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ORIGINAL RESEARCH ARTICLE

Effect of anaerobic bio digestion of cage layer manure and pig manure on digestate's proximate and mineral composition, microbial status and effect on performance of growing cockerels

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ABSTRACT

This study examined the effects of anaerobic bio-digestion of cage layer manure and pig manure on the digestates' proximate and mineral composition, microbial status, and the performance of growing cockerels fed graded levels of the digestates. Cage layer manure and pig manure were bio-digested for six weeks, after which the digestates were oven-dried, analysed for proximate and mineral composition, and incorporated into the diets of growing cockerels. The birds were randomly allotted to six dietary groups in a 2×3 factorial arrangement comprising two types of bio-digested manure (cage layer and pig manure) and three inclusion levels (0, 10 and 20%), with each treatment replicated thrice using ten birds per replicate. Proximate analysis showed that bio-digested pig manure had significantly higher crude protein content, whereas potassium, sodium, phosphorus and calcium were significantly higher in bio-digested cage layer manure. For the main effect of manure type, bio-digested pig manure supported higher weight gain than cage layer manure, and among inclusion levels, birds fed 10% bio-digested pig manure recorded the highest weight gain. These findings indicate that bio-digested pig manure can be included in the diet of growing cockerels at up to 10% without adversely affecting performance.

Key words: Bio digestion, cage layer manure, pig manure, performance

INTRODUCTION

The exploration of alternative feed ingredients for poultry nutrition has gained significant momentum due to the escalating cost associated with conventional feedstuffs like soya meal and corn, coupled with growing environmental concerns regarding manure disposal (Saleh et al., 2026). Given that traditional backyard farming has been replaced by concentrated animal feeding operations to meet the growing demands for animal products, there has been a significant increase in the number of livestock and poultry manure produced (Cheng and Cheng, 2024). Poultry and pig manure have been found to be rich in essential nutrients and organic matter and present a viable option for integration into poultry diets; this utilization also offers an additional benefit of waste management (Chisom et al., 2022).

Bio-digestion, an anaerobic process, transforms organic waste into biogas renewable energy source rich digestate which can be further processed and incorporated into livestock feed (Salendu et al., 2021; Castro – Ramos et al., 2022). According to Ankathi et al., (2024) the integration of bio-digested manure into animal diet requires a comprehensive understanding of its nutritional composition, potential bioavailability of nutrients and any inherent risk associated with pathogen transmission or accumulation of heavy metals. While digestate from livestock manure is widely recognized as a feed alternative in livestock nutrition, its potential as a feed component for poultry remains underexplored (Dhungana et al., 2022). In addition, Incorporation of digestate into growing poultry diets could supply supplemental protein, minerals, and probiotic organisms, but raises concern regarding palatability, nutrient bioavailability, and microbial safety (Parra-Orobio et al., 2021). To this end, detailed characterization of digestate proximate and mineral composition alongside rigorous microbial profiling is needed to assess its suitability in poultry nutrition. The integration of animal waste products into poultry diet represents a multifaceted approach to addressing both economic and environmental concerns within agricultural systems (Zhang et al., 2023). Understanding these dynamics will therefore enable integrated waste to resource strategies that permit renewable energy production with sustainable animal feeding practices. This study therefore aims to evaluate how anaerobic bio-digestion of cage layer and pig manure impacts digestate composition and microbial status, and to evaluate the effects of these digestates on the performance of growing cockerels.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta. The site is in the rain forest vegetation zone of south-western Nigeria on latitude 7° 13' 49.46"N, longitude 3° 26'

11.98"E and altitude 98 m above the sea level. The climate is humid with a mean annual rainfall of 1003 mm, mean annual temperature (31.9-34.8 ˚C) and humidity of 79.7-90.1% (Google Earth, 2025).

Collection of manure

Caged layer manure and pig manure were collected from the poultry and pig units of the directorate of university farms of the Federal University of Agriculture, Abeokuta.

Construction of biodigesters

Two biodigesters were fabricated, one for caged layer manure and the other one for pig manure using the following materials:

- Four plastic water tanks (two for digestion, two for gas collection)
- PVC pipes (for inlet, outlet, and gas line)
- Ball valves, elbows, and adapters
- Epoxy adhesive or solvent cement
- Teflon tape (for sealing threaded joints)
- Rubber washers and gaskets

Production of the digestates

Freshly voided caged layer manure and pig manure were collected from the poultry and pig units of the directorate of university farm of the Federal University of Agriculture, Abeokuta and were mixed with water separately to form slurry. The slurry for each manure was poured into the upright water tank through the inlet pipe. The otfher water tank which is smaller than the first one was turned upside down in the upright tank. Bacteria activities were noticed to build up inside the tank as the tank turned upside down began to rise.

The digestion was carried out under anaerobic conditions and environmental temperature, and atmospheric pressure conditions were maintained. Both the cage layer manure and pig manure were allowed to digest for six weeks. After six weeks of digestion, a transparent gas pipe was fixed at the outlet of the upside-down tank to collect the gas produced after which the tank was opened to collect the effluent. The digestate was packed in sacks and the water was drained using a jack press. The drained digestate was sundried for 3 days and afterwards oven dried for another 3 days at 70 °C. This procedure was done for caged layer and pig manure respectively. The oven dried digestates were then packed in envelopes and later taken to feed mill to compound the diets for the birds.

Proximate analysis of dried bio digested cage layer and pig manure

Proximate analysis (dry matter, protein, fat, ash, and fibre) of the digestate from bio digestion of caged layer and pig manure was carried out according to AOAC (2005).

Microbial analysis of dried bio digested caged layer and pig manure

Samples of bio digested caged layer and pig manure were collected for microbial analysis according to De et al., (2007). One gram (1g) of the bio digested manure was suspended in 9 mL of sterile normal saline and serially diluted from test tube 1 to test tube 8, then dispense 1 mL from test tube 8. MacConkey agar medium was prepared by suspending 47 g in 1 L of distilled water. This was brought to boil to dissolve completely and then sterilized by autoclaving at 121 ˚C for 15 minutes. After cooling to about 55 ˚C, it was poured into a petri-dish in which 1 mL of serially diluted faecal suspension in sterile normal saline was dispensed. The inoculated plate was then incubated at 37 ˚C for 24 hrs. The colonies on the plate were counted using a colony counter. Macconkey agar medium was used for coliform count, Nutrient agar medium was used for aerobic bacteria count, Potato Dextrose agar medium was used for fungi count while de Manns Rogosa and Sharp (MRS) agar was used for anaerobic bacteria count.

Experimental birds and their management

One hundred and eighty (8 weeks old) growing cockerels were used for the study. The birds were maintained on a commercial diet for seven weeks and at the eighth week the birds were balanced for weight and introduced into respective treatments. The experimental birds were raised on deep litter which was properly cleaned, disinfected and layered with wood shavings prior to the commencement of the experiment. The required vaccinations and medications were administered as at when due, following standard commercial poultry vaccination and biosecurity protocols (Stewart-Brown, 2024), and strict biosecurity measures were adhered to throughout the period of the study.

Experimental design

The birds were randomly allotted to 6 dietary groups. Each treatment was replicated thrice with ten (10) birds per replicate. The experiment was a 2×3 factorial arrangement comprising 2 types of bio digested manure (Caged layer and pig manure) and 3 levels of inclusion (0,10 and 20%).

Experimental diet

The dried digestate was included in the diet of growing cockerels as replacement for soybean at 0 (control), 10 and 20% for caged layer manure and 0 (control), 10 and 20% for pig manure (Table 1). The feeding trial lasted for seven weeks.

Data collection

Performance characteristics

Feed intake

A known quantity of feed was given per day to the birds; the leftover was collected and weighed for each day and subsequently each week. Feed intake was obtained from the difference between the feed given per day and the leftover.

Feed intake (g) = Feed given(g) - Feed left over(g)
Number of birds

Body weight gain

Birds in each replicate were weighed every week using a weighing scale.

Weight gain (g/week) = Final weight - Initial weight

Feed conversion ratio

The feed conversion ratio of each group of birds was determined by calculating the ratio of feed intake to weight gain and thus calculated as:

Feed conversion ratio= Total Feed intake (g)
Total body weight gain (g)

Protein efficiency ratio

This was determined by calculating the ratio of body weight gain to protein consumed of each group of birds. It was calculated using the formula:

Protein efficiency ratio = Total body weight gain(g)
Total protein consumed(g)

Statistical analysis

Data obtained for the chemical and mineral composition of bio digested cage layer manure and pig manure were subjected to one-way analysis of variance using SAS 2010, while data obtained for the growth performance were subjected to a 2x3 factorial arrangement in a completely randomized design as contained in SAS (2010) package. Significant differences among treatment means were determined using Duncan Multiple Range test within the same statistical package.

RESULTS AND DISCUSSION

The effect of bio digestion on the chemical and mineral composition of cage layer manure and pig manure

Bio-digested caged layer manure recorded a dry matter content of 94.33%, compared with 94.00% for bio-digested pig manure (Table 2); the difference was not significant ($P > 0.05$), indicating comparable dry matter content between the two digestates, consistent with Elizabeth et al. (2024), who reported consistent dry matter levels across anaerobically digested livestock manures. Crude protein was significantly higher ($P < 0.05$) in bio-digested pig manure than in cage layer manure. Feixing et al. (2025) reported that raw pig manure typically contains 16.89–25.79% crude protein on a dry matter basis, reflecting the comparatively inefficient protein digestion in pigs, which results in higher nitrogen excretion and a richer substrate for microbial protein synthesis during anaerobic digestion (Sun et al., 2023).

Table I: Composition (%) of grower mash containing varying levels of bio digested caged layer and pig manure

		Inclusion levels			
	Control	BCLM		BPM	
Ingredients	0%	10%	20%	10%	20%
Maize	50.00	50.00	50.00	50.00	50.00
Soybean meal	15.00	13.50	12.00	13.50	12.00
*BCLM	0.00	1.50	3.00	0.00	0.00
**BPM	0.00	0.00	0.00	1.50	3.00
Wheat offal	30.00	30.00	30.00	30.00	30.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00	2.00	2.00
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
***Vit/Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated analysis:					
Crude protein (%)	16.20	15.70	15.20	15.70	15.30
Ether Extract (%)	3.60	3.60	3.60	3.60	3.60
Crude fibre (%)	4.50	4.70	4.90	4.70	4.90
Calcium (%)	1.50	2.40	3.30	2.10	2.70
Phosphorus (%)	0.40	0.40	0.40	0.40	0.40
Lysine (%)	0.90	0.80	0.80	0.80	0.80
Methionine (%)	0.50	0.50	0.50	0.50	0.50
Energy (MJ/Kg)	11.01	10.94	10.87	10.93	10.87

***Contains: Vit A,10 000 000iu; D_3,2 000 oooiu; E, 12 500iu; K, 1.30g; B_1,1.30; B_2,4.00g; D Calcium-Pantothenate, 1.30g; B_6, 1.30g; B_{12},0.01g; nicotinic acid.15.00g; folic acid,0.05g, biotin,0.02g; Co, 0.20g; Cu,5.00g; Fe, 25.00g; I,0.06g; Mn,48.00g; Se,0.10g; Zn,45.00g; choline chloride,200.00g; BHT,50.00g.

*BCLM: Biodigested cage layer manure, **BPM: Biodigested pig manure

Table 2: Chemical and Mineral composition of bio digested cage layer and Pig manure

Parameter	BCLM	BPM	SEM	Pr>|t|
Dry matter (%)	94.33	94.00	0.94	0.8149
Crude protein (%)	10.27	13.13	0.12	<0.001
Crude fibre (%)	19.57	18.27	1.55	0.0010
Ether extract (%)	4.00	3.67	0.62	0.7247
Ash (%)	27.33	22.67	0.89	0.0352
Nitrogen Free Extract	38.83	42.27	3.02	0.2043
*Metabolizable Energy (MJ/kg)	16.73	16.09	0.24	0.0462
Potassium (g/kg)	42.22	25.74	1.11	0.0001
Sodium (g/kg)	10.32	3.88	0.45	<0.001
Phosphorus (g/kg)	2.15	0.49	0.08	<0.001
Calcium (g/kg)	60.63	40.67	5.90	0.0001

NS: Not Significant, BCLM: Bio digested Caged layer Manure, BPM: Bio digested Pig Manure, SEM: Standard Error of Mean

Estimated using the formula by Pauzenza (1985): ME (kcal/kg) = (37.7XCP%) + (81.8xEE%) + (35.5XNFE%). ME (MJ/kg) = ME (kcal/kg(4.18/1000))

Crude fibre was significantly higher ($P < 0.05$) in bio-digested cage layer manure than in pig manure, consistent with the higher inclusion of fibrous ingredients typical of layer diets (Johnson and Lee, 2020), whereas pig diets are formulated for higher digestibility and lower fibre content. Ramirez and Singh (2023) noted that the efficiency of fibre breakdown during bio-digestion remains closely linked to the original feed composition and the animal's digestive capacity. Ether extract did not differ significantly between the two digestates, in agreement with reports that anaerobic digestion tends to equalize lipid composition through consistent degradation pathways regardless of the original substrate (Ramirez and Singh, 2023; Holohan et al., 2022).

Metabolizable energy was statistically similar between the two digestates, consistent with the comparable methane content reported for chicken- and pig-manure biogas (55–64% and 60–64%, respectively; Hassan and Jaber, 2024; Vannasinh et al., 2023; Offon et al., 2024). Bio-digested cage layer manure had significantly higher ($P < 0.05$) potassium, calcium, phosphorus and sodium than pig manure, reflecting the higher dietary calcium and phosphorus fed to laying hens to support eggshell formation, with excess minerals excreted in manure, compared with the leaner, lower-mineral diets fed to pigs (Marques-dos-Santos et al., 2023). This is consistent with the view that bio-digestion concentrates minerals by breaking down organic matter while conserving the inorganic fraction, an effect more pronounced in poultry manure given its higher initial mineral content (Hansen, 2022).

The effect of bio digestion on the microbial status of cage layer manure and pig manure

Total aerobic count (including *Bacillus* spp, *Pseudomonas* spp, and *Flavobacterium* spp) was significantly higher (Table 3; $P < 0.05$) in bio-digested pig manure (5.9 cfu/g) than in cage layer manure (4.6 cfu/g), consistent with reports that pig manure generally carries a higher and more diverse bacterial load than poultry manure due to differences in digestive physiology and gut microbiome (Ayotunde et al., 2022; Wan et al., 2021). High aerobic bacterial counts in feed materials can serve as reservoirs for pathogens and may impair nutrient digestibility in poultry (Oluwadara et al., 2018; Katada et al., 2021). Coliform count was likewise significantly higher in bio-digested pig manure, suggesting that anaerobic digestion was less effective at reducing pathogens in pig manure than in poultry manure (New York State Department of Health, 2024). Fungal counts did not differ significantly between the two manures, likely because fungi are obligate aerobes that are suppressed under the oxygen-free conditions of anaerobic digestion regardless of substrate (Yang et al., 2022; Zhang and James, 2022).

Table 3: Microbial status of biodigested caged layer and pig manure

Parameter	BCLM	BPM	SEM	Pr>|t|		Organisms isolated
Total aerobic count (cfus/g)	4.9	5.6	0.12	0.0128	**	*Bacillus sp*, *Pseudomonas sp* and *Flavobacterium sp*
Total coliform count (cfus/g)	4.2	5.3	0.06	0.0002	***	*Aeromonas sp,* *Proteus sp* and *Enterobacter sp*
Total fungi count (cfus/g)	0.4	0.5	0.06	0.2875	NS	*Aspergillus sp* and *Penicillum sp*

, * respectively signifies significant difference at 1% and 0.1%

BCLM: Biodigested caged layer manure, BPM: Biodigested pig manure

Fungal counts did not differ significantly between the two manures, likely because fungi are obligate aerobes that are suppressed under the oxygen-free conditions of anaerobic digestion regardless of substrate (Yang et al., 2022; Zhang and James, 2022). Birds maintained on bio-digested pig manure recorded significantly higher weight gain and final weight than those on cage layer manure.

Main effect of bio digested caged layer and pig manure and levels of inclusion on the growth performance of growing cockerels

Bio-digested pig manure produced significantly higher crude protein content than cage layer manure (Table 4), which likely explains the higher weight gain recorded on pig-manure diets, since pig-manure digestate is associated with greater availability of essential amino acids following anaerobic digestion (Xiang et al., 2017), improving feed conversion and weight gain. Feed intake was also significantly higher for birds on bio-digested pig manure, suggesting better palatability than cage layer manure; digestate from anaerobic digestion of pig manure produces short-chain fatty acids that promote palatability and stimulate gastric secretion (Cheng and Wang, 2022), whereas the higher mineral ash and residual yolk lipids in bio-digested cage layer manure can generate bitter flavours that reduce appetite (Bhattarai et al., 2021).

Birds on 10% inclusion of either bio-digested manure performed comparably to those on the control diet, whereas 20% inclusion produced the lowest performance values. This is consistent with reports that residual fibre and ash in both digestates dilute dietary energy density and reduce amino acid digestibility at higher inclusion levels (Cheng and Wang, 2022). At moderate inclusion, digestate appears to promote beneficial gut microbes such as Lactobacillus, improving nutrient uptake, whereas higher inclusion levels may overload the gut with microbial metabolites and antimicrobial peptides, disrupting microbiota balance and triggering subclinical inflammation (Cheng and Wang, 2022). Protein efficiency ratio was significantly higher in birds on 10% inclusion of either manure than in those on 20% inclusion or the control, indicating better protein utilization at the 10% level, consistent with the associated lower feed intake and comparable weight gain relative to control birds (Li et al., 2022; Dairo et al., 2020).

The result of the interaction between bio-digested cage layer manure and bio-digested pig manure is presented in Table 5. Weight gain in birds on bio-digested cage layer manure declined significantly as inclusion increased from 0 to 20%, whereas among birds on bio-digested pig manure, the 10% inclusion level gave the highest weight gain and the 20% level the lowest. This pattern is consistent with reports that anaerobic digestion of poultry manure can increase the bioavailability of heavy metals such as copper, zinc and lead beyond safety thresholds (Zheng et al., 2022), which may impair nutrient absorption and depress performance with prolonged exposure at higher inclusion levels (Adekanmi, 2021).

Anaerobic digestion is reported to substantially reduce pathogenic bacteria and parasites in raw pig manure and to lower coliform counts to acceptable levels (Ndubuisi-Nnaji et al., 2022), while residual microbial biomass and enzymes in the digestate may act as prebiotics, promoting beneficial genera such as Lactobacillus and Bifidobacterium, short-chain fatty acid production, and suppression of pathogens such as Clostridium perfringens at moderate inclusion (Martinez-Lopez et al., 2021). At higher inclusion levels, however, residual pathogens may pose health challenges that interfere with growth, consistent with the better performance observed at 10% than at higher inclusion levels in this and previous studies (Martinez-Lopez et al., 2021).

Table 4: Main effect of bio digested caged layer and pig manure and levels of inclusion on the performance characteristics of growing cockerels

	Biodigested Manure				Levels of Inclusion				
Parameter	BCLM	BPM	SEM	P value	0%	10%	20%	SEM	P value
IW(g)	500.46	500.46	0.46	1.0000	500.00	500.70	500.70	0.47	0.7162
FW (g)	1126.1^{b}	1200.5^{a}	36.11	0.0181	1195.4^{a}	1180.7^{a}	1113.7^{b}	45.95	0.0550
ADFI (g/b/d)	59.82^{b}	60.47^{a}	0.81	<0.0001	62.70^{a}	59.00^{b}	58.73^{c}	0.61	<0.0001
TWG (g/b/d)	625.62^{b}	700.01^{a}	36.23	0.0180	695.44^{a}	680.03^{a}	612.98^{b}	46.08	0.0536
ADWG (g/b/d)	12.77^{b}	14.29^{a}	0.74	0.0186	14.19^{a}	13.88^{a}	12.51^{b}	0.94	0.0553
PI (g/b/d)	9.40^{b}	9.52^{a}	0.20	<0.0001	10.15^{a}	9.26^{b}	8.96^{c}	0.29	<0.0001
PER	1.62^{b}	1.78^{a}	0.12	<0.0001	1.39^{c}	1.98^{a}	1.72^{b}	0.11	<0.0001
FCR	4.78	4.35	0.26	0.2694	4.50	4.33	4.87	0.31	0.4921

^{a,b,c} Means in the same row with different superscripts differ significantly ($P < 0.05$)

BCLM: Bio digested Caged layer Manure, BPM: Bio digested Pig Manure, IW: initial weight, FW: Final weight, ADFI: Average daily feed intake, TWG: Total weight gain, ADWG: Average daily weight gain, PER: Protein efficiency ratio, FCR: Feed conversion ratio

Feed intake declined significantly as inclusion of bio-digested pig manure increased from 0 to 20%, likely reflecting off-odours and bitter compounds such as ammonia and short-chain fatty acids that reduce palatability at higher inclusion (Oladimeji et al., 2022). Despite lower feed intake, birds on 10% bio-digested pig manure achieved better weight gain than control birds, suggesting that the beneficial microorganisms and probiotic content of the digestate improved gut health and nutrient absorption rather than feed quantity alone (Lui et al., 2021). This indicates that digestive efficiency and nutrient quality, rather than feed intake per se, drove performance, with 10% inclusion representing the optimal balance between benefit and risk.

CONCLUSION

This study confirms that anaerobic bio-digestion of pig manure produces a digestate with superior crude protein content and enhanced growth performance in growing cockerels; however, inclusion should not go beyond the 10% level. Although cage layer manure digestate exhibited higher mineral concentrations, it was less effective in promoting weight gain. Microbial analysis revealed higher aerobic and coliform counts in pig manure digestate, indicating the need for microbial safety monitoring when incorporating bio-digested pig manure into the diet of growing cockerels. Overall, these findings suggest that bio-digested pig manure may be incorporated into the diets of growing cockerels at levels not exceeding 10%; however, as this conclusion is based on a single trial in growing cockerels, further studies across other poultry classes and production stages are recommended before the practice is more broadly recommended. This offers a potentially sustainable and cost-effective alternative to conventional feed ingredients while contributing to environmental waste management.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research

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