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Original Research Article

The characteristics of fungi contaminating chicken feed in Tegal, Bogor, West Java

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Abstract

Fungi can cause contamination in animal feed. Contamination of the feed may result in damage to feed quality and decrease the health of livestock. The aim of this study was to isolate and identify fungi strains in cage- and warehouse-based chicken feed. Fungal isolates was collected using the dilution plating method. A sample was taken from the cage and feed in Tegal village, Bogor, West Java. The numbers of sample taken were 10 g of each plot. The isolation, identification and characterization of mold, was based on its phenotype macroscopic and microscopic. Forty-six molds [7 sourced from cages (15.21%) and 39 from warehouses (84.78%)] were isolated from 10 samples of the cage and warehouse-based chicken feed. The selection of representative mold isolates amounted to 22 isolates, consisting of four mold isolates from cages and 18 isolates from the warehouse. Six genera were identified, based on their morphological characteristics: *Aspergillus* (3 isolates), *Penicillium* (8 isolates), *Fusarium* (3 isolates), *Trichoderma* (3 isolates), *Cladosporium* (1 isolate), Paecilomyces (2 isolates) and Mycelia sterilia (2 isolates). The highest occurrence of mold isolates in chicken feed was *Penicillium* (36.36%), and the lowest of it was *Cladosporium* (4.54%). The findings are in line with the information about occurrence fungi in the chicken feed of poultry. These results showed that a potential exists for fungi contamination in chicken feed can be found at cage- and warehouse poultry feeds. Occurrence of fungi gives information to farmers to prevent a health of their livestock from excessively contaminated fungi genera *Penicillium*. Controlling as prevented by early detection or visual inspection and good management is a better choice compared to curing.

Keywords: Fungal, *Cladosporium*, Isolation, *Penicillium*, Poultry feed contamination, Warehouse

Introduction

Feed is a source of organic and inorganic material used for the growth of livestock (Suprijatna et al., 2005; Kariyasa, 2003), the quality of which is correspondingly crucial to health of the livestock. Mold degrades the quality of the feed through contamination (Widhiastuti, 2006), which, in turn, negatively impacts on the livestock. Feed material, such as grains, can be infected by mold (fungus *Alternaria* spp. and *Fusarium* spp.). Fungus can contaminate corn that has not yet been ground off the cob and stored for a long time (*Fusarium* spp. and *Chaetomium* spp.) (Ahmad, 2009).

The contamination of mold is known to occur in fodder stored in warehouses. Fungi found in warehouse-stored fodder include *Aspergillus* spp. and *Penicillium* spp. Aliyu et al. (2016) reported a total 258 fungal was found at poultry feed contain genera *Aspergillus*. Genera *Aspergillus*, *Fusarium*, and

Penicillium could contaminate and produce mycotoxin as it is said by Rahman et al. (2015). Azarakhsh et al. (2011) mentioned that his study showed that the broiler feeds in Kermanshah province were highly contaminated with genus *Aspergillus*, the most toxigenic fungi found in feed.

Mold contamination is widespread in tropical countries where poultry production and processing are expanding rapidly (Kotinagu et al., 2015). The occurrence of fungal pathogen and mycotoxin in poultry feed came from their raw materials of animal feed (Kotinagu et al., 2015; Shama, 2015). Feed contamination by mold can lead to a decline in the health of the livestock. Mycotoxin as an example, affects chickens, in particular. A positive result was reported following research on isolates taken from 31 liver and chicken meat samples, in which mold was found in as many as 14 liver samples and 5 chicken meat samples following the identification of mycotoxin residue (Widhiastuti, 2006).

The danger of mycotoxin in chickens include a decrease in food intake, negative effect of immune response, a reduction in function of the lymphoid organs, especially the thymus (Shareef, 2010), a decline in breeding efficiency; the presence of neurotoxicity and impaired productivity in both livestock and humans (Labuda et al., 2006). A toxin in the mold can be transferred to liver and chicken meat samples. Isolation and identification of fungal contamination on the livestock feed might be important for sanitation.

The aim of this study was to obtain mold isolates in contaminated chicken feed taken from cages and warehouses in the village of Tegal chicken farm, Bogor, West Java. It was expected that the mold isolates found in the chicken feed would provide information to poultry farmers on mold genus that causes feed contamination. The study was conducted as a first step, to be developed further, in determining the type of mold at chicken feed.

Material and Methods

Chicken feed sampling

Samples of chicken feed were taken from poultry in the village of Tegal, Bogor, West Java. The sampling technique was done using purposive sampling. Samples were taken from two plots that were in the cage and storage warehouse fodder. Each plot consisted of five subplots. The first plot was the hen cage, sampling was conducted in three hours after feeding. The second plot, was the feed barn, sampling was done by following the feed storage in a warehouse. The numbers of samples taken were 10 g of each subplot. Then the samples were inserted into the plastic tip and labeled then stored in cooler box.

Mold isolation

Mold isolation was done using dilution plating method (Sukmawati et al., 2015; Sukmawati, 2016). A total of 1 g sample was poured in a tube containing 9 ml of sterile distilled water (dilution 10^{-1}). The tube was spun by vortex for ± 1 minute. Furthermore, another isolation was done using the same method above resulting different concentration (dilution 10^{-6}). Samples in different fluid concentration (dilution of 10^{-4}, 10^{-5} and 10^{-6}) were inserted into 2 petri dishes that have already contained MEA (Malt Extract Agar). Samples were incubated for 7 days at a room temperature (25–30ºC). Colonies of mold were isolated then purified by quadrant streak using the modified method (Cappuccino and Sherman 2001; Gandjar, 2006).

Mold identification

Mold identification was based on the observation of morphological characters both macroscopically and microscopically (Cappuccino and Sherman 2001). The macroscopic observation of mold was done on colony (granular, such as flour, mounting, slippery), texture, growing zone, the growing area, the radial and concentric lines, background color of the colony (reverse color), and drops of exudate (exudate drops). Microscopic observation was done using Henrici's slide culture method. Microscopic observation includes the presence or absence of sexual and asexual spores, sexual and asexual spore-producing structures, conidia shape and surface, whether there was a bulkhead on conidia, hyphae and whether there was any type of bulkhead on hyphae.

Results and Discussion

Mold isolation

Forty-six molds [7 taken from cages (15.21%) and 39 taken from warehouses (84.78%)] were isolated from 10 samples in the cage and on warehouse-based chicken feed in Tegal Village, Bogor, West Java. Based on the obtained data, the highest percentage of mold was found in plot 1 and the lowest was found in plot 4. The results demonstrate that the acquisition of mold isolates was higher in chicken feed taken from

warehouses than in that taken from cages. This was probably due to the physical conditions of the feed warehouses (temperature, humidity and wind speed) which support the mold growth. The study observed humidity from warehouse was 75.6- 78.9%.

The important factors for optimal feed storage conditions in a warehouse are certain suitable temperature and humidity, the need for cleanliness, the place to store feed which is away from pesticides and direct sunlight, and the place which protect the feed from water and damp (Olusola et al., 2013). The optimum temperature for mold growth ranged from 25 °C to 37 °C while the optimum humidity ranged from 65% to 90% regarding to Handayani, and Sulistyo (2000).

The percentage of cage-produced mold recorded was lower than that of warehouse-produced mold. It was because at the time that the cage-based chicken feed mold sampling took place, some of the tightly sealed feed intended for the warehouse was sampled directly, without first being stored for any length of time in the warehouse, so it was assumed it was not contaminated. Chicken feed contamination was avoided if it is stored in sewed plastic bags and tightly sealed (Aklilu et al., 2016). The damage caused was due to mold contamination in the feed renders unfits for animal consumption, as evidenced by changes to the color, taste and smell, and the decay process, as a result of the chemical composition been modified by Panda et al. (2010). Farm-based cages require good air circulation, exposed to sufficient sunlight and should be built on open land. The factors required for optimum cage conditions are suitable air circulation, adequate sunlight in the morning (while not being exposed to sunlight all the time), and good access of fresh air (Olusola et al., 2013).

Mold identification

After isolation, 22 representative mold isolates were obtained (4 cage-sourced isolates and 18 warehouse-sourced isolates). The mold isolates were selected and identified based on similarities in colony morphology. The identification results consisted of six genera of molds namely *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Cladosporium*, *Paecilomyces* and a sterile mycelia order.

Three isolates of the genus *Aspergillus*, were identified through mold isolation code 8, 19, and 59. The genus *Aspergillus* has greenish-brown colonies, a granular texture, a growing zone, an exudate drop and very dense associated spores as a result of macroscopic identification (Fig. 1). At the beginning growth, the yellowish-brown color quickly turns to greenish brown, with heavy sporulation (Embaby et al., 2015).

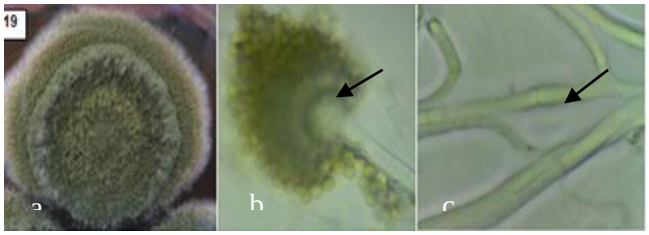

Fig. 1 (a-c): Macroscopic observation of *Aspergillus* in the MEA medium, incubation time of 7 days, the temperature of 25 ºC. a. Colony in petri dish; b. Conidiogenus cell;c. Branching hifa.

Rounded ends of *Aspergillus* hyphae appeared as conidiophores in our study, forming vesicles, metulae and fialid. Round-shaped, brown-colored conidia, with a smooth surface, were noted at the end of the fialid. The conidiophores appeared to be straight, and the head was a round circular shape (Fig. 2).

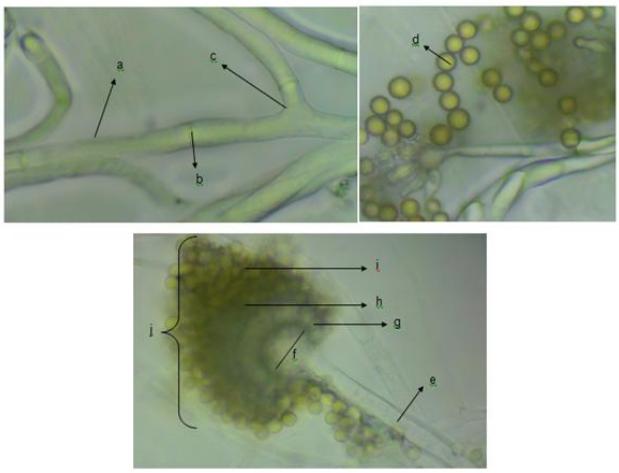

Fig. 2: The microscopic characteristic of *Aspergillus* from mold isolate code 19 (magnification 1000x) with methods of Henrici's slide culture, the incubation time of 7 days, 27 ºC. (a = hyphae; b = bulkhead, c = branching hyphae, d = conidia; e = conidiophores, f = vesicles, g = metula; h = fialid, i = conidia, j = conidia head).

The vesicle was formed at the end of the conidiophore due to swelling in the fertile hyphae that carry metulae or fialid. Fialids are conidiogenous cells that form conidia (Panda et al., 2010). The

conidia head is black and round-shaped. The conidia are round or semi-spherical in shape and dark brown in color. Conidia are created by fialid lodged in the metulae, forming a circular brush (radiate or columnar).

It is likely that *Aspergillus*, identified in our study in the chicken feed, was derived from the source of the feed materials as a result of physical conditions that supports the mold growth. Corn, rice and beans are usually contaminated with *Aspergillus*. Twenty-five species of *Aspergillus* have been identified in host plants, particularly maize, rice, sorghum and beans (Pakki, 2005). *Aspergillus* has also been identified in various phases (vegetative, generative and post corn harvest) (Pakki and Muis 2006). Physical conditions (temperature and humidity) on farms support its growth. The *Aspergillus* is xerophilic that can survive at a temperature of 27ºC and humidity of 70%. *Aspergillus* is found in a cosmopolitan habitat (Ilyas, 2007). Its growth can be avoided by adjusting the temperature of 15ºC and humidity of 61.5% for storage condition (Asvedo et al., 1993). Occurrence of pathogenic fungi has been determined. This pathogenic fungus produced toxin. Infection from pathogenic fungus causes reduced growth rate and lowered metabolism of animal. This pathogenic fungus produced by of fungal genera: *Aspergillus*, *Penicillium* and *Monasccus* (Achakzai, 2015).

Eight isolates of genus *Penicillium* were identified by mold isolation (mold isolate code 12, 40, 42, 45, 48, 50, 51 and 53). Based on macroscopic identification, in our study, the genus *Penicillium* has green to gray, blue and white colonies, a granular texture, the absence of a radial furrow (in general) and indicative exudate drop (Fig. 3). *Penicillium* mold colonies are whitish-bluish in color (Pakki, and Muis 2006).

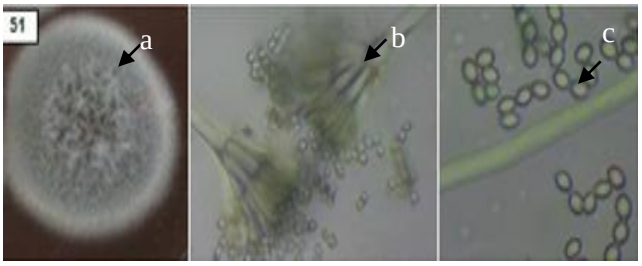

Fig. 3 (a-c): Macroscopic and microscopic of *Penicillium* on MEA medium, incubation time of 7 days, the temperature of 25ºC. a. Colony in petri dish; b. Conidiophore; c. Conidia (magnification 1000x).

In our study, the *Penicillium* hyphae were insulated with ramifications at the end of the conidiophores. Metulae, fialid and conidia were attached so that they looked like a chain. Lastly, the conidia varied in shape, being either round, semi-round or obovoid (Fig.4). *Penicillium* include the fact that it is arranged like a chain, produced by the conidiogenous cells, is called as fialids (Samson et al., 2004). Metulae in Penicillium can either be branched or unbranched. It is likely that *Penicillium*, identified in our study in the chicken feed, was derived from the source of the feed materials as a result of physical conditions which support mold growth. Corn is a feed that is most likely to be contaminated by *Penicillium*. *Penicillium* is a seed-borne pathogen, and corn is a major host. *Penicillium* can infect corn plants during the pre- and post-harvest phases. Physical conditions (temperature and humidity) on farms support the growth of *Penicillium* mold. *Penicillium* grows well at temperatures of 15–40 ºC and humidity of 80% (Mona et al., 2011).

Three isolates of the genus, *Fusarium*, were identified through mold isolation (molds isolate codes 10, 13, and 46). The genus *Fusarium* has white and pink colonies, a granular texture (like cotton), a radial furrow and exudate drops associated with Fusarium through macroscopic identification. The growth of white colonies of *Fusarium* becomes yellow, pink and brown, with a cottony mycelium (Sukmawati and Mieke 2017; Samson 2004). Figure 5 shows genus *Fusarium* has both insulated and branched hyphae. Macroconidia, shaped like a crescent moon, is a feature of cylindrical conidiophores included insulated hyphae and conidiophores, as well as insulated macroconidia, in a crescent moon shape.

The *Fusarium*, identified in our study in the chicken feed, was derived from the source of the feed materials as a result of physical conditions supporting the growth of mold. Corn is often contaminated by *Fusarium*. *Fusarium* is responsible for stem rot and leaf spot in maize (Styler and Cantlife 1984). It is predominantly found in corn, and infects the roots, stems, cob, and seeds (Schutless, 2002). Physical conditions (temperature and humidity) on farms support the growth of *Fusarium* mold, which grows well at temperatures of 5–20ºC and humidity of 80%.

Three isolates of genus *Trichoderma* were identified through mold isolation (mold isolate code 55, 56, and 60). The Trichoderma has transparent white mycelium and a greenish color with white edges as a

result of macroscopic identification. *Trichoderma* colonies are initially visible white on agar medium. Later, the mycelium changes to a greenish color, reflecting green-colored colonies surrounded by an oscillating white mycelium. Eventually, along with the growth, the entire medium becomes green (Nurhayati, 2014).

Trichoderma has both insulated and branched hyphae. The conidiophores are cylindrically shaped, have a fialid with oval- and obovoid-shaped conidia at the end (Fig. 6). It is also regularly branched with the conidiophores which has no bundle formation. The unicellular conidia are arranged in a small group, and the conidia group is green and blue in color. Trichoderma conidia have a round to oval shape and are formed at the ends of the fialid (Ilyas, 2007).

It is likely that the *Trichoderma* contaminated chicken feed in our study came from the source of the feed ingredients, i.e. the soil in which the feed ingredients were grown, and was facilitated by the physical conditions on farms that are supportive for the mold growth. Coconut cake and rice bran are examples of products containing feed ingredients in which *Trichoderma* is present. This is because the crude fiber in coconut cakes and rice bran is usually treated with *Trichoderma*, which is being intentionally added to improve the quality of the feed and to enhance the growth of chickens. Land on which feed ingredients are grown, such as wheat, grass and straw, is the ideal habitat for *Trichoderma* (Tengku et al., 2012). *Trichoderma* is an antagonist in the rhizosphere of wheat, grass and hay. Physical conditions (temperature and humidity) on farms support its growth. Trichoderma can grow well at temperatures ranging from 30–40°C and humidity of 80% (Akinyele et al., 2013).

One isolate of the genus *Cladosporium* was identified through mold isolation (mold isolate code 3) (Fig. 7). Dark green colonies, somewhat blackish in color, with a velvety texture and pleated like wrinkles, are identified as *Cladosporium* through macroscopic identification. In this study, *Cladosporium* have hyphae and unicellular conidia (Fig. 8).

The characteristics of *Cladosporium* are dark brown hyphae and unicellular conidia, arranged to form a chain and elliptical in shape, with thickening at the edges Samson et al. (2004). It is likely that the *Cladosporium* mold that contaminated the isolated chicken feed in our study came from the source of the feed ingredients and was facilitated by crop conditions in the field. Nuts are an example of a feed material that is often contaminated with *Cladosporium*. *Cladosporium* mold is one of the main microorganisms in peanuts and red beans. Plant fertilizer applied to improve soil fertility to assist with plant growth and to enhance microorganisms in the soil, nevertheless contributed to the presence of *Cladosporium* in the chicken feed (Ilyas, 2007).

Two isolates of the genus *Paecilomyces* were identified through mold isolation (mold isolate code 20 and 52) (Fig.9). The genus *Paecilomyces* was identified to have brownish-yellow colonies with a white edge and a smooth granular texture, like flour, based on macroscopic identification. The characteristics of *Paecilomyces* are insulated hyphae, direct conidiophores emerging from the hyphae, a fialid, and oval-shaped conidia at the end of the fialid, arranged in the form of a chain (Fig. 10).

It is likely that the *Paecilomyces* mold contaminated the isolated chicken feed in our study came from the source of the feed material and was facilitated by physical conditions on farms supporting the growth of mold. Corn is an example of a feed ingredient that is often contaminated with *Paecilomyces*. *Paecilomyces* is spread by maize aphids (Rhopalosiphum maidis) in Bogor (Milicevic, et al., 2010). *Paecilomyces* grows well at temperatures of 26–30 ºC, within a wide pH tolerance range and on a variety of substrates. In addition, it can be grown in a variety of habitats, including cultivated ones, as well as in forests, deserts and grasslands, and in different substrates, such as grass and silt (Gortari et al., 2008).

The percentage of cage-produced mold recorded was lower than that of warehouse-produced mold because at the time that the cage-based chicken feed mold sampling took place, some of the tightly sealed feed intended for the warehouse was sampled directly, without first being stored for any length of time in the warehouse, so it was assumed that it was not contaminated. Chicken feed contamination is avoided if it is stored in sewed plastic bags and tightly sealed (Aklilu et al., 2016). The damage caused was due to mold contamination in the feed renders unfits for animal consumption, as evidenced by changes in color, taste and smell, and decay process, as a result of the chemical composition been modified (Panda et al., 2010).

Mold occurrence

Based on the results of the isolation and identification of fungi in chicken feed, it can be seen that the highest occurrence of contamination in this study was

due to genus *Penicillium* with a frequency of 36.36%. The lowest occurrence of contamination was due to genus *Cladosporium* with a frequency of 4.54% (Fig. 11). The reason for the former is that it is likely that Penicillium came from the source of the plant feed materials, i.e. soil and root.

Conventional feed ingredients in poultry feed, such as corn, beans, bran, soybean meal, coconut meal, fish meal and straw, are good substrates for the growth of *Penicillium* (Aklilu et al., 2016; Milicevic et al., 2010; Gortari et al., 2008; Koteswara et al., 2011; Marham et al., 2016). Soil and root are habitat for widely available microorganisms, one of which is *Penicillium*. It is a fungus that lives in the soil and produces ligninase, an enzyme that can break down lignin and cellulose compounds (Yang et al., 2005). Five genera of *Penicillium* were isolated from the rhizosphere of plants in the province. The *Cladosporium* was the lowest occurring genus in this study owing to the unfavorable (for its growth) substrate and physical conditions on farms. Substrates, such as chicken feed, do not facilitate *Cladosporium* growth. *Cladosporium* is usually found on dead plants, timber plants, food, straw, soil and textiles, and does not grow well at 25°C (Tasic et al., 2007).

Mold contaminating feed and its constituent components have to be watched out for. When it is detected, it should be immediately controlled so it would not cause loss. Control can be done by prevention among other inspection visual of feed ingredients, sanitation, humidity, temperature, cleanliness on storage, and discard materials that have been contaminated. Treatment of livestock can be done to reduce toxins in the body by mycotoxin binder and medicine. In conclusion, the feed was a potential source of contaminating *Penicillium, Aspergillus, Fusarium, Cladosporidium, Trichoderma,* and *Paecilomyces*. Information about fungi contaminating feed will give contribution information to farmer to prevent their chicken from pathogenic fungi (Achakzai, 2015).

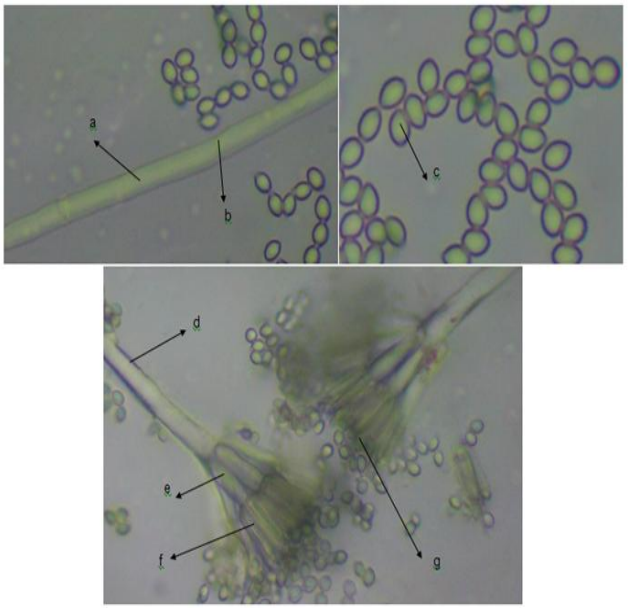

Fig. 4 (a-g): The microscopic characteristic of *Penicillium* (1000x magnification) with Henrici's slide culture method, incubation time of 7 days, 27 ºC. (a = hyphae, b = bulkhead, c = conidia, d = conidiophores, e = metula, f = fialid, g = conidia).

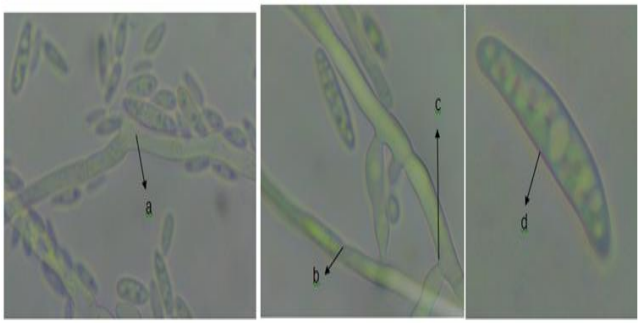

Fig. 5: The microscopic characteristic of *Fusarium* (1000x magnification) with Henrici's slide culture method, incubation time of 7 days, 27 ºC. (a = hyphae, b = bulkhead on hyphae, c = branching hyphae, d = macroconidia).

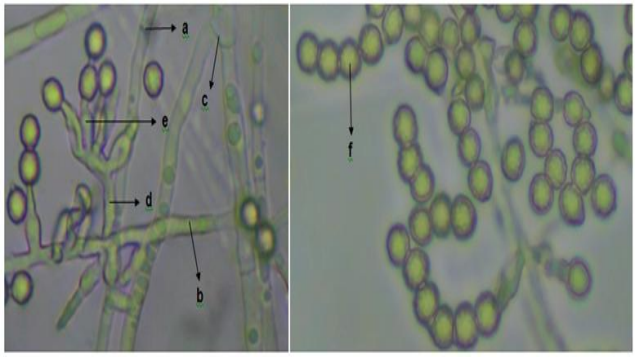

Fig. 6: The microscopic characteristic of *Trichoderma* (1000x magnification) with Henrici's slide culture method, incubation time of 7 days, 27 ºC. (a = septa, b = hyphae, c = branching hyphae, d = conidiophore, e = phialid, f= conidia).

Fig. 7: Macroscopic and microscopic observations of *Cladosporium* on MEA medium, incubation time of 7 days, the temperature of 25 ºC.

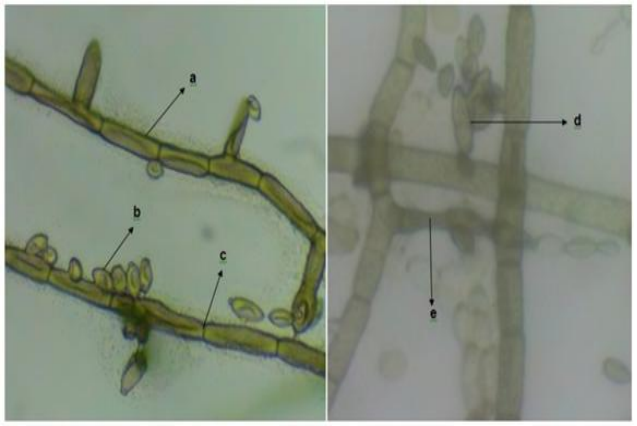

Fig. 8: The microscopic characteristic of *Cladosporium* (1000x magnification) with methods of Henrici's slide culture, the incubation time of 7 days, 27 ºC. (a = hyphae; b = conidia, c = bulkhead on hyphae, d = bulkhead on conidia and f = conidiophores).

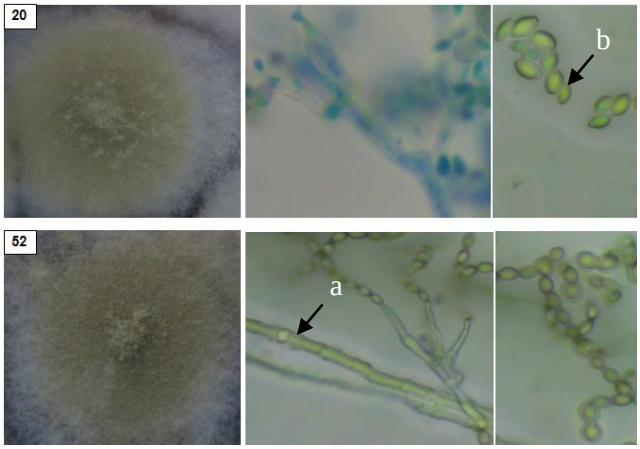

Fig. 9: Macroscopic and microscopic observations of *Paecilomyces* isolates on MEA medium with incubation time of 7 days and the temperature of 25ºC. 20ºC and 52ºC. These molds are isolates indicated Paecilomyces (a = hyphae; b = conidia).

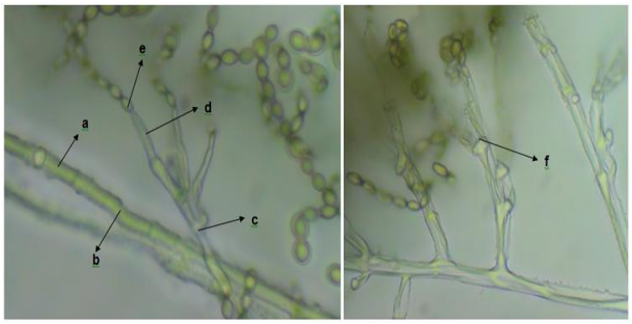

Fig. 10: The microscopic characteristic of *Paecilomyces* (1000x magnification) with Henrici's slide culture method, incubation time of 7 days, 27 ^{0}C. (a = hyphae, b = bulkhead, c = conidiophores, d = fialid, e = conidia; and f = branching conidiophores).

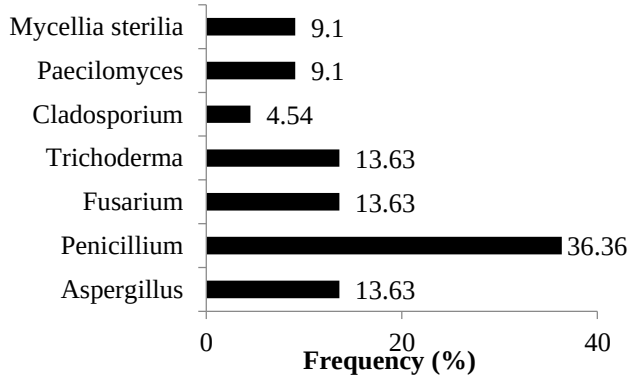

Fig. 11. The frequency occurrence of molds from cage and warehouse fodder in the village of Tegal chicken farm, Bogor, West Java.

Conclusion

In conclusion, Feed is a source of nutrients for molds. Environmental conditions such as temperature, humidity, pH suitable for mold growth will cause contamination of the feed. The genus *Penicillium* (36.36%) was accounted for the highest percentage of isolated fungi, and *Cladosporium* (4.54%) was the lowest of it. The presence of the genus *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. will poses potential health hazards to both human beings and animals.

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