



MORPHOLOGICAL IDENTIFICATION OF FUNGAL CONTAMINANT IN PIG FEED FARM IN BALI

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Abstract

Feed quality is a key determinant of pig health and productivity. Fungal contamination of feed not only reduces its nutritional value but may also result in the formation of mycotoxins that adversely affect animal health. Information on fungal contaminants in pig feed from Bali is still limited. This study aimed to identify the genera of fungi contaminating pig feed collected from several pig farms in Bali. Six feed samples were purposively collected from six pig farms and designated A1–A6. Fungal isolation was performed on Sabouraud Dextrose Agar (SDA) using the spread plate technique, followed by incubation at 25–28°C for 3–5 days. Fungal identification was conducted based on macroscopic colony characteristics and microscopic features observed using Lactophenol Cotton Blue staining. The results showed that all feed samples were contaminated with fungi. *Aspergillus* spp. and *Penicillium* spp. were the most frequently identified genera, detected in 5 of the 6 samples, whereas *Mucor* spp. and *Rhizopus* spp. were present in several samples. Microscopic examination revealed septate hyphae with characteristic conidial structures typical of *Aspergillus* and *Penicillium*, as well as aseptate hyphae with sporangia consistent with *Mucor* and *Rhizopus*. In conclusion, pig feed in Bali was contaminated by various fungal genera, with a predominance of potentially toxigenic fungi. Although identification was limited to the genus level and no mycotoxin analysis was performed, these findings highlight the need for improved feed storage management and regular feed quality monitoring.

Keywords: pig feed; fungal contamination; *Aspergillus* spp.; *Penicillium* spp.

INTRODUCTION

Feed quality is a critical determinant of livestock productivity and health, including in pigs. A high-quality feed must not only meet daily nutritional requirements but also remain free from biological contaminants (bacteria and fungi) and chemical hazards (mycotoxins) during production and storage processes (Granados-Chinchilla et al., 2021). Within the livestock industry, fungal contamination of feed represents a major concern, as it may occur at multiple stages of production, ranging from post-harvest handling of raw materials to storage and distribution (Pietsch et al., 2020). Suboptimal storage conditions, particularly high humidity, poor ventilation, and elevated temperatures, are known to markedly enhance fungal growth and mycotoxin production in animal feed (Geng et al., 2023; Hamasalim et al., 2021).

Globally, mycotoxins produced by fungi belonging to the genera *Aspergillus*, *Penicillium*, and *Fusarium* are among the most frequently detected contaminants in animal feed and feed

ingredients (Gajecki et al., 2020). These fungi are capable of rapid growth on cereal grain-based substrates that are widely used in feed formulation, including flour and its processed products (Deligeorgakis et al., 2023). *Aspergillus*, *Penicillium*, and *Fusarium* are well recognized as producers of major mycotoxins such as aflatoxins, ochratoxins, and fumonisins, whereas *Mucor* and *Rhizopus*, although rarely mycotoxigenic, may still compromise feed quality and contribute to spoilage (Nazareth et al., 2024; Snyder et al., 2019). The presence of molds in feed not only reduces its nutritional value but also poses a risk through the production of toxic metabolites that may adversely affect animal health.

Mycotoxins exert significant effects on animal health. These compounds are toxic secondary metabolites that commonly contaminate animal feed and can interfere with digestion, nutrient absorption, metabolism, and overall physiological function (Xu et al., 2022). In pigs, exposure to mycotoxins has been associated with hepatotoxicity, reduced performance, immunosuppression, and increased susceptibility to secondary infections (Tkaczyk & Jedziniak, 2021). Consequently, controlling fungal contamination in feed constitutes an essential component of livestock health management.

Several studies have documented the presence of fungal contaminants in animal feed across different regions. Alkhursan et al. (2021), reported the dominance of *Aspergillus* and *Penicillium* in poultry feed in Iraq, while Khalifa et al. (2022), highlighted a high diversity of toxigenic fungi in animal feed on a global scale. Nurtjahja et al. (2023), also identified *Aspergillus* as a major contaminant in feed samples from North Sumatra. However, most of these studies have focused primarily on poultry feed, leaving data on fungal contamination in pig feed in Bali, particularly studies involving isolation on Sabouraud Dextrose Agar (SDA) and identification based on macroscopic and microscopic morphology largely limited.

The scarcity of local information regarding the types of fungal contaminants in pig feed underscores the need for accurate microbiological identification. This study is therefore important as a basis for feed quality evaluation and as a preventive measure against the risk of mycotoxicosis. The findings are expected to provide fundamental data to support feed safety surveillance, inform feed management decision-making, and contribute to the development of strategies for preventing fungal contamination at both the farm level and within the pig feed industry.

MATERIALS AND METHODS

Ethical Clearance

This study did not involve experimental animals or invasive procedures; therefore, ethical approval was not required. The samples used consisted of feed collected from six pig farms in Bali, and sample collection was conducted with the consent of the farm owners.

Samples

Feed samples were obtained from six pig farms in Bali and consisted of commercial feeds commonly used for pig production. The six samples were coded A1 to A6. Sampling was performed purposively in the feed storage areas of each farm. Approximately 50 g of feed was collected using sterile equipment, placed into sterile sample bags, labeled accordingly, and transported to the laboratory in sealed conditions to prevent cross-contamination during transport.

Equipment and Materials

The equipment used in this study included Petri dishes, an analytical balance, test tubes, micropipettes, an autoclave, an incubator, sterile spatulas, a laminar airflow cabinet, glass

slides, cover slips, and a light microscope. The materials used comprised SDA, sterile distilled water, Lactophenol Cotton Blue (LPCB) staining solution, 70% alcohol, and sterile tissues as supporting supplies.

Fungal Isolation

Fungal isolation was performed by weighing 1 g of feed and suspending it in 9 mL of sterile distilled water to obtain a homogeneous suspension. A 1 mL aliquot of the suspension was then inoculated onto SDA medium using the spread plate method. The inoculated media were incubated at 25–28°C for 3–5 days until fungal colonies became clearly visible and suitable for observation (Nurtjahja et al., 2023; Yastanto, 2020).

Macroscopic Identification

Fungal colonies growing on SDA were examined macroscopically to assess colony surface morphology. The observed parameters included colony color on both the surface and reverse sides, colony texture (e.g., smooth, granular, powdery, or cotton-like), and colony topography, which may appear flat, wrinkled, or folded. Radial growth patterns were also evaluated to assist in distinguishing characteristics among fungal taxa (Bhargava & Tandon, 2019; Pitt & Hocking, 2009).

Microscopic Identification

Microscopic identification was carried out by preparing wet mounts of fungal colonies using 10% KOH solution or Lactophenol Cotton Blue (LPCB) stain. The preparations were examined under a microscope to identify characteristic fungal structures, including hyphae, conidiophores, phialides, vesicles, sporangia, and rhizoids when present. The observed microscopic features were subsequently compared with standard fungal identification keys to determine the genus of each isolate (Bhargava & Tandon, 2019; Pitt & Hocking, 2009).

Data Analysis

Data analysis was conducted descriptively based on the macroscopic and microscopic characteristics observed in each fungal isolate. The identification results were then compared with established fungal taxonomic literature to confirm morphological consistency and to determine the genera of fungal contaminants present in each pig feed sample.

RESULTS AND DISCUSSION

Results

Morphological identification on Sabouraud Dextrose Agar (SDA) revealed that all collected pig feed samples (A1–A6) were contaminated with mold species. *Aspergillus* spp. and *Penicillium* spp. were the most prevalent fungal genera, present in 5 out of the 6 samples, while *Mucor* spp. and *Rhizopus* spp. were identified in several samples. Macroscopically, *Aspergillus* spp. and *Penicillium* spp. exhibited powdery to granular textures ranging from yellowish-green to blackish with whitish-gray margins, whereas *Mucor* spp. and *Rhizopus* spp. formed thick, floccose, white-to-grayish colonies covering most of the agar surface (Figure 1). Microscopic observations confirmed these findings, displaying septate hyphae with characteristic conidial structures for *Aspergillus* and *Penicillium*, and aseptate hyphae bearing prominent sporangia for *Mucor* and *Rhizopus* (Figure 2). The high prevalence of these potentially toxigenic and spoilage fungi underscores a significant risk of mycotoxin accumulation, likely exacerbated by suboptimal storage conditions on local farms.

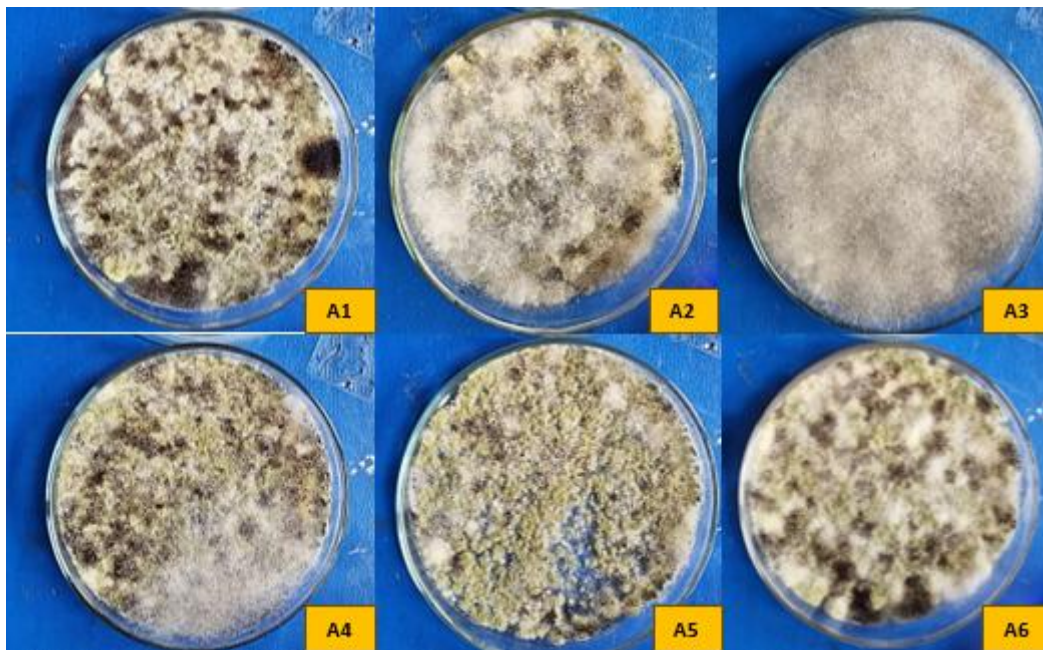


Figure 1. Macroscopic appearance of mold colonies on SDA medium. *Aspergillus* sp. and *Penicillium* sp. colonies appeared yellowish-green to blackish in color with whitish-gray margins and exhibited a powdery to granular texture (A1, A2, A4, A5, A6). In contrast, *Mucor* sp. and *Rhizopus* sp. colonies appeared thick, soft, and cotton-like (floccose), covering nearly the entire surface of the medium (A2, A3, A4, A5, A6).

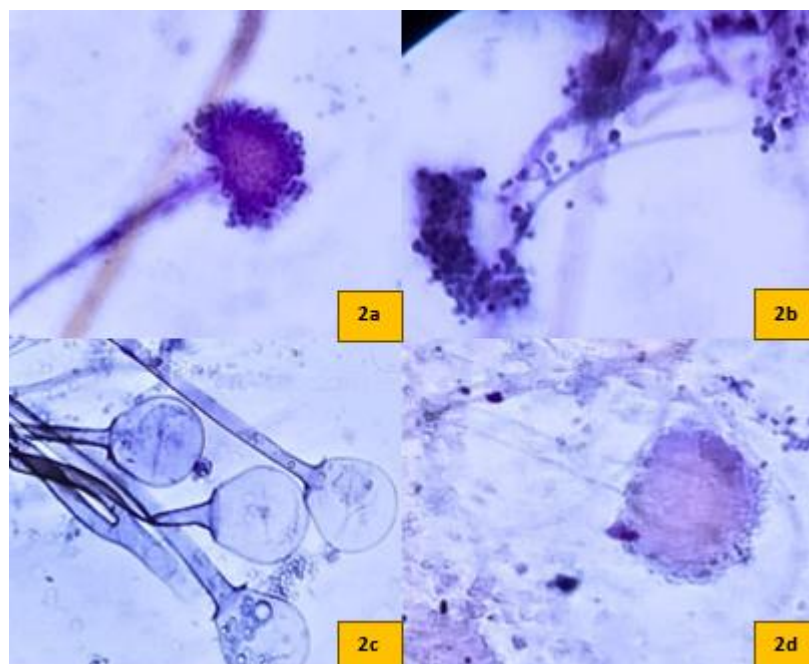


Figure 2. Mold genera isolated from pig feed on SDA medium: *Aspergillus* sp. (2a), *Penicillium* sp. (2b), *Mucor* sp. (2c), and *Rhizopus* sp. (2d).

Discussion

This study demonstrated that pig feed obtained from six farms in Bali was contaminated by various fungal genera, with a predominance of *Aspergillus* spp. and *Penicillium* spp., as well as the presence of *Mucor* spp. and *Rhizopus* spp. in several samples. Identification based on

macroscopic and microscopic morphological characteristics on SDA confirmed that this medium is effective as an initial screening method for detecting mold contamination in animal feed. Macroscopic and microscopic observations indicated that five of the six samples were contaminated with *Aspergillus* spp. and *Penicillium* spp.

The predominance of *Aspergillus* and *Penicillium* observed in this study is consistent with global reports identifying these genera as major contaminants throughout the production and storage chain of animal feed (Alkhursan et al., 2021; Khalifa et al., 2022). *Aspergillus* sections *Flavi* and *Nigri*, observed macroscopically, were characterized by yellowish-green to blackish colony coloration with a granular texture, reflecting intensive sporulation (Lučev et al., 2025; Salnus et al., 2020). *Penicillium* spp. were also identified in most samples, exhibiting macroscopic features of green to yellowish colonies with a velutinous texture (Noverita & Debby, 2023). Fungi belonging to the order Mucorales, namely *Mucor* spp. and *Rhizopus* spp., were detected in several samples and displayed cotton-like colonies ranging from white to gray in color (Figure 1). Macroscopically, *Mucor* typically forms large white or cream-colored colonies after 48–96 hours of incubation, which may subsequently turn grayish or brownish as sporulation progresses (Raposo et al., 2023). In contrast, *Rhizopus* sp. exhibits rapidly growing white, cottony colonies that later become grayish-brown during the sporulation phase (Rudramurthy et al., 2022).

Microscopic identification further supported the macroscopic findings (Figure 2). Microscopically, *Aspergillus* spp. were characterized by branched, septate hyphae; erect conidiophores bearing terminal vesicles; and chains of conidia, which constitute the principal diagnostic features of this genus (Tjampakasari et al., 2023). *Penicillium* spp. exhibited hyaline, septate hyphae with erect, branched conidiophores forming a penicillus structure, supporting flask-shaped phialides that produce chains of globose to ellipsoidal conidia (Noverita & Debby, 2023; Tarigan et al., 2025). *Rhizopus* sp. was characterized by aseptate hyphae, erect sporangiophores, and spherical sporangia with prominent columellae containing spherical to ellipsoidal sporangiospores (Rudramurthy et al., 2022). Meanwhile, *Mucor* sp. was identified by coenocytic hyphae (non-septate or sparsely septate) with simple or branched sporangiophores terminating in globose sporangia filled with spherical to ellipsoidal sporangiospores, and by the absence of distinct rhizoids and stolons (Ziaee et al., 2016).

The concordance between macroscopic and microscopic characteristics confirms that morphological approaches remain relevant and reliable for the initial surveillance of fungal contamination in feed, particularly in diagnostic laboratories with limited access to molecular facilities (Pitt & Hocking, 2009). The presence of *Aspergillus* and *Penicillium* in feed samples is generally associated with growth during the storage phase. Both *Aspergillus* spp. and *Penicillium* spp. are classified as storage fungi and commonly proliferate in stored feed materials under conditions of high humidity and inadequate ventilation, thereby increasing the risk of contamination (Khalifa et al., 2022). Environmental conditions and suboptimal storage practices significantly contribute to the occurrence of contamination by these genera in animal feed samples (Zeweld et al., 2025).

From a pig health perspective, the predominance of *Aspergillus* and *Penicillium* has important implications, as *Aspergillus*, particularly the *Flavi* group, is a major producer of aflatoxins with hepatotoxic and immunosuppressive effects that can impair growth performance (Ahmad et al., 2025). *Penicillium* spp., on the other hand, have the potential to produce ochratoxins that are nephrotoxic and, when chronically ingested through feed, may disrupt renal function, reduce feed efficiency and productivity, and increase susceptibility to infectious diseases (Malekinejad & Fink-Gremmels, 2020). Nevertheless, the presence of toxigenic fungi in this study does not necessarily indicate that the feed is hazardous, as previous studies have reported that detected

toxin levels are often well below the established maximum residue limits (MRLs) (Khalifa et al., 2022). In addition to the dominance of *Aspergillus* and *Penicillium*, *Mucor* spp. and *Rhizopus* spp. were also identified in pig feed, reflecting contamination by molds of the order Mucorales, a group of fungi commonly associated with feed materials during storage and processing (Rudramurthy et al., 2022). The growth of these molds is typically linked to a decline in feed nutritional value due to the utilization of carbohydrates and other nutrients by fungal mycelia, which may indirectly reduce feed intake and feed conversion efficiency as the feed becomes less nutritious and less palatable to livestock (Osman, 2024).

The high prevalence of fungal contamination observed in this study is likely influenced by suboptimal storage conditions, including high humidity, prolonged storage duration, and inadequate periodic quality control (Kulczyk-Małysa & Bogusławska-Wąs, 2025). Numerous studies have demonstrated that humid storage environments and poor ventilation create ideal conditions for mold growth and mycotoxin production in feed, and that the microbiological quality of feed is strongly affected by storage temperature and humidity (Kushwaha et al., 2025). Therefore, appropriate storage management is essential to maintain feed quality and reduce the risk of fungal contamination. Although this study successfully identified the genera of contaminating fungi, limitations remain, as identification was restricted to the genus level based on morphological characteristics. This approach does not allow for precise determination of toxigenic species or the actual risk of mycotoxicosis. Consequently, further studies employing molecular approaches and mycotoxin analyses are strongly recommended to complement these findings and to provide a more robust scientific basis for the control of pig feed safety.

CONCLUSIONS AND SUGGESTIONS

Conclusions

All pig feed samples collected from six farms in Bali were contaminated with fungi, predominantly *Aspergillus* spp. and *Penicillium* spp., with the presence of *Mucor* spp. and *Rhizopus* spp. detected in several samples. Morphological identification using SDA proved effective as an initial screening method for mold contamination in feed and indicated a potential risk of mycotoxin exposure associated with feed storage management.

Suggestions

Routine monitoring of feed quality and improvements in storage management are strongly recommended, particularly with respect to moisture control and ventilation. Further studies are suggested to include mycotoxin analysis and molecular-based confirmation of fungal identification to provide a more comprehensive assessment of feed safety.

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