First Case of Chronic Copper Toxicosis in Dairy Cows in Israel and its Remedial Management

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ABSTRACT

Chronic copper toxicosis in cattle has never been reported in Israel and very rarely abroad. Chronic copper toxicity in cattle results mostly from the ingestion of high copper containing feeds, or due to accidental administration of excessive amounts of soluble copper salts. In February of 2015, the complete feed for lactating Holstein-Friesian dairy herd was accidently supplemented with 250 mg copper /kg complete feed. By June of 2015, clinical signs of copper intoxication occurred in several cows followed by sudden death of twenty cows within 4 days. The mean liver and kidney copper level was 405±120 mg/kg and 29±16 mg/kg, respectively. The remaining herd was treated with 200 mg/cow/day of sodium molybdenum for 7 days added to the new feed from which the copper supplement has been removed. Subsequently, sodium molybdenum supplement was terminated and the herd was further fed on complete feed lacking any copper supplements for several months. About 2-3 months after the remedial management of the dairy herd, a full recovery of the animals was observed, with milk production and body condition returning to normal. The molybdenum therapy achieved a 50% reduction in mean liver copper level. In contrast, the mean kidney copper level was reduced to normal levels only 2-3 months post treatment. It was concluded that chronic copper poisoning of dairy cows can be successfully treated by the removal of all copper supplements from feed and oral administration of 200 mg molybdenum/cow/day, thereby reducing liver and kidney levels within 2-3 months to normal values.

Key words: Copper; Liver; Kidney; Chronic Copper Toxicity; Sodium Molybdenum.

BACKGROUND

Copper fulfils numerous vital functions in living organisms and is regarded as an essential micronutrient (1). Although copper does not belong to the highly toxic metal group commonly associated with major health hazards (e.g. arsenic, mercury, lead and cadmium) to animals and humans, chronic copper poisoning is encountered in most parts of the world, causing more health problems than the heavy metals altogether (2-5). Chronic copper toxicity in ruminants may result from the ingestion of high copper containing feeds, or due to accidental administration of excessive amounts of soluble copper salts (2-5). Chronic copper poisoning in cattle is reported to be associated with ingestion of feed containing >100 mg of copper/kg total mixed ration (TMR), whereas exposure to >5000 mg copper/kg TMR are acutely toxic in mature cattle (2-6). Copper toxicosis is clinically evident when blood copper concentrations increases suddenly due to liver storage overload, resulting in lipid peroxidation

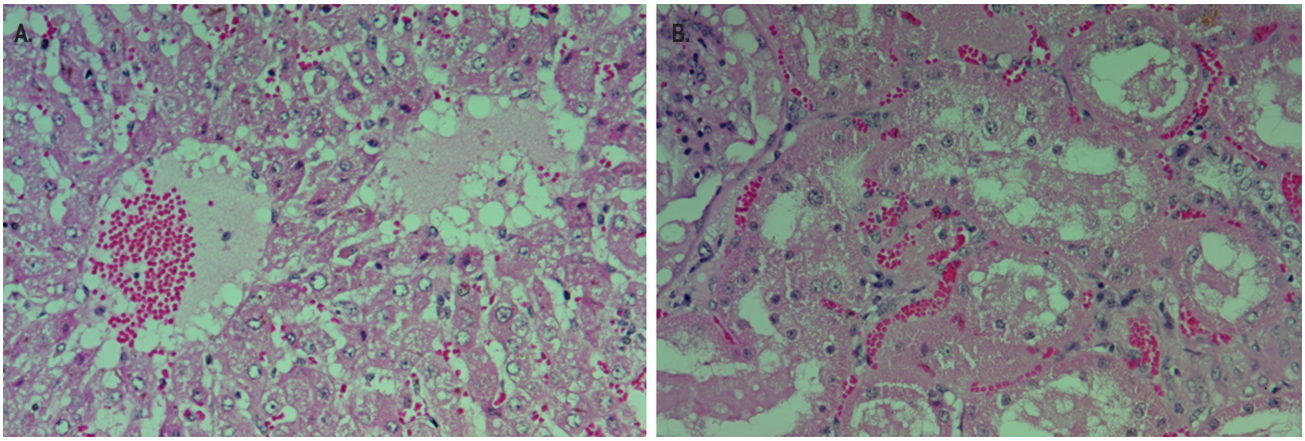

Figure 1. Histopathological liver (A) and kidney (B) samples of Holstein-Friesland dairy cow (4 years old) chronically exposed to toxic copper levels (250 mg/kg TMR) for 6 months.

(A) Centrolobullar area (zone 3) revealed dilated sinusoids, diffuse hydrophic changes, moderate fatty changes and single cell necrosis. Mild mononuclear infiltration in part of the portal areas was observed. Paraffin embedded; H&E X 100.

(B) In the kidneys multiple dilated tubules were observed, revealing eosinophilic proteinaceous material, interpreted as tubular nephrosis. Dilated tubules were lined by variably injured cells, characterized by hypereosinophilic cytoplasm and karyolytic nuclei, interpreted as necrotic tubular epithelial cells. Other cells had vacuolated eosinophilic granular cytoplasm, interpreted as degenerating tubular epithelial cells. Paraffin embedded; H&E X 100.

and intravascular hemolysis (2-5). In Israel, chronic copper poisoning in sheep is highly prevalent (7). Sheep, mainly in the East Friesan/Awassi bread crosses, are the most affected species diagnosed in Israel every year with copper toxicosis (7). The determination of copper concentrations in liver is considered the best biomarker for diagnosing copper disorders in animals, since no valid and reproducible biomarker indicating excess or deficient copper levels are available (8).

The aim of the present case study was to provide a clinical description of chronic copper toxicosis in dairy cows (3-5 years old), reported for the first time in Israel.

CASE REPORT

In February of 2015, the complete feed for lactating Holstein-Friesian dairy herd consisting of 350 head (age 3-5 years old, weighting on average 650 kg) was accidently supplemented with 250 mg copper /kg complete feed (as copper sulphate). The supplemented copper level was 10 times higher than the recommended level of 25 mg/kg complete feed (1, 6). By June of 2015, 6 months after daily ingestion of feed containing toxic copper levels, clinical signs of copper intoxication were present in several cows characterized by weight loss, reduced milk production, diarrhea, anorexia and dehydration. Within 4 days, twenty cows were found dead after displaying various degrees gastroenteritis, lethargy, recumbency, anorexia, thirst, dyspnea, pale mucous membranes, hemoglobinuria and jaundice. Of the twenty dead cows, 4 cows underwent full necropsy at the Kimron Veterinary Institute and histopathological as well as parasitological, bacteriological and virological examinations were carried out.

Laboratory Investigations

Total mixed ration (tmr) analysis

Samples of the TMR were screened for a wide range of pesticides, including organophosphates, carbamates, pyrethroids and organochlorides as well as pyrrolizidine alkaloids by GC-MS (Agilent Technologies, Santa Clara, CA), according to an in-house validated method (9). The TMR ingredients were also analyzed for doxycycline, oxytetracycline as well as the ionophores monensin, lasalocid, salinomycin, maduramicin, semduramicin and narasin by LC-MS/MS according to the previously described method (10). The analysis of the TMR ingredients for ochratoxin and aflatoxins B1, B2, G1 and G2 was performed by HPLC with post-column photochemical derivatisation and fluorescence detection, according to an in-house validated method (11). Our routine multi-residue screening analysis utilizing GC-MS of the TMR ingredients did not reveal any pesticide and pyrrolizidine alkaloid

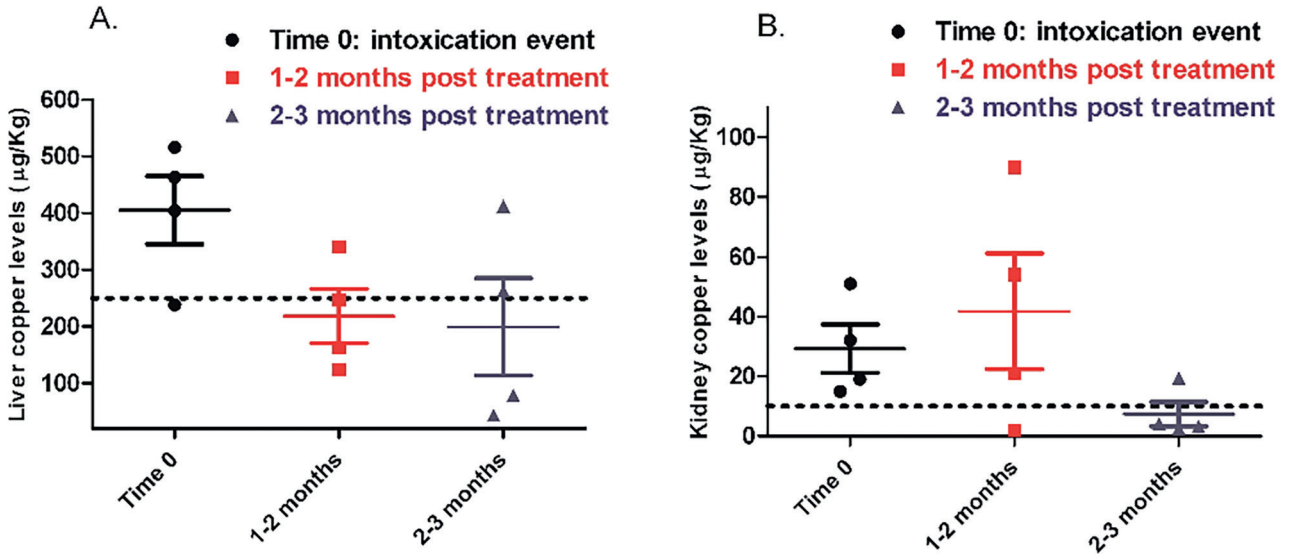

Figure 2. Concentrations of Cu in livers (A) and kidneys (B) of dairy cows prior to (Time 0) and after the removal of all Cu supplements, and after being fed 500 mg sodium molybdate (200 mg Mo/cow/day) as a solution sprinkled onto TMR for 7 days. The horizontal dotted line represents the lower boundary of toxic copper levels.

contamination. The antibiotics and ionophores analyzed by LC-MS/MS were below the detection limit of 60 µg/kg and the feed ingredients analyzed for aflatoxins and ochratoxin by HPLC-FLD were below the limit of quantitation (1 µg/kg).

Liver and kidney samples analysis

Liver and kidney samples of four cows submitted for full necropsy, were analyzed for the trace elements As, Cd, Co, Zn, Cu, Fe, Pb, Mn, Hg, Mo, Se, Tl and Zn, by utilizing an ARCOS ICP-AES (Spectro Analytical Instruments, Kleve, Germany) according to the EPA method 6010 (12). Except for copper, all of the elements analyzed were within the normal range. The mean liver and kidney copper levels determined in 4 intoxicated cows was 405±120 mg/kg wet weight in the liver samples and 29±16 mg/kg wet weight in the kidney samples. The kidney and liver copper levels were above the lower boundary for toxic copper levels of 10 mg/kg and 250 mg/kg wet weight respectively (6). However, mean molybdenum level (0.2 mg/kg) in the liver was found to be within normal range (1, 6).

Bacteriology and virology

Fresh samples of lung, liver, spleen and kidney from 4 dead cows were examined for evidence of bacteriological and viral infections. The analysis for botulism neurotoxin from feed, stomach content and plasma was performed by the Israeli Reference Laboratory, Department of Bacteriology, The Kimron Veterinary Institute, Israel, utilizing in-house validated mouse lethality assay (13). Brain tissues were tested for rabies virus (RABV) at the Israeli Reference Laboratory, Department of Rabies, The Kimron Veterinary Institute, Israel, utilizing the direct fluorescent antibody test according to an in-house validated method (14). No pathogenic bacteria (aerobic, anaerobic and Salmonella, Clostridium) and viruses of epidemiological significance were detected in the tissues examined.

Pathology and histopathology

On post-mortem examination, the intoxicated animals showed gastroenteritis and diffuse jaundice. The histopathological examination of the liver revealed mild dilatation of the sinusoids, multifocal random haemorrhages, various stages of hepatocyte degeneration as well as moderate diffuse vascular hydrophic and fatty changes (Figure 1A). Moreover, in the kidney, multiple dilated tubules were observed, revealing eosinophilic proteinaceous material, interpreted as tubular nephrosis (Figure 1B). Dilated tubules were lined by variably injured cells, revealing hypereosinophilic cytoplasm and karyolytic nuclei, interpreted as necrotic tubular epithelial cells. Other cells had vacuolated eosinophilic granular cytoplasm, interpreted as degenerating tubular epithelial cells (Figure 1B).

TREATMENT

Based on the aforementioned laboratory and postmortem findings, chronic copper poisoning in dairy cows was established. Immediately upon receipt of the diagnosis, the copper – supplemented complete feed was removed and replaced by a fresh complete feed composed of grass silage and hay, lacking any copper supplement, to which 500 mg/kg sodium molybdenum was added (200 mg/cow/day). The new feed supplemented with sodium molybdenum was given to the affected herd for 7 days, after which the sodium molybdenum supplement was terminated and the herd was further fed on complete feed lacking any copper supplements for several months. This treatment has been successfully implemented in several studies, which resulted in reduced copper absorption and enhanced copper elimination from the body (8, 15). About 2-3 months after the remedial management of the dairy herd, a full recovery of the animals was observed, with milk production (25-30 liter/day) and body condition returning to normal.

Following the molybdenum chelation therapy, a 50% reduction in mean liver copper level was observed in 4 cows 1-2 months post treatment (218±120 mg copper/kg wet weight). A comparable reduced mean liver copper level of 200±171 mg/kg wet weight in 4 additional cows was observed 2-3 months post treatment (Figure 2A). In both post-treatment periods, 3/4 of the cows displayed liver copper concentration below 250 mg/kg, which represents the lower boundary of toxic copper levels in cattle liver (Figure 2A). In contrast, the mean kidney copper level obtained 1-2 months post treatment determined in 4 cows (29±16 mg/kg wet weight), was not markedly different as compared to the pre-treatment mean level of 42±39 mg/kg wet weight, both of which exceeding the lower toxic boundary level of 10 mg/kg in the kidney (Figure 2B). Only 2-3 months post treatment, a marked decrease in the mean copper kidney level of 7.4±8 mg/kg was observed, with 3/4 of the cows revealing kidney copper levels below the lower toxic boundary level (Figure 1B).

DISCUSSION

Chronic copper toxicosis in cattle is rarely reported worldwide, while in Israel it has never been reported before (2-5). Clinical signs of chronic copper toxicosis in cattle are non-specific and may include weight loss, poor hair coats, dark diarrhea, loss of appetite and reduced milk production (2-5, 16, 17). The histopathological findings of chronic cupper toxicosis in cattle and ruminants in general, are non-pathognomonic and may include hepatocellular necrosis, biliary proliferation and periacinar fibrosis, rendering the diagnosis as extremely difficult (2-5). Hence, the best indicator of copper toxicosis in ruminants is measurement of the copper liver concentration (2-5, 16). An established safe range for Cu in liver is 5–100 mg/kg wet weight, while toxicity has been associated with concentrations of Cu above 200 mg/kg wet weight (6).

In ruminants, the metabolic balance of copper is determined mostly by molybdenum and copper levels within the feed (7). Cattle will usually perform normally when the copper to molybdenum ratio in feed is from 5:1 to 10:1 (18). Increasing the copper to molybdenum ratio may result in copper accumulation and possibly chronic copper poisoning, whereas decreasing the ratio may induce copper deficiency. In ruminants, dietary sulfate is reduced to sulfide in the rumen which can subsequently combine with molybdate to form thiomolybdates (7). Thiomolybdates can bind to copper before and after absorption, thereby leading to reduced copper levels (7). Molybdenum is the most frequent interacting element in copper metabolism, and so determination of copper and molybdenum, as well as sulphur in liver and feedstuffs, is recommended for diagnosis and for monitoring interactions (7).

In conclusion, chronic copper toxicosis occurred following 6 months exposure to 250 mg copper /kg complete feed, resulting in liver and kidney levels of 405 ± 120 mg/kg and 29 ± 16 mg/kg wet weight, respectively. A molybdenum treatment of 200 mg Mo/cow/day for 7 days together with removal of any copper supplementation resulted in a complete recovery of the dairy herd with milk production returning to normal range within 2-3 months.

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

ACKNOWLEDGMENT

The authors kindly thank Efrat Habusha and Faina Golender for their excellent technical assistance.

REFERENCES

1. National Research Council. Minerals: Copper. In: Nutrient Requirements of Dairy Cattle. 7th Ed. Washington, DC; pp. 133-136, 2001.
2. Bidewell, C. A., Drew, J. R., Payne, J. H., Sayers, A. R., Higgins, R. J. and Livesey, C. T.: Case study of copper poisoning in a British dairy herd. Vet. Rec. 170: 464, 2012.
3. Perrin, D. J., Schiefer, H. B. and Blakley, B. R.: Chronic copper toxicity in a dairy herd. Canadian Vet. J. 31: 629-632, 1990.
4. Stogdale, L.: Chronic copper poisoning in dairy cows. Australian Vet. J. 54: 139-141, 1978.
5. Tremblay, R. R. M. and Baird, J. D.: Chronic copper poisoning in two Holstein cows. Cornell Vet. 81: 205-213, 1991.
6. Puls, R.: Mineral levels in animal health. Diagnostic Data, 2nd edition, Sherpa International, Aldergrove, Canada, 1994.
7. Shlosberg, A.: Syndromes of Ill-health Associated with Copper. Israeli J. Vet. Med. 65, 170-171, 2010.
8. EFSA. Revision of the currently authorized maximum copper content in complete feed. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). EFSA J. 14: 4563, 2016.
9. Shimshoni, J. A., Evgeny, E., Lublin, A., Cuneah, O., King, R., Horowitz, I., and Shlosberg, A: Determination of brain cholinesterase activity in normal and pesticide exposed wild birds in Israel. Israeli J. Vet. Med. 67: 214-219, 2012.
10. Shimshoni, J. A., Britzi, M., Pozzi, P. S., Edery, N., Berkowitz, A., Bouznach, A., Cuneah, O., Soback, S., Bellaiche, M., Younis, A., Blech, E., Oren, P., Galon, N., Shlosberg, A. and Perl, S.: Acute maduramicin toxicosis in pregnant gilts. Food Chem. Toxicol. 68: 283-289, 2014.
11. Shimshoni, J. A., Mulder, P. P., Bouznach, A., Edery, N., Pasval, I., Barel, S., Abd-El Khaliq, M. and Perl, S.: Heliotropium europaeum poisoning in cattle and analysis of its pyrrolizidine alkaloid profile. J. Agri.c Food Chem. 63: 1664-1672, 2015.
12. EPA method, 6010c. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6010c.pdf . Accessed on January 14, 2019.
13. Elad, D., Yas-Natan, E., Aroch, I., Shamir, M. H., Kleinbart, S., Hadash, D., Chaffer, M., Greenberg, K. and Shlosberg, A.: Natural Clostridium botulinum type C toxicosis in a group of cats. J. Clin. Microbiol. 42: 5406-5408, 2004.
14. David, D., Yakobson, B., Rotenberg, D., Dveres, N., Davidson, I. and Stram, Y.: Rabies virus detection by RT-PCR in decomposed naturally infected brains. Vet. Microbiol. 87: 111-118, 2002.
15. Morgan, P., Grace, N. and Lilley, D.: Using sodium molybdate to treat chronic copper toxicity in dairy cows: A practical approach. New Zealand Vet. J. 62: 167-170, 2014.
16. Gummow, B.: Experimentally induced chronic copper toxicity in cattle. Onderstepoort J. Vet. Res. 63: 277-288, 1996.
17. Jolly, P. D., Clark, R. G. and Fraser, A. J.: Effect of copper status and copper supplementation on milk production and fertility in dairy cattle. The Fourth Animal Science Congress of the Asian-Australasian Association of Animal Production Societies. Hamilton, New Zealand, p. 420, 1987.
18. Maas J.: Copper nutrition of beef cattle. UCD VET Views California Cattleman, 1997. https://ucanr.edu/sites/UCCE_LR/files/151747.pdf . Accessed on January 20, 2019.