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Review Article
Feed Microbiology: A Forsaken Piece in Animal Nutrition Puzzle

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Abstract

The importance of microbial quality of the feed is not being considered in animal feeding till they affect the life or performance of the host. The animal feedstuffs-roughages and grains were exposed to various environmental conditions before they eventually reach the oral cavity of the animals. The microbes bacteria, virus and fungi along with harmful protein all affects the animal welfare and performance. These microbes not only affects the animals but also the humans with respect to *Salmonella*, scrapie, listeriosis. The contamination with fungus leads to not only change in colour, flavour and palatability, it also causes hepatic and kidney damages due to the production of mycotoxins. These toxins also possess tumour causing properties. The presence of microbial population in silage is very much important for the preparation of good quality silages. However, this area of animal nutrition is rarely under studied except the mycotoxins. The importance of microbiological quality is gaining importance over the past two decades since the report of bovine spongiform encephalopathy in Europe.

Key words: Bacteria, bovine spongiform encephalopathy, feed microbiology, mycotoxins, silage

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INTRODUCTION

The art of establishing a favorable microbial population in the animal body especially in the digestive system for better performance, feed utilization and immune status is getting wider importance. Now-a-days, everyone is in counsels/researches on Direct Fed Microbials (DFM) which involves the incorporation of selected microbes (Lactic acid bacteria, *Bacillus*, yeast, etc.) in the animal feeds due to their beneficial effect on host system[1]. In order to achieve this desired population of microbes in the gut, the studies went to the level of establishing them during embryonic stage challenging the proven concept of maintain sterile gut environment at the time of birth. This is being achieved through the method of *in ovo* incorporation in chickens with respect to broilers especially[2]. But the effect of environmental or plant attached microbes on the animal body which is mostly harmful/pathogenic of origin.

The quality of feed is assessed by its nutritional value, particle size, colour, its safety after consumption and microbial quality[3]. Among these, the nutritive value and microbial quality is more important and is the case for all categories of animals as other factor vary according to the size, age and physiological status of the animals[4]. The microbial quality of animal feeds has gained much importance than ever before ever since the occurrence of salmonellosis, coliforms, *Campylobacter* and Bovine Spongiform Encephalitis (BSE) in the European Union. The awareness on microbial characteristics in United Kingdom since 2001-2002 after epidemic incidence of foot and mouth disease. The animal feeds are being contaminated with microbes (bacteria, virus and yeast/fungi) of different origins[5]. However, the occurrence of fungal infestation in grains/forages is matter of concern due to its ubiquitous nature and also their secretory products. The incidence of fungal contamination in animal grains and feed stuffs is a regularly reported especially during winter, rainy and hot-humid seasons. The fungal infestation resulted in detrimental effects on almost all animal species worldwide. This detrimental effect of fungal infestation was due to the generation of mycotoxins from certain species and strains of moulds. The microbial contamination of feed might be due to numerous factors such as plat itself, soil from which it grows air of locality, water quality, animals grazing on the pasture and also during the periods of harvesting, storage and processing[6]. Similarly, the environmental contamination with bacteria (*Salmonella*, *E. coli*, *Listeria*, etc.) and viruses (FMD) are commonly encountered in almost all parts of the world[7]. The consumption of these infected feeds not only affects the host of animals but these animals will in turn act as source for environmental contamination through its faecal excretion. The quality of feeds got deteriorated when contaminated with microbes. The contamination includes about variety of species which is influenced by lots of factors namely pasture, environment, season, type of plant, method of extraction and feed preparation, etc. Similarly, the occurrence of contamination was reported to be varying with the ingredients. For instance, the presence of *Micrococcus* and *Bacillus* was predominantly higher among protein ingredients[8] against the predominance of *Enterobacter* and *Klebsiella* sp., as reported by Mdemu *et al.*[9] in commercial poultry diets. Studies by Product Board Animal Feed (PBAF)[10] and Kukier *et al.*[4] revealed that oilseeds and cakes, extracts (its derivatives) were termed as critical feed material in animal feeds based on its microbial quality assessment. Over the decades, the importance of microbial contamination received more attention worldwide[11,12]. So far so many in-feed additives such as antimicrobials, probiotics, prebiotics, organic acids and phytochemicals were being tried[1,13]. However, the addition of organic acids such as propionic, butyric acids[14] and also certain phytochemicals[15,16] during feed processing or storage had improved the microbial quality of feeds especially against fungal infestations. Hence, in the present review provides an overview about the occurrence, effects of microbial contamination in animal feeds and fodders.

MICROBIAL CONTAMINATION

Animal feeds get contaminated with harmful bacterial organisms like *Salmonella, Listeria* and *E. coli* and majority of contamination of *E. coli* originates from contamination from faecal sources and slurry in compound feeds and pastures. Water activity, oxygen tension, pH and nutrient composition of feed affect microbial diversity[17]. Silo storage of pellets where day and night temperature varies leads to condensation of silo walls causing humidity entry in silo favouring bacterial growth. Feeding of catering waste to pigs in UK caused the outbreak for FMD causing the ban of catering waste containing meat products feeding.

Bacterial contamination: Major threat in bacterial contamination in zoonoses aspect is *Salmonella* sp., following which other spore forming organisms like *Clostridium* sp., *Bacillus* sp. and other Enterobacteriaceae family[18,19]. European Union Regualtion No. 142/2011 gives that Enterobacteriaceae count cannot exceed 300 colony forming units (CFU g^{-1}) in five batches of feed samples derived from animal by-products. The bacterial contaminations of feeds were mainly of Gram negative organisms to be precise the microbes belongs to

Enterobacteriaceae being *E. coli* and *Salmonella* spp. were the major organisms in poultry feeds with occurrence about 16 and 13%, respectively[20]. One of the study by Al-Musawi *et al.*[21] stated that bacterial contamination of imported feed of poultry in Iraq has 13.6% of *Salmonella*, 16% of *E. coli, Klebsiella* at 2.4%, *Shigella* 3.2% and *Proteus* sp., 3.2% among the total Gram negative bacterial contamination of 38.4% of analyzed samples (n = 48). Important source of *Salmonella* in feed is barley among the cereals and blood with fish meals[11]. Spreading cattle slurry on grazing land and forage cultivable area act as potentially significant source of infection. The *E. coli* contamination in cattle feeds signifies faeces contact with either feed directly or at production point.

Fungal nemeses: Fungi from plant pathogens or developed during storage contaminates mostly grains and oilseed by-products. Mycotoxins, harmful compounds produced from secondary metabolism of fungi are the major cause for the effects of fungal contamination. Moisture content and ambient temperature are the major factors for fungal growth in the field of forages and cereals or during processing and storage. *Aspergillus* sp., grows in warm and humid conditions, whereas *Penicillium* proliferates in temperate foods. Cereals from tropical and sub-tropical conditions favour growth of *Fusarium* fungi[22]. *Fusarium graminearum* is worldwide regarded as most important fungal contamination in cereals and grains causing agricultural losses[23] which produces zearalenone mycotoxin which produces oestrogenic response in animals (Table 1).

Maize gets infected with *A. flavus* prior to harvest and remain viable even during storage. *Fusarium* species causing head blight in wheat, barley and ear rot in maize carries the contamination to feeds if contaminated feeds are used. Use of propionic acid prior to storage prevents the fungal contamination. Plant feed material contamination by fungi remains constant or increases in rainy years, when the total annual rainfall exceeds national average[12].

Bovine Spongiform Encephalopathy (BSE)/scrapie: The fatal neurological disease Bovine Spongiform Encephalopathy (BSE) was reported to be recently identified towards the final third of 20th century (1970) and subsequently 16 years were needed to confirm the same. The BSE belongs to the transmissible category[25,26], where the naming of condition changed according to the species it affects as scrapie in sheep and goats, Creutzfeldt-Jakob Disease (CJD) in humans and chronic wasting disease in wild animals (deer, elk, moose, etc.). The causative agent is an abnormal protein which

Table 1: Occurrence of fungal infestation among different feedstuffs and type of toxin produced

Fungal organisms	Infestation	Type of toxin produced
Aspergillus flavus	Peanut meal, cottonseed cake,	Aflatoxins
A. parasiticus	palm kernel cake, maize, compound feeds	
A. flavus	Oilseed meals, compound feeds	Cyclopiazonic acid
A. ochraceus,	Barley and wheat grains	Ochratoxin A
Penicillium viridicatum,		
P. cyclopium		
P. citrinum, P. expansum	Cereal grains	Citrinin
P. citreo-viride	Cereal grains	Citreoviridin
Fusarium culmorum	Cereal grains	Deoxynivalenol
F. graminearum		
F. sporotrichioides	Cereal grains	T-2 toxin
F. poae		
F. sporotrichioides	Cereal grains	Diacetoxyscirpenol
F. graminearum		
F. poae		
F. culmorum	Cereal grains	Zearalenone
F. graminearum		
F. sporotrichioides		
F. moniliforme	Maize kernels	Fumonisins
		Moniliformin
		Fusaric acid
Neotyphodium	Grasses	Ergopeptine alkaloids
coenophialum		
Phomopsis	Lupin stubble	Phomopsins
leptostromiformis		
Pithomyces chartarum	Pastures	Sporidesmin A
N. lolii	Grasses	Lolitrem alkaloids
Claviceps purpurea	Cereal grains	Ergot alkaloids

Adapted from D'Mello[24]

formed due to continuous accumulation of misfolded proteins-prion. Prion proteins came into limelight when scrapie infected sheep meat and bone meal was fed to cattle leading to Bovine Spongiform Encephalopathy (BSE). This is a progressive disease which gradually damages the nervous system and alters the behaviour of the animals and an apt name of "Mad cow disease" was given to this disorder. The occurrence/incidence was reported in animals fed with under processed animal by-products such as meat and bone meal. These prion proteins are highly resistant to the normal sterilization process of rendering. The consumption of affected animal's meat results in occurrence of disease syndrome in humans. Hence, the EU banned the use of animal by-products in feeding of food animals.

Exposure of animals to unhygienic environment and high microbial load in feed stimulates immune system and hampers the homeostatic pathways that regulate metabolism, nutrient partitioning, behaviour, thermoregulation and hypothalamic pituitary adrenocortical activity[27]. Mycotoxins affects animals by causing mutagenic, carcinogenic, teratogenic, neurotoxic, oestrogenic and immunosuppressive changes, whereas proteolytic and lipolytic bacteria lead to disintegration of lipids and proteins changing the nutritive value of feed ultimate goal with regard to microbial load in feed is not giving sterile feed but to give feed with 'safe contamination level'.

SILAGE MICROBIOLOGY

The process of silage making plays an important role in feeding of green forages during post-monsoon seasons throughout the continents. In this process of preservation of high-moisture green roughages depends upon how well the activities of microbes especially bacteria (Table 2). From the Table 2 it was clear that the majority of the crop microbes were aerobic, hence maintenance of strict anaerobic condition during each step of ensiling after rapid imposition is necessary. In the preservation process, the soluble plant sugars are fermented to lactic acid by the proliferating lactic acid bacteria. The attainment of pH as low as 4 will results in successful silage preparation[5]. When the fermentative process shits from lactic acid to other like ammonia which results in characteristics foul smelling. The preservation freshly cut/lush crops are too much demanding process as their fermentation favours the growth of undesirable microbes especially of *Clostridium* sp.

The maintenance of anaerobic condition and fermentation of plant sugars into lactic acids are the two important steps in conversion of green forage to silage[29].

Table 2: Microbial populations in crops for silage preparation

Organismss	Crop (CFU g^{-1})
Bacteria-aerobic	>1,00,00,000
Bacteria-anaerobic	10-10,00,000
Enterobacteria	1000-10,00,000
Yeast	1000-1,00,000
Molds	1000-10,000
Clostridia	100-1000
Bacilli	100-1000
Acetic acid bacteria	100-1000
Propionic acid bacteria	10-1000

Source: Pahlow *et al.*[28]

During the process of ensilage, there will be change in microbial population as the population of lactic acid bacteria dominates all other microbes especially of aerobic microbes. During ensilage either one of the two types of fermentation (homo- and hetero-fermentation) will takes place and ultimately determines the silage quality. The production of lactic acids results in drop in pH of the silage to about 4-5 against the scale of 14. The degree of drop in pH varies with plants that are being used for silage making. For instance, the pH drops to below 4 in plants that are having more soluble sugars (corn, grass) than the legumes (alfalfa)[30]. The fermentation pattern of both the homo- and hetero-fermentation results in lactic acid, acetic acid, ethanol and CO_2 based on the type of lactic acid bacterial populations (Table 3).

Among these two fermentation types, the homo-fermentative more desirable as maximum acidity could be obtained (lactic and acetic acid) which inhibits the growth of aerobic organisms, moulds and yeast and protect the silage from spoilage. The maintenance of anaerobic environment during silage making not only favours the growth of lactic acid bacteria, also the growth of obligate anaerobe clostridial organisms. These clostridial organisms all of three types, the proteolytics which ferments amino acids and results in ammonia and amines, *Clostridium butyricum* ferments sugars and *Clostridium tyrobutyricum*-sugars and lactic acids. The proteolytic fermentation not results in amines and ammonia generation which causes ammonia odour as well as increase the pH towards alkaline side (>5 pH). This raise in pH accompanied with high moisture facilitates the growth of fungus which changes the smell, taste and colour which in turn the acceptability by the animals. The production of butyric acid as a result of clostridial fermentation caused reduced feed intake in ruminants at a level of >5 g kg^{-1} dry matter[31]. Hence, the fermentation dynamics inside the silo should be maintained towards lactic acid production. The studies by various researchers, *Lactobacillus plantarum*[32], *Lactobacillus buchneri*[33,34] strongly substantiated the use of inoculation of lactic acid bacterial culture during the process of silage making as an additive.

Table 3: Homo- and hetero-fermentation during silage by lactic acid bacteria

Types	Fermentation end products
Homo-fermentation/facultative hetero-fermentation	1 6-carbon sugar→2 Lactic acid
Facultative hetero-fermentation	1 5-carbon sugar→1 Lactic acid+1 acetic acid
Hetero-fermentation	1 6-carbon sugar→1 Lactic acid+1 acetic acid+CO_2
	1 6-carbon sugar→1 Lactic acid+1 ethanol+CO_2
	1 5-carbon sugar→1 Lactic acid+1 acetic acid
	1 Lactic acid →1 Acetic acid+CO_2

Source: Muck[30]

DETECTION AND QUANTIFICATION OF EXTENSION OF MICROBIAL CONTAMINATION

The assessment of microbial contamination in animal feeds needs to be rapid, sensitive and representation of diverse population. The approaches included the complete enumeration by total plate count[35,20] (both aerobic and anaerobic organisms)[36-38], heterophil counts[39], fungal counts[40,41] and bacteriophages[42]. The Thin Layer Chromatography (TLC) quantify mycotoxins formed and indirectly measure the extent of fungal contamination which mainly during the post-harvest and storage periods. These conventional procedures are less sensitive and time consuming; hence, the modern techniques of polymerized chain reaction could be used in assessment of feed microbial quality[43]. This test will be rapid, more sensitive and also representation of diverse microbial contamination in the feed[44-46]. This protocol includes, DNA extraction, amplification of 16S DNA, amplification of the DNA and can be detection by electrophoresis[47,48].

CONCLUSION

Ultimate goal with regard to microbial load in feed is not giving sterile feed but to give feed with 'safe contamination level'. Evaluation of feed safety for microbiological contamination and establishing a safer level for allowing entry into feeding is needed.

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