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Biochemical, hematological and field studies on macro and micronutrients imbalances as a cause of hormonal, metabolic changes and oxidative stress in growing buffalo calves in Sharqia province.

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Abstract

One hundred clinically healthy buffalo calves of 6 months old were fed on total mixed ration according to NRC**, (**well fed buffalo calves) served as normal control group, the second group contained eighty five hazard fed buffalo calves poor growth group. Blood samples were collected for determination of some biochemical and hematological parameters. The obtained results revealed a significant decrease in serum glucose, insulin, glucagon, thyroid hormones triiodotyrosine (T3) and tetraiodotyrosine (T4), parathyroid (PTH), lipids and lipoprotein profilein addition to serum macro elementscalcium, phosphorus and magnesium (Ca, P, Mg) and micro elements copper, zinc, iron and manganese (Cu, Zn, Fe, Mn) concentrations were observed in hazard fed buffalo calves. Also, serum total protein, albumin and globulin concentration were significantly decreased in hazard fed calves while, serum non estratified fatty acids (NEFA) and liver marker enzymes activates aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were markedly increased. A significant increase in the biomarkers of oxidative stress L- malondialdehyde (MDA) and nitric oxide (NO) with marked decrease in total antioxidant capacity (TAC) were observed in hazard fed buffalo calves as compared with the well fed calves.

The hematological parameters revealed a significant decrease in red blood cells count (RBces), hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values while whitw blood corpuscles count (WBCes) was markedly increased. In conclusion, a state of poor fed buffalo calves was largely attributed to macro and micro elements disturbance causes metabolic and hormonal disturbance, hematological changes and oxidative stress in growing buffalo calves.

Keywords: *Buffalo calves, biochemical, hematological, metabolic changes, oxidative stress.*

Introduction

Minerals are essential for life, to satisfy the needs of growth and production and to replace quantities lost during the course of normal metabolism. Minerals participate in a range of biochemical reactions as components of enzymes, minerals have many important functions other than enzymes **(Radostits *et al.*, (2007).**

The trace elements are essential components of enzyme systems. Simple or conditioned deficiencies of mineral elements therefore have intense effects on metabolism and tissue structure. To assess the dietary intake and adequacy of minerals, information needs to be collected on mineral element content of foods and water **(Simsek and Aykut, 2007).**

Failure to gain weight is the main feature of this condition and chief complain of livestock

producersand has a drastic economic impact on livestock production as its affects the rate of animals body weight gain, marketing, day to the first calving, herd survivorship and future productivity **(Underwood and Suttle, 1999).**

Poor growth in calves is considered the widely mentioned complain among farmers in the world. Calf ill-thrift is a vaguely defined condition with a variety of causes however suboptimal growth are terms often used interchangeably **Radostits *et al.,* (2007).** Irregular, inadequate availability quality of feedstuffs and their utilization are the main causes of poor performance of buffaloes. **(Terramoccia *et al.,* 2000)**. **Wynn *et al.* (2009)** reported higher mortality and morbidity losses in buffalo calves and attributed this to poor feeding management of calves. Reduced reproductive performance and low growth rate have been reported in buffaloes suffering from macro and micro elements deficiency **(Wynn *et al.,* 2009).**

Growth is a dynamic process which is regulated by several factors including metabolic hormones (growth hormone, thyroid hormones, and insulin) and nutritionally related metabolites (protein, glucose, lipid, minerals, and vitamins) (**Abou-El Amaiem 2012).** Disturbances in metabolism of water, protein, energy and mineral balances, also enzymatic reactions, hormonal secretions and blood metabolites markedly changed. Such changes result in impairment of growth, production, and reproductive performance **(Ganaie *et al.,* 2013)**.

Moreover, stress of trace elements deficiencies increases the production of free radicals, leading to oxidative stress which has a negative impact on the calves live weight, mortality and health **(Saleh *et al.,* 2008)**.

The aim of the present study was designed to investigate the effect of bad feeding programs on various metabolic indices in buffalo calves to evaluate the role of some major and minor elements disturbance son some hormonal, oxidative stress markers, metabolic and hematological changes in growing buffalo calves compared to well fed calves under field conditions.

Materials and Methods

Animals:

A group of one hundred clinically healthy buffalo calves of 6 months old fed on total mixed ration TMR (concentrated ration and corn silage) according to **NRC (2007)** were selected from private farm at Sharkeia province, the selected calves were in good health, good nutritional condition and approximately have the same body scores served as control group. The second group contained eighty five poor growth and hazard fed (rice hay, bran and Alfalfa). Fresh and clean drinking water was supplied ad-libitum. During the period of the study all animals were proved to be free from external, internal and blood parasites.

Food composition:

Table (A). Ration for healthy buffalo calves according to **NRC (2007):**

Ingredients	Quantity (Kg / ton)
Corn	625
Bran	250
Soya mail(46%)	100
mineral mixture	5
Vitamins	2.5
Sodium chloride	12.5
Lime stone	5

Table (B). Ration for hazard fed buffalo:

Ingredients	Quantity (Kg / ton)
Bran	550
Rice hay	350
Alfalfa	100

Blood samples:
For determination of biochemical and hematological parameters, individual blood samples were collected from the jugular vein from Five animals from each group. Approximately 10 ml of blood samples were obtained in three clean, dry screw capped tubes, the first without anticoagulant for serum separation by centrifugation at 3000 r.p.m for 10 minutes. The clear serum was obtained and received in dry sterile sample tube using sterilized pipettes processed directly for determination of glucose, lipid profile and liver marker enzymes, then kept in deep freeze at -20^0 until used for the following parameters, hormonal profile, minerals, total protein and protein fractions. The second heparinized tubes where blood sample were collected for determination of malondialdhyde (MDA), nitric oxide (NO) and total antioxidant capacity (TAC). While the third 10 ml contained EDETA Tubes used for collected blood samples for hematological parameters, complete blood picture (RBCes, Hb, HCT, MCV, MCH, MCHC and WBCes).

Biochemical analysis:
Glucose was determined according to the method described by **Tietz, (1995).**Insulin by-**Wilson and miles, (1977),** according to **Okuno *et al*, (1993),** triiodothyronine (Tʒ), thyroxin (T4) and parathyroid (PTH) hormones using ELISA kits in all the blood serum samples using commercial Kits supplied by sigma **(Catalog number:** MDO28-96) according to **Chopra *et al.,* (1979).** Calcium by **Gindler and King (1972).** Phosphorus, by **Goldenberg, (1966),** magnesium, **by Gindler, (1971),** serumcopper, zinc, iron and manganese concentrations were determined using atomic Absorption Spectrometer **model 2380 (PERKIN-ELEMER)** AHRI. Moreover, serum total cholesterol was determined by the method described by **Meiattini, *et al.,* (1978),** triacylglyceroles by **Bucolo and David (1973),** HDL-cholesterol by **Grove, (1979),** LDL-c and VLDL - c were estimated according Formula described by **Friedewald, (1975),** NEFA by-**Schuster (1979).** Enzymatic activities of aspartate transaminase (AST) and alaninetransaminase (ALT) by **Reitman *et al.,* (1957),** alkaline phosphatase (ALP) **Kind and King (1954).** Hematological RBCes Hb, HCT, MCV, MCH, MCHC and WBCes. by using the automatic cell counter **sysmex (2000 IV) AHRI zagazig.** MDA, **Esterbauer *et al.,* (1982).** Nitric oxide by **Moshage *et al.,* (1995)** and TAC by **Koracevic *et al.,* (2001).**

Statistical analysis:
The obtained results were analyzed statistically with Independent Samples Test were used to evaluate the relationships between different parameters. T-test the obtained data were statistically performed on the data Usingpack-ageV.11.5 **(SPSS, 2002).**

Results

Table (1). The concentration of glucose, insulin, glucagon, T3, T4 and parathyroid hormone in growing buffalo calves (n=5).

Parameters	Group I	Group II
Glucose (mg/dL)	78.12±2.29	51.7±3.56***
Insulin (µIU/m)	30.86±1.28	28.8±2.37
Glucagon (µIU/ml)	78.12±3.60	68.60±1.91*
T3 (ng/dl)	5.29±0.43	4.21±0.26
T4 (ng/dl)	2.1±0.16	1.73±0.024*
PTH (pg/m)l	9.74±0.63	6.28±0.39*

*Represents statistical significant at P< 0.05 level.
***Represents statistical significant at P< 0.001 level.

Table (2). The concentration of calcium, phosphorous, magnesium, zinc, iron , Copper, manganese, MDA, Nitric oxide and TAC in growing buffalo calves (n=5).

Parameters	Group I	Group II
Calcium (mg/dL)	9.99±0.83	6.91±0.141**
Phosphorous (mg/dL)	5.002±0.45	3.24±0.27**
Magnesium (mg/dL)	2.84±0.08	1.70±0.21***
Zinc (µ mol/L)	80.24±2.58	57.14±2.88***
Iron (µ mol/L)	187.21±1.59	163.76±5.60**
Copper (µ mol/L)	160.12±1.24	150.60 ±1.70**
Manganese (µ mol/L)	52.60±2.54	43.40±2.16*
MDA (nmol/mL).	3.132±0.36	5.89±0.39***
Nitric oxide (mmol/L)	23.00±0.22	34.59±2.10***
TAC (u/gm Hb)	3.17±0.22	1.8±0.16***

*Represents statistical significant at P< 0.05 level.
**Represents statistical significant at P< 0.01 level
***Represents statistical significant at P< 0.001 level

Table (3). The concentration of total cholesterol, triacylglycerol, HDL, LDL, VLDL and NEFA in growing buffalo calves (n=5).

Parameters	Group I	Group II
Total Cholesterol (mg/dL)	97.62±4.55	91.30±5.10
Triacylglycerol (mg/dL)	89.12±3.32	81.44±3.89
HDL (mg/dL)	12.95±0.54	8.27±0.34***
LDL (mg/dL)	66.85±3.97	66.74±4.45
VLDL (mg/dL)	17.82±0.66	16.29±0.78
NEFA (mg/dL)	119.80±2.08	126.14±1.74*

*Represents statistical significant at P< 0.01 level.
***Represents statistical significant at P< 0.001 level

Table (4). The concentration of total protein, albumin, globulin, A/G, AST, ALT and ALP in growing buffalo calves (n=5).

Parameters	Group I	Group II
Total protein (g/dL)	7.27±0.41	4.80±0.34**
Albumin (g/dL)	3.96±0.11	2.78±0.19***
Globulin (g/dL)	3.31±0.42	2.02±0.28*
A/G ratio	1.30±0.22	1.46±0.21
AST(IU/L)	76.80±6.93	140.20±6.41**
ALT (IU/L)	63.20±3.93	92.20±3.90***
ALP (IU/L)	80.90±3.58	118.00±4.21***

*Represents statistical significant at P< 0.05 level.
**Represents statistical significant at P< 0.01 level
***Represents statistical significant at P< 0.001 level

Table (5). Blood picture of the control and investigated growing buffalo calves.

Parameters	Group I	Group II
RBces (10^6 /µL)	12.50±0.32	9.55±0.21***
Hb g/dL	13.36±0.61	8.61±0.39***
HCT (%)	46.29±0.76	38.26±0.78***
MCV (fL/red cell)	37.17±1.47	40.1±0.31
MCH (Pg)	10.67±0.29	9.05±0.55*
MCHC (%)	28.95±1.66	22.60±1.37*
WBces (10^3 /µL)	10.29±0.59	12.78±0.55**

*Represents statistical significant at P< 0.05 level.
**Represents statistical significant at P< 0.01 level
***Represents statistical significant at P< 0.001 level

Results

Buffaloes are the major source of good quality meat and milk production. Many buffalo calves suffering from malnutrition cause characterized by inferior productive and reproductive sings which is the main reason of high economic losses. Poor growth, wasting and weakness were the only obvious clinical signs. Also, poor coat and hair conditions were noticed. Biochemical and hematological studies on macro and micronutrients imbalances as a cause of hormonal, metabolic alterations, hematological changes and oxidative stress in growing buffalo calves in sharqia province.

The present study revealed a very high significant decrease in serum glucose, and a significant decrease inglucagon, T3, and PTH, meanwhile, a non-significant decrease showed in insulin and T_4 **(Table 1).** Serum calcium, phosphorous, iron, copper **(Table 2)** showed a high significant decrease, In addition, a very high significant decrease in serum magnesium and zinc was recorded. Moreover, a significant decrease showed in serum manganese concentration. Lipid profile showed a non-significant decrease in serum total cholesterol, triacylglycerol, LDL and VLDL. Moreover, a very high significant decrease showed in HDL concentration in hazard fed animals meanwhile a signifi-

cant increase showed in serum NEFA concentration **(Table 3).** A high significant decrease observed in serum total protein and a very high significant decrease showed in serum albumin concentration in hazard fed animals (**Table 4**) in addition**,** a significant decrease showed in globulin. Moreover, the study illustrated a high significant increase in serum liver marker enzymes AST, ALT and ALP **(Table 4).** Heamatological values revealed a very high significant decrease in RBces count, Hb and HCT and a non-significant increase showed in MCV, mean while a high increase showed in WBCes count **(Table 5)** furthermore, a significant decrease showed in MCH and MCHC values. The antioxidant status presented a very high significant increase in MDA, nitric oxide and a very high significant decrease in TAC (**Table 5).**

Discussion

Mineral requirements for buffalo calves were published by the **NRC (2007).** Since that time, considerable research has been published dealing with minerals and vitamins in buffalo nutrition. This research attempts to focus on mean role of minerals requirements or recommendations. The study of blood biochemical composition of the growing buffalo calves has received a great significance from the stand point of nutrition, because the levels of various blood constituents often serve as a valuable guide in evaluating the nutritional adequacy of the diet as well as the nutritional status of the animal.

The present study showed a marked decrease in serum glucose level **(Table 1)** in macro and micronutrients deficiencies in poor fed buffalo calves in comparison to the well fed animals. Glucose concentration is an indication of the energy status of individual animals. Reduced nutrient intake, periods of fasting and stress can cause reduction in blood glucose concentration**,** our results agree with **Khillare (2007)** who illustrated that the decrease of glucose concentration in calves probably due to the influence of hormonal changes primarily regulate metabolism and also decreased gluconeogenesis and peak basal metabolic rate value**.** The low levels of glucose and insulin provoke hypoglycemia **(Neama Ashmawy 2015).** Moreover, **Ingvartsen, (2006)** stated that the glucose concentration in calf decreased during gestational poor nutrition and stress. The decrease in energy misbalance by the process of mobilization of fats from body deposits as compensatory mechanisms can lead to disturbance in metabolic balance **(Šamanc *et al.*, 2000).**

The mean values of serum calcium, phosphorus and magnesium concentrations in buffalo calves are recorded in (table 2). The mean values of calcium, phosphorus and magnesium concentrations showed a significant decrease. the magnesium level which was accompanied with reduction in calcium level is agreeable by finding of **Randal *et al.* (2002)** who stated that hypomagnesaemia is often associated with hypocalcaemia and magnesium deficiency may influence calcium hemostasis. Magnesium increases calcium release from the bone by displacing it from the hydration shell and by stimulating processes which involve the simultaneous catabolism of the matrix and mineral phase, thus magnesium deficiency would lead to decrease in calcium release from the bone and hence reduce its concentration in blood **(Odette, 2005).** The results explained by the lower values of magnesium accompanied by decrease in the formation and activity of PTH, where hypomagnesaemia cause target organ resistance to the physiologic effects of PTH which ultimately results in hypocalcaemia **(Martens and Schweigel, 2000).**

In the present study, a trend towards reduced serum thyroid hormones in buffalo calves fed lower dietary protein levels revealed further points to an apparently positive relationship between protein nutrition and thyroid status. During the initial stage of growth, lower serum levels of both T3 and T4 were observed when compared with healthy calves **(Table 1)** it may be due to the decreased rate of oxidation and

the rate of continuous breakdown and formation of protein and fat **(Neama Ashmawy 2015).** The decreased T_3 and T_4 both produced in the thyroid gland attributed to the Lack of iodine which leads to a reduction of growth and disturbance in metabolic processes **(George, 2008).** These hormones accelerate reactions in most tissues and organs, thus increasing basal metabolic rate and accelerating growth, thiscommon problem affected by iodine deficiency leads to productive and reproductive disorders **(Paulikov *et al.,* 2011)**. Animals fed low protein diet tended to exhibit comparatively lower values for both thyroid hormones **(Pattanaik *et al.,* 2003).**

The mean values of parathermon hormone (PTH) concentration (table 1) in buffalo calves under experiment recorded hypoparathyriod hormone level Which explained by the low calcium level **(Radostits *et al.,* 2007).**

The judgment of the thyroid state under field practice was depending mainly on the values of thyroid hormones in the serum of farm animals **(McGavin *et al.,* 2001)** as an indicator of the thyroid activity. The decrease of iodothyronin deiodinase (D1 and D2) which convert T4 into T3, the conversion of thyroid hormones occurs by the action of iodothyronine-5-deiodinase enzyme, which is responsible for the deiodination of L-thyroxin (T4) to its more active form triiod-L-thyronine (T3). **(Behrad *et al.,* 2010).**

The low levels of T3, in this study may be attributed to the low minerals supplementation in the diet which may the main cause of hypothyroidism.

Moreover, **Huang *et al.,* (2004)** observed that the reduced levels of circulatory thyroid hormones in buffalo calves may attributed to fed on diet low in macro and microelements, The reduction in growth referred to the main role of these hormones in turn take over important control processes in the energy metabolism and are thus particularly important for growth **(Yuygal *et al.,* 2013). Pattanaik *et al.* (2003)** observed a reduction in the level of circulatory thyroid hormones in sheep and goats, when fed on protein restricted diets. Glucagon is a pancreatic peptide hormone that plays a critical role in glucose metabolism and homeostasis. Glucagon secretion is a response to hypoglycemia and acts as the counter regulatory hormone to insulin. The main action of glucagon is to stimulate hepatic glucose production by increasing glycogenolysis and gluconeogenesis while inhibiting glycogen synthesis. In addition, glucagon has many extrahepatic effects, including increased lipolysis in adipose tissue **(Fosgerau, *et al.,* 2011).**

Our data suggested that associated macronutrients deficiency in buffalo calves is largely due to significant reduction in the serum macroelements include calcium, phosphorous and magnesium. Macronutrients Ca, Ph and Mangnesium concentrations were significantly decreased in poor growth buffalo calves than good fed illustrated in **(table 2)** The significant decrease in serum calcium, phosphorus and magnesium levels in in poor growth buffalo calves could be attributed to their low concentration in the offered ration together with the decreased intestinal absorption of most nutrients. **Yugal *et al.,* (2013)** established a decrease of Ca concentration in poor fed buffalo calves. Low calcium concentrations also prevent insulin production, further exacerbating this situation**.**

Shetaewi and Ross (1991) have also recorded similar levels of calcium and inorganic phosphorus in the serum of cows fed protein restricted or adequate diets. Micronutrients deficiencies in buffalo calves are largely due to impair the conversion of food into energy and therefore Cu deficient calves go off their diets **(George and Fisher 2008).** Cu prevents lipid peroxidation via the activity of superoxide dismutase. SOD catalyzes both extracellular and intracellular super oxide anion to H_2O_2 and molecular oxygen. **(Slivkova *et al.,* 2009).**
The estimated blood levels of serum copper, zinc, iron, manganese calcium, phosphorus

and magnesium **(table 2),** showed a significant decrease the obtained results coincide with that of **Timothy (2001)** recorded a highly significant decrease in serum copper, zinc and iron concentrations in poor growth buffalo calves than healthy ones. Moreover, our data concerned with **Sikka *et al.* (2002)** who reported that micro-elements include such as Zn, Cu, Fe and Mn improve the efficiency of antioxidant system in lipid per oxidation prevention. Moreover, elevation of serum hydrogen peroxide and reduction of TAC in our research in ill-thrift calves suffering from trace element deficiencies **(Lykkesfeldt and Svendsen 2007).**

Poor Cu dietary status can be linked to specific biochemical steps such as, poor growth, reproduction, impaired immunity and antioxidant defense, and depigmentation resulting from Cu deprivation can be linked to specific enzymes such as Cu-Zn superoxide dismutase which catalyse the superoxide anion **(Keen *et al.,* 2003).**

Iron is an important components of catalase enzyme which is main antioxidant enzyme preventing fluctuations in ROS production and protecting the cellular structure and function against oxidative damage via activation of CAT enzyme which breaks H_2O_2 into O_2 and water (H_2O). The poor growth in buffalo calves, iron deficiency has an important role in the synthesis of nucleic acids and proteins, electron transport, cellular respiration, proliferation and differentiation **(Wise *et al.,* 2003).**

Results of our study coincided with **Spears and Weiss (2008)** who stated that micro-elements include zinc, copper and iron have significant roles in maintaining good health condition in farm animals as deficiency of these trace elements results in a case of calf ill-thriftiness, this also was in agreement with **Aref *et al.* (2009)** who reported that low blood copper and zinc concentrations in growing animals result in general weakness, stunted growth and anemia. The reported decrease in serum zinc and iron levels may be attributed to disturbance in their absorption from gastrointestinal tract or may be due to loss of appetite **(George and Fisher, 2008).** Moreover, the present study agree with **Ahmed and Ghada Nabil (2007)** who proved that the real cause of ill-thriftiness in buffalo calves was microelements deficiency including Cu, Zn and Fe. there is a tight relationship between antioxidant status and state of inferior vitality in buffalo calves as there was an elevation of oxidative stress markers malondialdehyde (MDA) and significant reduction in TAC **(Celi, 2010)**.

SOD is known to be an important factor in protection against harmful free radical activity and is considered the first defense mechanism against pro-oxidants **(Celi, 2010).** Our data suggested that a concomitant ill-thrift in calves is largely due to significant reduction in the serum calcium, phosphorous, magnesium, Iron, copper, zinc and manganese concentrations. There was a significant correlation between copper deficiency and ill-thrift in buffalo calves. These results were in concern with those reported by **Ghanem and Abd-El Raof, (2006).** These results could be attributed to Copper which is a cofactor of several metalloenzymes and other metalloproteins such as ceruloplasmin, superoxide dismutase, cytochrome oxidase, lysyl oxidase, and metallothionein **Minatel and Carfagnini (2002).**

Also these data were in agreement with **(Gressley, 2009)** who concluded that deficiency of copper results in metabolic disorder, poor growth, Unthriftiness, coat and skin abnormality.

Zinc is a critical nutrient of metabolic enzymes andimmunity, being involved in so many immune mechanisms including cell mediated and antibody mediated immunity, thymus gland function and thymus hormone action. Zinc is also important as an antioxidant. So zinc deficiency is implicated in the occurrence of ill thrift in association with copper, manganese and iron deficiencies **(Borghese, 2005). Nockles *et al.,* (1993)** attributed the role of zinc in

reducing transportation, feed and handling stress which affects animal growth rates. Our data findings were in agreement with **Abou El-Amaiem (2012)** who showed, a significant association between zinc, copper and iron deficiencies and poor growth of calves so, heindicated that nutritional deficiency has a significant impact on growth of buffalo calves. Also there was a proven relationship between ill thrift in buffalo calves and iron deficiency. These results could be attributed to the role of copper deficiency in decreasing the absorption of iron which has a great role in synthesis of certain enzymes related to oxygen utilization and plays a vital role in many enzyme systems as component of cytochrome oxidase, which is important in oxidative phosphorylation **(Radostits *et al.,* 2007).**

The reduction of antioxidant activity of SOD which is copper, zinc and manganese dependent enzymes is due to the deficiency of these element which lead to decrease in the activity of this enzymes in calves and may allow reactive oxygen species (ROS) such as superoxide to accumulate beyond their capacity to an extent that oxidative damage may occur **(Mullis *et al.,* 2003).** Previous studies of **Gaal *et al.,* (2006)** showed a tendency for leukocytes to release greater amounts super oxide ($O2^-$) in calves fed on copper deficient diet and elevated superoxide and diversion of nitric oxide (NO) by superoxide to peroxynitrite, a potent oxidizing agent, Moreover, **Dyavolova *et al.,* (2014)** showed substantial increasing of MDA levels with significant reduction in total antioxidants capacity poor growth calves in comparison with the good healthy calves**.** In addition our results were in concern with **Maurya, *et al.,* (2014)** who reported that Zn, Cu, Fe and Mn improve the efficacy of antioxidant system in lipid peroxidation prevention, as occur elevation of serum hydrogen peroxide and reduction of TAC in ill-thrift calves suffering from trace elements deficiencies.

Albumin serves as the major amino acid pool **(Ravindra and Dass 2006)** the catabolism of albumin provides protein precursors needed for growth or other physiological needs. They mentioned that albumin concentration has a relatively short half-life and can reflect protein deficiency problems over a period of a growth. The cause of these findings suggest that the correlations between the serum concentrations of trace elements with lipid profile in physiological concentrations may not be the same as the changes observed during deficiencies of the trace elements **(Al-Sabaawy, 2012)**.

Cholesterol and triglycerides table (3) showed significant decrease in poor growth buffalo calves. Cholesterol levels showed a significant decrease in poor fed buffalo calves compared with normal ones. These results were similar to previous report of **Ghanem and El-deeb (2010).** This could be attributed to mild liver asteatosis which cause reduction in cholesterol formation in the liver **(Grumer *et al.,* 2004).**

Moreover, HDL-cholesterol level showed a significant decrease in comparison with the control groups. These results coincide with those of **Turk *et al.* (2008)** these results may be attributed to reduction in cholesterol level. The decrease in serum cholesterol concentration due to nutritional deficiency may be attributed to the marked inhibition of fat utilization as **El-Barody *et al.* (2001)** mentioned that the insulin concentration in the blood is affected by various factors, serum insulin is a balance between its secretion stimulated by glucose, amino acids, hormones like glucagon. which affected by the nutritional status and hemostasis of various metabolic factors.

Our data were in agreement with the reports of **Turk *et al.* (2008),** who found that the serum glucose and cholesterol level generally exaggerated with thyroid hormone activity on cholesterol metabolism to increase the rate of its catabolism by the liver and enhance the liver ability to excrete it in the bile thereby lowering the cholesterol in hypothyroidism, the net effect is a decrease in cholesterol catabolism.

VLDL cholesterol being involved in transportation of triacylglycerol from liver to the adipose tissue a significant reduction in VLDL cholesterol levels observed during summer in control group could be attributed to the corresponding lower levels of triglycerides **(Satyanarayana and Chakrapani, 2006).**

Concentration of NEFA in the blood increase table (3), based on the findings of our study, the highest levels of NEFA may be related to negative energy balance **(Grummer *et al.* 2004).** Plasma NEFA showed significant increase in serum of poor fed Buffalo in comparison with well-fed ones reflect the plane of energy and protein nutrition which could be the reason for less negative energy balance reflected in buffalo calves under study, **(Paul *et al.*, 2003).**

Serum albumin, globulins, total protein and liver enzymes AST. ALT and ALP, showed a significant changes in poor growth buffalo calves in comparison with healthy ones (Table 4). The mean values of activities of serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphates (ALP) in apparently healthy and poor growth buffalo calves are presented in **(table 4).** The tabulated results showed significant increase of serum AST, ALT and ALP activities in poor growth buffalo calves compared to healthy ones. On the other hand, the mean values of blood serum total protein, albumin table (4), glucose, table (1) were significantly deceased, The significant decrease in serum total protein and albumin levels shown in table (4) in poor growth buffalo calves was in agreement with **Coles (1986)**. Data presented in **(table 4)** showed significant increase in AST, ALT and ALP activities in the buffalo calves fed on trace element deficient ration when compared with calves fed on TMR. Similar results were previously reported by **Morales *et al.,* (2000)** who observed elevation of liver enzymes in the blood of buffalo calves fed copper deficient diet, indicating hepatic dysfunction. The decrease in serum glucose level in the trace elements deficient animal as reported in table (1) may be due to copper deficiency **(Engle, *et al.,* 2001)**. The significant decrease in total serum cholesterol and triglyceride levels in poor growth buffalo calves than good healthy ones may reflect the reduced food intake. These results were similar to those previously reported by **Hamam *et al.* (1980)** who found reduction in serum cholesterol level in trace element deficient animals.

The obtained hematological picture of apparently healthy and poor growth buffalo calves in **(Table 5)** revealed that ill-thrift calves had a significant decrease in RBces count, Hb, HCT, MCV with a significant increase in WBces count. The mean corpuscular volume indicating the presence of normochromic normocytic anemia in poor growth buffalo calves as compared with the good health one (Table 5).

The mean values of total red blood corpuscles, hemoglobin, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration showed significant decrease in poor growth buffalo calves when compared with corresponding values in healthy ones. Our results agree with **Asati *et al.,* (2008)** who recorded that significant decrease in total RBCs Counts, Hb values, MCH, PCV, and MCHC than the values of healthy ones. They found that these results might be due to anemia with deficiency of minerals and indicators of erythrocytic normality and general health of the animal**.** The similar observations were made by **Mahboob *et al.,* (2014)** who recorded significant difference in WBCes count and Hb, however PCV values in poor fed calves.

Poor growth buffalo calves exhibited a significant increase in total WBCs count when compared with the values of healthy ones. The increase of total leucocytic count of poor growth buffalo calves may be attributed to the malnutrition **(Malik *et al.,* 2013).** This suggestion was supported by the results of **Mahboob *et al.*, (2014).** Regarding the results of some trace elements of apparently healthy and poor

growth buffalo calves presented in **(table 5),** the significant decrease in serum copper level of buffaloes may be attributed to its deficiency in the ration, which, reduce the availability of dietary copper as recorded by **Gropper *et al.*, (2005)** who also reported that the serum level of iron was significantly decreased, in poor growth calves to induce anemia was evident and represents an additional complicating factor for suboptimal growth**.** There was a significant decrease in the total RBCs count. These findings were in agreement with those reported by **Radostits *et al.*, (2007**) who reported that significant decrease in total RBCs counts, Hb values, MCH and MCHC than the values of healthy ones these results might be due to co-existence of clinical marginal anemia with deficiency of minerals**.**

Conclusion

In conclusion, a state of sub-optimal growth in calves was largely attributed to deficiency of macro elements including Calcium, Phosphorous and Magnesium in addition, micro elements, including zinc, copper, iron, and manganese deficiency that may cause decrease in metabolic hormone as insulin, glucagon, T_3,thyroxin and parathyroid and increase in liver marker enzymes. Furthermore, reduction in the total antioxidant capacity, with a lower ability to reduce oxidative compounds which have a negative impact on health and immunity status of poor fed buffalo calves. Our study investigates the association between trace elements, Biochemical parameters and total antioxidant activities. The nutritional factor could be controlled for buffalo calves by giving balanced ration to improve such parameters and consequently improving animal performance and kept a good health condition.

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