Pak. J. Agri. Sci., Vol. 54(3), 683-688; 2017
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/17.5179
http://www.pakjas.com.pk

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

Fazal Mahmood[1], Riaz Hussain[2], Ahrar Khan[1,*], Atia Basheer[3], Farah Ali[4] and Mohammad Farooque Hassan[4]

[1]Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan; [2]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur-63100, Pakistan;[3]Genetics and Genomics Laboratory, Department of Livestock Production, University of Veterinary and Animal Sciences Lahore Pakistan; [4]Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan.
[*]Corresponding author's e-mail: ahrar1122@uaf.edu.pk

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 antispermatogenic 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; Kirmizigul *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 a1., 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).

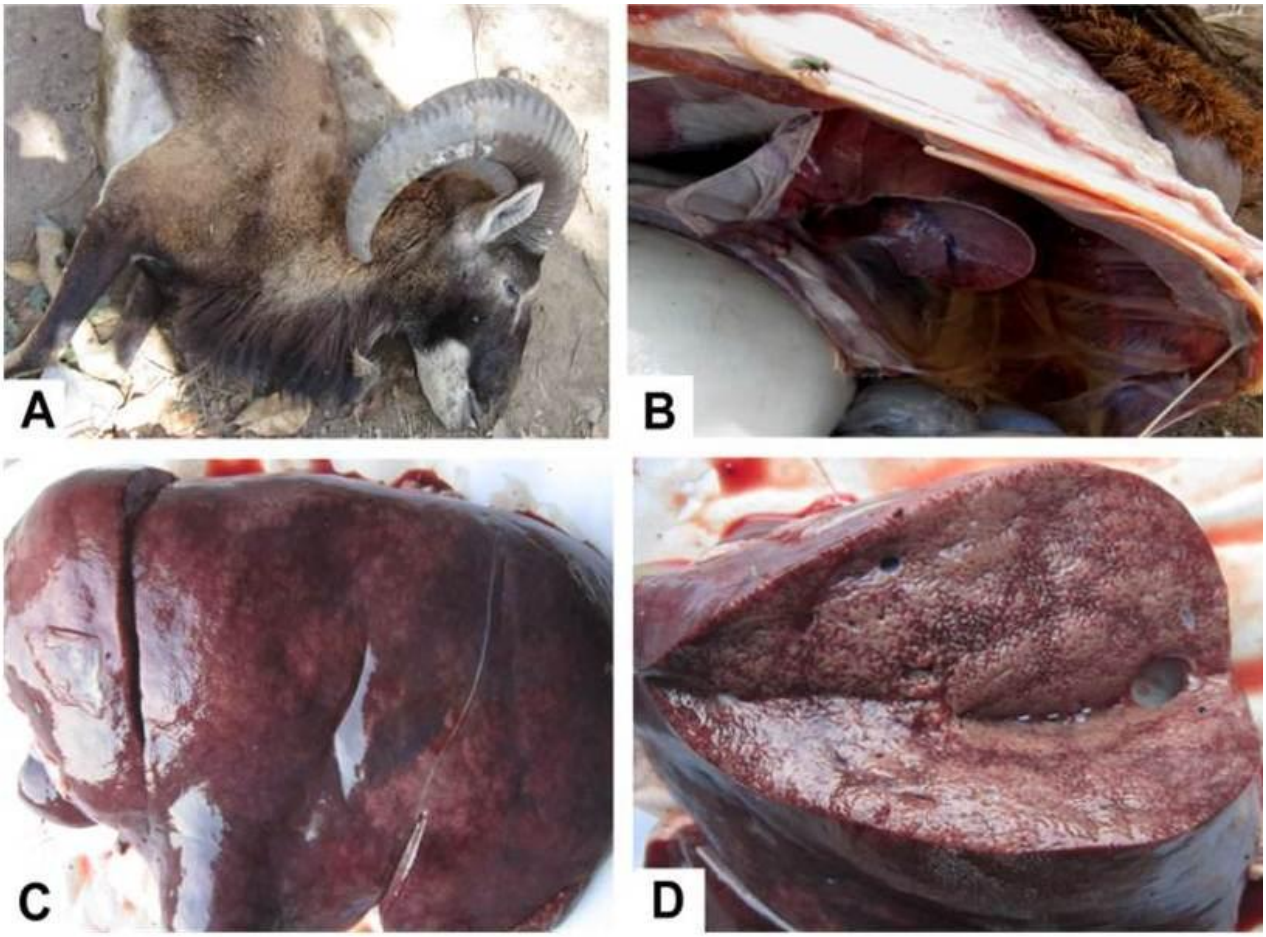

Figure 1. Photograph showing (A) Dead male Mouflon sheep, (B) Hydrothorax with large quantity of yellow color fluid and bloody flacks and (C&D) Hepatomegaly, round edges, dark color with reticular appearance "Nutmeg liver" and cut surface of liver.

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

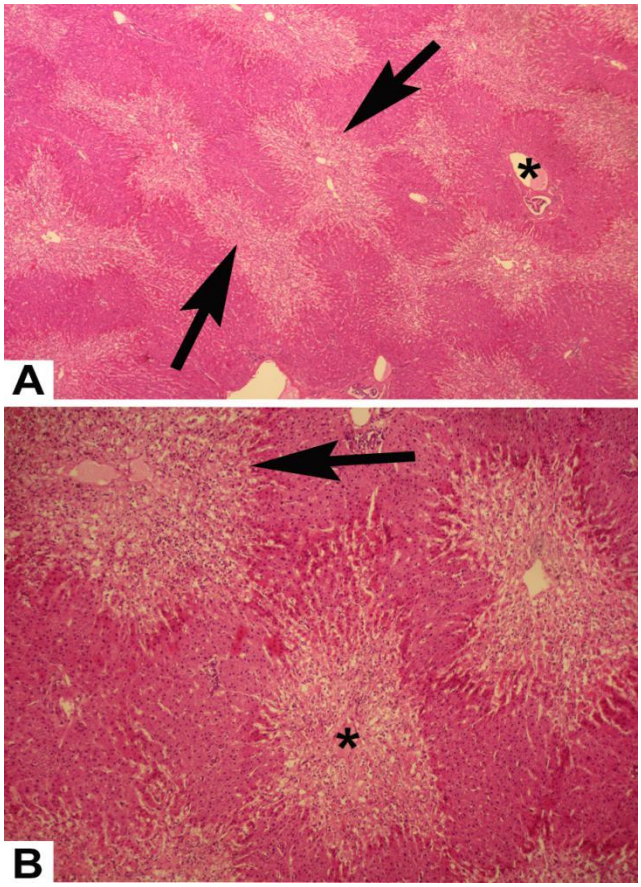

Figure 2. Photomicrograph of liver tissue showing centrilobular necrosis (arrows) with dilatation of central vein (asterisk); X-100 (A) and X-200 (B): H&E stain.

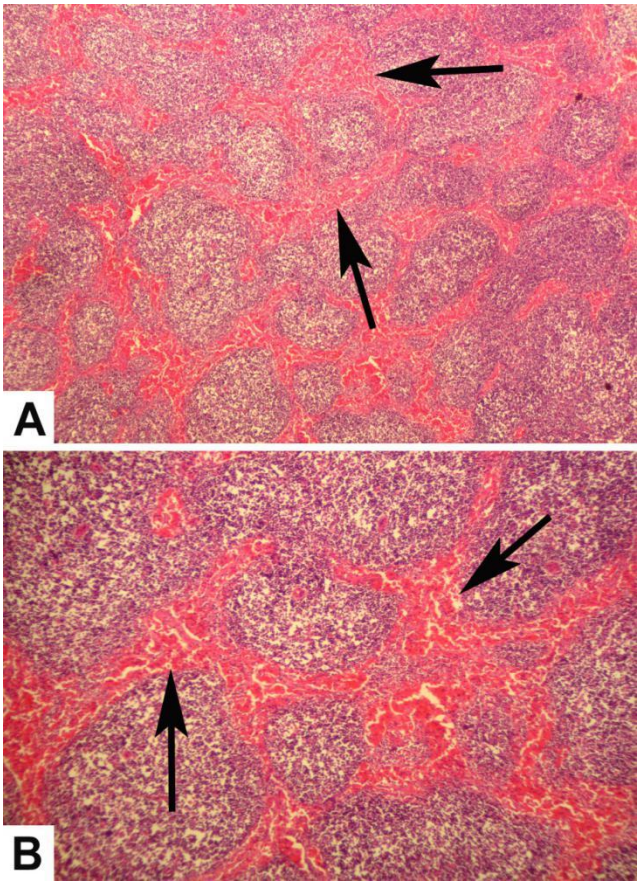

Figure 3. Photomicrograph of spleen showing severe congestion (arrows); X-100 (A) and X-200 (B): H&E stain.

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 "Gamna 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

Alexander, J., D. Benford, A. Cockburn, J.P. Cravedi, E. Dogliotti, A.D. Domenico, M.L. Férnandez-Cruz and P. Fürst. 2008. Gossypol as undesirable substance in animal feed. The EFSA J. 908:1–55.

Anonymous, 2008. http://www.wildlifeofpakistan.com/ Mammals of Pakistan/urial.htm. Wildlife of Pakistan. Accessed on January 31, 2016.

Anwar, M.S., M.Z Khan, I. Javed, A. Khan and M.K. Saleemi, 2008. Pathological effects of cottonseed meal with and without ferrous sulphate in male Japanese quails (*Coturnix japonica)*. Pak. Vet. J. 28:51-56.

Barba, E., E. Burri, A. Accarino, D. Cisternas, S. Quiroga, E. Monclus, I. Navazo, J.R. Malagelada, and F. Azpiroz. 2015. Abdominothoracic mechanisms of functional

abdominal distension and correction by biofeedback. Gastroenterology 148:732–739.

Brand, T.S., D. Squizzato, L.C. Hoffman and A.D. Zotte. 2012 Preliminary results on the effect of the inclusion of cottonseed oilcake meal on the feed intake and growth of slaughter ostriches (*Struthiocamelus* var. *domesticus)*. S. Afr. J. Anim. Sci. 42:520-523.

Câmara, A.C.L., A.M. do Vale, C.R.S. Mattoso, M.M. Melo and B.S. Blanc. 2016. Effects of gossypol from cottonseed cake on the blood profile in sheep. Trop. Anim. Health Prod. 48:1037–1042.

Clawson, A.J., J.H. Maner, G. Gomez, O. Mejia, Z. Flores and J. Buitrago. 1975. Unextracted cottonseed in diets for monogastric animals .1. effect of ferrous sulfate and calcium hydroxide in reducing gossypol toxicity. J. Anim. Sci. 40:640-647.

Cohn, L. 2015. Abdominal distension in dogs. http://www.petplace.com/article/dogs/diseases-conditions-of-dogs/symptoms/abdominal-distension-in-dogs. Accessed on January 15, 2016.

Cunha, M.G.G., C.I.M. Gonzalez, F.F.R. Carvalho and A.T. Soares. 2012. Effect of diets containing whole cottonseed on the quality of sheep semen. Acta Sci. Anim. Sci. 34:305–311.

Dağ, S., M. Sözmen, M. Cihan, R. Tunca, B. Kurt, A.K. Devrim and H. Ozen. 2016. Gastric helicobacter like organisms in stray cats: identification, prevalence, and pathologic association. Pak. Vet. J. 36:199–203.

Damián, J.P., J. Pacheco, M.J. Sabalsagaray and K. Rando. 2015. Osseous metaplasia in the lateral abdominal region of a dog. Pak. Vet. J. 35:114–115.

East, N.E., M. Anderson and L.J. Lowenstine. 1994. Apparent gossypol-induced toxicosis in adult dairy goats. Am. J. Vet. Med. Assoc. 204:642–643.

Furlan, F.H., C. Zanata, E. dos S. Damasceno, L.P. de Oliveira, L.A. da Silva, E.M. Colodel and F. Riet-Correa. 2014. Toxic myopathy and acute hepatic necrosis in cattle caused by ingestion of *Senna obtusifolia* (sicklepod; coffee senna) in Brazil. Toxicon 92:24–30.

Gadelha, I.C.N., N.B.S. Fonseca, S.C.S. Oloris, M.M. Melo and B.S. Blanco. 2014. Gossypol toxicity from cottonseed products. Sci. World J. 2014:231635.

Garcia-Abiado, M.A., G. Mbahinzireki, J. Rinchard, K.J. Lee and K. Dabrowski. 2004. Effect of diets containing gossypol on blood parameters and spleen structure in tilapia, *Oreochromis* sp., reared in a recirculating system. J. Fish. Dis. 27:359–368.

Guedes, F.C.B and B.S. Blanco. 2010. Sperm quality of sheep fed cottonseed cake. Acta Sci. Vet. 38:415–418.

Haschek, W.M., V.R. Beasley, W.B. Buck and J.H. Finnell. 1989. Cottonseed meal (gossypol) toxicosis in a swine herd. Am. J. Vet. Med. Assoc. 195:613–615.

Howard, R.S., A.G. Rudd, C.D. Wolfe and A.J. Williams. 2001. Pathophysiology of respiratory control in stroke. Postgrad. Med. J. 77:700–702.

Hudson, L.M., L.A. Kerr and W.R. Maslin. 1988. Gossypol toxicosis in a herd of beef calves. J. Am. Vet. Med. Assoc. 192:1303–1305.

Javed, S., J.A. Khan, T. Khaliq, I. Javed and R.Z. Abbas. 2015. Experimental evaluation of nephroprotective potential of *Calotropis procera* (Ait) flowers against gentamicin-induced toxicity in albino rabbits. Pak. Vet. J. 35:222–226.

Karishma, R., U.L. Sahithya, P. Suneetha, V.C.B. Naik and M.S.R. Krishna. 2016. Determination of total gossypol and free gossypol content in different varieties of BT and non-BT cotton seed extracts by high-performance liquid chromatography (HPLC). Res. J. Biotechnol. 11:70–74.

Kim, H.J. and H.M. Park. 2016. Effect of cyclosporine in canine atopic dermatitis: safety, clinical evaluation, and mechanism studies. Pak. Vet. J. 36:194–198.

Kim, H.L., M.C. Calhoun and R.D. Stipanovic. 1996. Accumulation of gossypol enantiomers in ovine tissues. Comp. Biochem. Physiol. Biochem. Mol. Biol. 113:417–420.

Kirmizigul, A.H., M. Ogun, H. Ozen, E.E. Erkilic, E. Gokce, M. Karaman and A. Kukurt. 2016. Oxidative stress and total sialic acid levels in sheep naturally infected with pox virus. Pak. Vet. J. 36:312–315.

Lindsey, T.O., G.E. Hawkins and L.D. Guthrie. 1980. Physiological responses of lactating cows to gossypol from cottonseed meal rations. J. Dairy Sci. 63: 562–573.

Lordelo, M.M., A.J. Davis, M.C. Calhoun, M.K. Dowd and N. M. Dale. 2005. Relative toxicity of gossypol enantiomers in broilers. Poult. Sci. 84:1376-1382.

Mahmood, F., M.Z. Khan, A. Khan, G. Muhammad and I. Javed. 2011. Lysine induced modulation of toxico-pathological effects of cottonseed meal in broiler breeder males. Pak. J. Zool. 43:357-365.

Marks, J.W. 2017. Intestinal gas (belching, bloating, flatulence). Available online with updates at http://www.medicinenet.com/intestinal_gas_belching_bloating_flatulence/article.htm. Accessed on May 1, 2017.

Mena, H., J.E.P. Santos, J.T. Huber, M. Tarazon and M.C. Calhoun. 2004. The effects of varying gossypol intake from whole cottonseed and cottonseed meal on lactation and blood parameters in lactating dairy cows. J. Dairy Sci. 87:2506–2518.

Morgan, S., E.L. Stair, T. Martin, W.C. Edwards and G.L. Morgan. 1988. Clinical, clinicopathologic, pathologic, and toxicologic alterations associated with gossypol toxicosis in feeder lambs. Am. J. Vet. Res. 49:493–499.

Nugali, T.A., S.K. Abunasef, S.A.M. Karim, F. Al-Qudsi and W. Iqbal. 2016. Expression pattern of stem cell markers in developing mouse pancreas. Pak. Vet. J. 36:35–40.

Fombad, R.B. and M.J. Bryant. 2004. An evaluation of the use of Cottonseed cake in the diet of growing pigs. Trop. Anim. Health Prod. 36:295-305.

Schneider, I.C., M.L. Ames, M. Rasmussen and J. Reilly. 2002. Fermentation of cottonseed and other feedstuffs in cattle rumen fluid. J. Agri. Food. Chem. 50:2267-2273.

Sein, G.M. 1986. The embryotoxic and immunodepressive effects of gossypol. Am. J. Chin. Med. 14:110–115.

Sijun, D., A. Pawlak and B. Pozniak. 2012. Effects of gossypol acetic acid on cellular and humoral immune response in nonimmunized and SRBC-immunized mice. Cent. Eur. J. Immunol. 37:11–19.

Uzal, F.A., B. Puschner, J.M. Tahara and R.W. Nordhausen. 2005. Gossypol toxicosis in a dog consequent ingestion of cottonseed bedding. J. Vet. Diagn. Invest. 7:626–629.

Willard, S.T., D.A. Neuendorff, A.W. Lewis and R.D. Randel. 1995. Effects of free gossypol in the diet of pregnant and postpartum Brahman cows on calf development and cow performance. J. Anim. Sci. 73:496–507.

Yusuf, S. 2011. Complaints against BT cotton grow. The Daily Dawn, December 19, 2011.

Zelski, R.Z., J.T. Rothwell, R.E. Moores and D.J. Kennedy. 1995. Gossypol toxicity in preruminant calves. Aust. Vet. J. 72:394–398.

Zhang, W.J., Z.R. Xu, X.L. Pan, X.H. Yan and Y.B. Wang. 2007. Advances in gossypol toxicity and processing effects of whole cottonseed in dairy cows feeding. Livest. Sci. 111:1-9.

Zubair, M., M. Ahmad, H. Jamil and F. Deeba. 2016. Dietary vitamin E supplementation: A strategy to combat arsenic induced toxicity in teddy bucks. Pak. Vet. J. 36:455–459.