Vet. Med. J., Giza 40, No. 1, 31-48 (1992)

FURTHER STUDIES ON VITAMIN E AND SELENIUM IN BUFFALO-CALVES 1. STUDIES ON BLOOD SELENIUM LEVEL IN NATURALLY OCCURRING AND DURING EXPERIMENTAL INDUCTION OF WMD USING COD LIVER OIL IN BUFFALO- CALVES IN EGYPT

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(Received: 30. 11. 1991)

INTRODUCTION

Nutritional muscular dystrophy "NMD" is the most frequent and from diagnostic point of view-the most important manifestation of vitamin E and/or selenium deficiency syndrome in domestic animals (Blood et al., 1989). It wasfirstly recorded among buffalo - calves in Egypt by Soliman et al.. (1965) in two farms where daily addition of cod liver oil "CLO" to the milk of calves was common practice for cont- rolling hypovitaminosis A (corneal opacity).

More recent work was presented also in Egypt by El-Neweehy (1982), included its clinical picture, blood chemistry and its P.M. picture as well as histopathological picture and expermental induction using CLO.

Information was lacking about blood selenium status not only in naturally occurring cases of NMD but also during its experimental induction. So the pres- ent study was planned to determine whole blood selenium level "states" in naturally occurring cases of the disease and during experimental inducation in buffalo-calves using cod liver oil.

MATERIAL AND METHODS

A total number of 46 suckling buffalo-calves were used in the present study. These animals were included in two major groups:

The first one was of 10 NMD naturally affected calves beside 20 apparently healthy calves acting as control group.

The second one was of 16 apparently healthy buffalo-calves. All above mentioned animals were put under the same nutritional (Table, 1) and environmental conditions. The second group was divided into 5 subgroups, both first and second one were of 5 calves, while the other groups, each was only of two calves. With exception of the animals of the first gorup which act as control one, all calves were given 30 ml cod liver oil orally daily with morning feeding, 3 days after natural suckling of colostrum and till end of the experment or death of the animal. Calves of the third sub-group were given, vitamin E (200 i.u. DL-o tocopherol acetate "Rovimix E" obtained from F. Hoffman La Roche Co Ltd) mixed with milk while calves of 4th sub-group were given selenium (1 ppm/calf/day). Calves of 5th sub-group were given both vitamin E (200 i.u/calf/day) plus selenium (1 ppm/calf/day). Grouped calves were kept in seperate pen of rice straw thick bedding covered the cement floor and were fed using coyner calf pail with rubber nipple on milk collected from the lactating buffaloes of the herd after warming it to body temperature. These calves were kept during experimental period under close clinical observation. Heparinized blood samples were collected and used for determination of whole blood selenium level according to the method of Olson (1969), in E. Hoffman La Roche Ltd laboratories. The detection limit of this method is approximately 15 ng/Se/gm substance. Statistical analysis of the present data was performed according to Kempthorne (1962).

RESULTS AND DISCUSSION

The results obtained are showed in Table (2-4) and represented graphically in Figs (1-3). Table (2) showed that whole blood selenium level in naturally occurring cases of NMD averaged (72.43±10.93 ng/ml), a level which is considered to be significantly low when compared with that of clincially normal calves (90.37±3.97 ng/ml).

These findings were generally in agreement with those reported by Whanger, (1970); Admas, (1972); Oldfield, (1974) and Jenkins et al. (1974), who considered blood selenium level approximating (40 ng/ml) is adequate for protecting offsprings aganist NMD.

However, very low levels were reported by Jacobsson et al. (1970), in NMD affected calves, while Linklater et al. (1977), reported very similler selenium levels in NMD affected calves to that in this investigation.

Van Fleet (1980), observed that blood selenium level below (50 ng/ml) are generally indicative of selenium-E deficiency.

Although Muth and Allaway (1963), attributed occurrance of NMD to distribution of naturally occurring selenium, Oskanen (1967), mentioned that incidence of NMD in livestock has been found to be related to low level of selenium in feed grown in soil of low selenium concentration.

As El-Neweehy (1982), reported adequate soil selenium level in Egypt is ($\leq$ 0.5 ppm), selenium deficiency in naturally occurring cases may be attributed as Hill, (1975); Jensen, (1975); Hill, (1976) ; Sandstead, (1977); Van Flett, (1977) and Howell,

Table (1): Feeding system given in Mehallet Mousa farm for buffalo-calves. from the records of the farm.

Age-seek.	Milk	Additions
0-7 days	dan colostrum by natural suckling	
1st week	5 lb at morning + 5 lb at evening	
2ed ,,	,, ,, ,, + ,, ,, ,,	2 kg rice straus
3rd ,,	6 ,, ,, + 5 ,, ,,	for all calves
4th ,,	6 ,, ,, + 6 ,, ,,	as bedding
5th ,,	6 ,, ,, + 5 ,, ,,	
6th ,,	6 ,, ,, + 5 ,, ,,	
7th ,,	5 ,, ,, + 5 ,, ,,	
8th ,,	4 ,, ,, + 4 ,, ,,	
9th ,,	4 ,, ,, + 3 ,, ,,	
10th ,,	6 lb. at morning	1 kg conc.+1 kg Barseem
11th ,,	5 ,, ,, ,,	,, ,, + ,, ,,
12th ,,	4 ,, ,, ,,	,, ,, + ,, ,,
13th ,,	4 ,, ,, ,,	,, ,, + ,, ,,
14th ,,	3 ,, ,, ,,	,, ,, + ,, ,,
15th ,,	2 ,, ,, ,,	,, ,, + ,, ,,

Table (2): Blood selenium levels (ng/ml) in buffalo-calves naturally affected with NMD.

NMD naturally affected calves			Apparently normal control group Whole blood Se (ng/ml)	
Calf No	Age/day	whole blood Se (ng/ml)		
1	50 days	42.439	95.68	
2	56 days	101.164	62.867	
3	74 days	90.858	95.428	
4	64 days	75.115	118.004	
5	104 days	78.108	64.140	
6	57 days	146.818	60.216	Average
7	64 days	31.135	69.219	± S.E.
8	60 days	22.680	91.087	90.377
9	105 days	75.115	78.154	±3.979
10	76 days	60.902	86.265	
			105.268	
			116.793	
			124.242	
Average			98.970	
± S.E.	71.00	72.433	97.736	
	±18.33	+10.936	86.997	
			88.633	
			91.429	
			90.504	
			85.850	

(1978), who suggested that selenium deficiency in animals may be induced by the incorporation of certain elements that antagonize the selenium.

On studying the pattern of whole blood selenium levels during experimental induction of NMD in buffalo-calves using cod lvier oil (CLO), Table (3) showed that although gradual weekly decrease was observed in its levels (in both CLO and CLO plus vitamin E treated group) of calves, its drop was significantly sharp (42.52 ng/ml) and occur somewhat earlier (at 3 weeks of administration) in the second when compared with the first one. Furthermore, whole blood selenium level was comparatively low in both groups.

On contrary, inclusion of selenium and selenium plus vitamin E with CLO in the 4th and 5th sub-groups of calves, were accompanied with gradual increase in whole blood selenium levels, reached its maximum level 5 weeks after administration in both groups. Its level was comparatively higher and remain high in 4th when compared with 5th sub-group. Moreover, its level was comparatively higher in both groups up to 10 weeks of administration.

In spite of the close clinical observation, as shown in Table (4), calf No. 1 died suddenly 7 weeks of CLO administration. Its whole blood selenium level prior death was very low (27.906 ng/ml) and its necropsy findings showed typical cardiac form of the disease. Dyspnea and severe respiratory distress were also observed in both calf No. 3 and calf No. 4, 8 weeks of CLO administration. Their whole blood selenium levels were (53.34 ng/ml) and (59.49 ng/ml) respectivelly. Death occurred one day after treatment trial using vitamin E and E-selenium preparation parentrally. P.M. Pictures were also mainly of cardiac form of the disease. Confirmative diagnosis of the disease in the before mentioned calves by

Table (3): Whole blood selenium level in cod liver oil treated groups of suckling buffalo-calves.

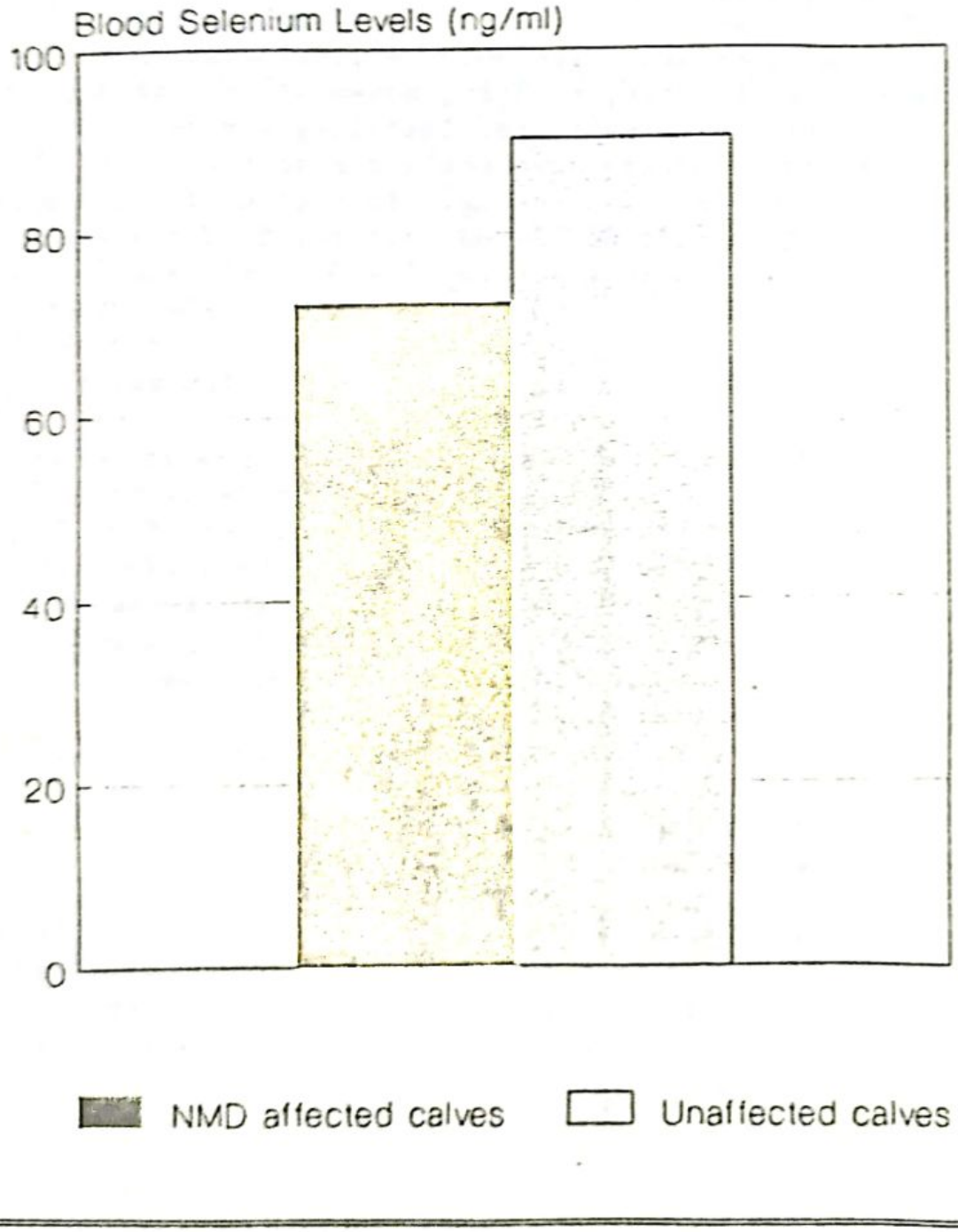
Type of treatment	No. of cases	Before CLO administ	1 week after	2 weeks after	3 weeks after	4 weeks after	5 weeks after	6 weeks after	7 weeks after	8 weeks after	9 weeks after	10 weeks after	11 week after
Control group	5	75.800 ±9.660	61.380 ±8.200	96.27 ±7.4	65.740 ±6.400	124.690 ±9.960	115.850 ±5.600	104.360 ±8.300	93.090 ±9.700	123.460 ±6.500	89.960 ±9.700	91.330 ±8.300	92.020 ±8.400
CLO only	5	90.933 +11.966	117.320 ±4.263	96.02 +11.545	84.050 ±6.750	96.830 ±6.450	85.733 ±6.390	82.820 ±4.980	72.160 ±6.402	63.208 ±3.818	66.780 ±8.520	211.660 ±4.808	192.560 ±3.650
CLO + Vit. E (200 i.u.)	2	67.437	101.857	106.602	42.520	44.701	58.228	65.677	82.228	82.515	82.274	100.204	104.497
CLO + Se (1 ppm)	2	77.458	122.345	157.077	147.435	174.809	284.398	224.234	178.534	143.322	137.520	117.913	116.039
CLO + Vit. E + Se	2	78.906	87.362	146.374	174.809	174.809	216.762	214.911	186.120	139.506	117.684	116.039	86.197

Table (4): Whole blood selenium level (ng/ml) in COD Liver oil (CLO) treated group of suckling buffalo calves.

	Before CLO adminis.	1 week after	2 weeks after	3 weeks after	4 weeks after	5 weeks after	6 weeks after	7 weeks after	8 weeks after	9 weeks after	10 weeks after	11 week after
Control	75.800	61.380	96.270	65.740	124.690	115.85	104.360	93.090	123.460	89.960	91.330	92.020
group	±0.660	±8.200	±7.400	±6.400	±9.960	±5.60	±8.300	±9.700	±6.500	±9.700	±8.300	±8.400
Calf No 1	123.854	128.012	156.120	90.858	114.851	85.63	61.953	27.906	-	-	-	-
Calf No 2	122.813	127.817	63.895	64.649	103.060	90.86	93.683	86.197	76.695	67.985	204.876	196.874
Calf No 3	65.677	117.602	125.123	105.025	106.602	80.66	82.854	54.138	53.347	WMD (CF)	-	-
Calf No 4	76.645	106.602	120.827	90.838	81.147	80.66	84.865	62.111	59.495	WMD (CF)	-	-
Calf No 5	65.677	106.602	65.123	68.864	78.496	90.86	90.757	86.197	63.347	65.575	218.464	188.246
Average	90.933	117.327	96.020	84.050	96.831	85.73	82.822	72.160	63.208	66.780	211.664	192.560
± S.E.	±11.966	±4.263	±11.545	±6.751	±6.453	±2.64	±4.982	±6.402	±3.818	±8.520	-	-

WMD White muscle disease
C.F. Cardiac form.

Fig. (1): Blood selenium levels (ng/ml) in buffalo calves naturally affected with (NMD) and control unaffected group



histopathological examination of skeletal, diaphragmatic and cardiac muscles. Which revealed typical coagulative necrosis (zenker's necrosis) of the cardiac muscle mainly.

On the other hand, locomotor disturbances including stiffness in gait, rotating movement in hock joints, seperation of hind limbs, liability for reucmbancy, with unwilling for movement and muscle tremors (which become evident when the calf is forced to walk) were observed on calf No. 2 and calf No. 5. Their whole blood selenium levels were (67.98 ng/ml) and (65.57 ng/ml) respectively. Treatment trials using vitamin E (Ephynil amp each one contain 100 mg DL ∝ tecopherol acetate), 5 ampoule were given I/M daily beside 5 ml injacome E-selenium (each ml contain 150 mg DL ∝ tocopherol acetate and 0.5 mg selenium as sodium selenite pentahydrate), given daily for 5 successive days. Both drugs were obtaiend from F. Hoffman La Roche Co Ltd. Basle, Switzerland. Clinical and laboratory improvement were indicated by both disappearance of above mentioned signs and marked decrease in muscle specific enzymes levels. In these calves, blood selenium levels, elevated markedly after treatment, reached (204.86 ng/ml, 196.87/ml) and (218.46, 188.24 ng/ml) for calf No. 2 and No. 5 during 10th and 11th week respectively as shown in **Table (4).**

Hamed and Decker (1959), reported that diet containing adequete tocopherol may mask selenium deficiency, while Schwarz (1961), suggested that selenium can correct many but not all-of symptoms of vitamin E deficiency, meanwhile Oskanen (1967), indicated that NMD induced expermentally using CLO could be delayed by prophylactic treatment with selenium. On the other hand, Jenkins et al. (1970), observed that vitamin E together with selenium has effective antidystrophic action.

Fig. (2): whole blood selenium levels (ng/ml) in CLO treated group of suckling buffalo-calves.

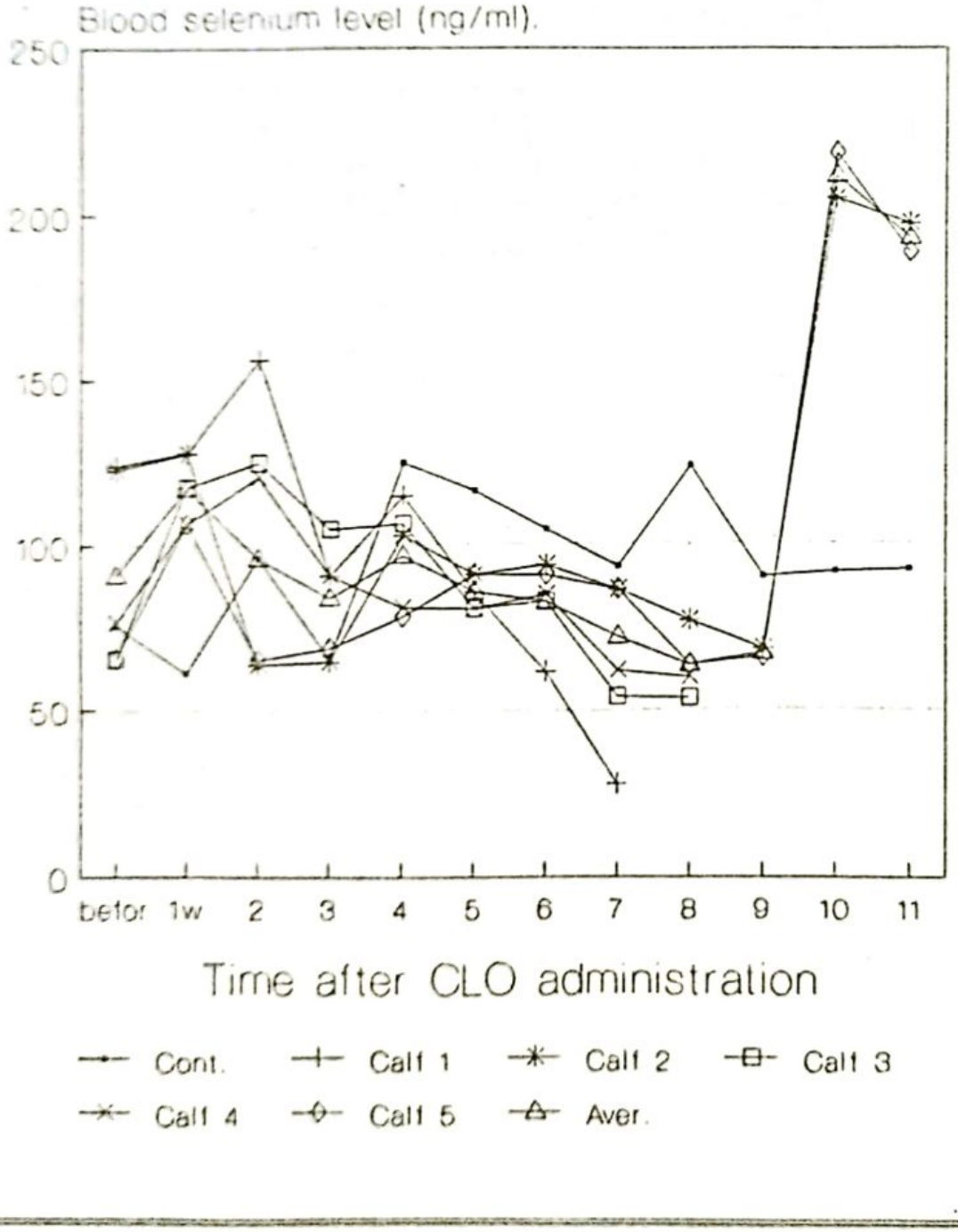
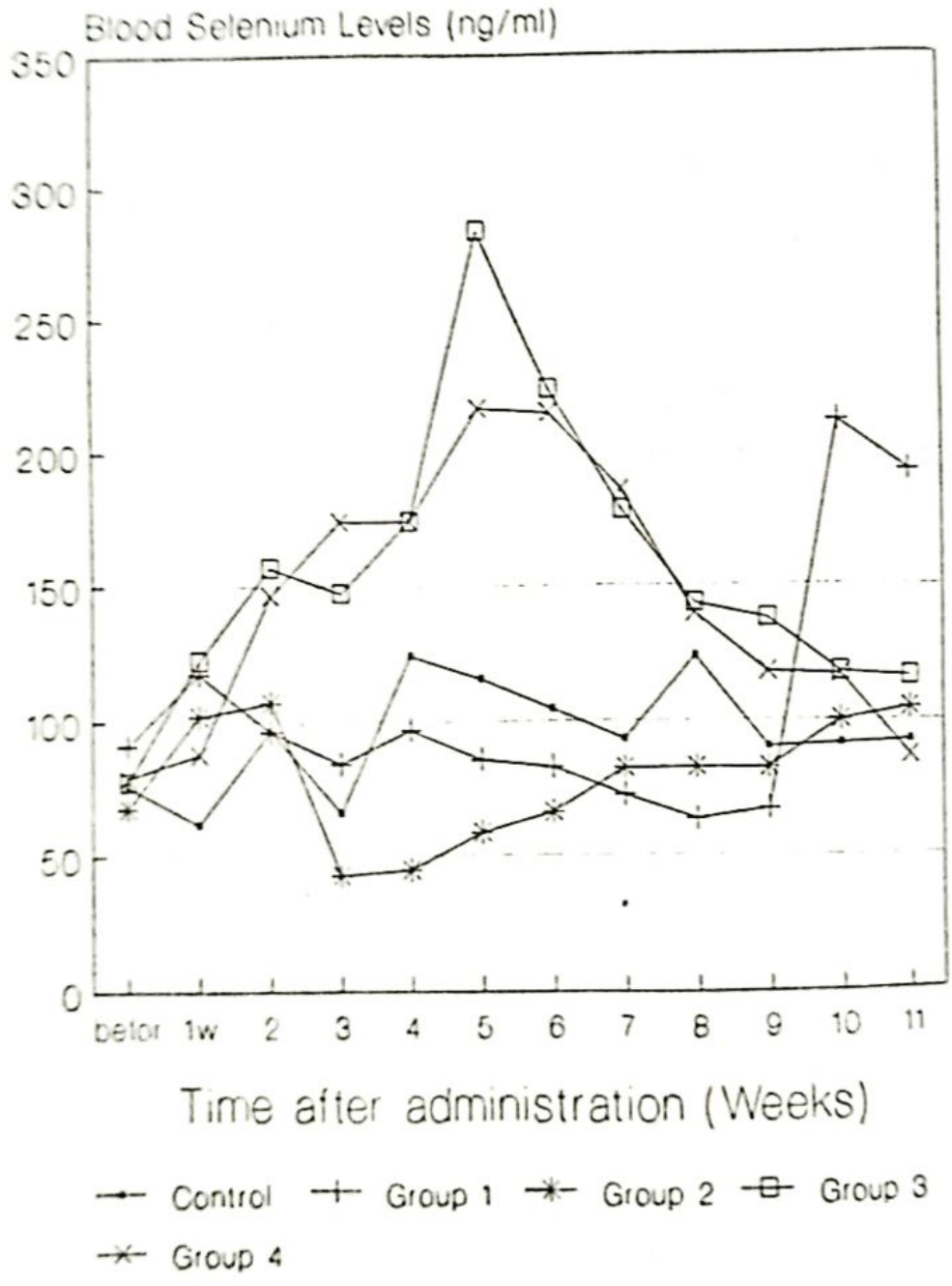


Fig. (3): Blood selenium levels (ng/ml) in Cod Liver Oil (CLO) treated groups of buffalo calves



Group 1 Control group
Group 2 CLO treated group
Group 3 CLO + Vit. E treated group
Group 4 CLO + Vit. E + Se treated group

Oskanen (1973) and McDowell (1989), mentioned that vitamin E is known to reduce selenium requirement in at least two ways. First by maintaining body selenium in an active form or preventing loss from the body and second by preventing destruction of membrane lipids within the membrane, thereby, inhibiting production of hydroperoxides and reducing the amount of glutathione peroxidase formed in the cells.

On contrary, Maplesden and Loosli (1960); Blaxter, (1962); Hartley, (1963); Sondergaard (1967) and Jenkins et al., (1970), mentioned that selenium is not effective against NMD produced experimentally using CLO. Therefore the reported elevation of whole blood selenium in the third sub-group of CLO plus vitamin E which begin 7 weeks of administration snd till end of the experiment may be attributed as Whanger (1970) who suggested, in the young prerumenants lambs and calves that selenium deficiency exert damaging effect more frequently than in older animals and with development of rumen affected animals may recover. McDowell (1989), emphasized that vitamin E is known to reduce selenium requirements.

From the above mentioned data, it could be generally concluded that whole blood selenium level was significantly low in NMD naturally affected buffalo-calves. Although gradual decrease was observed in whole blood selenium levels in both CLO and CLO plus vitamin E treated groups, inclusion of selenium and selenium plus vitamin E to CLO treated calves were on contrary accompanied with gradual increase in its levels. As it was expected that inclusion of vitamin E, protect the calves from dystrophic action of polyunsaturated fatty acid of CLO, one of the worthnoting unexpected finding in our study is that selenium plays the same role in protecting calves from dystrophic action of CLO. In cod liver oil treated group, it was found that while very low blood selenium level was associated with cases of NMD which either die suddenly or

even during treatment trials, with mainly the cardiac form of the disease, relative low blood selenium was accompanied with cases of the disease which respond to treatment and suspectd to be the skeltal form of the disease.

SUMMARY

1. Whole blood selenium level was found to be singificantly low in calves naturally affected with NMD.

2. On inducing the disease expermentally among suckling buffalo-calves, using cod liver oil (CLO), gradual decrease was observed in whole blood selenium levels in both CLO and CLO plus vitamin-E treated gorups.

3. Inclusion of selenium and selenium plus vitamin E to CLO treated groups, was accompanied with gradual increase in whole blood selenium level.

4. One of unexpected finding in our study is that selenium play the same role of vitamin-E in protecting calves from dystrophic action of cod liver oil.

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