Comparative Efficacies of Imarsil and Activated Charcoal in Reducing Aflatoxin M$_1$ in Cows’ Milk

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Comparative Efficacies of Imarsil and Activated Charcoal in Reducing Aflatoxin M₁ in Cows’ Milk

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Abstract— Many health risks associated with Aflatoxin M₁ (AFM₁) drive the demand for its control. Adsorption studies of AFM₁ were performed using activated charcoal (AC) and imarsil (0.5, 1 or 2%) at aflatoxin contamination rates of 9, 231 or 456 ng/L for 5 h at 4, 16, 28 and 32°C. The aflatoxin-adsorbing capabilities of the two adsorbents depend on the adsorbent, adsorbent concentration, contact time and treatment temperature. At 4, 16 and 28°C, Imarsil demonstrated significant reductions (p<0.05) at the highest contamination rate and adsorbent concentration; while at 32°C, significant reductions (p<0.05) were observed at all contamination rates and adsorbent concentrations. However, at all treatment temperatures AC exhibited a very poor adsorbent capacity, except at 32°C where a milder activity was only exhibited at the highest contamination rates and adsorbent concentration. Results from the present study indicate that imarsil demonstrates a potential for aflatoxin reduction in the developing tropical world.

Keywords: cow’s milk, aflatoxin M₁, HPLC, adsorbents, activated charcoal, imarsil.

I. Introduction

Milk is an important component of human diets. Milk plays an important role in nutrition, growth, development and immunity (Keira and Mao, 2004). The composition of milk varies from species to species (Kataoka, 2002). Although cow’s milk has continued to play an important role in human nutrition, growth and development, raw milk obtained from cows may contain a wide range of contaminants. Galvano et al. (2001) reported that milk, regarding mycotoxin, is mainly contaminated with aflatoxin M₁ (AFM₁), and consumption of such milk might be a principal route for entrance of AFM₁ into the developing tropical world.

Aflatoxins are fungal metabolites that contaminate the food supply in certain areas of the world (Gourama and Bullerman, 1995; Smela et al., 2001). The contamination of food with aflatoxins is more serious in tropical countries, where relative humidity is high, and the temperatures are conducive to the growth and production of aflatoxins by moulds. These toxins are produced by Aspergillus flavus, A. parasiticus and A. normius, which grow on improperly stored foods. Aflatoxin M₁ is a toxic metabolite of aflatoxin B₁ (AFB₁); it is formed by enzymatic hydroxylation of the B₁ carried over from contaminated feed, primarily cereal grains. It is normally excreted in the urine and also in the milk of dairy cattle (Creepy, 2002; Gurbay et al., 2006; Oliveira and Ferraz, 2007). In a related observation, AFM₁ is present in the milk of nursing women who eat food containing AFB₁ (Henry et al., 2001; Oluwafemi, 2012).

The occurrence of AFM₁ in milk is transitory in nature, usually reaching a peak within two days after the ingestion of the contaminated commodity and disappears within 4-5 days after the withdrawal from a contaminated food source (Henry et al., 2001).

The major concerns with aflatoxins are their potent carcinogenic, mutagenic and teratogenic effects in humans (Battacone et al., 2003, Kocaba and Sekerel, 2003). Although AFM₁ is less carcinogenic and mutagenic than AFB₁, it exhibits a high level of genotoxic activity and certainly represents a health risk due to its possible accumulation and linkage to DNA (Shundo and Sabino, 2006). Avoidance of contaminated food/feed is rarely possible, and feeds that contain relatively low concentrations of AFB₁ may still have deleterious effects on sensitive species such as poultry (Doer et al., 1983; Giambrone et al., 2005; Rauber et al., 2007). Unfortunately, residues of AFB₁ and AFM₁ in different animal products, such as meat, eggs and milk, intended for human consumption have been reported (Pattersen et al., 2005; Mumksgaard et al., 2007; Oluwafemi, 2012).

Recently, several approaches to preventing aflatoxins from entering the food chain such as decontamination or remediation of feed and feedstuffs have been proposed (Bailey et al., 1998; Ledoux et al., 1999). A variety of adsorbents such as bentonite (Rosa et al., 2001), zeolite (Miazzo Roso et al., 2000), hydrated sodium calcium aluminosilicate (HSCAS) (Kubena et al., 1993; Scheideler, 1993; Ramos and Hernandez, 1997), clinoptilolite (Oguz et al., 1994), dietary clay (Phillips, 1999), Saccharomyces cerevisiae (Celik et al., 2001) and activated charcoal (AC) (Jindal et al., 1994) have been used successfully to detoxify AFB₁ in contaminated feeds. In a very recent study, Manafi and Khosravining (2013) reported on the efficacy of an herbal mycotoxin binder (a unique combination of minerals,
antioxidants and enzymes) for the control of aflatoxin in broiler/breeder diets.

Little attention has been paid to studies on adsorption of AFM₁ from milk meant for human consumption. This study is reasonable given the understanding that the control of aflatoxin in feed will automatically translate into alleviation of AFM₁ in dairy cattle that are fed such remediated feed (Smith et al., 1994). However, it is pertinent to note that this wide assumption is hardly applicable to free-range dairy cattle because the diet of such cattle is difficult to monitor or control. Incidentally, a large portion of dairy cattle in many developing countries such as Nigeria are raised under a free-range system. As such, control measures for aflatoxin in products obtained from those animals should be designed, and this is the goal of the present study.

Imarsil™, an inexpensive synthetic absorbent obtained from oxidized natural polymer of Brachystegia nigerica (Akpan and Kareem, 2002). B. nigerica is a legume used especially in the eastern states of Nigeria as condiment to thicken soup. Its thickening characteristics have been attributed to the presence of hydrocolloid property or gelling property (Odum, 2000). Imarsil as an oxidized polymer of B. nigerica is considered as an efficient absorbent because of its quick and simple recovery approach especially in the clarification of microbial enzymes from fermentation broth (Kareem and Akpan, 2003).

The objective of the present study is to compare the efficacy of easily and locally accessible adsorbents, such as Imarsil and activated charcoal, in the reduction of AFM₁ contamination of milk.

II. Materials and Methods

a) Chemicals and standards
Imarsil™ was prepared as previously described by Akpan and Kareem (2002). Solvents such as acetonitrile, methylene chloride, and methanol were of HPLC grade. The standard AFM₁ was purchased from Chromogen (New Delhi, Delhi, India). Immunoaffinity column was Aflastar M1 R, (lot: AF 1011 1012), supplied by RomaLabs Diagnostic Technopark 13430, Tulln, Austria.

b) Adsorption Studies
The adsorption studies of aflatoxin were performed using a 4 x 4 x 2 x 3 x 3 factorial design involving four different contact times (2, 3, 4, or 5 h), four temperature levels (4, 16, 28, or 32°C), two adsorbents (activated charcoal or imarsil) at three different concentration (0.5, 1 or 2%) and three concentrations of aflatoxins (9, 231 or 456 ng/L AFM₁).

Recent report of survey of AFM₁ in cows’ milk from free-grazing cows in Nigeria indicated that toxin levels in positive samples ranged from 9.0 to 456.0 ng/L (Oluwafemi et al., and 2014). Therefore, milk samples (50mL) were spiked with AFM₁ at the three concentrations of 9, 231 and 456 ng/L AFM₁. These were passed through a separating funnel containing activated charcoal and imarsil at three different concentrations (0.5, 1 or 2 %). The experimental setups were in place for 5 hrs with samples taken at 2, 3, 4 and 5 hrs. The experiment was repeated at four different temperatures: 4, 16, 28 and 32°C. Quantification of AFM₁ in the remediated milk samples was performed by a modification of the method of Smith et al (1994) as recently described by Oluwafemi et al. (2014).

c) Nutrient analysis
The nutrient contents such as moisture, protein, fat, carbohydrates and saturated fatty acid were determined using the AOAC method (1993) before and after the administration of the adsorbents.

d) Statistical analysis
The comparisons between means were evaluated using Student’s t-test and analysis of variance. A value of p< 0.05 was considered significant.

III. Result and Discussion
The efficacy of the different tested adsorbents in reducing the AFM₁ in the contaminated milk samples is shown in Figures 1a -4b. The aflatoxin-adsorbing capabilities of the two investigated adsorbents depend on the adsorbent, adsorbent concentration, contact time and treatment temperature. At the investigated temperatures of 4, 16 and 28°C, Imarsil demonstrated a significant reductions (p<0.05) of AFM₁ contents of contaminated milk at the highest contamination rate (0.456 μg/l) and adsorbant concentration(2.0%) (Figures 1a, 2a and 3a). However, at 32°C significant reductions (p<0.05) were observed at all contamination rates and adsorbant concentrations (Figure 4a). On the contrary, at all treatment temperatures AC exhibited a very poor adsorbant capacity, except at 32°C where a mild activity was exhibited only at the highest contamination rates and adsorbant concentration (Figures 1b, 2b, 3b and 4b).
Figure 1a: Effect of Imarsil on AFM1 content of Contaminated milk treated for 5 hrs at 4C

KEY
A = 0.009 μg/l AFM1 + 0.5 % Imarsil;  B = 0.231 μg/l AFM1 + 0.5 % Imarsil;  C = 0.456 μg/l AFM1 + 0.5 % Imarsil
D = 0.009 μg/l AFM1 + 1.0 % Imarsil;  E = 0.231 μg/l AFM1 + 1.0 % Imarsil;  F = 0.456 μg/l AFM1 + 1.0 % Imarsil;
G = 0.009 μg/l AFM1 + 2.0 % Imarsil;  H = 0.231 μg/l AFM1 + 2.0 % Imarsil;  I = 0.456 μg/l AFM1 + 2.0 % Imarsil

Figure 1b: Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 4C

KEY
J = 0.009 μg/l AFM1 + 0.5 % Charcoal;  K = 0.231 μg/l AFM1 + 0.5 % Charcoal;  L = 0.456 μg/l AFM1 + 0.5 % Charcoal
M = 0.009 μg/l AFM1 + 1.0 % Charcoal;  N = 0.231 μg/l AFM1 + 1.0 % Charcoal;  O = 0.456 μg/l AFM1 + 1.0 % Charcoal;
P = 0.009 μg/l AFM1 + 2.0 % Charcoal;  Q = 0.231 μg/l AFM1 + 2.0 % Charcoal;  R = 0.456 μg/l AFM1 + 2.0 % Charcoal
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Figure 2a: Effect of Imarsil on AFM1 content of Contaminated milk treated for 5 hrs at 16°C

KEY
A = 0.009 μg/l AFM1 + 0.5 % Imarsil; B = 0.231 μg/l AFM1 + 0.5 % Imarsil; C = 0.456 μg/l AFM1 + 0.5 % Imarsil
D = 0.009 μg/l AFM1 + 1.0 % Imarsil; E = 0.231 μg/l AFM1 + 1.0 % Imarsil; F = 0.456 μg/l AFM1 + 1.0 % Imarsil;
G = 0.009 μg/l AFM1 + 2.0 % Imarsil; H = 0.231 μg/l AFM1 + 2.0 % Imarsil; I = 0.456 μg/l AFM1 + 2.0 % Imarsil

Figure 2b: Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 16°C

KEY
J = 0.009 μg/l AFM1 + 0.5 % Charcoal; K = 0.231 μg/l AFM1 + 0.5 % Charcoal; L = 0.456 μg/l AFM1 + 0.5 % Charcoal
M = 0.009 μg/l AFM1 + 1.0 % Charcoal; N = 0.231 μg/l AFM1 + 1.0 % Charcoal; O = 0.456 μg/l AFM1 + 1.0 % Charcoal;
P = 0.009 μg/l AFM1 + 2.0 % Charcoal; Q = 0.231 μg/l AFM1 + 2.0 % Charcoal; R = 0.456 μg/l AFM1 + 2.0 % Charcoal
Figure 3a: Effect of Imarsil on AFM1 content of Contaminated milk treated for 5 hrs at 28°C

KEY
A=0.009 μg/l AFM1 +0.5 % Imarsil; B=0.231 μg/l AFM1 +0.5 % Imarsil; C= 0.456 μg/l AFM1 +0.5 % Imarsil
D=0.009 μg/l AFM1 +1.0 % Imarsil; E=0.231 μg/l AFM1 +1.0 % Imarsil; F=0.456 μg/l AFM1 +1.0 % Imarsil;
G=0.009 μg/l AFM1 +2.0 % Imarsil; H=0.231 μg/l AFM1 +2.0 % Imarsil; I=0.456 μg/l AFM1 +2.0 % Imarsil

Figure 3b: Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 28°C

KEY
J=0.009 μg/l AFM1 +0.5 % Charcoal; K=0.231 μg/l AFM1 +0.5 % Charcoal; L=0.456 μg/l AFM1 +0.5 % Charcoal
M=0.009 μg/l AFM1 +1.0 % Charcoal; N=0.231 μg/l AFM1 +1.0 % Charcoal; O=0.456 μg/l AFM1 +1.0 % Charcoal;
P=0.009 μg/l AFM1 +2.0 % Charcoal; Q=0.231 μg/l AFM1 +2.0 % Charcoal; R=0.456 μg/l AFM1 +2.0 % Charcoal
Imarsil exhibited 0.4-87% reduction at 4 and 16°C, while 12-100 and 22-100% reduction in AFM were observed at 28 and 32°C, respectively. The efficacy of AC was significantly (p<0.05) lower than the observed values for imarsil; this adsorbent only achieved reductions of 0.4-22% at 4 and 28°C and 0.4-44 and 0.4-77% at 16 and 32°C, respectively. AFM removal is thus concentration-, time- and temperature-dependent.

Previous studies that evaluated the ability of AC to reduce AFB1 toxicity have produced conflicting results. Studies by Dalvi and Ademoyero (1984), Dalvi and McGowan (1984) and Hesham et al. (2004) indicated that AC was able to decrease aflatoxin toxicity, while Kubena et al. (1990), Edington et al., (1996) and Denli and Okan (2006) reported no significant differences in performance of birds following the addition of charcoal to rations that included aflatoxins. Denli and Okan (2006) had suggested that the conflicting results could have been due to the differences in chemical content, specifically the cationic compounds of the charcoal used. In the present study, it is evident that AC demonstrated a comparatively weak efficacy for AFM removal. Hence, it is also probable that the previously observed conflicting results regarding the efficacy of AC to adsorb aflatoxin could be as a result of failure to standardize the experimental conditions such as concentrations of aflatoxin, adsorbent and detection methods.

The moisture, protein, fat, carbohydrates and saturated fatty acid contents of the investigated milk before the adsorption studies were 87.8, 3.2, 3.9, 4.8 and 1.4% respectively. After remediation with both Imarsil and AC, the nutrient contents of remediated milk were the same, suggesting that these adsorbents had no effect on the essential nutrients of milk.

Earlier reports have demonstrated that imarsil possesses a flocculating ability, and hence it has been used for enzyme purification (Kareem and Akpan, 2003; Kareem and Adebowale, 2007). Economic and logistical considerations sometimes restrict the use of conventional techniques to detoxify aflatoxin (Smith et al. 1994). The use of high affinity adsorbents such as imarsil to significantly diminish the AFM content of contaminated milk as shown in the present study therefore represents a notable approach to the management of aflatoxicosis in the developing tropical world.

IV. Conclusions

The results from the present study indicate that imarsil demonstrates a potential to reduce aflatoxin M1 and by extension ameliorate the risk of aflatoxicosis in the developing tropical world. However, further studies to evaluate imarsil’s in vivo activity, its effect on biochemical indicators in both humans and animals and the organoleptic qualities of the remediated milk will be needed.

V. Acknowledgments

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Figure 4b: Effect of Charcoal on AFM1 content of Contaminated milk treated for 5 hrs at 32°C

KEY: As in Fig 3b

References Références Referencias


