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Evaluation of pollution potential in swine manure across growth stages: Impact of dietary nutrients and management strategies

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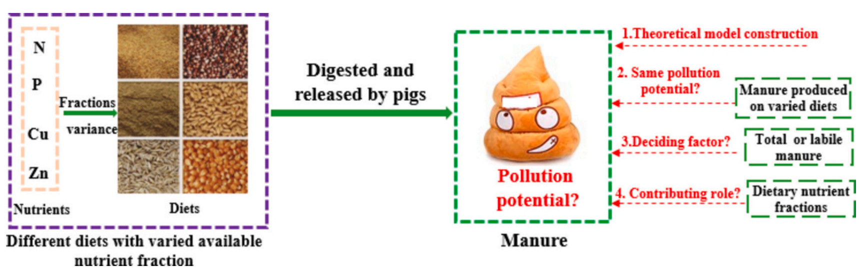
HIGHLIGHTS

- Total manure content did not accurately represent labile manure levels
- Swine growth stages had no significant impact on manure pollution potential
- Dietary nutrient fractions greatly influence nutrient retention rates
- Increased pollution potential is not a measure of manure release quantity
- Nutrient reduction potential model proves effective for pollutant management

GRAPHICAL ABSTRACT



Abbreviations: ADF, Acid detergent fiber; ADF-C, Carbon within ADF; ADE, Apparent digestible energy; AH-N, Alkaline hydrolyzable N; A_D, The amount of diet consumed by pigs; $A_{R\text{-}N}$, The retained amounts of nutrients; $A_{O\text{-}P}$, The output of typical elements; A_{ADFI}, The average daily feed intake of pigs; A_T, The total feed intake; $A_{I\text{-}N}$, Dietary nutrients intake by pigs from diets per day; $A_{O\text{-}P}$, The output dietary nutrient per day; $A_{LFP\text{-}E}$, The amounts of pollutants in labile fractions of excrement; $A_{T\text{-}M}$, Total manure pollutants; $A_{L\text{-}M}$, Total labile pollutants in manure; $C_{I\text{-}N}$, The average concentration of the consumed dietary nutrients in the diet; $C_{O\text{-}P}$, The average concentration of the output pollutants in pig urine and manure; CP, Crude protein; DC, Digestible carbon in diet; DP, Digestible phosphorus in diet; DPP, Dietary phytate phosphorus; DTC, Total dietary carbon; DTOC, Total dietary organic carbon; DTON, Total dietary organic nitrogen; DTN, Total dietary nitrogen; DTP, Total dietary phosphorus; FD, Feeding/fattening stage; FD-1, Treatment with high diet in FD; FD-2, Treatment with medium diet in FD; FD-3, Treatment with low diet in FD; FH, Finishing stage; FH-1, Treatment with HE diet in FH; FH-2, Treatment with ME diet in FH; FH-3, Treatment with low diet in FH; LE, High energy; LN, Manure labile nitrogen; LP, Labile phosphorus; LOC, Labile organic carbon; LFP-E, Labile fractions of pollutants in excrement; LFP-M, Labile fractions of pollutants in manure; LFP-U, Labile fractions of pollutants in urine; MC, Moisture content; non-LN, Non-labile N; Non-LOC, Recalcitrant organic carbon/non-labile organic carbon; Non-LP, Non-labile phosphorus; PP, Phytate phosphorus; RO-P, Release/output ratio of pollutants from one dietary nutrient; RLFP-E, The spreading rate of pollutants in labile fractions; RLFP-U, The contributing rate of urine in labile fractions present in the total excrement; RLFP-M, The contributing rate of manure in labile fractions present in the total excrement; RD-M, The diet to meat ratio of pigs; WN, Weaning stage; WN-1, Treatment with HE diet in WN; WN-2, Treatment with ME diet in WN; WN-3, Treatment with LE diet in WN; WTG, Total weight gained; WADG, The average daily weight gain; Wp, Gain one kilogram of body weight.

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ABSTRACT

Regulation of dietary nutrient fractions to control the release of labile manure pollutants in swine production remains a challenge. Feeding trials were conducted to assess the impact of dietary nutrient fractions on labile manure composition and pollution potential in pigs at different growth stages. The pigs were selected based on age (weaning = 60 days, feeding = 100 days, and finishing = 160 days), health, and average body weight (23.7 kg, 50.5 kg, and 109.0 kg respectively) and fed with (1) high, (2) medium, and (3) low energy diets twice daily in all three growth stages. Urine and feces were collected for analysis. The study utilized correlation, nutrient balance analysis, and theoretical models to evaluate the dietary impact on nutrient distribution and pollution potential. Results showed higher nutrient retention (N, P, Cu, Zn) with the high-energy diet across all growth stages compared to the other two diet energy levels. Correlation analysis revealed that pigs' weight gain does not reflect dietary efficiency nor indicate lower pollution potential from nutrient retention. However, dietary nutrient fractions played more significant role in labile manure release compare to the total manure component. Overall, the dietary regulatory approach offers a sustainable strategy to mitigate environmental pollution while supporting nutrient recycling using manure with lower pollutant loads.

1. Introduction

With the expansion and intensification of pig breeding farm in China, the pig breeding waste has experienced a significant increase. According to recent statistics, about 4 million tons of manure and urine are discharged annually for every ten thousand pigs slaughtered (Fan et al., 2021; Paul and John, 2022). Considering the additional leftover of pig breeding waste each year, the overall amounts of pig breeding waste become very large. The accumulated pig breeding waste (especially manure and urine) has caused severe and detrimental pollution for water, soil, and air (Zhang et al., 2024). Consequently, livestock waste production control research has become a pressing concern and a crucial aspect for environmental protection.

Manure source control is an effective and promising strategy to realize the reduction of manure production, which affords cost savings, advanced environment pollution control and resource conservation, compared with conventional treatment methods such as, composting and anaerobic fermentation, which solely generates manure (Trabue et al., 2022; Feng et al., 2024). Beneficial outcomes of implementing manure source control can be typically attained through the regulation of dietary ingredients and total dietary nutrient amounts, while assessing the potential for manure pollution is pivotal in appraising the environmental impact resulting from the regulation of dietary formulas for manure source control.

Such analysis can provide valuable insights for modification of dietary formulas with the aim of achieving the greatest possible reduction in manure production. For example, Trabue et al. (2021a, 2021b) observed that increasing dietary C levels could increase manure pH, total solids and total N, and pigs subjected to high-fiber diets exhibited enhanced performance in the reduction of ammonia, sulfide, volatile fatty acids, and phenol emissions in their manure when compared to pigs fed low-fiber diets. Hickmann et al. (2024) discovered that lowering crude protein amounts was beneficial for the reduction of the total nitrogen concentrations in manure ($p < 0.001$), which exerted varied influence on the anaerobic process. Recent studies on manure pollution potential all focused on the dietary nutrient amounts and the relevant manure pollutant amounts. However, dietary nutrients typically consist of fractions that include components readily accessible for utilization by animals and those that pose challenges for animal consumption. For instance, within the dietary phosphorus content, dietary phytate phosphorus (DPP) constitutes a fraction that is challenging to utilize effectively in feed, whereas the remaining phosphorus can be classified as readily bioavailable fractions. (Christian et al., 2023; Ran et al., 2023; Wise et al., 2024).

Similarly, the organic microelements, like organic copper (OCu) and organic zinc (OZn), are less accessible to animals when compared to their inorganic microelement component (Gourlez et al., 2024). Thus, dietary nutrient fraction availability in animals is the critical factors influencing pig growth performance and it's a vital aspect for manure source control (Almeida et al., 2021; McGhee and Stein, 2023). However, the potential influence of dietary nutrient fractions on manure pollutant source control is still unclear.

Manure pollutants (such as N, P, heavy metals) comprise labile and non-labile fractions. The labile fractions are the primary component in manure contributing to environmental pollution due to its availability, high inter-conversion capacity, and solubility in the environment (Dinkler et al., 2021). The labile fractions of manure pollutants include labile N fractions from acid-hydrolyzed nitrogen, labile P fraction present in forms of H_2O—P, $NaHCO_3$-P, NaOH-P and other compounds, and labile heavy metals (Dinkler et al., 2021; Ran et al., 2023). The non-labile fractions such as, the non-labile N fractions from acidolysis-resistant amide nitrogen and fatty amine, immobilized P fractions of HCl-P and residual P, and non-labile heavy metal present as sulfide bounded fraction and residual fraction. These fractions are generally stable and are slowly released into environment, making them a low-impact environmental pollutants and are suitable for recycling treatments such as composting and anaerobic fermentation (Dinkler et al., 2021; Wang et al., 2022; Ran et al., 2023). Thus, manure pollutant fractions are critical indicators of manure pollution potential and its reutilization prospect. These fractions could serve as a basis for assessing the environmental impacts of manure source control methods and determining necessary dietary formula modifications.

Nevertheless, until now, only few studies have explored the influence of dietary nutrient fractions on manure pollutant fractions. Furthermore, the extent to which manure pollutant fractions induce the potential for manure pollution remains incompletely understood, and the contributory effects of dietary nutrient fractions on effective control of labile manure sources is yet to be addressed. Therefore, in this study, feeding trials, correlation analysis, nutrient balance analysis and theoretical models were developed to explore the theoretical feasibility of manure source control from the dietary nutrient fractions regulation. We also evaluated the manure pollution potential in relation to the swine growth stages and identified the pollutant fractions of manure. The contributory effects of source-controlled dietary fractions on manure pollution potential were analyzed by correlation analysis. Moreover, occurrence of N, P, Cu and Zn pollutant fractions in manure produced by pigs in the different growth stages with varied dietary nutrient fractions were analyzed systematically. The distribution channels of N, P, Cu and Zn pollutant fractions from dietary nutrients to manure pollutants were assessed via nutrient balance analysis, which is a function of the dietary chain including diet input, pig nutrient retention and excreta amounts. This work offers new insights into potential diet-regulated composition for swine at different growth stages and suggests options for comprehensive manure management.

2. Materials and methods

2.1. Experiment setup and sample collection

A feeding trial was conducted at an intensive pig breeding farm (annual inventory of over 10,000 heads) using three treatment groups of pigs to evaluate the potential contribution of dietary N, P, Cu, and Zn fractions on labile manure, environmental effects of diets, and manure pollution potential based on the distinct manure pollutant fractions. The Duroc-Landrace-Yorkshire (DLY) crossbred pigs were selected as the subjects for this experiment due to their widespread popularity across various farms and regions in China and other regions around the world, which is attributed to their rapid growth rates, lean meat production, and associated economic benefits (Wang et al., 2024). The groups were arranged based on the primary developmental phases observed during the growth process of pigs. These phases included the weaning stage (WN), with growth period of 35–70 days, the feeding/fattening stage (FD), encompassing a growth period of 70–120 days, and the finishing stage (FH), covering a growth period of 120–210 days, respectively. Three pigs within each group were concurrently subjected to each treatment, and these pigs were matched in terms of age, health status, and body weight. The selected experimental pigs in this study were pigs in the growth age of 60 days (WN) with respective original weight of 23.54 kg, 23.87 kg and 23.66 kg. For pigs with growth age of 100 days (FD), the original weights were 50.49 kg, 50.55 kg and 50.53 kg, respectively. For pigs at growth stage of 160 days (FH), the respective original weights were 108.92 kg, 108.89 kg and 109.1 kg. The first, second and third diet treatments were carried out at each pig growth stages (WN, FD and FH stages), respectively.

In each group, three treatment regimens were implemented in each stage (WN, FD, and FH), utilizing diets containing distinct nutrient fractions, with “1” representing high energy diet, “2” representing medium energy diet, and “3” representing low energy diet. The experimental diets were formulated from popular market foods composed of primary ingredients such as corn, wheat bran, barley and wheat with some additional nutrient materials including protein, phosphorous and microelements (Cu, Zn) in different ratios (Table S1). As shown in Table 1, the selected diets in each stage comprises varying nutrient contents and available nutrient fractions, such as, DTOC, DTON and DP due to their easy utilization by pigs relative to other fractions (Ran et al., 2023; Peng et al., 2024). For dietary microelements, Cu and Zn are the available nutrient fractions, which can be derived from the difference between total dietary microelements (DTCu and DTZn) and dietary organic microelements (DTOCu and DTOZn) (Liu et al., 2022; Liu et al., 2023; Peng et al., 2024).

All feeding trials based on the different diet fractions were conducted for 7 days. Each pig group were fed in individual pens to facilitate the ease of collection of feces and urine daily (Trabue et al., 2022). The selected pigs of identical age had consistent health conditions and comparable levels of feed intake, and were all fed ad libitum twice daily at 7:00 and 18:00 h, respectively. In addition, pigs in all groups were fed under similar environmental conditions at room temperature of 23–25 °C, air humidity of 55 %–58 % and continuous ventilation conditions that are optimal for pig growth and necessary for enhancing production on pig farms (Duan et al., 2018). These established experimental conditions can maximally ensure the representativeness of the data for different regions and various scales of farming operations.

2.2. Urine and manure collection and analysis

Manure samples and urine samples were collected separately. Urine samples were collected using sterile containers by guiding the pigs to the designated area and the samples were transferred to the lab for analysis using urine collection bags. The urine samples were collected daily at 7:00, 12:00, 17:00 and 22:00, while manure samples were collected daily at 8:00 and 19:00. The collected samples were weighed on platform scales with maximum capacity of 20 kg. Nitrogen concentration in the urine samples was determined by the spectrophotometry using Ultraviolet photometer (Thermo Scientific Evolution 200, USA) (Drescher et al., 2020). The urine P was analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 7000DV, USA) (Malgorzata and Jolanta, 2022). The urine Cu, Zn were detected by ICP-MS (Agilent) (Zheng et al., 2021).

Approximately 300 g of manure were obtained for physicochemical analysis. The moisture content (MC) of mixed materials was determined by weight loss on drying the samples in vacuum oven at 105 °C for 24 h

Table 1
Nutritional content of the experimental diets on an ‘as-fed’ dry basis.

Parameters	WN			FD			FH		
	WN-1	WN-2	WN-3	FD-1	FD-2	FD-3	FH-1	FH-2	FH-3
DTC (g/kg)	452.3 ± 4.41	454.34 ± 2.75	442.9 ± 3.24	442.05 ± 2.51	417.89 ± 6.16	426.91 ± 3.82	450.92 ± 4.36	439.53 ± 4.33	443.27 ± 2.61
DTOC (g/kg)	440.4 ± 4.33	442.55 ± 3.01	433.4 ± 5.33	426.84 ± 4.39	410.26 ± 3.91	415.16 ± 4.16	444.72 ± 4.47	431.38 ± 4.35	435.42 ± 3.76
ADF-C (g/kg)	12.31 ± 0.14	16.52 ± 0.25	19.01 ± 0.12	23.11 ± 0.64	27.62 ± 0.21	30.71 ± 0.44	27.72 ± 0.29	30.29 ± 0.55	34.69 ± 0.27
ADF (g/kg)	30.62 ± 1.59	38.02 ± 2.19	43.39 ± 3.02	55.76 ± 5.06	63.58 ± 3.2	67.56 ± 4.15	63.82 ± 4.78	68.16 ± 3.74	76.35 ± 5.91
DTN (g/kg)	35.65 ± 1.54	32.94 ± 0.78	33.79 ± 2.17	29.39 ± 2.04	28.51 ± 0.74	27.53 ± 1.14	29.98 ± 1.22	30.29 ± 1.04	29.35 ± 1.35
DTON (g/kg)	35.36 ± 1.48	32.64 ± 1.54	33.62 ± 1.05	29.15 ± 1.49	28.28 ± 1.92	27.25 ± 1.47	29.89 ± 1.06	30.10 ± 1.83	29.08 ± 1.59
CP (g/kg)	216.6 ± 7.75	198.11 ± 2.42	202.1 ± 2.41	189.2 ± 3.6	175.6 ± 5.87	170.2 ± 2.92	182.8 ± 4.26	189.6 ± 2.82	176.58 ± 3.76
DTP (g/kg)	7.15 ± 0.06	5.81 ± 0.31	6.26 ± 0.07	7.4 ± 0.25	6.5 ± 0.35	10.68 ± 0.21	6.5 ± 0.09	5.1 ± 0.08	5.58 ± 0.23
DP (g/kg)	3.02 ± 0.01	3.57 ± 0.01	2.93 ± 0.02	2.79 ± 0.04	3.92 ± 0.25	6.9 ± 0.18	3.01 ± 0.08	3.48 ± 0.06	3.44 ± 0.78
DTCu (mg/kg)	80.68 ± 8.38	76.66 ± 5.63	70.68 ± 5.24	75.47 ± 5.15	70.99 ± 5.38	65.64 ± 3.93	65.16 ± 4.19	100.75 ± 6.29	98.63 ± 7.31
DTOCu (mg/kg)	80.64 ± 7.24	26.75 ± 2.82	0.68 ± 0.02	75.47 ± 5.19	30.99 ± 2.95	0.64 ± 0.03	65.15 ± 4.19	16.75 ± 4.72	0.63 ± 0.04
DTZn (mg/kg)	111.37 ± 8.22	108.15 ± 7.46	100.25 ± 8.51	104.46 ± 9.69	98.86 ± 6.65	92.84 ± 7.28	89.72 ± 4.09	107.66 ± 8.55	99.93 ± 6.81
DTOZn (mg/kg)	111.23 ± 8.21	38.19 ± 2.77	2.25 ± 0.21	104.46 ± 9.69	56.86 ± 4.75	1.84 ± 0.07	89.89 ± 4.34	23.66 ± 3.04	0.92 ± 0.05
ADE (MJ/kg)	14.31 ± 0.01	13.36 ± 0.02	12.37 ± 0.01	14.13 ± 0.02	13.17 ± 0.01	12.52 ± 0.05	14.25 ± 0.01	13.45 ± 0.03	12.73 ± 0.03

ADF – Acid detergent fiber, ADF-C – carbon fraction of acid detergent fiber.
CP – Crude Protein.
ADE – Apparent digestible energy.
“D” in DTC, DTOC, DTN, DTON, DTP, DP, DTCu, DTOCu, DTZn, and DTOZn – represents the dietary fraction.

(Fan et al., 2019). Manure labile N (LN), namely alkaline hydrolyzable N (AH-N) composed of AH-ON including amino acids (e.g., glutamine) and amino sugars (e.g., glucosamine) and inorganic N including NH_4^+-N and NO_3^{2-}-N were determined using direct steam distillation method (Roberts et al., 2009). The manure non-labile N (non-LN) was obtained as the difference between TN and AH-N (Drescher et al., 2020). CP, namely crude protein, was determined as the product of the dietary nitrogen content measured using the Kjeldahl method as described by Alejandro et al. (2002), and multiplication factor (6.25).

Manure TP was analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 7000DV, USA) (Malgorzata and Jolanta, 2022). Dietary phytate phosphorus (DPP) was extracted from the diet samples using trichloroacetic acid and quantified spectrophotometrically (UV6100, MAPADA) as described by Aureli et al. (2017). The P fractions were determined by the Hedley phosphate fractionation method from manure components based on inorganic P and organic P, including H_2O—P, $NaHCO_3$-P, NaOH-P, HCl-P and residual-P, which were extracted by water, 0.5 M $NaHCO_3$, 0.1 M NaOH, 1.0 M HCl and 10 ml H_2SO_4 and $HClO_4$ sequentially and separately (Dinkler et al., 2021). The Labile P (LP) included H_2O-P and $NaHCO_3$-P, while the non-LP comprises NaOH-P, HCl-P and residual-P (Dinkler et al., 2021).

The total heavy metals (Cu and Zn) in the manure were extracted by HNO3, HClO4 and H_2O_2 from dry manure samples and determined by ICP-MS (Agilent) (Liu et al., 2023). The labile fractions of the heavy metals (including the exchangeable fraction, reducible fraction and the oxidizable fraction) and the non-labile heavy metal fractions such as the organically bound fraction and the residual fraction were extracted using BCR sequential extraction method, and detected by ICP-MS (Agilent) (Zheng et al., 2021). The standard solutions of Cu and Zn were provided by the National Centre for Research on Standard Substances of China, which are all of high-grade purity.

2.3. Theoretical model

2.3.1. Pig growth performance metrics

The average daily feed intake (A_{ADFI}) of pigs in each treatment group, measured in kg, was calculated by dividing the total feed intake (A_T) for the group, in kg, by the total number of feeding days (t). This calculation provided an assessment of the average feed intake per pig per day. Thus, the average daily feed intake of pigs in each treatment group (A_{ADFI}) was computed as:

$$A_{ADFI} = A_T/t \quad (1)$$

The average daily weight gain of the pigs during the experimental period, measured in g, was calculated as the total weight gained (W_{TG}, kg) by the pigs, in kg, divided by the total number of days (t) during the feeding period, in days. The average daily weight gain (W_{ADG}, g) of the pigs during the experimental period was calculated by dividing the total weight gained (W_G, kg) by the pigs, measured in kg, by the total duration of the feeding period, measured in days (t). This provides a measure of the average weight gain per pig per day. The (W_{ADG}, g) was determined using the following formula:

$$W_{ADG} = W_{TG}/t \times 1000 \quad (2)$$

Where, W_{ADG}, g is the average daily weight gain per pig during the experimental period (g/day). W_{TG} equals total weight gained by all pigs in the group during the experimental period (kg) while t is the total number of days during the experimental feeding period. The factor of 1000 is used to convert kg to g.

The diet to meat ratio of pigs ($R_{D\text{-}M}$) represents the amount of diet consumed by pigs (A_D, kg) to gain one kilogram of body weight (W_p, kg), which is an important indicator for evaluating feed compensation (Tarek et al., 2022). The $R_{D\text{-}M}$ was determined as follows:

$$R_{D-M} = A_D/W_P \quad (3)$$

2.3.2. Model development for nutrient balance analysis

The nutrient balance was evaluated by the amounts of retained nutrients by pigs, the amounts of input nutrients and the amounts of output nutrients. The retained amounts of nutrients (such as N, P, Cu and Zn under different fractions) by pigs per day ($A_{R\text{-}N}$, g) were the difference between the average input of typical elements ($A_{I\text{-}N}$, g). This is represented as dietary nutrients intake by pigs from diets per day and the output/release of typical elements ($A_{O\text{-}P}$, g) represented as pollutants (such as N, P, Cu and Zn under different fractions) discharged by urine, manure and respiration per day. $A_{I\text{-}N}$ was determined as the average concentration of the consumed dietary nutrients in the diet ($C_{I\text{-}N}$, g/kg) and the average intake of diet by pigs per day ($A_{I\text{-}D}$, kg). $A_{O\text{-}P}$ was determined as the average concentration of the output pollutants in pig urine and manure ($C_{O\text{-}P}$, g/kg), and the average amounts of urine ($A_{O\text{-}U}$, kg) and manure ($A_{O\text{-}M}$, kg) produced per day. The equations were represented as follows:

$$A_{I-N} = C_{I-N} \times A_{I\text{-}D} \quad (4)$$

$$A_{O-P} = C_{O-P\text{-}U} \times A_{O-U} + C_{O-P-M} \times A_{O-M} \quad (5)$$

$$A_{R-N} = A_{I-N} - A_{O-P} \quad (6)$$

2.3.3. Manure pollution evaluation model

Release/output ratio of pollutants from one dietary nutrient (such as N, P, Cu and Zn under different fractions) is represented by $R_{O\text{-}P}$, which is a clear and quantitative response to the rationality and pollution potential of dietary intake in respect of nutrient/pollutant content. The $R_{O\text{-}P}$ is determined by the ratio of average nutrient input ($A_{I\text{-}N}$, g) per day and the output/released dietary nutrient per day ($A_{O\text{-}P}$, kg), which can be denoted as follows:

$$R_{O\text{-}P} = \frac{A_{O\text{-}P}}{A_{I\text{-}N}} \times 100 \quad (7)$$

The evaluation of the environmental threat level of excrement (manure and urine) among different treatments could be described as the amounts of pollutants in labile fractions of excrement ($A_{LFP\text{-}E}$, including $A_{LFP\text{-}M}$ - manure and $A_{LFP\text{-}U}$ - urine fractions, g), and the spreading rate of pollutants in labile fractions present in the total excrement ($R_{LFP\text{-}E}$, %). The evaluation of the threat level of one excrement (manure or urine) to the environmental threat level of the whole excrement among different treatments could be described as the contributing rate of specific excrement (manure or urine) in labile fractions present in the total excrement ($R_{LFP\text{-}M}$-manure, %; $R_{LFP\text{-}U}$-urine, %), which are denoted as follows:

$$A_{LFP-E} = \sum_{i=1}^{n\to+\infty} A_{LFP_i-M} + \sum_{i=1}^{n\to+\infty} A_{LFP_i-U} \quad (8)$$

$$R_{LFP\text{-}E} = \frac{A_{LFP-E}}{A_{O-P}} \times 100 \quad (9)$$

$$R_{LFP-M} = \frac{\sum_{i=1}^{n\to+\infty} A_{LFP_i-M}}{A_{O-P}} \times 100 \quad (10)$$

$$R_{LFP-U} = \frac{\sum_{i=1}^{n\to+\infty} A_{LFP_i-U}}{A_{O\text{-}P}} \times 100 \quad (11)$$

In this experiment, the pollutants mainly referred here are the typical pollutants (N, P, Cu and Zn), and thus the "n" in eq. (8), (10), (11) are equal to 4.

For the evaluation of the environmental threat level of solid manure without regard to urine among different treatments, the indicator could be described as R_{LFP} (%), which is the spreading rate of total labile pollutants ($A_{L\text{-}M}$, g) present in the total pollutants of manure ($A_{T\text{-}M}$, g). R_{LFP} is denoted as follows:

$$R_{LFP} = \frac{A_{L\text{-}M}}{A_{T\text{-}M}} \quad (12)$$

To evaluate dietary nutrient content relative to the typical nutrient requirements outlined in the NRC nutrient standards for pigs (NRC, 2012), we defined an indicator, $R_{ER\text{-}n}$ (%). This indicator was determined based on the daily nutrient supply from the experimental diets, $A_{ED\text{-}n}$ (g/d), in comparison to the NRC (2012) nutrient requirement, defined as $A_{NRC\text{-}n}$ (g/d). $A_{ED\text{-}n}$ (g/d) is the product of the nutritional content of the experimental diets on an 'as-fed' dry basis, CED-n (g/kg) and daily feed intake amounts, A_{ADFI} (kg/d). $R_{ER\text{-}n}$ (%) and $A_{ED\text{-}n}$ (g/d) are denoted as follows:

$$R_{ER-n} = \frac{(A_{ED\text{-}n} - A_{NRC-n})}{A_{NRC-n}} \times 100 \quad (13)$$

$$A_{ED-n} = C_{ED-n} \times A_{ADFI} \quad (14)$$

The theoretical regulated nutrient content per unit weight of the diet, denoted as $A_{U\text{-}TR}$ (g/kg), was determined based on the difference between the NRC nutrient requirement, $A_{NRC\text{-}n}$ (g/d), and the daily nutrient intake from the experimental diet, $A_{ED\text{-}n}$ (g/d), adjusted by the average daily feed intake, A_{ADFI} (kg/d). The $A_{U\text{-}TR}$ is determined as follows:

$$A_{U-TR} = \frac{A_{NRC-n} - A_{ED-n}}{A_{ADFI}} \quad (15)$$

2.4. Statistical analysis

All data were expressed as the mean ± standard deviation (SD). The significance of differences in experimental results, produced by varying treatments influenced by single independent factors such as total nutrient levels at respective growth stages, was evaluated using one-way ANOVA with SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Variances in the dependent experimental results from treatments categorized by independent influencing factors were analyzed using a Univariate ANOVA test. Post-hoc comparisons were then conducted using the Bonferroni method (assuming equal variances) and the Tamhane method (not assuming equal variances). The impact of variance on dependent variables, specifically pig growth performance and manure pollution potential, influenced by two independent variables (diet nutrient level and pig growth stage), as well as their interactions, was analyzed using two-way ANOVA with SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). The analysis includes the following steps: testing the independence, normality, and homogeneity of variance of the collected data to ensure validity. Subsequently, a two-way ANOVA was conducted, incorporating second-order effects analysis, simple effects analysis, and post hoc multiple comparisons. The results of the ANOVA were reported using *p*-values and described within a 95 % Confidence Interval, with $p < 0.05$ denoting statistical significance.

The impact levels of influencing factors on experiment results were analyzed using Redundancy analysis (RDA) based on correlation using CANOCO software version 5, in which the influencing factors (such as diet nutrient level, nutrient elements and ingredients) and the experiment results (such as pig growth, manure pollutant characteristics) were taken as explainable or response variables, respectively. The procedures were as follows: the data were analyzed firstly by direct gradient analysis with least squares method, and then the results obtained were interpreted with regression rule. The correlation level of influencing factors on the experiment result were determined by the projection point of influencing factor arrow line on experiment result arrow line and the angle between influencing factor arrow line and experiment result arrow line. The figures were drawn using Origin 8.0 software.

3. Results and discussion

3.1. Pig growth performance

The pig growth performance data is presented Fig. 1. The A_{ADFI} of experimented pigs were similar across all dietary treatment stage ($p>0.05$), but differed significantly among the group treatments WN, FD and FH ($p<0.02$), indicating the growth age played more significant role in the A_{ADFI} than diet characteristics stemming from the sufficient nutrients available to pigs at the different growth stages (discussed in details in section 3.3).

The W_{ADG} of pigs subjected to various dietary fractions in the different stages exhibited analogous trends, demonstrating a consistent decline in W_{ADG} from the initial diet treatment to the third diet treatment in each group. The FH group pigs had higher W_{ADG} (0.87 kg/day) than the FD (0.79 kg/day) and WN (0.62 kg/day) group, respectively. In the work conducted by Williams et al., 2019, they observed that the W_{ADG} for animals fed with high energy diet was the greatest but higher W_{ADG} does not necessarily translate into optimal W_{ADG}. The optimal W_{ADG} in animals age between 6 months and 18 months has been determined to be of about 1.0 kg/day (Tank and Monke, 2022). Thus, excessive W_{ADG} above the optimal is not beneficial for animal growth performance (Williams et al., 2019). In this study, W_{ADG} for all the diet treatment groups (WN, FD, and FH) were well within the optimal range with the FH pig growth stage showing the closest proximity to the optimum level. Conversely, the $R_{D\text{-}M}$ displayed an ascending trend from the first diet energy treatment to the third diet energy treatment at each respective stage, indicating a deterioration in diet efficiency across the successive dietary treatments.

3.2. Solid manure production

As shown in Table 2, the average weights of manure produced daily (namely manure solids) by experimented pigs in the respective growth stages (WN, FD and FH) increased from the first diet treatment to the third diet treatment in the proportion 8.89 % and 13.99 %, 6.58 % and 10.98 %, and 3.63 % and 5.99 %, respectively. The dry and wet weight of manure produced by the experimented pigs fed with different dietary fraction among groups of the three pig growth stages were characteristically similar.

The outcome of the statistical analysis revealed that the daily solid manure weight among the three pig growth stages were significantly different ($p<0.01$), while the weight of the solid manure produced by pigs fed by varied diets were different but not significant ($p>0.05$) in each of the respective growth stages. Moreover, the daily manure production weight were significantly positively correlated with dietary ADF ($r>0.91$, $p<0.02$), ADF-C ($r>0.9$, $p<0.01$) and $R_{D\text{-}M}$ ($r>0.93$, $p<0.01$) for all treatments, suggesting that the indigestible dietary ingredients such as ADF played an important role in solid manure production. This finding was similar with the result reported by Trabue et al. (2022) where higher fiber diets resulted in higher levels of manure solids. However, across all pig growth stages and treatments, daily manure production weight showed a significant negative correlation with CP ($r < -0.7$, $p < 0.035$) and TN ($r < -0.07$, $p < 0.03$). This could be because of the surplus N nutrient, which promoted the utilization of carbohydrate and other nutrients, and further lowered the production of solid manure as also observed by Rundle et al. (2023). Thus, the regulatory reduction of indigestible dietary nutrients (such as ADF) and increase of nitrogen-based nutrients (such as CP) could be an effective approach utilizable for the control of solid manure. However, the daily manure production weight had no significant correlation with dietary nutrient characteristics in the different growth stages ($p>0.05$). Therefore, manure production weight are greatly influenced by the digestibility of nutrient materials in diet.

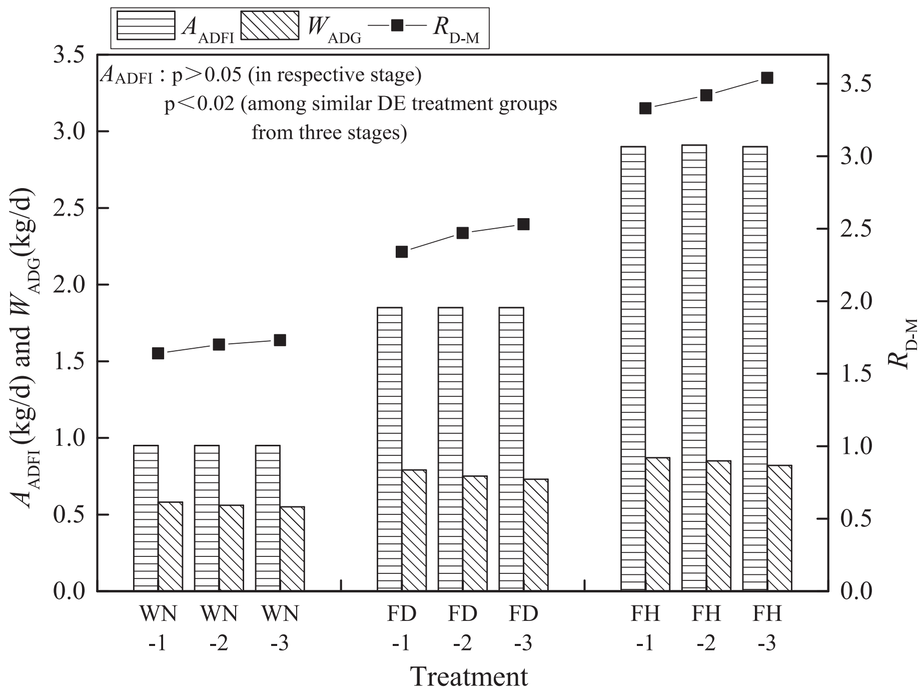

Fig. 1. Pig growth performance in respect of A_{ADFI}, W_{ADG} and R_{D-M} in the feeding trial of pigs fed by dietary nutrients in different growth stages.

Table 2
Weight and MC characteristics of manure produced by experimented pigs fed with distinct dietary fractions at different growth stages.

	Diet type								
	WN-1	WN-2	WN-3	FD-1	FD-2	FD-3	FH-1	FH-2	FH-3
Manure production weight (wet weight, kg)	1.23 ± 0.16	1.35 ± 0.18	1.43 ± 0.13	2.27 ± 0.14	2.43 ± 0.18	2.55 ± 0.19	3.45 ± 0.23	3.58 ± 0.11	3.67 ± 0.08
MC (%)	81	81	81	81.5	81.5	81.5	80	80	80

3.3. Characteristics of micro-nutrient fractions in diet and manure

3.3.1. Characteristics of N-fraction in diet and manure

As shown in Table 1, the dietary TON represent the main fraction of dietary N, which occupied >99 % of dietary TN, indicating the dietary N are mostly protein N, and CP, being the form of protein in the diet. The Dietary N was 9.07 %, 30.47 % and 57.07 % higher than the required N concentration specified in NRC (2012) for all diets in the WN, FD, and FH stages, respectively, indicating all diets met the N requirement of NRC (2012). The lowest TN concentration in the diets was found in the FH stage relative to other stages.

Manure N is the main causative factor for environmental pollution, leading to issues such as nitrogen-containing air contamination, eutrophication, and soil pollution (Zhang et al., 2018). Among these, manure LN represent the most crucial fraction responsible for environmental pollution, primarily attributed to its high mineralizability (Awasthi et al., 2020). In addition, swine manure reportedly harbors more LN than cattle manure (Hou et al., 2023). Consistent with the dietary TN concentration, manure TN concentration was lowest for WN-2 and FD-3. However, there was a disparity in TN concentrations during the FH stage, with FH-1 exhibiting the lowest TN concentrations in the manure. FH-1 TN concentration was 18.21 % and 14.44 % lower than FH-2 and FH-3 in the finishing stage, respectively. Overall, TN concentrations in the diets and manure were similar for all the growth stages ($p > 0.05$). However, the manure LN exhibited distinct concentration characteristics from manure TN (p>0.05). As shown in Fig. 2, the lowest manure LN concentrations were found in the FH stage, and unlike the TN, the manure LN varied significantly among the growth stages (p<0.04) and among feeding trials in each growth stages (p<0.02), indicating the

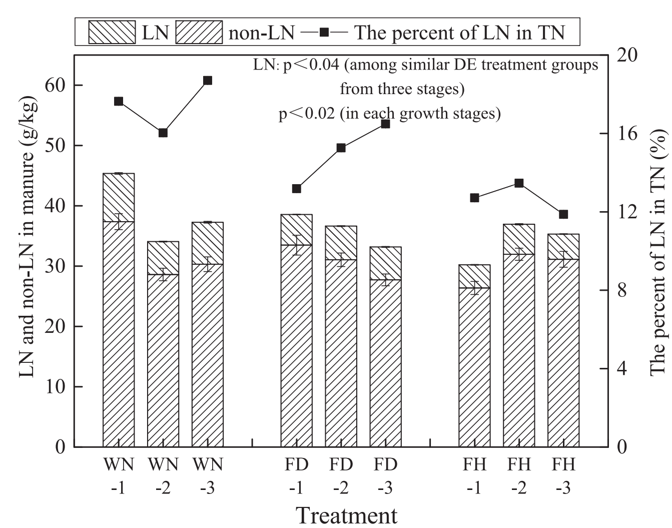

Fig. 2. Manure N fraction characteristics (a) manure LN and non-LN; (b) percent LN in TN (Manure TN was equal to the sum of manure LN and manure non-LN).

manure TN had no effect on the manure LN. Thus, manure TN cannot represent manure LN concentration characteristics, and also cannot decide the manure N pollution potential.

To gain in-depth understanding on the variation of manure TN and LN based on associated nutrients, correlation analysis was utilized and results showed that manure TN and LN produced showed varying characteristics with associated nutrients at different feeding stages. As shown in Fig. S1, the production of TN in manure was significantly increased by CP during all growth stages (WN, FD, and FH) ($p<0.01$, $r>0.8$), but was reduced when ADF was present in the diets during the WN and FD stages ($p<0.05$, $r>-0.7$), and by DTP during the FH stage ($p>0.05$, $r<0$). In contrast, the factors influencing LN generation in manure differed across the pig growth stages. The LN production was enhanced by the ratio of DC/DN/DP and CP during the WN and FH stages ($p<0.01$, $r>0.8$) (Fig.S1a and 1b), respectively, while ADF, and DP together promoted LN production during the FD stage ($p<0.01$, $r>0.82$) (**Fig. S1c**). Inhibitory effects on LN production were observed from DTP during the WN stage ($p<0.01$, $r<-0.8$), DC/DN/DP ratio and dietary microelements fractions of DTOCu and DTOZn during the FD stage ($p<0.04$, $r<-0.73$), and from ADF during the FH stage ($p>0.05$, $r>-0.56$). Thus, manure N (TN and LN) production was mostly introduced by the dietary protein ingredients (CP) in all the growth stages, and compared to the total dietary nutrients, the dietary nutrients of ADF and DP fractions played more significant role in the production of manure LN at all growth stages.

3.3.2. *Characteristics of P-fraction in diet and manure*

According to dietary requirement, the digestible P (DP) in diet is a true representation of the levels of phosphorus that could be utilized by animals compared to dietary TP (DTP), while the dietary phytate phosphorus (DPP) is the fraction of phosphorus that is hardly utilized by animals (Aureli et al., 2017). As shown in Table 1, DTP and DP showed distinct concentration characteristics among the diets in respective stage. For DTP, their lowest concentration in the diets was found in WN-2, FD-2 and FH-2 stages. However, for DP, the lowest concentration in the diets was found in WN-3, FD-1 and FH-3 stages. This suggest that the dietary energy content of the pig diet have direct effect on the levels of phosphorus released at all examined growth stages but differs with respect to the dietary energy levels for each stage.

Correlation analysis indicated that DTP and DP were not significantly correlated among treatments in the respective stages ($p>0.05$, $r<0.6$). By ANOVA analysis, the dietary TP ($p<0.05$) and DP ($p<0.01$) significantly varied among treatments in the respective stages. The release of phosphorus-rich manure into the environment represents a significant contributor to environmental phosphorus pollution, leading to issues such as water eutrophication and soil infertility (Ran et al.,2023; Aureli et al., 2017). Within this context, the predominant fraction responsible for environmental contamination is the labile phosphorus (LP) component, owing to its high mobility within the environment (Dinkler et al., 2021; Lv et al., 2013). As shown in Fig. 3a, for manure P, the lowest concentration was found in WN-2, FD-2 and FH-2. However, for manure LP, as shown in Fig. 3b, the lowest LP was observed in WN-1, FD-1 and FH-1. By analysis of variant, significant distinctions were observed in the levels of LP among treatments at each group and stages, respectively ($p<0.01$, $p<0.03$). However, a non-significant correlation between manure LP and TP levels were observed among treatments at each respective stage ($p>0.05$). This suggests that manure TP may not accurately represent the levels of manure LP. Research has indicated that nitrogen and phosphorus in fecal solutions readily interact with one another. For example, under certain conditions, nitrogen compounds (such as ammonium salts) may react with phosphate salts to produce precipitates (Hou et al.,2018; Cui et al., 2024), and the changes in the concentration and forms of phosphorus may affect the oxidation-reduction state of nitrogen (Fan et al., 2024). However, in this research, as shown in Fig. S4, manure LP and manure LN all showed non-significant influence on each other in respective stage ($p>0.05$, $r<0.6$), indicating the interaction of manure nitrogen and manure phosphorous in this study were not significant, and did not exert significant influence on the correlation relationship of diet nutrient fraction and manure pollutant fraction.

Furthermore, results from correlation analysis revealed that manure TP production was enhanced by DTP at all growth stages ($p<0.02$, $r>0.9$) as depicted in **Fig. S2**. Moreover, TP production was further enhanced by dietary Organic Zn (DOZn) and DP during the WN and FD stage ($p<0.01$, $r>0.8$), respectively (**Fig. S2a and 2b**). Factors enhancing manure TP production during the FH stage was somewhat different, which include DTC, dietary TOC (DTOC), dietary organic Cu (DOCu), and DOZn ($r>0.75$, $p<0.04$) (**Fig. S2c**). Significant inhibition of manure TP production was induced by ADF-C, DOZn, dietary inorganic Cu (DCu), and dietary inorganic Zn (DZn) during the WN, FD and FH stages ($p<0.05$, $r<-0.7$), respectively (**Fig. S2**). On the other hand, the production of LP in manure was influenced by DP in the WN stage ($p<0.01$, $r>0.9$), DP, DTP, ADF in the FD stage ($r>0.75$, $p<0.02$), and ADF-C and total dietary Cu (DTCu) during the FH stage ($r>0.7$, $p<0.03$) but was significantly inhibited by DTOC, DOCu and DOZn ($r<-0.7$, $p<0.05$)(**Fig. S2**). Hence, the various stages exhibited distinct contributing factors for manure TP and LP. However, the dietary nutrient fractions, specifically DP, ADF, and DTCu, played more significant role in the emission of P pollutants from manure.

3.3.3. *Characteristics of cu and Zn fractions in diet and manure*

Dietary Cu and Zn in diet are essential microelements for pig growth (Ding et al., 2021). Total Cu (TCu) and total Zn (TZn) represent the levels of dietary Cu and dietary Zn in diets. The dietary Cu content in all diets at each growth stage was as follows: 17.7 to 20.2 times, 18.8 to 21.6 times, and 21.7 to 33.6 times the recommended concentrations set by the NRC (2012). The NRC recommended Cu concentrations are 4 mg/kg for the weaning stage, 3.5 mg/kg for the feeding stage, and 3 mg/kg for the finishing stage. Similarly, the dietary Zn content in all diets at each growth stage was 1.7 to 1.9 times, 1.9 to 2.1 times, and 1.8 to 2.2 times the recommended concentrations according to NRC (2012). The NRC recommended Zn concentrations are 60 mg/kg for the weaning stage and 50 mg/kg for both the feeding and finishing stages. This

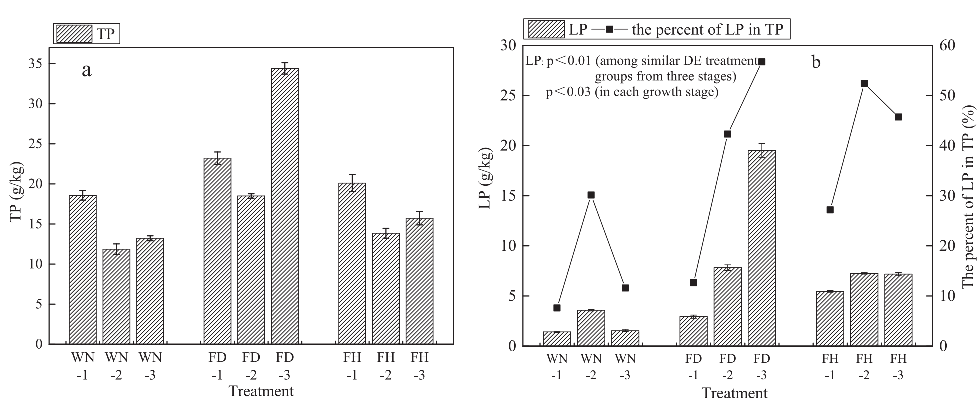

Fig. 3. Manure P characteristics (a) manure TP; (b) LP and percent LP in TP.

indicated that the dietary microelements (Cu and Zn) in all experimented diets were excessive. As shown in Table 1, the concentration characteristics of TCu and TZn were similar in the diets across the stages. The lowest DTCu and DTZn were found in WN-3, FD-3 and FH-1. Similarly, the lowest DOCu and DOZn were found in WN-3, FD-3 and FH-3, which were 0.68 ± 0.02 mg/kg and 2.25 ± 0.21 mg/kg, 0.64 ± 0.03 mg/kg and 1.84 ± 0.07 mg/kg, 0.63 ± 0.04 mg/kg and 0.92 ± 0.05 mg/kg, respectively. From ANOVA analysis, the dietary Cu and dietary Zn significantly varied among diets in all the stages ($p<0.01$) and among group diets in the three stages ($p<0.01$), respectively.

Manure-derived microelements, specifically Cu and Zn, primarily contributes to heavy metals pollution in the environment (Liu et al., 2022). Notably, the labile fractions of the manure microelements, denoted as LCu and LZn, constitute the predominant fractions responsible for inducing environmental pollution. As shown in Fig. 4a and b, the lowest manure-derived labile Cu and Zn (LCu and LZn) were found in pigs fed with high energy diet across the growth stages (WN-1, FD-1 and FH-1). Concentrations of the manured-derived labile heavy metals (LCu and LZn) and the percent of labile heavy metals in total heavy metals varied significantly among treatments in the respective stages ($p<0.02$).

As shown in Fig. 4, concentrations of LCu and LZn did not correlate with the concentrations of manure-derived total microelements of Cu and Zn (TCu and TZn) at $p>0.05$ in the respective stages, indicating that manure total microelements concentration is not representative of labile microelements concentrations in manure. Previous studies assessing the influence of dietary microelement characteristics on environmental pollution from manure relied on data derived from the total concentration of these microelements (including Cu and Zn) in manure, excluding the labile microelement fractions (Ding et al., 2021; Liu et al., 2020). These labile fractions primarily contribute to environmental pollution (Dourmad and Jondreville, 2007; Lv et al., 2013; Dinkler et al., 2021). Consequently, our work provides additional perspective on the importance of quantifying the labile fraction of microelements in both diet and manure to accurately assess the impact of manure on environmental pollution.

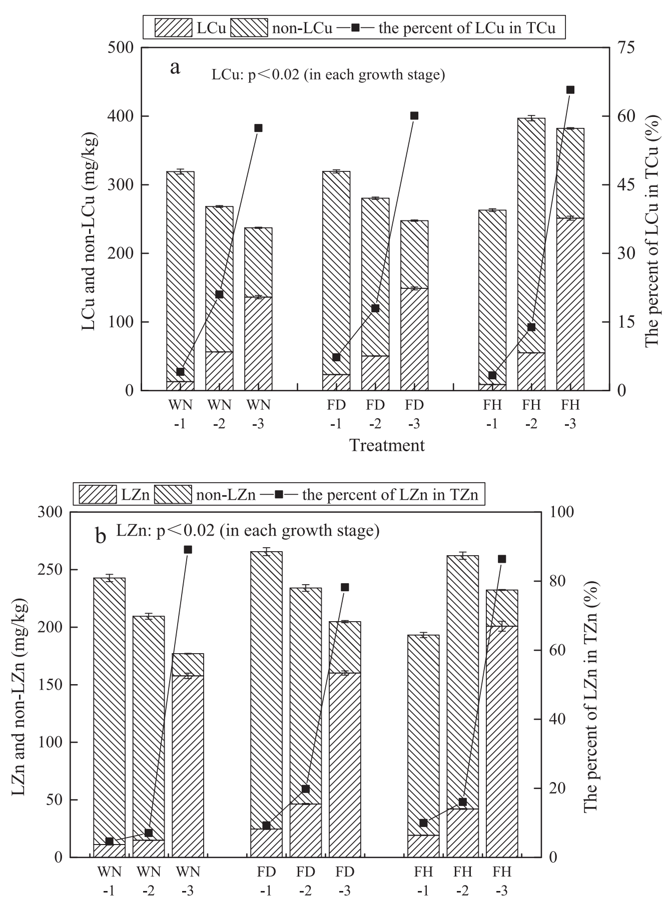

Fig. 4. Pig manure Cu and Zn characteristics (a) manure LCu, non-LCu and percent LCu in TCu; (b) LZn, non-LZn and percent LZn in TZn (Note: TCu = LCu + non-LCu; TZn = LZn + non-LZn).

Correlation analysis revealed that during the weaning stage, the production of manure-derived TCu and TZn was significantly enhanced, with the most substantial effect observed in response to DOCu ($p < 0.001$, $r > 0.921$) and DOZn ($p < 0.002$, $r > 0.877$), but significantly inhibited by ADF-C ($p < 0.001$, $r > 0.993$) (**Fig. S3**). Conversely, as shown in **Fig. S3**, the labile forms of manure-derived LCu and LZn was significantly promoted by ADF-C ($p < 0.012$, $r > 0.784$) and inhibited by dietary organic microelement fractions of DOCu and DOZn ($p < 0.017$, $r < 0.76$). In the feeding stage, the release/production of manure-derived TCu and TZn were significantly promoted by the DOCu ($p<0.006$, $r>0.896$) and DOZn ($p<0.002$, $r>0.913$), and inhibited by ADF-C ($p<0.001$, $r>0.993$), while the manure labile fractions LCu and LZn were enhanced by ADF-C ($p<0.003$, $r>0.865$), ADF ($p<0.004$, $r>0.845$) and DC/DN/DP ($p<0.01$, $r>0.79$), and mainly regulated by DOCu ($p<0.005$, $r<-0.835$), and DOZn ($p<0.005$, $r<-0.838$), respectively. In the finishing stage, discharge of manure-derived TCu and TZn were significantly promoted by DOCu ($p<0.04$, $r>0.75$), DOZn ($p<0.045$, $r>0.75$), DTCu ($p<0.05$, $r>0.7$) and DTZn ($p<0.05$, $r>0.7$), and limited by DTP ($p<0.005$, $r<-0.85$) and CP ($p<0.006$, $r<-0.83$), respectively. On the other hand, LCu and LZn were promoted by ADF ($p<0.02$, $r>0.81$), total dietary microelements ($p<0.05$, $r>0.67$) and microelements in organic fraction ($p<0.01$, $r>0.92$), respectively, but inhibited significantly by ADF-C ($p<0.01$, $r<-0.85$). Thus, the manure microelements characteristics were as a result of combination of multiple factors such as dietary microelements (DTZn, DOZn, DTCu and DOCu), DTC, DTOC, DTN, CP, DTP and indigestible components (ADF). However, compared with dietary microelements (DTCu and DTZn), the dietary microelements of different fractions played more distinctive role in the release of manure total and labile heavy metals. Although Cu and Zn in manure could interact with manure N and manure P by manure solution pH regulation (Viana et al., 2024), as shown in Fig. S4, the correlation of manure microelements (Cu and Zn) and manure pollutants (N and P) were not significant in the respective stages, suggesting that the interaction of manure microelements (Cu and Zn) and manure pollutants (N and P) did not influence the manure microelements source.

4. Nutrient balance analysis

The nutrients balance analysis (N, P, Cu and Zn) can be calculated by the input/added nutrient ($A_{I\text{-}N}$, namely, dietary nutrient intake amounts), the retained nutrient by pigs ($A_{R\text{-}N}$, namely, dietary nutrient retained by pigs) and the output/released nutrient ($A_{O\text{-}P}$, namely, the pollutants released by pigs). As shown in Table 3 and Table S2, the respective nutrient (N, P, Cu and Zn) input was not significantly different among treatments in the respective stages ($p > 0.05$).

Apart from manure release, the excretion of urine serves as an additional significant pathway for the release of nutrients such as N, P, Cu, and Zn. The quantities of urine excretion did not exhibit any statistically significant variance across the different treatments at each respective stage ($p > 0.05$). However, the amounts of pollutants (N, P, Cu, and Zn) present in the urine did demonstrate some significant difference among treatments at each stage ($p < 0.05$). This variation could be attributed to the significant correlation observed between the release of these pollutants and the levels of dietary nutrients available to the pigs at each respective stage ($p < 0.05$). This suggests that the fractions of nutrients available in the diet have significant influence on the release of pollutants in the urine of all pigs.

The reduction in output ($A_{O\text{-}P}$) of specific pollutants (such as N, P, Cu, and Zn) observed in certain treatments suggests that diets tailored in

Table 3
Nutrient balance analysis of manure and urine nutrient produced by pigs fed with different diets from different nutrient fractions.

	N				P				Cu				Zn			
	$A_{I\text{-}N}$ (g)	$A_{O\text{-}P}$ (g)		Average $A_{R\text{-}N}$ (g)	$A_{I\text{-}N}$ (g)	$A_{O\text{-}P}$ (g)		Average $A_{R\text{-}N}$ (g)	$A_{I\text{-}N}$ (mg)	$A_{O\text{-}P}$		Average $A_{R\text{-}N}$ (mg)	$A_{I\text{-}N}$ (mg)	$A_{O\text{-}P}$		Average $A_{R\text{-}N}$ (mg)
		M (g)	U(g)			M (g)	U (g)			M (mg)	U (μg)			M(mg)	U (μg)	
WN-1	33.86 ± 1.24	10.43 ± 0.78	8.37 ± 0.39	15.06	6.79 ± 0.73	4.27 ± 0.38	1.71 ± 0.67	0.81	76.65 ± 2.16	73.38 ± 4.29	4.27 ± 0.05	3.26	105.83 ± 4.62	55.83 ± 1.22	4.1 ± 0.05	49.99
WN-2	31.29 ± 0.74	8.860.42	7.97 ± 0.31	14.46	5.52 ± 0.08	3.08 ± 0.24	1.65 ± 0.03	0.79	72.89 ± 4.33	69.75 ± 3.54	6.05 ± 0.04	3.14	102.74 ± 6.34	54.45 ± 2.81	8.34 ± 0.03	48.28
WN-3	32.1 ± 2.16	10.06 ± 0.63	7.92 ± 0.08	14.12	5.95 ± 0.28	3.54 ± 0.12	1.64 ± 0.08	0.77	67.15 ± 1.82	64.05 ± 1.24	5.09 ± 0.02	3.09	95.24 ± 5.26	47.78 ± 1.51	4.53 ± 0.02	47.46
FD-1	54.37 ± 1.24	16.19 ± 1.06	8.19 ± 0.29	29.98	13.69 ± 1.01	9.75 ± 0.13	1.39 ± 0.07	2.55	139.62 ± 7.2	134.16 ± 6.34	3.28 ± 0.01	5.46	193.25 ± 7.22	111.55 ± 2.35	4.46 ± 0.04	81.70
FD-2	52.74 ± 0.74	16.48 ± 0.67	7.83 ± 0.59	28.43	12.03 ± 0.68	8.11 ± 0.05	1.46 ± 0.02	2.46	131.33 ± 5.12	126.15 ± 4.11	3.06 ± 0.04	5.18	182.91 ± 6.37	105.31 ± 4.21	4.83 ± 0.02	77.60
FD-3	50.93 ± 1.14	15.59 ± 0.17	7.91 ± 0.06	27.43	19.76 ± 0.61	15.89 ± 0.38	1.48 ± 0.01	2.39	121.43 ± 3.66	116.39 ± 4.92	5.59 ± 0.03	5.04	171.75 ± 5.18	96.24 ± 4.43	8.16 ± 0.05	75.50
FH-1	83.81 ± 1.22	20.84 ± 1.29	9.66 ± 0.41	53.31	18.85 ± 0.31	13.87 ± 0.59	1.32 ± 0.02	3.66	188.94 ± 6.27	181.40 ± 5.23	3.21 ± 0.05	7.53	260.68 ± 8.31	133.24 ± 7.72	4.42 ± 0.03	127.44
FH-2	88.14 ± 1.04	26.59 ± 1.57	9.64 ± 0.61	51.92	14.84 ± 0.61	9.94 ± 0.26	1.32 ± 0.03	3.58	293.18 ± 8.06	285.82 ± 7.24	4.38 ± 0.06	7.36	313.29 ± 8.66	188.71 ± 7.29	7.28 ± 0.04	124.57
FH-3	85.12 ± 1.35	25.78 ± 1.51	9.14 ± 0.05	50.21	16.18 ± 0.33	11.32 ± 0.59	1.42 ± 0.01	3.44	286.03 ± 7.03	278.88 ± 7.03	5.84 ± 0.02	7.14	289.81 ± 4.31	169.58 ± 2.76	7.94 ± 0.03	120.22

these treatments possess a lower potential for environmental pollution in terms of the quantity of pollutants released. As shown in Table 3, for N, the lowest mean $A_{O\text{-}P}$ values were observed in WN-2 (16.83 ± 0.31 g), FD-2 (24.31 ± 0.07 g), and FH-1 (30.5 ± 0.63 g). These values represent reductions of 6.44 %–10.52 % in the weaning stage, 0.28 %–3.33 % in the feeding stage, and 3.62 %–15.8 % in the finishing stage compared to other treatments within the respective stages. Regarding P, the lowest average $A_{O\text{-}P}$ was observed in WN-2 (4.73 ± 0.08 g), FD-2 (9.57 ± 0.05 g), and FH-2 (11.26 ± 0.13 g), reflecting respective decreases of 8.71 %–20.91 % in the weaning stage, 14.05 %–44.89 % in the feeding/fattening stage, and 11.61 %–25.85 % in the finishing stage relative to other treatments. For Cu, the lowest mean $A_{O\text{-}P}$ was recorded in WN-3 (64.06 ± 0.43 g), FD-3 (116.39 ± 2.13 g), and FH-1 (181.4 ± 2.25 g), indicating reductions of 8.16 %–12.71 % in the weaning stage, 7.74 %–13.24 % in the fattening stage, and 34.95 %–36.53 % in the finishing stage in comparison to other treatments. Lastly, for Zn, the lowest average $A_{O\text{-}P}$ was found in WN-3 (47.78 ± 0.23 g), FD-3 (96.25 ± 2.45 g) and FH-1 (133.24 ± 2.07 g), with decreases of 12.26 %–14.42 % in the weaning stage, 8.61 %–13.72 % in the feeding stage, and 21.43 %–29.39 % in the finishing stage relative to other treatments.

The higher retention of N, P, Cu, and Zn nutrients ($A_{R\text{-}N}$) in the feeding trial with high energy diet especially as shown in FH1 indicates the extent of nutrient utilization in pigs, surpassing that of other treatments. Similarly, Table 3 reveals that the highest $A_{R\text{-}N}$ for these nutrients (N, P, Cu, Zn) were consistently observed in WN-1, FD-1, and FH-1 for the respective growth stages. These were followed in sequence by the medium energy diets (WN-2, FD-2, FH-2) and low energy diets (WN-3, FD-3, FH-3) within each respective stage. Correlation analysis demonstrated a significant positive association of $A_{R\text{-}N}$ with W_{ADG} ($p < 0.05$, $r > 0.78$) and a significant negative correlation with $R_{D\text{-}M}$ ($p < 0.05$, $r < -0.75$), providing substantial evidence for the trend characteristics of W_{ADG} and $R_{D\text{-}M}$. However, $A_{R\text{-}N}$ for N, P, Cu, and Zn did not exhibit any significant correlation with the corresponding $A_{O\text{-}P}$. This implies that higher nutrient retention levels in the pigs do not necessarily indicate reduced manure pollutant release. In other words, enhanced nutrient utilization in the animal does not serve as an indicator for lower pollution potential in terms of specific manure pollutant release levels.

The redundancy analysis (RDA) depicted in Fig. S5 showed that the release/output nutrient of N, P, Cu and Zn (namely *AO-N*, *AO-P*, *AO-Cu* and *AO-Zn,* respectively) were influenced significantly by the total dietary nutrients (DTN, DTP, DTCu, and DTZn) and dietary nutrient fractions (CP, DP, DOCu, and DOZn). As revealed in Fig. S5, the input amount of all nutrients ($A_{I\text{-}N}$) was identified as the primary determinant influencing the efflux of pollutants including N, P, Cu, Zn from the manure ($p < 0.04$, $r > 0.85$). Additionally, the proportion of dietary nutrients significantly influenced the release of contaminants from manure ($p < 0.04$, $r > 0.8$), as shown in Fig. S6. Moreover, the proportions of the dietary nutrients rather than the total nutrient inputs as shown in Fig. S7 significantly influenced the amount of nutrients retained.

5. Evaluation of pollution potential

5.1. Manure pollutants and source control factors analysis

The release ratio of pollutants from one nutrient (N, P, Cu and Zn) ($R_{O\text{-}P}$) at different growth stages varied according to the stage characteristics. Lower $R_{O\text{-}P}$ from dietary nutrient resulted in higher retention rate of nutrients by pigs in the treatments, which suggests that a higher nutrient retention rate will result in lower environmental pollution potential. As illustrated in Fig. 5, the lowest $R_{O\text{-}P}$ for N (*RO-N*), corresponding to the highest N retention rates, were observed in WN-3 at 43.98 % among WN stage, in FD-3 at 53.85 % among FD stage, and in FH-1 at 58.90 % among FH stage. These values are, on average, 1.13 %–4.87 %, 0.1 %–2.37 %, and 0.14 %–7.4 % lower than the N retention rates in other WN, FD, and FH stages, respectively. Similarly, for P (*RO-*

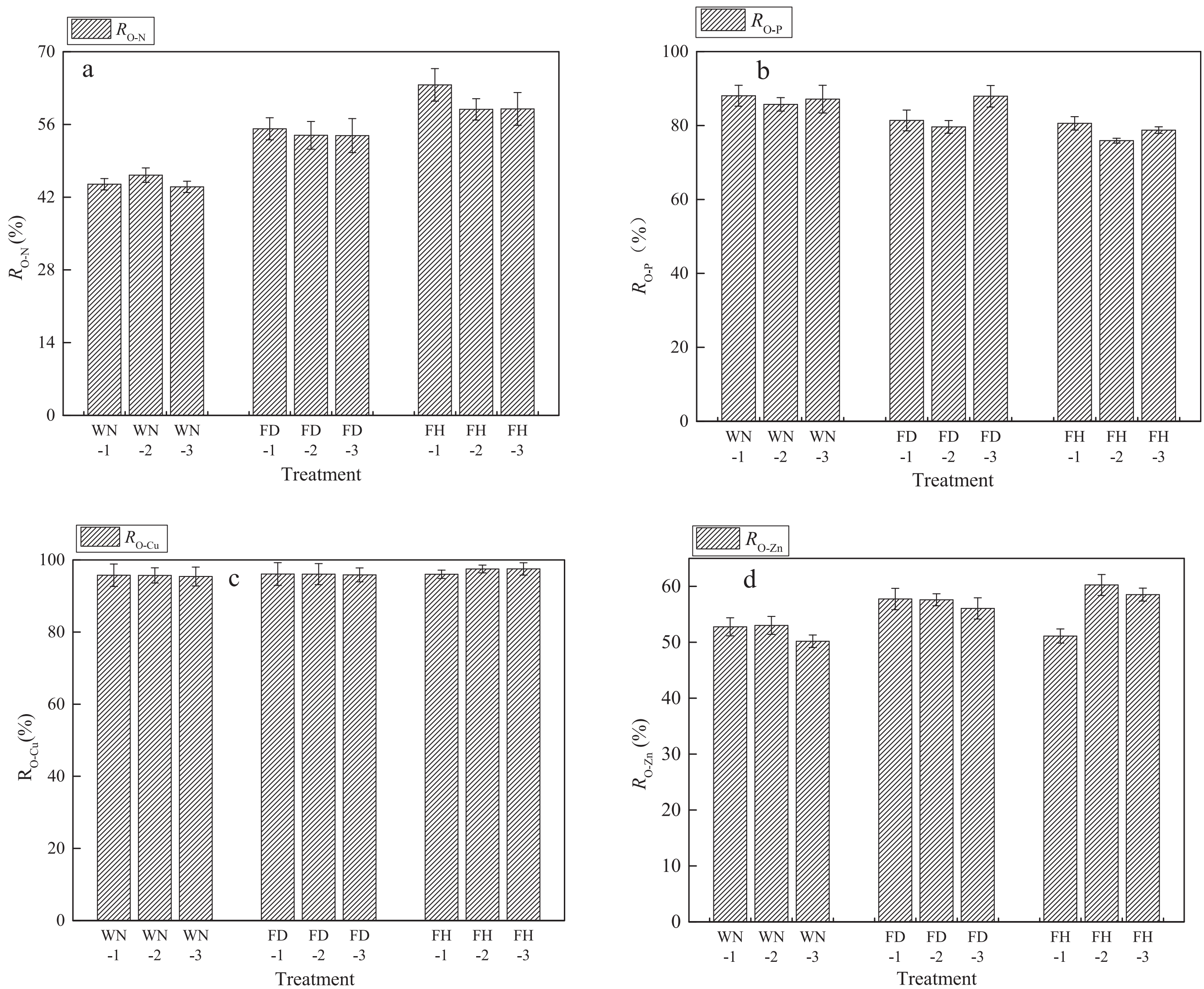

Fig. 5. The R_{O-P} of nutrients (a) N, (b) P, (c) Cu and (d) Zn in all treatments.

P), the minimum average R_{O-P} occurred in WN-2, FD-2, and FH-2 with retention rates of 85.71 %, 79.61 %, and 75.89 %, respectively (Fig. 5). The least release ratio of Cu (*RO-Cu*) and Zn (*RO-Zn*) were observed in WN-3, FD-3, and FH-1, with retention percentages of 95.41 % and 50.71 %, 95.85 % and 56.04 %, and 96.01 % and 51.11 %, respectively for Cu and Zn (Fig. 5).

From correlation analysis, the R_{O-P} correlated negatively with A_{O-P}, indicating that higher nutrient retention rate may not translate into lower environmental pollution potential, which could be as a result of the extra nutrient inputs (A_{I-N}). However, manure characteristics from similar dietary nutrient level demonstrates that higher nutrient retention rate could lower manure pollution risk when considering the total pollutant released amounts. Thus, dietary nutrient levels greatly influence manure pollution potential.

From analysis of correlation, as shown in Fig. S8, the R_{O-P} is directly affected by a combination of dietary nutrients such as, DTN, CP, DTP, DPP, DCu, DOCu, DZn, DOZn, DC/DN/DP and ADF, but mostly influenced by dietary nutrient ($r>0.7$, $p<0.04$) and dietary indigestible fractions of DPP, DOCu and DOZn ($r>0.85$, $p<0.01$). Compared to other dietary nutrient fractions, dietary P, Cu and Zn played more significant role in R_{O-P} production due to the higher correlation coefficient of R_{O-P} values of these dietary nutrient fraction ($0.7<r<0.85$) relative to other dietary nutrient ($0.85<r<0.93$). This indicates that the dietary nutrients of P, Cu and Zn were critical to R_{O-P} levels.

5.2. Labile manure pollutants and management strategies

As earlier described, labile manure pollutants are true representation of manure environmental pollution potential, due to their high mobility and toxicity to the environment. To effectively manage labile manure fractions, the A_{LFP-E} and R_{LFP-E} are good indicators for the comprehensive evaluation of the environmental pollution potential for manure released by pigs from the different treatments. The pollutants N, P, Cu and Zn in urine were represented as urea nitrogen, inorganic phosphorus and inorganic microelements (Cu, Zn), respectively. These were labile in the environment; thus, the urine-based pollutants could be regarded as the labile pollutant fractions.

As shown in Fig. 6a, the lowest A_{LFP-E}, namely, the treatment with the lowest environmental pollution potential occurred in WN-2, FD-2 and FH1, consistent with previous stages treatment pattern earlier evaluated. With difference analysis, the A_{LFP-E} was significantly different among treatments in respective stage ($p<0.01$). As shown in Fig. 6b, except for FD-3, percentage A_{LFP-M} in A_{LFP-E} in other treatments were all <50 %, indicating that urine played a more significant role in A_{LFP-E}

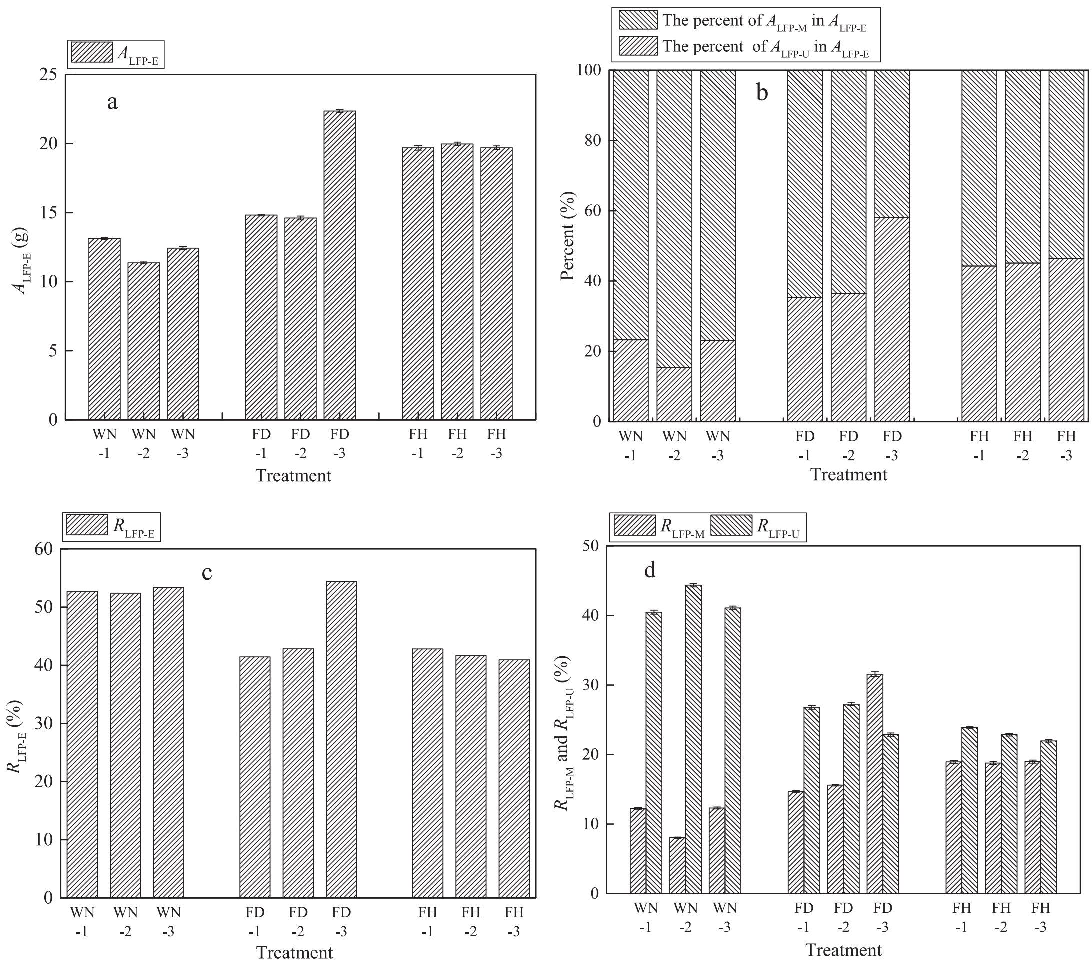

Fig. 6. Labile pollutant characteristics in different excrement (manure and urine) from different treatments based on diets: (a) $A_{LFP\text{-}E}$ of all treatments; (b) percent $A_{LFP\text{-}M}$ in $A_{LFP\text{-}M}$ and percent $A_{LFP\text{-}U}$ in $A_{LFP\text{-}M}$, (c) $R_{LFP\text{-}E}$ of all treatments; (d) the $R_{LFP\text{-}M}$ and $R_{LFP\text{-}U}$ of all treatments.

release than manure. However, the difference of $A_{LFP\text{-}M}$ ($p = 0.005$–0.01) among treatments based on different diets were larger than the difference of $A_{LFP\text{-}U}$ among treatments ($p = 0.03$–0.054) in the three stages, indicating the potential for reduced pollutant release in *LFP-M* (labile fractions of pollutants in manure) compared to *LFP-U* (labile fractions of pollutants in urine).

The pollution potential and recycling potential of manure is related to $R_{LFP\text{-}E}$. A lower $R_{LFP\text{-}E}$ indicates that the manure possesses lower pollution potential and higher recycling potential when used as biological resource treatments such as, composting and anaerobic fermentation. The $R_{LFP\text{-}E}$ is composed of $R_{LFP\text{-}U}$ and $R_{LFP\text{-}M}$. Both displayed distinct characteristics during treatments in the respective stages. As shown in Fig. 6c, in weaning stage, the lowest $R_{LFP\text{-}E}$ occurred in WN-2, FD-1 and FH-3 with average values of 52.39 %, 41.43 %, and 40.92 %, respectively. Excluding the weaning stage, the lowest $R_{LFP\text{-}E}$ and $A_{LFP\text{-}E}$ were observed at different dietary energy treatment levels, which could be caused by the significant difference of $A_{O\text{-}P}$. Thus, this indicates that when manure production amounts vary, lower labile manure production rate is not representative of lower environmental pollution and higher recycling capacity. However, when $A_{O\text{-}P}$ is not significantly different among treatment levels, lower labile manure production rate in manure could lower the manure pollution potential and increase the manure recycling capacity. As shown in Fig. 6d, the $R_{LFP\text{-}M}$ were all lower than $R_{LFP\text{-}U}$ in all treatments, which provides substantial support that labile manure component (urine) played greater contributing role to *LFP-E* (labile fractions of pollutants in excrement) than manure. However, the difference of $R_{LFP\text{-}M}$ ($p = 0.004$–0.01) among treatments was larger than the difference of $R_{LFP\text{-}U}$ among treatments ($p = 0.037$–0.061) in the respective stage, which provide another evidence that there was a greater potential for the controlled release of *LFP-M* than that of *LEP-U* by regulation of critical diet factors.

As shown in Fig. S9, the labile pollutant fractions from excrement ($A_{LFP\text{-}E}$), which include manure ($A_{LFP\text{-}M}$) and urine ($A_{LFP\text{-}U}$) were all derived from the comprehensive diet nutrient factors, including the dietary C, dietary N dietary microelements (Cu, Zn), indigestible ingredients (ADF) and DC/DN/DP. Each total dietary nutrients (DTC, DTN, DTP) acted as the positive and significant contributors to $A_{LFP\text{-}E}$ ($r>0.75$, $p<0.02$) and $A_{LFP\text{-}M}$ ($r>0.8$, $p<0.01$) in WN stage, while they exerted negative but non-significant contributory role in $A_{LFP\text{-}E}$ ($r>-0.6$, $p>0.05$) and $A_{LFP\text{-}M}$ ($r>-0.65$, $p>0.05$) during the FD and FH stage. This could be as a result of the different digestibility of pigs at different ages, as also described by Zentek and Goodarzi, 2020. The dietary nutrient fraction of DP, DOCu and DOZn also played important role in $A_{LFP\text{-}E}$ and $A_{LFP\text{-}M}$ at all pig growth stages, for example, DP, DOCu and DOZn exerted positive and significant role in the spread rate and amount of labile pollutants *RLFP-E* ($r>0.65$, $p<0.05$) and $A_{LFP\text{-}M}$ ($r>0.75$, $p<0.03$), released in WN and FH stage, while DOCu and DTOC played negative but significant role in $A_{LFP\text{-}E}$ ($r<-0.75$, $p<0.02$) and $A_{LFP\text{-}M}$ ($r<-0.65$, $p<0.05$) in the FD stage. This may be because the DC/DN/DP requirement for pigs at different growth stages varies ($p<0.01$). Thus, the effect of dietary nutrient fraction on manure labile fraction can be determined by DC/DN/DP ratio (NRC, 2012). The indigestible ADF also played significant role in the release of labile manure and urine, which enhances the production of labile manure, as shown in Fig. S9b and 9c, but was favorable towards the inhibition of the release of urine pollutants.

As shown in Fig. S10, the occupation/spreading rate of pollutants of labile fractions in total excrement (R_{LFP-E}), manure (R_{LFP-M}) and urine (R_{LFP-U}) were derived from the comprehensive diet nutrient factors. In WN stage, the factors responsible for R_{LFP-E} and R_{LFP-M} were DC/DN/DP ($r>0.7$, $p<0.02$) and DPP ($r>0.65$, $p<0.04$), while the least contributing factor was DCu ($r<-0.6$, $p<0.05$). However, for R_{LFP-U}, the major and least contributing factors are DTOC ($r>0.7$, $p<0.03$) and DPP ($r<-0.6$, $p<0.05$), respectively. In the FD stage, the major influencing factors for R_{LFP-E} and R_{LFP-M} spreading rate are DC/DN/DP ($r>0.8$, $p<0.02$), ADF ($r>0.7$, $p<0.04$) and ADF-C ($r>0.8$, $p<0.02$), while the least contributing factor are the dietary organic microelements (DOCu, DOZn) ($r<-0.6$, $p<0.05$). However, dietary organic microelements (DOCu, DOZn) ($r>0.7$, $p<0.04$) positively enhanced R_{LFP-U}, while DC/DN/DP ($r<-0.85$, $p<0.01$) slowed down the spreading rate. In the FH stage, R_{LFP-E} and R_{LFP-M} were enhanced by DPP ($r>0.8$, $p<0.02$), and significantly inhibited by DOCu and DOZn ($r<-0.7$, $p<0.03$). Similarly, R_{LFP-U} was positively enhanced by DPP ($r>0.7$, $p<0.04$), and significantly inhibited by DOCu ($r<-0.85$, $p<0.01$) and ADF-C ($r<-0.7$, $p<0.02$). Thus, these differences in influencing factors for R_{LFP-E}, R_{LFP-M}, and R_{LFP-U} across the growth stages (WN, FD, and FH) suggest variability in pigs' digestibility at different growth stage, influenced by dietary nutrient fractions, which plays an important role in the release of labile manure, urine and labile excrement (Zentek and Goodarzi, 2020).

To study the effect of dietary nutrient on labile pollutant release, excluding the urine fraction, the manure may be treated solely for nutrient (N, P, Cu and Zn) output/release management. As shown in Fig. 7a, the $A_{O\text{-}M}$ (namely $A_{O\text{-}P}$ in eq. 9, measured in kg) characteristically varied among treatments in the respective stages. Using ANOVA analysis, a clear variation in the significant difference for $A_{O\text{-}M}$ was observed among treatments (WN ($p = 0.03$), FD ($p = 0.008$), and FH ($p = 0.042$)). As shown in Fig. 7b, the $A_{LFP\text{-}M}$ was significantly different among treatments in the weaning ($p = 0.02$) and feeding stages ($p = 0.013$), but was not significantly affected in the finishing stage ($p>0.05$). The R_{LFP} also showed similar correlation characteristics with $A_{LFP\text{-}M}$ among treatments in the respective stages. This indicated that the dietary nutrient characteristics played more significant role in the amounts of labile pollutant present in manure. The percentage of labile pollutant fraction in the total manure pollutant (relative to the dietary nutrient) was more significant than pig growth days for pigs in the weaning and feeding stage, but was inversely proportional for the finishing stage. That is, the dietary nutrients were less involved in the release of labile pollutant in manure and percent labile pollutant in total manure compared to the pig growth days. Thus, the potential for release of manure labile pollutant for treatments in weaning and feeding stage is higher than for the finishing stage.

As shown in Fig. S11, the $A_{T\text{-}M}$, $A_{L\text{-}M}$ and R_{LFP} significantly correlates among all treatment stages due to the composite dietary nutrient factors. In the weaning stage (as shown in Fig. S11a), DTP ($r>0.7$, $p<0.05$), dietary nutrient in non-easily-utilized fractions (DPP, DOZn) ($r>0.75$, $p<0.03$) and DC/DN/DP ($r>0.6$, $p<0.05$) all significantly contributed to $AT\text{-}M$, $AL\text{-}M$ and R_{LFP}. However, dietary C ($r<-0.65$, $p<0.05$) and ADF-C ($r<-0.65$, $p<0.05$) negatively influenced $AT\text{-}M$, $AL\text{-}M$ and R_{LFP}. In the FD stage (as shown in Fig. S11b), ADF ($r>0.7$, $p<0.05$), ADF-C ($r>0.75$, $p<0.03$) and DC/DN/DP ($r>0.7$, $p<0.04$) significantly contributed to $A_{T\text{-}M}$, $A_{L\text{-}M}$ and R_{LFP}, while DTOC ($r<-0.8$, $p<0.02$) and DCu ($r<-0.65$, $p<0.05$) negatively influenced $A_{T\text{-}M}$, $A_{L\text{-}M}$ and R_{LFP}. In the FH stage (as shown in Fig. S11c), the dietary microelements (DZn, DCu) and ADF significantly contributed to $A_{T\text{-}M}$ ($r>0.8$, $p<0.03$) and $A_{L\text{-}M}$ ($r>0.75$, $p<0.04$), while DTC and DC/DN/DP negatively influenced $A_{T\text{-}M}$ ($r<-0.8$, $p<0.03$) and $A_{L\text{-}M}$ ($r<-0.7$, $p<0.05$). Thus, it is clear that factors contributing to $A_{T\text{-}M}$, $A_{L\text{-}M}$ and R_{LFP} in different pig growth stage are different, which may be due to the different diet digestibility of pigs from different growth stages (Zentek and Goodarzi, 2020). Similarly, the dietary nutrient fraction played great role in the release of the total and labile manure. Enhancement of the positive contributors and inhibition of the negative factors may be an effective way to manage the release of labile manure and reduce the percentage of labile manure in total manure.

6. Advisory for swine manure management strategy

The primary goal of dietary regulation is to enhance nutrient retention while simultaneously reducing both total manure and labile manure discharge. For instance, the FD-1 diets (as shown in Table S6) contained nutrient levels of CP, TN, TP, Cu, and Zn that were 94.46 %, 80.03 %, 24.12 %, 1527.27 %, and 57.76 %, respectively higher than the NRC Swine Nutrition Guidelines (2012) requirements. From the standpoint of total manure management, the reduction potential (AU-TR) of CP, TN, TP, Cu, and Zn can reach up to 91.90 g/kg, 13.06 g/kg, 1.44 g/kg, 70.83 mg/kg, and 38.24 mg/kg, respectively. However, as analyzed in section 4, dietary nutrient fractions and components significantly impacted nutrient retention by pigs (AR-N) but had no substantial effect on nutrient released in form of pollutants (AO-P). Furthermore, correlation analysis between diet and manure pollutants indicates that dietary nutrient fractions greatly influence nutrient retention rates (AR-N) and labile manure discharge. Therefore, regulating dietary nutrient fractions is essential based on AU-TR and correlation results (Table S4),

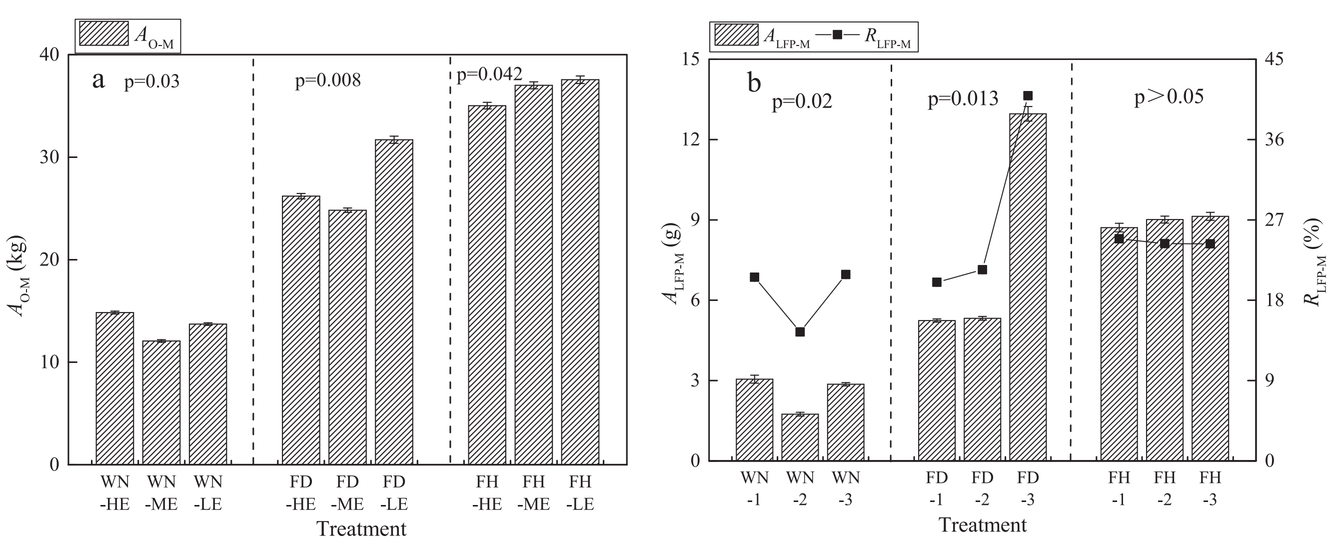

Fig. 7. The manure pollutant characteristics (a) $A_{O\text{-}M}$, and (b) $A_{LFP\text{-}M}$ and $R_{LFP\text{-}M}$, excluding urine in the respective stage.

although more in-depth investigation is required to determine the precise method for nutrient fraction regulation.

Adopting a coordinated management strategy for both total and labile manure, while ensuring the normal growth of pigs, can yield better economic benefits. Firstly, optimizing nutrient levels using AU-TR to align with the recommended amounts based on NRC guidelines, as opposed to excessive levels, can lead to reduced feed usage and lower feed procurement costs. Secondly, controlling manure discharge with a lower proportion of labile manure reduces the risk of pollutant migration and makes the manure more suitable for resource recovery processes like aerobic composting. This results in manure being converted into organic fertilizer, which is beneficial for plant growth. Consequently, it lowers manure treatment costs and mitigates the expenses associated with managing secondary pollution from the immediate transfer of labile manure, such as air, water, and atmospheric pollution. Additionally, recycling manure with reduced labile components as fertilizer offers a viable alternative to chemical fertilizers, which is vital for the development of sustainable agriculture.

7. Conclusion

Dietary nutrient fractions play a critical role in labile manure release compared to the total dietary nutrient consumption. Nutrient fractions such as phytate phosphorus, organic copper and zinc are more critical components for nutrient utilization than the overall nutrient amounts consumed. The diet to meat ratio of pigs does not accurately represent the level of manure release. The theoretical model indicates that manure pollution potential is determined by labile manure components rather than the total manure composition. High pollutant accumulation in manure across the growth stages does not necessarily translate into high environmental pollution potential; it is influenced primarily by labile manure pollutants. Therefore, regulating dietary nutrient fractions is a novel and feasible approach for effective swine manure management.

CRediT authorship contribution statement

Hongyong Fan: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chenghao Li:** Writing – review & editing, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Wenxuan Zhang:** Writing – review & editing, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Chaoxiang Liu:** Resources, Methodology, Funding acquisition, Conceptualization. **Olusegun K. Abass:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Lin Liu:** Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xu Huang:** Software, Project administration, Formal analysis, Data curation. **Yingjie Sun:** Software, Project administration, Formal analysis, Data curation. **Huawei Wang:** Software, Project administration, Formal analysis, Data curation. **Maureen W. Gesiye:** Writing – review & editing, Software, Formal analysis, Data curation. **Wushan Chen:** Resources, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177942.

Data availability

Data will be made available on request.

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