

STUDIES ON THE LEVEL OF SOME TRACE ELEMENTS IN CATTLE SERA AT DIFFERENT GOVERNORATES

By

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ABSTRACT

Two hundred and fifty field blood and serum samples were aseptically and randomly collected from young pastured beef cattle located at different private farms in five governorates (Aswan, Minufiya, Al-Fayoum, Matrouh and Alexandria) during winter season. Green berseem that constitutes the main constituent of diet was also collected at the same time from these locations, in which the animals grazed. Blood, sera and berseem samples were subjected to biochemical analysis for trace elements. Haemogram was also done for these calves. The results provided that the level of trace elements in berseem and blood sera of calves varies between the different districts which gave a remarkable and valuable information about the effect this alteration on blood picture and animal health. This may serve as a useful reference measure in treatment of different immunological, reproductive, and early growth problems that occur due to nutritional imbalance in these regions.

INTRODUCTION

There are four main areas influencing production from a flock nutrition, genetics, reproduction and disease. Of these, nutrition is the most significant, having not only direct effect on output, but also significance on the three other factors (**Hindson and Agnes, 2002**).

Nutrients are primary factors in the regulation of the immune response. Both macronutrients and micronutrients derived from the diet affect immune system function through action at several levels in the gastrointestinal tract, spleen, regional lymph nodes and immune cells of circulating blood (**Chandra, 1997; Cunningham-Rundles and Lin, 1998; Wallace et al., 2000 and Cunningham-Rundles, 2001**).

Micronutrient such as trace elements and vitamins, are present in the local environment and have important regulatory effects on adaptive immune

cell function (**Frankenburg et al., 1998**). Serious nutrient imbalance will ultimately compromise the development of the immune response. Malnutrition promotes susceptibility to pathogen, even subclinical infection directly affects nutrient intake and metabolism. This cascade of events can be altered by nutritional intervention (**Jeevanandam et al., 1999**). Immune deficiency and susceptibility to infection are often directly linked with malnutrient (**Cunningham-Rundles, 1993**).

FAO/WHO animal health year books indicated that of the countries providing information on animal diseases, 80% report nutritional diseases of moderate or high incidence and trace elements deficiencies or toxicities are involved in more than half of those whose causes were identified (**Radostits et al., 2000**).

The main objective of the present study was to estimate the level of seven trace elements (Cu, Co, Fe, Zn, Se, Mo and Mn) in cattle sera and berseem diet in addition to the hemogram picture in five governorates.

MATERIAL AND METHODS

Cattle:

250 Balady young pastured, yearling old calves, weighing about 130 kg from five governorates [Aswan (Edfow), El-Fayoum (Sannoures / Matretares), Menufiya (Shanshour), Alexandria (Baheeg) and Matrouh (El-Hammam/El-Nahda)] were fed diet consisting mainly of the locally available berseem and hay, without any additives. Diet and water were offered ad libitum.

Blood and serum samples:

A total of 250 field blood and serum samples were aseptically collected from conventionally raised calves during the winter season from the five governorates. The blood samples were taken from the jugular vein on EDTA as anticoagulant for determination of hematological picture according to **Coles, (1986)**, and by plastic syringes, in sterile plastic tubes and transported on ice bag to the institute. Sera was separated from the whole coagulated blood samples (without EDTA), and preserved in plastic tubes, at -20°C till being analyzed for determination of 7 trace elements. Fifty samples were collected from each locality. All samples were obtained before the a.m. feeding.

EDTA:

Ethylenedinitrilo tetra acetic acid, disodium salt Boehringer Mannheim GmbH, Germany was used for whole blood.

Berseem samples:

They were collected synchronously from the same localities, of cattle pasture in the different governorates.

Haematological assay:

Hematological assay was done according to **Archer and Jefcot, (1977)**, while erythrocytes, total and differential leucocytic counts were according to **Schalm et al., (1975)**.

Estimation of trace elements:

A clear serum samples were analyzed for determination of copper (Cu); iron (Fe); zinc (Zn); manganese (Mn), cobalt (Co) and molybdenum (Mo) using atomic absorption spectrophotometer (Hitachi, Z-6100 Polarized Zeeman) according to **Parsons, (1992)**, selenium content was determined by the fluorometric method of **Olsen et al., (1975)** and the buffer solution of **A.O.A.C., (1980)**. The trace elements in berseem were determined according to the method described by **A.O.A.C., (1980)**.

The obtained data statistically analyzed according to **Snedecor and Cochran, (1982)**.

RESULTS AND DISCUSSION

Table (1): Trace elements (ppm) in berseem of some Governorates of Egypt (M±SD)

Governorate	Aswan	Al-Fayoum	Minufiya	Alexandria	Matrouh
Element					
Copper (Cu)	68±11.26*	103±22.43	74±11.36*	89±21.62	106±23.01
Iron (Fe)	345±44.9	297±35.59	251±51.88*	238±43.68*	289±60.87
Cobalt (Co)	0.69±0.09	0.52±0.05*	0.93±0.08	0.76±0.07	0.43±0.09*
Zinc (Zn)	89±10.36	63±10.81*	94±13.18	78±9.39	68±10.10*
Selenium (Se)	0.45±0.15	0.33±0.02*	0.39±0.01	0.48±0.02	0.28±0.04*
Manganese (Mn)	30±2.81*	49±5.35	27±2.92*	50±7.39	52±4.9
Molybdenum (Mo)	2.87±0.31*	1.08±0.58*	2.56±0.82*	2.24±0.63	1.86±0.46*

M: Mean SD: Standard Deviation

* Significant at P < 0.01

Table (2): Trace elements (ppm) in sera of calves in some Governorate of Egypt (M_±SD)

Governorate Element	Aswan	Al-Fayoum	Minufiya	Alexandria	Matrouh
Copper (Cu)	0.340±0.15*	0.855±0.44	0.300±0.18*	0.611±0.39	0.756±0.39
Iron (Fe)	380±140.8	486±117.0	301±101.2	348±100.1	452±116
Cobalt (Co)	0.62±0.009	0.65±0.012	0.70±0.012	0.75±0.018	0.73±0.017
Zinc (Zn)	44±3.19	30±1.19*	46±2.23	29±3.12*	32±4.26*
Selenium (Se)	0.146±0.13	0.152±0.14	0.132±0.10	0.185±0.21	0.242±0.44
Manganese (Mn)	62±12.53	90±12.3**	59±9.4	76±9.55**	87±7.5**
Molybdenum (Mo)	0.126±0.05	0.96±0.026	0.138±0.04	0.114±0.10	0.100±0.01

M: Mean SD: Standard Deviation

* Significant at P < 0.01

** Significant at P < 0.05

Good nutrition is necessary for resistance to illness and for optimal recovery from medical and surgical procedures. In addition to the basic food substances (Carbohydrates; fats and proteins), water and energy necessities, requirements for nine essential amino acids, one fatty acid and essential micronutrients (vitamins and minerals). Trace elements (microminerals); iron, zinc, cobalt, copper, manganese, molybdenum, chromium, selenium, fluoride and nickel serve as crucial component of different tissues and enzyme system (McClatchey, 2002). In this study, blood samples were collected by plastic syringes and sera were preserved in plastic tubes to avoid the collection apparatus which may contain comparable amounts of trace elements (Jain, 2000).

Samples were collected from the different governorates in the same season (winter) and before the a.m. feeding, to avoid the effect of the changes in the ambient temperature on the concentration of blood constituents (Paape, 1973; Fisher *et al.*, 1980 and Nancy, 2003). EDTA is the anticoagulant of choice, it is suitable for a full haematological examination (Hindson and Agnes, 2002). Serum is usually chosen for analysis because it avoids the cost and possible analytical complications of adding an anticoagulant, gives a more stable (haemolysis-free) form for transportation. The use of whole blood brings complications and new possibilities into the assessment of mineral status (Underwood and Suttle, 2001).

Table (1) indicated that there was a deficiency in copper level ppm in berseem of Aswan (68 ± 11.26) and Minufiya (74 ± 11.36) governorates. **NRC (1996)** reported that the normal requirements for cattle should not less than 100 ppm. On the other hand, the molybdenum levels were significantly high in these governorates (2.87 ± 0.31) and (2.56 ± 0.82), respectively. **Engle et al., (2001)** reported that primary Cu deficiency is due to inadequate levels in diet and secondary due to conditioning factors such as excess molybdenum (Mo) than the normal requirement which equal (0.2 ppm DM) according to **NRC (1996)** which explains primary and secondary Cu deficiency reported in Aswan and Minufiya and also explain why the Cu level was significantly decreased sera in these governorates (Table 2). Moreover, **Smart et al., (1986)** reported that Cu deficiency may be induced in sheep and cattle by using sulphur fertilizers. The nature of soil and its treatment are thus important determinants of the value of seed and forages as sources for minerals for animals.

Cu is essential for growth and prevention of a wide range of clinical and pathological disorders in all types of farm animals (**Underwood and Suttle, 2001**). The low serum Cu and anaemia in Aswan and Minufiya (Table 2, 3) may be referred to malabsorption due to the presence of excess (Mo) in the food supplied which interferes with Cu absorption. These results are in coincidence with **Suttle and Field, (1983)** who suggested that the primary consequence of exposure to Mo is the formation of unabsorbable copper. **Suttle (1991)** reported that there was a synchronicity of release of copper and its potential antagonists, molybdate, sulphide and iron from the diet. **Miltimore and Mason, (1971)** indicated that determination of copper in the diet or pasture has no diagnostic value in ruminants unless other elements with which Cu interact are determined.

Copper deficiency is endemic world wide and causes diseases of economic importance that may be severe enough to render large area of otherwise fertile land unsuitable for grazing by ruminants of all ages, but primarily young, growing ruminants (**Radostits et al., 2000**).

Cu is well-absorbed from diets low in fiber, such as cereals and brassicas, but poorly absorbed from fresh forage. Conservation of grass as hay or silage generally improves its availability. This explains why Cu deficiency is a problem of the grazing animal and seen only rarely in housed ruminants receiving diets that are commonly adequate in copper (**Smart et al., 1992**). **Suttle et al., (1999)** reported that Mo concentrations increase and can double during the grazing season. **Hartmans and Bosman, (1970)** induced hypocuproses in grazing animals which are therefore essentially "green-sward" problems. **Underwood, (1973)** reported that Cu is an essential element required for iron utilization and for the activity of several enzymes which may explain the results obtained for estimation of the iron level (Table 1, 2).

The results indicated that although the level of iron (ppm) in Aswan berseem (345 ± 44.90) was higher than that of Al-Fayoum and Matrouh (297 ± 35.59 and 289 ± 60.87) respectively, its level in the animal sera of the latter two governorates (486 ± 117.0 and 452 ± 116.0) was higher than that of Aswan's calves sera (380 ± 140.8), which reflect on the blood picture (Table 3), that indicated a significant decrease of Hb level in Aswan (anaemia). These results coincided with **Nancy, (2003)** who reported that iron required for a wide variety of metabolic processes in the body, while the largest amount is utilized for hemoglobin synthesis and anaemia is the most prominent finding in iron deficiency. On the other hand, iron deficiency recorded in berseem of Alexandria and Minufiya governorates (238 ± 43.68 and 251 ± 51.88) (Table 1), reflected on its level in calves sera (Table 2) that was (348 ± 100.1 and 301 ± 101.2), respectively. Table (3) also indicated iron-deficiency anaemia in which the Hb levels and RBCs were significantly decreased in these two governorates in comparison with the normal level recorded by **Jain, (2000)**, in addition to presence of neutropenia and leucopenia in these governorates. These results were in agreement with **Moore and Bender, (2000)**. **Sherman, (1998)** reported that the iron deficiency is the most common nutritional deficiency in populations around the world, it appears to be active in the immune response. In Egypt, **Abou-Hussein et al., (1970)** reported that the mean concentration of iron for Egyptian green-cut cloven and cloven hay were of low value, between 90 and 110 mg Fe Kg⁻¹ DM.

In the governorate in which cattle sera indicated iron deficiency, their treatment by injection of iron dextran or oral supplements should be applied after insurance that there are no infection, because iron is required for growth and proliferation of microorganisms and it is rapidly redistributed in the host to stunt proliferation of bacteria by removing iron from circulation and storing it in organs, such as the liver and spleen. Redistribution of iron during infection can be misinterpreted as iron deficiency anaemia by hemoglobin and hematocrit measurement (**Sherman, 1998**).

The cobalt (Co) level ppm in berseem ranged from (0.43 ± 0.09) in Matrouh to (0.93 ± 0.08) in Minufiya and in calves sera its concentration ranged from (0.62 ± 0.09) in Aswan to (0.75 ± 0.18) in Alexandria, which means that Co intake was sufficiently high according to **Smith, (1987)**. These results were in agreement with **Espinoza et al., (1991)** who suggested that forage Co deficiency in cattle is (0.1 ppm).

Yang and Lewandrowski, (2002) performed that the only known function of cobalt is as the metal component of vitamin B12 and the average diet contains a surplus of cobalt (about 300 mcg).

In all species, zinc deprivation is characterized clinically by inappetence, retardation or cessation of growth, lesions of the integument and its outgrowths-hair, wool or feathers and decreased efficiency of feed

utilization (**Kennedy et al., 1998**). Zinc concentrations in blood serum or plasma are the most widely used indicator and determination of Zn in the diet (**Engle et al., 1997**). Tables (1 and 2) showed that there are a significant deficiency in Zn level (ppm) in berseem in Al-Fayoum (63 ± 10.81) and Matrouh (68 ± 10.10) and a significant elevation of its level in Minufiya berseem (94 ± 13.18). These results coincide with those recorded levels in the animal blood sera of these governorates (30 ± 1.19 and 32 ± 4.46) respectively, which referred to its presence in pasture. **Legg and Sears, (1960)** found that Zn deficiency has been encountered under practical conditions with growing and mature cattle where pasture or feeder contained 18-42 ppm zinc. **Demertzis and Mills, (1973)** observed lesions of Zn deficiency in young bulls on rations containing 30-50 mg Zn/kg; and signs of deficiency have been reported in cattle where the pasture or fodders contain 19-83 mg/kg DM (**Dynna and Havre, 1963**).

On the other hand, Table (2) indicated that Zn level was also low in calves sera of Alexandria governorate (29 ± 3.12 ppm/100ml) while it was nearly normal in berseem (78 ± 9.39) which may be referred to the effect of manganese and Cu present in berseem. The present results coincided with **Bonomi, (1999)** who reported that the high manganese level in ration lead to secondary Zn deficiency (decrease in plasma Zn content). **Bremner, (1993)** found that absorption of Zn is impaired by elements such as copper and cadmium.

Underwood and Suttle, (2001) concluded that serum or plasma zinc values must obviously be used with caution in the diagnosis of Zn deficiency in farm animals. Since serum iron (Fe) declines and copper (Cu) rises under the influence of most stressors, abnormal Zn:Cu ratio but normal Zn:Fe ratio should distinguish such cases from those of trace Zn deficiency.

Table (3) indicated an increase in WBCs count in Al-Fayoum; Alexandria and Matrouh governorates which are in agreement with **Miller et al., (1968)** who reported that some abnormalities as poor growth are due largely to poor appetite while others (parakeratosis and raised white cell count) are due solely to lack of zinc. On the other hand, monocytes % was decreased in the hypozencemia cattle in the previously mentioned governorates.

Table (3) also indicated a mild lymphopenia in Al-Fayoum; Alexandria and Matrouh governorates due to Zn level deficiency. **Beisel, (1998)** reported that zinc deficiency consistently leads to atrophy of the thymus and other lymphoid organs, to reduce lymphocyte numbers, which lead to reversible dysfunction of T-lymphocytes, and adverse effects on β -cell function. Zinc deficiency also reduces the production of monocytes and macrophages. Such combined problems make the zinc-deficient host extremely susceptible to infectious diseases, and greatly increased the severity of infectious that do occur in these governorates.

The RBCs count was reduced in Al-Fayouin; Alexandria and Matrouh governorates in which Zn deficiency was recorded. This was in agreement with **Bremner, (1993)** who found that erythrocyte levels were reduced in Zn deficiency.

The selenium level in different governorates, either in berseem or in animal sera was fairly normal in which its level (ppm) in berseem ranged from 0.28 ± 0.04 to 0.48 ± 0.02 and from 0.132 ± 0.10 to 0.242 ± 0.04 in cattle sera (Tables 1 and 2).

Chenoweth and Sanderson, (2001) indicated that the Se requirements for beef cattle was 0.1 ppm which agreed with the present results. **Binnerts and Viets (1989)** found that Se content was higher in winter than summer on all soil types and the differences were significant. On the other hand, the present results disagree with that obtained by **Szabo, (1989)** who estimated selenium in winter pasture grass and in blood sera of cattle and the results indicated a deficient Se supply of cattle kept on the pasture.

Espinoza et al., (1991) suggested that forage trace mineral deficiency in cattle are as follows, Co (0.1 ppm), Mn (40 ppm), and Se (0.2 ppm). **Bires et al., (1991)** reported that the daily Cu and Se intake from the emission and food were 46.6 ppm and 0.26 ppm respectively.

According to the obtained results (Tables 1 and 2) manganese (Mn) level (ppm) in berseem ranged from 27 ± 2.92 in Minufiya to 52 ± 4.90 ppm in Matrouh and in blood sera of calves was from 59 ± 9.4 ppm in Minufiya to 90 ± 12.3 in Al-Fayoum Governorate. A significant high level was reported in sera of animals in Al-Fayoum (90 ± 12.3) and Matrouh (87 ± 7.5 ppm/100ml), which causes a secondary deficiency of Zn in these governorates. According to **NRC, (1996)**, the Mn requirements for cattle is 20 ppm. The current results coincided with **ARC, (1980)**, that reported no well defined deficiency syndrome in Mn and few pastures fail to meet Mn requirement for cattle.

The inferential effect of Mn on Zn was in agreement with **Bonomi (1999)** who found that the high Mn level in ration leads to secondary Zn deficiency in blood.

Tables (1 and 2) established that molybdenum (Mo) level (ppm) was significantly high nearly in all governorates in berseem and sera whereas its level in berseem ranged from (1.08 ± 0.58) in Al-Fayoum to (2.87 ± 0.31) in Aswan and in calves sera from (0.96 ± 0.26) in Al-Fayoum to (0.138 ± 0.04 ppm/100ml) in Minufiya. The present results were in agreement with **McClatchey, (2002)** who reported that there was no well-recognized Mo deficiency syndrome. According to **NRC, (1996)**, the normal requirement ranges from 0.0 to 0.2 ppm DM. **Higgins et al., (1956)** reported that the Mo requirements of animals are extremely low. High dietary Mo intake result in drastic scouring, powerful antagonists of Cu metabolism and weight losses. **Ferguson et al., (1938)** found that treatment with Cu sulphate through intravenous injection or via the drinking water stop the scouring.

Table (3) indicated that the RBCs values of calves were lower than normal all over the different governorates, which may be due to their raising on a low protein diet; but it was highly significant decreased in the governorates with poor Fe content (Minufiya and Alexandria) or with abnormal content of Cu that interfere with the Fe metabolism (Aswan). This was in agreement with **Manston, (1975)**.

The results indicated a significant decrease of Hb level in Aswan which means secondary iron deficient anaemia due to Cu deficiency which affect the iron absorption while a significant deficiency of both Hb level and erythrocyte count was recorded in Alexandria and Minufiya that means primary iron deficient anaemia which is in full agreement with **Nancy (2003)**. The leucogram picture showed a significant ($P < 0.05$) leucocytosis in Al-Fayoum and Matrouh which may refer to lack of Zn. These results were in agreement with **Miller et al., (1968)**.

Neutrophilia and lymphopenia were reported in Aswan and Minufiya which occurred from lack of Cu; Zn and/or increasing of molybdenum. These results coincided with **Beisel, (1998)**.

Monocytes % was significantly increased and reached (9.2 ± 1.88 and 10.3 ± 2.30) in Aswan and Minufiya, respectively, which reflects the effect of Cu deficiency and increasing of Mo in these governorates. No significant variation in eosinophil and basophil % was reported within the different governorates.

The hemogram picture (Table 3) render the animal health condition and confirm the results obtained from studying the level of some trace elements in cattle sera at these governorates (Tables 1 and 2).

In conclusion, the results indicated that there is an intelligible interaction between the trace elements which influence the nutritive value of a particular source, which reflect on its level in the animal sera. This help in prognostication with the problems or diseases probably present and help in prevention and/or controlling of them. The soil and fertilizers used also play an important role in increasing or decreasing of certain elements which performed the important role of the agronomists in treatment of the soil and selection of the suitable fertilizers.

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Table (3): Hemogram of calves in different governorates (M ± SD).

Parameter	(a) Normal blood values	Aswan	Al-Fayoum	Minufiya	Alexandria	Matrouh
Total RBCs (10 ⁶ /μl)	8.36±1.05	7.0±0.9	7.9±0.4	6.2±0.7*	7.0±1.02	7.7±0.8
Hemoglobin (Hb) (gm/dl)	11.4±1.7	8.6±1.85**	10.3±0.52	8.4±1.03**	8.2±1.14**	10.6±1.43
Total WBCs (10 ³ /μl)	9.623±3.453	8.400±1.87	11.620±1.94**	9.120±2.55	9.415±2.68	11.400±1.08**
Neutrophils (%)	31±8.8	48.50±2.35**	37.30±3.51	45.60±3.91**	38.50±2.84	37.90±2.76
Lymphocytes (%)	56±7.9	37.40±3.99**	49.80±4.01	38.30±3.97**	49.10±5.83	49.10±3.15
Monocytes (%)	5.0±2.9	9.20±1.88**	5.80±1.51	10.3±2.30**	6.1±1.72	5.5±1.59
Eosinophils (%)	7.0±1.9	4.50±1.29	6.90±1.51	5.10±1.42	5.6±1.38	6.4±1.32
Basophils (%)	0.50±0.06	0.40±0.01	0.50±0.05	0.60±0.08	0.5±0.06	0.8±0.07

M: Mean

SD: Standard Deviation

* Significant at P < 0.01

** Significant at P < 0.05

(a) According to Jain (2000)

المخلص العربي

دراسات على مستوى بعض العناصر النادرة في سيرم دم الماشية في محافظات مختلفة

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تم جمع مائتين وخمسين عينة دم، سيرم الدم عشوائياً تحت ظروف نقيه من عجول بقرى صغيرة من مزارع خاصة فى خمس محافظات (أسوان، المنوفية، الفيوم، مطروح والاسكندرية) خلال فصل الشتاء. كما تم جمع عينات من البرسيم الأخضر المكون الرئيسى لغذائها فى نفس الوقت، ومن نفس الأماكن التى ترعى فيها هذه الحيوانات. تم إجراء التحليل البيوكيميائى والهيماطولوجى لإستبيان صورة الدم والعناصر النادرة فى كلا من السيرم والبرسيم. أثبتت النتائج إختلاف مستوى العناصر النادرة فى البرسيم وسيرم دم الماشية ما بين المواقع المختلفة التى أعطت معلومات قيمة جديدة بالملاحظة عن تأثير هذه الإختلافات على صورة الدم وصحة الحيوانات. هذه النتائج تخدم كقياسات مرجعية مفيدة فى علاج المشاكل المناعية، التناسلية، والخاصة بالنمو المبكر التى تحدث من عدم التوازن الغذائى فى هذه المناطق.