Histopathological Studies on Mycotoxins (Ochratoxin A) in Camels

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ABSTRACT

This study was applied on a camel herd of 200 heads with a good healthy condition aging 1-12 years in Saudi Arabia at 2011. The present work aimed to reveal the prevalence of ochratoxin A in two types of feed stuffs commonly used in camel feeding in the farm. The animals divided into 2 flocks in 2 yards, the first flock contains 150 camels and other flock contains 50 camels. The animals were raised in a desert environment and consume a complimentary ration at night. The ration of first flock was changed while no changes were done in ration of second flock. Within 20 days 79/150 animals (52.66%) were affected of which 60/150 (40%) were died of which 34 camel calves (22.67%) and 26 adult camels (17.33%). With the beginning of the clinical signs, the affected animals were unable to stand. lied in sternal position and off food. Within one to three days the affected animals lies on lateral side and voided bloody urine and died. Eight cases of abortion were occurred in 8 she camels in different stages of pregnancy. Postmortem exploration of sacrificed moribund animals revealed lung emphysema, flabby heart with hydropericardium, granular surface of both liver and spleen, ascitis with blood tinged exudates. Also, the kidneys were enlarged with subcapsular petechial and ecchymotic haemorrhages with pale cortex in cut surface. The urinary bladder contained red urine. The ration nutrient analysis revealed high crude protein level (18%), while mycotoxin analysis reveals six times increase in ochratoxin A level (30 ppb) than the permissible limit (5 ppb). Histologically, kidneys reveal prominent degenerative changes in renal tubules, hyaline and granular casts and haemorrhages were also detected. Liver shows vacuolar degeneration with necrobiotic changes and fatty changes. Lung reveals mild congestion of inter-alveolar blood vessels with areas of emphysema and collapse. Also, subcapsular haemorrhages in spleen, and
degeneration and oedema in cardiac muscles were seen. In conclusion, the study revealed the clinical and pathological picture of ochratoxicosis in camels. In addition, the study appeared the important of food safety and storage for preventing the incidence of mycotoxicosis. Thus many companies are adopting fodder quality assurance programs and making efforts to ensure that animal feeds are mycotoxins free.

INTRODUCTION

Nowadays camels, “ships of the desert” have been dying in large numbers in Saudi Arabia. The reports show that at least 5000 camels have died and thousands more have become sick. The affected camels lose control of their movements and suffer from cerebral hemorrhage and total paralysis (Bokhari, 2010).

Mycotoxins are fungal secondary metabolites that have been associated with severe toxic effects to vertebrates (mycotoxicosis). It represents a difficult problem (Barly and Vadehara, 1999). Mycotoxin-induced disease syndromes can be confused with other diseases caused by pathogenic microorganisms. Mycotoxins produced by many important phytopathogenic and food spoilage fungi including Aspergillus, Penicillium, Fusarium, and Alternaria species (Anon, 1980). They produce aflatoxins, patulin, ochratoxin A and zearalenone. The contamination of foods and animal feeds with these mycotoxins is a worldwide problem (Ngindu et al., 1982 and Dokhan and Rabab, 2008). Sixty-three isolates of Aspergillus, Penicillium and Fusarium, isolated from corn grains and sunflower seeds in Egypt. Eleven different known mycotoxins (aflatoxin B1, B2, G1 and G2, sterigmatocystin, ochratoxin A, citrinin, and zearalenone) were detected in the extract of these isolates (Abdel-Mallek et al., 1993 and Duarte et al., 2011).

Ochratoxin A (OTA) is a mycotoxin produced by some strains of Penicillium and Aspergillus molds that naturally contaminate food and feed under all climatic conditions (Fung and Clark, 2004). It is a nephrotoxic and carcinogenic mycotoxin, formed during the storage of cereal grains and other plant-derived products. The permissible limit of mycotoxins in animal ration as ochratoxin is 5 ppb, aflatoxin is 20 ppb, zeralinone is 100
ppb and fumensine is 2000 ppb (EFSA, 2004). WHO (1990) and Fatma et al. (2012) stated that the ochratoxin A has been found as a contaminant in foods with a frequency in the range of 2-30% in all countries where attempts to perform food analysis have been made. Field cases of ochratoxin A and associated nephropathy in farm animals have been encountered in many countries, underlining the nephrotoxic potential of this compound, also ochratoxin A, is thought to be responsible for Balkan endemic nephropathy (Anzai et al., 2010). Gareis and Wernery (2010) studied the mycotoxicosis in camels which characterized by clinical symptoms such as diarrhoea, haemorrhage and death. These cases observed in breeding camels in U.A.E. Hay samples, body fluids and intestinal contents were investigated for the presence of mycotoxins and revealed gliotoxin and trace amounts of ochratoxin A. Houwelingen et al. (2008) describe the first successful isolation of recombinant alpaca single-domain antibody fragments with high affinity to the mycotoxin ochratoxin A based on genetically engineered recognition elements.

The present work was designed to reveal the prevalence of ochratoxin A in two types of feed stuffs commonly used in camel feeding in a farm in Saudi Arabia.

MATERIALS AND METHODS

Animals:
A camel herd of 200 heads with a good healthy condition aging 1-12 years in Saudi Arabia at 2011 divided into 2 flocks in 2 yards (150 camels in one and 50 camels in the another yard). The animals were raised in a desert environment and consume a complimentary ration at night. The animals showed no external parasites and they were received regularly drugs for internal, external and blood parasites. Then, there is changing of dry ration in the flock of 150 camels and the other flock was still consuming the old dry ration and did not introduced to the new ration.

Sampling and Examinations:
1. Collection of blood samples which stained with Giemsa for detection of blood parasites and Pasteurella microorganisms (Bancroft and Gamble, 2008).
2. Tissue samples from liver and kidneys were taken for bacteriological examination (Cruickshank et al., 1975).
3. Tissue samples from liver, kidneys, heart, lung, spleen and uteri were taken for histopathological study.
4. The ration nutrient analysis was applied (NRC, 1989).
5. Examination of feedstuffs for presence of mycotoxins using The Vicam Series-4 Fluorometer (Model VicamV1.0) was used (Benford, et al., 2001).
6. Examination of feedstuffs for presence of insecticides (organic phosphorus compounds and pyrethroids) using QuEChERS were used (Lehotay, 2004).
7. Analysis of water for presence of lead and copper toxicity was applied (EPA, 2010).

Pathological examination:
Postmortem examination of sacrificed moribund animals was applied. Then histopathological examination is applied on tissue specimens from liver, kidneys, heart, lung, spleen and uteri. Tissue specimens were fixed in 10% neutral-buffered formalin and were routinely processed in an automated tissue processor, embedded in paraffin, sectioned at 3-5 μm, and stained with Haematoxylin and Eosin (Bancroft and Gamble, 2008).

**RESULTS**

A camel herd of 200 heads with a good healthy condition (1-12 years in age) suddenly showed signs of disease in 30 heads after three days of the complimentary ration changing in the flock of 150 camels. Within 20 days 79/150 animals (52.66%) were affected of which 60/150 (40%) were died of which 34 camel calves (22.67%) and 26 adult camels (17.33%). Firstly the affected animals were unable to stand and lied in sternal position, refused eating and stopped rumination. In one to three days the affected animals lies on lateral side and voided bloody urine and died. The body temperatures of the diseased animals were not significantly altered. Eight cases of abortion were occurred in 8 camels in different stages of pregnancy. The rate of deaths declined from three or more to two or less by ten days and nearly stopped by 20 days of onset of signs after stoppage of the contaminated ration and veterinary interference.

Postmortem examination:
Postmortem exploration of sacrificed animals at moribund state revealed lungs emphysema, flabby heart with hydropericardium, granular surface of both liver and spleen, ascitis with blood tinged exudates that also laden with albumin as it
get clotted after transport. The kidneys were enlarged with subcapsular petechial and ecchymotic haemorrhages were seen (Fig. 1), while the cut surface showed pale cortex (Fig. 2). The urinary bladder contained red urine. No prominent lesions were observed in the gastrointestinal tract. Also, no obvious lesions in the 8 uteri after abortion were observed.

**Bacteriological and parasitological examinations:**

Blood smears stained with Giemsa stain revealed absence of both blood parasites and pasturella microorganisms. Bacteriological examination of liver and kidneys on blood agar reveals absence of pathogenic bacterial growth. Also, absence of leishmanial forms of *Trypanosoma cruzi* in cardiac muscle.

**Ration analysis:**

The ration nutrient analysis revealed high crude protein level (18%), while mycotoxin analysis reveals six times increase in ochratoxin A level (30 ppb) than the permissible limit (5 ppb). On the other hand aflatoxin (4.7 ppb), zeralinone (85.5 ppb) and fumensine (144 ppb) were within the permissible limits (20, 100 and 2000 ppb, respectively). The analysis also revealed absence of insecticides (organic phosphorus compounds and pyrethroids) in feed samples.

**Water analysis:**

Water analysis revealed that lead and copper levels were 0.04 ppm and 0.01 ppm, respectively that was under the permissible values (0.05 and 1 ppm, respectively).

**Histopathological examinations:**

Examination of H&E stained paraffin sections of organs revealed prominent degenerative changes in renal tubules of kidneys as pyknotic nuclei, vacuolar and hydropic degeneration within tubular epithelial cells (Figs. 3, 4, 5&6) with hyaline and granular casts within tubular lumina (Fig. 6). Marked dilation and engorgement of intertubular blood vessels of kidneys were present.

The liver showed vacuolar degeneration with necrobiotic changes, chromatolysis, in some hepatocytes (Fig. 7) and multiple sporadic necrotized hepatocytes throughout the hepatic parenchyma were present. Fatty change was recognized in the cytoplasm of hepatocytes which had foamy appearance due to the presence of minute fat globules, thus the amount of fat increases these globules coalesced producing larger globules (Fig. 8).

Mild degeneration and oedema were present in the heart in between muscle fibers of cardiac muscle (Fig. 9).
The lung tissues revealed only mild congestion of inter-alveolar blood vessels with areas of emphysema and collapse (Figs. 10&11).

The spleen has subcapsular and peritrabicular haemorrhages in some areas. Sinusoids were congested and engorged with blood (Fig. 12) and the white pulb showed depleted lymphoid follicles.

Normal picture of uterine tissues with dilated glands and moderate congestion of uterine vessels were observed.
FIGURES

Figs. 1&2: Gross photo of camel kidneys showing subcapsular haemorrhages (Left) and pale cortex compared with medulla (Right).

Fig. 3: Camel kidney showing marked degeneration of renal tubules and the intertubular blood vessels were dilated and engorged with blood (H&E, X 100).

Fig. 4: Camel kidney showing dilated blood vessels and engorged with blood in intertubular areas (H&E, X 100).
Fig. 5: Camel kidney showing marked degeneration of proximal tubules and highly congested glomerular tufts (H&E, X 100).

Fig. 6: Camel kidney showing marked degeneration and necrosis of lining epithetium and dilatation of renal tubules with presence of renal cast in some tubules (H&E, X 100).

Fig. 7: Camel liver showing vacuolar degeneration with necrobiotic changes and chromatolysis in hepatocytes (H&E, X 400).

Fig. 8: Camel liver showing fatty changes of hepatocytes (H&E, X 100).
**Fig. 9:** Camel heart showing mild degeneration and oedema in between muscle fibers of cardiac muscle (H&E, X 100).

**Fig. 10:** Camel lung showing giant and dilated alveoli (emphysema) (H&E, X 40).

**Fig. 11:** Camel lung showing congestion of perialveolar blood capillaries (H&E, X 400).

**Fig. 12:** Camel spleen showing the sinusoids are congested and engorged with blood (H&E, X 40).
DISCUSSION

Many owners have attributed the deaths of thousands of camels to the bran used in animal feeding instead of barley, whose price has been spiraling and due to “toxic fodder” contaminated with mycotoxins. Vet team regarded the high deaths of clinical cases to renal failure produced by the nephrotoxic effect of ochratoxine A that was increased six times as permissible limit (WHO, 1990; Bokhari, 2010 and Fatma et al., 2012). This comes in harmony with the present study. Moreover, the case was aggravated by the increased level of crude protein 18% in the toxic ration compared with 13% in the previous ration, as its metabolites impresses kidney function. Supported this point the histopathological examination revealed absence of inflammatory changes that caused by infectious diseases and presence of degenerative and necrobiotic changes that observed in acute toxicosis. This effect on kidneys comes in agreement with those recorded by Kuiper and Goodman (1990); WHO (1990); Diekman and Green (1992) and EFSA (2004) who suggested that mycotoxins induce renal failure. Moreover, Plestina et al. (1990) found that ochratoxin is nephrotoxic in human. Anzai et al. (2010) described the molecular mechanism of ochratoxin A transport in kidneys and liver as the excretion of OTA into urine is thought to be mainly by tubular secretion presumably via the organic anion transport system (OAT). These renal transporters mediate the transmembrane transport of OTA and play an important role in the development of OTA-induced nephrotoxicity. Also, Bennett and Klich (2003) and Luhe et al. (2003) explained how OTA inhibits respiration in mitochondria where it acts as a competitive inhibitor of the carrier’s proteins localized on the inner membrane of mitochondria.

In our study, Pathological alterations in liver were multiple sporadic necrotized hepatocytes throughout the hepatic parenchyma, this result was supported by Cheo (1991) and Amer et al. (2007) who stated that, mycotoxins causes hepatotoxicity due to an increase in the permeability of liver cell membrane.

In the present study the level of ochratoxin A in foodstuffs is 30 ppb that exceed the permissible limit (regulatory limit) 5ppb and <20 ppb recorded with EFSA (2004) and FDA (1997), respectively.

In conclusion, food safety has become an important issue for all sectors of the fodder industry. To promote safety, a growing
number of fodder producing companies are adopting fodder quality assurance programs, which stimulate actions for all aspects of fodder production to reduce the risk of fodder becoming contaminated with mycotoxins. These actions include making efforts to ensure that animal feeds are mycotoxin free and reflected the need for routine surveillance of agricultural commodities to minimize potential hazards to human health.

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المستخلاص العربي

دراسات هستوباثولوجية للسموم الفطرية (الأوكراتوكسبين 1) في الجمال

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تمت الدراسة على قطيع من الجمال مكون من 200 جملا بصفة عامة جيدة بتراوح السن من 1-12 سنوات بالمملكة العربية السعودية عام 2011. تهدف هذه الدراسة لتوضيح تأثير الأوكراتوكسبين على نوعين من الأعلاف المستخدمة في المزرعة. قسمت الحيوانات إلى مجموعتين مختلفتين (150 جملا في مكان و 50 جملا في مكان أخر). تعرف الحيوانات في بيئة صحراوية وتتناول علفية مجهزة ليبلا. ثم حدث أن تغيرت العلية المجهزة المقدمة لمجموعة الحيوانات ال 150 جملا ولم تغير للمجموعة الأخرى. في خلال 20 يومًا أصيب 150 جملا (65.26%) منهم 60/150 جملا نافقا (40%) منهم 34 جملا صغيرًا (22.67%) والسم و 26 جملا كبيرا (17.13%). وبدأت تظهر الأعراض المرضية بداية لا تستطيع الحيوانات المصابة الوقوف وترقد وضع عظمة الفص وتمتيع عن الأكل. خلال يوم 3 أيام ترقد الحيوانات المصابة على جانبيها وبصاحب الحاله أفرز بول مدمم ثم يحدث النفق. ظهر أيضا 8 حالات من الإجهاض لثمانية جمال عشار في مختلف مراعل الحمل. تم إجراء الصفة التشريحيه للجمال النافقة وأظهر الفحص وجود انفخاخ هواي رئوي (أفيزيما) وقلب مترهل وتجمع منى بالغشاء التاموري للقلب. سطح ذو مسمى خشن لكلا من الكبد والطحال وجمع ماني من الدم بالغشاء البريتي. أشياء الكلى ظهرت ممتاخمة وبها نقاط نفخة صغيرة وكبيرة تحت الجدار الخارجي مع وجود هتان بالقشرة بعد القطع بالكلى وكذلك وجود بول مدمم بالكثافة البولية. تحليل المكونات الغذائية بالعالية المجهزة وجد أن نسبة البروتين الخاص 18% بينما تحلل السموم الفطرية للفصية وجد أن نسبة (5 ppb) الكيتي النصفي (30 ppb) الأوكراتوكسبين (أفيزيما) الكلي بها اسحاحات بالأنياب البولية مع وجود قوالب داخل الأنياب البولية. أظهرت انتفاخ هواي رئوي (أفيزيما) حالة أظهرت انتفاخ هواي رئوي (أفيزيما) بشكلي تحت القشرة الخارجية للطحال وإرتفاع ماني يسبط واستحالات بين أثواب عصا حسب القلب. وقد أظهرت الدراسة المصورة الباثولوجية لتمسح بالأوكراتوكسبين في الجمال. كما بدت الدراسة أهمية سلامة الأعلاف وتخزينها للوقاية من السموم الفطرية ولذا وضعت كثير من الدول برامج لرفع كفاءة تصنيع الأعلاف ومحاولات جادة لتأمين وسلامة الأعلاف من السموم الفطرية.