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A review of filamentous fungi in broiler production



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ABSTRACT

The relationship between filamentous fungi and broiler chicks has long been recognized. In the past, filamentous fungi have been attributed to the disease occurrence and feed contamination causing substantial economic loss in broiler production. Currently, the relationship is expanded to the use of filamentous fungi as probiotics, fermentation starters, antioxidant sources and enzyme producers that can exert beneficial impacts on broiler production. This present review provides a summary of the role of filamentous fungi in broiler production.

1. Introduction

Filamentous fungi have long been known to play a significant roles in food processing. In broiler production, filamentous fungi had previously been attributed to disease occurrence, feed contamination and production of mycotoxins. These conditions may consequently result in big economic loss. Several species of the filamentous fungi have been identified as disease agents and feed contaminants (Dhama et al., 2013; Ghaemmaghami et al., 2016). Hence, the fungal decontamination program should be conducted to ensure the health of birds as well as the quality and palatability of feed. Apart from their harmful effects, recent studies showed the potential of some filamentous fungi as probiotics (Saleh et al., 2011a; Sugiharto et al., 2017). This property is useful in improving the growth performance and health of broilers in the post-antibiotic era. The potential of filamentous fungi as fermentation starters has also been recently documented (Lateef et al., 2008). It seems that fungal fermentation is an effective means to improve the nutritional qualities of particularly inconventional feed ingredients for broiler chicks. In this regard, fungal fermentation may help to reduce the use 10 expensive conventional feed ingredients in broiler rations. Today, synthing antioxidants have commonly been used in broiler production. However, the excessive use of synthetic antioxidant may imply in carcinogenic effect on human as consumers (Fellenberg and Speisky, 2006). Several studies have confirmed the potential of filamentous fungi as antioxidant sources (Sugiharto et al., 2015, 2016, 2017), and this may thereby answer the concern related to the use of synthetic antioxidant in broiler production. The use of exogenous enzymes has commonly been practiced in broiler production to increase the digaribility of chicks. Today, almost half of the commercial enzymes are produced by the filamentous fungi through fermentation (McKelvey and Murphy, 2017). This present review aimed to provide the comprehensive insight of filamentous fungi in broiler production.

2. A brief about filamentous fungi

Fungi are special type of microorganisms that are plenteous and widespread in nature. In general, fungi are 4 pwn as decomposers and also parasites of animals and plants. Unlike other microbes, fungi have no ability to produce their own food as they are devod of chlorophyll. To maintain their existences, fungi obtain food either from living plants and animals (living host) or from dead and decaying plants and animals. The fungi secrete extracellular enzymes that can break down complex organic compounds into their monomeric compounds. These simple compounds are eventually utilized by the fungi as sources of energy, carbons and other nutrients (Cole, 1996; Madigan et al., 2009). Hence, fungi are essential for recycling the dead plants and animals in nature (Rawat, 2015). Recently, approximately 100,000 species of fungi have been described, including mushrooms (macrofungi), yeast and molds (microfungi/filamentous fungi) (Madigan et al., 2009). By definition, yeasts are unicellular microorganisms that reproduce by budding. With regard particularly to molds, these microscopic fungi are characterized by multicellular filaments termed hyphae. Such filaments are essential to support spores for reproduction and propagation. Walker and White (2017) described that hyphae extend and branch within the supporting substratum as a network, called a mycelium that can be seen without microscope. The hyphae may grow by an apically extending network of mycelium to search, exploit and translocate available nutrients. In general, apically growing hyphae have a diameter of about 1–30 μm or more, depending on the species and growth conditions of fungi.

The filamentous fungi has long been known to reproduce by sexual as well as asexual means, but Madigan et al. (2009) pointed out that

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most of filamentous fungi reproduce asexually. Nuclear fusion and subsequent production of spores (sexual spores) through meiotic processes are the principal process involved in the fungal sexual reproduction. While, the production of propagules such as conidia (asexual spores) through mitotic processes is the primary activity of filamentous fungi during the asexual reproduction (Dyer and Paoletti, 2005; Walker and White, 2017). In the favourable environment, asexual or sexual spores can germinate and grow into a new hypha and mycelium. In general, the methods of reproduction in filamentous fungi depend largely on nutritional and environmental conditions. Dyer and Paoletti (2005) noticed that some fungi reproduce asexually when the nutritional and environmental conditions are favourable, but they reproduce by sexual means when the nutrient availability is low. In the laboratory, filamentous fungi may be grown on liquid or solid media, but the fungi seem to better grow on solid media (Rawat, 2015; Walker and White, 2017). Most of the filamentous fungi grow at a pH of 3 to 8 and some of them may survive at very low water activity level (i.e., 0.7 to 0.8) (Rawat, 2015). According to Meletiadis et al. (2001), there are five different phases in the growth of filamentous fungi. These phases are lag phase, the first transition period, the log phase, the second transition period, and the stationary phase. With regard particularly to the log phase, this phase is characterized by a substantial increase in cell mass and therefore determines the production of the fungal bio-

3. Filamentous fungi as feed contaminants

Feed is one of the most important constituents in broiler production. Beside the quantity, the quality and palatability of feed have been a considerable concern for long time. Among the factors affecting the quality of feed, feed contamination by the filamentous fungi has been reported to negatively influence the organoleptic properties and the qualities of broiler feed (Greco et al., 2014). The filamentous fungi may assimilate, grow and utilize the readily availate nutrients in broiler feed (Ghaemmaghami et al., 2016). The latter condition may therefore result in nutrial loss and thereby imbalance nutrients in the feed. breover, the growth of the filamentous fungi may lead to spoilage, producing sour odour and thus adversely affecting the organoleptic characteristics of the feed (Schnürer et al., 1999). This condition may eventually reduce the palatability and thus feed intake and production performance of broiler chickens. Several studies have been conducted to identify the common species of the filamentous fungi that often contaminate the broiler feeds. These filamentous fungi include Aspergillus sp., Penicillium sp., Mucor sp., Rhizopus sp., Epicoecum sp., Cladosporium sp., Fusarium sp. and Mortierella sp., with Aspergillus sp. seems to be more dominant than the other filamentous fungi (Okoli et al., 2006, 2007; Kmjaja et al., 2014; Vera et al., 2016). In respect particularly to Aspergillus sp., A. flavus and A. niger are the most contaminants in broiler feeds (Vera et al., 2016). Indeed, fungal contamination of broiler feed is more prone in tropical countries than in the other regions (Okoli et al., 2007). It seems that tropical regions with high environmental temperature and humidity favour the growth of filamentous fungi. Likewise, seasonal variation is also seen with regard to the fungal contamination in broiler feeds. Investigation conducted by Okoli et al. (2006) in Nigeria confirmed that fungal contamination in broiler feed is more frequent during the rainy season (with high humidity level) than during the dry season. Study showed that the raw material feeds with high content of carbohydrate such as maize and bran seem to be more susceptible to the contamination by filamentous fungi than those with relatively low carbohydrate content (Vera et al., 2016). In such case, carbohydrate rich feedstuffs may be a good medium for the growth of the filamentous fungi.

In broiler farm, feed storehouse has an important function to store the feeds before being given to the chicks. To maintain the nutritional quality and organoleptic properties of broiler feed during the storage, optimal temperature and humidity within the storehouse is crucial as under inappropriate storage condition the filamentous fungi may grow and thereby damage the feed. Beside contamination during storage, most of raw feed ingredients are contaminated by the filamentous fungi before entering the processing unit in feed plants. Vieira (2003) reported that some filamentous fungi contaminate the crops in field and may continue to extend through post-harvest, processing and formulation of finished feeds. Other than generating significant damage in feed, the growth of filamentous fungi on feed may result in the production of allergenic spores and mycotoxins (Schnürer et al., 1999), which can harm the health of chicks. Owing to this fact, it is therefore important to monitor and eradicate the grown filamentous fungi on the feeds before and after entering the feed processing plants. To alleviate the fungal contamination in crops (raw feed ingredients) prior to harvesting, de Oliveira et al. (2018) suggested to implement the good farming practices and to select the plants that are resistant to fungal infections. It is also necessary to harvest the crops at the right stage (mature stage) and not to damage the grains as the damaged grains may easily be contaminated by the filamentals fungi due to the lack of protective capacity of the cell wall (Filazi et al., 2017; de Oliveira et al., 2018). Moreover, the grains should be dried properly following the harvesting to reduce the moisture content (de Oliveira et al., 2018). Indeed, humidity exceeding 11% may support the growth of filamentous fungi in feed. Taking these conditions into the consideration, it is therefore essential to ascertain the good ventilation of the storehouse so that the humidity inside the storehouse could be kept low. Furthermore, storing the feed in the farm storehouse only for a short period seems to be helpful in preventing the fungal growth and mycotoxins production in feed during the storage in storehouse (Filazi et al., 2017).

To produce the good and safe feed for broilers, the fungal decontamination program has commonly been conducted by the feed mill industries. The program is crucial to eradicate the fungi originated from the field as well as from contamination during the harvesting and transportation. Some decontamination procedures may be implemented, including biological (using microorganisms, through fermentation), physical (heating, radiation) and/or chemical (adding specific chemical compounds functioning as antifungal agents or antimolds) methods. Of the procedures, de Oliveira et al. (2018) suggested that decontamination program based on the biological methods was the best as the physical and chemical decontamination procedures may be costly and result in nutrient loss. In respect particularly to chemical procedure, antimolds and mycotoxin binders have commonly been incorporated in the finished feeds. Antimolds or antifungal agents are intended to inhibit the fungal growth, whereas mycotoxin binders may reduce the toxicity of mycotoxins in broiler chickens. The mycotoxin binders may strongly bind to the mycotoxins and thereby the exposure of mycotoxins to the digestive tract of the chickens could be prevented. Some mycotoxin binders may also inactivate and neutralize the mycotoxins in the gastrointestinal tract of broiler chickens (Filazi et al., 2017; de Oliveira et al., 2018).

4. Filamentous fungi as disease agents

It has long been known that although less prevalent than other microbes, filamentous fungi may cause disease in domesticated animals (Madigan et al., 2009). In poultry, several studies have documented the role of filamentous fungi as disease agents. Aspergillosis (caused by Aspergillus fumigatus), dactylariosis (caused by Dactylaria gallopava) and favus (caused by Microsporum gallinae) are among the diseases in broiler chickens caused by the filamentous fungi (Beernaert et al., 2010; Arné et al., 2011; Dhama et al., 2013). The aetiology, diagnosis, treatment and control of these diseases are reviewed elsewhere (Dhama et al., 2013). The fungal-related diseases may cause a substantial economic loss in broiler industry as such diseases are attributed to high morbidity and mortality particularly in young broiler chicks. The diseases also cause diarrhoea and compromise growth performance in broilers (Dhama et al., 2013). In general, Friend (1999) proposed three modes

Table 1
Examples of filamentous fungi producing mycotoxins.

Filamentous fungi	Mycotoxins produced	References
A. flavus	Aflatoxins B ₁ and B ₂ , cyclopiazonic acid, kojic acid, beta-nitropropionic acid, aspertoxin,	Goto et al. (1996); Yu (2012); Liew and Mohd-
	aflatrem and aspergillic acid	Redzwan (2018)
A. parasiticus	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Yu (2012): Frisvad et al. (2019); Liew and Mohd
		Redzwa 5 2018)
A. pseudotamarii	Aflatoxin B ₁ and B ₂	Frisvad et al. (2019)
A. togoensis	Aflatoxin B ₁ and B ₂	Frisvad et al. (2019)
A. aflatoxiformans	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. cerealis	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. austwickii	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. arachidicola	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. minisclerotigenes	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. mottae	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. luteovirescens	14toxins B ₁ , B ₂ , G ₁ , G ₂ and tenuazonic acid	Frisvad et al. (2019)
A. nomius	Aflatoxins B1, B2, G1, G2 and tenuazonic acid	Pitt (2000); Frisvad et al. (2019)
A. novoparasiticus	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. pseudocaelatus	14toxins B ₁ , B ₂ , G ₁ , G ₂ and tenuazonic acid	Frisvad et al. (2019)
A. pseudonomius	Aflatoxins B ₁ , B ₂ , G ₁ , G ₂ and tenuazonic acid	Frisvad et al. (2019)
A. sergii	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. transmontanensis	Aflatoxins B ₁ , B ₂ , G ₁ and G ₂	Frisvad et al. (2019)
A. alliaceus s. str.	Ochratoxin A	Frisvad et al. (2019)
A. neoalliaceus	Ochratoxin A	Frisvad et al. (2019)
4. vandermerwei	Ochratoxin A	Frisyad et al. (2019)
A. bertholletius	Tenuazonic acid	Frisvad et al. (2019)
A. caelatus	Tenuazonic acid	Frisvad et al. (2019)
A. pseudotamarii	Tenuazonic acid	Frisvad et al. (2019)
A. tamari	Tenuazonic acid	Frisvad et al. (2019)
Penicil lium viridicatum	Ochratoxin A	Pitt (2000)
A. ochraceus	d4 atoxin A	Pitt (2000); Liew and Mohd-Redzwan (2018)
A. carbonarius	Ochratoxin A	Pitt (2000); Liew and Mohd-Redzwan (2018)
usarium monilifornte	Fumonisins	Pitt (2000)
F. graminearum	Deoxynivalenol, nivalenol, trichothecenes and zearalenone	Pitt (2000); Liew and Mohd-Redzwan (2018)
Penicil lium citrinum	Citrinin	Ames et al. (1976)
Penicil lium expansum	Patulin	Hammami et al. (2017)
Fusarium moniliforme	Fusaric acid, fumonisins and moniliformin	Porter et al. (1995)
Fusarium langsethiae	T-2 toxins	Krska et al. (2014)
изи ит индзении: 7. poae	T-2 toxins	Krska et al. (2014)
. poue . sporotrichioides	T-2 toxins	Krska et al. (2014) Krska et al. (2014)
Phomopsis leptostromiformis	Phomopsins	Battilani et al. (2011)
Phomopsis teptostromijormis Claviceps purpurea	Ergot alkaloids	Grusie et al. (2011)
Neotyphodium coenophialum	Ergopeptine alkaloids	
Neotypnoa:um coenopnia:um Penicil lium citreoviride	Citreoviridin	Sobhani Najafabadi et al. (2010) Nishie et al. (1988)
Peniculium citreoviriae Fusarium verticillioides	Fumonisins	
rusariam veracunoides	rumonisins	Murugesan et al. (2015); Liew and Mohd-Redzw
Cooklinkship has and	O	(2018)
Cochliobolus kusanoi	Oosporein	Ramesha et al. (2015)

of actions by which filamentous fungi cause diseases in poultry, i.e., invading directly into animal tissues, inducing host hypersensitivity (leading to allergic response) and producing toxins. Among the body organs, the pathogenic fungi primarily hvade the respiratory and nervous system of broilers. This fungal invasion may induce specific physiological alterations such as inflammations, lesions and thus illness (Dhama et al., 2013). Naturally, immune defence systems of broiler chickens are activated in response to incoming fungal antigens (spores) (Nichita et al., 2010). This immune activation is a normal response to condition that may threaten the body of chickens. However, an excessive immunologic response may cause hypersensitivity leading to allergic diseases and tissue damage (Friend, 1999). The production of toxins (mycotoxins) by the filamentous fungi has been attributable to mycotoxicoses. The toxins are mainly produced by the toxigenic filamentous fungi 13 h as Aspergillus, Fusarium, Penicillium and other species (Table 1). Ingestion of high levels of mycotoxins may result in high mortality, whereas moderate levels of mycotoxins can suppress the immune systems of broiler chickens. The latter condition makes the chickens prone to several bacterial and viral infections (Asfaw and Dawit, 2017). In addition, mycotoxins also weaken the vaccine responses in broiler chickens and thereby compromise the immune defence against specific pathogens (de Oliveira et al., 2018). Table 2 shows some examples of mycotoxicoses in broiler chickens.

As previously mentioned, the filamentous fungi are widespread in

nature and by their spores, the pathogenic fungi may spread the diseases across the animals. In poultry house, Nichita and Tirziu (2008) and Nichita et al. (2010) found a high concentration of the fungal spores particularly from the species of A. funigatus, A. flavus, Penicillium crysogenum, Cladosporium cladosporiodes and Scopulariopsis. These fungal species are known as allergenic fungi, and therefore exposure to these spores may trigger health problem in broiler chickens. In response to these fact, eliminating the pathogenic filamentous fungi from the broiler house can avoid the chicks from the fungal-related diseases.

5. Filamentous fungi as growth inhibitor

The impaired quality and palatability of the fungal contaminated-feeds has been attributed to the reduced feed intake and nutrient utilization by broiler chickens. Such condition may therefore lead to malnutrition and impaired growth performance in broiler chickens (Grenier and Applegate, 2013; Greco et al., 2014). As mentioned in the earlier section that filamentous fungi may act as disease agents in broiler chickens. Considering that infected animals may allocate more energy for maintenance and recovery from disease, the fungal infected chickens may therefore reduce the energy for growth and thereby compromise the growth performance. The harmful effect of mycotoxins should always be monitored and anticipated by the broiler producers. Besides impairing the immune defence, mycotoxins have been shown to

Table 2
Examples of mycotoxicoses in broiler chickens.

Mycotoxicoses	Mycotoxins causing mycotoxicoses	References
Aflatoxicosis	Aflatoxins	Rashid et al. (2013)
Fusariotoxicosis	Trichothecenes	Katkiewicz et al. (1991)
Ochratoxicosis	Ochratoxins	Elaroussi et al. (2006)
Ergotism	Ergot alkaloids	Dänicke (2017)
Trichothecene mycotoxicosis	Trichothecene mycotoxins	Hoerr et al. (1982)
Citrinin mycotoxicosis	Citrinin	Mehdi et al. (1981)
Oosporein mycotoxicosis	Oosporein	Rottinghaus et al. (1989)

induce inflammation in broiler chickens. This condition may consequently reduce feed intake and, concomitantly, energy is directed to cellular defence, instead of for growth (Wang and Hogan, 2019). Study showed that mycotoxins may attenuate the intestinal functions leading to poor productivity in broiler chickens (Grenier and Applegate, 2013). The latter workers documented that mycotoxins decreased the digestive enzymes activity in the intestine resulting in lower nutrient digestibility. Moreover, mycotoxins disturbed the balance of intestinal microflora and integrity of the intestinal mucosa, which may therefore negatively affect the nutrient absorption capacity of broiler chickens. In agreement with the previous authors, Wang and Hogan (2019) reported that mycotoxins shortened the villi height and shallow the crypt resulting in depressed nutrient digestion and absorption and thus growth rate in broilers. At the level of cell, Murugesan et al. (2015) and Filazi et al. (2017 13 cumented that mycotoxins may down-regulate the expression of genes associated with energy production and fatty acid 13 abolism (carnitine palmitoyl transferase). The genes responsible for growth and development (insulin-like growth factor 1), antioxidant protection (glutathione S-transferase) and immune protection (interleukins) are also downregulated when the chickens ingest feed containing mycotoxins. Moreov13 mycotoxins may inhibit the protein synthesis in broiler chickens by interfering with transcription and other factors of protein synthesis (Murugesan et al., 2015; de Oliveira et al., 2018). Overall, the reduction rate in protein synthesis and changes in energy metabolism may result in impaired growth performance of broiler chickens.

6. Filamentous fungi as probiotics

Probiotics have attracted a considerable interest among the poultry nutritionists as an alternative for in-feed antibiotics (Sugiharto, 2016). Today, several types of probiotics have been used in broiler production, among which bacteria-based and yeast-based prolinities are the most popular. Many filamentous fungi have a 🛂 been used as probiotics in broiler production. Saleh et al. (2011a) reported that Aspergillus awamori-based probiotic improved growth performance, increased muscle vitamin E content, modified the skeletal muscle fatty acid profile and decreased lipid peroxidation in the muscle of broiler chickens. In addition to th⁹ growth promoting effect, Saleh et al. (2011b) demonstrated the capability of A. awamori in alleviating the muscle protein breakdown, abdominal fat content and plasma cholesterol of broilers. Corresponding results were also confirmed by Yamamoto et al. (2007) when using A. awamori as probiotic for broiler chickens. They found that A. awamori improved growth performance, nutrient digestibility and carcass weight of broiler chicks. Aspergillus niger is another filamentous ligus exhibiting probiotic properties. Saleh et al. (2011b) revealed that feeding A. niger as probiotic resulted 111 mproved growth performance in broiler chicks. The treatment also decreased saturated fatty acid and increased unsaturated fatty acids in the muscle fat of broilers. In agreement with this, earlier study by Saleh et al. (2010) demonstrated the potentia of A. niger in improving the growth performance, decreasing the muscle protein breakdown, abdominal fat content and cholesterol content in plasma. The latter workers also noticed an antioxidative activity of A. niger, and that feeding such filamentous fungus resulted in increased muscle α-tocopherol content and decreased concentrations of thiobarbituric acid reactive substance (TBARS) in muscle and glutamic-oxalacetic transaminase activity in the liver of broilers. Aspergillus oryzae is another filamentous fungus that has widely been used as probiotics in poultry. Lee et al (2006) documented that administration of this fungus was able to improve the growth performance, nutrient digestibility and intestinal health of broilers. Another example of the filamentous fungus with probiotic properties is Chrysonilia crassa. In broilers, feeding with C. crassa was beneficial in improving the physiological conditions and antioxidant status of broilers exposed to heat stress (Sugiharto et al., 2017). In the later study, dietary supplementation with C. crassa improved the growth, immune responses, and intestinal bacterial populations of broilers (Sugiharto et al., 2018a). Acremonium charticola is other filamentous fungus that has been tested to have probiotic potential (Sugiharto et al., 2015). Feeding such fungus has been reported to improve the intestinal microbial balance as well as protect the broiler chicks from infections (Sugiharto et al., 2018a). Scytalidium acidophilum is other filamentous fungus that has been used as probiotic for improving the growth performance in broilers (Huang et al., 2004). Likewise, Rhizopus oligosporus has been confirmed to exhibit probiotic potential. In pigs, treatment with R. oligosporus resulted in higher body weight gain, improved nutrient digestibility, intestinal bacterial population and immune defence (Park et al., 2016). Yet, the data on broiler chickens is scarce.

In general, there are some characteristics that make filamentous fungi-based probiotic suitable as in-feed antibiotic substitute for broilers. These characteristics include antimicrobial activity, gut microbiota modulating capacity, digestive stimulating activity, growth promoting activity and cholesterol lowering capacity (Lee et al., 2006). In controlling the pathogenic microorganisms in the intestine, Sugiharto et al. (2015) suggested that the filamentous fungi may produce various form of antimicrobials that can impair the biological functions of pathogenic microbes. In term of digestive stimulation, Lee et al. (2006) pointed out that the filamentous fungi may produce proteolytic and amylolytic enzymes that may help to digest the ingested feed. The fungal probiotics may also elevate the digestive enzyme activities resulting in improved digestion and feed utilization by the birds (Saleh et al., 2014). A number of studies have confirmed the mechanisms through which the fungal probiotics improve the growth performance of broilers. It appears that fungal probiotics im 10 ve feed digestibility and thereby increase the nutrient availability for broiler chickens (Saleh et al., 2014; Park et al., 2016). Moreover, Yamamoto et al. (2007) suggested that fungal probiotics may produce growth promoters and thereby boost the growth rate of broilers. Further, the reduced degradation of skeletal muscle protein and the increased muscle protein synthesis may also responsible for the increased growth rate in broilers treated with the fungal probiotics (Saleh et al., 2014). In the study of Saleh et al. (2010, 2014), it was apparent that the fungal probiotics decreased the concentration of plasma 3-methylhistidine (biomarker for muscle protein turnover) and gene expressions of proteolysis-related factors in muscle, and on the other hand increased mRNA expressions of myosin and actin and thus protein synthesis in broilers. Finally, the capability of fungal probiotics in alleviating the infections in broilers (Sugiharto et al.,

2018a) seems also to contribute for the growth as more energy is allocated for growth rather than for maintenance and recovery.

Today, meat quality of broilers has been a concern as the consumers believe that consumption of meat with high saturated fat content can induce health problems. Dietary supplementation of fungal probiotics has been a part of the nutritional approach to improve the fat profile in broiler chickens (Saleh et al., 2010, 2011b). It is largely unknown how the fungal probiotics interfered fat composition in broilers, but Saleh et al. (2014) proposed that fungal probiotics may modify the mRNA expression of delta-6 fatty acid desaturase (responsible for the synthesis of polyunsaturated fatty acids) in the muscle of broilers. With regard to the cholesterol reducing effect of the fungal probiotics, Hajjaj et al. (2005) showed that filamentous fungus A. oryzae produces compounds inhibiting cholesterol biosynthesis. Furthermore, Mersmann (1998) documented that some filame 18 us fungi such as Asperyillus sp. decreased the fatness in animals by influencing the activities of hormone sensitive lipase and malate dehydrogenase enzyme in adipose tissues.

7. Filamentous fungi as fermentation starters

Fermentation has become a popular method in broiler industry to improve the nutritional contents of feed. Fermentation has also been directed to produce functional properties that may be beneficial for broiler chickens. The detailed explanation regarding to the fermented feed is reviewed elsewhere (Sugiharto and Ranjitkar, 2019). To proceed and control the fermentation process, the presence of starter cultures (microorganisms) is crucial. There are various types of microorganisms that can be employed as starter cultures during the fermentation process. Besides bacteria and yeast, filamentous fungi are other microbes that may be used as fermentation starters. Several filamentous fungi have been reported to improve the nutritional contents of feed or feed ingredients. For instance, Lateef et al. (2008) reported that fermentation with filamentous fungus Rhizopus stolonifer LAU 07 decreased the 16 e and cyanide contents, while increased the content of protein of cocoa pod husk, cassava peel and palm kernel cake. Other study showed that fermentation using A. niger increased crude protein and essential amino acids, while decreased crude fibre, cellulose, hemicellulose, neutral detergent fibre and nitrogen free extract of palm kernel cake (Marini et al., 2008). Earlier study also showed that fermentation using A. niger could lower the fibre a 10 elevate the crude protein content of cassava solid waste (Obadina et al., 2006). In accordance with this, Ramin et al. (2011) noticed the capacity of A. niger in increasing the crude protein content of cassava. Our study further documented that A. charticola and R. oryzae were capable of increasing the crude protein and lowering the fibre contents of cassava pulp (Sugiharto et al., 2015). Recently, we also found that fermentation with R. oryzae or C. crassa as starter cultures was capable of decreasing the fibre contents of rice bran (Sugiharto et al., 2017). Solid-state fermentation using C. crassa also decreased crude 12 re and elevated crude protein and amino acids contents of some agro-industrial by-products such cassava pulp, banana peel and rice bran (Sugiharto et al., 2018b). Another fungal species that has commonly been used as fermentation starter is Trichoderma sp. In the study of Obadina et al. (2006), it was noted that Trichoderma sp.fermentation increased and decreased the contents of crude protein and fibre, respectively, of the cassava solid waste. In accordance with this, fermentation using Trichoderma viride resulted in increased crude protein, true protein and ash content in cassaya peel (Ezekiel and Aworh, 2013). The latter fermentation also decreased crude fibre, crude fat and cyanide in cassava peel. Likewise, T. viride-fermentation was associated with the reduced fibre and increased crude protein contents in copra meal (Hatta et al., 2014). Apart from the successful attempt to improve the nutritional metrics of feed ingredients, some studies reported differently. For instance, Ramin et al. (2011) failed to increase protein content in cassava through fermentation using R. oryzae as a fermentation starter. Also, fermentation using R. oryzae or C. crassa did not substantially increase the crude protein content of rice bran (Sugiharto

et al., 2017). The differences in fungal species, nature of substrates and fermentation conditions seem to be responsible for the above discrepancy.

In terms of the nutritional improvement, decreasing and increasing of fibre and protein contents of the substrates seem to be the main focus of fermentation (Lateef et al., 2008; Sugiharto et al., 2017, 2018b). It was confirmed by literature that filamentous fungi were able to break down the less accessible lignocellulosic materials (Kazda et al., 2014) and thereby help to lower the fibre content of particularly inconventional feed ingredients. Through their extracellular lignocellulolytic enzymes, the filamentous fungi may degrade lignin, which is the most recalcitrant substance of the lignocellulosic stuffs (Dashtban et al., 2010; Znameroski and Glass, 2013). The fibrolytic activity of the fungi may also be assisted by rhizoids that can penetrate deep into the recalcitrant components of plant cells. Upon the penetration, the fungal enzymes can then degrade the plant cell v12 structures (fibre fraction). In term of protein content enhancement, Liang et al. (2009) suggested that the increased production of the fungal extracellular protein during fermentation process may contribute for the increased protein content of the 16 strates. Indeed, the fungal hyphae growing during fermentation may account for the increased protein content of the substrates as the fungal hyphae can serve as single cell protein (Lateef et al., 2008). Moreover, the ability of the fungi to produce enzymes that can hydrolyse amylum and non-starch polysaccharides to monosaccharides may also help to increase the protein content in the fermented products, as monosaccharides are conveniently processed to protein (Bayitse et al., 2015).

8. Filamentous fungi as natural antioxidant sources

Antioxidants are molecules that can alleviate the detrimental effect of oxidative stress. In broiler industry, synthetic antioxidants have commonly been used as feed sup 17 nents particularly during heat stress and around the vaccination. However, the excessive use of synthetic antioxidants may implicate in carcinogenic and/or mutagenic effects on consumers (Fellenberg and Speisky, 2006). In this response, nutritionists are now searching for the natural antioxidants for commercial broiler chickens. Among the source of natural antioxidants, filamentous fungi have 6 great potential. Smith et al. (2015) showed that filamentous fungi Grifola frondosa, Monascus purpureus, Pleurotus spp., Lentinula edodes and Trametes versicolor are good sources of natural antioxidants. Likewise, Sugiharto et al. (2015, 2016, 2017) noticed the potential of A. charticola, R. oryzae and C. crassa as good sources of natural antioxidants. Also, Hameed et al. (2017) revealed the antioxidant potential of the filamentous fungus Mucor circinelloides. In addition to the above mentioned fungi, other filamentous fungi such as Aspergillus PR78 and Aspergillus PR66 (Arora and Chandra, 2010), Aspergillus candidus, Penicillium roquefortii, Emericella fal 6 ensis, Mortierella, Colletotrichum gloeosporioides (Rios et al., 2006), Chaetomium sp., Cladosporium sp., Torula sp., Phoma sp. (Huang et al., 2007), Antrodia camphorata (Song and Yen, 2002) and Mycelia sterilia (Moon et al., 2006) also possess antioxidant properties.

Phenolic compounds, flavonoids and condensed tannins have generally been attributed to the antioxidant properties. With regard to filamentous fungi, Sugiharto et al. (2016) suggested that the antioxidant capacity of *A. charticola* was contributed mainly by the phenolic and tannins contents in the fungal mycelium. In agreement, Arora and Chandra (2010) noted the attribution of phenolic compounds to the antioxidant activity of *Aspergillus* sp. extracts, while Hameed et al. (2017) documented the role of phenols, flavonoids and condensed tannins in determining the antioxidant capacity of *Mucor circinelloides*. Moreover, the presences of antioxidant enzymes (such as superoxide dismutase and catalase), ascort $\frac{10}{10}$ cid, γ -linolenic acid, β -carotene, tocopherols and polysaccharides may also contribute to the antioxidant properties of the filamentous fungi (Prior et al., 2005; Tosi et al., 2010; Smith et al., 2015; Hameed et al., 2017).

Besides being used as antioxidant sources, the filamentous fungi have also been exploited to increase the antioxidative properties of feed or feed ingredients by means of 16 mentation. Lateef et al. (2008) reported that fermentation using R. stolonifer [16] 07 increased the antioxidant activities (scavenging capacity) of cocoa pod husk, cassava peel and palm kernel cake. Likewise, other study showed the increase in total phenols, flavonoids and antioxidant activities of oat following solid-state fermentation using A. oryzae var. effuses, A. oryzae and A. niger (Cai et al., 2012). In line with this, Dev and Kuhad (2014) demonstrated the enhanced antioxidant potentials in wheat after solidstate fermentation using R. oryzae RCK2012. In addition to the increased mycelium (rich in antioxidative properties) density in the substrates, the increased antioxidant activity of the substrates see 12 o be attributed to the increased release of antioxidative compounds from their complex bindings as well as the increased synthesis of antioxida 10 components during the fermentation process (Hur et al., 2014). In contrast to the above studies, Sugiharto et al. (2018b) reported that solid-state 12 rmentation with C. crassa reduced the levels of polyphenols, tannins, and antioxidant activity of banana peel meal. The latter worker sugilated that prolonged period of fungal fermentation may damage the antioxidant components and thus antioxidant activity of the substrates. Hence, the appropriate period of fungal fermentation is crucial for the content of antioxidative properties of the substrates.

9. Filamentous fungi as enzyme producers

Dietary supplementation of enzymes has been practiced in broiler production to improve the digestibility and thus nutrient utilization by the chicks. In addition to bacteria and yeast, filamentous fungi have long been exploite to produce enzymes. Compared to other microbes, filamentous fungi have attracted more interest as an enzyme producer. This because filamentous fungi are easy to be grown and produce higher e 🛂 acellular enzymes (Guimarães et al., 2006; Jun et al., 2011). Indeed, among the enzymes available in the market today, almost half are produced by the filamentous fungi (McKelvey and Murphy, 2017). Several species and strains of the filamentous fungi have been elucidated for their potential to produce enzymes. For example, Guimarães et al. (2006) screened some filamentous fungi including Aspergillus caespitosus, A. phoenicis, Rhizopus stolonifer, A. versicolor, Mucor rouxii, Paecilomyces variotii, A. phoenicis and Rhizopus microsporus var. rhizopodiformis for their capacity to produce xylanase, glucose-oxidase, alkaline phosphatase, acid phosphatase, phytase, pectinase and amylase. In dition, McKelvey and Murphy (2017) revealed that fungal strains of Aspergillus, Rhizopus and Penicilium were capable of producing protease, cellulase, xylanase, lipase, amylase and phytase that are essential in broiler production. Moreover, A. niger and T. viride were exploited to produce cellulase during fermentation (Mrudula and Murugammal, 2011; Hatta et al., 2014). It was shown from the literature that each fungal species/strain may be better in producing particular enzyme. Guimarães et al. (2006) noticed that A. caespitosus and A. phoenicis was good producer of xylanase, while R. stolonifer and A. versicolor were best producer of glucose-oxidase. Hence, the screening of filamentous fungi for the most relevant producer of specific enzyme is necessary.

Fungal enzyme production has been conducted through solid-state and sub-merge fermentation (Mrudula and Murugammal, 2011; Viniegra-González et al., 2003). However dudy showed that solid-state fermentation seems to yield better result when using filamentous fungi (Mrudula and Murugammal, 2011). This because filamentous fungi are better to grow on solid materials with low water activity (Rawat, 2015; Walker and White, 2017). In addition to the fungal species/strains and fermentation methods, the production of enzyme may also be affected by the cultural conditions such as the nature of substrates, temperature, oxygen, etc.

10. Conclusion

The role of filamentous fungi is not limited to disease agents and feed contaminants, but the fungi may also bring beneficial effects on health and growth performance of broilers. The filamentous fungi may act as fermentation starters, antioxidant sources and enzyme producers.

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