CHRONIC COPPER INTOXICATION IN VEAL CALVES

Chronische kopervergiftiging bij mestkalveren

S. Croubels[1], K. Baert[1], T. Torck[3], P. Deprez[2], P. De Backer[1]

[1]Department of Pharmacology, Pharmacy and Toxicology

[2]Department of Internal Medicine and Clinical Biology of Large Animals,
Faculty of Veterinary Medicine, Ghent University,
Salisburylaan 133, B-9820 Merelbeke, Belgium

[3]Opaaigem 76, B-9420 Erpe-Mere, Belgium
siska.croubels@rug.ac.be

ABSTRACT

The addition of excessive amounts of copper to commercially prepared milk replacers caused chronic copper toxicity in veal calves from two different Belgian farms. A possible mixing error by the feed company resulted in copper levels ranging from 120 to 159 mg/kg in the milk powder. On one farm, four animals died showing the typical clinical signs of chronic copper toxicity, including weakness, anorexia and severe icterus. Pathology and biochemistry of the calves and chemical analysis of the blood, milk powder and liver were performed to establish the diagnosis. In the liver, copper concentrations of 297 and 500 mg/kg fresh liver were found, which are indicative of a chronic copper intoxication.

Keywords :Copper - Chronic intoxication - Milk replacer - Calf

SAMENVATTING

De aanwezigheid van te hoge hoeveelheden koper in commerciële kalverkunstmelk veroorzaakte een chronische kopervergiftiging bij mestkalveren afkomstig van twee verschillende Belgische bedrijven. Waarschijnlijk zorgde een mengfout binnen het mengvoederbedrijf voor een kopergehalte van 120 tot 159 mg/kg in de kunstmelk. Op één bedrijf stierven vier kalveren die de typische klinische symptomen van intoxicatie vertoonden, namelijk zwakte, anorexie en erge icterus. Pathologisch en biochemisch onderzoek van de kalveren en chemische analyse van het bloed, de kunstmelk en de lever werden uitgevoerd om de diagnose te kunnen stellen. In de lever werden gehalten van 297 en 500 mg/kg vers materiaal teruggevonden, die wijzen op een chronische koperintoxicatie.

INTRODUCTION

Copper poisoning, acute or chronic, is encountered in most parts of the world. Sheep are affected most often, though other animals such as cattle and to a lesser extent pigs are also susceptible. Acute poisoning is usually observed after accidental administration of large quantities of soluble copper salts, which may be present in mineral mixes or improperly formulated rations. The clinical signs associated with acute copper poisoning in calves include anorexia, depression, icterus and gastroenteritis with dehydration, ascites and diarrhoea (Humphreys, 1988). Currently, acute copper toxicosis is not common in veterinary medicine. Chronic poisoning is seen when elevated amounts of copper are ingested over a prolonged period. The toxicosis remains subclinical until the copper that is stored in the liver is released in massive amounts. Blood copper concentration increases suddenly, which causes severe hemolysis. Affected animals exhibit signs of depression, weakness, anorexia, hemoglobinuria and jaundice, but no additional gastrointestinal disturbances.

Copper poisoning has been reported in calves receiving milk substitutes (Humphreys, 1988). Experimental daily feeding of milk substitutes containing 50, 100, 200 or 300 mg/kg of copper has shown that calves receiving those containing 100-300 mg/kg died within 16-75 days (Weiss *et al.*, 1967). The principal clinical biochemical changes associated with

copper poisoning in cattle include increases in the serum bilirubin level, in the copper concentration and in the activities of the liver-derived enzymes. Osweiler (1996) reported that an increase in the circulatory activities of the enzymes aspartate aminotransferase and alanine aminotransferase was an early indicator of copper poisoning. A release of copper from the liver occurs 6-10 days before the haemolytic crisis, with levels of 19 μmol/l blood or above being significant (Humphreys, 1988). Chronic copper poisonings have been reported to be accompanied by dry-weight basis liver copper levels ranging from 560 mg/kg to 3000 mg/kg (Humphreys, 1988).

CASE REPORT

History

Two veal calves, originating from two different farms, were presented at the Faculty of Veterinary Medicine. They were 3 and 4 months old and showed depression and weakness. The oldest calf was apathetic and anorectic and had strong yellow coloured mucosae. The blood of the other calf was dark brown coloured (chocolate colour), indicating a possible methaemoglobinemia. In one farm, other 8-day-old calves were affected. They exhibited tremor and lay down in decubitus immediately after drinking the milk. Remarkably, they all exhibited continuous compulsive tongue movements, also immediately after drinking the milk on the farm.

A history check on the farms revealed the presence of blue spots floating on the surface of the milk after the commercial milk replacer powder had been dissolved in hot water. This raised the suspicion of a possible copper intoxication, since copper sulfate crystals are blue. In addition, copper sulfate has a very bad taste, which would explain the tongue movements of the new-born animals.

The milk replacers used on the two farms had been purchased from different dealers, but in both cases the powder was from the same manufacturer and the information leaflet did not mention the presence of copper. The calves were fed about 8 litres of milk per day over a period of three weeks. One litre of milk contained about 50 g of milk powder. Since the apparent methaemoglobinemia was suspected to be due to nitrate poisoning, one calf was treated with methylene blue. In addition, antibiotics, corticosteroids, Ringer solution and glucose were administered as supportive treatment. These treatments did not improve the calf's

health. The other calf was treated with EDTA, also without results. When both calves died, post-mortem histological examinations of the liver were performed. Blood from the oldest calf, both livers and milk replacer samples from both farms were sent for laboratory examination.

Finally, four calves died on one farm. Six months after the use of the milk replacer was terminated, other calves that had been exposed to copper still showed distinct growth disturbances. Although the animals had a good appetite and no diarrhea and were free of any other clinical symptoms of disease, some were still emaciated, had not gained weight and had a rough hair coat.

Haematology, biochemistry and pathology

Haematological examination of the blood originating from the oldest calf showed normal levels of leukocytes. However, the total bilirubin concentration was 343 μmol/l, whereas normal levels vary between 17 and 51 μmol/l. The activities of γ-glutamyl aminotransferase (γ-GT) and aspartate aminotransferase (AST) in the serum were evaluated and found to be very high, viz. 130 mU/ml γ-GT and 846 mU/ml AST. Normal levels of γ-GT range between 1 and 18 mU/ml and between 127 and 427 mU/ml for AST. These results clearly indicate liver cell degeneration.

Autopsy revealed that the carcass and all organs had icteric aspects and that the liver was pale and degenerated. Histological examination of the liver showed vacuolar degeneration, hepatocellular necrosis and severe bile duct proliferation.

Copper quantification: analytical procedures and results

Copper was quantified in the blood of the four-month-old calf, in the liver samples of both calves and in both commercial milk replacer powders purchased from the two different dealers (dealer 1 and dealer 2). The copper content in the serum was determined photometrically using a commercial test kit (Copper Merckotest, Merck-Belgolabo n.v., Overijse). Copper concentrations in the liver and in the milk powder were determined by flame atomic absorption spectrophotometry (AAS). About 4 g of fresh, homogenised liver were ashed overnight in a muffle furnace at 450 °C. After cooling, the samples were moistened with 1 ml of 20% ammonium nitrate and carefully ashed again in a muffle furnace at 450 °C for about two hours until the remaining ash was white. Thereafter, 5 ml of 6 N nitric acid and 5 ml of 3 N nitric acid were ad-

Table 1. Concentration of copper in the blood of one calf, in the liver samples of both calves and in the commercial milk replacer powders.

Sample	Concentration copper
3-month-old calf	
liver	500 mg/kg on a fresh-weight basis
4-month-old calf	
liver	297 mg/kg on a fresh-weight basis
blood	24 μmol/l
Milk replacer	
dealer 1	159 mg/kg powder (lab 2)
(fed to the 3-month-old calf)	SD=31.2 mg/kg (n=5)
dealer 2	
(fed to the 4-month-old calf)	120 mg/kg powder (lab 1)
	124 mg/kg powder (lab 2)

Table 2. Maximum limits of copper in feed for calves and pigs (Anonymous, 1999).

Feed for	Cu (mg/kg)
Calves	
milk replacer	30
Fattening pigs	
up to 16 weeks	175
from 17 weeks	35

ded and the ashes were intermediately dried on a hot plate for 10 min. The samples were then filtered, dissolved in bidest water and diluted to 50.0 ml. Copper concentrations were determined by flame AAS at 324.7 nm.

The milk powders were sent to two different independent laboratories (lab 1 and lab 2).

Table 1 shows the results of the copper quantification. In the blood, copper concentrations of 19 μmol/l or above are reported to be sufficient to initiate a haemolytic crisis.

Chronic copper intoxication is observed in calves when copper concentrations are higher than 200 mg/kg fresh liver (Weiss *et al.*, 1967).The maximum allowable concentration of copper in animal feed for calves is presented in Table 2, having been set at 30 mg/kg milk replacer powder (Anonymous, 1999).

DISCUSSION

Occurrence of copper poisoning in animals

Chronic copper poisoning is the most common form of intoxication and results from the successive ingestion of non-toxic doses of copper, which have a cumulative effect. It can be caused by excessive copper sulfate supplementation for prolonged periods of time as a prophylaxis for copper deficiency (copper oxide is less toxic, since it is resorbed to a lesser extent) and by feeding calves milk substitutes that are too rich in copper. The term 'chronic' is something of a misnomer. It refers to the time it takes to produce clinical signs, but once these appear, the condition follows a very acute course, with death occurring within one to four days.

Interestingly, only a few case reports have been published about the occurrence of copper toxicity in the bovine species, in contrast to the many reports on sheep.

Tokarnia *et al.* (2000) report an outbreak of copper poisoning caused by the consumption of litter from poultry that had been fed a ration treated with copper sulfate to avoid aspergillosis. Iatrogenic copper toxicosis induced by administering copper oxide boluses to neonatal calves has also been reported (Hamar *et al.*, 1997, Steffen *et al.*, 1997). The addition of excessive copper to a commercially prepared dairy ration caused chronic copper toxicity in a dairy herd (Blakley *et al.*, 1982, Perrin *et al.*, 1990, Thoonen *et al.*, 1963). Copper toxicosis was also experimentally in-

duced by Jenkins and Hidiroglou (1989) and by Gummow (1996). The trial of Gummow was designed to determine the effects in cattle of continuous exposure to low doses of copper administered *per os*. From the results it was concluded that subclinical damage to the liver, and perhaps also copper toxicity, can occur when cattle are continually exposed to oral doses of 12 mg of Cu/kg BW/day. He also concluded that cattle can tolerate oral doses of 0.6 mg Cu/kg BW/day for an indefinite period, provided that there are no other sources of copper, or provided no other mineral interactions occur, such as with molybdenum, which forms unabsorbable complexes with copper. Cattle appear to be more resistant than sheep to copper toxicity and it may require a considerable length of time for typical clinical and pathological features to appear. This is probably caused by the limited absorption (only 10%) of the ingested copper in ruminants (Suttle, 1974). However, monogastric animals and calves absorb 45-55% of the dietary copper, the high absorption in calves possibly being due to the very low iron content in their feed or to the formation of insoluble copper sulfide from the reduction of sulfate to sulfide in the rumen. In addition, veal calves, which are fed milk replacer throughout their entire life, resemble monogastric animals with respect to digestion (Groot and Gruys, 1993). It was also found that there are differences between a number of cattle breeds concerning copper toxicity. Claus and Dierenfeld (1999) report an unusually high requirement for copper in the yak.

Treatment

Auza *et al*. (1999) suggest that the best treatment for ruminants with copper poisoning is oral administration of ammonium molybdate combined with sodium sulfate, which appears to remove copper from the lysosomes and cytosol of copper-loaded hepatic cells.

Blood copper concentration

In this reported case, a level of 24 µmol/l copper was found, whereas levels below 19 µmol/l are reported to occasion no risk whatsoever for hemolytic crisis. However, nowadays it is accepted that blood levels of copper are a poor indicator of the copper loading of the liver. Perrin *et al*. (1990) found cows that were in the 'low normal' to 'deficiency' range of blood copper concentration but upon liver biopsy and post-mortem examination they were nonetheless found to be at or near a toxic state. In addition, infections of various origins can be accompanied with copper levels of as

much as 30 µmol/l, though still without the presence of haemolysis. One example of the latter can be a *Rhodococcus equi* infection in a horse. Gummow (1999) suggests that liver copper concentrations in cattle are probably the most sensitive indicators of exposure to high oral concentrations, and analysis of the copper content in hepatic biopsy may therefore still be the best diagnostic tool currently available. The post-mortem lesions of the present case, such as hepatocellular necrosis and vacuolar degeneration of the hepatocytes, are not necessarily indicative of copper poisoning, however, since other hepatotoxic injuries such as aflatoxicosis must also be considered and excluded by laboratory analysis.

Perrin *et al*. (1996) interpreted various degrees of bile duct proliferation and hepatic fibrosis to be associated with copper toxicity. However, they concluded that it was not possible to correlate the analytical findings with the histological findings. Nevertheless, tissues which are discoloured by icterus and methaemoglobinemia are considered to be characteristic for chronic copper poisoning.

Liver copper concentration

Hapke (1988) establishes the normal copper content in the liver as being between 10 and 50 mg/kg on a fresh weight basis. Hadrich (1996) suggests that normal copper concentrations in calf's liver range from 50 to 150 mg/kg fresh weight. Chronic copper intoxication in calves could only be seen at levels higher than 200 mg/kg fresh liver (Weiss *et al*., 1967). Other investigators (Hadrich, 1996) reported levels of between 500 and 800 mg copper/kg liver on a dry weight basis in cases of poisoning. Taking into account an average water content of 73% in liver, this corresponds to concentrations of between 135 and 216 mg/kg on a fresh weight basis. In this reported case, copper concentrations of 297 and 500 mg/kg fresh liver were found, clearly indicating a chronic copper intoxication.

Milk replacer copper concentration

Regulatory limits for copper in animal feed are set at 30 mg/kg milk replacer powder (Table 2). However, no limits have been set for copper in food of animal origin for human consumption. Because there is no direct correlation between the colour or superficial condition of a calf's liver and the amount of copper stored, possible consumer health hazards must be taken into consideration. Recently, the German health aut-

horities established a limit of 300 mg copper/kg fresh liver (Hadrich, 1996).

Under normal conditions, milk replacers have a mean copper content of 5 to 8 mg/kg (Anonymous, 1995, Jilg et al., 1997). It is clear that the excessive amounts of 120 and 124 mg/kg milk powder of dealer 2 and 159 mg/kg powder of dealer 1 can originate from a formulation error. It is possible that the manufacturer erroneously mixed a porcine feed premix, which was intended to feed fattening pigs up to 16 weeks, with the milk replacer for calves. Table 2 shows that a maximum of 175 mg/kg copper can be added as a growth promoter to feed pigs up to 16 weeks of age. Table 1 also shows that the copper concentrations found in both livers correlate well with the amounts of copper found in both milk replacers. Weiss et al. (1967) and Jilg et al. (1997) studied the influence of copper supplementation in milk replacers on the copper concentration of calf livers. Weiss et al. found that calves receiving milk substitutes containing 100-300 mg/kg of copper died within 16-75 days. Jilg et al. reported that the feeding of milk replacer based on a milk powder containing 15 mg copper/kg led to mean copper levels of up to 305 mg copper per kg fresh weight of liver tissue (n=5). A group fed milk replacer containing only 5 mg copper/kg had a mean liver copper concentration of 138 mg/kg fresh weight (n=5). Therefore, in the interests of consumer and animal protection, they proposed setting a legal limit for copper of 6 mg/kg milk replacer instead of the current limit of 30 mg/kg. These recommendations are in accordance with recent European discussions on lowering the maximum limit from 30 mg/kg to 10 mg/kg milk replacer.

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