ANAEROBIC DIGESTION OF UNTREATED MANURE: ENVIRONMENTAL RISK ASSESSMENT OF RESULTANT DIGESTATES

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ABSTRACT

Anaerobic digestion (AD) of poultry and goat manure was performed to estimate the biochemical methane potential (BMP) and biosafety of the digestates using standard analytical and microbiological methods. The effects of residence time (RT) on BMP, process performance indicators, potential pathogens and indicator bacteria reduction as well as heavy metals concentrations were determined. The experiment was performed in a semi-batch mode at mesophilic temperature of $30 \pm 0.2^{o}C$ using 20 L prototype biodigesters over 45 days RT. The cumulative biogas yield from goat manure (31,703 ml/gVS) was > yield from poultry manure (30,275 ml/gVS). The process performance indicators after digestion revealed a minimal variation in pH (6.0 to 7.5) with notable reduction in total solids (55.0% in goat manure >50.6% in poultry manure) and volatile solid (56.1% in goat manure > 44.2% in poultry manure). Besides methanogens (*Methanothrix, Methanobacterium* and *Methanosarcina* species), *Bacillus* (100%) and *Clostridium* (87.5%) species were the most predominant bacterial genera. Sanitary assessment revealed a significant reduction ($p < 0.05$) of indicator and potentially pathogenic bacteria at residence time ≥ 30 days. At 45 days RT, faecal coliform, *Staphylococcus* and *Vibrio* species were undetected in both poultry and goat manure digestates, while total coliforms (3.6 log CFU/ml) and *Salmonella* count (3.2 log CFU/ml) in poultry manure digestate were above tolerable limit. A negligible amount ($p < 0.05$) of heavy metals was observed with higher zinc and copper concentrations in poultry and goat manure respectively. Extension of residence time and/or further treatment is critical to ensure digestate meets the United States EPA/EU permissible limit of 3.0 log CFU/ml before farmland application

KEYWORDS: Anaerobic digestion, livestock manure, digestate, biosafety, BMP.

INTRODUCTION

Along with the intensive development of domestic animal husbandry, livestock manure production has increased dramatically. It has been recognized that wastes generated from livestock production has great potential for environmental degradation (Coelho *et al.*, 2018) and where untreated or not properly managed, livestock manure becomes a potential source of hazard to the environment and public health (Alburquerque *et al*., 2012; Qi *et al.*, 2018). In Akwa Ibom State and many parts of Nigeria, livestock manure particularly poultry and goat manure are usually applied in its raw or untreated form as biofertilizers for the cultivation of fluted pumpkin, cucumber, garden egg and many other crops (Ndubuisi-Nnaji *et al.*, 2022). However, little attention has been given to the chemical and microbiological quality status of these manures. While livestock manure serves as sources of emission of the greenhouse gases, manure-based methane contributes about 4% of all anthropogenic methane sources (Kafle and Chen, 2016). Again, large volume of gases, organic material, bacteria and other substances generated during livestock activities poses a biosafety risk in the ecosystems (Mathias, 2014).

Anaerobic digestion (AD) provides a promising route for treating organic wastes such as agricultural and livestock wastes with cogeneration of a renewable energy (biogas) source and enriched organic remains in the digester with biofertilizer potential (Ndubuisi-Nnaji *et al.*, 2022; Ndubuisi-Nnaji *et al*., 2021; Qi *et al*., 2019 and Shah *et al.*, 2015). However, after AD, the existence and high concentrations of indicator bacteria, potential pathogens, heavy metals etc in anaerobic digestates have been reported by researchers (Ndubuisi-Nnaji *et al.*, 2020a; Qi *et al.*, 2018, Resende *et al.*, 2014; Thomas *et al.*, 2019). The high cost, nitrate pollution and loss of soil carbon stemming from intensive soil fertilization with inorganic mineral fertilizers has generated tremendous interest in the search for organic fertilizers including anaerobic digestates creating biosafety risk which could pose a threat to the environment and human health. Hence the assessment of livestock manure digestates for bacterial indicators, pathogens and heavy metals to ensure their safety when used as soil conditioner/amendment cannot be overemphasized. Livestock manure has served as an excellent feedstock for anaerobic digestion because of its high total nitrogen content, fermentation stability and insensitivity to acidification during the fermentation (Zhang *et al.*, 2013). Alfa *et al.* (2014) and Anjum *et al.* (2016) reported that livestock manure contains many pathogenic and non-pathogenic bacteria such as *Pseudomonas, Klebsiella, Salmonella, Bacillus, Shigella, Clostridium* and other microorganism which may survive AD processes and persist in digestate. Since heavy metals are generally used as feed additives to promote livestock growth, and their contents is found to be increasing in livestock manure which is used as feedstock for anaerobic digestion (Zhu and Guo, 2014), the heavy metal contents in the digestate should be considered when applied to soils as spectroscopic techniques have recently demonstrated that anaerobic digestates inherits the chemical attributes of the feedstock from which they are produced (Bonetta, *et al*., 2014; Tambone *et al*., 2017; Coelhe *et al*., 2018). Inappropriate storage or application of anaerobic digestates can lead to gaseous nitrogen emission (ammonia and nitrous oxide) and/or nutrient leaching and runoff into surface and ground waters (Nkoa, 2014).

According to Kucharczak *et al.* (2010), toxic compounds in waste that are used as organic fertilizers can be assimilated by plants and accumulated in harmful concentrations. This study evaluated the biomethane potential of untreated chicken and goat manure during AD as well as examined the environmental risk/profile and hygienic status of the resultant digestate in terms indicator bacteria and potentially toxic metals.

MATERIALS AND METHODS

Sample Sources and Collection

Fresh and untreated poultry manure was obtained from a commercial farm: Vika farms limited, located at Mbak Etoi, Uyo and goat manure sample was sourced from a loafing shed located in a farm outbuilding (barn) in Ikot Ukot Anang, Ukanafun, L.G.A, both in Akwa Ibom State, Nigeria. The manure samples were collected into sterile containers and transported to the Laboratory. All samples were stored at 4 °C before further processing.

Experimental Design and Anaerobic Digestion Assay

The experiment was conducted by wet anaerobic digestion in a semi-continuous system. A slightly modified method of Tayyab *et al*. (2019) was adopted in this paper. The reactors were fed at a feedstock/inoculum ratio of 1:1 based on volatile solids. Detailed experimental setup and reactor design have been described elsewhere (Ndubuisi-Nnaji *et al.*, 2022). The experiment was operated for a duration of 45 days and digesters kept at mesophilic temperature (28 ± 2ºC). The reactors were stirred daily to enable efficient waste mixing and sludge stabilization. To determine the effect of residence time (RT) on pathogen reduction, digestate effluent was sampled at 15 days interval respectively for microbiological and potentially toxic metal (PTM) analyses. The volume of biogas produced daily accumulated in the reactor headspace and the biochemical methane potential (BMP) was determined daily via downward liquid displacement technique using 2 % lime water solution.

Analytical Methods

Influent slurry pH was measured at zero (0) residence time, and the digestates at 15-, 30- and 45-days interval using a functional pH meter (HI 98107 pHep). The available total solids (%) in the substrates was determined as per standard methods for the examination of water and waste water (APHA, 2005). The concentrations of potentially toxic metals (Mn, Zn, Cu, and Ni) were determined as described by Dong and Liu (2013). After digestion of dried samples in $HNO_3/HClO_4$ (2: 1v/v) at 180ºC, samples were filtered with 0.45 mm filter and heavy metals concentrations were measured using inductively coupled plasma – optical emission spectrometer (ICP – OES, Perkin Flmer Inc. USA).

Microbiological and Biosafety Risk Assessment

The sanitary quality and microbial profile of livestock manure was assessed by standard plate count. The plates were incubated aerobically for the isolation of heterotrophic bacteria and putative pathogens while anaerobic bacterial count was obtained from cultures in anaerobic jar containing the gaspak and indicator strip for 24-48 hours at 37 °C. The experiment was performed in duplicates and distinct colonies were counted and recorded as CFU/ml. For the detection and enumeration of indicator bacteria and potential pathogens, the pour plate method was performed using selective, and differential media. Eosine methylene blue (EMB) for faecal coliform (*Escherichia coli*), *Salmonella Shigella* agar (SSA) for *Salmonella* and *Shigella*, MacConkey agar for coliforms, Manitol salt agar (MSA) for *Staphylococcus* and thiosulfate-citrate-bile salt (TCBS) agar for *Vibrio* species. Typical colonies after incubation were counted and recorded as CFU/ml. Morphological, biochemical and presumptive identification of isolates was done according to Cheesbrough (2006) and Brenner *et al*. (2005). Low phosphate basal medium (LPBM) enriched with some catabolic substrates and organic growth factors was used for the isolation and characterization of methanogens as described by Zeikus (1977).

Data Analysis

All data were obtained from duplicate experiments and reported as mean values ± standard deviation. Confidence interval was fixed at 95% probability level where $p \leq 0.05$ was designated significant.

RESULTS AND DISCUSSION

Biomethane Potential of Poultry and Goat Manure

The daily and cumulative biogas yield of both poultry and goat manure during anaerobic digestion was investigated and results shown in Figures 1 and 2. The biomethane potential efficiency was significantly higher in goat manure than in poultry manure ($p < 0.05$). The minimum biogas yield for both poultry and goat manure recorded at the start of the experiment (day 1) was 280 and 75 mL/gVS.d respectively while the maximum yield for poultry manure recorded on day 23 was 1500 mL/gVS.d, that of goat manure was recorded on day 45 was 2450mL/gVS.d. in terms of cumulative biogas yield, our results indicated that goat manure produced sustained biogas that was 1.05 times more than poultry manure. This report corresponds with the result of Hanafiah *et al.* (2017) that goat manure produced 1.08 times more biogas than poultry manure while contradicting the report of Kafle *et al.* (2016) where poultry manure produced 1.62 times more biogas than goat manure while comparing batch anaerobic digestion of five different livestock wastes. As stated by Kigozi *et al.* (2014) and Kafle *et al*. (2016), the variation in quality and quantity of biogas may be ascribed to the nature and composition of feedstock.

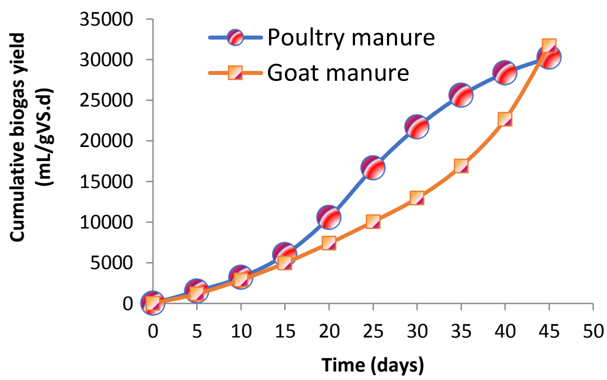

Figure 1: Daily biogas yield from AD of livestock manure

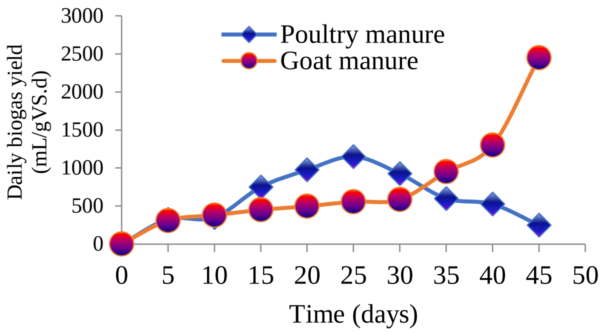

Figure 2: Cumulative biogas yield from AD of livestock manure

Performance Indicators during AD of Livestock Manure
The results presented in Table 1 indicates that the pH, volatile solids (VS) and total solids (TS) concentrations of goat manure were significantly higher ($p<0.05$) than poultry manure. However, the pH values in both goat and poultry manure digestate were not significantly different ($p > 0.05$) although it reduced minimally from 7.4 to 6.0 in poultry manure and 7.5 to 6.8 in goat manure thus contributing to the continuous biogas production from both digesters. Other researchers (Mao *et al*., 2015 and Khalid *et al*., 2011) had observed that the optimal pH for methanogenesis during anaerobic digestion ranged between 6.5 and 7.5 which was consistent with this study. The buildup of volatile fatty acids (VFAs) which is inimical to the AD process could be responsible for the drop in pH and subsequent biogas yield. The pH of goat manure was optimum for the continuous biogas production even at day 45day. It is worthy to note that pH is a critical factor during anaerobic digestion as fluxes in pH could negatively affect the microbial group present in the digester. At the end of digestion, a significant reduction ($p < 0.05$) in total and volatile solids was recorded in poultry (TS = 50.6, VS = 56 %) and goat manure (TS = 55, VS = 44.2 %). This may have been responsible for the low volume of biogas noted at the beginning of the goat manure digestion as volatile solids consumption results to a corresponding increase in biogas production.

Table 1: Process parameters during AD of poultry and goat manure

Substrate (livestock manure)	Process parameters	Days 0	15	30	45
Poultry manure	pH	7.5±0.4	7.3±0.6	7.1±0.3	6.8±0.5
	VS (%)	64.2±0.6	52.6±0.4	40.8±0.8	28.2±0.6
	TS (%)	81.6±0.5	72.5±0.2	66.4±0.5	40.3±0.8
Goat manure	pH	7.4±0.2	7.2±0.4	7.0±0.1	6.0±0.2
	VS (%)	47.5±0.7	40.4±0.5	34.4±0.3	26.5±0.1
	TS (%)	67.8±0.2	60.7±0.6	56.5±0.4	30.5±0.1

Key: VS = volatile solids; TS = total solids

The total solids contents obtained in the study were generally considered appropriate for dry AD at high solid content (Motte *et al.*, 2013). Comparable degradation in the TS and VS contents during anaerobic digestion have been documented in previous literature (Ndubuisi-Nnaji *et al*., 2021, Ndubuisi-Nnaji *et al*., 2020b, Lu, *et al*., 2016; Qi *et al*., 2018; Panjicko *et al*., 2015).

Hygienic status and Microbial (Safety) Profile during AD of Manure
An overall decrease in microbial load profile was observed for all bacterial groups in both poultry and goat manure digestate (Figures 3a and b). *Vibrio* sp were eliminated before day 30 of anaerobic treatment of both poultry and goat manure while total coliforms, faecal coliform, *Salmonella Shigella* and *Staphylococcus* sp were not detected at the end of the digestion of goat manure (45 days). This manifest reduction ($p < 0.05$) ranged between 1.63 – 4.4 log CFU/ml in goat manure, agreeing with the report of Cote *et al*., (2006) who recorded 1.62 – 4.23 log CFU/ml in population of indicator bacteria while studying efficiency of pig slurries AD.

European Union (EU) limit for indicator bacteria in manure-based product according to Animal by-product regulation (EU No. 1069 / 2009). In goat manure digestate effluent, indicator bacteria (coliforms and faecal coliform) were entirely eliminated at termination of digestion whereas in poultry manure digestate, total coliforms (3.6 log CFU/ml) and *Salmonella* sp (3.2 log CFU/ml) with and respectively was recorded after digestion. The figures are slightly above the European Unit permissible limit of 3.0 log CFU/ml for land application of digestate in agriculture (McCarthy *et al*., 2003). Earlier report by Alf*a et al*. (2014) corroborates our findings. The authors reported the total removal of *Escherichia coli* and *Shigella* spp after anaerobic digestion while species of *Salmonella* and *Klebsiella* persisted in the digester. Analogous results of pathogen detection, persistence, survival and elevated level of Enterobacteriaceae in digestate effluent even after a residence period of 60 days have been documented elsewhere (Bonetta, *et al*., 2014; Coelho *et al*., 2018; Qi *et al*., 2018 and Resende *et al*., 2014). So, the practice of using untreated manure and/or ineffectively treated digestates poses greater risk to the environment (humans, air, soil, water and animals). Post AD hygienization will guarantee digestate safety and can be obtained at elevated temperature over an extended period (Astals *et al.*, 2012).

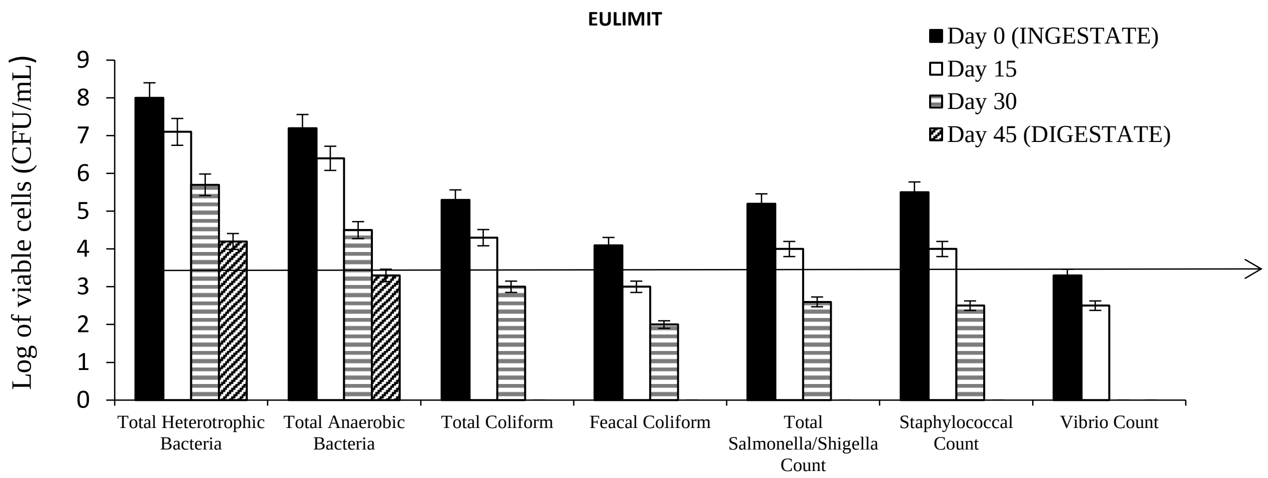

Figure 3a Microbial count of goat manure during AD

European Union (EU) limit for indicator bacteria in manure-based product according to Animal by-product regulation (EU No. 1069 / 2009)

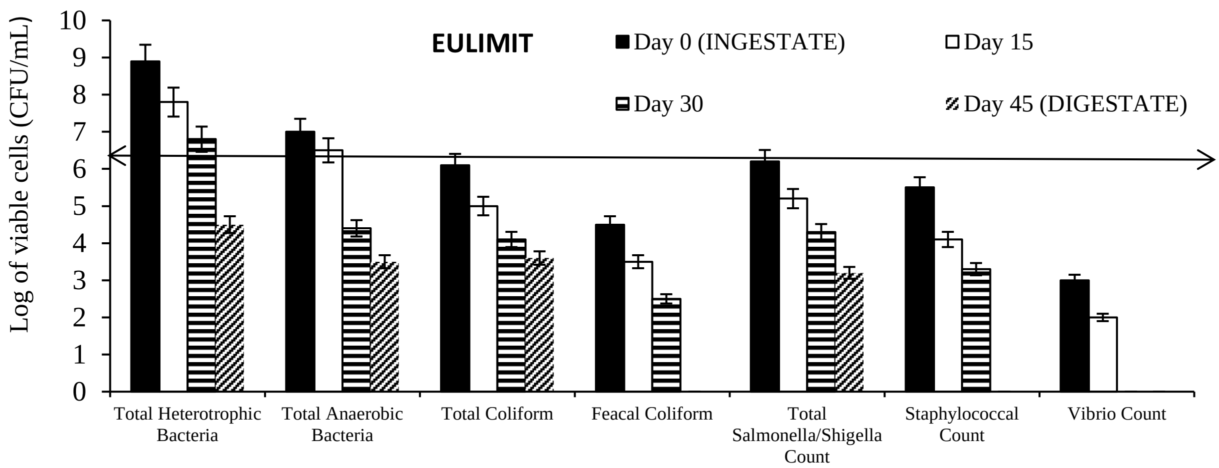

Figure 3b Microbial Count of Poultry Manure during AD

The microbial reduction rate basically rests on the bacterial type and its initial feedstock concentration. For instance, in the study, there was no significant reduction in initial and final heterotrophic and anaerobic bacteria concentration. Difficult-to-destroy endospore-forming mesophiles and strict anaerobes and during AD treatment process were also detected in this study. With *Clostridium* (16 %) and *Bacillus* (14 %) being the most predominant in goat and poultry manure respectively (Figure 4), the microorganisms encountered in the study were members of Enterobacteriaceae, Clostridia, Bacillales, Vibrionales and methanogens. These organisms have been isolated and implicated with different stages of AD (Diego-Diaz *et al.*, 2018; Campanaro *et al.*, 2016).

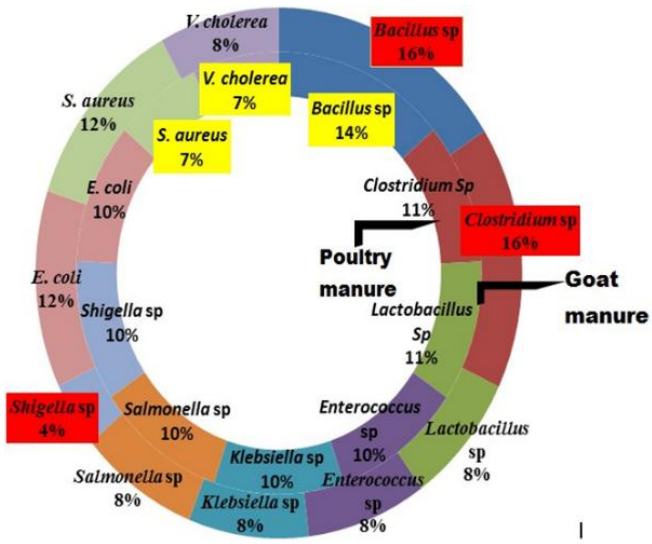

Figure 4 Percentage Occurrence of Microbial Isolates during Anaerobic Digestion

Trace Metals Concentrations during AD of Manure

Examination of metals fractions in manure samples and anaerobic digestate can provide useful information required to forecast their bioavailability and toxicity potential for environmental contamination (Muhammad *et al*., 2011). The concentration of potentially toxic metals (zinc, copper, nickel, mercury, cadmium and chromium) presented in table 2 were generally lower in the digestate than (undigested) raw feedstock although these differences was not statistically significant (p > 0.05). Comparatively, zinc recorded the highest abundance in poultry manure digestates (41.5 mg/kg) while Copper (36.7 mg/kg) was the most abundant metal in goat manure. The high concentration of zinc in poultry digestate can be due to the fact that poultry (layers) at Vikas farm where the poultry manure sample was collected were fed with top poultry feed. High zinc concentration has been reported in top poultry feed (Okoye *et al*., 2011). Again, corresponding increase in manure-based product is usually the result of initial metal concentration in poultry feed (Muhammad *et al*. 2011) However, zinc has been shown to enhance methane formation during AD (Bozym *et al*., 2015). In contrast, copper (36.7 mg/kg) concentration was highest in goat manure digestates. The high concentration of copper in goat manure was also reported by Muhammad *et al*., 2011. Source of copper in goat manure (feedstock) may be linked to those of goat feed/diet (leaves, nuts and seeds which the goats). It is pertinent to state that the concentration of all potentially toxic metals ions of both feedstock and digestate were within the recommended limits (WRAP, 2010) of publicly available specifications (PAS – 110). Besides its environmental risks on biota, trace concentrations of these metals can stimulate plant growth and development.

Table 2: Heavy metal concentration of feedstock and digestates

Metals		Con-centra-tions	(mg/kg)		**PAS**
	PM_i	PM_f	GM_i	GM_f	
Zinc	86.1	41.5	30.5	15.6	400
Copper	40.3	22.5	150.1	36.7	200
Nickel	20.5	15.1	15.8	8.5	50
Mercury	BDL	ND	BDL	ND	1.0
Cadmium	BDL	ND	BDL	ND	1.5
Chromium	20.5	8.5	BDL	ND	100

Key: PAS = Publicly acceptable specification; BDL = Below detection level; ND = Not determined; PMi = Initial concentration in poultry manure; GMi = Initial concentration in goat manure; PMf = Final concentration in poultry manure; GMf = Final concentration in goat manure.

CONCLUSION

The biochemical methane potential (BMP) in goat manure was significantly higher than in poultry manure ($p < 0.05$). The indicator bacteria and putative pathogens recorded in the feedstock gradually decreased towards complete elimination in the digestates from both manures. The complete destruction of indicator bacteria and potential pathogens in goat manure digestates indicates its safety when used as a soil conditioner/amendment. However, the presence of total coliforms and *Salmonella* species in poultry manure digestate suggests it may pose an environmental risk and attendant public health implications if used as a biofertilizer without further treatment, as the interest in digestate (biofertilizer) for agriculture is reliant on compliance with quality standards.

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