MICROBIAL CONTAMINATION OF POULTRY FEED AND THE EFFECTS ON BIRDS' PERFORMANCE

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ABSTRACT

Poultry feeds are made with ingredients from different raw materials; therefore contamination with microbial agents is very common. When contaminated poultry feeds are consumed by birds, they may serve as reservoirs for many human pathogens. Bacterial pathogens seem to be the most prevalent, viral and fungal pathogens are also hazardous in poultry feeds. When microorganisms colonize poultry feeds, they utilize the readily available nutrients in the feed, thereby reducing its nutritional quality. Therefore, information on the diversity of microbial contaminants in feeds is important in designing effective feed monitoring and hazard control strategies. Some of the established contaminants of poultry feeds are bacterial and fungal agents and their secretory products. These microbes are released in the feed through raw feed ingredients, stored feed products, during feed processing and handling as well as through other environmental sources. Contaminated poultry feed adversely affects feed intake, feed conversion ratio, weight gain, organ function and alters blood and clinical chemistry parameters of birds. This review provides background information on the various microbes that contaminate poultry feeds, their sources, the adverse impacts on the health and performance parameters of birds as well as their control strategies.

Keywords: Bacteria, Feed contamination, Fungi, Microbes, Poultry

INTRODUCTION

The presence of pathogenic microbial contaminants in livestock feed is a food safety issue because it constitutes a huge threat to animal and human health. Due to the burgeoning global population, increasing incomes and preference for white meat, especially in developing countries, the poultry industry has become the most rapidly growing livestock sector, which is projected to be the major industry to provide animal protein for the global consumption as from 2025 (Mottet and Tempio, 2017). The role of poultry in providing cheap quality protein (122.5 million tons/year), economic benefits, alleviating poverty and creating gender equality (especially

in developing countries) is well recognized (Mottet and Tempio, 2017). However, microbial-associated diseases, especially those involving viruses and pathogenic antimicrobial-resistant organisms, remain constraint to the poultry industry. The acquisition from feed is an established route through which livestock get colonized or infected by pathogenic microbes (Crump *et al*., 2002). Diverse microorganisms, including bacteria, fungi, protozoa and viruses can be acquired from the feed by livestock and humans (Roy *et al*., 2019). Microorganisms in poultry feed originate from diverse sources such as contaminated feed stuff/ingredients that are of plant and animal origin, handlers (preparers, and those that feed the animals) of livestock feed, vectors (which pick and deposit organisms on stored feed stuff/feed), and containers used in the preparation and packaging of feed (Maciorowski *et al*., 2007). Also, contamination of feed may happen during raw material processing, transport, storage, processing, or even from the bird housing.

Although dry, the poultry feed is a good substrate for microbial growth because it contains nutrients such as carbohydrate (carbon source), protein (nitrogen source), minerals (including limiting ones like iron), vitamins and essential amino acids, which support microbial growth, particularly fungi and bacteria (Ezekiel *et al*., 2012). Environmental factors such as moisture and elevated temperature also make the poultry feed/feed stuff suitable for microbial growth (Ezekiel *et al*., 2012). Bacteria that produce spores enter survival state until the moisture is high enough for germination to vegetative state (Maciorowski *et al*., 2007). However, molds are particularly adapted to the small amount of available moisture and grow actively within stored feed stuffs (especially seeds and grains) and feeds (Ezekiel *et al*., 2012). Nutrients in the feed are destroyed when used by organisms for growth, thus the nutrients become unavailable for the birds (Chattopadhyay, 2014). As microbes (bacteria and fungi) proliferate in poultry feed, they release metabolites (toxins) that elicit damaging effects on poultry birds following contaminated feed ingestion (Ezekiel *et al*., 2012). These metabolites are tasteless, odourless and heat-stable, enabling them to be undetected and consumed by poultry birds (Cegielska-Radziejewska *et al*., 2013).

When poultry feeds are contaminated with microbes, three major events are likely to take place; (i) decrease in feed composition and quality, which decreases performances, (ii) synthesis of toxins, which can decrease performance or alter the health depending on the toxins and the dose and (iii) colonization of the animal that ingest the feed leading to complex effects from beneficial effect (gut colonization and development of gut microbiota) to detrimental effects such as reduced feed efficiency. These negative effects may result to financial loss to the farmers due to poor feed conversion and reduced growth rate/weight gain, damage to body organs/tissues, organoleptic changes, reduced egg productivity, poor quality egg/meat, and mortality (Ezekiel *et al*., 2012).

Humans are also affected when these toxins (which have teratogenic and carcinogenic conditions) are ingested from poultry meat/egg (Cegielska-Radziejewska *et al*., 2013). It was recently demonstrated that a contaminated poultry feed-derived organism (specifically *Bacillus cereus*) aggravates viral disease (avian influenza) in poultry birds (Zhang *et al*., 2019). Although viruses do not proliferate outside host cells, they infect poultry birds following ingestion of feed contaminated by droplets from infected individuals (humans/animals) (Serbessa and Tucho, 2017).

Contaminated livestock feed is also a potential source for antimicrobial-resistant (AMR) organism; hence, AMR in livestock feed is also a food safety issue. Increasing the intensification of poultry remains one of the major causes of increased use of antimicrobial agents (Van Boeckel *et al*., 2015). In the feed mills/integrated feed manufacturing companies, poultry feed are fortified with antibiotics (feed additives) (unregulated, especially in developing countries), including the last-line antibiotics like colistin, for prophylactic control of microbial (especially bacterial and protozoan) infection and enhancing the growth rate (Chattopadhyay, 2014; Manikandan *et al*., 2020). Premixes, which are mixtures of inorganic minerals and

vitamins used in poultry feed contain antimicrobial agents. Thus, individuals who feed these birds are at huge risk of acquiring resistant organisms from these contaminated poultry feed and potentially disseminating them to the public.

Thus, there is a need to understand the occurrence and effects of microbial contaminants in poultry feed. Information on the diversity of microbial contaminants and metabolites/toxins produced by them, the effect of the metabolites on different body systems in birds, and the public health impact of microbial contaminants in poultry feed is important in designing effective feed monitoring and hazard control strategies. In this review, the objective is to describe findings of studies on microbial contaminants in poultry feed, with emphasis on the diversity of microbial isolates from poultry feed, sources of organisms, environmental factors facilitating the growth of the organism, the metabolites elaborated by them, clinical and histopathological effects elicited by the organisms/their metabolites as well as the control strategies for these contaminants.

MATERIALS AND METHODS

A comprehensive internet search of related literature on microbial contamination of poultry feed was undertaken using Google search, ScienceDirect and PubMed databases. The download papers were examined in detail and cited accordingly.

RESULTS

Sources of Microbial Contaminants in Poultry Feed: Poultry feed contains plant-derived (e.g., carbon source – grains, nitrogen source - groundnut, lentil, sunflower, cotton seed, pea seed meals; fibre source – grain offal), animal-derived (e.g., fish, animal/meat, blood, feather and bone meals) ingredients, mineral (calcium source - limestone, shell grit, bone meal, dicalcium phosphates, mineral premix - sodium salt, sodium bicarbonate) and essential amino acids (methionine, lysine) (Ezekiel *et al*., 2012; Manikandan *et al*., 2020).

Plant-derived feed ingredients can become contaminated pre-harvest in the field by organisms originating from the soil, untreated /insufficiently-treated manure, sewage, vectors (wild birds, rodents, insects) and even air particulate matters (dust) (Maciorowski *et al*., 2007; Rossato *et al*., 2019). Although composting is effective in eliminating microbes (including antimicrobial-resistant strains) from animal manure before application in farmlands (Gao *et al*., 2019), some organisms survive the composting temperature and find their way into the finished feed ingredient (spore-formers can survive without moisture) and subsequently into the poultry feed (Maciorowski *et al*., 2007). These dangerous organisms are disseminated to humans, other animals and environment through various pathways (Figure 1). Spore-bearing bacteria such as *Clostridium*, have been shown to be able to survive in manure/soil after composting (Maciorowski *et al*., 2007). Gazal *et al*. (2015) reported the escape and survival of *Escherichia coli* (a non-spore-former) in organic fertilizer after the composting process.

Post-harvest contamination of plant-derived feed ingredient originate from hands of persons involved in the handling, processing, packaging and transportation of feed ingredients/feed, vectors (rodents, birds and flies) that pick organisms and deposit into feed stuff/feed during processing (such as grain drying, pelletization), and storage (Roy *et al*., 2019). The drying process is done by subjecting the feed ingredients to high temperatures to remove moisture, thereby making the feed stuff unsuitable for microbial growth. However, some organisms survive drying and processing temperature and find their way into the finished feed. Dargatz *et al*. (2005) demonstrated tolerance to adverse conditions of *E. coli* to survive in dry environments, such as dried corn. Eighteen diarrhoeagenic *E. coli* were isolated from samples of vegetable meal used as poultry feed ingredient in Brazil (Rossato *et al*., 2019), suggesting that pathogenic organisms in poultry feed originate from anthropogenic/animal setting, and they can escape processing (thermal treatment) conditions.

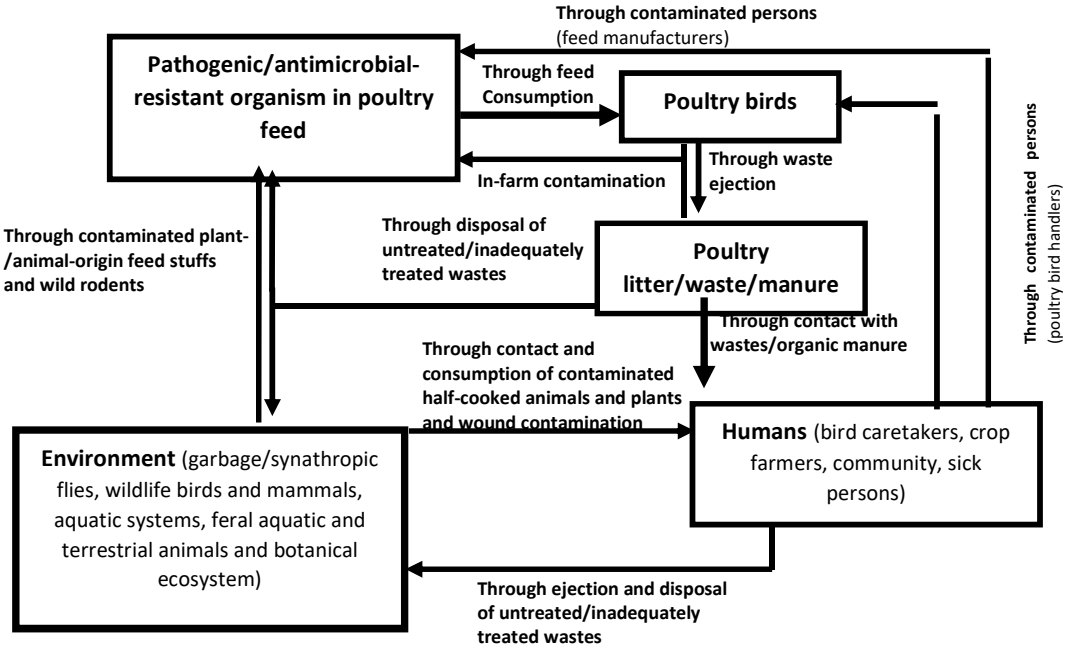

Figure 1: Pathways through which pathogenic and antimicrobial-resistant organisms in poultry feed can disseminate to humans, other animals and environment

Animal-derived poultry feed ingredients such as blood, meat, feathers, bone and fish meal have also been reported as sources of microbes in poultry feed. In Brazil, diarrhoeagenic *E. coli* was isolated from animal meal used as poultry feed ingredient (Rossato *et al.*, 2019), suggesting that pathogenic microbes enter poultry feed from animal-derived feed ingredients. Feed ingredients from terrestrial animals are contaminated in slaughterhouses, during storage from contaminated holding-containers, environment, vectors, and handlers (Rouger *et al.*, 2017). Fish meal is potentially contaminated by organisms originating from the aquatic environment, containers and handlers involved in processing and transportation (Han *et al.*, 2017). However, animal ingredients are often heated (cooked) at high temperature to destroy contaminating microbial agents. Nevertheless, the organisms escape the heat treatment (thermal failure) after processing, cooling, storage, transportation and handling. Torres *et al.* (2011) attributed the contamination of feed to cooling after the pelleting process, storage and transportation.

The mineral ingredients derived from rocks get contaminated by soil microflora, which may constitute commensal and pathogenic organisms.

Microbial Contaminants and their Secretary Products

Fungal contaminants of poultry feed: Poultry feed is considered one of the important sources of contamination of poultry products (Brown *et al.*, 2001). Identification of the microbiota contaminating poultry feed is essential for quality control and feed safety. Data on the microbiota in feed provides information on potential production of mycotoxins and other microbial metabolites; and such data is a helpful indicator of the hygienic quality of feed (Khosravi *et al.*, 2007). Admissible limits for fungal contamination in animal feeds vary from country to country (Cegielska-Radziejewska *et al.*, 2013). However, fungal load of 1×10^5 CFU g^{-1} is the permissible limit which ensure the hygienic quality of feed (Magnoli *et al.*, 2002). Nonetheless, poultry feed sampled across the globe had varying fungal load (Table 1).

Table 1: Studies on microbial contamination of poultry feed

Region	Country	Number of feed sample analyzed	Target organism	Microbial load (CFU g^{-1})	Organism isolated	Reference
Europe	Serbia	230	Mycoflora	0–6x10^5	*Fusarium**, *Aspergillus*, *Rhizopus*, *Penicillium*, *Mucor* and *Alternaria*	(Krnjaja *et al*., 2008)
	Turkey	82	Mycoflora	1x10^2–1x10^3	*Mucor**, *Absidia*, *Fusarium**, *Cladosporium*, *Aureobasidium*, *Aspergillus**, *Eurotium*, *Penicillium**, *Scoploriopsis*, *Ulocladium*, *Trichoderma* and *Acremonium*	(Heperkan and Alperden 1988)
	Poland	45	Microbiota	Fungi: 5.5×10^1–8.5x10^1 Bacteria: 1.1×10^3–7.2×10^3	Fungi: *Aspergillus**, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus** Bacteria: Enterobacteriaceae/Aerobic bacteria	(Cegielska-Radziejewska *et al*., 2013)
		6552	Microbiota	Bacteria: 1x10^2–1x10^6 Fungi: >1x10^5	*Salmonella*, *E. coli*, *Clostridium perfringes* and fungi	(Kukier *et al*., 2012)
		648	Mycoflora	1x10^2 –8.2 x10^4	*Fusarium**, *Aspergillus**, *Mucor** and *Rhizopus**	(Kubizna *et al*., 2011)
	Slovakia		Mycoflora	1×10^3–200×10^5	*Absidia*, *Mucor*, *Rhizopus*, *Syncephalastrum*, *Zygorrhynchus*, *Emericella*, *Eurotium*, *Monascus*, *Acremonium*, *Alternaria*, *Aspergillus*, *Fusarium*, *Geosmithia*, *Paecilomyces*, *Penicillium**, *Scopulariopsis*, *Stachybotrys*, *Trichoderma*, *Ulocladium* and *Wardomyces*	(Labuda and Tancinová, 2006)
South America	Argentina	49	Mycobiota	1x10^1–1x10^6	*Aspergillus*, *Cladosporium*, *Penicillium*, *Eurotium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Scopulariopsis*, Yeast* and others	(Greco *et al*., 2014)
		130	Mycobiota	6.6 x 10^3–6.3 x10^5	*Aspergillus**, *Fusarium*, *Penicillium*, *Mucor*, *Eurotium*, *Phytophtora*, *Cladosporium*, *Trichoderma*, *Alternaria*, *Absidia*, *Paecilomyces*, *Circinella*, *Wafermia*, *Ulocladium*, *Scarpularlospsis*, *Tsiaromyces*, *Acreabasidium* and *Rhizopus*	(Dalcero *et al*., 1998)
		300	Mycobiota	1x10^4–1x10^6	*Penicillium**, *Aspergillus*, *Fusarium*, *Rhizopus*, *Paecilomyces*, *Absidia*, *Mucor*, *Emericella*, *Alternaria*, *Trichoderma*, *Ulocladium*,	(Dalcero *et al*., 1997)

					Cladosporium Rhodotouria and *Acremonium*	
		35	Mycoflora	4×10^4–8×10^4	*Aspergillus**, *Penicillium*, *Fusarium*, *Cladosporium*, and *Eurotium*	(Astoreca *et al.*, 2011)
		120	Mycoflora	2.0×10^3– 3.0×10^5	*Fusarium**, *Penicillium**, *Aspergillus* and Yeast	(Magnoli *et al.*, 2002)
	Brazil (Embaby *et al.*, 2015)	480	Mycoflora	$2.18x10^3$–$3.27x10^3$	*Penicillium**, *Aspergillus* and *Fusarium*	(Oliveira *et al.*, 2006)
		90	Mycoflora	$1.15x10^3$–$6.11x10^2$	*Aspergillus**, *Penicillium*, *Fusarium*, *Cladosporium*, *Eurotium*, *Mucor*, *Scopulariopsis*, *Chaetosartorya*, *Trichosporium*, *Phoma*, *Alternaria* and *Curvularia*	(Rosa *et al.*, 2006)
Africa	Egypt	-	Mycoflora	-	*Aspergillus**, *Penicillium*, *Fusarium* and *Alternaria*	(Embaby *et al.*, 2015)
	Nigeria	50	Microbiota	Bacteria: $2.19x10^5$– 5.4×10^5 Fungi: $5.7x10^5$–$8.1x10^5$	Bacteria: *E. coli*, *S. aureus**, *Bacillus*, *Lactobacillus* and *Salmonella* Fungi: *Aspergillus*, *Rhizopus**, *Penicillium* and *Mucor*	(Osaro *et al.*, 2017)
		120	Mycoflora	$1x10^6$–$7x10^5$	*Aspergillus**, *Penicillium*, *Fusarium*, *Mucor* and Yeast	(Nwiyi *et al.*, 2019)
		50	Mycoflora	-	*Aspergillus*, *Rhizopus** and *Fusarium*	(Osho *et al.*, 2007)
		16	Microbiota	Bacteria: 1.27×10^7– 2.70×10^7 Fungi: 3.00×10^4– 9.60×10^5	Bacteria: *S. aureus**, *E. coli*, *Salmonella*, *Pseudomonas*, *Klebsiella*, *Streptococcus* and *Listeria* Fungi: *Aspergillus**, *Fusarium*, *Penicillium* and *Rhizopus*	(Ukaegbu-Obi *et al.*, 2017)
		4	Microbiota	Bacteria: $1.46x10^4$–$6.60x10^2$ Fungi: $1.50x10^2$–$7.40x10^2$	Bacteria: *Aerobacter aerogenes*, *Bacillus cereus*, *Erwinia amylovora*, *Micrococcus luteus* and *S. aureus** Fungi: *Aspergillus**, *Claudosporium*, *Acaulopa*, *Dotchiza populare*, *Fusarium*, *Geotrichum*, *Pleurophrigmium*, *Candida*, *Rhizopus* and *Saccharomyces*	(Arotupin *et al.*,2007)
		300	Mycoflora	-	*Aspergillus**, *Mucor*, Dermatophyte, *Rhizopus*, *Penicillium*, *Fusarium* and Yeast	(Ibrahim *et al.*, 2017)
		180	Mycoflora	-	*Aspergillus**, *Mucor*, Yeast and *Rhizopus*	(Habib *et al.*, 2015)
		239	Mycoflora	-	*Penicillium*, *Aspergillus** and *Fusarium*	(Aliyu *et al.*, 2012)
		60	Mycoflora	$1.0x10^3$–$8.0x10^3$	*Aspergillus**, *Fusarium*, *Candida*, Yeast and others	(Anifowose and Bakre, 2021)

		100	Mycoflora	-	*Aspergillus**, *Fusarium*, *Alternaria*, *Cladosporium*, Mucor, *Penicillium*, *Rhizopus*, *Torula* and Yeast	(Adeniran *et al*., 2013)
		-	Mycoflora	-	*Mucor**, *Aspergillus*, *Epicoeceum*, Yeast, *Penicillium*, *Gymnoaeseus*, *Cladosporium*, *Mortierella*, *Rhizopus* and bacteria	(Okoli *et al*., 2006)
Asia	Bangladesh	189	Mycoflora	-	*Aspergillus**, *Fusarium* and *Rhizopus*	(Islam *et al*., 2016)
	Indonesia	-	Mycoflora	-	*Penicillium**, *Aspergillus, Fusarium, Cladosporidium, Trichoderma,* and *Paecilomyces*	(Sukmawati *et al*., 2018)
	Pakistan	119	Mycoflora	-	*Aspergillus**, *Fusarium*, *Penicillium* and *Alternaria*	(Saleemi *et al*., 2010)
	Saudi Arabia	100	Mycoflora	$3.181x10^3$	*Aspergillus* and *Penicillium**	(Gherbawy *et al*., 2020)
	India	15	Mycoflora	-	*Aspergillus** and *Penicillium*	(Sivakumar *et al*., 2014)
	Iraq	180	Mycoflora	$5x10^1$–$2.1x10^6$	*Aspergillus**, *Penicillium*, *Rhizopus*, *Cladosporium*, *Mucor*, *Alternaria* and *Fusarium*	(Alkhursan *et al*., 2021)
	Iran	44	Mycoflora	-	*Aspergillus*, *Fusarium**, *Penicillium*, *Mucor*, *Scopulariopsis*, *Chrysosporium*, *Rhizopus*, Yeast, and others	(Ghaemmaghami *et al*., 2016)
		20	Mycoflora	1×10^1–$63x10^3$	*Aspergillus*, *Fusarium**, *Penicillium*, *Mucor*, *Rhizopus*, Yeast, *Scopulariopsis* and others	(Ghaemmaghami *et al*., 2018)

*-: not reported, *: predominant organism(s)*

Contamination of poultry feed by microbes is a potential pathway for entry of pathogens into human food supply (Maciorowski *et al*., 2007). Microbial contamination of poultry feeds may be of fungal or bacterial origin and their secreted toxins and metabolites. High fungal and bacteria counts in feed are potential hazard to the animal. The presence of fungi in poultry feeds affects the nutritional quality of the feed as well as its organoleptic attributes (Shareef, 2010). Moulds like other microorganisms utilize the readily available nutrients in the feed and their activity may result in loss of nutrients in the feed (Okoli *et al*., 2006). Detection of fungi as major contaminant of poultry feed dates back to 1960 when an outbreak that claimed the lives of over 100,000 turkeys in Southern England was discovered to be caused by *Aspergillus* species (Asao *et al*., 1963).

Aspergillus species are the major fungal contaminants of poultry feeds in the tropics (Klich *et al*., 2000; Peterson *et al*., 2001; Mgbeahuruike, 2016; Ibrahim *et al*., 2017). Among the *Aspergillus* species known for fungal contaminants of poultry feed, *A. flavus* and *A. parasiticus* are of major concern in poultry production and the most common producers of aflatoxin (Magnoli *et al*., 2011; Othman and Al-Delamiy, 2012). *A. flavus* is the most frequently encountered fungal contaminant of feeds in most tropical countries (Varga *et al*., 2011; Fapohunda *et al*., 2012;Davari *et al*., 2015; Ibrahim *et al*., 2017). *A. flavus, A. parasiticus* and *A. nomius* are known aflatoxigenic species and if present in feed, can be passed to poultry meat or egg and this may have a negative effect on human health. Similarly, *A. fumigatus*, *A. parasiticus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. nomius* and *A. caelatus* have been found as contaminants of poultry feeds at different amounts (Ibrahim *et al*., 2017; Mgbeahuruike *et al*., 2018). Other fungi such as *Rhizopus, Mucor*, *Fusarium, Cladosporium, Penicillium* and yeast have been reported as contaminants of poultry feed (Table 1) (Saleemi *et al*., 2010; Sivakumar *et al*., 2014). *Penicillium* species and *Fusarium* species are mycotoxigenic species and have been isolated from poultry feeds (Table 1) (Ibrahim *et al*., 2017).

Mucor has not been linked with any metabolite or toxin secretions in feed, although they are commonly isolated from contaminated poultry feed. However, they may constitute a major source of infection to animals and humans when consumed through feed. Other fungi that have been isolated as poultry feed contaminants are *Trichosporium* (Rosa *et al*., 2006), *Stachybotrys* (Labuda and Tancinová, 2006)*, Trichoderma* (Heperkan and Alperden, 1988) among others (Table 1). These fungi have been linked with the production of different mycotoxins (Tiemann and Dänicke, 2007; Rodrigues and Naehrer, 2012; Queiroz *et al*., 2013). Mycotoxigenic fungi are capable of producing more than one mycotoxin in feed because many different species of fungi develop in the feed at the same time, especially in feed prepared using multiple feed ingredients (Streit *et al*., 2012). These mycotoxins are regulated in feed in different countries. However, in Nigeria there are no regulations on mycotoxin levels in feed. Therefore, there is every likelihood of mycotoxins existing in the Nigerian poultry sector since the common feed ingredients, like maize and groundnut cake, are major reservoirs of mycotoxins (Kpodo and Bankole, 2008).

Fungal toxins and metabolites: Most fungal contaminants in feed secrete either mycotoxins or toxic metabolites that have adverse effects to birds. Over 56 fungal metabolites/toxins have been identified in poultry feeds using LC/ESI–MS/MS multi-toxin analysis (Ezekiel *et al*., 2012). Some of these metabolites are regulated, while some are known as non-regulated metabolites. The regulated metabolites commonly found in poultry feeds include; aflatoxins (AF), ochratoxin (OTA), trichothecenes, fumonisins (FUM), zearalenone (ZEN), deoxynivalenol (DON) (Iqbal *et al*., 2014). The permissible level for aflatoxin in most countries where regulations exist is 20 $mgkg^{-1}$ of feed (Van Egmond and Jonker, 2004). For DON, OTA and ZEN, limits of 1000 $mgkg^{-1}$ in feed may be accepted. Aflatoxins are structurally related fungal metabolites with hepatocarcinogenic properties, and they are produced on nuts and cereals by fungi of the *Aspergillus* species (Mgbeahuruike, 2016; Mgbeahuruike *et al*., 2018; 2020). Aflatoxins

have four major fractions; aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Monbaliu *et al*., 2010; Lereau *et al*., 2012; Mgbeahuruike *et al*., 2018). Aflatoxin AFB1 is the most potent and is derived from sterigmatocystin, a naturally occurring carcinogen (Xu *et al*., 2000). Aflatoxin M1 and M2 are metabolites and derivatives of AFB1 formed and excreted in the milk of humans and animals following ingestion of feedstuffs contaminated with AFB1(Xu *et al*., 2000). A recent survey of the aflatoxin level in feed mills in Nigeria showed that AFB1 was the most predominant aflatoxin in all the feed mills sampled (Mgbeahuruike *et al*., 2020). However, other fractions of aflatoxin were also found in the feed mills but at a relatively lower quantity. OTA is an important mycotoxin in poultry nutrition produced by *Penicillium* and *Aspergillus* species (Hassan *et al*., 2012). ZEN mycotoxins or F-2 toxins are produced by *Fusarium* species and they occur as natural mycotoxin contaminant in corn, wheat, barley, oats and sorghum (Tiemann and Dänicke, 2007; Rodrigues and Naehrer, 2012). ZEN is produced by different strains of *Fusaria*, *F. avenaceum*, *F. equiseti, F. gravinearum*, *F. culmorum, F. lateritium* (Shi *et al*., 2016). Furthermore, *Fusarium* species are also known to produce the mycotoxins, T-2 toxin and diacetoxyscirpenol (DAS) (Shi *et al*., 2016). Fuminosins are produced by *F. verticillioides* (moniliforme) and *F. proliferatum* in grains and feed (Proctor *et al*., 2003; Brown *et al*., 2005). However, some strains of *A. niger* have been demonstrated to produce fumonisins B_2, B_4 and B_6 but not FB_1 (Frisvad *et al*., 2007; Månsson *et al*., 2010). Fumonisin B1 (FB1) is the most common and the most studied and causes high level of toxicities in animals. A recent study on fungal and bacterial metabolites in commercial poultry feed from Nigeria showed that fusarium toxins were more frequent in the feed than those of *Aspergillus, Penicillium* or bacteria (Ezekiel *et al*., 2012). Trichothecenes are groups of fungal metabolites with similar basic backbone structure, and they include T-2 toxins, HT-2 toxins, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), neosolaniol, 8-acetoxyneosolaniol, 4-deacetylneosolaniol, nivalenol, 4-acetoxynivalenol (Fusarenone-X), DON (vomitoxin), and 3-acetyldeoxynivalenol. The non-regulated metabolites occur in poultry feed in very low amounts and they include cytochalasins, aurofusarin (ARF), 3-nitropropionic acid (3NPA), enniatins, ergot alkaloids, anthraquinoid and xanthone dimers, RUG and S-A-D, produced by *Penicillium* species (Schmeda-Hirschmann *et al*., 2008; Ezekiel *et al*., 2012).

Bacterial contaminants in feeds and their metabolites: Bacterial contamination of poultry feed is an important public health issue just as the presence of fungi in feed constitutes a serious challenge to poultry health. Bacterial contamination of poultry feed may come from the stock feed, raw materials or from the farm (Maciorowski *et al*., 2007). Food-borne bacterial pathogens are common contaminants of poultry feeds (Table 2) (Ezekiel *et al*., 2011; Atere *et al*., 2015; Mamman and Ndakotsu, 2015; Islam *et al*., 2017; Hossain *et al*., 2020). *Salmonella* is a major contaminant of poultry feed and it is the most important cause of bacterial infection in poultry (Borland, 1975). About 82 serotypes of *Salmonella* species have been identified, out of which 45 isolates were isolated from poultry feeds (Borland, 1975). *Salmonella enterica,* a non-typhi serotype of *Salmonella* has also been documented as a major contaminant of animal feeds (Kidd *et al*., 2002). *Salmonella pullorum* and *S. gallinarum* are important poultry pathogens and both were isolated from poultry feeds produced inside poultry farms (Borland, 1975; Ahmed, 2010). Other gram-negative bacteria, such as *E. coli* and *Klebsiella pneumoniae* have also been isolated from poultry feeds at varying degrees (Table 2) (Ukaegbu-Obi *et al*., 2017; Hossain *et al*., 2020). *Shigella* species were found to be the most abundant pathogenic bacteria present in poultry feed samples from feed mills in Bangladesh, while *Vibrio* species was the second most abundant pathogen isolated from the feeds (Roy *et al*., 2019). Several other bacterial species such as *Bacillus* species, *Campylobacter* and *Clostridium perfringens* have been isolated from poultry feeds as contaminants (Bryan and Doyle, 1995; Kukier *et al*., 2012; Osaro *et al*., 2017).

Table 2: Studies on antimicrobial resistance among bacteria isolated from poultry feed

Country	Number of feed sample analyzed	Bacterial load	Bacteria isolated (number resistant/number isolated, % resistance)	Antimicrobial resistance profile (resistance genes)	Reference
Portugal	22	0.15 log–6.00 log	*Enterococcus** (52/414, 12.6%) and *E. coli* (13/105, 12.4%)	RIF, ERY, NIT, CIP, GEN, AMP, TET, VAN, CHL, ENR, SXT, KAN, APR	(da Costa *et al.*, 2007)
Kenya	150	3.1×10^{5}–3.0×10^{6}	*E. coli** (62/87, 71%) and *Salmonella* (17/42, 40.1%)	AMP, SXT, CTR, TET, CHL (*bla*$_{TEM}$, *bla*$_{SHV}$, *dfr* and *strB*)	(Ngai *et al.*, 2021)
Nigeria	12	1.9×10^{6}–3.6×10^{7}	*E. coli* (22/24, 91.7%), *Klebsiella* (11/12, 91.7%), *Pseudomonas* (8/8, 100%), *Bacillus* (8/9, 88.9%) and *Staphylococcus* (8/10, 80%)	CAZ, CRX, GEN, ERY, CLX, OFX, AMC	(Atere *et al.*, 2015)
	52	-	*Citrobacter** (12/50,24%), *Morganella* (8/16, 50%), *E. coli* (7/39, 17.9%), *Klebsiella* (10/13, 76.9%), *Salmonella* (0/4, 0%) and *Staphylococcus* (0/1, 0%)	AMC, ENR, ERY, NIT, SXT, TET	(Mamman and Ndakotsu, 2015)
	58	-	*E. coli** (14/15, 93.3%), *K. pneumoniae* (17/18, 94.4%), *Enterobacter* (32/32, 100%), *Salmonella* (32/32, 100%) and *Yersinia* (11/11, 100%)	AMC, CTR, NIT, GEN, SXT, OFL, AMX, CIP, TET, PEF	(Ezekiel *et al.*, 2011)
Bangladesh	9	1.2×10^{8}–8.0×10^{4}	*E. coli*, *Klebsiella**, *Pseudomonas**, *Vibrio*, *Staphylococcus*, *Salmonella* and *Bacillus**	NOV, PEN, CEF, CRX, RIF, ERY	(Hossain *et al.*, 2020)
	15	$23.40\pm2.88\times10^{12}$–$33.20\pm1.30\times10^{12}$	*Sphingobacterium daejeonense* and *Bacillus*	AMP, BAC, GEN, SUL, TET	(Islam *et al.*, 2017)

*-: not assessed, *: predominant organism(s), resistance profile: pooled profile of all isolates, RIF: rifampicin, ERY: erythromycin, NIT: nitrofurantoin, CIP: ciprofloxacin, GEN: gentamicin, AMP: ampicillin, TET: tetracycline, VAN: vancomycin, CHL: chloramphenicol, ENR: enrofloxacin, SXT: sulphamethoxazole-trimethoprim, KAN: kanamycin, APR: apramycin, CAZ: ceftazidime, **CRX:** cefuroxime, CLX: cloxacillin, OFX: ofloxacin, AMC: amoxicillin-clavulanate, BAC: bacitracin, CTR: ceftriaxone, AMX: amoxicillin, PEF: perfloxacin*

Bacillus species are soil bacteria that are commonly found in several food substances such as eggs, meat, dairy and plant products as contaminants. *Bacillus cerues* is known to be the cause of over 25% of food borne intoxications in animals and humans due to its secretion of emetic toxins, enterotoxins and resistance of its spores to heat treatment (Pal *et al*., 2014). The presence of the different groups of microorganisms in poultry feed can cause food borne infections (European Commission, 2011). *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus gallinarum* have been identified as major public health risk when consumed by birds through contaminated feeds (Maciorowski *et al*., 2007). The EU Commission Regulation act has indicated the bacterial limit for animal feed products for acceptable count of specific microbes in the feed. For example, the acceptable count for the *Enterobacteriaceae* species is 300 CFU/g, while zero count is recommended for *Salmonella* species (European Commission, 2011). In a study by Ezekiel *et al*. (2012), seven bacterial metabolites were found in poultry feed samples collected from different feed vendors in 17 states of Nigeria. However, most of the studied bacterial metabolites appeared to be beneficial to poultry since they are antibiotics, and they were found in relatively low levels. However, unintended exposure to very low levels of antibiotics may be responsible for the increase in antimicrobial resistance to the conventionally used antibiotics. Nevertheless, unhygienic feed manufacturers and/or poultry bird caretakers constitute potential sources of antimicrobial-resistant organisms isolated from poultry feeds (Table 2).

Effects of microbial contaminated feed on production parameters: Optimum productivity in birds requires adequate supply of protein, energy, lipids, vitamins minerals and water in adequate and balanced proportions (Gillespie and Flanders, 2009; Damerow, 2012). Any imbalance or deficiency of these would result in poor growth performance and if prolonged, death. When fungi and/or bacteria colonize poultry feeds, they utilize readily available nutrients in the feed for their metabolism, growth and propagation, leading to nutrient degradation and a loss of between 5 – 100% of nutrients in the feed (Okoli *et al*., 2006). This reduces the nutritional quality of the feed, with the energy, protein and lipid contents being more affected (Wang and Hogan, 2019). Greco *et al*. (2014) sampled poultry feeds in important broiler producing regions of Argentina, for presence of fungi and mycotoxins. Their results showed that all feed samples analyzed were contaminated with fungi and mycotoxins. About 56% of the feed samples had fungal counts below 3.10^4UFC/g and were regarded as good quality feeds, 7% had 3.10^4 – 7.10^4 UFC/g counts and were termed regular, while 37% had counts above 7.10^4 UFC/g and where classified as bad quality feeds (Gimeno and Martins, 2007). Over 90% of these samples had at least one type of nutritional deficiency or the other.

Fungi and bacteria in feed also reduce feed quality through physical damages to the feed, adversely affecting the organoleptic properties of the feeds in the process (Cegielska-Radziejewska *et al*., 2013). Through degradative changes such as oxidation, putrefaction, fermentation and rancidity, microorganisms can bring undesirable changes in the appearance, flavour, odour, taste and other features that affect feed acceptance by animals (Amit *et al*., 2017). Some bacteria are also able to synthesize pigments and slime on the feed, reducing further the feeds' appeal to the animals (Kamala and Kumar, 2017). Wang and Hogan (2019) conducted trials to determine the effect of *Fusarium*-contaminated diets on 308 male Ross broilers feeding behaviour, feed preference and growth performance. For the feed preference trial, birds preferred the control diets without contamination over the low and high contaminated diets. Furthermore, reduced feed intake and poor growth rates were observed in broilers fed ochratoxin A (OTA) contaminated diets (Elaroussi *et al*., 2006). The study suggested that the poor feed consumption observed in the treated birds may be due to the adverse effects of the toxins on the organoleptic properties of the feed. Fungi and bacteria in feed are also known to elaborate harmful toxins

which can cause significant production losses due to their effects on performance and health. Fungal and bacterial toxins have been shown to cause reduction in growth rate, feed consumption, poor feed conversion and a wide range of adverse health conditions in birds (Binder *et al*., 2007; Venancio and Paterson, 2007). Ross broilers fed ochratoxin-contaminated diets had reduced growth rate, reduced feed consumption and increased mortality (Elaroussi *et al*., 2006). Wang and Hogan (2019) reported suppressed growth performance when male Ross broilers were fed *Fusarium* mycotoxin (deoxynivalenol, DON) contaminated feeds. Birds fed the DON contaminated diets also had shorter villi and shallower crypts than the control birds and this had adverse effect on proper nutrient digestion and feed utilization, resulting in poor weight gain (Wang and Hogan, 2019). Microbial toxins apparently alter the intestinal morphology of birds, this is evident in reduced villi height, crypt depth and villi surface area (Awad *et al*., 2012; 2014; Maresca, 2013; Pinton and Oswald, 2014; Ghareeb *et al*., 2015). Other studies have shown that feeding broilers with diets moderately contaminated with DON mycotoxin, may adversely affect the morphology of the small intestines (Awad *et al*., 2019). Wang and Hogan (2019) also observed that although there were no growth changes for the first 14 days, growth performance was suppressed in Ross broilers fed DON-contaminated diets during the grower period (22 to 34 days) and further histopathological analysis of the ileum region revealed that birds offered the DON diets throughout the entire trial (1 to 34 day) had shorter villi and shallower crypt than the control birds. Since the gastrointestinal tract is the site for nutrient digestion and absorption, a fully functioning and healthy intestine is essential for broilers to achieve maximum growth with superior feed efficiency (Denbow, 2015). Reduced villi height, crypt depth and villi surface area would therefore mean an impairment of nutrient uptake in affected birds and ultimately, reduced growth performance (Pinton and Oswald 2014; Ghareeb *et al*., 2015). Microbial toxins may also induce feed refusal in birds through the release of certain neurotransmitters which regulate appetite and digestion. Swamy *et al*. (2004) in their study to evaluate the effects of *Fusarium* mycotoxins on brain neurochemistry, recorded increased levels of serotonin, a strong satiety neurochemical, following long-term (1 to 56 days) feeding of *Fusarium* mycotoxin-contaminated diets to broilers. Pathogenic bacteria in poultry feeds may also cause production losses when they are directly ingested with the contaminated feed. Broilers fed artificially contaminated feed with non-Typhi serotypes of *S. enterica* developed infection with the organism (Crump *et al*., 2002).

Furthermore, some studies reported that feeds contaminated with mycotoxins at levels close to the maximum permissible level may decrease weight gain and feed consumption rate (Elaroussi *et al*., 2006; Sakhare *et al*., 2007; Hanif *et al*., 2008; El-Barkouky *et al*., 2010), others researchers observed that almost the same concentrations caused no effects on birds' performances (Biró *et al*., 2002; Politis *et al*., 2005). Andretta *et al*. (2011) reported a greater adverse effect of mycotoxins on growth in younger broilers than older birds, while Wang and Hogan (2019) observed greater reduction in feed intake and growth in older birds than younger ones. The authors suggested that the greater adverse effects observed in the older birds may be due to higher feed intake (more mycotoxins consumption) than the younger ones and consequently, higher feed conversion ratio (FCR), since FCR increases with age in broilers (Zuidhof *et al*., 2014; ROSS, 2014). The differences observed in the results may be as a result of the duration of exposure to the contaminants, the level of exposure, timing of exposure as well as whether the poultry feed was spiked with the toxin/mycotoxin or whether naturally-contaminated grain and/or feeds were used (Awad *et al*., 2012; Wang and Hogan, 2019).

Effects of microbial contaminants in poultry feed on haematology and serum biochemistry of birds: Haematology and serum biochemistry are the cornerstone of disease diagnosis in veterinary medicine (Harr,

2002). Values generated from analysis of haematology and serum biochemistry parameters of animals and birds can be used as physiological indicators (Hrabčáková *et al*., 2014). In addition, blood analyses are widely used to diagnose and monitor general health and diseases in the avian species (Han *et al*., 2016). In birds, analysis of haematological and serum biochemistry parameters plays vital role in assessing the pathophysiological and nutritional status, as it provides the opportunity to evaluate the level of certain blood and serum metabolites in the body of animals (Etim *et al*., 2014). Alterations in the level of these body metabolites or blood constituents when compared to normal values and in combination with other laboratory tests may help make a diagnosis, prognosis and determine the efficacy of instituted therapy (Harr, 2002; Ibrahim, 2012). Specifically, evaluation of the haematology and serum biochemistry enables the veterinarian to monitor a bird's response following consumption of contaminated feeds in its environment.

Microbial contaminants in feed (fungi and bacteria) and their toxic metabolites have great deleterious impacts on poultry productivity (Aravind *et al*., 2003; Uwaezuoke and Ogbulie, 2010; Sultana *et al*., 2017; Danbappa *et al*., 2018; Chat *et al*., 2019; Fouad *et al*., 2019). Aflatoxins are considered unavoidable fungal metabolite that contaminates poultry feed (El-katcha *et al*., 2017). The consumption of multiple mycotoxin contaminated diet by broilers has been reported to alter haematological and serum biochemistry parameters of birds (Rezar *et al*., 2007; Gowda *et al*., 2008; Che *et al*., 2011). Azizpour and Moghadam (2015) reported a decrease in serum uric acid concentration, cholesterol and triglycerides as well as increases in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in 42-day old broilers fed 250 ppb of aflatoxin. A decrease in leukocyte count and increased serum activities of AST, glutathione peroxidase (GPx) and catalase (CAT) were reported in birds fed aflatoxin contaminated feed without treatment with mycotoxin adsorbents (bentonite and fuller's earth) (Mgbeahuruike *et al*., 2018). Mold-contaminated poultry feed caused leukocytosis, low haematocrit and high serum activities of AST and gamma glutamytransferase (GGT), and low red blood cell counts. Hypoglobulinemia and low urea nitrogen concentration was recorded in broilers fed aflatoxin mold-contaminated feeds (Che *et al*., 2011). Liver superoxide dismutase (SOD) activity was reduced while myeloperoxidase activity of affected birds was increased following consumption of mold-contaminated diet (Che *et al*., 2011). Aflatoxin level was positively correlated to packed cell volume (PCV) and haemoglobin while a negative relationship exists between aflatoxin levels and white blood cell counts in two weeks old broilers fed poorly processed feed which was stored for a long time (Ejioffor *et al*., 2018). Also, a decrease in serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and creatinine were notable findings in avian aflatoxicosis (Kana *et al*., 2014). Decrease in serum cholesterol and total protein in 35-day old broiler chickens was reported by Raju and Devegowda (2000) but inconsistent results were obtained for the blood urea nitrogen, ALT and AST activities throughout the 35 days of the study. Broiler chickens from 1 – 3 weeks of age showed significant decrease in serum total protein, albumin, inorganic phosphorus, uric acid and total cholesterol, haematocrit, Hb content, mean corpuscular haemoglobin, thrombocyte counts, lymphocyte and monocyte counts, heterophil and WBC count in aflatoxicosis (Keçeci *et al*., 1998). Therefore, in poultry, aflatoxicosis is mainly manifested by decrease in total protein, albumin, serum cholesterol, glucose, uric acid, inorganic phosphorus and calcium. Decreased total protein and albumin alongside increased serum hepatic enzyme activities are consistent indicators of the hepatotoxicity of aflatoxins in chickens and turkeys (Keçeci *et al*., 1998). Prolonged coagulation time and reduced blood haemoglobin content were observed in experimental ochratoxicosis (Raju and Devegowda, 2000). Furthermore, eexperimental ochratoxicosis in 5-week-old broiler chickens induced anaemia manifested by a significant

decrease in red blood cell count, PCV and haemoglobin concentration, and leukocytosis. A significant increase in serum triiodothyronine concentration was also observed in the ochratoxin A-fed broiler chickens (Elaroussi *et al*., 2006). Ten (10) mg deoxynivalenol mycotoxin/kg of feed induced a significant increase in plasma corticosterone and a higher heterophil: lymphocyte ratio in broiler chickens (Ghareeb *et al*., 2014). Chronic consumption of *Fusarium* mycotoxin contaminated feed by laying hens induced slight decreases in haematocrit values, total leukocyte count, mean lymphocytes counts including $CD4^+$ and $CD8^+$ T lymphocytes, and biliary IgA concentration (Chowdhury *et al*., 2005). A significant increase in serum GGT and lactate dehydrogenase (LDH) activities, monocyte and heterophil counts are also associated with aflatoxicosis in broilers (Kaki, 2012). Zearalenone-contaminated diet consumed by young broiler chickens also resulted in decreased serum total protein and albumin concentration and increased ALT and AST activities (Xu *et al*., 2018). Increase in serum, liver and kidney total SOD activity and malondialdehyde level, and decreased glutathione level were also recorded (Xu *et al*., 2018). A combination of aflatoxin (168 ppb), ochratoxin (8.4 ppb), zearalenone (54 ppb), T-2 toxin (32 ppb) in naturally contaminated poultry feed induced significant decreases in urea nitrogen and haematocrit values alongside altered GGT activity after a 35-day study in broilers (Aravind *et al*., 2003).

Several bacteria organisms have been implicated in poultry feed contamination, however, not much is known on the haematology and serum biochemistry of birds fed bacteria-contaminated feed. Nevertheless, studies have shown that birds orally infected with *Salmonella enterica serovar gallinarium* (causative agent of fowl typhoid) showed decreased body weight, reduced feed intake, with haematological abnormalities such as low Hb concentration, PCV and RBC count, leukocytosis due to heterophilia and lymphocytosis, and significant increases in ALT and AST activities (Shah *et al*., 2013). One hundred and ninety-two (192) day old broiler chicks showed significant increases in serum AST, ALT and LDH activities, hyperglobulinemia and decrease in ALP activity, hypoproteinemia and hypoalbuminemia post-infection with *E. coli* (Sharma *et al*., 2015). *S. aureus* co-infection with *E. coli, Proteus mirabilis* and *Pseudomonas aeruginosa* resulted in anaemia, leukocytosis, lymphocytosis, monocytosis, significant increases in serum activities of AST, ALT and uric acid, creatinine, alpha- and gamma globulins, IL-6 and TNF-α levels, hypoproteinemia and hypoalbuminemia in naturally infected chicks (Youssef *et al.*, 2019).

Gross and histopathological lesions: The type of microbe involved determines the severity, nature, and morphology of the lesions. Extensive effect on the lungs, kidneys, air sac and heart can occur before any apparent clinical signs are noticed. Gross lesions seen in feed microbial induced liver damage include pale, enlarged and friable liver (Ortatatli *et al*., 2005; Pandey and Chauhan, 2007), multiple necrotic foci have also been reported (Omer *et al*., 2010). Bacteria like *E. coli* and *Salmonella* from feeds present mild congestion and haemorrhages in the lungs and air sac (Deshmukh *et al*., 2007; Martin *et al*., 2007). Multi-foci grayish to yellowish mycotic granuloma have been reported in birds exposed to feeds contaminated by fungi (Monson *et al*., 2015; Ahamad *et al*., 2018). The sizes of the granuloma may range from 2 cm and above, and may be located either in the lungs, air sacs, oesophagus, proventriculus, gizzard, small intestine, liver, kidney, spleen, skin, trachea, peritoneum, brain, eye, muscle or heart (Akan *et al*., 2002; Throne Steinlage *et al*., 2003; Martin *et al*., 2007; Singh *et al*., 2009). Consolidation in the lungs of birds have also been reported (Talha *et al*., 2001; Islam *et al*., 2003; Ghosh *et al*., 2006). Enlarged and congested kidneys have been reported in birds exposed to feeds contaminated with both bacterial and fungi microbes (Kumar *et al*., 2004; Ahamad *et al*., 2018). Congested and haemorrhagic kidneys caused by *E. coli* present in feeds have been reported (Dutta *et al*., 2013; Srinivasan *et al*., 2014). *E. coli* contaminated feeds fed to birds causes formation of a thin fibrin layer over the pericardium (Manimaran *et*

al., 2003). Enlarged and discoloured spleens have been identified in birds fed *Salmonella* contaminated feeds (Holt *et al*., 2006; Msoffe *et al*., 2006; Saha *et al*., 2012). Haemorrhagic to catarrhal enteritis have been reported in birds fed to *Salmonella* contaminated feeds (Hafeji *et al*., 2001; Islam *et al*., 2003; Deshmukh *et al*., 2007). *Aspergillus* from feeds presents with nonulcerative dermatitis swelling and exudates on the eyelids and conjunctival sac (Akan *et al*., 2002).

For histopathological lesions of birds that consumed feeds contaminated with fungi, the mycotic granuloma in the lungs and air sacs have characteristic necrotic center with interlacing fungi hyphae surrounded by mononuclear cells, giant cell and fibrous tissues (Kumar *et al*., 2004; Cortes *et al*., 2005; Zafra *et al*., 2008). *Aspergillus* infection presents degenerative changes, haemorrhage and necrosis around the central vein in the liver (Klein *et al*., 2002; Ortatatli *et al*., 2005; Pandey and Chauhan, 2007). There is also fibrinous perihepatitis, leukocyte infiltration and proliferation in the liver. Hyperplasia of the bile duct, epithelial and periportal fibrosis presents in *Aspergillus* infection from feeds (Pandey and Chauhan, 2007). Small generalized perivascular and inter-septal oedema and bronchial alveolar haemorrhages with serous exudates are features associated with *E. coli* infection from feeds (Kumar *et al*., 2004). *Aspergillosis* has been reported to cause variation in crypt and villus height in the both jejunum and duodenum (Applegate *et al*., 2009; Yang *et al*., 2012). Microscopic lesion seen in the kidney caused by *E. coli* include swollen proximal convoluted tubules, focal interstitial nephritis, degeneration of the tubular epithelium and cellular infiltrations (Kumar *et al*., 2004). Extensive depletion and focal necrosis have been reported in the spleen due to salmonellosis and colibacillosis arising from contaminated feeds (Holt *et al*., 2006; Msoffe *et al*., 2006; Saha *et al*., 2012; Abalaka *et al*., 2017). Intestinal degeneration, necrosis and desquamation of mucosal epithelia have been associated with *E. coli* infections from feeds (Islam *et al*., 2003; Ghosh *et al*., 2006).

Strategies for mitigating microbial contaminants in poultry feed: Risk mitigation strategies are necessary for safe feed production in any feed mill. Producing a pathogen-free feed may be difficult because the pathogens are invisible to the naked eye, and they can be transferred through the feed mill to the potential animal feeds. At the feed mill, improved microbial control can be accomplished by following good manufacturing practices, employee training, cleaning/sanitation and quality assurance. In recent times, ingredient suppliers and animal food producers have made significant progress in reducing feed pathogens like *Salmonella* by 40% using the approaches of Li *et al*. (2012). Most feed mills apply conventional methods to remove bacterial contaminants in feeds and these methods are limited to control of spore formers (da Costa *et al*., 2007). Control of microbial contaminants in feeds can be achieved through either physical or chemical methods. In most feed mills, antimicrobial drugs are used to improve feed safety, enhance the shelf-life of feeds and growth of birds, however this may add additional risk to the food chain as most antimicrobial resistant bacteria are selected against and it may have adverse consequences to humans and animals (da Costa *et al*., 2007). Aside from biosecurity plans and preventative measures, physical methods of mitigating microbial contaminants in feed such as irradiation and thermal processing abound (Darwish *et al*., 2013). Irradiation of feeds using gamma rays from cobalt-60 appears to be a better alternative to the conventional methods as it improves the safety, quality, and shelf-life of feeds (Trudeau *et al*., 2016; Jayathilakan *et al*., 2017). The method also eliminates *Salmonellae*, *Enterobacteria*, molds and insect pests from feeds (Jayathilakan *et al*., 2017). Irradiation breaks down feed particles into digestible forms thereby improving feed nutrient digestibility and utilization (Daghir and Murtada, 2018). Thermal processing of feeds which is done via pelleting using temperature and time to ensure the lethality of the process on biological hazards (Jones, 2011).

Chemical mitigation such as addition of feed additives in feeds is necessary to decrease the risk of cross-contamination after thermal processing and irradiation. Some known chemical additives include organic acids and formaldehyde, essential oils, medium chain fatty acids, and acidulants like sodium bisulfate. Organic acids such as propionic, formic, lactic and acetic acids have been shown to reduce the presence of *Salmonella* in feeds (Amado *et al*., 2013; Koyuncu *et al*., 2013; Menconi *et al*., 2013). It has been proposed that organic acids mitigate *Salmonella* contamination by penetration of the cell membrane into the bacterial cell's cytoplasm, thereby causing increase in pH and cell atrophy (Brul and Coote, 1999). Essential oils such as oils of oregano, rosemary, garlic, turmeric, and capsicum have been found to have antimicrobial properties. It is thought that some essential oils contain phenol compounds which interact with and disrupt the cell membrane of bacteria, causing the cell to lose functional properties and leak the inner cell materials (Rasooli *et al*., 2006). The quantity of *Salmonella* colonization of feeds fed to broiler chicks was found to be reduced when caprylic acid was added to the feed (Johny *et al*., 2009). Inclusion of sodium bisulfate in poultry feeds reduces enzyme activity in the feed and was effective in reducing microbial contamination (Kassem *et al*., 2012). Although the mitigation measures are not exhaustive in the present review, the list however ensures that microbial contaminants in feeds are reduced to the barest minimum, if not eliminated and the produced feeds are safe for poultry consumption.

Conclusion: Poultry feeds are prepared from different plant and animal ingredients blended to provide the nutritional needs of birds. These ingredients undergo many manufacturing processes including grinding, mixing, pelleting, and extrusion, drying and packaging. Because of the varied sources of raw materials used in feed production, as well as the different stages of production process involved, contamination with microorganisms is very common. Although bacterial contamination appears to be more common, fungal and viral contaminants are also problems associated with feed safety. Feed contamination has a serious one health implication as the feed acts as reservoir to many bacterial (including antimicrobial-resistant strains) and fungal pathogens of both human and animal. Control measures to reduce microbial contamination of poultry feeds involve both physical and chemical approaches. The physical method which includes proper feed mill biosecurity plans, irradiation and thermal processing gives satisfactory results; it requires a combination with chemical additives because feeds which have undergone irradiation and thermal treatment may likely be contaminated as the feed passes through different stages of processing. Although manufacturers apply strict measures to ensure that poultry feeds released to the markets are safe, complete elimination of microbial contaminants in feeds is still a difficult task to most manufacturers.

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