

## **EFFECT OF TRACE ELEMENT SUPPLEMENTATION TO SUCKLING BUFFALO CALVES**

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### **SUMMARY**

*A study was conducted to evaluate supplementation of buffalo calves with trace elements up to weaning on performance, blood metabolites and trace element concentrations in blood and tissues. Twenty buffalo calves at the age of 7 days with an average body weight (BW) of 37.8 kg were assigned into two groups: 1) Control (C) received no supplement; 2) Trace elements (TE) supplemented group. The trace elements mixture supplied 100 ppm Fe; 10 ppm Cu; 40 ppm Zn; 40 ppm Mn (as sulfate compounds); 0.32 ppm Se (as sodium selenite) and 0.5 ppm I (as potassium iodide). Calves were fed on buffalo milk at the rate of 10% of birth weight from 1<sup>st</sup> - 4 weeks of age and then was reduced to 7.5% from 5<sup>th</sup> - 8 weeks, thereafter it was reduced to 5% from 9<sup>th</sup> to weaning at 15 weeks. Calf starter and berseem hay were available to animals ad libitum from 2<sup>nd</sup> week until weaning.*

*Average daily gains were 0.40 and 0.42 kg for C and TE groups, respectively without significant difference. However, the final daily gain was higher in TE than C group (0.94 vs. 0.67 kg/d). Serum glucose in TE was greater ( $P<0.05$ ) than C group (100.0 vs. 90.2 mg/dl). Serum albumin was lower and globulin higher in TE as compared to C calves ( $P<0.10$  and  $P<0.05$ , respectively). Activity of alkaline phosphatase was higher ( $P<0.05$ ) in TE than C group, whereas activities of either AST or ALT were not altered. Trace elements supplementation did not affect hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH and MCHC). Red blood cell counts (RBC), white blood cell counts (WBC) and their fractions were not influenced by TE supplementation.*

*Serum Cu was higher ( $P<0.05$ ) in TE than C, but serum Mn and Fe tended to decrease. In general, tissue contents of Cu, Zn, Mn and Fe tended to increase in TE group with significance only for liver Cu, spleen Zn and lung Fe. Hair TE content did not differ between groups. It can be concluded that TE supplementation to suckling buffalo calves had no added value growth rate, blood hematology or metabolites, whereas, Cu and Zn status was improved, as well as the immune status of calves.*

**Keywords:** *Trace element, supplementation, buffalo calves*

### **INTRODUCTION**

During pregnancy, trace elements deficiency adversely affects development of the fetus, health and survival of neonates. Among the most limiting trace elements are copper (Cu), zinc (Zn), selenium (Se), and manganese (Mn). Postnatal mortality and

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low birth weights of calves are attributed to Cu deficiency in beef breeds (Michel, 1976). Anemic calves have higher rates of mortality due to failure of maintaining body temperature (Adams *et al.*, 1993). Zinc deficiency in pregnant ewes decreased body weight of born lambs and reduced Zn stores in the body (Masters and Moir, 1983).

Milk contents of trace minerals vary widely (Gustafson, 2000 and Hermansen *et al.*, 2005). Milk is deficient in iron, manganese and copper, therefore milk contents from these elements are faraway from covering requirements (NRC, 2001). The contents of Zn, Se and I in milk are not ample and deficiency may appear on suckling calves. The problem of trace element nutrition of suckling calves appears in veal calves fed solely on milk for extended periods. Methods tried to improve trace element nutrition of calves include either supplementation to dams pre- and postpartum or providing calves with the required element(s).

Bremner and Dalgarno (1973) supplied milk- fed calves with up to 100 ppm iron. They observed improvements in weight gain, raised hemoglobin (Hb), packed cell volume (PCV) and plasma Fe levels as the levels of Fe increased. Also, Kume and Tanabe (1994 and 1996) found improvements in Fe status of suckling calves by Fe supplementation. Plasma Mn and glucose was not affected by Mn supplementation to growing and finishing steers (Legleiter *et al.*, 2005).

Selenium supplementation to dams prenatally proved to be efficacious in alleviating deficiency symptoms in calves and enriching milk (Gunter *et al.*, 2003 and Abd El-Hady *et al.*, 2005). Administration of 100 mg Cu to deficient cows during late pregnancy increased plasma Cu concentration in calves (Suttle *et al.*, 1980); although Muehlenbein *et al.* (2001) found that Cu supplements (200 mg as CuSO<sub>4</sub> or 100 mg as organic Cu) to beef cows fed diets low in Cu pre and postpartum did not improve Cu in serum, liver or health and growth of the calves. Abd El-Hady *et al.* (2006) supplied pregnant buffaloes for 60 days prepartum with 50 ppm Zn as ZnSO<sub>4</sub>, Zn methionine or both. They observed improvements in body weight gains (BWG) of calves and in immune status of dams and calves. Mee *et al.* (1995) found that TE supplement (Cu, I, Se and Co) for pregnant cows for 10 weeks prepartum increased TE in blood of cows and calves.

Supplementation of neonatal calves with minerals was performed in several studies. Dietary supplementation with Zn over 40 mg/kg DM and up to 100 mg/kg DM from inorganic or organic sources had no effect on growth rate from birth up to 90 days (Arrayet *et al.*, 2002). Provision of suckling buffalo calves for the first 69 d of life with a mixture of major (Ca, Mg, Na and Cl) and minor (Cu, Zn, Mn, Fe, I, Co and F) elements tended to improve weight gains but not change Zn, Cu concentration in blood serum (Sikka *et al.*, 2002).

The objective of the present study was to evaluate the influence of soluble mixture of trace elements supplementation to suckling buffalo calves on performance and mineral concentrations in blood serum, hair and tissues.

## MATERIALS AND METHODS

### *1- Animals and management*

This study was conducted on twenty on-week-old buffalo calves at the Buffalo Research Station, Mahallet Mousa, belonging to Animal production Research Institute, Ministry of Agriculture. Calves were left with their dams for the first 3 days

after birth to get colostrum, thereafter they were separated and kept in individual concrete pens (140 cm x 120 cm x 106 cm) which were bedded with rice straw. All calves were kept indoors for 3 weeks, after that they were allowed to have exercise from 9:00 am to 2:00 pm daily. Calves were fed whole buffalo milk at 10% of birth weight (divided into two meals) till the 4<sup>th</sup> wk of age and then the amount of milk was reduced to 7.5% till the 8<sup>th</sup> wk, thereafter was reduced to 5% until weaning at 15 wk of age to allow calves consume dry feeds. Calf starter and berseem hay were available to calves *ad libitum* from the 2<sup>nd</sup> wk of age in their pens and play yard. Clean drinking water, also, was available to calves twice daily (11:00 am and 2:00 pm). All calves were injected with antiparasitic and vitamin AD<sub>3</sub>E. Calves were weighed at the beginning of the experiment and thereafter biweekly until weaning.

## 2- Experimental design

The animals were randomly assigned according to body weight and sex of calf to either a control group (C) which was not supplemented by trace elements (n=10, 5 males and 5 females; body weight  $\pm$  SE = 37.9  $\pm$  1.5 kg) or a supplemented group (n=10, 5 males and 5 females; body weight  $\pm$  SE = 37.6  $\pm$  1.44 kg). The second group was supplemented with a soluble trace element mixture (TE) in drinking milk. This mixture supplied 100 ppm Fe; 10 ppm Cu; 40 ppm Zn; 40 ppm Mn (as sulfate compounds); 0.32 ppm Se (as sodium selenite) and 0.50 ppm I (as potassium iodide).

## 3- Blood, hair and tissues collection:

Blood samples were collected at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> wk of age. Blood samples were collected via jugular venipuncture from each calf using trace mineral evacuated tubes. After a minimum 30 min, samples were centrifuged at 3000 r.p.m for 15 min. to separate blood serum, which was stored frozen at -20°C until determination of trace elements and blood metabolites. Other blood samples were withdrawn into heparinized tubes to determine blood hematology (as RBCs, WBCs, Hb, PCV, leucocytes and differentiation) using auto blood counter (Cell-Dyn 1800; Abbott Co., USA). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH and MCHC) were calculated as follow: MCV= mean corpuscular volume (PCV/RBC\*10); MCH= mean corpuscular hemoglobin (hemoglobin/RBC\*10); MCHC= mean corpuscular hemoglobin concentration (hemoglobin/PCV\*100).

Blood serum glucose, total protein, albumin, creatinine and alkaline phosphatase (Alk-ph) were determined colorimetrically using commercial kits (Spinreact, Spain; Fluitest, Germany; Dialab; ABC Diagnostics, Egypt, respectively) according to the procedures outlined by the manufacture. Also, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined colorimetrically at 505 nm according to Reitman and Frankel, (1957). Some physical examination of calves included determination of rectal temperature, diarrhea, anemia, alopecia and white muscle disease (WMD) were specifically evaluated.

Hair samples were collected from the neck and shoulder area of every calf using stainless steel scissors at 6<sup>th</sup> wk and 15<sup>th</sup> wk of age and kept frozen in polyethylene bags until preparation for analysis. At the end of the experiment, 3 calves of each group were slaughtered to obtain all carcass data and tissue samples (i.e. liver, spleen, heart, lung, kidney, eye muscle) for mineral analysis.

#### 4- Hair samples preparation

Every hair sample was cleaned manually then cut to small parts (0.5 - 1.0 cm) and washed 3-4 times by distilled water using small beaker. After that, they were soaked in distilled acetone for 1 hr. and washed 3 times with distilled water. Finally, the sample was soaked in acetone for 15 min and then dried in the oven at 70°C for 1 hr and kept in a desiccator (Wasiak *et al.*, 1996).

#### 5- Digestion of samples

Blood serum samples were treated with 12% of trichloroacetic acid (TCA) at the ratio 1:3 and shaken by vortex and centrifuged at 3000 r.p.m for 15 min. The clear layer was separated and kept until mineral analysis. Tissues and hair samples were soaked in a digestion mixture containing concentrated HNO<sub>3</sub>, HClO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub> (70 : 21 : 9%) over night. After that, samples were heated on hotplate for about 2 hr. until obtaining clear and colorless solution. Samples were transferred quantitatively to 50 ml volumetric flasks using 0.1 N HCl and the volume was completed with distilled water and kept in polyethylene containers until mineral analysis. The trace elements Fe, Zn, Mn and Cu were determined in digested samples using Atomic Absorption/Flame emission Spectrophotometer (AA-640-13, Shimadzu, Japan). Chemical analysis of feedstuffs (Table 1) was determined according to A.O.A.C. (1990).

**Table 1. Chemical composition (%) and trace element contents (ppm) of the experimental feedstuffs**

Ration	DM	On dry matter bases							
		CP	CF	EE	Ash	Cu	Zn	Mn	Fe
<b>Calf Starter*</b>	90.15	17.2	10.49	5.7	9.52	7.9	138.4	47.3	161.0
<b>Clover Hay</b>	87.7	12.6	34.6	2.1	11.46	7.8	21.3	41.3	245.0

\*Composition: Yellow corn, soybean meal (44%), linseed cake, wheat bran, molasses, calcium carbonate and sodium chloride

#### 6- Statistical analysis

The data of trace element concentrations (of serum and hair), blood metabolites, hematology, growth rate and rectal temperature were analyzed using the general linear models procedure (GLM) of SAS (1996) with a model that included TE (treatment), time of samples and their interaction. Trace element concentrations of tissues were analyzed using proc T-test of SAS. The overall means were compared using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

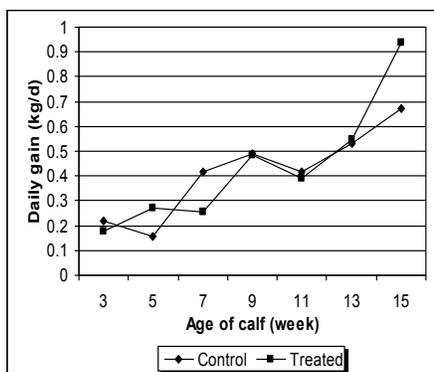
### 1- Growth rate

Least square means of initial, final body weight, total gains and daily gains are presented in Table (2). The treated group tended to be higher ( $P>0.05$ ) in weaning weight and total and daily gains compared with the untreated group. Mean of daily gain of treated calves in the period from 13 to 15 weeks of age was higher than control (0.94 vs. 0.67 kg; Fig. 1). Body weight increased linearly ( $P<0.01$ ) with age (Fig. 1 & 2). Many studies of trace mineral supplementation revealed non significant

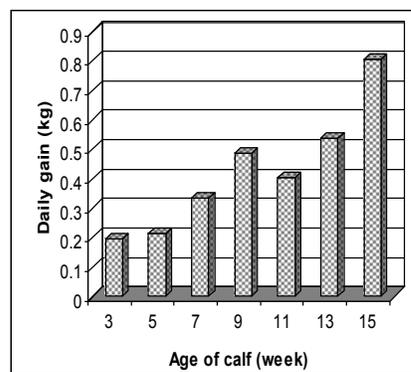
improvements of growth rate (Zn, Arrayet *et al.*, 2002; Cu, Muehlenbein *et al.*, 2001; Se, El-Ayouty *et al.*, 2003 and Mn, Legleiter *et al.*, 2005). Hemingway *et al.* (1997) found that growth rates did not differ significantly in dairy weaned calves or suckling beef calves, when they were supplemented with sustained-release boluses containing TE (Cu, Zn, Mn, Se, I and Co) and vitamins (A, D<sub>3</sub> and E). They found that mean daily gains were 0.59 kg and 0.53 kg for supplemented and unsupplemented dairy calves, respectively. Likewise, non significant improvements in BWG were noted by Sikka *et al.* (2002) who used mineral mixture and El-Ayouty *et al.* (1996) who used Se and vitamin E.

**Table (2): Body weight and gains of suckling buffalo calves.**

Items	Control	Treatment	SE
<b>Body weight (kg): Initial (1<sup>st</sup> wk)</b>	37.9	37.7	2.52
<b>Final (15<sup>th</sup> wk)</b>	76.7	78.6	2.40
<b>Total Gain (kg)</b>	39.0	40.9	3.1
<b>Daily Gain (kg)</b>	0.40	0.42	0.029
<b>Daily Gain (kg): Initial (2<sup>nd</sup> wk)</b>	0.22	0.18	0.076
<b>Final (15<sup>th</sup> wk)</b>	0.67	0.94	0.076



**Fig. 1. Daily gain of buffalo calves as affected by TE supplementation at intervals**



**Fig. 2. Daily gain of buffalo calves at consequent age intervals**

Abd El-Hady *et al.* (2006) fund that Zn supplementation to late pregnancy buffalo cows tended to increase BWG of their calves and the effect was significant with ZnSO<sub>4</sub>+Zn methionine mixture. The BWG values reported herein are similar to the values given by Abd El-Hady *et al.* (2006) but lower than the values of El-Ayouty *et al.* (2003).

**2- Blood criteria:**

Blood metabolites are shown in Table (3). Trace elements supplementation increased (P<0.05) plasma glucose levels from 90.2 to 100.0 mg/dl. The increase in glucose concentration was gradual during the course of study. However, Legleiter *et al.* (2005) found that glucose concentration was not affected by Mn supplementation to growing calves.

**Table 3. Blood Parameters of buffalo calves as affected by trace elements supplementation**

Parameter	Control	Treatment	SEM
Glucose (mg/dl)	90.2	100.0**	2.87
Total Protein (g/dl)	5.85	6.05	0.11
Albumin (g/dl)	3.16	3.00*	0.06
Globulin (g/dl)	2.64	3.06**	0.12
Alk-ph (U/dl)	110.6	138.7**	7.83
AST (U/l)	46.1	47.7	3.97
ALT (U/l)	15.8	13.1	2.22
Creatinine (mg/dl)	0.79	0.80	0.021

\*P<0.1; \*\*P<0.05

Total protein content was 5.85 and 6.05 g/dl in C and TE calves, respectively, without significant differences. Levels of albumin were decreased (P<0.1) in TE than C group (3.00 vs. 3.16 g/dl). Consequently, globulin content was higher (P<0.05) in TE than C group (3.06 vs. 2.64 g/dl). Therefore, the treated calves had higher immunity compared with untreated calves. Selenium supplementation to buffalo calves did not affect protein albumin or globulin in blood plasma (El-Ayouty *et al.*, 2003). The values of total protein, albumin or globulin found herein are lower than the values reported by El-Ayouty *et al.* (2003). Kessler *et al.* (2003) mentioned that serum total protein and albumin were not affected significantly by Zn supplementation.

The overall mean of Alk-ph activity was elevated in TE than C calves (138.7 vs. 110.6 KU/dl; P<0.05). The activity of Alk-ph was slightly declined up to 8<sup>th</sup> wk of age, then increased significantly (P<0.01) until weaning (Fig. 3 and 4). The result indicates an effective Zn supplementation (within TE mixture) in improvement of Alk-ph activity. Therefore, Zn is a constituent of Alk-ph and the activity of the enzyme has been used as a good indicator of Zn status in the body (Riordan and Vallee, 1976). Our findings are in agreement with those reported by Hatfield *et al.* (2001) and Kessler *et al.* (2003). Swinkels *et al.* (1996) reported that serum Alk-ph activity falls during Zn deficiency. Furthermore, Abd El-Hady *et al.* (2006) found that Zn supplementation to late gestation buffaloes improved Alk-ph activity in dams (P<0.01) and their calves (P>0.05). On the other side, Wright and Spears (2004) found that Zn supplementation at 20 mg/kg DM did not affect plasma Alk-ph activity in Holstein calves. Struharikova *et al.* (1989) observed that Alk-ph of calves tended to decline with the advancement of age.

Activities of transaminases (AST and ALT) and creatinine concentration were not influenced by TE supplementation. The activity of AST was elevated (P<0.01) with the advancement of age of calf and arrived to the highest level at 8<sup>th</sup> week of age (Fig. 5). However, creatinine concentration was declined (P<0.01) gradually until weaning (Fig. 6).

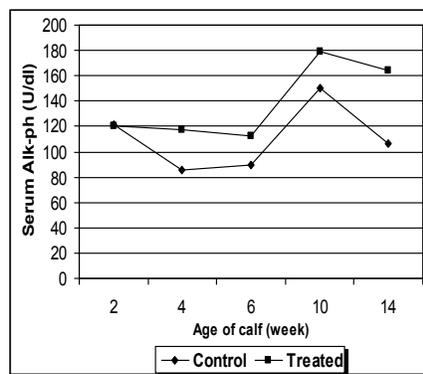


Fig. 3. Serum Alk-ph activity of buffalo calves as affected by TE supplementation

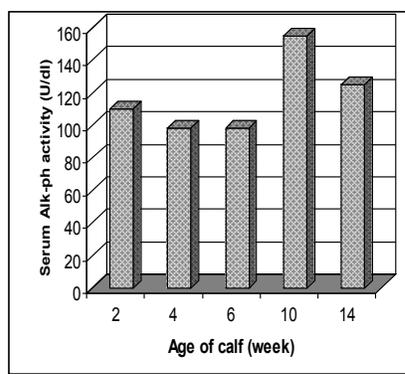


Fig. 4. Serum Alk-ph activity of buffalo calves at consequent age intervals

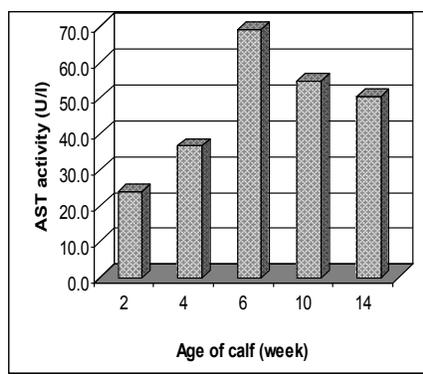


Fig. 5. Serum AST activity of buffalo calves at consequent age intervals

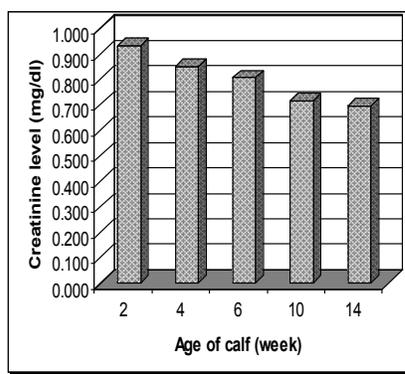


Fig. 6. Serum creatinine levels of buffalo calves at consequent age intervals

### 3- Blood hematology and cell counts

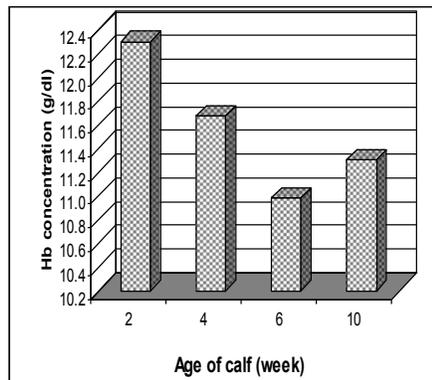
Least square means of blood hematology of suckling buffalo calves are shown in Table (4). Trace elements supplementation did not affect hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH and MCHC). Likewise, white blood cell counts (WBC) and their fractions (lymphocytes, neutrophil) were not different between TE and C calves. Red blood cell counts (RBC) were not influenced by TE supplementation. Blood Hb, PCV and MCV levels were decreased linearly with the advancement of age (Fig. 7 and 8). The results are in agreement with Roy *et al.* (1964) who observed that supplementation with 100 ppm Fe to Ayrshire suckling bull calves did not affect PCV, Hb, RBC, MCV, MCHC, WBC and its differentiation. They also found that Hb, PCV and MCV declined gradually with the advancement of age. However, Bremner and Dalgarno (1973) found that Fe supplementation to suckling calves

improved blood Hb, PCV and serum Fe but did not affect RBC or WBC. El-Ayouty *et al.* (2003) found that selenium and/or vitamin E supplementation to suckling buffalo calves did not affect Hb or PCV. As well as, the results are in agreement with Kessler *et al.* (2003), who found that organic and inorganic forms of Zn supplementation to Holstein crossbred bulls did not affect metabolic profile as Hb, PCV, RBC, MCV, MCH and MCHC.

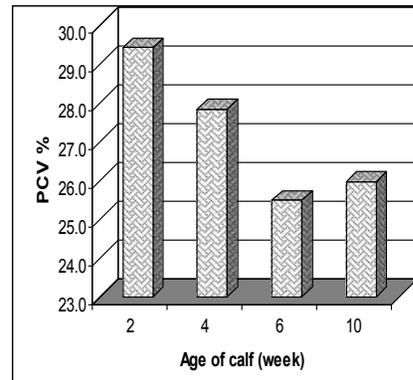
**Table 4. Blood hematology of buffalo calves affected by TE supplementation**

Items	Control				Treatment				SE
	Wk 2	4	6	10	Wk 2	4	6	10	
WBC $\times 10^3$	9.8	10.8	10.1	8.6	9.1	10.1	9.8	8.7	0.92
Lymph%	54.1	53.1	53.2	52.2	49.4	52.0	53.5	57.8	2.57
Neutrophil%	25.1	26.0	28.6	27.5	29.6	25.1	26.4	25.8	2.31
RBC, $10^6 \times \mu\text{l}$	6.81	6.54	5.97	6.53	6.58	6.83	6.44	6.42	0.26
Hb, g/dl <sup>a</sup>	12.4	11.6	10.4	11.1	12.2	11.8	11.5	11.5	0.48
PCV, % <sup>b</sup>	29.7	27.4	24.7	26.4	29.1	27.9	26.3	25.6	1.22
MCV, fl <sup>b</sup>	43.7	42.2	41.1	40.3	45.0	40.9	40.8	39.8	1.27
MCH, pg/cell	18.6	17.6	17.4	17.0	18.8	17.4	18.2	18.0	0.77
MCHC, %	42.6	41.5	42.5	42.2	42.4	41.6	44.6	45.5	1.81

<sup>a</sup> Age,  $P < 0.05$ ; <sup>b</sup> Age,  $P < 0.01$ ; WBC= white blood cell count; RBC= red blood cell count; Hb= hemoglobin; PCV= packed cell volume; MCV= mean corpuscular volume ( $\text{PCV}/\text{RBC} \times 10$ ); MCH= mean corpuscular hemoglobin ( $\text{hemoglobin}/\text{RBC} \times 10$ ); MCHC= mean corpuscular hemoglobin concentration ( $\text{hemoglobin}/\text{PCV} \times 100$ ).



**Fig. 7. Blood hemoglobin concentrations of buffalo calves at consequent age intervals**



**Fig. 8. Blood packed cell volume (PCV%) of buffalo calves at consequent age intervals**

#### 5- Rectal temperature and clinical observations

No significant differences of rectal temperature (RT) was found between control and supplemented groups. The interactions between treatment and both time and sex of calf were significant ( $P < 0.05$ ). So that, the highest variation of RT between control and supplemented groups was observed in 1<sup>st</sup> week of age ( $39.3^{\circ}\text{C}$  vs.  $38.4^{\circ}\text{C}$ ; Fig. 9). Also, the highest value of RT was in female of control (Fig. 10). It was observed three cases of diarrhea and one case of white muscle disease (WMD) in control group, whereas the supplemented group had not any cases. The WMD case died and it was replaced.

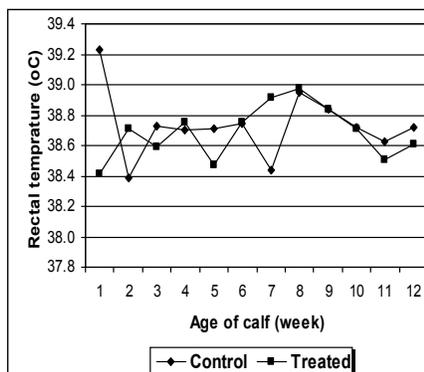


Fig. 9. Rectal temperature ( $^{\circ}\text{C}$ ) of buffalo calves as affected by TE supplementation

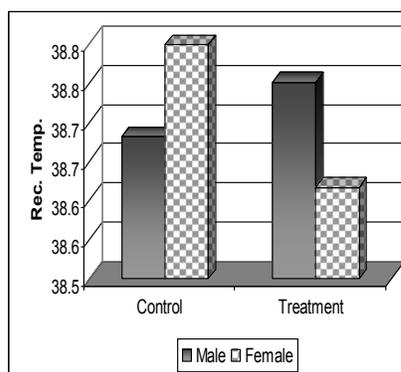


Fig. 10. Rectal temperature ( $^{\circ}\text{C}$ ) of buffalo calves as affected by TE supplementation and sex of calf

#### 4- Serum trace elements

Copper levels in blood serum (Table 5) increased significantly ( $P < 0.05$ ) from  $79.9$  in C to  $88.8$   $\mu\text{g}/\text{dl}$  in TE group, but were not affected by the advancement of age. A similar trend was found by Hemingway *et al.* (1997). Serum Zn concentration was not altered but both of Mn and Fe tended to decrease ( $P > 0.05$ ) in TE group compared with untreated group. Effect of age was not evident on serum trace minerals except the lower level ( $P < 0.05$ ) of Zn at 4 weeks of age. From the above results it appears that TE concentrations in serum are not sensitive enough to supplementation with TE especially when the level of supplementation is physiologic. Similar results were reported by Salyer (2000) who found that plasma Zn concentration of growing heifers was not affected ( $P > 0.05$ ) when they were supplemented with  $75$  mg Zn/kg DM as  $\text{ZnSO}_4$  or Zn polysaccharide. Underwood and Suttle, (1999) reported that plasma Zn concentration is not a reliable indicator of Zn status unless animals are severely deficient in Zn. As well as, Mn in blood plasma did not respond to supplementary Mn up to  $120$  ppm (Legleiter *et al.*, 2005). Sikka *et al.* (2002) found that buffalo calves supplemented with mineral mixture tended to show lower concentrations of Zn and Cu in blood plasma.

**Table 5. Blood serum trace elements of buffalo calves as affected by TE supplementation and age of calf**

Main effects	Serum trace elements ( $\mu\text{g}/100\text{ml}$ )			
	Cu	Zn	Mn	Fe
Control	79.9	87.6	171.7	331.7
Treatment	88.8*	89.2	160.8	299.4
SE	3.01	3.8	14.1	13.9
Age, week: 2	87.1	101.2 <sup>a</sup>	177.3	309.0
4	88.8	74.7 <sup>b</sup>	177.7	343.6
6	84.8	93.1 <sup>a</sup>	152.9	321.3
10	81.3	88.2 <sup>ab</sup>	151.3	303.0
14	79.7	84.7 <sup>ab</sup>	171.9	301.1
SE	4.7	6.1	22.3	21.9

\* The difference between control and treated cows was significant at  $P < 0.05$

<sup>a, b</sup> Means with differing superscripts within column are significantly different at  $P < 0.05$

##### 5- Tissue trace elements

Trace element concentrations in different tissues are given in Table (6). Copper in liver was increased significantly ( $P < 0.1$ ) from 6.37 in C to 10.30  $\mu\text{g}/\text{g}$  fresh tissue in TE group. Also, Cu concentration in eye muscle and heart was higher in TE than C group, but without significant differences between them; whereas Cu concentration in lung and spleen was little changed. Engle and Spears (2000a,b) found that Cu supplementation to growing beef steers increased Cu in the liver, whereas Cu in eye muscle was not altered. Also, Olson *et al.* (1999) found that supplementation of cows from calving to breeding with Cu, Co, Mn and Zn increased Cu in the liver.

**Table 6. Tissue trace element concentrations ( $\mu\text{g}/\text{g}$ ) of buffalo calves affected by TE supplementation**

Tissue		Tissue trace elements ( $\mu\text{g}/\text{g}$ )			
		Cu	Zn	Mn	Fe
Eye M.:	C	0.65 $\pm$ 0.54	14.7 $\pm$ 0.5	1.4 $\pm$ 1.2	12.3 $\pm$ 1.2
	TE	1.23 $\pm$ 0.54	17.2 $\pm$ 6.4	1.7 $\pm$ 1.2	16.1 $\pm$ 1.2
Heart:	C	1.13 $\pm$ 0.95	13.7 $\pm$ 0.55	1.4 $\pm$ 0.55	29.3 $\pm$ 1.04
	TE	2.20 $\pm$ 0.95	14.1 $\pm$ 0.86	4.1 $\pm$ 1.5	31.6 $\pm$ 3.55
Kidney:	C	0.19 $\pm$ 0.005	12.7 $\pm$ 0.7	2.0	25.4 $\pm$ 1.5
	TE	0.17 $\pm$ 0.003	15.1 $\pm$ 1.9	3.8	28.5 $\pm$ 4.2
Liver:	C	6.37 $\pm$ 0.29	18.2 $\pm$ 2.3	3.3 $\pm$ 1.3	43.5 $\pm$ 13.8
	TE	10.3 $\pm$ 1.54*	17.3 $\pm$ 0.7	5.8 $\pm$ 0.3	51.9 $\pm$ 8.1
Lung:	C	0.10	13.2 $\pm$ 0.1	Trace	36.8 $\pm$ 1.7
	TE	0.13	13.9 $\pm$ 0.7	Trace	45.8 $\pm$ 1.2**
Spleen:	C	0.40 $\pm$ 0.3	14.4 $\pm$ 0.45	3.9 $\pm$ 3.8	75.2 $\pm$ 8.8
	TE	0.43 $\pm$ 0.3	16.0 $\pm$ 0.45*	2.9 $\pm$ 0.8	71.2 $\pm$ 4.6

\*  $P < 0.1$

\*\* $P < 0.05$

Zinc content in spleen tended to be higher ( $P<0.1$ ) in TE than C group (16.0 vs. 14.4  $\mu\text{g/g}$ ). Whereas, Zn contents in other tissues were not affected by TE supplementation. The levels of Mn in liver and heart were higher ( $P>0.05$ ) in TE than C group but in other tissues there were no differences between groups. These results agree with Olson *et al.* (1999) who found no effect of TE supplementation on Zn and Mn in the liver. Also, Legleiter *et al.* (2005) found that Mn levels up to 120 ppm did not influence Mn in liver. On the other side, Jenkins and Hidiroglou, (1991) found that calves fed milk replacer based on skim milk powder that contained either 40 or 200 ppm of Zn resulted in 10.4 and 25.0  $\mu\text{mol/g}$  of DM of hepatic Zn, respectively.

Iron level in lung was significantly higher ( $P<0.05$ ) in TE than C group (45.8 vs. 36.8  $\mu\text{g/g}$ ). While, Fe contents in eye muscle, heart, kidney and liver showed non significant increases in TE group. Bremner and Dalgarno, (1973) found that Fe supplement up to 100 ppm increased non-significantly Fe in liver, kidney, spleen and significantly in heart and muscle. Similar results were found by Miltenburg, *et al.* (1991) when they supplemented 100 ppm of Fe in milk replacer to newborn Frisian calves. They noted that muscle and liver Fe concentrations did not differ significantly in supplemented group. On the other side, Roy *et al.* (1964) found that Fe supplementation to suckling calves increased ( $P<0.01$ ) liver Fe concentration but did not affect significantly Fe in pectoral muscle.

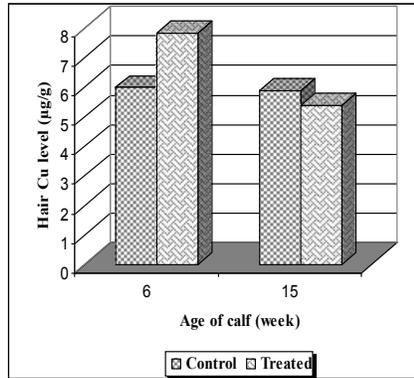
#### 6- Hair trace elements

Least square means of Cu, Zn, Mn and Fe concentrations of calf's hair are presented in Table (7). No significant differences were found between treated and control groups for all elements. The level of Cu in the TE group was higher ( $P<0.1$ ) in 6<sup>th</sup> week of age than C group (Fig. 11). On the other hand, Mn concentration in C group declined ( $P<0.05$ ) at 15<sup>th</sup> week of age but increased in treated group (Fig. 12). The concentration of Cu and Zn of calf's hair was greater ( $P<0.05$ ;  $P<0.1$ , respectively) at 6 week of age than at 15 wk, likewise with Mn contents but without significant differences. However, Fe concentration was higher ( $P<0.05$ ) at weaning (15 wk of age). There is no or a few studies are available concerning hair mineral contents as affected by TE supplementation to ruminants or livestock.

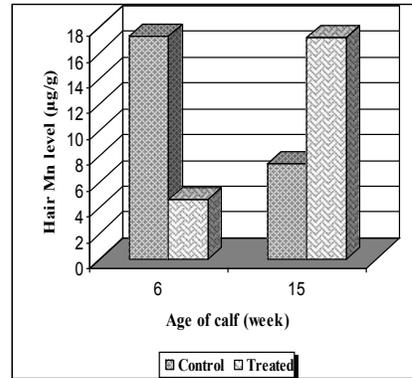
**Table 7. Hair trace element concentrations ( $\mu\text{g/g}$ ) of buffalo calves affected by TE supplementation**

Main effects	Hair trace elements ( $\mu\text{g/g}$ )			
	Cu	Zn	Mn	Fe
Control	5.9	128.9	12.3	71.4
Treatment	6.6	119.1	10.9	64.6
SE	0.4	7.7	3.4	3.2
Age, week: 6	6.9*	129.8	10.9	63.2
15	5.6	118.2	12.3	72.9*
SE	0.4	7.7	3.4	3.1

\*  $P<0.05$



**Fig. 11. Hair Cu concentration of buffalo calves as affected by TE supplementation**



**Fig. 12. Hair Mn concentration of buffalo calves as affected by TE supplementation**

## CONCLUSION

It can be concluded that the trace elements supplementation to suckling calves did not affect growth rate, blood hematology and metabolites, as well as manganese and iron concentrations in serum, tissues or hair. However, the trace elements supplementation improved Cu and Zn status; and the immune status of calves against diarrhea and white muscle disease.

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## تأثير إضافة العناصر المعدنية النادرة لعجول الجاموس الرضيعة

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أجريت هذه الدراسة لتقييم تأثير إضافة مخلوط أملاح معدنية للعجول الجاموسى الرضيعة فى اللبن حتى الفطام على الأداء ومقاييس الدم وتركيزات هذه العناصر فى الدم والأنسجة المختلفة. تم اختيار ٢٠ عجل جاموسى عند عمر ٧ أيام بمتوسط وزن جسم ٣٧.٨ كجم ووزعت إلى مجموعتين على أساس الوزن والجنس: (١) مجموعة المقارنة بدون معاملة، (٢) مجموعة المعاملة يضاف إليها مخلوط أملاح معدنية فى وجبة اللبن كل صباح. هذا المخلوط يحتوى على ١٠٠ جزء فى المليون من الحديد، ١٠ جزء فى المليون من النحاس، ٤٠ جزء فى المليون من الزنك، ٤٠ جزء فى المليون من المنجنيز (فى صورة سلفات)، ٠.٣٢ جزء فى المليون من السيلينيوم (فى صورة صديوم سيلينيت)، ٠.٥ جزء فى المليون من اليود (يوديد بوتاسيوم). كانت تغذى العجول على اللبن الجاموسى بمعدل ١٠% من وزن الميلاد من الاسبوع الأول حتى الاسبوع الرابع من العمر ثم تخفض الكمية الى ٧.٥% من الاسبوع الخامس الى الثامن ثم تخفض الى ٥% من الاسبوع التاسع وحتى الفطام على ١٥ أسبوع. وقد كان بادئ العجول ودريس البرسيم متوفر أمام الحيوانات من الاسبوع الثانى وحتى الفطام.

كان متوسط معدلات النمو اليومى ٠.٤٠، ٠.٤٢ كجم لمجموعتى المقارنة والمعاملة على التوالى بدون فروق معنوية. وان كان متوسط معدل النمو اليومى النهائى ٠.٩٤ و ٠.٦٧ كجم لمجموعتى المقارنة والمعاملة على التوالى. وكان مستوى الجلوكوز فى سيرم الدم لمجموعة المعاملة أعلى منه فى مجموعة المقارنة وذلك بفروق معنوية ( $P<0.05$ ). وقد كان مستوى الألبومين أقل ( $P<0.1$ ) وبالتالي مستوى الجلوبيولين أعلى معنويا ( $P<0.05$ ) فى مجموعة المعاملة عنه فى مجموعة المقارنة. وقد ازداد نشاط انزيم الفوسفاتيز القاعدى معنويا ( $P<0.05$ ) فى مجموعة المعاملة عنه فى المقارنة، فى حين لم يكن هناك اختلاف فى نشاط انزيمات الكبد (AST, ALT) وكذلك مع قياسات الدم الأخرى مثل الهيموجلوبين، الهيماتوكريت، كرات الدم الحمراء والبيضاء. وقد كان مستوى النحاس فى سيرم الدم أعلى ( $P<0.05$ ) فى مجموعة المعاملة من المقارنة. فى حين أن المنجنيز والحديد كانا يميلان إلى الانخفاض. بشكل عام كانت مستويات عناصر النحاس والزنك والمنجنيز والحديد تميل الى الزيادة فى أنسجة الحيوانات المعاملة عنها فى المقارنة، مع وجود فروق معنوية فقط للنحاس فى الكبد، الزنك فى الطحال، والحديد فى الرئتين. ولم يكن هناك فروق معنوية فى تركيزات العناصر المختلفة فى الشعر بين المجموع.

نستخلص من هذه الدراسة أن إضافة مخلوط الأملاح المعدنية النادرة للعجول الرضيعة لم يؤثر على معدلات النمو أو مقاييس الدم فى حين أنه قد أدى الى تحسن حالة الزنك والنحاس فى الحيوانات المعاملة كما حسن من الحالة المناعية للحيوانات.