

Effects of Dietary Cobalt Deficiency on Performance, Blood and Rumen Metabolites and Liver Pathology in Sheep

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Abstract: The present study aimed to determine changes in serum vitamin B₁₂ (VitB₁₂) and folate concentrations, live body weight development, haemogram and serum and rumen biochemistry as well as liver pathology in sheep fed on cobalt (Co) deficient diet and also to document the responses to Co supplementation. 14 male Egyptian Baladi lambs were assigned randomly into two groups; the first group (n=9) was fed a Co-deficient whole barely-based diet (55 µg Co/ Kg DM) and the second group (control) of lambs (n=5) received the same diet after it had been supplemented with Co to a total of 1000 µg /Kg DM. After 20 weeks of experimental period, the Co-depleted lambs were transformed to the Co-sufficient diet and administered orally with 2mg Co/head twice weekly for 4 weeks. The results showed that serum VitB₁₂ and folate concentrations decreased significantly at the 6th week and fell below the lower limit of normality after 10 weeks on deficient diet, preceding the onset of loss of condition and clinical signs of the disease. The Co-depleted lambs exhibited significantly lower body weight development and clinically showed inappetence, lacrimation, alopecia and pale mucous membranes. At the 20th week experimental period, Co-deficient group exhibited significant decline in haematological parameters compared to control ones. Serum concentrations of total proteins, albumin, globulin and cholesterol were markedly decreased, while the activity of AST and ALT and blood urea nitrogen levels were increased in the Co-deficient group. Rumen fluid analysis of the Co-deficient lambs revealed that propionic acid was markedly decreased, while acetic acid elevated significantly. After 4 weeks of Co-supplementation, VitB₁₂ status and the metabolic variables showed significant improvement and the body weight development of deficient - lambs responded positively. From Co-deficient group, two lambs (22.22%) died on days 126 and 138 which developed pathological changes in liver. It could be concluded that feeding of young sheep on Co-deficient diet, clearly diminished VitB₁₂ status in serum and developed consistent signs of clinical Co-deficiency, metabolic disturbances and hepatic fatty changes.

Key words: Sheep • Cobalt deficiency • Vitamin B₁₂ • folate • Haematology • biochemistry • Rumen volatile fatty acids • Ovine white liver disease

INTRODUCTION

The trace element; cobalt is a dietary essential element for ruminants, allowing synthesis of VitB₁₂ by rumen microorganisms [1]. In higher animals, VitB₁₂ is a cofactor for two enzymes; methyl malonyl-Co A mutase and methionine synthase. The former catalyzes the inter conversion of methyl malonyl-Co A to succinyl-Co A [2], an important step in gluconeogenesis, whereas the latter acts to remethylate homocysteine in the terminal step of

methionine synthesis [3]. In ruminants, Co-induced VitB₁₂ deficiency limits the activity of these two enzymes and therefore it disturbs normal energy and protein metabolism [1,4].

Ruminants normally do not have any dietary source of VitB₁₂, therefore their supply of this vitamin has to be ensured by continuous adequate supply of dietary Co [5]. Therefore, VitB₁₂ deficiency can be induced by long-term consumption of Co-inadequate diets.

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Sheep tend to be extremely susceptible to Co deficiency because their Co requirement is about twice that of cattle [6]. Levels of dietary Co less than 0.07 mg/kg DM are likely to lead to VitB₁₂ deficiency in sheep[7], which is clinically manifested as anaemia, inappetence, weight loss, poor production, lacrimation, photosensitivity, alopecia and immune deficiency [8, 9].

A deficiency of VitB₁₂ is accompanied in many species by symptoms of folic acid deficiency, but the mechanism by which VitB₁₂ ensures normal folate metabolism remains uncertain. Vitamin B₁₂ deficiency has been shown to cause depletion of intracellular folate concentrations in sheep liver [10].

Cobalt deficiency has also long been incriminated as the cause of fatty hepatic degeneration that has been termed ovine white liver disease (OWLD) [11,12] or chronic hepatitis [13].

The present study aimed to determine the effects of dietary - Co deficiency on serum VitB₁₂ and folate concentrations, live body weight development and haematology as well as serum and rumen metabolites of sheep. Also, this study aimed to document whether the low dietary Co in the present model could induce the OWLD in Egyptian baladi sheep. In addition, the efficacy of the supplementary treatment to correct Co deficiency as evaluated by the improving of all the studied parameters was another target.

MATERIALS AND METHODS

Experimental Diets: The basal diet, which consisted of naturally Co-deficient whole barley, was purchased from a farm in Sadat city, Menoufia, Egypt. The Co- sufficient and Co- deficient whole barley diets were prepared as described by [14] and the composition of the diet is shown in Table 1.

The Co- contents of the sufficient and deficient diets were 1000 and 55µg Co/kg DM, respectively, as measured by Varian Spectra AA220 graphite furnace atomic absorption spectrophotometry [15].

Animal studies: The experiment began in early September 2005 and finished in October 2006, using fourteen male Egyptian baladi lambs (aged 4 - 6 months and weighed 18-20 kg). Lambs were raised in the farm of Faculty of Veterinary Medicine, Menoufia University. The animals were protected against parasitic infections by drenching of albendazole (Pharma-Seed) at dose level of 2ml/20kg Bwt and injection of ivermectin (Ivomec super, Merial) subcutaneously at dose level of 1ml/50kg Bwt, before beginning of experiment and every 3 months.

Table 1: Composition of diet used for feeding of the experimental lambs

Ingredient	Amount (per kg)
Whole barley	970.0 g
Urea	14.0 g
Vitamin A and D3 (AD3)	10.0 mg
Vitamin E	40.0 mg
Calcium carbonate	15.0 g
Sodium sulfate anhydrous	400.0 mg
Zinc sulfate heptahydrate	150.0 mg
Manganous sulfate tetrahydrate	50.0 mg
Potassium iodate anhydrous	1.0 mg
Sodium selenite anhydrous	175.0 µg
Cobalt chloride hexahydrate *	4.0 mg

* Added to cobalt - sufficient diet only.

The experimental animals were randomly divided into two feeding treatment groups. The first group was consisted of five lambs fed on the basal diet supplemented with Co and was kept as control, while the second group was of nine lambs, fed the same diet without Co-supplementation. The lambs were maintained indoor in two separate groups and fed *ad libitum* the specified diet for 20 weeks and had free access to water. After 20 weeks, all lambs in the group of Co deficiency were then transformed to the Co- sufficient diet for a period of 4 weeks. In addition, each lamb in this group was administered orally with a dilute Co solution as Co- chloride hexahydrate (Sigma-Aldrich Co Ltd-UK), at a dose level of 2mg/head twice weekly for 4 weeks, as recommended by [16]. All animals were weighted during sampling periods of the experiment. On daily basis, the clinical picture of animals was observed.

Samples and analytical techniques

1. Blood sampling: Blood samples were collected by jugular venipuncture on day 0 and every two weeks thereafter for 20 weeks and once at the 24th week, on EDTA for haematological evaluation and in plain vacutainer tubes for serum biochemistry and VitB₁₂ and folate analyses.

Haematological parameters: Red blood cell counts (RBCs), packed cell volume (PCV), haemoglobin (Hb) and white blood cell counts (WBCs) were determined according to [17]. Red blood cell indices; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated.

Serum vitamin B₁₂ and folate levels: Radio assay kit Vitamin B₁₂ (⁵⁷Co) /Folate (¹²⁵I), purchased from Simul TRAC-SNB-Biochemicals, Germany was used for

simultaneous quantitative determination of VitB₁₂ and folate in serum according to [18].

Serum biochemistry: Sera were analyzed spectrophotometrically for determination of total proteins [19], albumin [20], cholesterol [21] and transferases (Aspartate amino transferase; AST and alanine amino transferase; ALT) [22], creatinine [23] and blood urea nitrogen [24] using test kits supplied by Stanbio -Texas, USA

2. Ruminal fluid samples: On day 0, at the end of the experimental period of deficiency (week 20) and after treatment (week 24) ruminal fluid samples were obtained by clean, dry stomach tube from all animals. Each sample was acidified with 1ml of a 50% sulfuric acid solution and frozen until analyzed for volatile fatty acids (VFAs); acetic, propionic, n-butyric, isovaleric and n-valeric acids, by gas chromatography according to [25].

3- Pathological studies: Tissue specimens from liver were obtained immediately from the freshly dead lambs and from two control lambs slaughtered by the end of the 20- week experimental deficient period and fixed in 10% neutral formalin solution dehydrated, cleared and embedded in paraffin blocks. Paraffin sections of 5µ thickness were prepared, stained by haematoxylin and eosin (H&E) and examined microscopically for detection of histopathological alterations [26].

Statistical analysis: All data were subjected to statistical analysis including the calculation of the mean and standard error. Significance between data of treated groups was evaluated by Student t-test at levels $P < 0.05$ according to [27] using SPSS version 10 computer programme.

RESULTS

Clinical signs: Clinically, most of the Co-deficient lambs exhibited pale mucous membranes and had varying degrees of reduced feed intake appeared after 8-12 weeks on Co-deficient diet. Additional symptoms were seen 2-8 weeks later, including listlessness, bilateral ocular discharge, alopecia and diarrhea in some lambs. From these lambs, two were found died on days 126 and 138 of the experiment and they were severely emaciated. Control lambs were clinically normal at all times of the experiment.

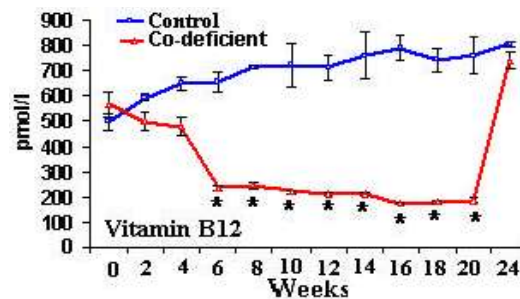


Fig. 1: Mean of serum vitamin B₁₂ values in lambs fed on cobalt sufficient (Control) and cobalt deficient diets (Co-deficient) for 20 weeks then after treatment for 4 weeks (week 24). * = Significant at $P = 0.05$.

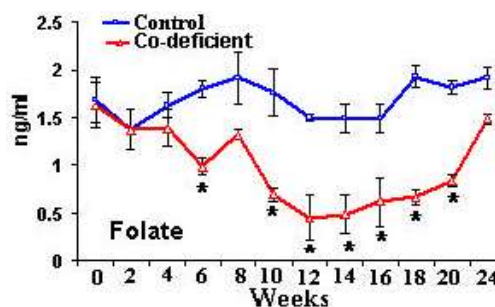


Fig. 2: Mean of serum folate values in lambs fed on cobalt sufficient (Control) and cobalt deficient diets (Co-deficient) for 20 weeks then after treatment for 4 weeks (week 24). * = Significant at $P = 0.05$.

Serum vitamin B₁₂ and Folate: The serum VitB₁₂ values of the Co-deficient lambs dropped significantly ($P < 0.05$) from the 6th till 20th weeks of the study compared to control ones. However, after treatment, serum VitB₁₂ concentrations were markedly elevated ($P < 0.05$) and approach value of the control group (Fig.1). Serum folate values of the Co-deficient lambs behaved similar to VitB₁₂ which dropped significantly from the 6th till 20th weeks (Fig. 2).

Body weight development: The live body weight of the animals fed the Co-sufficient diet increased steadily during the course of study (Fig.3). In contrast, the body weight of lambs fed the Co-deficient diet increased more slowly and was lower significantly ($P < 0.05$) than control at the 12th till 20th weeks of experiment. After treatment, at the 24th week, the body weight of the depleted lambs returned to increase, but still significantly lower ($P < 0.05$) than those in the control group.

Table 2: Changes in haemogram of lambs fed on cobalt-sufficient (control) and cobalt- deficient diets (Co- deficient) on day 0 and at the end of the experimental deficient period (week 20) then after treatment for 4 weeks (week24)

Groups parameters	Periods					
	Day 0		Week 20		4 weeks after treatment (week 24)	
	Control	Co- deficient	Control	Co- deficient	Control	Co- deficient
Red blood corpuscles (RBCs- $\times 10^6$ μ l/l)	12.43 $\pm$ 0.62	13.48 $\pm$ 0.35	13.73 $\pm$ 0.31	11.03* $\pm$ 0.32	14.15 $\pm$ 0.18	13.90 $\pm$ 0.32
Packed cell volume (PCV%)	32.75 $\pm$ 1.89	31.97 $\pm$ 1.76	40.50 $\pm$ 0.65	32.83* $\pm$ 1.49	40.00 $\pm$ 0.71	39.33 $\pm$ 0.55
Haemoglobin (Hb -g/dl)	8.68 $\pm$ 0.20	9.03 $\pm$ 0.46	13.53 $\pm$ 0.28	8.55* $\pm$ 0.18	13.00 $\pm$ 0.58	12.98 $\pm$ 0.37
Mean corpuscular volume (MCV- fl)	29.07 $\pm$ 2.87	30.22 $\pm$ 2.96	29.55 $\pm$ 0.87	25.99* $\pm$ 1.98	28.30 $\pm$ 0.65	30.56 $\pm$ 0.66
Mean corpuscular haemoglobin (MCH-pg)	7.63 $\pm$ 0.30	9.28 $\pm$ 0.86	9.88 $\pm$ 0.37	7.79* $\pm$ 0.32	9.45 $\pm$ 0.49	9.64 $\pm$ 0.40
Mean corpuscular haemoglobin concentration (MCHC-g/dl)	31.85 $\pm$ 2.12	31.90 $\pm$ 1.89	33.40 $\pm$ 0.31	26.27* $\pm$ 1.15	32.95 $\pm$ 0.96	31.49 $\pm$ 0.88
Total leukocytic count (WBCs- $\times 10^3$ μ l/l)	8.43 $\pm$ 0.11	8.5 $\pm$ 0.12	11.87 $\pm$ 0.17	8.10* $\pm$ 0.11	11.23 $\pm$ 0.40	9.67 $\pm$ 0.36

Mean $\pm$ SE SE = Standard Errors. * = Significant at p= 0.05.

Table 3: Changes in serum biochemical parameters of lambs fed on cobalt-sufficient (control) and cobalt- deficient diets (Co- deficient) on day 0 and at the end of the experimental deficient period (week 20) then after treatment for 4 weeks (week24)

Groups parameters	Periods					
	Day 0		Week 20		4 weeks after treatment (week 24)	
	Control	Co- deficient	Control	Co- deficient	Control	Co- deficient
Total proteins (g/dl)	6.98 $\pm$ 0.37	7.17 $\pm$ 0.14	7.30 $\pm$ 0.19	4.03* $\pm$ 0.07	7.00 $\pm$ 0.27	7.13 $\pm$ 0.19
Albumin (g/dl)	2.70 $\pm$ 0.14	2.48 $\pm$ 0.13	2.78 $\pm$ 0.05	1.63* $\pm$ 0.08	2.80 $\pm$ 0.12	2.75 $\pm$ 0.08
Globulin (g/dl)	4.28 $\pm$ 0.26	4.68 $\pm$ 0.11	4.53 $\pm$ 0.17	2.40* $\pm$ 0.12	4.20 $\pm$ 0.32	4.40 $\pm$ 0.17
Albumin/Globulin ratio	0.64 $\pm$ 0.03	0.53 $\pm$ 0.03	0.62 $\pm$ 0.02	0.70 $\pm$ 0.07	0.69 $\pm$ 0.06	0.63 $\pm$ 0.03
Aspartate amino- transferase (AST-IU/l)	125.37 $\pm$ 8.15	122.08 $\pm$ 6.86	129.48 $\pm$ 10.21	383.44* $\pm$ 17.97	137.69 $\pm$ 18.17	199.85* $\pm$ 14.06
Alanine amino- transferase (ALT- IU/l)	17.25 $\pm$ 0.85	16.17 $\pm$ 0.75	17.00 $\pm$ 0.71	43.00* $\pm$ 1.83	17.50 $\pm$ 0.65	18.50 $\pm$ 0.62
Cholesterol (mg/dl)	44.25 $\pm$ 4.07	46.50 $\pm$ 0.89	51.00 $\pm$ 2.08	24.5* $\pm$ 0.92	49.00 $\pm$ 2.45	47.33 $\pm$ 1.71
Urea nitrogen (mg/dl)	12.25 $\pm$ 1.70	11.98 $\pm$ 1.02	11.32 $\pm$ 0.39	16.23* $\pm$ 1.13	11.53 $\pm$ 1.04	12.67 $\pm$ 0.88
Creatinine (mg/dl)	1.40 $\pm$ 0.17	1.55 $\pm$ 0.08	1.63 $\pm$ 0.09	1.49 $\pm$ 0.09	1.63 $\pm$ 0.14	1.58 $\pm$ 0.15

Mean $\pm$ SE SE = Standard Errors. * = Significant at p= 0.05.

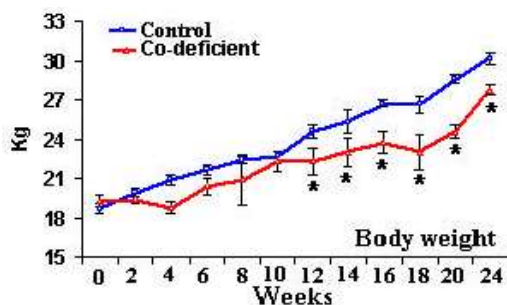


Fig. 3: Mean of body weight development of lambs fed on cobalt sufficient (Control) and cobalt deficient diets (Co-deficient) for 20 weeks then after treatment for 4 weeks (week 24). * = Significant at P= 0.05.

Haemogram: At the 20th week, lambs in the Co-deficient group had significantly decreased values of RBCs, PCV,

Hb, MCV, MCH, MCHC and WBCs (P<0.05) than those in the control group. After treatment of the Co-deficient lambs, these parameters returned to be near to the control values (Table, 2).

Serum biochemistry: At the 20th week of experiment, lambs fed on the Co-deficient diet had significantly lower (P<0.05) values of total proteins, albumin, globulin and cholesterol than those fed on the Co-sufficient diet, whereas after treatment -at the week 24th- no differences were detected. At the week 20th, the activities of AST and ALT and the concentrations of blood urea nitrogen showed marked increases (P<0.05) in the Co-deficient lambs as compared to control, while post treatment, at the week 24th, no significant variances were observed in the values of these parameters. No significant differences were observed in the values of creatinine among both groups during the period of experiment (Table, 3).

Table 4: Changes in rumen volatile fatty acids of lambs fed on cobalt-sufficient (control) and cobalt- deficient diets (Co- deficient) on day 0 and at the end of the experimental deficient period (week 20) then after treatment for 4 weeks (week 24)

Groups parameters	Periods					
	Day 0		Week 20		4 Weeks after treatment (week 24)	
	Control	Co-deficient	Control	Co-deficient	Control	Co-deficient
Acetic acid (mol/100 mol)	63.76± 0.35	63.33±0.27	63.13±0.09	66.63*±0.09	63.47±0.15	63.77±0.09
Propionic acid (mol/100 mol)	17.90±0.06	18.00±0.06	18.41±0.20	14.07*±0.88	18.23±0.15	17.63±0.18
n-Butyric acid (mol/100 mol)	15.11±0.15	15.20±0.17	15.40±0.25	15.85±0.12	14.80±0.21	15.33±0.20
Isovaleric acid (mol/100 mol)	1.53±0.12	1.60±0.06	1.60±0.12	1.60±0.15	1.50±0.12	1.63±0.09
n-Valeric acid (mol/100 mol)	1.70±0.36	1.90±0.31	1.47±0.52	1.85±0.17	2.00±0.21	1.67±0.18

Mean±SE SE = Standard Errors. * = Significant at p= 0.05.

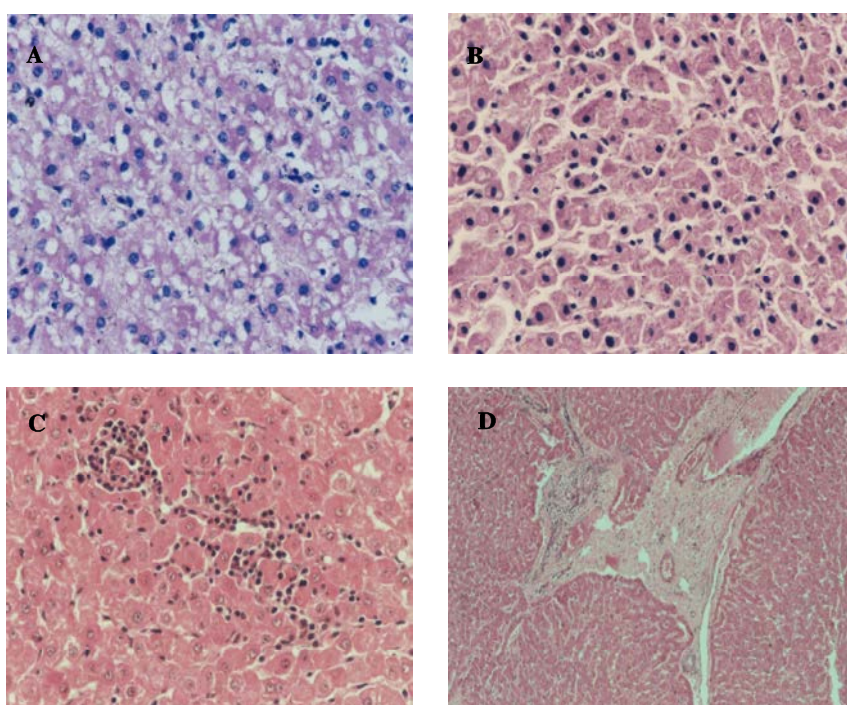


Fig. 4: Liver showing: A: Hydropic degeneration fatty change, apoptosis and kupffer cell hyperplasia. B: Apoptosis and necrosis of the hepatocytes. C: Focal necrosis and kupffer cell hyperplasia D: Portal cirrhosis with lymphocytes infiltration and congestion of the blood vessel at the portal area (X40 H&E).

Ruminal volatile fatty acids: The Co-deficient lambs had higher molar percentage of acetic acid ($P<0.05$) and lesser propionic acid ($P<0.05$) than control. After treatment, at the week 24, these parameters showed no significant differences compared to control. No observable changes were detected in the levels of n-butyric, isovaleric or n-valeric acids among both groups (Table, 4).

Pathological examination of livers: *Post mortem examination:* the bodies of the freshly dead Co- depleted lambs were extremely emaciated with total absence of

body fat. The livers were characterized as uniformly pale throughout the parenchyma, swollen and distinctly friable.

Histopathological examination: livers of Co-depleted lambs exhibited diffuse fatty changes, apoptosis and necrosis of hepatocytes and kupffer cell hyperplasia. One liver showed portal cirrhosis with lymphocytes infiltration and congestion of the blood vessel at the portal area (Figure, 4). No gross or histopathological alterations were seen in livers of control.

DISCUSSION

Cobalt deficiency in sheep is of major economic importance in several countries [13]. Dietary Co requirements have been established at 0.1 to 0.2 ppm [28]. Experiments with sheep suggest an oral requirement for growing lambs of some 200 µg/day, about 10 times the reported oral requirement for other species per unit of food intake [29]. Furthermore, more recent studies also indicate the necessity to increase the amount of dietary Co for growing ruminants up to a level of 300-500 µg/Kg DM for optimum microbial activity, fermentation and VitB₁₂ synthesis [30, 31]. Signs of Co deficiency develop in sheep fed diets that contain less than 70 µg Co/Kg DM [29].

In this study, chemical analysis showed that basal diet contained 55 µg Co/Kg DM. Based on the clinical observations on lambs that fed on the basal diet without Co-supplement, it is apparent that the level of Co provided was inadequate and animals were in a seriously depleted state. The observed signs on these lambs that developed inappetence, lacrimation and loss of condition were similar to those reported in spontaneous outbreaks of ovine Co deficiency [32, 33] and those induced experimentally by [13, 34, 35]. In contrast, [36] suggested that feed contain 40- 70 µg/Kg DM of Co is considered moderately deficient diet for sheep and developed no consistent signs of clinical Co deficiency. The contradiction may be explained by suggesting that Egyptian lambs are quite susceptible to Co deficiency. The induced loss of appetite attributed to the impaired propionate metabolism resulting from Co deficiency, leading to higher blood propionate which inversely correlated to voluntary feed intake [37].

In this study, serum VitB₁₂ of the lambs fed on the Co-deficient diet declined rapidly and was significantly lower than that of Co- sufficient controls after 6 weeks. It was fallen below the lower limit of normality of sheep (220 pmol/L) after 10 weeks, preceding the onset of loss of condition and clinical signs and remained below that level for the remainder of the deficient period of the experiment. As Co deficiency becomes progressively more severe, serum VitB₁₂ fall to values indistinguishable from those in control. In contrast, the control group maintained levels that were normal throughout the study. The values in Co-deficient animals of the present study were consisted with those found in Co-deficient lambs, which are reported to have values <250 pmol/L [13, 34, 35].

Although VitB₁₂ is required for normal folate metabolism [38, 39], this response variable has not

hitherto been used to estimate the dietary requirement for Co. [39] recommended that levels of dietary Co for normal folate metabolism can be set to be 150-200 µg/kg DM. It was obvious from this study that serum level of folate could be used as a valid variable to estimate the Co-status of sheep. The obtained result coincided with that recorded by [1], who reported that plasma folate concentration was lower in cattle- deficient in VitB₁₂. However, the present results do not support those of [39, 40], who reported that hepatic folate appeared to be sensitive to Co-status than folate plasma levels. The relative importance of each of these parameters requires further investigation.

The interrelationship between VitB₁₂ and folate is best explained by the methyl trap hypothesis stating that VitB₁₂ deficiency can lead to lowered levels of methionine synthase, which results in a functional folate deficiency by trapping an increased proportion of folate as the 5-methyl derivative [39, 41].

In the present study, the significant decrease in live body weight development of Co-deficient lambs is likely due to a number of reasons. Cobalt- deficient diets have been reported to reduce the number of rumen microorganisms, leading to lower rumen digestibility [42]. Lambs receiving inadequate levels of Co might also experience a decrease in intestinal absorption of nutrients. [43] demonstrated that animals with VitB₁₂ deficiency experienced a shortening of the intestinal villi. Lower levels of growth rate may also be likely linked to two VitB₁₂ - dependent enzymes, methyl malalonyl Co- A mutase and methionine synthase which are important for protein and energy metabolism [44]. The body weight development of deficient lambs responded positively to Co supplementation, which is similar with findings of [35].

In the current study, there was significant decrease in erythrogram parameters; RBCs, PCV, Hb, MCV, MCH and MCHC in Co-deficient group compared to control ones at the end of the deficient period of experiment. The Co-depleted lambs showed mild microcytic hypochromic anaemia as manifested by reduced MCV and MCHC. In this respect, animals behaved similar to lambs with ovine white-liver disease [45] and goats [46] fed Co-deficient diets. The present results strongly suggest that decreased RBCs and Hb might be prominent feature of Co deficiency. The obtained results also confirm the essential roles of VitB₁₂ and folate for the production of haem [39]. Reduced activity of haem-enzyme catalase may possibly result from a decreased formation of succinyl- Co A, necessary for haem synthesis, via the cobalamine - dependent methyl- Co A mutase [47]. Vitamin B₁₂ cooperates with folate in the synthesis of

deoxyribonucleic acid (DNA). A deficiency of either compounds lead to disturbed production of DNA and impaired production of red blood cells and causes anaemia. In addition, folic acid deficiency causes a reduction in biosynthesis of nucleic acids essential for cell formation and function [17].

In the present study, the Co-deficient lambs exhibited significantly lower WBCs compared to those in the control group. This depression in WBCs may be attributed to the stress of Co deficiency which caused nutritional and metabolic disturbances and stimulate the adrenal gland to secrete corticosteroid hormone resulting in sustained change in leukocyte numbers [48]. The present findings don't support those of [12] in lambs and [46] in goats, who found no significant differences in total WBCs or in any of the individual white blood cell types. The Co-deficient lambs responded positively to treatment with Co and restored the normal values of haematological parameters.

Changes in blood chemistry were examined in Co-deficient lambs and were compared with values of Co-supplemented lambs on the same diet. All blood changes were partly regarded as reflections of the inappetence or hepatic injury. The obtained results showed that the Co-deficient lambs exhibited a significant decrease in total serum proteins, albumin and globulin. Similar results were obtained in lambs [45] and goats [46] fed on Co-deficient diet. Although VitB₁₂ plays a significant role in overall protein metabolism and a deficiency could result in serum protein deficiency, it is likely that the lower serum protein levels observed in the lambs in the present study are more likely the result of reduced feed intake. It cannot be ruled out however that the decrease in protein levels are not partially a direct result of developing hepatic lipidosis resulting in impaired protein metabolism [49].

In this study, Co-depleted lambs had elevated serum activities of AST and ALT. These findings are indicative of primary hepatocyte damage [13]. The histopathological findings as reported later also support primary hepatocyte damage as the main lesion in liver of Co-deficient lambs. Although AST isn't a liver specific enzyme, it has been reported to be elevated in sheep with OWLD [50-52], cattle [53] and goats [46] with hepatic lipidosis.

The observed significant depression in serum cholesterol levels in Co-deficient lambs may be attributed partly to the inappetence or due to increase lipolysis and decrease lipogenesis as a result of failure of energy metabolism induced by Co-deficiency [44]. Similar results were obtained by [34-45] in sheep.

Significant elevation of blood urea nitrogen, as observed in the present study has also reported by [54] in Co-deficient cattle. This result may be attributed to the disturbance in protein metabolism due to liver damage, which leads to accumulation of urea in blood [55]. However, some authors have reported no differences in total proteins, albumin and globulin [12] and elevation in cholesterol [56] and a decrease in urea [45] in Co-deficient animals.

Rumen fluid of lambs was analyzed for VFAs as an indication of the effects of Co-deficiency on rumen fermentation. Cobalt supplementation may increase propionate production by certain ruminal bacteria that are dependent upon VitB₁₂ in order to convert succinate to propionate [57].

In Co-deficient lambs of the present study, the mean rumen propionic acid had fallen which was accompanied by an increase in the mean rumen acetic acid. When the animals were once again supplemented with Co, the normal mean rumen propionic and acetic acids restored. Similar results were obtained by [36] who suggest that growth of propionate – producing bacteria in the rumen be inhibited in Co-deficiency. As a consequence succinate – a normal end product of several cellulolytic and amylolytic bacteria – would accumulate. This accumulation could entirely be accounted for by decline in rumen propionate concentration [58]. However, an earlier report from another laboratory [49] showed that there was no differences in the propionate concentration in the rumen fluid of animals that were either Co-deficient or Co-sufficient. This contradiction may be explained by suggesting that some other factors caused the different findings as Co content of the deficient diet.

At necropsy, no abnormalities were found in any of the control animals, while many Co-deficient lambs developed liver lesions known as ovine “white liver” disease, but the etiology of these lesions is controversial. It has been suggested that cofactors such as plant or fungal hepatotoxins [50] or interactions with other minerals [8] are required for development of liver damage in Co-deficient sheep. The experimental diets used in the present study were similar in all respects except Co-content. Absence of lesions in Co-supplemented control animals indicates that Co deficiency was the primary cause of hepatic alteration in Co-deficient lambs. Liver damage consequent to primary Co deficiency may exacerbate the effects of plant or fungal hepatotoxins in grazing sheep. [13] suggested that reduced activities of the VitB₁₂–dependent enzymes, methyl molonyl Co A

mutase and methionine synthase and lipid peroxidation are of likely pathogenetic importance in the development of these lesions.

It could be concluded that prolonged feeding of Egyptian lambs on a diet low in Co (55 µg Co/Kg DM) for 20 weeks, produced severe Co deficiency as judged by subnormal serum concentrations of VitB₁₂ and folate, together with the low voluntary feed intakes, growth development and decrease in molar percentage of ruminal propionic acid. In addition, Co-depleted lambs exhibited mild anemia and metabolic disturbances. On the basis of the recorded activities of AST and ALT and the pathological findings in the livers Co-deficient lambs were likely associated with developing fatty hepatic degeneration. Co-deficient animals would probably have responded positively to treatment with Co for 4 weeks.

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