Indian Journal of Experimental Biology
Vol. 62, March 2024, pp. 199-205
DOI: 10.56042/ijeb.v62i03.2120

Changes in microbiota and parasitic load of poultry manure undergoing value addition through different techniques for their safe disposal or utilization

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Received 31 May 2023; *revised 27 October 2023*

There is scanty information about the microbial composition of poultry manure processed through several bioprocessing interventions such as anaerobic digestion (AD) and vermicomposting. Here, we investigated the changes in microbial population and parasitic load of poultry manure (both litter and excreta separately) undergoing AD and vermicomposting . The study was conducted for a period of three months. Six biogas digesters and seven vermicompost units were used comprising of different treatments. Broiler litter was pretreated with 0.1 and 0.2 mol/L NaOH prior to AD while the layer excreta was subjected to different dilutions in water (1:6, 1:8, 1:10) for the production of biogas. Further, the substrates were also used for vermicompost production wherein similar treatment was given to the broiler litter while different carbonaceous materials (16-20 kg of leaves, rice husk, saw dust and wheat straw per 100 kg of poultry waste) were incorporated to the layer excreta. The microbiota changes were studied which comprised of changes in total bacterial count (TBC), coliform count and *Salmonella* spp. count in all the treatments at regular intervals. The parasitic load was also measured by counting eggs per gram (EPG) in all the treatments at regular interval. The results of the study showed a significant (P <0.01) reduction in the microbial populations of pathogens (upto 44-54%) as well as parasitic load (92-100%) with the advancement in time and proved AD and vermicomposting of broiler litter and layer excreta at the defined pretreatment conditions as an effective technique to treat poultry waste prior to safe disposal and utilization.

Keywords: Anaerobic digestion, Broiler industry, Excreta, Litter, Vermicomposting

Poultry production is one of the fastest growing sectors all around the world. Poultry is a major source of protein and the demand for poultry products has been steadily increasing due to population growth, urbanization, and changing dietary habits. The top five poultry meat producers in the world are the United States, Brazil, China, the European Union and India[1]. Asia has been the fastest-growing region in terms of poultry meat production, with China, India, and Indonesia leading the way[1]. The broiler industry has been expanding rapidly, with more countries investing in modern production methods and technology. Similarly, demand for the egg is also increasing all around the globe and Asia is the largest egg-producing region, with more than 64% of the global output[2]. With such a huge production rate of poultry industry the amount of waste generated from this sector is also increasing day by day. Different types of wastes are generated by poultry industry includes hatchery wastes, litter and excreta, feathers, dead birds, and abattoir waste[3,4]. Out of these waste reserves, poultry litter and excreta were mostly dumped into the agricultural fields without any treatment. This poultry waste (excreta and litter) contains large number of pathogenic microbes and parasitic eggs exhibiting zoonotic concerns[5]. Also, contamination of environment with such wastes leads to introduction of pathogens in the food chain and ultimately affecting human health[6].

Growing interest has been shown in zoonotic pathogen transmission, their persistence in soils, and the relationship between the presence of pathogens and the security of agricultural goods in recent years[7,8]. Different pathogenic microbes were reported in poultry manure including *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella* spp., *Citrobacter* spp., *Salmonella* spp., *Serratia marcescens*, *Shigella dysenteriae*, *Proteus* spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Micrococcus luteus*[9]. Also, parasitic eggs are present in poultry manure which pose potential health threats. These parasites

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may include *Coccidia* spp., *Strongylata* spp., *Ascaridia* spp. and *Capillaria* spp.[10]. Out of these microorganisms, *E. coli* and *Salmonella* spp. were highly studied in other animal wastes and considered as indicator organisms for environmental contamination[11].

Anaerobic digestion (AD) performed for biogas production and vermicomposting are the two value added techniques which are now-a-days utilized to manage poultry manure along with establishing new avenues for revenue generation[12]. The main advantages of anaerobic digestion and vermicomposting includes stabilization and reduction of organic matter, energy production in the form of biogas, environment-friendliness, and biofertilizer production[13,14]. Anaerobic digestion involves rise in temperature and change in pH during conversion of substrate into end product (biogas and slurry) which is responsible for the destruction of harmful microorganisms[15]. Similarly, in the case of vermicomposting, due to enzymatic action of earthworm's digestive tract, the microbial population got reduced[16]. Costa *et al*.[11] reported a significant decrease in pathogenic microorganisms on processing swine and dairy excreta through anaerobic digestion. Suppression of parasitic eggs (*Ascaris suum*) was also reported through anaerobic digestion in the case of pig manure[17]. Monroy *et al*.[18] studied the impact of pig slurry vermicomposting by earthworm *Eisenia fetida* on total coliform and reported a significant reduction in their numbers. They also highlighted the fact that reduction in bacteria number was dependent on the dose of pig slurry. Karimi *et al*.[19] also showed significant reduction in faecal coliforms and *Ascaris* eggs on AD of municipal solid waste and cattle manure.

In literature, only limited information is available on the changes in microbiota of poultry manure undergoing anaerobic digestion and vermicomposting. In this context, and also considering the health concerns related to handling of poultry waste, here, we evaluated the effect of anaerobic digestion and vermicomposting on microbial population and parasitic load of poultry manure. Further, we explored whether the treatment strategy adopted in the current investigation can be used for safe disposal and sustainable utilization of poultry waste.

Material and Methods

Experimental location and design

The setup for biogas production from poultry litter and excreta was established at the poultry farm of the Central Avian Research Institute (CARI), Izatnagar, UP, India. The farm is located at 28°22' N, 79°24' E, is 169.2 m above mean sea level, and lies in the Upper Gangetic Plain (India's agroclimatic zone). At this farm around 30,000 birds of different species were kept, out of which around 6,000 layers and 7,000 broiler chicken were kept. Layers were housed in the cage system whereas broilers were kept in the deep litter system. Experimental biogas production unit from layer excreta and broiler litter was setup at the farm consisting of six biogas producing digesters each subjected to a different treatment. The three digesters that were fed daily with broiler litter (B) were named as B_0, B_1 and B_2 whereas the other three digesters were fed daily with layer excreta (L) and named as L_0, L_1 and L_2. The layer excreta were supplemented with 16-20 kg of leaves, rice husk, saw dust and wheat straw per 100 kg of poultry waste whereas the same quality of leaves was added to the broiler litter in order to adjust the C:N ratio. B_0 was taken as control and broiler litter was added without any pretreatment whereas in B_1 and B_2 alkali pretreatment was given at 0.1 and 0.2 mol/L, respectively. In case of layer excreta, different dilutions were taken for biogas production, wherein L_0 was taken as control with dilution of l:10 whereas L_1 (1:8) and L_2 (1:6) had lower dilutions. At the same farm, a vermicompost unit consisting of seven vermi pits was also established utilizing layer excreta and broiler litter subjected to different treatments. For vermicomposting (V) using broiler litter, VB_0, VB_1 and VB_2 were the three treatments in which VB_0 was kept as control whereas VB_1 and VB_2 were given alkali pretreatment at the rate of 0.1 and 0.2 mol/kg, respectively. In case of layer excreta, four different types of digestion materials were taken for vermicomposting i.e., VL_0 having leaves, VL_1 having wheat straw, VL_2 having rice husk and VL_3 having wooden straw as digesting material. Broiler litter and layer excreta was collected from the farm manually for use in these experiments.

Sampling methodology

The duration of the trial was of three months and samples were taken in triplicates at regular intervals. Approximately, 10-15 mL samples of slurry from all the biogas digesters were collected in sterile sampling tubes at a monthly interval. Three samples were taken at a time and were freshly processed for bacteriological and parasitic eggs examination. Similarly, 10-15 g samples of vermicompost at

different intervals from all the treatments were collected and processed for bacterial as well as parasitic examination.

Microbiological analysis

Samples were processed for TBC (total bacterial count), coliform count (*E. coli*) and *Salmonella spp.* using standard protocols. The media used for TBC was nutrient agar (NA, HiMedia® Mumbai)[20], Hektoen-Enteroagar media (HEA, HiMedia® Mumbai) for *Salmonella* spp.[21] and Eosin methylene blue (EMB, HiMedia® Mumbai) for coliform count[22]. The method used to count bacterial numbers was pour plate method[23] and it involved taking 1 g of slurry/vermicompost material and then suspending it in 10 mL of PBS followed by thorough mixing. The prepared solution was serially diluted followed by adding 0.1 mL of the solutions from each tube of serially diluted sample to the Petri plate. The plates were whirled properly to ensure even distribution of suspension and media mixing. The plates were left undisturbed to let the contents cool and solidify. The plates were then incubated for 24 h to allow the bacterial colonies to grow on them. Manual counting of grown bacterial colonies were done and total bacterial load was calculated based on serial dilutions. The final result was expressed as Log_{10} values/g of fresh sample.

Parasitic examination

Fresh samples were evaluated under microscope for the presence of eggs and for the quantification of parasitic load modified McMaster technique was employed[24]. Approximately, two grams of sample (slurry/vermicompost) was placed in the mortar and triturated with pestle after addition of saturated salt solution. The mixture was then strained through a filter and transferred to a centrifuge tube and some more solution was added until it was completely fill. To ensure that the clean cover slip was in contact with the liquid, it was slipped sideways over the top of the centrifuge tube so that the upper meniscus of the contents touched the cover slip. It was left undisturbed for 10-15 min following which the cover slip was gently picked and placed over the McMaster slide. Microscopic examination of the slide was done and eggs per field was counted and the results were expressed as eggs per gram (EPG).

Statistical analysis

Analysis of data was done with the help of JMP software (SAS Institute Inc., USA). The significant means among various treatments and days were compared using Tukey post hoc test at 1 and 5% level of significance.

Results

Changes in microbiota and parasitic load of broiler litter undergoing anaerobic digestion

The results of the total bacterial count, coliform count and *Salmonella* spp. count have been presented in Fig 1A. The values for total bacterial count ranged from 9.33 ± 0.024 to 9.52 ± 0.014 log_{10} CFU/g between different treatments during anaerobic digestion. It was observed that there was significant reduction in total bacterial count (P <0.01) as the period of anaerobic digestion advanced. However, the effect of treatment was not significant. In case of *E. coli* count and *Salmonella* spp. count there was significant (P <0.01) difference within days as well as within treatments. Highest reduction of coliforms and *Salmonella* spp. was observed in B_1 treatment.

The parasitic load was determined as eggs per gram (EPG) and it was significantly (P <0.01) reduced with the advancement in anaerobic digestion irrespective of the treatment (Table 1). The values for EPG ranged from 190.00 ± 65.95 to 10.00 ± 10.00 for all the treatments.

Changes in microbiota and parasitic load of layer excreta undergoing anaerobic digestion

In case of layer excreta, similar trend was observed showing reduction in total bacterial count, coliforms and *salmonella* spp. in all the treatments as anaerobic digestion progressed [Fig. 1A].The parasitic load in the slurry produced from anaerobic digestion of layer excreta was significantly reduced as the time progressed (P <0.01) (Table 2). It was also observed that the rate of reduction in EPG value was highest in the first month of anaerobic digestion in all the treatments.

Changes in microbiota and parasitic load of broiler litter undergoing vermicomposting

Mean values for the total bacterial count, coliform and *Salmonella* spp. numbers present in the vermicompost samples during and after competition of process were presented in Fig. 1B. As the time progressed, the microbial population decreased significantly in all the treatments (P <0.01). In coliforms, significant difference was observed between treatments and it was found that reduction was highest in VB_2 i.e., 6.30 ± 0.023 to 4.07 ± 0.033 log_{10} CFU/g.

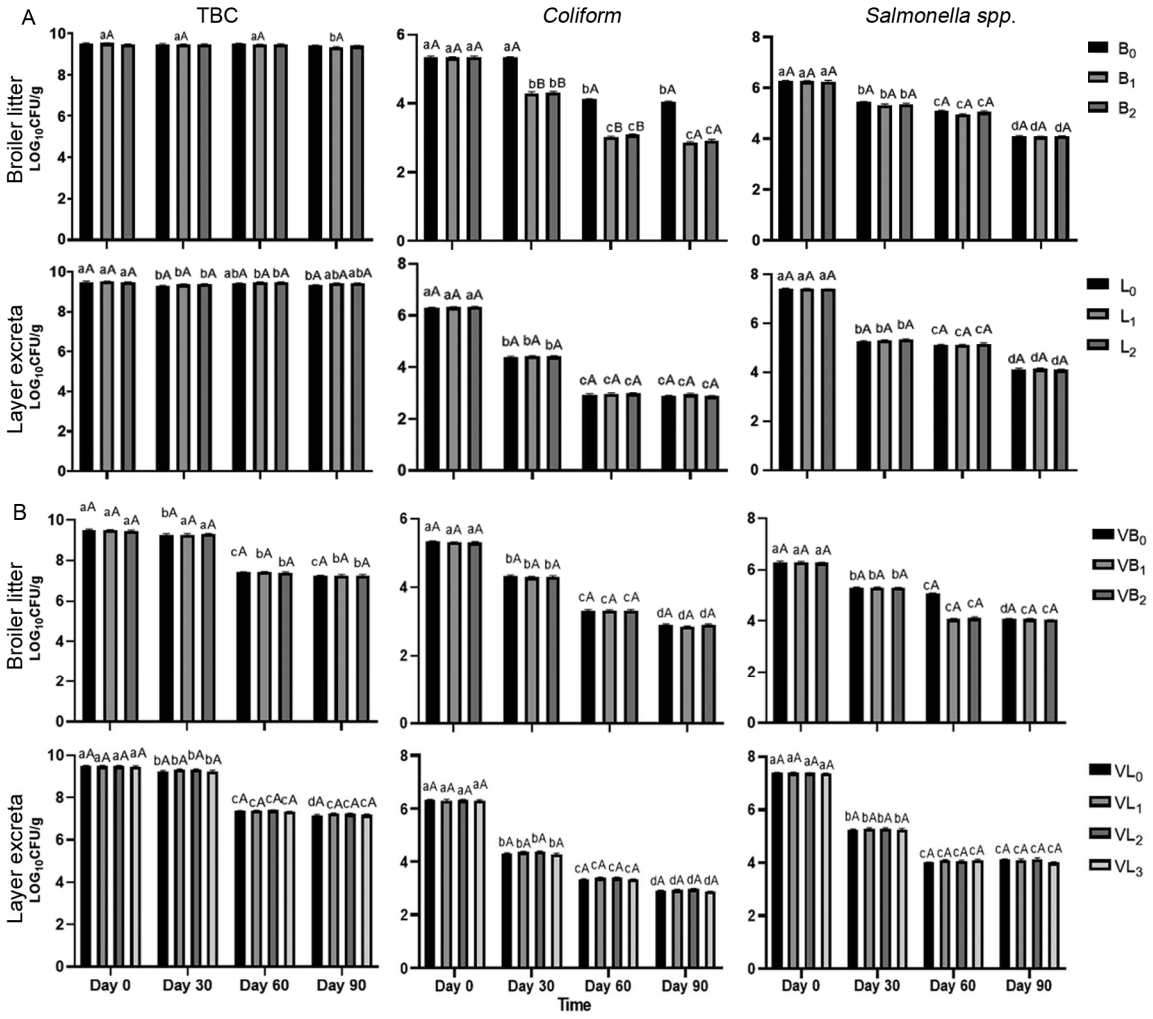

Fig. 1 — TBC, Coliform count and *Salmonella* spp. (Log_{10} CFU/g) count for broiler litter and layer excreta undergoing (A) Anaerobic digestion; and (B) Vermicomposting through different treatments

Table 1 — Eggs per Gram (EPG) of broiler litter slurry undergoing anaerobic digestion through different treatment

Time duration	B_0	B_1	B_2
Day 0	190.00^{a}±65.95	160.00^{a}±36.74	170.00^{a}±25.50
Day 30	40.00^{b}±10.00	30.00^{b}±12.25	50.00^{b}±0.00
Day 60	20.00^{b}±12.25	10.00^{b}±10.00	10.00^{b}±10.00
Day 90	20.00^{b}±12.25	10.00^{b}±10.00	20.00^{b}±12.25

[Values bearing different superscripts a,b vary significantly (P <0.01) between rows]

Table 2 — EPG of layer excreta slurry undergoing anaerobic digestion through different treatments

Time duration	L_0	L_1	L_2
Day 0	100.00^{a}±22.36	110.00^{a}±18.71	130.00^{a}±12.25
Day 30	30.00^{b}±12.25	30.00^{b}±12.25	30.00^{b}±12.25
Day 60	10.00^{b}±10.00	20.00^{b}±12.25	10.00^{b}±10.00
Day 90	10.00^{b}±10.00	20.00^{b}±12.25	20.00^{b}±12.25

[Values bearing different superscripts a,b vary significantly (P <0.01) between rows]

Table 3 — EPG of broiler litter undergoing vermicomposting through different treatment

Time duration	VB_0	VB_1	VB_2
Day 0	190.00^{a}±65.95	160.00^{a}±36.74	170.00^{a}±25.50
Day 15	70.00^{bc}±12.25	60.00^{ab}±10.00	90.00^{ab}±18.71
Day 30	30.00^{c}±12.25	20.00^{b}±12.25	30.00^{c}±12.25
Day 60	20.00^{c}±12.25	ND	20.00^{c}±12.25
Day 90	ND	ND	ND

[Values bearing different superscripts a,b vary significantly (P <0.01) between rows]

The values for parasitic load were measured at day 0, day 15 and then at monthly intervals (Table 3). Parasitic eggs number were significantly (P <0.01) reduced with time. The reduction was highest within first month in all the treatments and also on the completion of process no parasitic eggs were detected in the samples.

Table 4 — EPG of layer excreta undergoing vermicomposting through different treatments

Time duration	VL_0	VL_1	VL_2
130.00^{a}±12.25	170^{a}±60.42	130.00^{a}±12.25	110.00^{a}±18.71
100.00^{ab}±22.36	110.00^{abc}±18.71	100.00^{ab}±15.81	80.00^{a}±12.25
30.00^{b}±12.25	30.00^{bc}±12.25	20.00^{b}±12.25	20.00^{b}±12.25
ND	20.00^{c}±12.25	20.00^{b}±12.25	ND
ND	ND	ND	ND

[Values bearing different superscripts a,b,c vary significantly (P <0.01) between rows]

Changes in microbiota and parasitic load of layer excreta undergoing vermicomposting

Microbial population decreases significantly with the advancement in time duration and the values at different intervals were presented in Fig. 1B. At the end of vermicomposting process that on day 90 least number of coliforms (4.01 ± 0.028) and Salmonella spp. (2.88 ± 0.013) number was found in VL_3.

The parasitic load was also decreased with the increase in time duration and almost eggs were completely disappeared from day 60 onwards (Table 4).

Discussion

In the present study, significant effect of anaerobic digestion and vermicomposting was evident on the microbial population as well as parasitic load. Anaerobic digestion was responsible for significantly decreasing the number of pathogenic microbes due to the changes in temperature and pH[15]. The total bacterial count decreased significantly with time but its value remained higher throughout the anaerobic digestion as AD harbors a dynamic and varied community of microbiota throughout, which was dominated by bacteria (82-88%) and a sizable proportion of archaea (8-15%) depending on the stage of the production of biogas[25]. In the present study, the number of pathogenic microbiotas reduced significantly but they did not completely disappear as the feeding of substrate was done regularly to the digester. The results indicating lower values of pathogenic bacteria such as coliforms and *Salmonella spp.* on anaerobic digestion in our studies were supported by the reports presented by Salsali *et al*.[26] on anaerobic digestion of municipality wastewater. Thermophilic AD (50-55°C) and mesophilic AD (30 – 42°C) are two broad categories for AD. At mesophilic temperatures, as opposed to thermophilic temperatures, the majority of infections can survive longer. The fluidity and permeability of the cell membrane increase with temperature (from 37 to 70°C), which speeds up the diffusion of harmful substances into the cytoplasm and slows down cell growth and hence inhibits pathogenic microbial population[26]. Similarly, Watcharasukarn *et al*.[27] showed reduction in *E. coli* numbers from 6×10^4 to below detection limit in case of cow slurry undergoing AD in a batch type digester. In continuous type of digester, the reduction in coliform numbers of pig slurry was also reported by Massé *et al*.[28]. The results of our study were also in accordance with the results of Costa *et al*.[11] who investigated the influence of anaerobic digestion process and storage on indicator microorganisms in swine and dairy excreta and reported decrease in indicator microorganisms (*lactobacilli*, coliforms and *streptococci*) with advancement in AD. Ma *et al*.[29] also highlighted the fact that anaerobic digestion offers a valuable chance to reduce biosafety risks associated with biowaste and minimize human exposure to pathogens linked to foodborne illnesses.

The parasitic load was also significantly reduced within 1 month of AD but it does not completely disappear because of the continuous addition of fresh poultry manure in the digesters. In accordance with our above results, significant reduction in the parasitic load (helminths eggs) in municipality waste handled by AD was showed by Cabirol *et al*.[30]. Seruga *et al*.[31] also showed significant inactivation of the pathogens (*Salmonella Senftenberg* W775, *Enterococcus* spp. and *Ascaris suum* eggs) after AD of food waste. Patil and Mutnuri[32] also reported similar results to our study in which they have showed that the AD process yielded a 98% reduction in *A. suum* egg viability, with their concentration decreasing from 0.15 eggs per milliliter to 0.14 eggs per milliliter over a 35-day period.

Vermicomposting was also proved successful in reducing the total bacterial count, pathogenic microbiota and parasitic load of poultry waste in the present study. The enzymatic action of earthworm's digestive tract and reduction in the moisture content of slurry during vermicomposting were responsible for reduction in microbial population as well as parasitic load[16]. Other possible mechanisms responsible for reduction in pathogens during vermicomposting of organic waste includes microbial inhibition in gut, secretion of antibacterial fluids, stimulation of endemic microbes, competition and antagonism, and aeration through burrowing activity[33,34]. In the present study, earthworms were introduced after 15 days of pre-digestion as a standard

protocol and the rate of reduction of pathogens were higher after day 15. Also, parasitic eggs were completely eliminated by the end of the vermi-composting process. Similar to our study, reports have been published on vermicomposting of sewage sludge and cow dung in combination with sawdust which showed greater and quicker reduction in the parasite's population[35]. The results for the reduction in number of coliforms and parasitic eggs were also supported by the reports of Karimi *et al*.[19] who reported that the number of faecal coliforms in cow manure as organic waste reduced significantly from 350,000 MPN/g in the raw sample to 800 MPN/g within an 8-week period, while the parasite eggs were totally eliminated in the second week undergoing vermicomposting. Another report by Aira *et al*.[36] highlighted reduction in number of pathogenic microbes during vermicomposting of cow manure and proved vermicomposting as a suitable technology for processing different organic wastes.

The results of the study indicate that the process developed in this investigation provides an innovative and more effective strategy to dispose off poultry waste with minimal harm to the environment with better use efficiency.

Conclusion

Anaerobic digestion (AD) and vermicomposting of broiler litter and layer excreta have been demonstrated to be effective value-added techniques that helps in reducing the pathogenic microbes as well as parasitic eggs. The significant reduction in *E. coli* count, *Salmonella* spp. count (44-54%) and EPG (92-100%) was evident both during AD and vermicomposting. It was also evident that number of pathogens decreases with the increase in time duration in both AD and vermicomposting providing substantial evidence that the developed process is a more sustainable and ecofriendly approach for poultry waste disposal/utilization.

Acknowledgement

The authors thank the Indian Veterinerary Research Institute (IVRI), Izatnagar and Central Avian Research Institute (CARI) for financial support. The authors also express gratitude to Dr. ZB Dubal (Principal Scientist, IVRI) for assisting in bacteriological count.

Conflict of interest

Authors declare no competing interests.

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