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*Review*

# Aflatoxin Contamination of the Milk Supply: A Pakistan Perspective

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**Abstract:** Improving both quality and quantity of food available is a pressing need especially when one eighth of the world's population consumes less energy than is required for maintenance and is exposed to contaminated food, both of which lead to greater susceptibility to diseases. The Pakistani population depends heavily on milk for nutritional needs and 10% of household income is spent on milk. This commodity requires continuous monitoring and care from its site of production by smallholder dairy producers through to urban consumers along tradition milk marketing chains. Feed ingredients used as concentrate feed to enhance milk production are often contaminated with mycotoxins, which, after ingestion, are transferred into milk. Aflatoxins can contribute to the causation of liver cancers, immune system disorders, and growth-related issues in children. Moreover, deaths in both humans and animals have also been reported after ingestion of aflatoxin-contaminated food. Studies have shown contamination of food and feed ingredients with mycotoxins, especially aflatoxins. This review places the dairy industry into context, summarizes how milk and milk products are contaminated with aflatoxins, and discusses the present legislative regulation of milk quality implemented in Pakistan. There is a need to eliminate fungus-susceptible animal feed ingredients, which are the source of mycotoxins so prevalent in the milk marketed to the consumer in Pakistan.

**Keywords:** aflatoxins; AFM1; AFB1; milk marketing chains; hepatocellular carcinoma

## 1. Introduction

The nutritional status and health of human populations depend heavily upon the economic status of nations. Pakistan is still facing problems of nutritional deficiencies and diseases, both acute/chronic and infectious/noninfectious among its ever-burgeoning population of 185 million [1]. Malnutrition affects children and women in particular [2,3]. Stunting and severe wasting affects 37% and 13%, respectively, in a population where 36% is under 15 years of age [4]. This situation is exacerbated by the fact that 60% of workers in the country earn less than $2 a day [5].

Milk and milk products are important in providing nutrition to the poor population in Pakistan and one fourth of the total household budget that goes into the food, is spent on milk [6]. The smallholder dairy production system is the prevailing enterprise throughout the country, providing around 95% of the total milk production [7]. Thirty-seven percent (8.8 million) of Pakistan's households rear livestock for milk production [8]. Maintaining the quality of this valuable commodity with poor on-farm hygiene standards and rudimentary marketing chain infrastructure for transferring the product to the consumer is a challenge for all involved [9]. These traditional informal marketing chains carry more than 30% of the country's production to big cities in the absence of the cool chain facilities associated with production in more advanced economies [10].

Since milk forms an integral part of the diet of Pakistani population to provide its nutritional requirements, its purity and wholesomeness is imperative. Some reports have shown the presence of adulterants [11–13] and contaminants [14–16] in milk, resulting in the product being a hazard to the health of consumers. Among milk contaminants, mycotoxins, especially aflatoxins [17] and antimicrobial residues [18], are at the top of the list of chemical hazards to human health. Mycotoxins are transferred to milk through animals fed with contaminated forages [17,19], which are highly prevalent in the commercial feed industry in Pakistan.

Aflatoxins (AFs) are thought to be one of the major causes of hepatocellular carcinomas (HCC) in humans [20]. Furthermore, liver cancer is ranked as the third most common cause of death due to cancer worldwide. The major burden of HCC cases (82%) is shared by developing countries [21]. The high prevalence and rapid increase in cases of HCC in Pakistan from 1970 to 2011 has been discussed in detail by Butt *et al*. [22]. Exposure to AFs has been reported to increase the risk of developing HCC by 5.5 times [23]. Synergism between hepatitis B virus and AFs has also been reported to cause HCC [24] and the risk is increased around 30-fold in the presence of both of them [25]. Apart from liver cancers, chronic exposure to aflatoxins interferes with metabolism of proteins and various micronutrients and affects immunity. An estimated 4.5 billion people in developing countries are exposed to uncontrolled amounts of mycotoxins. The toxic effects of aflatoxins, together with immunity and nutrition, negatively affect the health of the poor population [26].

Milk, being an essential part of the daily diet in the Pakistani population, could be one of the most important factors if contaminated with aflatoxins. This review focuses on the risk of contamination of milk with aflatoxins and its impacts in Pakistan.

## 2. Prevalence of Mycotoxins in Animal Feed in Pakistan

Apart from the intentional chemical adulteration, fungal toxins and veterinary drug residues are also important contaminants that can be transferred to milk. Major fungal toxins include AFs, zearalenone (ZEA), deoxynivalenol (DON), fumonisins (Fs), and ochratoxins (OTs). In a survey conducted by Binder *et al*. [27], more than half of the samples ($n$ = 1507) collected from European and Mediterranean markets and one third of the samples ($n$ = 1291) from the Asia-Pacific region were found to be positive for DON, T-2 toxin, ZEA, Fs, and AFs. Exposure to mycotoxins may compromise the production and health status in animals and humans, respectively. Toxicity of mycotoxins in animals affects mainly production and chronic problems; it rarely causes death. On the other hand, contaminated animal products that are used for human consumption may cause serious health problems [28]. The growth rate in pigs and poultry was depressed by 16% and 5% for each mg/kg of aflatoxins added into the feed [29]. Various mycotoxins, especially AFs, have been reported in animal feed in Pakistan (Table 1): their health effects are discussed in detail below.

**Table 1.** Prevalence of various mycotoxins in animal feed in Pakistan.

| Mycotoxins | Type of Feed | Province | Maximum Concentration (ppb) | References |
|---|---|---|---|---|
| AFB1 | Poultry feed and poultry feed ingredients | Punjab, Sindh, KPK, Baluchistan, GB | 78.0 | [30] |
| AFB1 | Poultry feed and poultry feed ingredients | Punjab | 156.0 | [31] |
| AFB1 | Poultry feed | Khyber Pakhtunkhwa | 266.6 | [32] |
| AFB1 | Poultry broiler feed | Baluchistan | 166 | [33] |
| AFB1 | Poultry feed | Khyber Pakhtunkhwa | 191.65 | [34] |
| AFB2 | Poultry feed | Khyber Pakhtunkhwa | 86.85 | [34] |
| AFG1 | Poultry feed | Khyber Pakhtunkhwa | 167.82 | [34] |
| AFG2 | Poultry feed | Khyber Pakhtunkhwa | 89.9 | [34] |
| OTA | Poultry feed ingredients | Sindh | 84.4 | [35] |
| AFB2 | Poultry feed and feed ingredients | Punjab | 39.2 | [36] |
| OTA | Poultry feed and feed ingredients | Punjab | 111.2 | [36] |
| AFs | Feed ingredients | N/A | 57.0 | [37] |
| AFB1 | Poultry feed and feed ingredients | Punjab | > 12.5 | [38] |
| AFB1 | Concentrate feed | Punjab | 554 | [19] |
| AFB2 | Concentrate feed | Punjab | 50 | [19] |
| DON | Concentrate feed | Punjab | 166 | [19] |
| FB1 | Concentrate feed | Punjab | 230 | [19] |
| OTA | Concentrate feed | Punjab | 31.2 | [19] |
| ZEA | Concentrate feed | Punjab | 18 | [19] |

## 3. Aflatoxin M1

Aflatoxins have been comprehensively investigated for the mechanism of action, carcinogenicity, and mutagenicity. This was paralleled by the development of biomarkers of metabolism of aflatoxins.

In addition, metabolism studies of aflatoxins in animals and humans have provided an opportunity for chemoprevention approaches [39]. Overall, integrated, multidisciplinary research has served as the scientific basis for setting minimum acceptable standards for aflatoxins to reduce human exposure [39,40]. The toxicity of aflatoxin M1 (AFM1) is 2%–10% that of AFB1 [41]. It has been classified by the International Agency for Research on Cancer (IARC) as a group 2B toxin, which is considered a possible carcinogen for humans known to have genotoxic and cytotoxic potential [42]. Aflatoxins influence cell-mediated immunity and phagocytic cell function due to their immunomodulatory capacity [43]. Depending upon the genetics, lactation stage, milk production, milking process, and health status of the animal, 0.3%–6.2% of the AFB1 in feed is metabolized and excreted into milk in the form of AFM1 [44–46]. Pasteurization and even Ultra High Temperature (UHT) treatments are ineffective in destroying it due to its stability at high temperatures [47]. Thus the dairy industry can only prevent contamination of milk products by testing the milk; the real challenge that remains in the management of commercial feed resources is to prevent mycotoxin-contaminated materials from entering the animal feed chain at the industry level.

Chronic exposure to AFM1 is believed to be a serious health risk due to its possible accumulation and linkage to DNA. Furthermore, chronic aflatoxicosis causes hepatocarcinoma, immunosuppression, and retarded growth in children. As milk has a key role in the human diet, especially in the development and growth of infants, its contamination with AFM1 poses a serious hazard for public health and food safety [48].

Poor economic status of countries, climatic change, and variation in environment and agricultural malpractices all contribute to increases in the concentration of AFM1 in milk and milk products [49]. Seasonal differences in concentrations of AFM1 in milk samples have also been reported [50]. A recent study [51] has shown the maximum concentration of AFs in milk marketed from small-holder dairy farms in the monsoon season in Punjab Pakistan was 2.59 ppb, whereas the lowest concentration was observed in summer (1.93 ppb). Animals are fed on pasture grasses, fresh green fodder, and weeds in summer, resulting in lower concentrations of AFM1 in milk: stored concentrate feeds are less prevalent in the feed base at this time of year. On the other hand, concentrate feed is provided to animals in winter due to a lack of availability of fresh fodder; it is these concentrates that are more susceptible to the growth of fungus resulting in higher concentrations of AFM1 in milk and milk products during this season across Pakistan [17]. Furthermore, the use of preserved green fodder is becoming more common in the form of hay and silage. Lack of cool chain systems and high temperature and humidity during the monsoon season often facilitate the growth of *A. flavus* and *A. paraciticus* [52]. The presence of these higher levels of AFs in milk available to consumers, including children and women, is alarming in developing countries like Pakistan where public awareness is lacking and health facilities are primitive, particularly for the poor.

*Aflatoxin M1: Pakistan's Perspective*

Lack of education for dairy farmers and insufficient financial and infrastructural facilities are two of the most important issues [53]. Quality checks in formal and informal milk marketing chains are perhaps the most neglected area. No test is in routine use by the largest milk processing companies for mycotoxins [54], although this has changed in the past two years as some companies are providing specially made concentrate feed to farmers and giving them incentives for the production of good quality milk. The European Commission regulation (EC) 1881/2006 [55] has set 0.05 ppb and 0.025 ppb as the maximum permissible levels of AFM1 in milk consumed by adults and infants, respectively. On the other hand, the USA has set an upper limit of 0.5 ppb, which is 10 times higher than the standards set by the EC [56]. Recent studies (Table 2) have clearly shown the prevalence of AFM1 in milk in Pakistan. Pakistan is one of those countries where the government has not imposed any limits for AFM1 in milk and milk products [57]. The Pakistan Standard and Quality Control Authority has recently set the maximum limit for aflatoxins in milk as 10 ppb [58]. This limit is still too high for such an important commodity used on a daily basis by a broad population of all ages. No further limits have been set for AFM1 in milk products and other mycotoxins in milk and milk products. A comprehensive set of limits needs to be formulated and adopted to ensure the product's safety for consumers.

**Table 2.** Summary of some recent studies for aflatoxin M1 in milk in Pakistan.

| Species | No. of Samples Analyzed | Mean Concentration ppb | Range of Concentration ppb | Samples Exceeding EU Limits (0.05 ppb) | References |
|---|---|---|---|---|---|
| Mix (cattle & buffalo) | 168 | 0.371 | 0.01–0.70 | 99.40% | [57] |
| Buffalo | 97 | 0.091 in winter<br>0.042 in summer | 0.050–0.200 in winter<br>0.025–0.105 in summer | 55% of winter<br>38% of summer | [17] |
| Cattle | 76 | 0.089 in winter<br>0.022 in summer | 0.065–0.150 in winter<br>0.014–0.095 in summer | 56% of winter<br>33% of summer | [17] |
| Goat | 62 | 0.069 in winter<br>0.018 in summer | 0.008–0.090 in winter<br>0.009–0.088 in summer | 32% of winter<br>21% of summer | [17] |
| Sheep | 75 | 0.079 in winter<br>0.024 in summer | 0.010–0.088 in winter<br>0.012–0.069 in summer | 58% of winter<br>36% of summer | [17] |
| Camel | 46 | 0.058 in winter<br>0.010 in summer | 0.012–0.064 in winter<br>0.005–0.081 in summer | 27% of winter<br>14% of summer | [17] |
| Mix (cattle & buffalo) | 107 | 0.151 | 0.00–0.845 | 41% | [15] |
| Mix (cattle & buffalo) | 104 | 0.049 | 0.00–0.89 | 25% | [59] |
| UHT Milk | 84 | 0.07 | 0.00–0.51 | 24% | [59] |
| Buffalo | 360 | 0.027 | NA | 13.90% | [60] |
| Cow | 120 | 0.044 | NA | | [60] |
| Mix (cattle & buffalo) | 21 | 0.018 | 0.00–0.040 | 0% | [61] |
| Cow | 84 | 0.037 | 0.00–0.084 | 15.50% | [53] |
| Buffalo | 94 | 0.043 | 0.00–0.350 | 17.00% | [53] |
| Mix (cow & buffalo) | 84 | 17.38 | 0.69–100.04 | 100% | [12] |
| Mix (cow & buffalo) | 232 | 0.252 | 0.00–1.9 | 32% | [14] |
| Mix (cow & buffalo) | 485 | 2.23 | 0.00–7.28 | 96.50% | [51] |

Milk products like yogurt, butter, cheese, and ice cream are very commonly used in Pakistan. Table 3 provides a short summary of studies conducted on the prevalence of AFM1 contamination of milk products in Pakistan.

**Table 3.** Studies reporting the presence of aflatoxin M1 in milk products in Pakistan.

| Product | No. of Samples Analyzed | Mean Concentration of AFM1 | Range of Concentration of AFM1 ppb | Samples Exceeding EU Limits (0.05 ppb) | References |
|---|---|---|---|---|---|
| Sweet | 138 | 0.48 | 0.00–1.5 | 78% | [14] |
| Yogurt | 10 | 0.007 | 0.00–0.013 | 0% | [61] |
| Butter | 10 | 0.003 | 0.00–0.007 | 0% | [61] |
| Yogurt | 96 | 0.090 | 0.00–0.616 | 29% | [15] |
| White cheese | 119 | 0.148 | 0.00–0.595 | 12% | [15] |
| Cheese cream | 150 | 0.103 | 0.00–0.456 | 6.67% | [15] |
| Butter | 74 | 0.070 | 0.00–0.413 | 23% | [15] |
| Yogurt | 96 | 0.037 | 0.00–0.88 | 22% | [59] |
| Butter | 70 | 0.026 | 0.00–0.78 | 27% | [59] |
| Ice cream | 79 | 0.017 | 0.00–0.67 | 11% | [59] |

## 4. Risk Management and Controlling Strategies

Best management practices include preventing mycotoxin synthesis from the field by strategically rotating crops and administrating fungicides [28]. While mycotoxin binders have been proved to be relatively effective, alternative strategies such as enzymatic or microbial detoxification have also been recently used [62]. In addition, application of a specific hazard analysis critical control point (HACCP) system would also help in controlling this risk. The presence of mycotoxins should be monitored continuously. If action is necessary, binders can be added to detoxify or limit the availability of specific mycotoxins in the gastro-intestinal tract [62]. Studies had focused on amending or supplementing diet to reduce the harmful effects of aflatoxins [43]. Weight gain depression was reduced in pigs when they were fed with DL-methionine for 28 days [63]. The effect of *Staphylococcus aureus* protein A was also investigated to prevent aflatoxin-induced immunotoxicity in rats [64]. Protein A proved effective in partially or completely negating the effects of immunotoxicity. Coffey *et al.* [65] developed a simulation model for mycotoxins in dairy milk and its potential for human exposure. The risk to consumers in developed countries is low. On the other hand, recent data from a developing country like Pakistan shows an alarming situation where a high concentration of aflatoxins is detectable in milk.

## 5. Legislation/Policies in Pakistan Relative to the Rest of the World

Punjab, the most progressive province in Pakistan, has not developed any regulations regarding AF contamination in milk. The presence of AFM1 in milk and milk products in Pakistan [14,15,17] clearly shows that immediate measurements need to be implemented to reduce the level of exposure of the milk-consuming population to AFs. According to Ashiq [58], the maximum limit for milk AFs has recently been set as 10 ppb in Pakistan, a limit much higher than the maximum permissible levels allowed in most countries. There is still a need to set more rigorous limits for mycotoxins including AFs in milk and milk products. Research has shown that the removal of contaminated maize and cottonseed products from the diet of cows can reduce the concentrations of aflatoxin in milk to acceptable levels within 72 h [66]. The maximum concentration of AFs in milk should not exceed 0.05 ppb, according to EU standards [67]. These permissible limits have been reduced further to 0.025 ppb in milk used for infants. Details of these limits worldwide have been documented by the FAO [68].

Thus a mass awareness campaign backed by enforceable government regulations has the potential to minimize the risk to human health very quickly. It remains to be seen whether the government would be willing to implement such measures and then enforce them. This would require a quick and easy method for mass screening of animal feeds and then an incentive system for their use by small-holder farmers and their feed suppliers. These incentives must be in the form of a financial reward for high-quality dairy products, or punitive measures in the form of fines for farmers and companies consistently marketing contaminated products.

It is imperative to have clear and enforceable regulations showing the standards for mycotoxin content including AFs in milk. Further research is required to identify the deleterious effects of the ever-growing family of mycotoxins and their metabolites on animal health and, in particular, reproductive efficiency, quite apart from their impact on human health.

## 6. Conclusion

The importance of milk as a dietary source of protein and energy for the malnourished population of Pakistan is not going to decrease. The quality of milk is very much dependent on its handling during transport via traditional milk supply chains after its production on smallholder dairy farms. The intake of AFs by milk-producing animals must be controlled if consumers are to be protected. Thus it is important that AF contamination of feeds is monitored carefully; alternatively, the most commonly contaminated feeds, cotton seed products and cornmeal, need to be eliminated from the dietary regime of dairy animals if fungal contamination cannot be avoided. This, in turn, can help in improving the health status of the underprivileged poor population of Pakistan.

## Author Contributions

Both authors contributed equally.

## Conflicts of Interest

The authors declare no conflict of interest.

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