MORTALITY AND EVIDENCE OF CLINICAL-PATHOLOGICAL ABNORMALITIES IN CAPTIVE MOUFLON SHEEP (*Ovis orientalis*) AFTER INCLUSION OF COTTONSEED CAKE IN THEIR RATION

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Necropsies of three Mouflon sheep (*Ovis orientalis*), died with the history of labored breathing, dyspnea, anorexia, depression, pendulous abdomen, enteritis and sudden death were performed. The animals had a history of consuming diet containing cottonseed cakes made from seeds of Bt (*Bacillus thuringiensis*) cotton after ginning. Postmortem examination revealed hydrothorax, hydropericardium, congested and edematous lungs, ascites, hepatomegaly and splenomegaly. Microscopic examination of the morbid liver revealed severe congestion and centrilobular necrosis with marked atrophy of hepatocytes around the central vein. Histologically, the lungs were congested and edematous. Marked congestion was also observed in splenic parenchyma. The clinical picture, gross and microscopic findings and history of inclusion of cottonseed cake in feeding regime were suggestive of cottonseed cake toxicity in Mouflon sheep and could have led to death.

Keywords: Mouflon sheep, clinical picture, pathology, cottonseed meal.

INTRODUCTION

Mouflon sheep (*Ovis orientalis*) are kept in different parts of the world, including central Asia, Sardinia (Italy), Corsica (France), Konya (Turkey) and Himalayan and Karakurum range (Pakistan). These sheep are also found in a number of Mediterranean countries that could have been intruded by migratory people from Corsica or Sardinia few centuries ago. The Mouflon sheep are wild and highly adaptable species to different habitats throughout the world. It is believed that these animals are descendants of feral sheep and have been widely introduced as exotic species to many countries (Anonymous, 2008). Domestic sheep and Mouflon sheep habitually graze the same pastures which make them susceptible to mutual infections.

Cottonseed cake, a by-product of cottonseed after oil extraction, is a good source of protein with high metabolizable energy (Guedes and Blanco, 2010). However, incorporation of cottonseed cake in concentrate ration of domestic and wild animals is toxic in nature (Alexander et al., 2008). After absorption, the polyphenolic compound (gossypol) in cottonseed cake accumulates in liver (Lindsey et al., 1980) and kidneys (Kim et al., 1996). Use of cottonseed and its derived products in the feed of animals cause poor weight gain, associated with decreased feed efficiency and increased mortality. In Pakistan, most of the farmers had left old kind of cotton varieties and sow 90% Bt (*Bacillus thuringiensis*) cotton, which has turned to be the most profit-earning crop. *Bacillus thuringiensis* cottonseed cake in feeding regimen of cattle in Pakistan caused adverse effects in terms of reduce milk production, lack of appetite, premature births and acute deaths (Yusuf, 2011). Moreover, administration of free gossypol from cottonseed has shown antispermatic activity (Anwar et al., 2008; Mahmood et al., 2011) and hepatotoxicity (Brand et al., 2012).

Cottonseed cakes produced from different types of cotton seeds contain high quantity of gossypol. However, the deleterious effects of gossypol in feeding regimen of different animals depend on the type of cottonseed cake and period of ingestion (Gadelha et al., 2014). Gossypol toxicity has been reported in different animals and birds such as cattle (Schneider et al., 2002; Zhang et al., 2007), goats (East et al., 1994), pigs (Haschek et al., 1989; Fombad and Bryant, 2004), sheep (Morgan et al., 1988), birds (Lordelo et al., 2005) and monogastric animals when the gossypol overwhelms detoxification capacity (Clawson et al., 1975; Willard et al., 1995).

Wild ruminants are highly sensitive to oxidative stress induced by the naturally occurring polyphenols in their diets, particularly the ration containing cottonseed meal (Javed et al., 2015; Zubair et al., 2016). Gossypol intoxication has occasionally been reported under natural conditions, however,
it is reported that animals ingesting higher level of gossypol in their feed showed ascites, anasarca, hydrothorax and centrilobular necrosis of liver (Furlan et al., 2014; Câmara et al., 2016). Endotheliotoxic properties of the polyphenolic compounds have been associated with congestive heart failure and these compounds also enhance the erythrocytes fragility by impairing the oxygen exchange and lipid peroxidation of the membrane phospholipids (Lindsey et al., 1980; Kirmizigül et al., 2016). Scanty information is available about the susceptibility of Mouflon sheep to cottonseed cake toxicity. Therefore, in the present study, clinical signs, history and gross lesions following necropsy of Mouflon sheep was recorded to establish a presumptive diagnosis.

MATERIAL AND METHODS

Case Description: We conducted the necropsies of three Mouflon sheep which were kept at the Wild Life Park, Gutwala, Faisalabad, Punjab, Pakistan. These animals were fed cottonseed cake made from Bt (Bacillus thuringiensis) cotton @ 0.5 kg/animal/day. After a few days of incorporation of cottonseed cake in their ration, two Mouflon sheep suddenly died without showing any clinical and behavioral signs, except lethargy for few hours before death. After one week, another sheep died with the history of clinical signs of lacrimation, salivation, lethargy, fever and respiratory arrest. Clinically sick animals were treated with antibiotics (CTC-20: 10 mg/kg BW, Amoxicillin: 10mg/kg BW and Tribersen: 15 mg/kg BW), antipyretics in case of fever, anti-allergic and multivitamin. In spite of treatment, the animals did not recover and died. In this study, gossypol contents of cottonseed cakes could not be determined, but the published literature shows that free gossypol contents among Bacillus thuringiensis varieties range from 1153 to 3887 mg/kg (Karishma et al., 2016). However, mortality in lambs has been reported at much lower dose of gossypol (409 mg) (Morgan et al., 1988).

Necropsies, gross and histopathological investigations: The necropsies of the three Mouflon sheep were conducted within 1-2 h after death. All the visceral tissues were examined for lesions. The tissue samples were collected and fixed in 10% neutral buffered formalin (Damián et al., 2015). For microscopic observations, standard histopathological techniques including fixation, dehydration and embedding were applied (Kim and Park, 2016). Briefly, 4-5 µm thick sections were stained with hematoxylin and eosin (Nugali et al., 2016).

Impression smears made from liver and other tissues, stained with Gram’s stain did not reveal any pathogenic bacteria. Moreover, inoculation of nasal swab and tissue samples collected at the time of necropsy, cultured on agar medium supplemented with 5% sheep blood did not show any kind of pathogenic bacterial growth (Dağ et al., 2016).

RESULTS AND DISCUSSION

Traditionally, the cottonseed and its byproducts are frequently used as a vegetable protein source in the domestic animals ration (Cunha et al., 2012). Cottonseed byproducts have variable quantity of gossypol, depending upon the genetics of varieties of cotton and extraction techniques. Different factors affect the free gossypol contents in cottonseed, thus making it difficult to know its exact quantity to be used in the animals feed. Prolonged consumption of gossypol-containing ration exerts undesirable effect on different species of domestic and wild animals (Gadelha et al., 2014). In Pakistan, livestock farmers frequently use cottonseed cake in animals feed as a substitute for soybean meal without realizing their deleterious effects on animal health and performance (Guedes and Blanco, 2010). The main reason that makes this case study interesting is the absence of any previous report on the lethal consequences of gossypol in Mouflon sheep. Many cases showing adverse effects of cottonseed meal in domesticated animals are seen, as it is easily available in the market at low price and has high protein contents. Despite treatment with broad spectrum antibiotics, the health of surviving animals continuously deteriorated. The Mouflons had access to cottonseed cakes as concentrate prepared from Bacillus thuringiensis cotton in feed for the last few weeks. Addition of cottonseed cake in the feed did not affect the appetite and health of the sheep until the appearance of signs of toxicity.

The clinical signs of toxicity in Mouflons might have been unobserved during peracute phase. Although the sick sheep exhibited signs of lethargy, severe dyspnea, loss of appetite, and diarrhea. Similar clinical signs due to gossypol toxicity have been observed in cattle (Hudson et al., 1988) and goats (Morgan et al., 1988). Previously, clinical signs of gossypol intoxication in cattle (Schneider et al., 2002), goats (East et al., 1994), pigs (Haschek et al., 1989), sheep (Morgan et al., 1988) and monogastric animals have been reported (Uzal et al., 2005).

In the present study, accumulation of fluid in the abdominal cavity in affected sheep lead to ascites (Cohn, 2015). Fluid also gathered in the thoracic cavity which lead to respiratory distress (Patel et al., 2011). Any condition that impedes diaphragm movement, such as abdominal distention and decreased abdominal wall compliance, can contribute to respiratory distress (Barba et al., 2015). Due to abdominal distension, upward pressure on the diaphragm and lungs can also cause shortness of breath, leading to respiratory distress (Marks, 2017). As a result of ascites and respiratory distress, diaphragmatic movements are impaired (Howard et al., 2001), which could have taken place in animals included in the present study.

Previously, in Pakistan clinical signs including anorexia, low milk yield, premature births and sudden deaths in cattle due to Bt cottonseed cake toxicity have been reported (Yusuf, 2011).
Necropsy examination in the present study revealed subcutaneous edema in the neck region (Fig. 1A), hydrothorax with yellowish tinged fluid (Fig. 1B) and hydropericardium in affected sheep. Abdominal organs showed congestion, the liver was enlarged and dark in color with reticular appearance of the surface “Nutmeg Liver” (Fig. 1C and 1D), splenomegaly with severe congestion and multiple white to pale color necrotic areas. Yellowish color fluid was present in the abdominal cavity (Fig. 1B). The lungs were congested and edematous. Microscopically, the alveolar capillaries were severely dilated in association with micro-hemorrhages. Heart showed white areas of severe focal necrosis over myocardium, both the ventricles were filled with un-coagulated blood. Presence of fluid in body cavities and the changes observed in the liver have also been reported in calves (Zelski et al., 1995). Hepatomegaly observed in the present study could be the result of severe right-side heart failure, as a result of cottonseed cake toxicity that transfer the vascular pressure to the central veins and into the sinusoidal spaces, resulting in marked dilatation of the central vein and the sinusoidal spaces. The central vein dilatation causes pressure atrophy of the adjacent hepatocytes, leading to centrilobular necrosis of hepatocytes (Fig. 2A and 2B). Due to increased hydrostatic pressure within the vascular meshwork, the dilated microvascular endothelial pores become more permeable to water and proteins to the extracellular spaces and accumulation into body cavities of the Mouflons. Previously, different clinical signs such as anorexia, poor weight gain, respiratory distress, weakness, heart failure and death have been reported after gossypol toxicity in calves and lambs (Morgan et al., 1988; Mena et al., 2004). Ascites, severe pneumonia, acute centrilobular hepatocyte necrosis and cardiovascular lesions in affected calves have been reported (Zelski et al., 1995). Histologically, liver tissues revealed collagen fiber proliferation in the peri-sinusoidal space (Gadelha et al., 2014). Liver of affected sheep also exhibited severe fibrous tissue proliferation and marked hepatocellular necrosis. The fibrosis was predominantly present at peri-lobular area, thus...
obliterating not only the portal circulation but also marked destruction in the hepatocytes. This destruction could be the result of persistent ingestion of free gossypol containing ration and development of oxidative stress due to the free gossypol, as membranes of hepatocytes are more vulnerable. Splenomegaly with multiple white areas of necrosis is suggestive of old calcified microhemorrhage “Gamma Gandi bodies”. Red pulp exhibited severe congestion and hemorrhage (Fig. 3A and 3B). Similar necropsy lesions have also been reported previously (Sein et al., 1986; Garcia-Abiado et al., 2004; Sijun et al., 2012). Some areas of spleen showed hemosiderin deposition and depleted splenocytes.

**Conclusions:** Clinico-pathological findings of this study indicated that Mouflon sheep were suffering from ascites and respiratory distress. Postmortem examination revealed hydrothorax, hydropericardium, congested and edematous lungs, ascites, hepatomegaly with reticular appearance and splenomegaly. It was concluded that clinico-pathological findings could have developed due to inclusion of cottonseed cake in feeding regime of Mouflon sheep which might have led to death.

**REFERENCES**


Mortality in Mouflon sheep


