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Abstract

The objective of the present study was to detect and quantify aflatoxins in liver and milk of camels. HPLC method was used to detect different aflatoxins. Liver samples were collected from slaughterhouses and milk samples from breeder's sites in Eastern Province, Saudi Arabia. Samples of camel livers and milk (250 samples of each) were collected and analyzed. About 26.4 and 16% of livers showed a contamination level in the range of 0.4-1 ng/g aflatoxin B1 and B2, respectively, but there were no detectable levels of G1, G2, M1 and M2. About 29.7% of milk samples had a range of aflatoxin M1 of 15-50 ng/l and 6% had a range of 50-100 ng/l. However, M2 was not detected in any milk sample. Based on regulatory limits, the present values of aflatoxin in livers and milk were within the internationally accepted limits (permissible limits of aflatoxins are 0.5 ppb for liver and liquid milk, according to the Saudi Organization for Standardization, Meteorology and Quality, (SASO).

Keywords: *Aflatoxins; Liver; Milk; Camel; Saudi Arabia.*

1. Introduction

Aflatoxins, a group of chemical molecule of difuronocumarines are fungal metabolites produced by certain fungi such as *Aspergillus flavus* and *A.Parasiticus (insert arefrence here).* Four Aflatoxins are currently recognized and are designated as Aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) (Ref?). These mycotoxins are worldwide contaminant of common livestock feedstuffs such as corn and wheat (Bigirwa et al, 2006; Jimoh and Kolapo 2008; Youssef 2009; Muthomi 2009 and Zahoor-ul-Hassan et al., 2010). Ingested Aflatoxins may be deposited in edible tissues of animals fed contaminated rations and could constitute a great risk to both human and animal health, causing diseases in both populations (Sandra *et al.,* 2006, Qazi and Fayyaz, 2006). Furthermore, Aflatoxins M1 and M2, a well-known hydroxylated metabolite of B1 and B2 respectively, arise by metabolism in the liver of lactating animals including humans (Allcroft and Carnaghan 1963, JECFA, 2001), and therefore excreted in milk (Scudmore 1994, Pittet 1998). AFB1 is listed as group1 carcinogen by the international agency for Research on Cancer (IARC) and is implicated as being the cause of human primary hepatocellular carcinoma (IARC 2002). The objective of the present study was to detect and quantify aflatoxin B1, B2, G1, G2, M1 and M2 in camel liver, and M1 and M2 in camel milk in order to evaluate the risk they may bring to the health of humans and animals.

2. Materials and Methods

Collections of Samples:

250 Camel liver samples were collected from slaughterhouses and supermarkets in different areas of Eastern Province. A total of 250 Camel raw milk samples were collected from breeder's sites at the suburbs of Eastern Province. One 100g each of liver and milk were collected monthly over 6 month's period between April and December 2020. All samples were collected into polyethylene bags and stored at -20°C until analysis.

Chemical and regents for HPLC:

All solvents were used in high performance liquid chromatography (HPLC) of high grade (Merck, Darmstadt Germany). The crystalline aflatoxins B1, B2, G1, G2, M1 and M2 were obtained from Sigma Chemicals (St. Louis, MO, and USA) and each stock standard solution was prepared in methanol at concentrations of 10 µg/ml and stored at -20°C. Working solutions were diluted in acetonitrite and stored at 4°C in darkness.

Sample extraction:

Aflatoxin B1, B2, G1, G2, M1 and M2 in liver were extracted and analyzed according to AOAC (2000). A 50-g portion of each liver was extracted with 100ml acetonitrite: water (90:10, v/v) for 3 min. The mixture was filtered through Whatman no.4 filter paper. The mixture was introduced into a 100x9mm i.d. glass chromatographic column with a coarse frit No.2 and covered with a plug of silanized glass wool in the top of the column. Purified extract (0.5ml) was collected in column reservoir. An aliquot (200/uL) was derivatized with 700/µl trifluoro acetic acid: acetic acid: water (20:10:70, VV) ready to be injected into the HPLC system.

Aflatoxins M1 and M2 in milk:

Aflatoxins M1 and M2 were extracted from milk on RP-18 column (Merck, Darmstadt, Germany) eluted with ether onto a silica column, eluted with dichloromethane: alcohol and derivatized with trifluoroacetic acid:

HPLC analysis:

The liquid chromatography peaks of the derivative aflatoxins were detected using Shimadzu (Kyoto, Japan) SCL-6A system equipped with an LC workstation, LC-6A solvent pumps, a shimadzu RF-535 fluorescence detector, and 7725i Rheodyne injector. The mobile phase for B1, B2, G1, G2 was water: acetonitrile (25:75V) with a flow rate of 0.7ml/min and for M1 and M2 was water: isopropyl alcohol: acetonitrile (80:12:8) with a flow rate of 0.5 ml. The recoveries were 91, 92.1, 93, 90.1, 92.3 and 96.5% for B1, B2, G1, G2, M1 and M2 respectively. The limits of detection were 0.04, 0.04, 0.15 and 0.75ng/g for B1, B2, G1, G2, respectively. The limit of detection for both M1 and M2 was 15ng/L.

3. Results

The occurrence of aflatoxins in liver samples of camels is shown in Table 1. About 73.6% and 84% of samples showed no detectable aflatoxin B1 and B2, respectively. About 26.4% and 16% of livers showed a contamination level in the range of 0.04-1 ng/g of aflatoxin B1 and B2, respectively. Neither of livers have shown aflatoxin levels greater than 1ng/g, nor have shown any detectable levels of G1, G2, and M1and M2.

The levels of aflatoxin M1 and M2 found in milk samples are summarized in Table 2. About 65.6% of samples were negative for M1, 29.7% had a range of aflatoxin M1 of 15-50 ng/L and 6% had a range of 50-100 ng/L. M2 was not detected in any milk sample.

Table (1): Occurrence of aflatoxins in Liver samples (N-250)

Range of aflatoxin concentration (ng/g)	No. of samples (%)
Aflatoxin B1	
<0.04*	184 (73.6)
0.04-1	66 (26.4)
>1	0
Aflatoxin B2	
<0.04*	210 (84)
0.04-1	40 (16)
>1	0

*Detection limits of toxins. G1, G2, M1 and M2 were not detected in liver samples.

Table (2): Occurrence of aflatoxin M1 and M2* in milk samples (N=128)

Rang of Aflatoxin M1 Concentrating (ng/L)	No of samples (%)
Not detected	84 (65.6)
15-50	38 (29.7)
50-100	6 (4.7)
>100	0

* M2 was not detected in any sample.

4. Discussion

About 66 (26.4%) of camel livers have shown levels in the range of 0.4-1.0 of aflatoxin B1 and 184 (73.3%) of camel livers have shown a level of aflatoxin B1 equivalent to <0.04. However, none of the liver samples has shown any contaminants with G1, G2 M1 and M2. These results are comparable with that reported by Al-Hizab et al. (2015) who have evaluated the potential effects of high AFB1 residues on the histological alterations of livers of camels slaughtered at AL-Ahsa abattoirs, Kingdom of Saudi Arabia. Standards for allowable limits of aflatoxin in food for human consumption vary from country to country. The allowable level is 5-20ng/g in Canada and USA, 4ng/g in France, and the Netherlands and 30ng/g in India (Henry et al 1999, FAO 2004). Generally, 4-30ng/g is widely recognized as acceptable limit of aflatoxin in food (Williams et al 2004) and 20ng/g an internationally recommended maximum limit of aflatoxin contamination (FAO 2004). In Saudi Arabia, permissible limits of aflatoxins are 0.5 ppb for liver and liquid milk, according to the Saudi Organization for Standardization, Metorology and Quality, (SASO). Based on these regulatory limits, the present results on aflatoxins in camels' liver were within the internationally accepted limits.

A low percentage of aflatoxin positive liver samples were reported world- wide in surveys carried out in calves (Vanerlinde et al 1964 Sabino et al 1995), poultry (Rodricks and Saoloff 1977, Sabino et al 1996) and pig (Honstead 1992).

Regarding camel milk, about 29.7% of samples were positive and had a range of aflatoxin M1 of 15-50ng/L only 6% of sampled had a range of 50-100 ng/L above the 50 ng/L limit permitted by European union (European Commission 2001). If the codex alimentarius (WHO, 2001) permitted limits which is 500ng/L are to be considered here, then all camel milk samples were well within the international recommendations for M1.

The present study agrees with data published in other countries like Greece Markaki and Melissari 1997, Italy (Galvano et al 1998), Brazil (Sylos et al 1996), Korea (Kim et al 2000) and Kenya (Lanyasunya et al 2005) where there was high incidence of aflatoxin M1 but at low concentration.

Unfortunately, at this stage of the study it was not possible to collect data on aflatoxin occurrence in the feed consumed by camels subjected to our objective and consequently establish a relationship between feed contamination and toxin incidence in liver and milk samples. Thus, the low liver and milk toxin levels found in our study might be explained taking into account not only the contaminated fodder (Bokari 2002, Alwakeel and Nasser 2011) but also the contribution of other feed ingredients which are not addressed in this study. One possible explanation for low incidence and levels is that most of samples collected from slaughtered camels or dairy camels in different locations in Saudi Arabia graze all year round being rarely fed with rations. Nevertheless, the presence of low levels of contamination of aflatoxin in meat and milk may still lead to nutrient loss, alteration of organoleptic properties and diminishment of product shelf-life in the market. Therefore, it is internationally agreed that all kind of food ought to be free from aflatoxin (Commission Regulations 257/2002). Previous reports have shown that, substance like oltipraz could be of use in reducing aflatoxin B1 hepatic toxicity (Li et al., 2000) and inhibit M1 production by animal hepatic cells (Kuilman, 2000). However, treatment of rats with a variety of compounds, including the synthetic dithiolthione oltipraz and the antioxidant ethoxyquin, protects these rodents from AFB-induced hepatocarcinogenesis (Bammler,et.,al.,

2000) . In order to monitor the level of aflatoxin in feedstuff milk, and meat we recommend the following proceedures:

- To keep fodder, meat and milk under obligatory mycotoxins regulation.
- Routine detections of AFB1 residues should be carried out in slaughterhouses.
- The health and veterinary authorities showed check all camel products before marketing.

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