Copper toxicity in confinement-housed ram lambs

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Abstract — Fourteen Suffolk rams (6 mo) were diagnosed with chronic copper poisoning. Preliminary results indicated that a combination of serum aspartate aminotransferase, gamma glutamyltransferase, and copper could be used as a test so that high risk lambs could be treated more aggressively.

Résumé — Toxicité au cuivre chez des agneaux mâles gardés en stabulation libre. Un empoisonnement chronique au cuivre a été diagnostiqué chez 14 béliers suffolk âgés de 6 mois. Des résultats préliminaires indiquent que la combinaison des activités sériques de l'aspartate aminotransférase et de la gamma glutamyl transférase ainsi que de la concentration en cuivre du sérum permettrait d'identifier les agneaux à risques élevés qui pourraient par la suite être traités de façon plus énergique.

(Traduit par docteur André Blouin)

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Chronic copper poisoning results from the accumulation of copper in hepatic tissues over a period of a few weeks to more than a year (1). During the accumulation phase, liver damage is occurring as indicated by increased levels of serum lactic dehydrogenase and aspartate aminotransferase (AST) (2). Stress is a major predisposing factor in the induction of a hemolytic crisis (1,3,4). Any stressful condition, such as, shearing, transportation, or exertion, can act as a triggering mechanism for the sudden release of hepatic copper stores (5). Increased plasma copper levels (37.8 to 314.8 µmol/L) result in an increased copper concentration in red blood cells and hemolysis (6). Hemoglobinuric nephrosis and death usually occur within 4 d (5).

Sheep are more vulnerable to the effects of copper toxicity than are other species of food-animal because of their less efficient excretory mechanism (3). This vulnerability is exacerbated in sheep housed indoors and on pelleted feeds, because of their reduced copper requirement (5). Suffolks are particularly at risk (1,7). The current recommended dietary copper requirement for sheep is 5 mg/kg of diet (8,9). Subclinical toxicity has been recorded in lambs on diets containing 12 mg/kg dry matter, a level often exceeded in commercial feeds (7,10). If molybdenum, which decreases absorption of copper is low, copper levels as low as 8–11 mg/kg of dry matter can produce toxicity (5,8,10).

Diagnosis of chronic copper poisoning is usually made following a hemolytic crisis in 1 or more of the exposed animals. During the accumulation phase, animals generally appear normal, making earlier diagnosis and subsequent accurate prognosis problematic. This paper describes the development of a biochemical profile for use in early diagnosis and individual prognosis in high risk lambs, which resulted from the following case.

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Table 1. Complete blood cell count from a serum sample taken from lamb #11 <18 h before death due to chronic copper poisoning

Parameter	Value	Normal Range
Red blood cells	8.22×10^{12}/L	8 to 15×10^{12}/L
Packed cell volume	0.281 L/L	0.24 to 0.50 L/L
Mean corpuscular volume	35 fL	23 to 48 fL
Mean corpuscular hemoglobin	12.8 pg	9 to 12 pg
Mean corpuscular hemoglobin concentration	377 g/L	310 to 380 g/L
White blood cells	16.6×10^9/L	4 to 12×10^9/L
— segmented neutrophils	7.5×10^9/L	0.7 to 6.0×10^9/L

Ram lamb #11 was presented for examination following a hemolytic crisis. This lamb was 1 of a group of 14 Suffolk ram lambs, 6 mo of age, weighing 60–74 kg, in a study on thyroid involvement with the onset of puberty. They had been housed indoors, in 4 pens (3 × 2 m), for 4 mo at the time of presentation. Physical examination of lamb #11 showed mild dehydration, severe icterus, and severe lethargy, with a temperature of 39.1°C, heart rate of 156 beats/min, and respiration rate of 68 breaths/min. Although urinalysis was not possible, hemoglobinuria was suspected, as the lamb was reported to be "urinating blood." The clinical signs were consistent with those seen in chronic copper poisoning, which include accelerated breathing, weakness, excessive thirst, hemoglobinuria, an arched back due to renal pain, icterus and recumbency (1,4,5,8). A blood sample was taken for a complete blood cell count and serum biochemistry analysis. Supportive care was provided, but this lamb died overnight. The diagnosis of chronic copper toxicity was confirmed on blood sample analysis and postmortem examination.

The complete blood cell count, submitted <18 h before death, showed few abnormalities in the red cell parameters (Table 1). The serum albumin concentration (39 g/L) was within normal limits (normal, 27 to 39 g/L), indicating that dehydration was unlikely to have markedly affected these values. Indications of a regenerative anemia included anisocytosis 1+, polychromasia 2% to 4%, poikilocytosis 3+, presence of

Table 2. Serum biochemical profiles from lamb #11, taken 17 wk to <18 h before death due to chronic copper poisoning

	Age in weeks									
	8	10	12	14	16	18	20	22	24	Final[a]
Cu[b]	22.5	16.5	17.8	14.3	22.8	18.3	20.3	20.5	34.8	38.7
gGT[c]	123	99	105	99	145	132	172	280	511	351
ALP[d]	386	322	223	183	266	199	266	185	302	N/A
AST[e]	77	77	120	105	226	351	697	1310	2155	4730
dBili[f]	N/A	2.0	2.0	2.0	2.0	2.0	3.0	N/A	14.0	232.0
iBili[g]	N/A	0.0	0.0	0.0	0.0	0.0	0.0	N/A	8.0	226.0
BUN[h]	6.9	9.0	9.5	11.7	8.9	12.9	10.2	9.4	7.3	14.5
Creat[i]	70	60	60	60	70	60	70	80	90	220

Underlined values indicate above normal levels
[a]The final blood sample was drawn on physical exam, just before 25 wk of age
[b]Cu — serum copper levels (adequate: 11 to 31.5 µmol/L; high: 15.74 to 78.7 µmol/L; toxic: 51.9 to 314.8 µmol/L)
[c]gGT — serum gamma glutamyltransferase (normal, 16 to 40 U/L)
[d]ALP — alkaline phosphatase (normal, 0 to150 U/L)
[e]AST — serum aspartate aminotransferase (normal, 0 to 125 U/L)
[f]dBili — direct bilirubin (normal, 0 to 4.6 µmol/L)
[g]iBili — indirect bilirubin (normal, 0 to 3.0 µmol/L)
[h]BUN — urea nitrogen (normal, 3.8 to 7.1 mmol/L)
[i]Creat — creatinine (normal, 100 to 170 µmol/L)

basophilic stippling, and 24 metarubricytes/100 white blood cells (WBC). The WBC count was mildly increased due to an increase in segmented neutrophils. Serum biochemistry indicated hepatic and renal damage (Table 2, Final).

At postmortem, the liver was yellow-orange and slightly fatty, the kidneys were dark bluish-gray, and the tissues were icteric. Histological examination showed biliary fibrosis, bile duct hyperplasia, apoptosis, and centrilobular degeneration, compatible with chronic copper toxicity. Kidney lesions were consistent with hemoglobinuric nephrosis. Copper levels (wet basis) in liver and kidney were 3368.4 µmol/L, toxic range 3935 to 15 740 µmol/L (9), and 239.2 µmol/L, toxic range 283.3 to 4092.4 µmol/L (9), respectively. Liver and kidney copper concentrations as low as 2361 µmol/L and 236 µmol/L, respectively, have been found in sheep with chronic copper poisoning (6).

It was assumed that all of the lambs on the experiment were affected and 3 actions were initiated. First, a search for the copper source was instituted. Simultaneously, each lamb was drenched daily with 100 mg ammonium molybdate and 1 g anhydrous sodium sulphate to reduce copper absorption until the source of copper could be eliminated. Finally, because of the ongoing experiment, it was necessary to determine a reliable prognostic indicator for the remaining at-risk lambs. Blood samples for biochemical analysis were taken from all at-risk lambs prior to the 1st treatment to act as a baseline and for prognostic purposes.

Feed analysis showed both the hay (314 µmol/L) and pellets (330 µmol/L) to be high in copper. Water was not found to be a source of copper (0.9 µmol/L) and copper fixtures were not present in the pens. All of the hay samples analyzed were found to have a high copper content (lowest 283 µmol/L). The copper level in the pellets could not be substantially reduced due to high levels in the grain. Consequently, the pellets were supplemented at a rate calculated to provide 100 mg ammonium molybdate and 1 g anhydrous sodium sulphate per lamb per 24 h, until the experiment ended 3 mo later.

Six weeks after initiation of diet supplementation, blood samples from all of the at-risk lambs were analyzed for AST and gamma glutamyltransferase (gGT). Serum AST levels ranged from 136 to 668 U/L and gGT levels ranged from 146 to 332 U/L, indicating continuing liver damage. Serum AST values were decreasing; however, gGT values remained similar to or above the initial values. It is uncertain from this data whether the ongoing liver damage was due to the copper already absorbed or whether there was continuing damage due to a failure of the supplementation to prevent further copper absorption.

In order to develop a biochemical panel useful for early diagnosis and improved prognosis, serum samples from lamb #11 and the at-risk lambs were analyzed. Serum samples from lamb #11 had been collected every 2 wk for the previous 4 mo as part of an experiment on thyroid function, and these were subjected to standard biochemical tests (Table 2). Serum copper levels fluctuated in the adequate to high range from 8 to 24 wk of age. Serum gGT and alkaline phosphatase (ALP) levels were already elevated at 8 wk of age, 17 wk prior to clinical signs. Serum AST reached above normal values at 16 wk of age, 9 wk before clinical signs. Indirect and direct bilirubin reached above normal levels at 24 wk of age. Urea nitrogen was elevated from 10 wk of age, but creatinine was normal to low until just prior to death. This represents a limited data base (lamb #11 only) and the above results should be considered preliminary.

Serum AST and gGT levels were elevated in all 13 of the at-risk lambs. Serum AST levels ranged from 242 to 1415 U/L (normal high, 125 U/L) and gGT ranged from 97 to 322 U/L (normal high, 40 U/L). Serum ALP levels ranged from 129 to 430 U/L (normal high, 150 U/L). Urea levels were consistently but mildly high, ranging from 7.6 to 10.6 mmol/L (normal high, 7.1 mmol/L). This was probably due to the high protein diet, rather than renal compromise, as creatinine levels were mildly

decreased, ranging from 70 to 100 µmol/L (normal low, 100 µmol/L). Serum copper levels ranged from 13.7 to 31.5 µmol/L, which are adequate to high (9).

During this survey sampling, serum from lamb #6 was observed to be icteric. Both direct bilirubin 8.0 µmol/L (normal, 0 to 4.6 µmol/L) and indirect bilirubin 6.0 µmol/L (normal, 0 to 3.0 µmol/L) levels were elevated. This lamb was placed on IV fluids for 4 d (1 L 0.9% NaCl, q8h) in an attempt to protect renal function. Although this treatment was not expected to have observable effects, the level of AST dropped gradually over a 5-day period (1655 U/L to 1360 U/L). The level of serum gGT dropped over the same period (392 U/L to 311 U/L). Serum ALP (202 to 219 U/L) and BUN (6.8 to 7.6 mmol/L) levels fluctuated but did not drop, and copper levels remained relatively constant between 30.2 and 33.4 µmol/L. Lamb #6 recovered uneventfully but died 5.5 wk later in a presumptive hemolytic crisis. A 2nd lamb (#4) was observed to have mildly icteric serum 1 wk later. The direct bilirubin level, 1.0 µmol/L (normal, 0 to 4.6 µmol/L), was normal but the indirect bilirubin level, 4.0 µmol/L (normal, 0 to 3.0 µmol/L), was elevated. Fluid administration was not felt to be necessary and lamb #4 did not have an acute episode in the next 3 mo. These 2 lambs were considered to be at highest risk for a subsequent hemolytic crisis.

The elevated serum values in lamb #11 provided a starting point for developing a prognostic biochemical panel. These values included AST, gGT, ALP, serum copper, BUN, and bilirubin. Bilirubin was not elevated until 1 wk before the hemolytic crisis; hence we felt it was of limited early diagnostic or prognostic value. Since creatinine was not elevated, the high BUN was probably due to high protein levels in the feed rather than renal problems and hence had no prognostic value. The 2 highest serum copper levels were found in lambs #6 and #4, determined to be at highest risk, and might be useful if combined with other information. Serum ALP, although elevated early in lamb #11, was inconsistent in that 1 (8%) of the at-risk lambs did not show elevated serum levels. Half of the lambs had ALP levels higher than lamb #6 and 1 had a value higher than lamb #4. Given this, we felt that ALP levels were of little value for differentiating affected from severely affected lambs. For these reasons and because ALP levels did not drop during fluid therapy, we considered that ALP level was unreliable for prognostic purposes. Serum AST and gGT levels were consistently able to provide prognostic information. Both enzymes rose to above normal levels at least 9 wk before clinical signs in lamb #11. This is in agreement with the results of MacPherson and Hemmingway (2), which indicated that serum glutamic-oxaloacetic transaminase (AST) rose at least 3 wk before death. The 2 highest values for AST were from the lambs showing icteric serum on survey sampling, lamb #4 (1415 U/L) and lamb #6 (1360 U/L). The highest values for serum gGT were also from lambs #6 (311 U/L) and #4 (271 U/L).

These results suggest that a combination of AST, gGT, and possibly serum copper values might be used as a low-cost biochemical panel to select lambs with an increased risk of death due to acute toxicity. This panel might also be useful for surveying flocks in areas in which high copper levels are known to be a problem.

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