk
		Fermented wheat	Aspergillus niger GB-125	15.00% 5.00%, 10.00%,	Growing pigs	48	L×Y×D	29.6	6 wk
Feng et al.	China	Fermented soybean meal	A. Oryzae	15.00%, 20.00% 26.50%	Weaned piglets	60	$L \times Y \times D$	8.6	17 kg
(2007) Hung et al. (2008)	China	Fermented soybean mealA/B	Streptococcus thermophilus, Lactobacillus acidophilus, Aspergillus awamori, Bifdobacterium thermophilus, Saccharomyces cerevisiae, Aspergillus niger and	22.90%	Growing pigs	32	Taoyuan×D	30	80 kg
			Irichoderma koningii		Finishing pigs	32	Taoyuan×D	80	110 kg
Jeong and Kim (2015)	Korea	Fermented medicinal plants	Lactobacillus plantarum, Saccharomyces cerevisiae, and Bacillus licheniformis	0.05%, 0.10%, 0.20%	Growing pigs	60	L×Y	25.5	6 wk
Jones et al. (2010)	American	Fermented soybean meal	NA	3.75%, 6.00%, 7.50%	Weaned piglets	84	NA	6.8	4 wk
Kim et al. (2006a)	Korea	Fermented persimmon shell	NA	3.00%, 5.00%, 7.00%	Finishing pigs	48	Berkshire	61	103 kg
Kim et al. (2006b)	Korea	Fermented persimmon shell	NA	3.00%, 5.00%, 7.00%	Finishing pigs	48	Berkshire	61	103 kg
Kim et al.	Korea	Fermented soy protein	A. Oryzae	3.00%, 6.00%	Weaned piglets	120	$L \times Y \times D$	5.56	5 wk
Kraler et al. (2015)	Austria	Fermented wheat	Lactobacillus paracasei and Lactobacillus plantarum	15.00%	Weaned piglets	24	L × Piétrain	8.36	6 wk
Lee et al. (2009)	Korea	Fermented apple diet	NA	2.00%, 4.00%, 6.00%	Finishing pigs	60	Berkshires	81	5 wk
Li et al. (2011)	China	Fermented potato pulp	S. thermophilus (CGMCC No. 1.2471), B. subtilis (MA193), and S. cerevisae	5.00%	Growing pigs	222	D×L×L	25.6	4 wk
				5.00%	Finishing pigs	191	$D \times L \times L$	86.4	109 kg

Table 1. Characteristics of the included studies $^{\rm 1}$

Table 1. Continu	ed								
						Sample		Initial	Trial duration or final BW at trial
Study	Country	Treatment group	Bacteria	Add amount	Growth stage	size	Crossbreed	BW, kg	end
Liu et al. (2017)	China	Fermented corn bran	B. subtilis MA139, Enterococcus faecium, and S. cerevisae	10.00%	Finishing pigs	40	L×Y×D	65.73	21 days
Park and Kim (2018)	Korea	High-density fermented	B. Subtilis	11.60%	Growing pigs	64	$L \times Y \times D$	29.59	6 wk
(0-0-)		Low-density fermented corn	B. subtilis	10.40%	Growing pigs	64	$L \times Y \times D$	29.59	6 wk
Rafai et al. (2011)	Hungary	Fermented wheat germ extract	NA	1.00%, 2.00%	Growing pigs	16	Hungarian L	13.5	47 days
Shi et al. (2016)	China	Fermented rapeseed meal	A. niger	10.00%	Growing pigs	48	NA	40.8	6 wk
Wang et al. (2007)	China	Fermented soybean meal	Lactobacillus plantarum	5.00%, 10.00%	Weaned piglets	48	L × Y × Beijing Black	7.29	7 wk
Wang et al. (2014)	China	Fermented soybean meal	B. subtilis MA139 with S. thermophilus and S.cerevisiae	6.00%, 12.00%	Weaned piglets	72	$D \times L \times L$	8.0	4 wk
Tian et al. (2017); Xu et al. (2017)	China	Fermented biogas residue	Yeast	5.00%, 10.00%, 15.00%	Growing pigs	64	D×L×Y	40.24	60 days
Yan et al. (2011)	Korea	Fermented garlic powder	Weissella koreensis	0.20%, 0.40%	Finishing pigs	48	(Y×L)×(Hampshire×D)	55.8	12 wk
Yan et al. (2012b)	Korea	Fermented garlic powder	Weissella koreensis	0.10%, 0.20%, 0.40%	Growing pigs	40	$L \times Y \times D$	50.7	77 kg
		Fermented garlic powder	W. koreensis	0.10%, 0.20%, 0.40%	Finishing pigs	40	$L \times Y \times D$	77	106 kg
Yan et al. (2012a)	Korea	Fermented chlorella	Baker's yeast and lactic acid bacterium	0.10%, 0.20%	Growing pigs	48	$L \times Y \times D$	26.58	53.1 kg
Yan and Kim (2013)	Korea	Fermented garlic powder	W. koreensis	0.05%, 0.10%, 0.20%	Weaned piglets	72	D×Y×L	5.5	5 wk
Yuan et al. (2017)	China	Fermented soybean meal	B. subtilis, Hansenula anomala and Lactobacillus casei	3.75%, 7.50%	Weaned piglets	100	NA	10.8	23.3 kg
Zhang et al. (2018)	China	Fermented soybean meal	B. subtilis BS12	10.00%	Weaned piglets	192	$D \times L \times Y$	9.35	24 days
Zhao et al. (2016)	Korea	Fermented medicinal plants	Lactobacillus plantarum, Yeasts and Bacillus licheniformis	0.05%, 0.10%, 0.20%	Weaned piglets	60	$L \times Y \times D$	6.08	6 wk
Zhou et al. (2015)	China	Fermented Ginkgo biloba L. residues	Candida tropicalis and A. oryzae	5.00%, 10.00%, 15.00%	Weaned piglets	48	D×L×Y	7.6	6 wk

¹D, Duroc; L, Landrace; Y, Yorkshire; NA, not applicable.

Study	Time	Temperature	Starter cultures
Ao et al. (2010)	48 h	NA ¹	Aspergillus oryzae GB-107
Ao et al. (2011)	5 d	NA	Bifidobacterium H-1
Chamorro- Ramirez (2017)	72 h	28 to 32 °C	Yeast
Cho et al. (2007)	48 h	NA	A. Oryzae GB-107
Cho et al. (2013)	36 h for oat and wheat; 42 h for corn	NA	Bacillus subtilis 2-19cx (oat and corn) or Aspergillus niger GB-124 (wheat)
Fend et al. (2007)	42 h 101 com	NIΔ	A 071/7.00
Hung et al. (2007)	72 h	32 ± 2 °C	Prohiotic
Jeong and Kim. (2015)	5 d	30 °C	Bacillus licheniformis species
Jones et al. (2010)	NA	NA	NA
Kim et al. (2006a)	60 d	NA	NA
Kim et al. (2006b)	60 d	NA	NA
Kim et al. (2006c)	NA	NA	Fungal and bacterial
Kim et al. (2010)	48 h	NA	A. oryzae GB-107
Kraler et al (2015)	NA	NA	Lactobacillus paracasei and
			Lactobacillus plantarum
Li et al. (2011)	4 d	25 °C	Streptococcus thermophilus (CGMCC No.1.2471), B. subtilis (MA 193), and Saccharomyces cerevisae (CGMCC No.2.1793).
Liu et al. (2017)	14 d	30 ∘C	B. subtilis MA139, Enterococcus faecium, and S. cerevisae
Park et al. (2018)	42 h	NA	B. subtilis 2-19cx
Rafai et al. (2011)	18 h	30 ℃	S. Cerevisiae
Shi et al. (2016)	72 h	32 °C	A. niger
Wang et al. (2007)	72 h	37 °C	Lactobacillus plantarum NF8
Wang et al. (2014)	5 d	40 °C	B. subtilis MA139 with Streptococcus thermophilus and S. cerevisiae
Xu et al. (2017)	48 h	NA	NA
Yan and Kim. (2013)	2 d to 5 wk	25 °C	Weissella koreensis
Yan et al. (2011)	24 h	25 °C	Weissella koreensis
Yan et al. (2012a)	72 h	NA	Carlina vulgaris
Yan et al. (2012b)	24 h	25 °C	Weissella Koreensis
Yuan et al. (2017)	48 h	37 °C	Lactobacillus casei (CGMCC1.62), B. subtilis (CGMCC1.504), and Hansenula anomala (CGMCC2.881)
Zhang et al. (2018)	24 h	37 °C	B. Subtilis
Zhao et al. (2016)	2 d	30 °C	Bacillus licheniformis species
Zhou et al. (2015)	96 h	28 to 30 °C	Candida tropicalis and A. oryzae

Table 2. The methodology of FF preparation for pigs	s aiets
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¹ NA, not applicable.

biloba L. residues) with concentrations greater than 1% and four fermented additives (red ginseng, *Gynura procumbens*, garlic powder, and Chlorella) with concentrations <0.5%. The study quality assessment based on SQANR is shown in Supplementary Table S2. The number of studies rated as "high," "moderate," and "low" were 7, 16, and 7, respectively. Unreported withingroup standard deviation was the main reason for downgrading studies.

Effects of Fermentation on Nutritional Components of Feed Stuffs

As shown in Fig. 2, fermentation improved the CP of ingredients with substantial heterogeneity (SMD [95% CI] 1.209 [0.501, 1.917], $I^2 = 86.50\%$, $P_Q < 0.001$), but fermentation had no effect on DM, crude fat, or crude fiber with substantial heterogeneity, DE, and crude ash with no evidence of heterogeneity.

Effect of FF on Pig Growth Performance

As shown in Fig. 3, compared with the basal diet, the increments (i.e., the WMDs) of weaned piglets' ADG, ADFI, and G:F with FF supplementation were 20.522 g/day, 9.716 g/day, and 0.026,

respectively. The increments of growing pigs' ADG and G:F with FF supplementation were 29.566 g/day and 0.013, respectively. The increment of finishing pigs' G:F was 0.014. There was almost no publication bias observed. In subgroup analyses (Supplementary Table S3), fermented ingredients had no effect on weaned piglets' ADFI (P > 0.05) and finishing pigs' ADG and G:F (P > 0.05), but fermented additives improved weaned piglets' ADFI (WMD 8.45 g/day [3.041 to 13.859]) and finishing pigs' ADG and G:F (ADG: WMD 50.046 g/day [23.078 to 77.013]; G:F: WMD 0.024 (0.015 to 0.034)).

Dose-Effect Relationship between FF and Growth Performance

Figure 4 shows the dose–effect relationships between the dose of FF and growth performance of pigs, many of which are nonlinear with no sign of a plateau. When the supplementation dose of the fermented ingredients was ~8%, ADG and G:F showed a peak simultaneously, but ADFI remained in the plateau phase. Under the condition that the supplementation dose of the fermented additives decreased to 0.15%, G:F exhibited a climax and ADG maintained a gently rising trend,

while ADFI slowly decreased without a significant difference compared with the basal diet treatment.

Effects of FF on Pig Nutrient Digestibility

Figure 5 shows that FF improved DM, N, and energy digestibility in weaned piglets and growing pigs (P < 0.05), but FF had no effect



Figure 1. Study selection process.

on EAA and NEAA digestibility in weaned piglets and DM, N, and energy digestibility in finishing pigs (P > 0.05). Three outcomes were detected to have publication bias. In the subgroup analyses (Supplementary Table S4), fermented additives had no effect on N and energy digestibility in growing pigs (P > 0.05), but fermented ingredients improved the above outcomes (P < 0.05).

Effects of FF on Meat Quality

As shown in Fig. 6, compared with the basal diet, FF improved lightness, redness, marbling, and flavor and reduced drip loss in finishing pigs (P < 0.05). Four outcomes were detected to have publication bias. In the subgroup analyses (Supplementary Table S5), compared with the basal diet, fermented ingredients significantly increased lightness, redness, and flavor and reduced drip loss and yellowness (P < 0.05), and fermented additives significantly reduced redness and increased marbling (P < 0.05).

Meta-Regression

We used sensitivity analyses to exclude individual data with large deviation from the overall value. As shown in Table 3, the different types of FF had a significant impact on ADG and redness (P < 0.05), the different growth stages had a significant impact on G:F and N digestibility (P < 0.05), and the association of the dose of FF supplement with a range of outcomes had no observed significance (P > 0.05).

Discussion

The meta-analyses suggest that CP significantly improved after fermentation. Overall, FF improved nutrient digestibility and growth performance in weaned piglets and growing pigs but had little or no effect on these outcomes in finishing pigs. Moreover, FF improved meat quality in finishing pigs. Findings from the subgroup analyses showed that fermented ingredients had no effect on ADFI and had significant effects on weaner and grower pigs. Fermented additives positively promoted growth at all stages. The result of the dose–effect relationship suggested that the optimal amount of fermented ingredients was ~8% and that of fermented additives was ~0.15%.



Figure 2. Summary forest plot of the effects of fermentation on ingredients. CI, confidence interval; SMD, standard mean difference; *P*_{egget}, *P*-value of Egger's test. The red circle represents the point estimate for each individual trial, and the horizontal line extending from each solid diamond represents the upper and lower limits of the 95% CI. If the 95% CI contains a zero value, there was no difference.



Figure 3. Summary forest plot of the effects of FF on pig growth performance. CI, confidence interval; SMD, standard mean difference; P_{egger}, P-value of Egger's test. The red circle represents the point estimate for each individual trial, and the horizontal line extending from each solid diamond represents the upper and lower limits of the 95% CI. If the 95% CI contains a zero value, there is no difference.



Figure 4. Dose-effect relationship between FF and growth performance. (a) Fermented ingredients and ADG. (b) Fermented ingredients and ADFI. (c) Fermented additives and ADFI. (f) Fermented additives and ADFI. (f) Fermented additives and G:F.

Producers and animal nutritionists regard growth performance as a very important economic trait. Consumers regard meat quality as an important quality indicator to decide whether to purchase the meat. Growth performance and meat quality depend on the nutritional value of feed and nutrient digestibility.

In terms of the nutritional value of feed (Fig. 2), we only observed some nutritional components of feed stuffs, such as DM and CP, due to the lack of studies reporting changes in nutritional components after fermentation. If a sufficient number of studies had reported changes in multiple antinutritional factors, we might have detected changes in phytates and protease inhibitors. Furthermore, substantial heterogeneity in many of the outcomes suggests that different ingredients, fermentation technologies, and fermentation parameters can cause considerable changes in nutritional components of feed stuffs. However, changes in CP and crude ash did not exhibit heterogeneity, resulting in convincing evidence. We speculated that fermentation increases the ratio of small to large peptides and reduces antinutritional factors. Therefore, we suggest that further studies should pay attention to changes in multiple antinutritional factors after fermentation.

With regard to nutrient digestibility (Fig. 5 and Table 4), we observed positive effects of FF on main nutrient digestibility (DM, N, and energy digestibility) in weaned piglets and growing pigs, but FF had no effect on this parameter in finishing pigs. We

		Pooled Estimation	ates	Hetero	geneity	Bias
Outcomes	Summary forest plot	SMD (95% CI)	P-value	P _Q	1 ²	$P_{_{\mathrm{Egger}}}$
Weaned piglets						
DM		SMD 1.067(0.62 to 1.514)	<0.001	<0.001	91.10%	<0.001
N		SMD 0.796(0.545 to 1.047)	<0.001	<0.001	80.00%	0.865
Energy		SMD 0.699(0.347 to 1.05)	<0.001	<0.001	77.50%	<0.001
EAA -		SMD 0.231(-0.024 to 0.487)	0.076	0.025	58.40%	0.370
NEAA		SMD 0.366(-0.08 to 0.813)	0.108	<0.001	85.90%	0.004
Growing pigs						
DM		SMD 0.748(0.503 to 0.994)	<0.001	<0.001	77.90%	0.397
N		SMD 0.352(0.124 to 0.58)	0.003	<0.001	75.70%	0.039
Energy	_	SMD 0.324(0.126 to 0.522)	0.001	<0.001	64.70%	0.093
Finishing pigs						
DM —	•	SMD 0.613(-0.081 to 1.306)	0.083	<0.001	91.90%	0.084
N		SMD 0.199(-0.056 to 0.455)	0.127	0.084	44.20%	0.169
Energy	<u> </u>	SMD -0.003(-0.205 to 0.199)	0.976	1.000	0.00%	0.978

Figure 5. Summary forest plot of the effects of FF on pig nutrient digestibility. CI, confidence interval; SMD, standard mean difference; P_{esset}, P-value of Egger's test. The red circle represents the point estimate for each individual trial, and the horizontal line extending from each solid diamond represents the upper and lower limits of the 95% CI. If the 95% CI contains a zero value, there is no difference.



Figure 6. Summary forest plot of the effects of FF on meat quality. CI, confidence interval; SMD, standard mean difference; P_{egger}, P-value of Egger's test. The red circle represents the point estimate for each individual trial, and the horizontal line extending from each solid diamond represents the upper and lower limits of the 95% CI. If the 95% CI contains a zero value, there is no difference.

Table 3	3.	Regression	analy	ses of	the	covariat	es
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Outcomes	Type of FF P-value ¹	Growth stages P-value	Dose P-value
ADG	0.017	0.628	0.088
ADFI	0.704	0.328	0.269
G:F	0.084	0.015	0.648
DM digestibility	0.234	0.189	0.241
N digestibility	0.957	0.040	0.742
Energy digestibility	0.137	0.436	0.359
Lightness	0.377	NA	0.736
Redness	< 0.001	NA	0.800
Yellowness	0.148	NA	0.827
Drip loss	0.749	NA	0.630

¹P-value of regression, P < 0.05 indicates a significant difference.

speculate that the developed digestive system of finishing pigs determines the upper limit of nutrient digestibility, as nutrition uptake ability is related to the intestinal villi height and the ratio of villi height to crypt depth (Wang et al., 2007). These findings show that fermentation promotes an increase in protein and energy utilization, which may be due to the elimination of trypsin inhibitors and the degradation of large-size protein (Feng et al., 2007). We observed that FF had no influence on EAA and NEAA digestibility with substantial heterogeneity in weaned piglets. However, the association of FF with certain AA digestibility is not yet clear and should be considered in further studies.

Figure 3, Table 5, and Fig. 4 show the effects of FF on pig growth performance and their dose–effect relationship. Together with the findings on the effect of FF on nutrient digestibility, the improvement in nutrient availability and utilization contributes to promoting the growth performance of

Type of FF	Dose	Outcomes	Growth stage of commercial pigs	Health status	Findings
Fermented ingredients	2% to 15%	DM, N, energy	Weaned piglets and growing pigs	There were no differences in RBC, WBC concentrations, and lymphocyte percentage among different groups	The fermented ingredients significantly improved DM, N, and energy digestibility of weaned piglets and growing pigs
		EAA, NEAA	Weaned piglets	Compared with the basal diet, fermented ingredients significantly increased the levels of serum IgG, IgA, and IgM of weaned pigs. Fermented ingredients significantly increased intestinal Lactobacillus number and decreased intestinal <i>Escherichia</i> coli number of weaned piglets compared with the basal diet group. The villous height to crypt depth ratio of weaned piglets was not affected by fermented ingredients	The fermented ingredients have no effect on EAA and NEAA digestibility of weaned piglets
Fermented additives	0.05% to 0.40%	DM, N, energy	Weaned piglets	Compared with the basal diet, fermented ingredients significantly increased the RBC, WBC concentrations, and significantly decreased intestinal Escherichia coli number of weaned piglets	The fermented additives significantly improved DM digestibility and notably increased N and energy digestibility of weaned piglets
		DM, N, energy	growing pigs	Compared with the basal diet, fermented ingredients significantly increased the WBC concentrations of growing pigs. Fermented ingredients significantly increased intestinal Lactic acid bacteria number and decreased intestinal <i>E. coli</i> number of growing pigs compared with the basal diet group	The fermented additives significantly improved DM digestibility and had no effect on N and energy digestibility of growing pigs
FF		DM, N, energy	Finishing pigs	The fermented additives and fermented ingredients have no effect on health status of finishing pigs	The fermented additives and fermented ingredients have no effect on DM, N, and energy digestibility of finishing pigs

Table 4. The findings of the effects of FF on pig nutrient digestibility

N, nitrogen; EAA, essential amino acids; NEAA, non-essential amino acids; RBC, red blood cell; WBC, white blood cell.

Table 5.	The	findings	of the	e effects	of FF	on r	nig g	prowth	performance	٤
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Type of FF	Dose	Outcomes	Growth stage of commercial pigs	Findings
Fermented ingredients	2% to to 15%	ADG, G:F	Weaned piglets and growing pigs	The fermented ingredients significantly improved ADG and G:F of weaned piglets and growing pigs. The optimal dose of fermented ingredients is 8%
		ADFI	Overall growth stage	The fermented ingredients have no effect on ADFI of pigs
		ADG, G:F, ADFI	Finishing pigs	The fermented ingredients have no effect on the growth performance of finishing pigs
Fermented additives	0.05% to to 0.40%	ADG, ADFI, G:F	Overall growth stage	Overall, the fermented additives notably increased growth performance of pigs. The optimal dose of fermented ingredients is 0.15%

weaner and grower pigs. The findings of the subgroup analyses suggest that fermented ingredients are appropriate for weaned piglets and growing pigs, and fermented additives are applicable to all growth stages of pigs. Fermented additives are usually fermented medicinal plants added at low levels; these additives can increase oxidation activity and other properties. Furthermore, considering the cost of FF together with the lack of significant effects on ADFI, the optimal amount of fermented ingredients and fermented additives was ~8% and 0.15%, respectively.

Table 6.	The	findings	of the	effects	of FF	on meat	quality
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Type of FF	Dose	Carcass weight of commercial finishing pigs	Meat quality (treatment group)	Meat quality (control group)	Outcomes	Findings
Fermented ingredients	2% to to 15%	103 to 110 kg	Mean of lightness, redness, yellowness, drip loss (%), marbling, and flavor were 46.41, 7.25, 4.85, 4.50, 6.17, and 6.30, respectively	Mean of lightness, redness, yellowness, drip loss (%), marbling, and flavor were 45.93, 6.70, 5.11, 5.14, 5.80, and 5.72, respectively	Lightness, redness, yellowness, drip loss, marbling, and flavor	The fermented ingredients significantly improved meat color, marbling, and flavor, and decreased drip loss
Fermented additives	0.05% to to 0.40%	106 to -108 kg	Mean of lightness, redness, yellowness, drip loss (%), and marbling were 52.72, 16.00, 7.31, 7.47, and 1.69, respectively	Mean of lightness, redness, yellowness, drip loss (%), and marbling were 45.93, 16.84, 7.04, 7.83, and 1.56, respectively	Redness and marbling	The fermented additives significantly improved marbling, and decreased redness of meat
					Lightness, yellowness, drip loss, and flavor	The fermented additives have no effect on lightness, yellowness, drip loss, and flavor

Figure 6 and Table 6 show the effects of FF on meat quality. FF improved meat color, such as lightness and redness, the content of intermuscular fat, and meat flavor, which are important appearance parameters to attract customers. Moreover, FF reduced drip loss, possibly because of its antioxidant activity (Ao et al., 2011). FF has a low pH environment and relatively high organic acid content that promotes the secretion of gastric juices and improves the absorption of iron combined with myoglobin, enhancing the red color of the muscles (Lee et al., 2009; Plumed-Ferrer and Von Wright, 2009). The intramuscular fat content also influences tenderness, succulence, and flavor (Lee et al., 2009; Tian et al., 2017). Flavor-associated AAs such as Ala, Gly, Glu, Asp, and Ser, which are precursor AAs for flavor development, added to feed might influence meat favor. However, in the present meta-analyses, we did not analyze changes in flavor-associated AAs in feed or meat or their digestibility due to the limited number of articles. Further studies should focus on what components of FF affect the flavor-associated AAs in meat and their efficiency in conversion and deposition.

A major strength of the present study is that we performed a comprehensive overview of FF ingredients and their effects on pig growth performance and meat quality; moreover, we demonstrated the logical relevance and consistency of the results and provided an optimal supplement dosage and use strategy. One of our important innovations is SQANR, which is used to assess the quality of primary studies on nonruminants. Compared with Cochrane collaboration's tool (Higgins et al., 2011) and other measurement tools targeted for medical animal trials (STAIR [Fisher et al., 2009], CAMARADES [Crossley et al., 2008], and ARRIV [Schulz et al., 2011] methodologies), SQANR was more suitable for studies on nonruminants because we considered the differences among humans, medical animals, and pigs. We also considered experimental design, information exhibition, error reporting, and sample size.

The main limitation in the present study is that there were many outcomes with substantial and unexplainable

heterogeneity. The results of meta-regression suggest that factors not used as a subgroup category were not relevant to a range of outcomes and covariates, such as the type of FF and growth stages of pigs, in the analysis of heterogeneity sources, significance and direction. Therefore, we tentatively believe that the heterogeneity resulted from uncontrollable factors such as environmental conditions and experimental targets. Publication bias might have resulted from missing negative reports and samples, which are inevitable and unexplainable. We failed to explore effects of fermentation of different strains on nutritional components of feed stuffs because of the low number of articles. Further studies should focus on the effects of different strains on antinutrients and organic acids in ingredients.

In conclusion, our findings indicate that FF is capable of improving the ADG and G:F of weaning piglets and growers; this improvement is related to the beneficial effects of the fermentation process on the nutritive value and utilization of feed. Given the cost of substrate feed, the optimal doses of fermented ingredients and fermented additives in the basal diet are 8% and 0.15%, respectively. Moreover, FF can improve meat quality (lightness, flavor, and intramuscular marbling) and reduce drip loss. The current analysis, which is comprehensive and systematic, favors FF supplementation in pig feed.

Supplementary Data

Supplementary data are available at Journal of Animal Science online.

Acknowledgments

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Conflict of interest statement

None declared.

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