MICROBIAL ISOLATES ASSOCIATED WITH POTENTIAL HOUSEFLY LARVAE MEAL (HFLM) PRODUCTION SUBSTRATES IN SOUTHERN GHANA

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ABSTRACT
Seven potential HFLM producing substrates,: donkey droppings, chicken manure, Camel droppings, sheep manure, palm kernel oil waste, brewery waste and pito waste, were analyzed to determine their microbial status. In donkey manure Escherichia coli (E. coli), Corynbacterium spp, Bacillus cereus, Rhizopus spp and Aspergillus fumigatus (As. fumigatus) were isolated. In pito waste, coliform, Proteus, Corynbacterium spp and B. cereus were isolated. In chicken manure isolates were coliform, Corynbacterium spp and B. cereus. In camel manure, E. coli, Coliform and B. cereus were isolated whilst Coliform and Corynbacterium spp, B. cereus, Aspergillus niger and A. fumigatus were isolated in sheep manure. Corynbacterium spp and Rhizopus spp were the isolates in Palm kernel extract waste whilst in brewery waste, E. coli, coliform spp and A. flavus were isolated. With all the identified microbes, only E.coli is associated with disease in animals. The presence or absence of these microbes in substrate would depend on source production process hygiene.

Keywords: *Brewery waste, palm kernel oil waste, isolates, camel, substrates*

INTRODUCTION

Global demand for meat is accelerating rapidly due to population growth and economic development in developing countries (Godfray *et al*., 2010). Cost of feeding livestock represents the highest cost in the meat value chain. Fishmeal and crops such as soybean are key protein sources for animal feeds but they are not economically and ecologically sustainable (Godfray *et al*., 2010). Indigenous poultry farming is practiced by almost all smallholder farmers who suffer from the increasing cost of feed in particular protein sources such as fishmeal, groundnut cake and soybean meal (Omole *et al*., 2005). Scavenging poultry farming suffers from quantitative and qualitative food shortages (Dankwa *et al*., 2004; Pousga *et al*., 2007a) affecting production of meat and eggs and thereby reducing family income.

For sustainable household poultry farming systems, the use of untapped local easily available and affordable protein sources must be used. Insects, which are natural food source for poultry are one such source and FAO now strongly recommends the use of insects for human food and animal feed as a tool for poverty alleviation (FAO, 2010; van Huis *et al*., 2013). Insect larvae and pupae are rich in protein (40-70% dry

weights) as well as other valuable nutrients such as iron, vitamin A and B and essential amino acids (Defoliart, 1995; van Huis, 2010; van Huis *et al*., 2013). These can be mass produced locally and on farm. Fly larvae feed on organic waste material and can even be used for waste management (Diener *et al*., 2011). The remaining digestate can also be exploited for various purposes including composting (Kavala and Borkovcava, 2013).

One of the most promising and commonly used species for feed is the housefly (*Musca domestica*) which has a protein content and amino acid composition which are comparable to traditional plant and fishmeal source (Heuze and Tran, 2013; Tran *et al*., 2013). The housefly is easier to rear because it can be reared on a wide range of plant and animal waste (Bouafo, 2011). In West Africa, extensive studies have demonstrated the suitability of its larvae as a poultry feed ingredient (Teguia *et al*., 2002; Agunbiade *et al*., 2007; Adesina *et al*., 2011; Okah and Onwujiari, 2012). Housefly larvae can be obtained simply by exposing adequate substrates in rearing beds, and observations suggest that the system does not increase housefly populations on farm (Kone, 1998). The system has been used successfully in experimental farms for many years (Nzamujo, 1999).

Although the use of housefly larvae for poultry nutrition is promising several issues on the safety of housefly larvae rearing systems need to be assessed. In addition, *M. domestica* is capable of transmitting disease causing pathogens from contaminated waste to humans hence this study to assess and to ascertain safety of some identified potential waste sources for housefly larvae production (Charlton *et al*., 2015).

MATERIALS AND METHODS

Substrates

The substrates used for the study were collected or purchased from identified sources and markets. These were, horse, camel, poultry and goat manure, brewery spent malt, rice and maize bran, pito manure and palm kernel waste. The substrates were brought to the experimental site at CSIR- Animal Research Institute, Frafraha for analysis.

Sample preparation for analysis

Substrates (2kg) were weighed separately into culturing plastic bowls of equal sizes and mixed thoroughly with 2L of water. For each substrate prepared for culturing, 5g were collected aseptically into sterile sample tubes and sent to the laboratory for analysis.

Test sample preparation

Samples were analyzed using the method by (Jugita *et al.*, 2009). Into a sterile McCartney bottles containing 9ml of 0.1% sterile peptone water (Merck, Darmstadt-Germany) was dispensed 1g of substrate to form the neat. The suspension produced was incubated briefly at 37^{o}C for 10-15 min using Wagtech bacteriological incubator (Wagtec, Wagtec International Ltd., UK). The samples were serially diluted using 10-fold serial dilution into 5 other sterile MacCartney bottles containing 9ml of 0.1% peptone water.

Bacterial load counts

Total viable count (TVC) was determined using the pour plate count method. A ml of each dilution (10^4-10^6) was aseptically added to 9ml of molten standard plate count agar (Merc, Darmstadt-Germany) and incubated in a water bath set to 45-50^{o}C (Grant, OLS 20). This was mixed by rotation and poured into 9cm sterile petri dishes. It was allowed to cool and incubated at 37^{o}C for 18-24hrs.

Total coliform count (TCC) was determined by the plate count method, 1 ml of each dilution was aseptically put into 9cm petri dish. A 9ml of molten violet Red agar (EOS Laboratories) kept at 45-50^{o}C in water bath was added, mixed and allowed to cool and set. The plates were subsequently incubated at 37^{o}C for 24-48hrs.

Faecal coliform counts (FCC) values were enumerated using the pour plate count method. Between 0.1 to 0.5ml of each suspension was aseptically put into 9 cm petri dish. Then 9ml of Eo-

sin mixed by swirling, allowed to cool and set was added. The plates were then incubated at 45^0C for 24- 48hrs.

For *Escherichia.coli* counts (ECC), colonies showing metallic green colour were counted after using the same pour plate count method as done for coliforms.

Salmonella spp counts were enumerated by adding 1ml neat sample to 2ml of double strength Selenite F broth (SF) (Oxiod, CM 395 and L121 Hampshire, England). It was then mixed thoroughly and then incubated at 37^0C overnight. One ml of the culture was then serially diluted using 10- fold serial dilution into 5 other sterile MacCartney bottles containing 9ml of 0.1% peptone water. With the pour plate technique, one ml of diluent was aseptically added to 9ml of molten Salmonella Shigella Agar (SSA) (Oxoid CM 533, Hampshire, England) kept at 45- 50^0C in a water bath. It was then mixed by rotation and incubated at 37^0C for 24hrs. For bacteria load count, plates showing between 30- 300 colonies were selected and counted.

Culture methods

From each sample, a sterile loop full of the neat was aseptically streaked onto blood agar (Merck, Darmstadt- Germany) using the plate count technique. Cultures were then incubated aerobically and anaerobically at 37^0C for 18- 24hrs. Impure cultures on primary media were purified by subculturing onto selected secondary media.

Isolation and identification

Colonial morphology of organisms based on their physiological characteristics were examined for size, shape, outline, colour, etc. Standard microbiological techniques including staining, cellular morphology and biochemical test such as Motility Indole Urea (MIU), Catalase, Triple Sugar Iron (TSI), carbohydrate O/F test among others were used to isolate and identify food poisoning organisms.

Data analysis

All bacterial count values determined were transformed to $\log_{10}$ cfu/g and values compared with Ghana Standards Authority regulatory values.

RESULTS AND DISCUSSION

Donkey manure had all parameters studied above, (Table 1.) TVC, TCC, EC, MYC values were above regulatory limit of 5 (GSA, 2003). Loads determined for pito, camel, sheep and palm kernel substrates were also above the re-

Table 1: Microbial isolates in experimental substrates

Substrate	TVC ($\log_{10}$ cfu/g)	TCC ($\log_{10}$ cfu/g)	ECC ($\log_{10}$ cfu/g)	MYC ($\log_{10}$cfu/g)	Isolates
DM-01	10	8.7	7.3	7.6	*E. coli, Coliform, Corynbacterium Spp, Bacillus cereus, Rhizopus spp Aspergillus fumigatus*
PW-01	8.8	7.3	-	-	*Coliform spp. Proteus spp C. spp, B. cereus*
CM-01	3.9	2.1	-	-	*Coliform, Corynbacterium spp, B. cereus*
CD-01	7.2	7.2	5.4	-	*E.coli, Coliform, B.cereus*
SH-01	5.9	5.7	-	4.5	*Coliform, Corynbacterium spp. B. cereus , Aspergillus nigger A. fumigatus*
PK-01	6	-	-	0.6	*Corynbacterium spp, Rhizopus spp*
BW-01	1.5	5.5	0	0.6	*E.coli, Coliform, A. flavus*

DM-01 Donkey manure, PW-01 Pito waste, CM-01 Chicken manure, CD-01 Camel manure, SH-01, Sheep manure, PK-01 Palm kernel waste, BW-01 Brewery spent malt waste.

quired limit of 5.0 cfu/g, (Table 1.) Coliforms were found in all the waste analyzed except Palm kernel waste.

Escherichia coli is the only species isolated that is also identified with some important pathogens of animals. *E. coli* are usually commensals of the intestinal tract, especially the large intestine. They may be opportunistic as well as primary pathogens (Moxley, 2013). They cause diarrhea in pigs, lambs and calves. In the poultry industry, Avian– pathogenic *E. coli*, (APEC) cause colibacillosis of fowls. They are invasive, extraintestinal strains of *E. coli* of certain serotypes with many virulence genes similar to the human strain of disease causing *E. coli* (01:K1:H7). This disease may come in many forms. When eggs are infected, the surface may be contaminated with potential pathogenic strains at the time they are laid. The bacteria then penetrate the shell to infect the yolk sac. This may result in the death of the embryo before birth or the chick dies after hatching (Moxley, 2013).

Corynbacterium can be found in many environments. These include the soil, water, plants and animals. Many species of the genus exist but only 2 species are identified with diseases; *C. pseudotuberculosis* and *C. renale*. The former is facultatively intracellular causing abscess in ruminants and horses and the latter, causes urinary tract infection in sows (Nagaraja, 2013).

Bacillus anthracis, *B. cereus* and *B. thuringiensis* are currently viewed as one species (Steward and Thompson, 2013). They occur all over varied ecosystems in nature, soils, water and animals. They number averagely 6 to 7 counts per gram in the environment (Stewart and Thomson, 2013). They are associated with anthrax, food poisoning and noted for pathogenesis in lepidopteran flies. They can survive in harsh environments forming spores with which they can be transmitted from one environment to another.

Fungi are key components of poultry feed as a result of ingredients used in the feed production. *Aspergillus flavus*, *A.parasiticus*, *A. ochraceus* have been identified in animal feed (D' Mello, 2000). *A.niger*, *A. fumigatus* and *A. flavus* were the fungi isolated from the wastes. They are found in peanut, cotton seed cake, palm kernel and maize feed formulations (D' Mello, 2000).

CONCLUSION

Some of the microbes isolated from the experimental substrates were as a result of the contaminated cereals used as feed ingredients. Feed producers should avoid the use of contaminated grains. Animal droppings targeted for housefly larvae production should be properly handled so as to avoid contact with bare ground to avoid getting contaminated with microbes not associated with original waste.

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