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Clinical Manifestations and Liver Pathology in Lambs Fed on Ration Deficient in Cobalt

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Abstract: The present study aimed to investigate the effects of experimentally induced cobalt-deficiency in sheep on clinical manifestations and pathological changes in liver. Thirteen clinically healthy male lambs, 4-6 months old, were randomly divided into two groups, each was placed in a separate pen. The first group consisted of four animals fed cobalt-sufficient diet and was kept as control group. The second group was consisted of nine lambs fed cobalt-deficient diet. The experiment lasted for 20 weeks. Cobalt concentration in the deficient-diet was 65 μg kg^{-1} dry matter. The clinical signs appeared gradually after 9-20 weeks on cobalt-deficient diet group and were accurately identified through careful clinical examination of the experimental animals. The cobalt-deficient lambs showed reduced feed intake, emaciation, depression, lacrimation, alopecia and pale mucus membranes. The animals appeared scruffy and two of them died. The pathological studies showed macroscopic and microscopic alterations in livers of cobalt-deficient lambs in the form of enlargement in size of livers with swollen and tense capsule and rounded borders. Its cut surface was yellowish with greasy bulged and friable fatty liver. Microscopically, there were diffuse fatty changes, karyopyknosis, apoptosis and necrosis of the hepatocytes, focal necrosis, kupffer cell hyperplasia, portal cirrhosis with infiltration of portal area with lymphocytes. Also, congestion of portal blood vessel and hydropic degeneration were detected. It is concluded that feeding of lambs on diet containing less than 70 μg kg^{-1} dry matter of cobalt, introduced severe ill thrift and fatty liver with high mortality rate.

Key words: Cobalt · deficiency · sheep · clinical signs · pathology · liver

INTRODUCTION

The trace element, cobalt (Co) is an essential dietary element for ruminants. It is essential for the synthesis of vitamin B_{12} by rumen microorganisms [1]. Vitamin B_{12} (cyanocobalamin) has two distinct but interdependent co-enzymatic functions. It assists the enzymes methylmalonyl-coenzyme A mutase in the formation of glucose and methionine synthase needed for methane, acetate and methionine synthesis [2]. Since cyanocobalamin is not present in feedstuffs of plant origin, the vitamin B_{12} supply of ruminants has to be ensured by a sufficient Co-supply [3]. In sheep, if the Co-content of forage falls below a level of about 70 μg kg^{-1}, the ability of the rumen microorganisms to produce sufficient vitamin B_{12} is reduced and the animal develops the signs of cobalt deficiency [4].

The Co-deficient animals appear as if they had been starved, except that the visible mucous membranes are blanched and the skin is pale, dry and fragile and eventually death occurs [3, 5-7]. Sheep tend to be extremely susceptible to Co-deficiency than cattle and goats and develop a normocytic and normochromic anaemia, anorexia, reduced weight gains, diarrhea and photosensitivity [8, 9]. Lacrimation, scaly ears and discoloration at the base of the wool [10], cardiovascular lesions [11] and cerebrocortical necrosis [12] have also been associated with low dietary levels of Co.

Cobalt deficiency had also long been incriminated as the cause of ovine white liver disease (OWLD) [13, 14]. Experimentally, the clinical symptoms of OWLD were varying degrees of reduced weight gain or loss of weight appeared after 6-12 weeks on Co-deficient pasture at an age of 10-15 weeks. Additional symptoms were seen

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2-4 weeks later, including in appetence, listlessness and often serous ocular discharge, crusty ears and acute photosensitization. From these lambs, 18% were died or euthanized because of OWLD [8].

Hepatic lesions referred to (OWLD) [15, 16] or "chronic hepatitis" [17], had been reported in some spontaneous outbreaks of Co deficiency in sheep, but the aetiology of these changes was controversial. It was not known whether they result from Co deficiency alone or was due to the combined effects of Co deficiency and unknown cofactors [18, 19]. Experimentally, the affected lambs with OWLD, at necropsy, had pale swollen, friable fatty livers and showed marked accumulation of lipid droplets and lipofuscin in hepatocytes as well as hepatocytes necrosis and billiary hyperplasia [20]. Cobalt analysis revealed that normal livers has six times more cobalt and a 3-fold less fat content than those measured in the fatty livers [21].

The present work aimed to study the effect of experimentally induced Co deficiency on the clinical manifestations and liver pathology in sheep.

MATERIALS AND METHODS

This experiment was started at September 2005 and terminated at the end of the March 2006 in the farm of the Faculty of Veterinary Medicine (Sadat City), Menoufia University, Egypt.

A total number of thirteen male lambs of 4-6 months old were used in the present experiment. Lambs were randomly divided into two groups; each was placed in a separate pen. The first group consisted of four animals fed Co-sufficient diet and was kept as control. The second group was consisted of nine lambs fed Co-deficient diet. Animals were protected against parasitic infections by drenching of Albendazole (Pharma-Sweed) at dose 2ml/20kg BW and injection of Ivermectin at dose 1ml/50kg BW S/C at zero time and every 3 months.

Ration: Lambs were fed a cobalt whole barley diet for 20 weeks. The Co-sufficient and cobalt-deficient whole barley rations were prepared as described by [22] and the composition of the ration is shown in Table 1.

Representative sample was taken from the ration for determination of their chemical composition according to the methods described by [23]. The cobalt contents of the deficient and sufficient rations werer measured by graphite furnace atomic absorption spectrophotometry according to [24].

Table 1: Chemical composition of the ration of the experimental

Ingredien	Amount (per kg/diet)
Whole barley	970.00 gm
Urea	14.00 gm
Vitamin A and D_3 (AD_3)	10.00 mg
Vitamin E	40.00 mg
Calcium carbonate	15.00 gm
Sodium sulfate anhydrous	400.00 mg
Zinc sulfate heptahydrate	150.00 mg
Manganous sulfate tetrahydrate	50.00 mg
Potassium iodate anhydrous	1.00 mg
Sodium selenite anhydrous	175.00 µg
Cobaltus chloride hexahydrate *	4.23 mg

*Added to cobalt sufficient ration only

Clinical examination: All the experimental animals were subjected for complete clinical examination including measuring of the rectal temperature, pulse and respiratory rates with palpation of lymph nodes. Clinical examinations were conducted according to [25].

Pathological studies: Liver specimens were obtained from freshly dead and slaughtered experimental (control and deficient) animals at the end of experiment for detection of the pathological alterations in liver due to cobalt deficiency. Tissue specimens were obtained from liver then fixed in 10% formol saline, histologically processed and stained with hematoxylin and eosin for histopathological examination [26].

RESULTS

- Cobalt concentration in the Co-deficient ration was 65 µg kg^{-1} dry matter.
- During the period of experiment, clinical signs appeared after 9-20 weeks in the Co-deficient group and include reduced feed intake (anorexia), reduced weight gain (emaciation), lacrimation (serous ocular discharge) that stains the face wool, easily detached wool, alopecia, diarrhea in some lambs, depression and pale mucous membranes. The animals appeared scruffy, as opposed to Co-supplemented lambs (photos 1-3).
- wo lambs of the Co-deficient group died.

Pathological studies:

- The macroscopical examination of the livers of Co-deficient lambs showed enlargement in size and

Photo 1: Cobalt-deficient lamb showed sever alopecia and sever emaciation

Photo 2: Cobalt-deficient lamb showed emaciation, Diarrhea, depression and loss of condition

Photo 3: Cobalt-deficient lamb showed profuse serous ocular discharge.

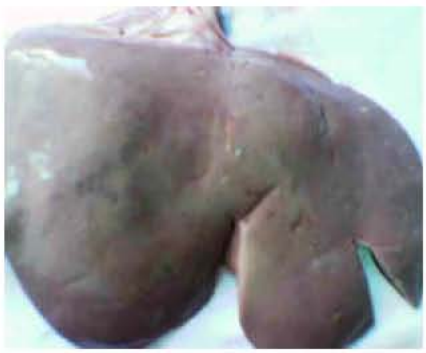

Fig. 1: liver of cobalt deficient lambs enlarged and appeared fatty (yellowish & friable), swollen and uniformly pale

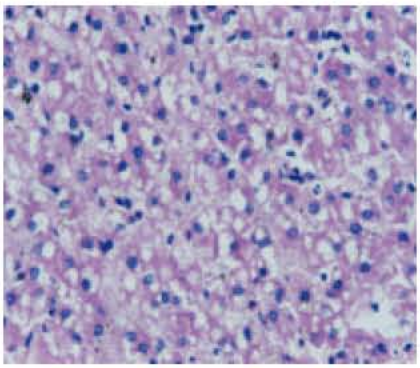

Fig. 2: Liver of cobalt deficient lamb showing hydropic degeneration, fatty changes and Kupffercells hyperplasia in (X40 H&E)

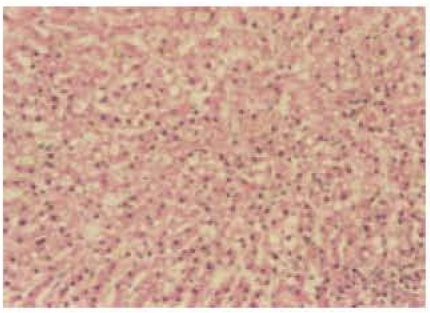

Fig. 3: Liver of cobalt deficient lamb showing diffuse fatty change and karyopyknosis (X20 H&E)

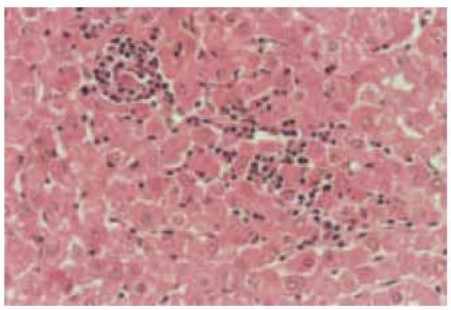

Fig. 4: Liver showing focal necrosis and Kupffer cell hyperplasia (20, "X40" & 21, "X20") (H&E)

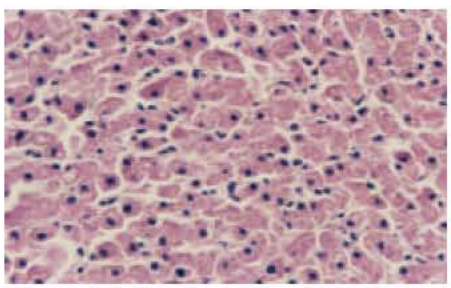

Fig. 5: Liver showing apoptosis and necrosis of the hepatocytes (X40 H&E)

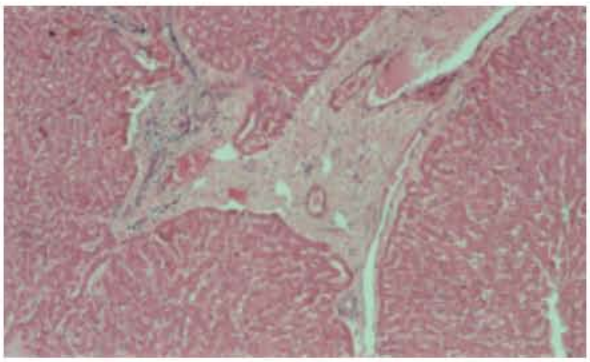

Fig. 6: Liver showing portal cirrhosis with infiltration of portal area with lymphocytes, congestion of portal blood vessel (X40 H&E)

swollen with tense capsule and rounded borders. Its cut surface is yellowish greasy bulged and friable fatty liver and uniformly pale throughout the entire parenchyma (Fig. 1).

- The histopathological examination of the liver of Co-deficient lambs revealed diffuse fatty changes, karyopyknosis, apoptosis and necrosis of the hepatocytes, focal necrosis, kupffer cell hyperplasia, portal cirrhosis with infiltration of portal area with lymphocytes, congestion of portal blood vessel and hydropic degeneration (Fig. 2-6).

DISCUSSION

Diseases caused by cobalt deficiency in ruminants are of major economic importance in several countries. The present study was planned to investigate the effects of experimentally induced Co-deficiency in lambs on clinical manifestations and histopathological changes in liver.

In the present study, feeding of lambs on the Co-deficient diet induced sever ill thrift and loss of condition with mortality rate of 22.2%. The clinical signs appeared gradually after 9-20 weeks on cobalt-deficient ration and were accurately identified through careful clinical examination of the experimental animals. Similar signs were recorded by [4, 5, 7, 8, 20, 27]. Loss of appetite may be due to increase urinary excretion of methylmalonic acid and increase blood propionate level caused by cobalt and vitamin B_{12} deficiency. The impairment in propionate metabolism inversely correlated to voluntary feed intake (rapidly depresses appetite) which result in decrease of body weight [28, 29]. During the period of experiment a gradual decrease in body weight was observed in Co-deficient group in comparison with the control one. This result may be due to loss of appetite as well as the effect of Co deficiency on protein and carbohydrate metabolism [30]. Impairment of protein synthesis may be the principal reason for the growth depression frequently observed in these animals. It may be also due to lower digestibility coefficients for dry matter. In addition, it may be attributed to a decrease capacity to absorb nutrients as a result of a reduction in numbers of microorganisms in rumen [31] as well as causing a shortening of the intestinal villi [32].

Concerning the pathological studies, the macroscopical and histopathological examination of the livers of Co-deficient lambs revealed hepatic fatty changes. The pathological alterations in liver of Co-deficient lambs in the present study were similar with those recorded by [33]. Ulvund [13] concluded that OWLD is a manifestation of B_{12} deficiency worsened by factors triggering early hepatic fatty change resulting in a more severe liver damage with loss of intracellular homeostasis rendering the hepatocytes vulnerable to other elements, like copper. The same author reported that the liver changes could be earliest be found after one month on ovine white liver disease pasture and include extensive fatty change with large spherical vacuoles in hepatocytes, varying size of hepatocytes and nuclei and formation of councilman bodies. Later come ceroid deposits, billiary hyperplasia and mesenchymal proliferation.

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