Egyptian J. Anim. Prod. (2003)40(2):73-87.

STUDIES ON SELENIUM STATUS OF SUCKLING BUFFALO CALVES: II- EFFECT OF EXERCISE TIME AND FEEDING LEVELS ON PLASMA SELENIUM CONCENTRATIONS, ENZYME ACTIVITIES AND THE INCIDENCE OF WHITE MUSCLE DISEASE.

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SUMMARY

Two trials with newborn buffalo calves were conducted to determine the effects of exercise time and feeding levels on selenium (Se) status and the incidence of white muscle disease (WMD). In trial I, twenty six newborn buffalo calves (one week old) were assigned to three groups: group 1 was allowed to exercise from 2nd week of age; group 2 was exercised from 5th week and group 3 was exercised from 7th week. The exercise time was for 5 hours daily. The calves were fed whole milk, starter and berseem. The experiment continued until weaning calves at 105 days of age. In trial II, twenty-four newborn buffalo calves (one week old) were divided into three groups. Group 1 was fed whole buffalo milk at 7% of live body weight (as low level of milk feeding), group 2 was offered 10% milk (as a control) and group 3 was fed 13% milk (as a high level of milk feeding) in addition to calf starter and berseem. Blood samples were collected from the jugular vein at 1, 5, 9 and 13 weeks of age. In the exercise trial, the Se concentration in the 3rd group was higher than those in other groups at 5 and 9 weeks of age but without significant differences among groups. Plasma creatine phosphokinase (CPK), lactic dehydrogenase (LDH) and glutamic oxaloacetic transaminase (GOT) were not affected significantly by exercise times, however the group exercised from 2nd week had higher level of CPK, LDH and GOT activities than other groups. Clinical symptoms of white muscle disease (WMD) had appeared on two calves in the first group and three calves in the third group. All these cases were treated with Se and vitamin E and recovered. In the feeding trial, the daily gains of calves increased significantly with increasing milk feeding levels (0.45, 0.52 and 0.61 kg, respectively). Plasma Se concentrations were higher in the first group (fed 7% milk) than other groups at 5 and 9 weeks of age but without significant differences among groups. The CPK, LDH and GOT did not differ significantly among groups and the lower activity of CPK and GOT were found in the first group. The risk of WMD symptoms appeared on one case in the third group (fed 13% milk) and it responded to Se and vitamin E treatments and recovered.

Keywords: Selenium, feeding level, enzyme activities, white muscle disease, buffalo cows

INTRODUCTION

The suckling period is considered to be the most critical period in which most of the cases of white muscle disease (WMD) appear in animals (Pehrson, 1993). There are some managerial and nutritional factors, which provoke the risk of Se and vitamin E deficiency symptoms. It is proposed that unaccustomed exercise after confinement has a major role in the etiology of clinical myodegeneration in cattle (McMurray *et al.* 1983). Physical exercise increases the oxygen consumption of muscles several folds that of the rest state, thus leading to oxidative stress and increasing free radical generation (Clarkson, 1995).

Rapid growth has been implicated as a provoking factor in the etiology of white muscle disease (Pehrson, 1993). However, no systematic experimental data has been conducted to test the effect of growth rates or exercise on Se and vitamin E deficiency symptoms of suckling animals. Therefore, the main objective of this study was to investigate the effect of exercise times or varying growth rates on plasma Se concentrations, enzyme activities and the appearance of WMD during the suckling period of buffalo calves.

MATERIALS AND METHODS

Trial I: "Exercise Time":

Twenty sex newborn calves were assigned to three experimental groups according to the body weight. They were allowed start exercising either from the second week of age (group 1), the fifth week (group 2) or the seventh week (group 3). The exercised animals were left in yards after morning suckling from about 9 a.m. until 2 p.m. Sometimes the animals were stimulated to move.

The calves were kept in individual concrete floor pens (140 x 120 x 106 cm) which were layered with rice straw. The animals were fed on whole buffalo milk at the rate of 10% of body weight from the starting till 5th week of age and then the amount of milk was reduced until weaning at 15 weeks of age (Table 1). The daily amount of milk was divided into two equal portions fed at 8.0 a.m. and 3.30 p.m. using plastic buckets and teats. The amount of milk fed to each calf was adjusted weekly according to the changes in body weight. Calf starter and green berseem, *Trifolium alexandrinum*, (or berseem hay) was offered for all calves *ad libitum* from the 2nd week of age.

Trial II: "Feeding Rates"

Twenty-four newborn buffalo calves (12 *males* and 12 *females*) were assigned at the age of one week according to sex and body weight to three experimental groups (8 animals in each) as following:

1- Group (1) (low feeding) fed milk at the rate of 7% of their body weight with *ad. Libitum* feeding on calf starter and green berseem or berseem hay.

2- Group (2) (control group) fed milk at the rate of 10% of body weight with *ad libitum* feeding on calf starter and green berseem or berseem hay.

3- Group (3) (high feeding), the animals were fed milk at the rate of 13% of body weight with *ad libitum* feeding on calf starter and green berseem or berseem hay.

Table 1. Feeding regimen on whole buffalo milk during the 1st (as in group 2) and 2nd trials from the first week of age until weaning

Age (Weeks)	Amount of milk, % of body weight					
	1st group (7%)		2nd group (10%)		3rd group (13%)	
	a.m	p.m	a.m	p.m	a.m	p.m
1	*Colostrum*					
2-4	4	3	5	5	7	6
5	3	3	5	4	6	6
6	3	3	4	4	6	5
7	3	2	4	3	5	5
8	3	2	3	3	5	4
9	2	2	3	2	4	4
10	3	1	2	2	4	3
11	3	-	3	1	5	1
12	3	-	3	-	5	-
13	2	-	2	-	4	-
14	2	-	2	-	3	-
15	1	-	1	-	2	-
16	*Weaning*					

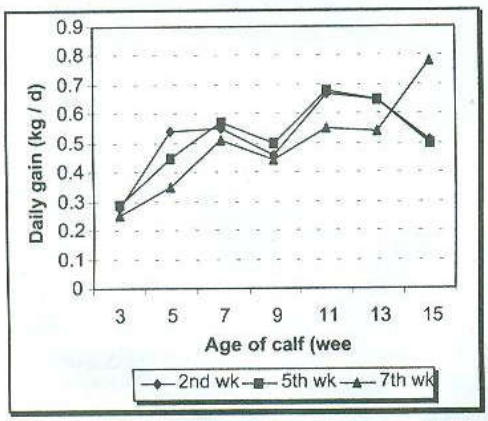

Fig. 1. Effect of exercise time on daily weight gains (kg/d) of buffalo calves.

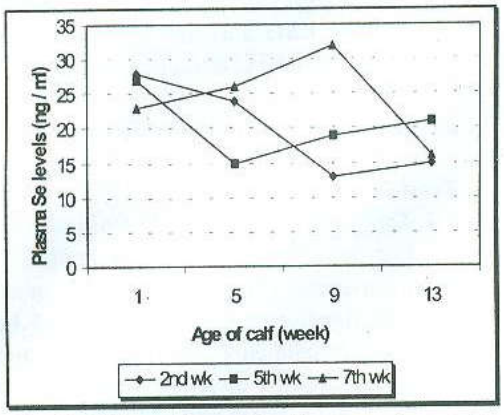

Fig. 2. Effect of exercise time on plasma Se concentration (ng/ml) of buffalo calves.

After the fourth week of experiment milk feeding rates were gradually decreased in all groups until weaning at the age of 15 weeks (Table 1).

Housing and system of feeding was similar to the first trial. After the fourth week of age the animals of all groups were allowed to have exercise for 5 hours daily except in bad weather days as the calves were kept indoors. The animals were weighed at one week of age and then at biweekly intervals until weaning.

Blood samples were collected (in both trials) from the jugular vein in heparenised tubes at start of experiment and then at 3, 5, 9 and 13 weeks of age. Blood samples were centrifuged at 2500 r.p.m. for 15 minutes. The obtained plasma was utilized immediately to assay creatine phosphokinase (CPK), and lactic dehydrogenase (LDH) using CPK and LDH colourimetric endpoint kits (Stanbio Laboratory Inc., USA). Glutamic oxaloacetic transaminase (GOT) activity was measured according to Reitman and Frankel (1957).

Selenium was determined in plasma, milk, starter and forage samples by fluorometric method according to Olson *et al.* (1975), with slight modification since buffer solution (the hydroxyl amine-EDTA) was used as recommended by the A.O.A.C. (1980). The excitation of Se complex was performed at 365 nm., and the emission was measured at 525 nm. using a fluoro-spectrophotometer (Model ANA-40, Japan).

The data of daily weight gains in trial I (exercise starting time) were analyzed using the factorial analysis of variance 3 (exercise starting time) x 2 (sex of calf) x 7 (times) utilizing MSTATC computer package (1984). The selenium concentration data were analyzed using factorial analysis of variance 3 (exercise starting time) x 4 (sampling times), as well as the enzyme activity of CPK, LDH and GOT (3 x 5) using the same program package.

The data of daily weight gains and plasma CPK in trial II (milk feeding rates) were analyzed using the same program used in the exercise trial. Whereas, the data of plasma Se levels, GOT and LDH activity were analyzed by factorial analysis of variance 3 (milk feeding rates) x 5 (sampling times) utilizing model (1) in Harvey's program (1990).

The overall means were compared using Duncan's multiple range test (Duncan, 1955). The LSD and the correlation coefficient between enzymes were calculated using the MSTATC computer package (1984).

RESULTS AND DISCUSSION

Trial I:

1. Daily weight gains of buffalo calves

The overall means of daily gains were 0.53, 0.52 and 0.49±0.03 kg/day for the groups exercised from second, fifth, or seventh week of age till weaning, respectively without significant differences among them. Slight differences were found between male and female calves in different groups in this respect. Also, the mean daily gains increased with the advancement of age, but without significant differences. Whereas, the daily gain increased significantly ($P<0.001$) in all groups from the second week till weaning and the highest increase was between 11-13 weeks old. The interaction between treatment and age of calves is shown in Fig. (1). It was clear that the third

group was the lowest group in daily gain at different times, but it increased significantly (P=0.108) than other groups at weaning. Salama and Mohy El-Deen (1993) and Abd El-Hady (1996) found similar trends with suckling buffalo calves.

3. Plasma Selenium concentration

Means of Se levels were 20.0, 20.3 and 24.0±1.8 ng/ml for groups exercised from 2nd, 5th and 7th week, respectively without significant differences among treatments. Whereas, the interaction between exercise time and age of calf was significant (P=0.008) since in the first group Se level declined gradually from second week till 13th week of age, while in the second group Se level declined in fifth week then elevated thereafter (Fig. 2). However, in the third group, Se concentration increased gradually from first week till 9 week old then it declined. Confinement of calves of third group during the first six weeks of age might caused low Se exhaustion with higher levels in blood plasma. The overall mean of Se levels decreased significantly (P=0.035) with age of calf (26.0 *vs.* 17.0 ± 2.1 ng/ml). These results are in agreement with those recorded by Weiss *et al.* (1983) who found that serum Se levels declined linearly from 24.0 to 18.8 ng/ml from birth to day 56 of age. A similar trend was found by El-Ayouty *et al.* (1996). In the present trial, it was observed that the overall mean of Se concentration was 22.0±1.0 ng/ml and it declined to less than 20.0 ng/ml in most times. This may indicate that most animals were in Se deficient status. Bostedt and Schramel, (1990) Van Saun (1990) and Gerloff (1992) suggested that serum Se level in calves or cattle lower than 40 ng/ml is indicative of Se deficient status.

4. Plasma enzymes activity

It was observed that, the activities of CPK, LDH and GOT were not significantly affected by exercise time (Fig 3, 4 and 5). However, the obvious trend was with the first group (exercised from the second week) since it was higher than other groups in CPK (as 562.7 *vs.* 538.3 and 524.0 IU/l) and GOT (as 86.8 *vs.* 72.2 and 67.3 IU/l) activities. While, the second group (exercised from 5th week) was the highest in LDH activity (as 576.0 *vs.* 525.7 and 484.3 IU/l). On the other side, the lowest group in all enzymes was the 3rd group that was exercised from 7th week till weaning. This may be a result of exercise time since the longer exercise time the higher enzymes activity and *vice versa*. The enzyme activity of CPK, GOT and LDH increased gradually and reached the maximum levels at 5-9 weeks old then declined to the minimum level at the last sampling time.

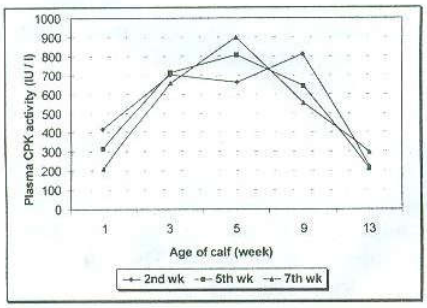

Fig. 3. Effect of exercise time on plasma CPK activity (IU/l) of buffalo calves

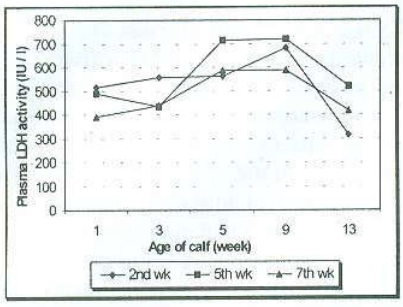

Fig. 4. Effect exercise time on plasma LDH activity (IU/l) of buffalo calves.

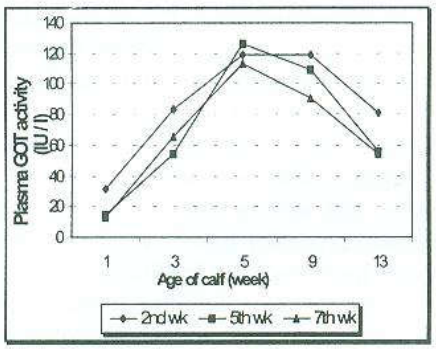

Fig. 5. Effect of exercise time on plasma GOT activity (IU/l) of buffalo calves.

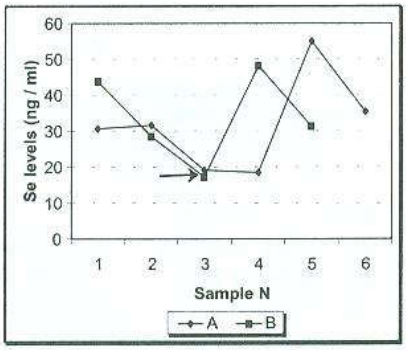

Fig. 6.The course of plasma Se concentrations in different clinical cases of WMD (Calf No. A and B) (the arrow indicates the time of symptoms beginning)

Pehrson *et al.* (1986) reported that serum CPK activity between 200-300 IU/l is indicative of subclinical WMD and the activity of 1000 IU/l is indicative of clinical status of myopathy. Also, Maas (1990) showed that GOT activity of 300-500 IU/l is associated with clinical stage of WMD, whereas the normal level of GOT activity in calves is usually less than 100 IU/l (Blood *et al.*, 1988). According to these studies, most of the calves in this trial were under Se deficiency and in subclinical status of WMD.

5. Clinical Signs of WMD

During the course of the exercise trial, symptoms characteristic of WMD had appeared on a number of calves. Table (2) shows the number of animals affected by WMD and responded to Se and vitamin AD_3E injection and oral supplementation. In the group exercised from 2nd week, two clinical cases (No. A and B) appeared (representing about 22.2% of the total number of this group). The symptoms of WMD in this group were manifested in listlessness, difficulty in standing without assistance, inability to walk, stiffness, unsteady gait, lameness and pulling the hind legs. Also, swelling was noticed in the rump muscle accompanied with pain. Body temperature was elevated to 39.4°C and 39.9°C in both cases, respectively. However, the affected animals were able to suckle. Plasma Se concentration was declined to 18.5 and 17.2 ng/ml in cases No. A and B, respectively at first appearance of the symptoms (Fig. 6). The GOT activity was elevated to a maximum level in both cases (160 and 180 IU/l, respectively) also, LDH was elevated to about 900 IU/l in case No. (A), whereas CPK activity was about 600 IU/l (Fig. 7-8). They responded to Se injection and oral vitamins AD_3E supplementation. The mean of recovery duration was 8 days. In the 3rd group (exercised from the 7th week), 3 clinical cases of WMD (C, D and E) had appeared representing about 33% of the group. The first case (No. C) showed the same mentioned symptoms with more swelling of rump muscle, arched back, quick breath and rise in the body temperature to 39.9°C. The plasma Se level at onset of the symptoms was 18 ng/ml (Fig. 9), whereas CPK, LDH and GOT were elevated to levels of 1107, 888 and 187 IU/l, respectively (Fig. 10-12). This

case responded to treatment with Se and vitamin E and recovered within 12 days, but after about 25 days from recovery, it manifested WMD symptoms again (swelling of rump, listlessness, lameness, stiffness and the animal remained in recumbence and prostrate state).

Table 2. The clinical case number of WMD (% of incidence of group) recovered case number and the duration recovery (day) in both exercise and feeding rates trails of buffalo calves

Items	Exercise from			Feeding rate		
	2nd wk	5th wk	7th wk	7%	10%	13%
No. of animals	9	8	9	8	8	9
Clinical cases	2	0	3	0	0	1
Male	1	0	3	0	0	1
Female	1	0	0	0	0	0
% of incidence	22.2	0	33.3	0	0	11.1
Recovered cases No.	2	0	2	0	0	1
Mean of duration recovery (day)	8	0	18	0	0	9

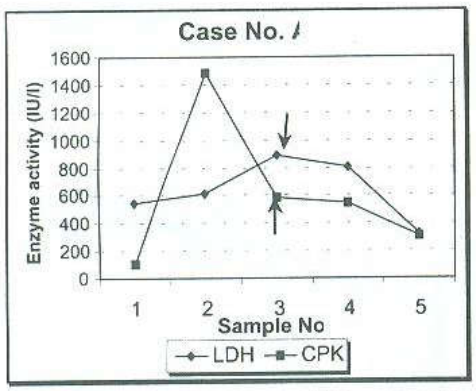

Fig. 7. The course of plasma CPK and LDH activity in clinical case of WMD (Calf No. A)(the arrow indicates the time of symptoms beginning).

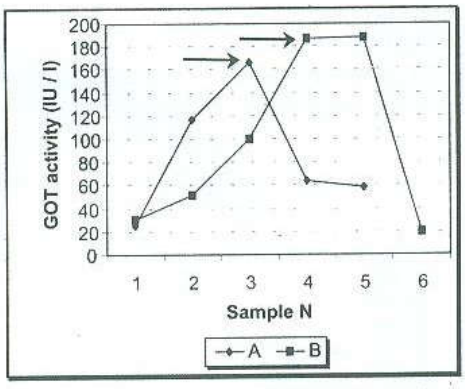

Fig. 8. The course of plasma GOT activity in different clinical cases of WMD (Calf No. A and B) (the arrow indicates the time of symptoms beginning).

At this time Se level was 45 ng/ml and the activities of CPK, LDH and GOT were 1300, 549 and 75.3 IU/l, respectively. The animal was treated with "Se, Neuril, as diazepam to muscle relaxation, and Duphalyte, as a source of vitamin B complex, electrolytes, dextrose and amino acids" but it did not respond and died in the 2nd day. The second case (No. D) showed slight myopathy symptoms as listlessness, lameness, unwilling to move and reduced growth rate. The Se level was 21.5 ng/ml

and CPK, LDH, GOT activities were 1134, 723 and 222 IU/l, respectively (Fig. 10-12). The animal recovered after five days without any treatment. The third case (No. E) in this group showed the same symptoms, as well as swelling in rump muscle and between thighs and increased respiratory rate. The body temperature was 39°C. The Se concentration was 20.6 ng/ml whereas, CPK and GOT activities were 1375 and 226 IU/l respectively (Fig. 9, 10 and 12). This case remained in a recumbency and prostration state and it was slower in the response to Se and vitamin AD_3E treatment. Ulcers appeared between legs. This animal recovered after 32 days.

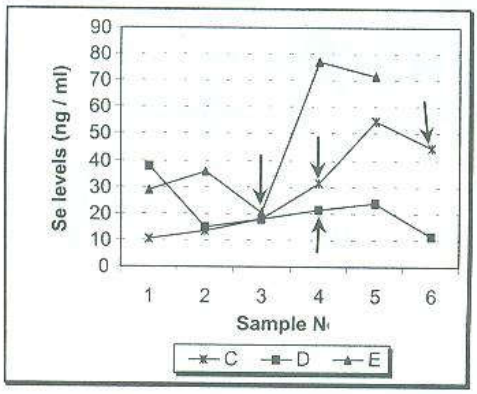

Fig. 9. The course of plasma Se concentration (ng/ml) in different clinical cases of WMD (Calf No. C, D and E) (the arrow indicates the time of symptoms beginning).

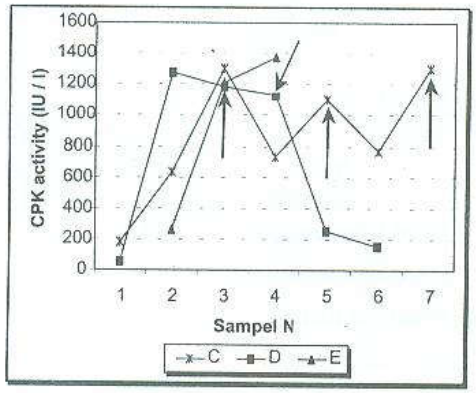

Fig. 10. The course of plasma CPK activity in different clinical cases of WMD (Calf No. C, D and E)(the arrow indicates the time of symptoms

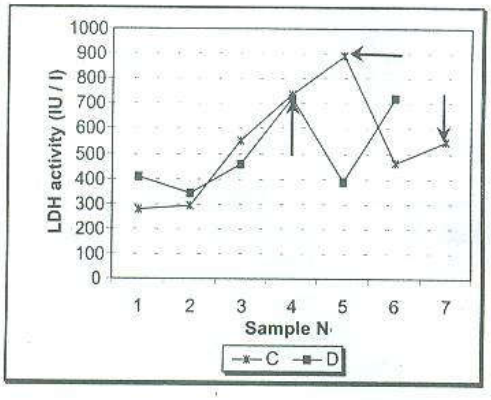

Fig. (11): The course of plasma LDH activity in different clinical cases of WMD (Calf No. C and D) (the arrow indicates to the time of symptoms beginning).

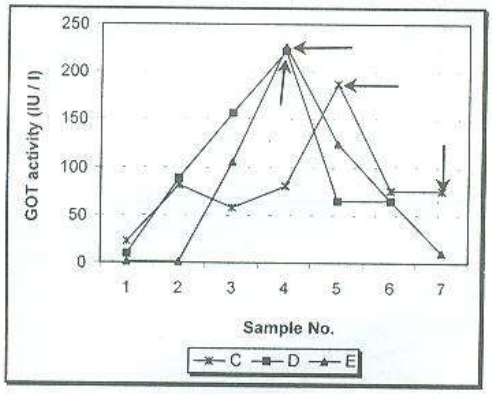

Fig. (12): The course of plasma GOT activity in different clinical cases of WMD (Calf No. C, D and E) (the arrow indicates to the time of symptoms beginning).

These findings are in agreement with those given by Pehrson *et al.* (1986), Blood *et al.* (1988) and Abd El-Hady (1996) in clinical findings and pathology of myopathy. Also, these results are in agreement with Allen *et al.* (1975) and McMurray *et al.* (1983) who reported that unaccustomed exercise have a major role in the development of clinical myopathy. On the other hand, Siddons and Mills (1981) showed that limited exercise about 1 hour/day for 2 weeks to bull calves (at 4 weeks old) did not induce clinical myopathy but only a transient small increase in serum CPK activity occurred. This may disagree with the obtained results which showed that CPK, GOT and LDH activities were increased gradually ($P<0.001$) in the mid exercise period (3-9 weeks old). It may be explained by increasing in exercise time that was at least 3 hours/day.

Trial II:

1. Body weight of buffalo calves:

At beginning of this trial, body weights were not significantly different among the experimental groups. Body weights of calves increased gradually to the end of experiment (Fig. 13). Male calves were more responsive to feeding rates than females. At the end of experiment, the differences in body weight between 7% and 10% level, and 10% and 13% level for male calves were 9.7 and 18 kg, respectively, whereas the corresponding values for females were 8 and 3.7 kg. The averages body weight as affected by milk feeding rate during the experiment were 56.9, 62.9 and 70.0 kg for groups fed at 7, 10 and 13% milk feeding rates with significant ($P<0.001$) differences among them. Male calves exhibited higher body weight than females (68.0 against 58.6 kg, $P<0.001$).

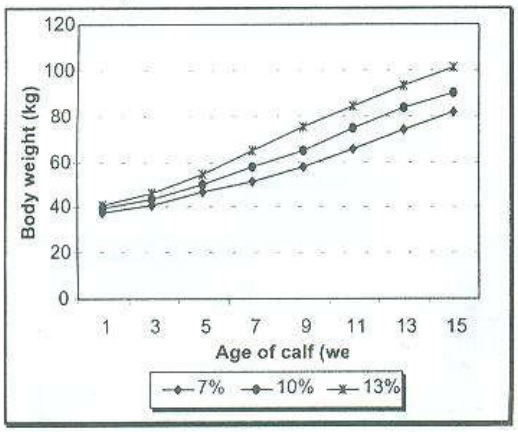

Fig. 13. Effect of milk feeding rate on body weight of buffalo calves.

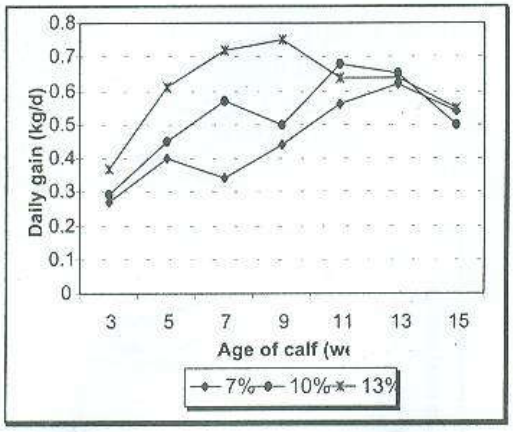

Fig. (14): Effect of milk feeding rate on daily weight gain (kg/d) of buffalo calves.

The average body weight of males surpassed significantly ($P<0.001$) body weight of females in the groups given 7 or 13% milk, although the difference in the group given 10% milk was not statistically significant compared with other groups. Thus it could be suggested that males could tolerate up to 13% milk feeding level, whereas it

is appropriate not to increase the feeding level of females more than 10%. These results agree with the findings obtained by Omer, (1998) in suckling Friesian calves.

2. Daily weight gains:

The effect of feeding rates on daily gains of buffalo calves is illustrated in Fig. (14). Daily gains increased paralleling the increase in milk feeding rates up to the ninth week of age. Afterwards, the differences in daily gains due to milk feeding rates decreased specially at 13 and 15 weeks of age. The mean daily gains recorded during the experimental period were 0.45, 0.52 and 0.61 kg for the groups fed 7%, 10% and 13% of milk (P<0.0001). The group fed 13% milk showed higher daily gains than the group fed 7% milk by about 35.5% whereas the group fed 10% milk was higher by about 15.5% only. Male calves exhibited higher daily gains than females (0.56 against 0.49 kg, P=0.027). The differences in daily gains between males and females increased as the milk feeding rate increased (the difference was 0.06 and 0.15 kg for 7, 10 and 13% groups, respectively.

Malik and Pasha (1988) found that the daily gains of buffalo calves was 0.50 kg/day when the calves were fed milk at 7% of body weight. Salama and Mohy El-Deen (1993) with buffalo calves and Omer, (1998) with Friesian calves found higher gains by increasing milk feeding rates. However, Abou-Selim *et al.* (1991) showed that the average daily gain of the suckling buffalo calves was not affected significantly by the amount of suckling milk (10% and 14% of body weight).

3. Plasma Selenium Concentration:

The average Se concentrations in blood plasma were 21.4, 20.4 and 20.7 ng/ml for groups fed 7, 10 and 13% milk, respectively without significant differences among groups. In the calves fed 10% and 13% milk there were decreases in plasma Se levels up to the ninth week of age whereas in the group fed 7% milk Se levels in plasma showed a rise at 5 weeks of age followed by decreases thereafter. These results might indicate that increasing feeding rates might cause increasing the requirement of Se. This agrees with the results of Kincaid and Hodgson (1989).

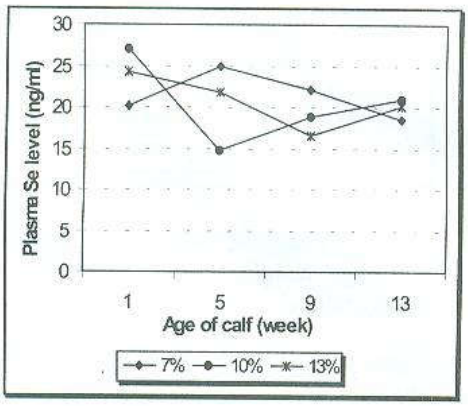

Fig. 15. Effect of milk feeding rate on Se concentration (ng/ml) of buffalo calves.

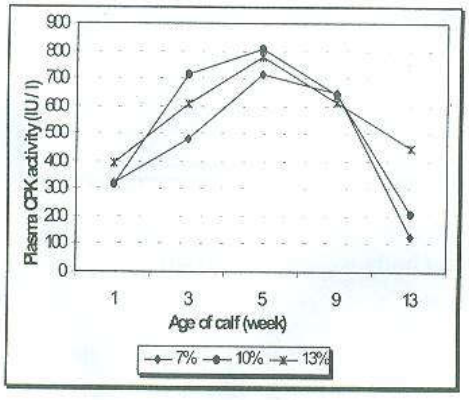

Fig. (16): Effect of milk feeding rate on Se concentration (ng/ml) of buffalo calves.

4. Plasma Enzyme Activity:

The activities of plasma CPK, LDH and GOT are illustrated in Fig. (16-18). There were no significant differences in CPK activity among feeding groups. Although, there was a tendency for higher CPK activity as the feeding rate increased. The mean CPK activity was increased by 17.8% as the milk feeding rate was elevated from 7 to 10% and a further increase of 5.5% by elevating the rate to 13%.

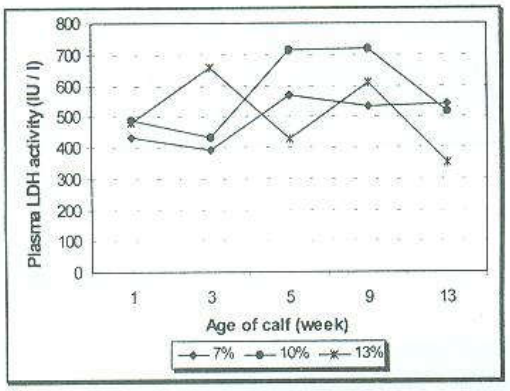

Fig. 17. Effect of milk feeding rate on plasma LDH activity (IU/l) of buffalo calves.

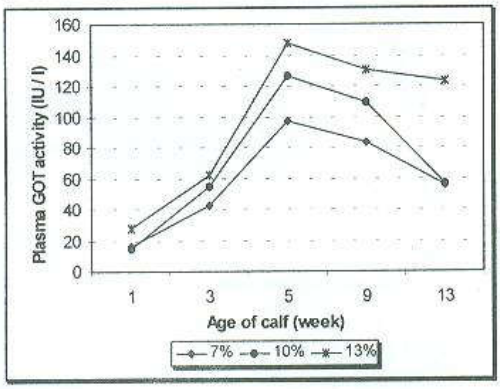

Fig. 18. Effect of milk feeding rates on plasma GOT activity (IU/l) of buffalo calves.

The highest LDH activity was noticed in the calves given 10% milk, but without significant differences compared with the other two groups. The activity of GOT was elevated as the milk feeding level increased and the differences between the groups fed 7% and 13% was significant. The activities of CPK, LDH and GOT reached the highest levels between 5^{th} and 9^{th} weeks of age and decreased afterwards.

From the previous results it appeared that CPK and GOT activities increased as the feeding rate was elevated. Thus the risk of incidence of subclinical and clinical white muscle disease increased as the feeding rate increased. A clinical case of WMD appeared in the group fed 13% milk. These results are in agreement with those given by Reddy *et al.* (1985); Kincaid and Hodgson, (1989) and Pehrson (1993) who reported that rapid growth rate increase the need for Se and vitamin E.

The symptoms started at the ninth week of age and were manifested in listlessness, lameness and dragging of the hind legs. The rectal temperature was elevated to 39.4°C. The activity of plasma GOT was elevated to 176 IU/l whereas Se levels decreased to 16.8 ng/ml (Fig. 19). The clinical case was injected with intramuscular dose of Se and a dose of vitamins AD_3E in milk. The animal responded within 2-3 days after the treatment.

From these results, it can be concluded that the high feeding rates and consequently high growth rates may provoke the appearance of the white muscle disease. However, the risk of sudden exercise is much greater in this respect. Attention to supplementation of Se and vitamin E may be recommended during the suckling period to avoid such disturbances.

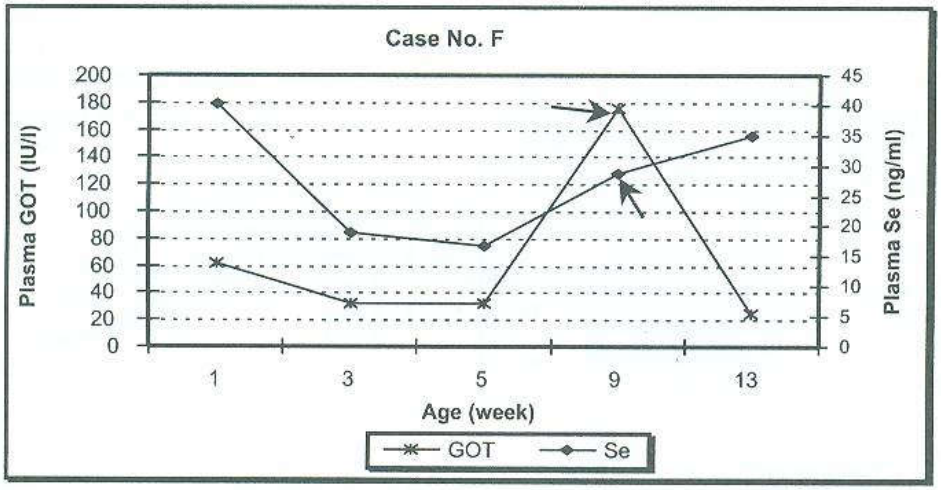

Fig. (19): The course of plasma Se concentration and GOT activity in a clinical case of WMD (Case No. F) (the arrow indicates the time of symptoms beginning).

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دراسات على السيلينيوم فى العجول الجاموسى الرضيعة ٢- تأثير وقت التريض ومستوى التغذية على اللبن على تركيز السيلينيوم ونشاط الإنزيمات فى بلازما الدم وعلى حدوث مرض العضلة البيضاء

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أجريت تجربتين على العجول الجاموسى الرضيعة لدراسة تأثير بدء وقت التريض ومستويات التغذية على اللبن على حالة السيلينيوم وحدوث مرض العضلة البيضاء، فى التجربة الأولى: تم توزيع ٢٦ عجل جاموسى (عمر أسبوع) على ثلاث معاملات: المجموعة الأولى سمح لها بالتريض من الأسبوع الثانى والمجموعة الثانية من الأسبوع الخامس والمجموعة الثالثة من الأسبوع السابع من العمر، وكان وقت التريض ٥ ساعات يوميا، وكانت العجول تغذى على اللبن والبادئ والبرسيم، واستمرت التجربة حتى الفطام على عمر ١٠٥ يوم. وفى التجربة الثانية وزع ٢٤ عجل جاموسي رضيع (عمر أسبوع) على ثلاث معاملات على أساس التغذية على اللبن بمعدل ٧ ،١٠ أو ١٣% من وزن الجسم بالإضافة الى التغذية على البادئ والبرسيم، واستمرت التجربة حتى الفطام على ١٠٥ يوم، وفى التجربتين تم تقدير وزن الجسم كل أسبوعين، وجمعت عينات دم من الوريد الوداجى عند ١، ٥، ٩،١٣ أسبوع من العمر لتقدير السيلينيوم (Se) ونشاط إنزيمات: الكرياتين فسفوكينيز (CPK) ، اللاكتيك ديهيدروجينيز (LDH) وكذلك الجلوتاميك أكسالوأسيتيك ترانس أمينيز (GOT) فى البلازما، بالإضافة الى تتبع حدوث حالات مرض العضلة البيضاء واستجابتها للعلاج.

وقد بينت نتائج التجربة الأولى أن تركيز السيلينيوم فى بلازما الدم فى عجول المجموعة التى تريضت من الأسبوع السابع من العمرأعلى عن القيم المماثلة للمجموعتين الأخرتين عند أعمار ٥ و٩ أسابيع ولكن الفروق لم تكن معنوية احصائيا، ولم يتأثر نشاط CPK ، LDH، GOT بتأثير وقت التريض، ولكن المجموعة التى تريضت من عمر أسبوعين قد مالت لإظهار نشاط إنزيمى أكبر عن المجموعتين الأخرتين، وقد ظهرت أعراض مرض العضلة البيضاء على حيوانين فى مجموعة التريض الأولىوثلاثة حيوانات فى مجموعة التريض الثالثة، وقد شفيت كل هذه الحالات بعد إعطاء السيلينيوم وفيتامين E .

وفى التجربة الثانية زادت معدلات النمو بزيادة اللبن المعطى فكانت: ٠,٤٥ ، ٠,٥٢، ٠,٦١، كجم/يوم للمجاميع المعطاة ٧، ١٠ ، ١٣% لبنا على الترتيب($P<0.01$) ،وكان تركيز السيلينيوم فى بلازما الدم مرتفعا فى المجموعة المعطاة ٧% لبنا عن المجموعتين الأخرتين عند أعمار ٥ و ٩ أسابيع؛ولكن بدون وجود فروق معنوية بين الجاميع، ولم يكن هناك فروق معنوية فى نشاط الإنزيمات بين المجاميع؛ ولكن المجموعة المعطاة ٧% لبنامالت لإظهار نشاط أقل من CPK و GOT ، وقد ظهرت حالة مرضية لمرض العضلة البيضاء فى المجموعة المعطاة ١٣% لبنا ولكنها استجابت لإعطاء السيلينيم وفيتامين E .

وقـــد استنتج من هذه الدراسة أن التريض المفاجئ للعجول الرضيعة قد يؤدى إلى إظهار حالات نقص عنصر السيلينيوم، وبالمثل فإن رفع معدل التغذية على اللبن يؤدى إلى تأثير مشابه؛ وبالتالى فينصح بإعطاء السيلينيوم وفيتامين E خلال فترة الرضاعة لتلافى هذه المشاكل.