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# STUDIES ON SELENIUM STATUS OF SUCKLING BUFFALO CALVES: I- EFFECT OF SELENIUM AND/OR VITAMIN E ADMINISTRATION ON GROWTH PERFORMANCE, PLASMA SELENIUM CONCENTRATIONS, ENZYME ACTIVITIES AND THE PREVENTION OF WHITE MUSCLE DISEASE

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## SUMMARY

*Thirty two newborn buffalo calves with an average live body weight of 40 kg were divided into four experimental groups: control group, without any treatment; selenium group, was injected with 5 mg selenium (Se)/head biweekly; vitamin E group, supplemented with 2000 IU of vitamin E weekly in milk and Se+E group, treated with both Se and vitamin E. All calves were fed whole milk, concentrate starter and berseem. Blood samples were collected from jugular vein of calves at 1, 3, 5, 9 and 13 weeks of age. The experiment continued until weaning calves at 105 days of age. The average daily gains were improved nonsignificantly by 6.4, 14.5 and 8.1% for Se, vitamin E and Se+E groups, respectively compared with control. Also, the respective treatments improved feed conversion by 17.99, 17.45 and 18.83%. Plasma Se concentrations of calves injected with Se or Se+E were significantly ($P<0.01$) higher than those treated with vitamin E or control group (32.9, 31.1 vs. 19.6, 18.7 ng/ml). Plasma creatine phosphokinase (CPK) and glutamic oxaloacetic transaminase (GOT) activities of the control group were significantly ($P<0.05$) higher than those of the treated groups. Lactic dehydrogenase (LDH) activity was fluctuated among groups. The hemoglobin, hematocrit, total protein, albumin and globulin levels were unaffected significantly by treatments. No clinical signs of white muscle disease appeared on any experimental animals.*

***Keywords:** Sukling, Vitamin E, Selenium, growth, while muscle disease, buffalo*

## INTRODUCTION

Selenium (Se) has been identified as trace element essential for life as it is involved in the composition of the enzymes glutathione peroxidase and tetraiodothyronine 5 -deiodinase (Awadeh *et al.*, 1998b). The glutathione peroxidase plays an important role in the breakdown of hydroperoxides, thus protecting the animals from damages of tissues as is manifested in the occurrence of nutritional myopathy (white muscle disease, WMD). This disease appears to be confined to the first period of life. The neonates walk with a stiff gait and arched backs, avoid movement, lose condition and die. They may have some degeneration of skeletal muscle but they do not always have cardiac lesions (Campbell *et al.*, 1990 and Abd El-Hady, 1996). This disease is characterized by an increase in the activity of creatine phosphokinase (CPK) and glutamic oxaloacetic transaminase (GOT) in plasma and may result in lameness or sudden death (Kennedy *et al.* 1987 and Blood *et al*, 1988).

Selenium concentration in most of feedstuffs in Egypt may be lower than the sufficient levels set at 0.3 ppm, which was recommended by NRC (1988). Thus, the transferred Se from dams to calf through placenta during the embryonic stage or through milk after birth is low.

Vitamin E has a synergistic relationship to Se in protecting tissues from damage of hydroperoxides as it terminates the free radical reactions and is considered as a lipophilic free radical scavenger (Rice and Kennedy, 1988). However, the placental transfer of vitamin E from the dam to its embryos is very limited (Van Saun *et al.*, 1989).

Several methods have been successfully used to supplement Se and vitamin E to animals. Selenium has been added to total ration, parenteral administration, ranged from 0.08-0.20 mg Se/kg body weight, added to the drinking water or by intra-reticulorumenal pellets (Weiss *et al.*, 1983; Campbell *et al.*, 1990). All these methods increased significantly serum and blood Se concentrations in cattle.

No or little studies have been conducted on the relationship between Se and vitamin E of suckling buffalo calves in Egypt. Therefore, the main objective of this study was to evaluate the influence of Se and/or vitamin E administration to suckling buffalo calves on their body weight gain, Se concentrations and some blood enzymes activity related to Se and vitamin E status and their values in protecting animals from WMD.

## MATERIALS AND METHODS

Thirty-two new born buffalo calves (16 males and 16 females) with an average initial live body weight of 40 kg at one week of age were used. They were divided into four groups according to sex and body weight (8 calves in each group). The first group was kept without any supplementation as control. The second group was injected intramuscularly with 5 mg selenium (Se)/head as sodium selenite (2.19g $Na_2SeO_3$/L bidistilled water equivalent to 1g Se/L) at biweekly intervals from the initiation of experiment until weaning. The third group was supplemented orally in milk with 2000 IU vitamin E/head as *dl-alpha*-tocopheryl acetate (Hydrovit E 15%, France) weekly. The fourth group was treated with Se and vitamin E at the same doses.

The calves were left with their dams for the first 7 days after birth to get colostrum. The calves were kept in individual concrete floor pens (140 x 120 x 106 cm) which were layered with rice straw. After the fourth week of age, all animals were allowed to have exercise for 3-4 hours daily, except in the case of bad weather times the animals were kept indoors. The animals were fed individually on whole buffalo milk using plastic buckets and teats. From the age of 2-4 week, each calf was fed on milk at the rate of 10% of its body weight. The daily amount of milk was divided into two equal portions fed at 8.0 a.m. and 3.30 p.m. The amount of milk was adjusted weekly according to the change of calf body weight. Starting from the fifth week, the amount of milk was reduced gradually by about 1% of body weight until weaning at 15 weeks of age. Calf starter and berseem *"Trifolium alexanrinum"* either fresh or as hay was offered for each calf *ad libitum*. Feed consumption was recorded daily for each calf. Water was available to calves twice daily. The ingredients and dry matter, crude protein and Se levels of calf starter, berseem and its hay are presented in Table (1). Weighing of the animals was performed weekly till weaning. Regular feed samples were analyzed for chemical composition according to A.O.A.C. (1980). Also, feed, milk and water samples were collected for Se determination according to Olson *et al.* (1975).

**Table 1. Chemical composition of experimental feeds**

| Parameters | Calf starter * | Clover hay | Berseem (Clover) | Buffalo milk | Drenching Water |
|---|---|---|---|---|---|
| **Dry matter (%)** | 89.0 | 91.0 | 16.0 | 16.8 | |
| **Crude protein (%)** | 17.6 | 13.3 | 15.5 | | |
| **Selenium (ppm)** | 0.058 | 0.047 | 0.061 | 0.028 | 0.009 |

* Composition: Yellow corn, 61.0%; Soybean meal, 25.5%; Wheat bran, 12%; Calcium carbonate, 1% and Sodium chloride, 0.5%.

Blood samples were collected from jugular vein of calves before treatment (1 week) then at 3, 5, 9 and 13 weeks of age in heparinized tubes after two hours of suckling and after one hour of starter and hay administration. Plasma was separated by centrifugation at 2500 r.p.m. for 15 min. and stored at -20°C till analysis. Selenium concentration was determined by the fluorometric method according of Olson *et al.* (1975). Creatine phosphokinase (CPK), lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT) activities were determined in fresh blood plasma, spectrophotometrically using CPK and LDH colorimetric endpoint kits at 500 nm (Stanbio Laboratory Inc., USA) according to the procedures outlined by the manufacturer. Also, plasma GOT activity was determined at 505 nm according to Reitman and Frankel (1957). Plasma total proteins and albumin content were determined in fresh samples using colorimetric kits (Stanbio Laboratory Inc, USA) at 550 nm according to the procedure outlined by the manufacture. Hemoglobin and hematocrit values were determined in fresh whole blood. All calves were observed for any health problems mainly the signs of WMD.

The data of live body weight were analyzed using the factorial analysis of variance 4 (Se and/or vitamin E treatment) x 2 (sex of calf) x 8 (times) utilizing MSTATC computer package (1984). Also The data of daily gains, total DM intake and feed conversion were analyzed by using the model: 4 (treatment) x 2 (sex of calf) x 7 (times). Plasma total proteins, albumin, globulin, A/G ratio, blood hemoglobin and hematocrit were analyzed using the model: 4 (treatment) x 2 (sex of calf) x 5 (times).

The data of selenium concentration and enzyme activity of CPK, LDH and GOT were analyzed using factorial analysis of variance 4 (Se and/or vitamin E treatment) x 5 (times) using model (1) in Harvy's program package (1990). The overall means were compared using Duncan's multiple range test (Duncan, 1955). The LSD and the correlation coefficient between enzymes were calculated by using the MSTATC computer package (1984).

## RESULTS AND DISCUSSION

### 1. Body weights and daily gains of buffalo calves

The results in Table (2) show that weaning weight of female calves was higher than male calves in all groups except for Se+E group, but without significant differences among them (106.8, 107.3, 110.5 and 103.8 kg *vs.* 94.5, 102.5, 108.8 and 107.5 ± 5.4 kg for control, Se, vitamin E and Se+E groups, respectively). The treatment with either Se or vitamin E or both had increased body weights of the male calves but had no effect on body weights of female calves. The overall mean of male calves was lower significantly ($P<0.05$) than females (67.8 *vs.* 70.7 ±1.0 kg). Whereas, the interaction between sex and treatment was not significant ($P=0.371$). There were no differences in body weight among groups with the advancement of age. However, the overall mean of body weight increased linearly and significantly ($P<0.0001$) with the advancement of age.

**Table 2. Effect of selenium and/or vitamin E administration on final body weight, daily gain, TDMI and feed conversion of buffalo calves**

| Items | Treatments | | | | SEM |
|---|---|---|---|---|---|
| | Control | Se | Vit.E | Se+E | |
| Initial body weight(kg): | | | | | |
| Male | 39.3 | 39.0 | 40.8 | 38.8 | 5.4 |
| Female | 40.3 | 40.8 | 39.5 | 41.8 | 5.4 |
| Weaning weight (kg): | | | | | |
| Male | 94.5 | 102.5 | 108.8 | 107.5 | 5.4 |
| Female | 106.8 | 107.3 | 110.5 | 103.8 | 5.4 |
| Daily gains kg/day: | | | | | |
| Male | $0.56^{B}$ | $0.65^{A}$ | $0.69^{A}$ | $0.70^{A}$ | 0.03 |
| Female | $0.68^{A}$ | $0.68^{A}$ | $0.72^{A}$ | $0.63^{AB}$ | 0.03 |
| Total DMI (kg/day): | | | | | |
| Male | $1.16^{c}$ | $1.17^{c}$ | $1.19^{c}$ | $1.28^{abc}$ | 0.05 |
| Female | $1.33^{ab}$ | $1.37^{ab}$ | $1.41^{a}$ | $1.25^{bc}$ | 0.05 |
| Feed conversion (kg DM/kg gain): | | | | | |
| Male | $2.83^{d}$ | $1.83^{e}$ | $2.00^{e}$ | $1.85^{e}$ | 0.22 |
| Female | $1.95^{e}$ | $2.10^{e}$ | $1.95^{e}$ | $2.03^{e}$ | 0.22 |

A and B values with different superscripts in the same row within item differ significantly ($P=0.064$).
a, b and c values with different superscripts in the same row within item differ significantly ($P=0.068$).
d and e values with different superscripts in the same row within item differ significantly ($P=0.042$).
SEM = Standard Error of Means

These findings are in agreement with those reported by El-Ayouty *et al.* (1991) who found that the body weight of calves injected with 5 mg Se/head at monthly intervals was higher than the untreated calves without significant differences. A similar trend was reported by Zachara *et al.* (1993) with lambs and El-Ayouty *et al.* (1996) with suckling buffalo calves. Whereas, Campbell *et al.* (1990); Maas *et al.* (1993 and 1994) and Awadeh *et al.* (1998b) found that supplementation with Se by different methods (reticulorumen bolus, parenteral or oral supplementation) did not affect body weight of calves.

The overall means of daily weight gain were 0.62, 0.66, 0.71 and 0.67 ± 0.02 kg for control, Se, vitamin E and Se+E, respectively. The daily gains were improved by Se, vitamin E and Se + vitamin E supplement by 6.4, 14.5 and 8.1% over control group, respectively. The difference in daily gains between vitamin E and control group was significant ($P=0.08$), whereas other differences were not significant. These results are in agreement with some studies in which Se supplementation increased (not significantly) body weight gains in different farm animals (Peter 1980; El-Ayouty *et al.* 1991 and 1996 and Zachara *et al.* 1993). Lee *et al.* (1985) found that supplementation of beef calves with 450 IU vitamin E increased body weight gains ($P<0.08$). Also, Reddy *et al.* (1985) found that oral supplementation with 1400 or 2800 mg/week or intramuscular injection of 1400 mg/week improved weight gains ($P=0.134$) by 25, 20 and 17.5% over untreated Holestein heifer calves, respectively. A significant improvement in body weight gains due to Se and/or vitamin E supplementation was noticed by Moser *et al.* (1977) in calves, Oh *et al.* (1976 a,b) and Rotruck *et al.* (1969) with lambs. Metry *et al.* (1998) found that Se injection (0.125 mg/kg of life B.W.) and/or 400 mg vitamin E in buffalo calves after weaning improved significantly body weight gains.

The daily weight gains of calves in all groups (Fig. 1) increased significantly with the advancement of age till weaning ($P=0.00$). The average weight gains of males was significantly ($P<0.05$) lower than females (0.65 vs. 0.68 kg/day). The males of control group exhibited lower gains than females in the same group and also less gain than calves in other groups.

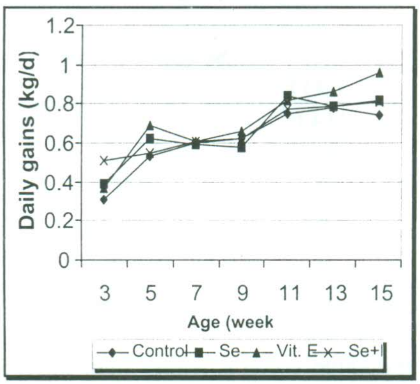


Fig. 1. Effect of Se and/or vitamin E treatment on daily weight gains (kg/d) of buffalo calves.

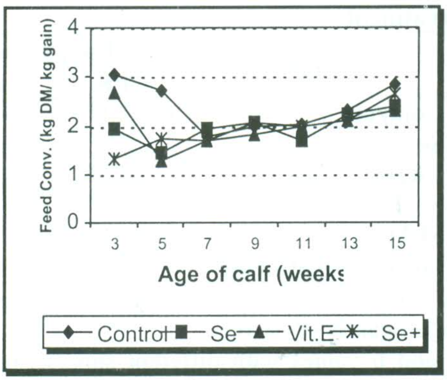


Fig. 2. Effect of Se and/or vitamin E treatment on feed conversion (kg DM /kg gain) of buffalo calves.

**2. Feed intake and feed conversion**

Total dry matter intake (TDMI) was not affected by treatments (Table 2). The obtained values were in the ranges reported for suckling Frisian calves (Omer, 1998). Weiss *et al.* (1983) found that Se and/or vitamin E treatment did not affect feed intake of neonatal heifer calves. However, Reddy *et al.* (1985) reported that vitamin E supplementation improved starter consumption (P=0.124)

The overall means of calculated feed conversion (FC) of buffalo calves was 2.39, 1.96, 1.98 and 1.94±0.16 kg DM/kg gain for control, Se, vitamin E and Se+E, respectively. The treatments with Se, vitamin E and Se + vitamin E improved (P=0.136) FC by 17.99, 17.15 and 18.83%, respectively compared with control group. These results agreed with those of Hicks (1985) who found that vitamin E supplementation improved feed conversion of calves by about 19%. However, Ochoa *et al.* (1992) reported no improvements in feed conversion in castrated rams treated with vitamin E.

**3. Selenium concentration in blood of calves**

The average Se concentration in the control group was 18.7 ng/ml, which was raised to 32.9 and 31.1 ng/ml in the groups treated with Se and Se+E, respectively as shown in Table (3). The average Se concentrations in blood plasma of (Se) and (Se + vitamin E) groups were significantly (P<0.01) higher than the Se unsupplemented animals (control and vitamin E groups). Selenium levels in blood plasma increased in Se supplemented groups during the course of experiment, whereas it decreased in Se unsupplemented groups at 5 and 9 weeks of age (Fig.3). Weiss *et al.* (1983) observed decreases in blood Se levels by advancement of age in calves.

Zachara *et al.* (1992); Mass *et al.* (1993) and Gant *et al.* (1998) observed increases in blood Se levels due to Se supplementation in different farm animals. However, Simpson *et al.* (1998) found that plasma Se concentration of calves was not affected by dl-α-tocopheryl acetate supplementation.

**Table 3. Least squares means of plasma CPK, LDH, GOT activities (IU/l) and Se concentrations of buffalo calves which affected by Se and/or vitamin E treatment**

| Groups | Enzyme activity (IU/l) | | | Se concentration (ng/ml) |
|---|---|---|---|---|
| | CPK | LDH | GOT | |
| **Control** | 447.6$^{a}$ | 470.5 | 114.6$^{A}$ | 18.7$^{B}$ |
| **Se** | 359.3$^{b}$ | 443.7 | 66.1$^{B}$ | 32.9$^{A}$ |
| **Vit. E** | 283.3$^{b}$ | 465.7 | 39.7$^{B}$ | 19.6$^{B}$ |
| **Se+E** | 283.0$^{b}$ | 440.6 | 46.0$^{B}$ | 31.1$^{A}$ |
| **SEM** | 30.0 | 22.4 | 9.3 | 1.7 |
| **Mean of treatments** | 343.3 | 455.1 | 66.6 | 25.6 |

A and B values with different superscripts in the same column within item differ significantly (P<0.01).

a and b values with different superscripts in the same column within item differ significantly (P<0.05).

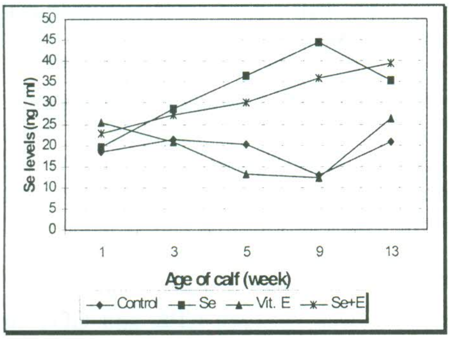


Fig. 3. Effect of Se and/or vitamin E on plasma Se concentration of buffalo calves with age advanced.

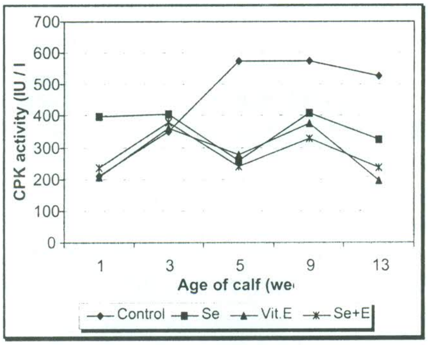


Fig. 4. Effect of Se and/or vitamin E on plasma CPK activity of buffalo calves with age advanced.

It is worth noting that even in the Se supplemented groups plasma Se levels were below the levels considered to reflect normal Se status (40 ng/ml) as suggested by Bostedt and Schraml (1990) and 70 ng/ml as proposed by Van Saun, (1990) and Gerloff, (1992). Thus, the treatment with 5 mg Se/animal at biweekly intervals appears to be insufficient dose for the suckling buffalo calves. However, the animals remained clinically normal during the course of experiment.

**4. Plasma enzymes activity**

The activity of CPK, LDH and GOT are presented in Table (3). The activity of CPK was lower in vitamin E and Se + vitamin E groups ($P<0.01$) than in control group, as well as in Se group ($P<0.05$) and was also lower in (Se) group than in control ($P<0.05$). The differences among treated groups were not significant, although vitamin E supplemented groups tend to reflect lower level than (Se) group. Whanger *et al.* (1977) found that vitamin E was more effective in reducing CPK activity than Se treatment. The results agreed with those given by Horton *et al.*, 1978; Reddy *et al.*, 1985 and Norton and McCarthy, 1986 who showed that Se and/or vitamin E decreased significantly ($P<0.05$) CPK activity. Figure (4) shows the changes in CPK activity during the experiment. Activity of CPK in the control group increased linearly and significantly ($P<0.05$) to reach the maximum levels at 5 and 9 weeks of age (above 570 IU/l) then it decreased slightly. Although no clinical cases of WMD were observed in this experiment, the higher values in control group are considered to reflect subclinical WMD (Pehrson *et al.*, 1986 and Walsh *et al.*, 1993). Figure (5) shows the changes in LDH activity during the experiment. The differences among groups in LDH activity were not significant.

The activity of plasma GOT in control group was significantly higher than other groups. The differences among other treated groups were not significant. However, it appeared that vitamin E alone was more effective than Se in reducing GOT which agreed with the results of Whanger *et al.* (1977). Treatments with Se and/or vitamin E were shown to decrease GOT in plasma (Whanger *et al.*, 1977 ; Horton *et al.*, 1978 and El-Ayouty, 1992). Figure (6) depicts the changes in GOT activity throughout the trial. The GOT activity in plasma of control group increased linearly and arrived to the maximum level (208.4 IU/l) at 9 weeks of age. This might indicate subclinical WMD according to Blood *et al.* (1988) and Maas (1990).

The correlation among enzyme activities was analyzed statistically in each group. The correlation coefficient between CPK and LDH in either vitamin E or Se+E groups was not significant. The correlation between CPK (X) and GOT (Y) activities was highly significant (P=0.000) in both control and Se groups, as in the following equations, respectively:

$Y = 21.9 + 0.207\ X$ (n = 40) (r =0.56±0.05)

$Y = 2.2 + 0.178\ X$ (n = 40) (r=0.62±0.037)

It may be concluded that the highest correlation was between CPK and GOT in control and Se groups, consequently, they may be more sensitive to detect clinical and subclinical myopathy in buffalo calves. These results are in agreement with those reported by Blood *et al.* (1988).

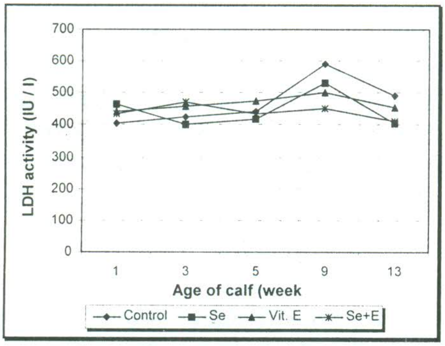


Fig. 5. Effect of Se and/or vitamin E treatment on plasma LDH activity of buffalo calves with age advanced.

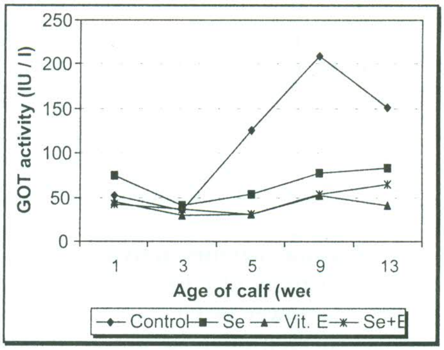


Fig. 6. Effect of Se and/or vitamin E treatment on plasma GOT activity of buffalo calves with age advanced.

**5. Blood constituents**

Total proteins in blood plasma, albumin (A), globulin (G) and A/G ratio values were not significantly affected by either Se and/or vitamin E supplementation (Table 4). This is in agreement with the results of Reedy *et al.* (1985) who found no effect of vitamin E supplementation on plasma total proteins and albumin of calves. Also, the obtained results agree with those given by Awadeh *et al.*, ($1998_a$). On the other hand, Metry *et al.* (1998) found that the treatment with vitamin E had more obvious effect on albumin and consequently increased A/G ratio. Both total proteins and globulin contents decreased ($P<0.05$ and $P<0.01$, respectively) with the advancement of age (Fig. 7 and 9), while albumin fraction increased significantly (Fig. 8 and 10, $P<0.001$).

Hemoglobin concentrations and hematocrit values were not affected significantly by Se and/or vitamin E supplementation (Table 4). Both hemoglobin and hematocrit values were higher at the older ages (9 and 13 weeks) than at earlier ages. This might reflect higher consumption of starter at the later ages. These results are in agreement with those reported by Abd El-Hady (1996). The female calves had more hemoglobin concentrations than males (12.9 vs. 11.7 g/dl, P=0.004). The females exhibited higher values of hematocrit than males (35.9 vs. 34.2%, P=0.058).

**Table 4. Effect of Se and/or vitamin E supplementation on some blood constituents of buffalo calves**

| Items | Treatments | | | | SEM |
|---|---|---|---|---|---|
| | Control | Se | Vit. E | Se+E | |
| **Total protein (g/dl)** | 6.8 | 7.0 | 6.8 | 7.0 | 0.18 |
| **Albumin (g/dl)** | 3.6 | 3.6 | 3.6 | 3.7 | 0.10 |
| **Globulin (g/dl)** | 3.2 | 3.4 | 3.2 | 3.2 | 0.17 |
| **A/G ratio** | 1.3 | 1.2 | 1.3 | 1.4 | 0.11 |
| **Hemoglobin (g/dl)** | 12.5 | 11.9 | 12.7 | 12.2 | 0.42 |
| **Hematocrit (%)** | 34.4 | 35.6 | 34.4 | 35.8 | 0.91 |

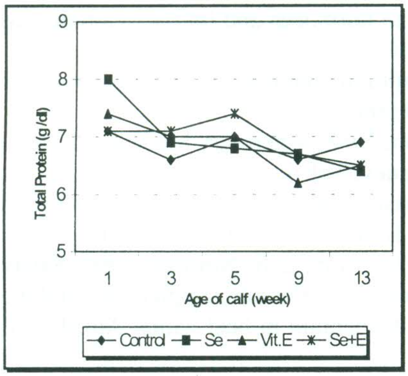


Fig. 7. Effect of Se and/or vitamin E treatment on plasma total protein content of buffalo calves with age advanced.

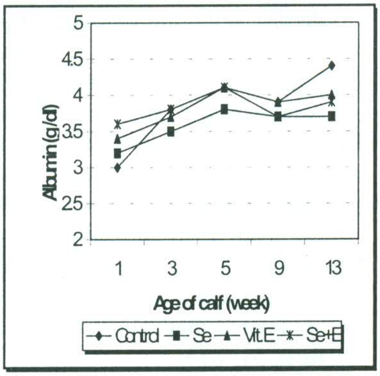


Fig. (8): Effect of Se and/or vitamin E treatment on plasma albumin content of buffalo calves with age advanced.

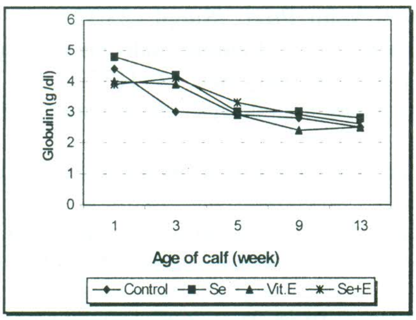


Fig. 9. Effect of Se and/or vitamin E treatment on plasma A/G ratio of buffalo calves with age advanced.

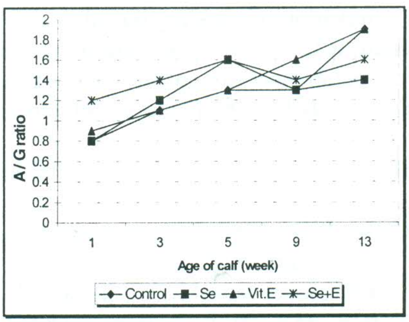


Fig. 10. Effect of Se and/or vitamin E treatment on plasma A/G ratio of buffalo calves with age advanced.

From the forgoing results, it could be concluded that suckling buffalo calves treated by 5 mg Se doses at biweekly intervals with or without vitamin E increases significantly plasma Se levels. But it appears that higher doses may be needed to further elevate plasma Se levels to the recommended adequate range (40 - 70 ng/ml) given by Van Saun (1990).

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# دراســات علــى السيلينيوم فى العجول الجاموسى الرضيعة ١- تأثير إعطاء السيلينيوم و/أو فيتامين هــ على كفاءة النمو وتركيز السيلينيوم ونشاط الإنزيمات فى بلازما الدم وعلى الوقاية من مرض العضلة البيضاء

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اســتخدم فــى هذه التجربة ٣٢ عجل جاموسى حديث الولادة بمتوسط وزن ٤٠ كجم تم توزيعها على أربع مجامــيع تجريبية، المجموعة الأولى (المقارنة) لم تعط أى معاملة ؛ المجموعة الثانية (مجموعة السيلينيوم) حقنــت فى العضل بخمسة ملجم Se كل أسبوعين؛ المجموعة الثالثة (مجموعة فيتامين هــ) أعطيت ٢٠٠٠ وحــدة دولية من فيتامين هــ مذابا فى اللبن مرة أسبوعيا؛ المجموعة الرابعة (مجموعة السيلينيوم وفيتامين هــ) أعطيت المعاملتين معا، وتغذت كل المجاميع على اللبن الكامل وبادئ العجول والبرسيم، جمعت عينات دم مــن الوريــد الوداجى عند ١،٣، ٥، ٩، ١٣ أسبوع من العمر،وقد استمرت التجربة حتى فطام العجول على عمر ١٠٥ يوم.

وقد بينت النتائج أن المعاملات الثانية والثالثة والرابعة قد أدت الى تحسن معدلات النمو بمقدار ٦,٤، ١٤,٥، ٨,١% على الترتيب عن مجموعة المقارنة، كما تحسنت معاملات تحويل الغذاء بتأثير هذه المعاملات بمقدار ١٧,٩٩، ١٧,٤٥، ١٨,٨٣% على الترتيب عن مجموعة المقارنة، وكان تركيز السيلينيوم فى بلازما الدم فى المجاميع التى أعطيت السيلينيوم (الثانية والرابعة) أعلى عن مثيله فى المجاميع التى لم تعط سيلينيوم (الأولى والثالــثة)حيث كانت التركيزات ٣٢,٩، ٣١,١ بالمقارنة ب ١٩,٦، ١٨,٧ نانوجرام/مل على الترتيب، وكان نشــاط إنــزيم الكرياتين فسفوكينيز (CPK) وكذلك الجلوتاميك أكسالوأسيتيك ترانس أمينيز (GOT) أعلى معــنويا ($P<0.05$) فــى مجموعــة المقارنــة عــن أى مــن المجاميع المعاملة، أما نشاط إنزيم اللاكتيك ديهيدروجينــيز (LDH) فقــد تذبــذب بــدون اتجاه واضح للفروق بين المجاميع، ولم يتأثر تركيز كلا من الهيموجلوبيــن أوالهيماتوكريــت أو البروتين الكلى أو أيا من الألبيومين أو الجلوبيولين معنويا بتأثير أيا من المعاملات، ولم تلاحظ أى حالات للإصابة بمرض العضلة البيضاء على أي من حيوانات التجربة.