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An Incidence of Aflatoxicosis in Hand-fed Ewe Lambs Exhibiting Icterus Subsequent to Hepatic Failure and Hemoglobinuria

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Abstract

Aflatoxin intoxication has been noted in most species; however, it is infrequently documented in sheep. In this report, three seven-month-old Romane ewe lambs were found with severe icterus, tachypnea, slight fever, diarrhea, anorexia and

lethargy. Two of them were culled, and one recovered after treatment. Necropsy findings included jaundice, ascites, diffuse hemorrhage on the peritoneum, gall bladder enlargement, and alteration in the color and texture of the liver and kidney. Histopathological evaluation revealed hepatocellular necrosis, megalocytosis and centrilobular to bridging fibrosis in the liver, and hemoglobinuria without nephrotic degeneration in the kidney. The Polymerase Chain Reaction test for leptospirosis as the main differential diagnosis was conducted, which was negative. Clinical signs and characteristic microscopic lesions were suggestive of aflatoxicosis in the lambs described in this investigation. As sheep flocks with intensive rearing systems are becoming more common in Iran, the prevalence of aflatoxicosis due to fungi-contaminated feedstuff can be of serious health concern in such farms.

Keywords: aflatoxin, lamb, hemoglobinuria, icterus, intensive rearing, hepatic failure, kidney

Case History

Aflatoxins are a kind of coumarins produced by *Aspergillus* fungi, including *Aspergillus flavus*, *A parasiticus,* and *A nomius* (Constable, Peter D. *et al*., 2017, Zachary, 2021). These toxins usually can be found on cereals,

especially corn (maize), rice, and cottonseed, either in the field or during storage when environmental conditions are favorable for mold growth (G. M. Maxie, 2015b). In tropical regions like Iran, aflatoxicosis poses more threats owing to climatic factors (Gowda *et al*., 2007)

All animal species are vulnerable to aflatoxicosis, but outbreaks usually occur mostly in pigs, sheep, and cattle (Constable, Peter D. *et al*., 2017). The clinical signs and disease severity are different in mammals and poultry because of species, sex, age, production status, and the duration of intake and level of the toxins in the ration (Aiello & Moses, 2016). Accordingly, lactating, pregnant, and growing animals are most likely to be seriously affected (Constable, Peter D. *et al*., 2017).

While ruminants are comparatively resistant to aflatoxicosis, intensively reared livestock, in which a large part of feed consists of stored grain, tend to be more at risk (Bingol, N. T., *et al*.). Acute aflatoxicosis is seldom an issue in adult cattle, sheep, and goats; however, they represent susceptibility if toxic diets are fed over long periods (Aiello & Moses, 2016) or with very high doses (Edrington, T. S. *et al*.).

Animals' exposure to aflatoxin is manifested by chronic and acute hepatocellular damage (Wang *et al*.). High dosages of aflatoxins give rise to necrosis of hepatocytes, while prolonged low dosages cause reduced growth rate, immunosuppression, and liver enlargement (Ramos, A. J. *et al*.). In lambs, consumption of aflatoxins may result in becoming susceptible to disease due to the suppression of some humoral and cellular immune responses (Fernandez *et al*.).

The diagnosis of aflatoxicosis in sheep is based on witnessing mortality, gross lesions on the mucosa, cyanosis, and petechial hemorrhage in the liver, associated with weakness and diarrhea (Barbour *et al*.). The diagnosis is confirmed by detecting aflatoxins in the feed and bloodstream and the characteristic gross and histopathological lesions in the liver and nervous tissue (Constable, Peter D. *et al*., 2017).

This report describes an incidence of aflatoxicosis in an intensive rearing Romane sheep flock near Tehran.

Clinical presentation

In December 2021, a seven-month-old ewe lamb of the Romane breed was found depressed and lethargic in the barn. The lamb was panting and reluctant to move. A thorough examination was conducted immediately. Slight hyperthermia (39.7 degrees Celcius), severe icterus, tachypnea, and dark brown diarrhea were evident. For welfare considerations, the lamb was culled. At necropsy, the most eye-catching findings were extreme jaundice, ascites (figure 1-A), and diffuse hemorrhage on the peritoneum (Figure 1-B). The liver was yellowish-brown and firm, and the gall bladder was heavily swollen (Figure 1-C). The kidneys were abnormally large with dark red color (Figure 1-D). Samples from the liver, kidneys, lung, heart and intestine were taken, put into a 10% formalin solution, and submitted to the lab for histopathological evaluation. Two other ewe lambs from the same barn were found ill within a week, representing the exact clinical signs. One of the latter lambs was slaughtered, and the other recovered after receiving supportive treatment for several days. Treatment included fluid therapy and administration of dexamethasone, vitamin B-complex and Phosphorus+B12 components.

Diagnostic testing

To perform the histopathological examination, 1.5×1.5×1.5 cm liver and kidney samples were taken and placed in 10% neutral buffered formalin. After 48 h, tissue samples were fixed and routinely processed, dehydrated, and embedded in paraffin wax, sectioned at 5 µm thickness (Rotary Microtome RM2 145; Leica), and stained with hematoxylin-eosin (H&E) staining. Additional sections of the liver and kidney were stained histochemically with Masson's trichrome and Prussian blue staining. Finally, the sections were evaluated via a light microscope, and micrographs were taken.

Microscopic examination of the liver section represented micro and macro vesicles of lipid within some hepatocytes (fatty change), bile retention (Figure 2-C), portal/central bridging fibrosis (including necrosis of hepatocytes and replacement with collagen fibers and proliferated fibroblasts and hyperplastic bile ducts (Figure 2-E), ductular reaction, cellular and nuclear atypia, infiltration of some neutrophils and mononuclear inflammatory cells (Figure 2-B), and some large hepatocytes with giant nuclei (15.06 µm in size) called megalocytes (Figure 2-C).

Histologically, the kidney revealed the existence of numerous casts of hemoglobin (Figure 2-F) and RBCs within renal tubules, proteinaceous materials

within urinary spaces and renal tubules, some vacuolated renal tubular epithelial cells, and scant neutrophils and mononuclear inflammatory cells (without remarkable inflammation- Figure 2-D). Interestingly, despite the substantial number of tubular casts, no remarkable degenerative alterations in renal tissue were seen.

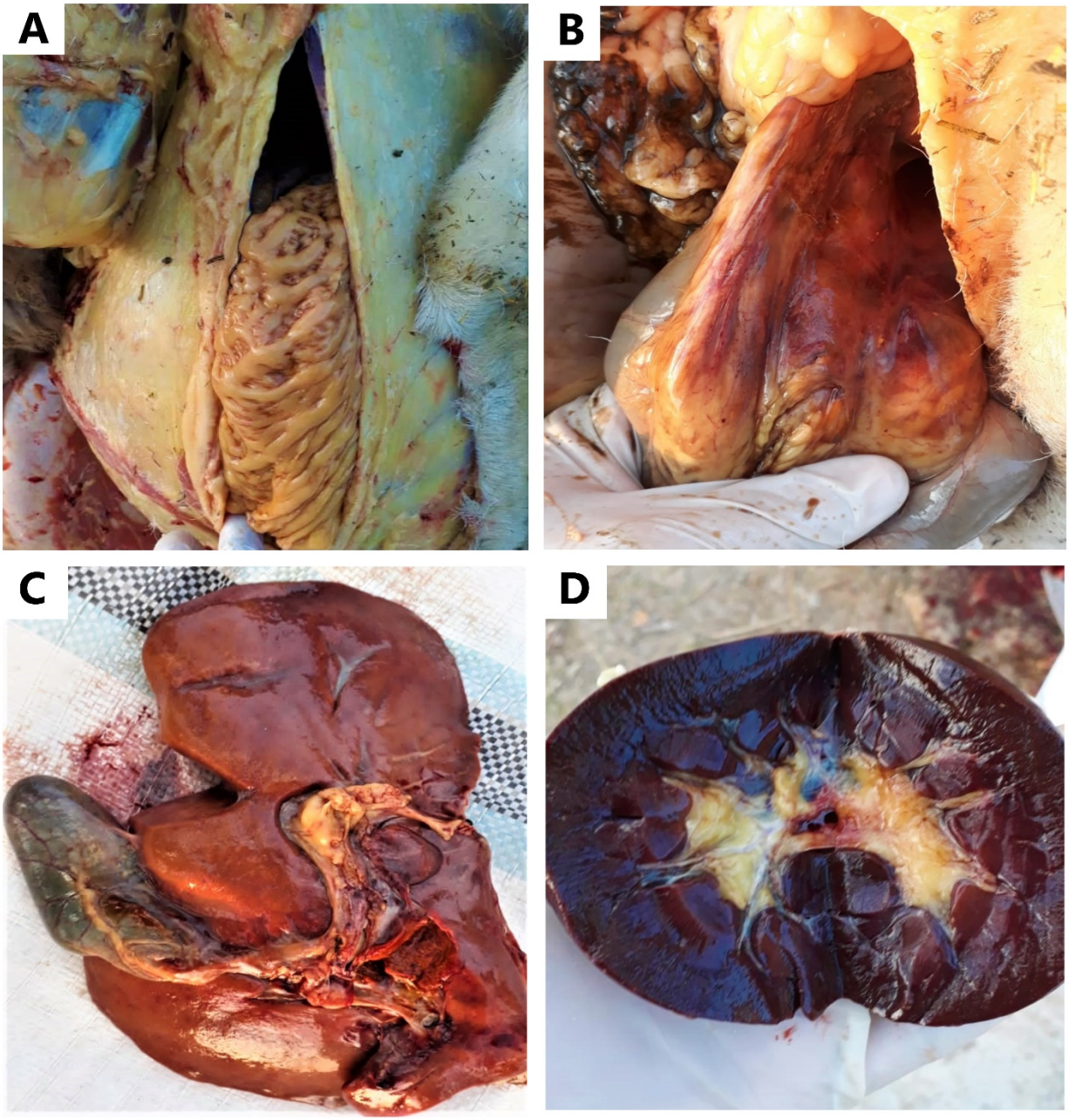

Figure 1- A) Extreme jaundice of the carcass, B) Diffuse hemorrhage on the peritoneum, C) Enlarged yellowish-brown liver with swollen gall bladder, D) Dark red kidney

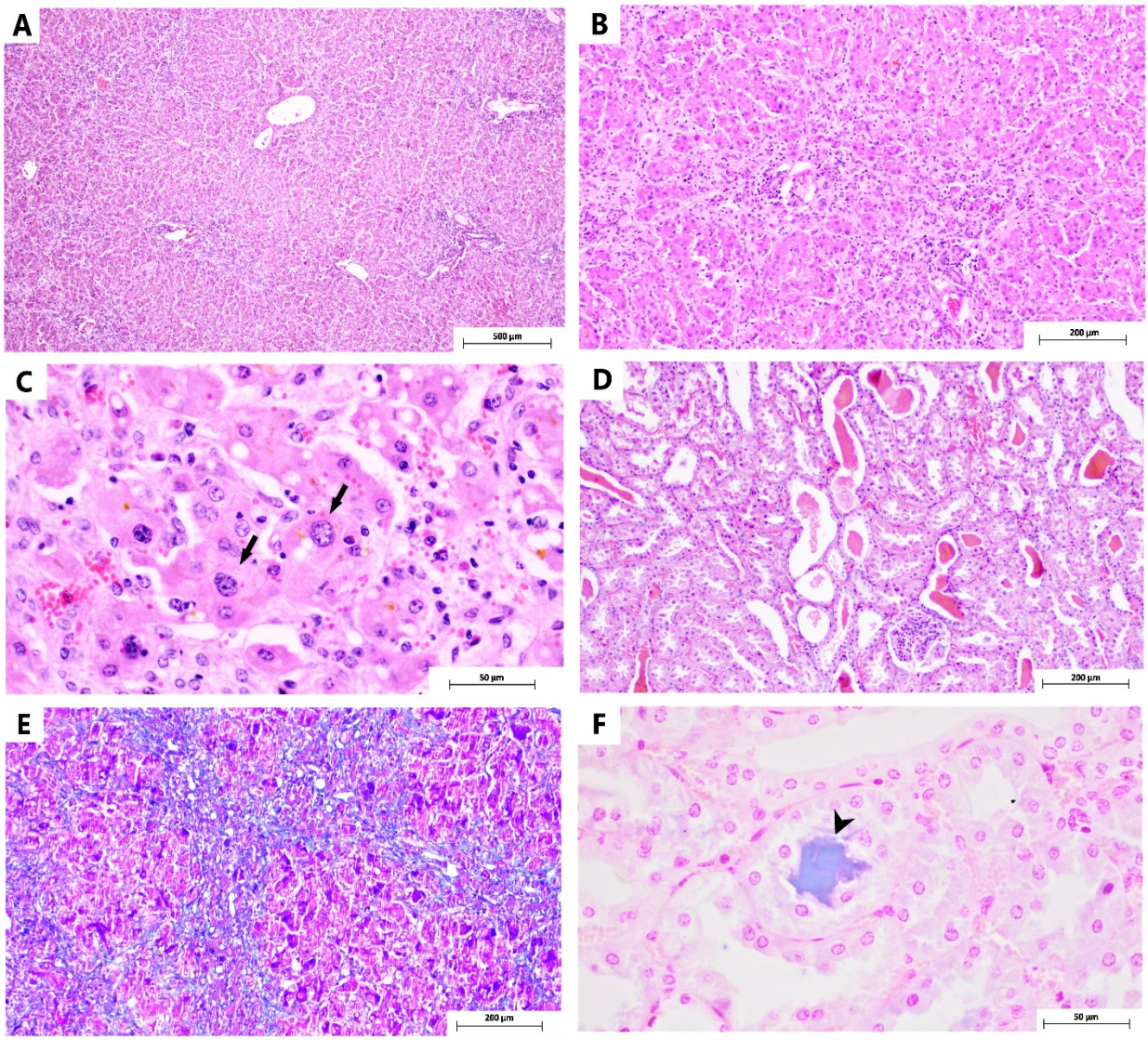

Figure 2- Histopathological micrographs A) Liver, H&E staining, 4X. B) Hepatocellular necrosis, Liver, H&E staining, 10X. C) Hepatocyte degeneration and necrosis, Megalocyte formation (arrows) Liver, H&E staining, 40X. D) Tubular urinary casts, Kidney, H&E staining, 10X. E) Blue areas of collagen deposition indicating bridging fibrosis, Liver, Masson's trichrome staining, 10X. F) Hemoglobin cast (arrowhead), Prussian blue staining, 40X.

Assessments

The FAO presumes that nearly 25% of the world's food commodities are contaminated with mycotoxins, mainly aflatoxin (Cao *et al.*). Sheep are quite resistant to aflatoxins (Edrington, T. S. *et al*.) as such aflatoxicosis is uncommon in sheep herds (G. M. Maxie, 2015b). However, in the housed flocks, in which animals are hand-fed with stored cereals and hay, the risk of the disease may rise significantly (Constable, Peter D. *et al*., 2017). The cases described in this report were kept in the barn the whole year, receiving a ration consisting of barley, corn, soybean meal, soybean oil, corn silage and alfalfa. Field investigation revealed that some parts of the alfalfa used in the feed had been moldy. On the other hand, the farmworkers used a feeder mixer machine to prepare animals' feed, whose container was rarely cleaned. It was observed that a bunch of feedstuffs was trapped under the mixer axis so that it could provide a favorable environment for fungal growth and, ultimately, mycotoxin production.

According to *Cammilleri et al.*, Eighteen aflatoxins have been classified to date, of which Aflatoxin B1 (AFB1) is the most potent. The major impacts of perpetuated aflatoxin consumption in livestock are hepatosis and hepatic insufficiency (Wang *et al.*), leading to poor growth rates, unthriftiness, icterus and death (Aiello & Moses, 2016). A survey performed by *Cao et al.* showed that Aflatoxins interrupt the normal microbiota of the ovine intestine. All three animals in this study exhibited poor performance, depression and jaundice at the onset of the disease. They were unwilling to move; they had no appetite for food, and their conjunctival and vaginal mucosa was severely icteric. Respiratory inadequacy was an apparent finding in all of them as well. In addition, in one of the lambs, chocolate diarrhea was seen.

In histopathology, lesions such as hepatocellular necrosis, megalocytosis and centrilobular to bridging fibrosis in the liver (Figure 2-A, B, C, E), and hemoglobin cast formation without nephrotic degeneration in the kidney (figure 2- D, F) were most prominent. In the affected lambs, hepatic dysfunction might have resulted in protein synthesis failure and subsequently ascites and erythrolysis due to low oncotic pressure. On the other hand, damage to hepatocytes could have contributed to the sudden release of their copper reservoir, leading to hemoglobin oxidization and destruction (G. M. Maxie, 2015a). Erythrolysis consequent to low oncotic pressure and copper release from damaged hepatocytes can be two possible justifications for hemoglobinuria and intense icterus (Figure 1-A) in the reported lambs.

Some disorders share identical clinical signs with aflatoxicosis in sheep. Given the farm condition, leptospirosis was considered the principal differential diagnosis in this case. Thus, PCR for leptospirosis from the fresh kidney specimen was performed, which was negative. Therefore, the characteristic hepatic and renal histopathologic changes can be confirmatory for the diagnosis of aflatoxicosis in this survey.

All three ill lambs underwent treatment with the following prescription for three consecutive days. Vitaforte B (Razak co.) 5 ml IM, Multivitamin (Abureihan co.) 5 ml SC, Vetacoid (Abureihan co.) 4 ml IV, Cobaject (Royandaru co.) 5 ml IV, Duphalyte (Zoetis co.) 25 ml IV, Oxivet 20% (Razak co.) 5 ml IV (one day only) and 500 ml of sterile normal saline infusion. Eventually, one of the lambs recovered after treatment, while two others were culled due to poor prognosis.

Conclusion

In the literature, sheep are considered resistant to aflatoxin intoxication, and reports on aflatoxicosis in sheep are scarce. Nevertheless, as ovine housed flocks with the hand-feeding system are becoming increasingly common in Iran, the prevalence of aflatoxicosis can emerge as a serious problem in such farms if not properly addressed.

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References

Afsah Hejri, S., Badiei, K., Pourjafar, M., Oryan, A., Keshavarzi, B., Chalmeh, A., Mesbah, G. (2019). Seasonal Variation and Relationships Between Copper of Serum and Various Tissues in Copper Poisoned Sheep in Kerman Province, Iran. *Iranian Journal of Veterinary Medicine*, 13(2), 163-173. https://dx.doi.org/10.22059/ijvm.2018.261446.1004911

Aiello, S. E., & Moses, M. A. (2016). The Merck Veterinary Manual (11th ed.). Merck Manuals.

Alizadeh, S., Mohammadi, T. (2019). Ultrasonographic Liver Findings in a Sheep Flock Involved in Chronic Fasciolosis. *Iranian Journal of Veterinary Medicine*, 13(1), 37-43. https://dx.doi.org/10.22059/ijvm.2018.254143.1004888

Barbour, E. K., Abou-Alsaud, M. E., Gheith, N. A., Abdel-Sadek, M. A., Heba, H. M. A., Harakeh, S., & Karrouf, G. I. A. (2014). Evaluation of a Diagnostic Model for Aflatoxicosis in Sheep: A Prerequisite for Future Adoption of National Surveillances. International Journal of Applied Research in Veterinary Medicine, 12(2). URI: http://hdl.handle.net/10938/20374

Bingol, N. T., Tanritanir, P., Dede, S. E. M. I. H. A., & Ceylan, E. (2007). Influence of aflatoxin present in forages and concentrated feedingstuffs on milk and some serum biochemical parameters in goats. BULLETIN-VETERINARY INSTITUTE IN PULAWY, 51(1), 65. http://www.piwet.pulawy.pl/bulletin/images/stories/pdf/20071/20071065070.pdf

Cammilleri, G., Graci, S., Collura, R., Buscemi, M. D., Vella, A., Macaluso, A., ... & Ferrantelli, V. (2019). Aflatoxin M1 in cow, sheep, and donkey milk produced in Sicily, Southern Italy. *Mycotoxin research*, *35*(1), 47-53. DOI: https://doi.org/10.1007/s12550-018-0329-y, PMID: 30215192

Cao, Q. Q., Lin, L. X., Xu, T. T., Lu, Y., Zhang, C. D., Yue, K., ... & Jian, F. C. (2021). Aflatoxin B1 alters meat quality associated with oxidative stress, inflammation, and gut-microbiota in sheep. Ecotoxicology and Environmental Safety, 225, 112754. DOI: https://doi.org/10.1016/j.ecoenv.2021.112754

Constable BVSc MS PhD Dipl ACVIM, Peter D., Hinchcliff BVSc MS PhD DACVIM (Large Animal), Kenneth W, Done BA BVetMed PhD DECPHM DECVP FRCVS FRCPath, Stanley H., & Gruenberg, W. (2017). Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats - two-volume set (11th ed.). Saunders Ltd.

Edrington, T. S., Harvey, R. B., & Kubena, L. F. (1994). Effect of aflatoxin in growing lambs fed ruminally degradable or escape protein sources. *Journal of Animal Science*, *72*(5), 1274-1281. https://doi.org/10.2527/1994.7251274x

Fernandez, A., Hernandez, M., Verde, M. T., & Sanz, M. (2000). Effect of aflatoxin on performance, hematology, and clinical immunology in lambs. Canadian Journal of Veterinary Research, 64(1), 53. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1189581/, PMID: 10680657

Gowda, N. K. S., Suganthi, R. U., Malathi, V., & Raghavendra, A. (2007). Efficacy of heat treatment and sun drying of aflatoxin-contaminated feed for reducing the harmful biological effects in sheep. Animal Feed Science and Technology, 133(1-2), 167-175. https://doi.org/10.1016/j.anifeedsci.2006.08.009

Hassan Abood, A., Khteer Al-Hadraawy, S., Abdulhur Alibraheemi, N., Zabibah, R. S., & Khalfa, H. M. (2022). Histological Changes and Ghrelin, P-Selectin, and Leptin Parameter in Patients with Hepatic Hydatidosis in Iraq. *Archives of Razi Institute*, *77*(2), 585-590. https://dx.doi.org/10.22092/ari.2021.356856.1927

Khorrami, R., Pooyanmehr, M., Nooriyan Soroor, M., Gholami, S. (2022). Evaluation of Some Aflatoxins in Feed Ingredients of Livestock and Poultry by HPLC Method, A Local Study in Kermanshah Province. *Iranian Journal of Veterinary Medicine*, 16(3), 298-310. https://dx.doi.org/10.22059/ijvm.2022.329690.1005192

Maxie, G. M. (2015). Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 2 (6th ed.). Saunders Ltd.

Maxie, G. M. (2015a). Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 1 (6th ed.). Saunders Ltd.

Ramos, A. J., & Hernandez, E. (1997). Prevention of aflatoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feedstuffs: a review. Animal Feed Science and Technology, 65(1-4), 197-206. https://doi.org/10.1016/S0377-8401(96)01084-X

Shakir Alkhafaji, R., Muhsin Khalfa, H., & LF Almsaid, H. (2022). Rat Hepatocellular Primary Cells: A Cellular and Genetic Assessment of the Chitosan Nanoparticles-Induced Damage and Cytotoxicity. *Archives of Razi Institute*, *77*(2), 579-584. https://dx.doi.org/10.22092/ari.2022.357103.1974

Wang, J., Lin, L., Jiang, Q., Huang, W., & Liu, N. (2019). Effect of supplemental lactic acid bacteria on growth performance, glutathione turnover and aflatoxin B1 removal in lambs. Czech Journal of Animal Science, 64(6), 272-278. http://dx.doi.org/10.17221/5/2019-CJAS

Zachary, J. F. (2021). Pathologic Basis of Veterinary Disease. Mosby.

گزارش موردی :وقوع آفلاتوکسیکوزیس در مزرعه پرورش صنعتی گوسفند

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خلاصه

مسمومیت با آفلاتوکسین در اکثر گونه های حیوانی مشاهده شده است. با این وجود، وقوع آفلاتوکسیکوز به ندرت در گوسفند ثبت شده است. در این گزارش، سه راس بره میش هفت ماهه نژاد رومن مبتلا به زردی شدید، تاکی پنه، تب خفیف، اسهال، بی‌اشتهایی و بی‌حالی مشاهده شد. دو راس از آنها به علت عدم پاسخ به درمان ذبح شده و یکی پس از درمان بهبود یافت. یافته های کالبدگشایی شامل زردی، آسیت، خونریزی منتشر در چادرینه، بزرگ شدن کیسه صفرا و تغییر رنگ و قوام کبد و کلیه بود. در ارزیابی هیستوپاتولوژیک، نکروز سلول های کبدی، مگالوسیتوز و فیبروز سانتریلوبولار در کبد و هموگلوبینوری بدون دژنراسیون نفروتیک در کلیه مشاهده شد. برای لپتوسپیروز به عنوان تشخیص افتراقی اصلی، آزمون PCR انجام شد که منفی بود. علائم بالینی و ضایعات میکروسکوپی مشخصه، حاکی از آفلاتوکسیکوزیس در بره‌هایی بود که در این بررسی توضیح داده شد.

کلمات کلیدی: آفلاتوکسین، مسمومیت، گوسفند، پرورش صنعتی، خوراک دستی، هیستوپاتولوژی، زردی، نارسایی کبدی