EFFECT OF AGE ON MINERALS LEVEL IN MILK AND SERUM OF CAMEL (*Camillus dromedaries*)

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DEDICATION

To my lovely parents father mother.

To my brothers, sisters

To my uncle Abu- elgasim.

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ABSTRACT

The aim of this study was to determine the effect of age on milk and serum concentrations of iron,copper, magnesium and iodine and the activity of the plasma Cerulopasmin enzyme in she camel.

Thirty tree ranging animals belonging to the Elrashida tribe in Kassala state of eastern Sudan were used. The animals were divided into three groups A,B and C according to their age, 9, 12 and 14 years respectively.

The data obtained revealed that highly significant ($P<0.01$) decrease in milk Fe, also significant ($P<0.05$) in milk Cu, Mg concentration were observed in older animals (group C) when compared with the two other groups (A and B). No significant differences were noticed in the iodine level in the milk between the three groups. Ash contents of milk showed no significant differences among three groups. Significant decreases ($P<0.05$) were demonstrated in plasma Fe, Cu, and Mg levels in group C when compared with groups A and B. Also significant decrease ($P<0.05$) in plasma Caeruloplasmin activity in group C was demonstrated when compared with the two other groups.

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Introduction

In the Sudan camels play an important role in nomads life. They provide an excellent source of income and transportation.

Lactational period in camels vary from 9 to 18 months (Karimi and kimenye, 1988). The annual milk yields range from 800 to 3600 litres (Shalash, 1984), and the daily mean milk production ranged from 2 to 6 litres under desert conditions (Yagil and Etzion ;1980; Yagil *et al* , 1984 and Farah 1993), and up to 12 to 20 liters under intensive breeding system (Knoes 1977, Knoes *et al* 1986, and karimi and Kimenye,/ 1988).

Camel's milk can certainly play a far more important role in prevention of malnutrition than it does today. Growing and raising food stuff for the rapidly increasing human population is specially precarious in the non and arid zones of the world.

In Southern Arabia camels is used for it’s milk and meat (Bullet, 1975). Camels can produce an adequate amount of milk in drought areas where other domestic animals can difficultly manage to survive.

Data reported concerning the composition of milk vary greatly. This can be attributed to the inherited capabilities of the animal, but also the age , stage of lactation and number of calving play a role. Of special significance to the quality of produced milk are the type of food and water quantity and quality that animal can consume.

The main constituents of milk include, water, fat and solid non fat (SNF). The later include minerals protein, non protein – nitrogen, lactose and vitamins.

Most camel milk is drunk fresh. It is also consumed slightly sour or strongly soured. Camel milk is generally opaque white (Dihanyan, 1959 Kheraskov, 1993, Yagil and Etzion, 1980). Normally it has a sweet and sharp taste, but some time its salty (Rao, 1970). Sometimes milk taste watery.However in certain countries there are prejudices among the urban population concerning camel milk. It is considered as having an unpleasant taste (Yasin and Wahid, 1957). It is frothy when shaken slightly (Shalash, 1979).

Fresh camel milk has a high pH (Grigor' Yants, 1954; Ohri and Joshi, 1961). The pH of milk is between 6.5 – 6.7 (Shalash 1979) this is similar to sheeps' milk. Ohri and Joshi, (1961) repoted that, the acidity rapidly increased when camel milk was left to stand

The first milk (Colostrum) is white and slightly diluted as compaired with the colostrums of cow (Yagil and Etzion, 1980).

Minerals are inorganic compounds being essential to health. However there are about 17-20 (depending on source) minerals seemed essential to human nutrition, with another nine recognized as probably being essential. Every living cell depends on minerals for proper structure and function. Minerals are needed for the formation of blood

and bones, and the proper composition of body fluids. They are grouped into two categories bulk or essential mineral also called macrominerals and microminerals (Joel, 2001)

The aim of the present study was to investigate the effect of age on some milk and serum constituents in camel. Three age groups in early lactation period of Elrashida (diaili) camels in Kassala province of eastern Sudan were investigated to estimate iron, copper, magnesium, iodine and caeruloplasmin activity in serum and milk.

CHAPTER ONE
LITERATUR REVIEW

1.1 Minerals function:

The function of Minerals is classified as either structural or regulatory. Structural as some mineral plays an integral part of cellular component. Regulatory with regard to the maintenance of water acid-base balance, muscle contraction, nerve impulses and cofactors for enzyme systems. Some enzymes cannot function in energy production without magnesium and still other require copper or iron.

1.2 Digestion of forage:

The yield and quality of milk produced by an animal depend on the composition of the food available including liquids. The lactating camel in an arid area must not only overcome the shortage of drinking water, but also the shortage of forage. The fodder that is available can also affect the composition and taste of milk. When camels subsist mainly on *Atriplex*, the milk acquires a salty taste while feeding on *Schowia Purpurea* gives the milk an odour similar to that of cabbage (Gast, *et al*., 1969). Fodder composition also directly affects the fat and protein content of milk. The availability of drinking water was shown to have a direct effect on milk fat content, with limited drinking water causing a decrease in milk fat and protein content (Yagil and Etzion, 1980a). One of the advantages of the camel in drought areas is its ability to utilize plants that grow well under arid conditions which are in the

main unacceptable to other grazing animals (Knoess, 1977; Sharma *et al*, 1963; Williamson and Payne, 1965).

The utilization of available fodder is also much higher in camels than in other domestic animals in the same area (Farid, *et al*., 1979) The camel ability to utilize the scanty fodder resources of the arid zones of the world for the body maintenance, growth and the production makes this animal a potentially important source of food.

1.3 Trace elements in camel's milk:

Studies on camel's milk whether with respect to concentration or bioavailability of trace elements are limited and warrant have further investigation.

The concentration of manganese and iron in camels milk was remarkably higher (7.20 fold and 4-10 fold, respectively) than in human, milk, cows’ milk and infant formula. Zink content of camels’ milk was higher than that of human milk but slightly lower than cows’ milk and infant formula. The concentration of copper in camel milk was similar to that of cows’ milk but lower than in human milk and infant formula (Farida, 2001). Aproximalty 50-80% of zinc, copper and manganese in camels’ milk were associated with the casein fraction, similar to that of cows’ milk. The majority of selenium and iron in camels’ milk were in association with the low molecular weight fraction (Farida, 2001).

Camel's milk is considered as a potential source of manganese, iron and selenium not only for infants but also for other groups suspected of milk deficiency of these elements.

Deficiency of trace elements such as those of zinc, copper, manganese, selenium and iron might occur due to their low intake and impaired bioavailability. Carry *et al*., (1981), Gross *et al*., (1998) reported that, dialy intake of zinc and iron from human milk is below the recommended level.

1.4 Iron:

1.4.1 Tissue distribution and function:

Iron is distributed in many of different compartments including hemoglobin and tissue iron. Iron primary function in both muscle and milk is the transport of oxygen to the cell. In blood iron forms hemoglobin, in muscle myoglobin. Transferrin also found within the cytosol of many cells and thought to serve as intracellular iron transport protein within the cell (Carl *et al*., 2000). Iron also is a component of enzyme systems that are involved in the oxidation of glucose to produce energy. Underwood, *et al*., (1971) reported that liver and spleen carry the highest iron concentration, followed by kidney heart, skeletal muscles. Pancreas normally contains only half to one tenth of the concentration of the liver. Spleen, iron is involved in the immune system

(Lynne, 1997). Significantly higher values of serum iron were recorded in camels> 4 years of age than in young camels (Sukhadia *et al*., 1987). Shekhawat, *et al*, (1987) reported that 2-6 years age of dromedaries, the mean serum iron was 110.11± 7.07mg/100ml. Barakat and Abdel Fattah (1970) found the level of iron in plasma to be 44.4mg/100ml

1.4.2 Absorption and Excretion:

Dietary iron is composed of two forms, nonheme and heme iron Lynne, (1997). Gastric acid is important for the solublization of non heme iron in food (Bezwoode, *et al*., 1978). Heme iron is quickly absorped into the small intestine, primarily in the duodenum. Non heme iron is absorped slowely because its ingested in the ferric form and reduced to ferrous iron.

Hallberg, (1980) reported that, several dietary factors may act either as enhancers or inhibitors of absorption from the common non-heme iron pool form in the stomach and the upper small intestine.

The heme iron is independent of meal composition and little affected by enhancers and inhibitors which alter non-heme absorption, while other components are necessary for adequate absorption (Cornad and Cortell, 1966). As iron enters the blood it is oxidized by caeruloplasmin, bound to transferrin, and carried to the liver and body tissue. Excess iron is stored in the form of ferritin-milk proteins, albumin (Todd’s 1976).

1.5 Copper:

1.5.1 Tissue distribution:

Church and Pond, (1988) stated that the liver, brain, kidney, heart, pigment part of the eye and hair or wool have highest Cu concentration in most species, pancreas, spleen, muscle, skin and bone are intermediate and the thyroid, pituitary, prostate and thymus are the lowest. Young animals have higher concentration of cu in their liver than adults (Mc Cosker, 1968). In the blood 90% of the cu is associated with globin, caeruloplasmin and 10% in the red blood cells as ethro cuprein (Under wood *et al*., 1977).

1.5.2 Function:

Cu functions in utilization of iron at an early stage of hematopoiesis (Hartam and Were, 1977). Also it functions in the normal myelination of the brain cells and spinal cord and for normal hair and wool pigment (O' Dell, 1979). Oxidation of iron from ferrous to ferric required for its transfer from tissue to plasma, Cu containing caeruloplasmin required for this oxidation.

1.5.3 Absorption:

Absorption varies among species. The main site of Cu absorption in dogs is the upper jejunum, in pigs and rats is small intestine and colon (Bowland, *et al*, 1961). Under Wood, (1977) stated that, some forms of Cu are absorbed more readily than others. Cupric sulphate is more readily absorbed than cupric sulphide. Cu absorption is done by two mechanisms passive diffusion and energy- dependent transport (Church and Pond, 1988).

1.5.4 Excretion:

Cu is mainly excreted in the bile, small amounts are lost in urine negligible amount in sweat under normal conditions (Hazelrie *et al.,* 1966). Radio active copper studies indicate that the main source of urinary cu is the one which loosely bound to albumin in the plasma,(NRC, 1980).

1. 6 Caeruloplasmin activity:

Caeruloplasmin is copper containing protein found in the plasma. Depending on the species the Cu in Caeruloplasmin constitutes 60-90% of the total plasma Cu (Evans and Wiederanders, 1967). However, ingested Cu disappears rapidly from the plasma and concentrates in the liver (Cartwright and Wintrobe, 1964). After that a secondary increase occurs in plasma Cu, accompanying the discharge of Caeruloplasmin from the liver (Bearn and Kunkel, 1954). While Caeruloplasmin is

synthesized in the liver (Hazerlrie and Owen, 1966), Cu is not incorporated into the globulin until the metal permeates the hepatic cells. Carl *et al*., (2000) stated that, specific enzymatic activity of Caeruloplasmin, estimated by ratio of enzymatically measured to immunoreactive of Caeruloplasmin may be more sensitive indicator of copper status than other plasma copper. Osakai *et al*., (1966), and Roeser *et al*., (1970) showed biological role for Caeruloplasmin of serum promoting the rate of iron saturation of transferrin and in stimulation iron utilization and the disgnation of the enzyme as serum ferroxidase was proposed. Srivastava and Dwaraknath, (1971) stated that, the average of Caeruloplasmin activity in units defined as increase in absorbance of 0.001/0.1ml serum in 1h was 25.00±10.54 in camels. It was reported that Caeruloplasmin has been found to be in substantial amount in the sera of many different animal species (seal, 1964).

1.7 Magnesium

1.7.1 Tissue distribution:

It is widely distributed in the body. About half of body Mg is in the bone at concentration of 0.5-0.7% of the bone ash Wacker and Vallee (1964). It is concentrated mainly within the cells in the soft tissues. Mg concentrated mainly in the liver and skeletal muscles. In the

blood 75% of Mg distribution is in the red blood cells and 25% in the serum (Church and Pond, 1988).

In milk Mg concentration was lower at the beginning of lactation than at the end of lactation (Brendehaug and Alzrahamson, 1986).

1.7.2 Function:

Mg is required for normal skeletal development as a constituent of bone. It is required for oxidative phosphorelation by mitochonderia, it is also required for activation of enzymes concerned in the reaction involving ATP (McDell *et al*., 1993).Also Mg acts as a Co-factor in decarboxylation and is required for the activation of certain peptidases (Wacker and Vallee, 1964).

1.7.3. Absorption:

Mg absorption occurs mostly from small intestine. No carrier is known for absorption and no effect of vitamin D on it's absorption (Phillipson and Storry, 1965). An increase in Ca concentration in cattle gradually decreases Mg absorption (Timent and Stojevic, 1989). Also Neathery, *et al* (1990) stated that, Mg absorption was reduced by aluminum in the diet.

1.7.4 Excretion:

Mg excretion occurs through urine and faeces. 95% of absorption is lost in urine, excretion in faeces account for the remainder (shraga, 1999). Todd's, (1976) reported that the secretion of Mg in milk may be important factor in of hypomagesemia.

1.8 Iodine

Iodine reaches the animal mainly with food and water, though it can easily be absorbed through the skin or lungs from the atmosphere (Pitt-Rivers and Tata, 1959; Brown, 1961). The dietary iodine is absorbed mainly in the form of inorganic iodide from gastrointestinal tract.

The thyroid gland accumulates iodide very efficiently and iodide is the only chemical form in which thyroid can take up iodine (Halmi, 1964).

Iodide is oxidized immediately after its entry in the gland to elemental or active iodine (Degroot, 964). Abdel Wahab, *et al*., (1971) stated that thyroid uptake of iodine by camels was much less than by sheep and goats. 1.7% of dose at three hours and 10.1% at 48 hrs compaired with 4.3 and 5.3 at three hrs and 19.5 and 23.1 at 48 hrs. Serum values also were much less of Protein bound iodine (PBI) 0.05%

of dose compaired with 0.27 and 0.25 in sheep and goats Abdel Wahab, (1971).

1.8.1 Role and importance in the body:

Iodine is essential for synthesis of the thyroid hormones which control the metabolism of the body, affecting growth rate, digestion, and the burning up of excess fat. It regulates cholesterol level, prevents cretinism in newborns when taken by the pregnant women, it is essential for energy production, prevention of anemia, necessary for lymphatic system (Michelli, 1982). Manganese and magnesium assimilation is also defective when iodine is missing. This is interesting because heart beats with calcium and relaxes with magnesium, (Michelli, 1982).

1.8.2 Iodine in the body:

About 20% of all iodine in animals is in the thyroid gland. In the Thyroid gland iodide is oxidized to iodine by coupling to the amino acid L-tyrosin into hormones like Tyrosin (T4) and triiodtyrosin, thyroid influences many functions of organs metabolism like maturation of tissues, muscle function and fertility performance (Indergaad and Minsaas, 1991). Recent studies have shown that, uptake of iodine is controlled via an intramembrance protein of nervous system which co transports iodo chemical gradient (Daig *et al*., 1996).

1.8.3 Excretion:

Iodine is exclusively excreted in urine in the form of inorganic iodide. Studies with labeled T4 have revealed that considerable amount of T4 is excreted in faeces and urine either free or conjugated (Irivine, 1969). Radioactive iodide was also detected in faeces after its oral administration to goats (Ayuob, 1966).

1.9 The effect of the physiological status of animals on the plasma mineral concentrations:

The concentration of minerals in the blood of cattle vary with the age of the animal, stage of pregnancy, milk yield, management factors and seasons of the year (Rowlands *et al*., 1974a and Kitchenham *et al*., 1975). Few studies have also provided evidence of genetic variation in the concentrations of some of the constituents (Rowl ands *et al*., 1974 and Kitchenham and Rowlands, 1976).

In a study carried to determine the association between breed, and carcass composition and status of liver Cu, Zn and Flour as well as plasma Cu, Zn, Ca and Mg of mature cows representing nine breeds of cattle, it has been observed that there was no breed difference in plasma concentrations of Cu or Zn, but there were differences in plasma Ca and Mg concentrations (Little Dike *et al*., 1995). One hundred and seventy female Ayrshire cattle approximately 6 months of age were sampled on

one occasion to determine the plasma concentrations of Cu, P, Ca, Mg, K and glucose. The combinations of age and stage of pregnancy or lactation significantly affected variation in all the constituents. Glucose and inorganic phosphate decreased with age, while copper and calcium increased (Wiener *et al*., (1980). Phosphorus, calcium, magnesium, copper, cobalt, molybdenum, selenium and zinc were measured in blood plasma of pregnant, non-pregnant and lactating animals (carabaos), levels of most minerals were influenced by lactation and reproductive status. But no significant differences in the groups of animals. Plasma calcium in pregnant animals was higher than in non-pregnant and lactating carabaos, but the difference was not statistically significant. Plasma Mg levels of pregnant and non-pregnant carabaos was similar (Obsioma *et al*., 1994).

In a survey of 260 cows of the pabna, 4-12 years of age, plasma mineral concentrations were: sodium 141.67±3.85 meq/L, potassium 5.14±0.53 meq/L, calcium 9.04±1.34 mg/100 ml and Mg 2.60±0.61 mg/100 ml for lactating cows and for non-lactating cows 140.77±2.87, 5.03±0.53, 9.27±1.06 and 2.4±0.55 respectively, no significant differences between groups were observed (Rahman and Begi, 1985).

Bone marrow samples from cattle about 2 to over 7 years of age, were analyzed by atomic absorption spectrometry. Ca concentrations were higher in the bone marrow than in muscle tissue, while those of K,

Mg, Zn, P, Na, Cu and Co were lower than in muscle. Bone marrow of young cattle had less P, Ca, Mn and Co and more Na than older animals, (Nowakowski, 1989). 89 cross-bred Angole cows 2.6 years of age were divided into 5 groups; oestrus, nearing parturition, anoestrus, recently calved and in early pregnancy. Plasma Ca and P values did not differ significantly (Sivaih *et al*., 1986).

Blood plasma Ca and P were determined in groups of 12 black Bengal goats at different stage of pregnancy and lactation, Ca and P were significantly higher in pregnancy than in lactation, plasma Ca was significantly higher in early than in late pregnancy and in late than in early lactation, (Uddin and Ahmed, 1984). Calcium transport in the intestine was measured in vitamin-D-replete and vitamin D-deficient rats during pregnancy and lactation, also plasma Ca concentration and 1-25 dihydroxy vitamins D were measured and correlated with their transportation. During the late stage of pregnancy and during lactation the concentration of Ca in the plasma is reduced (Halloran and Delucan, 1980).

Plasma calcium concentration was determined in 144 cows in late pregnancy, just after calving, in the second month of lactation or in the fifth month of lactation. The lowest value was found in cows that had just calved and the highest value found in late pregnancy (Ivanov *et al*., 1990). Knuthove (1986) observed that the magnitude of change in Ca

fluxes from the non-lactating to lactating state is dramatic with a three to six fold increase in Ca losses from extracelluar fluid. Lactation with the movement of Ca from the plasma into the milk causes a decrease in the concentration of Ca in plasma (Tovenrud *et al*., 1976 and Ballantine and Herben, 1989).

Miller *et al*. (1985) reported that the define oppositional rate in the rat incisor increased during pregnancy and decreased in lactation. By 4 weeks after parturition most pups had been weaned, and dentine oppositional rate was similar to that of controls. These changes may be indicative of generalized changes in mineral skeletal metabolism to accommodate foetal skeletal mineralization and milk production. Analysis of skeletons from 84 gilts slaughtered in groups on days 25, 50 or 103 of gestation and day 56 of lactation and comparison with those from non-pregnant gilts reared under the same conditions indicated that during gestation, minerals are deposited in the skeleton in exchange for water and fat, with this mineral reserve being mobilized during lactation (Farries *et al*., 1984). Fild and Scuttle, (1968) reported that, during lactation ewes gained 10 kg empty-body weight, but this gain was associated with a further loss of Ca, little change in P, Mg or Na and a considerable increase in K equivalent to 3 g/Kg gain. Buhman, (1985) reported that there was no relationship between the Ca status of a cow and the content of blood and urine. Plasma concentrations of Ca, P,

Mg, K, Cu and Zn in 21 heifer and 20 cows were determined during months 2-3, 5-6 and 7-8 of pregnancy, immediately before and after parturition, and during months 1-2 of lactation. There was a significant decrease in plasma P concentration at parturition in both age groups, the concentration of plasma P increased again during lactation (Agnes *et al*., 1986).

Naito *et al*. (1990) found that the concentration of plasma 1.25 dihydroxy vitamin D increased from calving to 3 days post-partum, in 5 Holstein Friesian cows, ages 3 to 5 years examined from 5 days before to 15 days after calving. The number of these receptor significantly decline with increasing age (Goff *et al*., 1989), so the intestinal absorption of Ca decreased accordingly, resulting in a decrease plasma Ca with increasing age.

To show changes in erythrocyte sodium and erythrocyte potassium blood samples from 54 dairy cows (mainly friesian and Jersey) during the periods 8 weeks before and 8 weeks after calving. The cows were divided into two age groups; group 1 consisting of cows aged 2-5 years and group 2 consisting of 28 cows aged 5-9 years. In the older cows the concentration of erythrocyte sodium (ENa) decreased while that of erythrocyte potassium (EK) increased after calving. Both the ENa and EK concentrations varied significantly between cows in the two age groups (Mulei and Daniel, 1990).

Chapter Two

Material and Methods

Field survey was included Rashayda camels (Diaili) in Kassala area of eastern Sudan. Thirty samples from both milk and serum were collected through August to September (2002).

2.1 Animals:

All investigated animal were mature, lactating she – camels, in early lactation period . Three different age groups of 9 years 12 years and 14 years were investigated. All animals graze freely Hantoot (*Convolvulus arvensis*) , Dirasa R*ibutlus*. *territories*) and Soleep (*Sesbenia sesbenia)*.

2.2 Designation of the milk and serum samples:

Letter coding was used to represent the samples of milk and serum obtained from the three different age groups.

Group A: samples obtained from age 9 years

Group B: samples obtained from age 12 years

Group C:samples obtained from age 14 years

2.3 Sampling Techniques for Milk and Blood:

Ten samples of milk and blood were taken from each age group of animals for analysis.

Milk samples were collected using hand milking in clean bottles and stored frozen at -20ºC until analyzed.

Blood samples were taken form the jugular vein by vein punctures. The serum was separated by centrifugation of blood samples at 3000 rpm for 10 minutes. Serum samples were stored at-20ºC until mineral and enzymic analysis was carried out.

2.4 forage samples

Fresh sample of camels feed which consist of *Convolvulus arvensis* (Hantoot) was weighted, then analyzed.

2.5 Preparation of Glass Ware:

All glassware was washed with soap, followed by rinsing with tap water. The glassware was then saocked in 10% nitric acid and left overnight before being washed several times with tab water followed by rinsing with double distilled water, oven dried and ready for use.

2.6 Analytical Methods:

2.6.1 Preparation of milk and forage samples for Analysis:

Empty, clean dry crucibles were numbered and weighted accurately using sensitive balance. 10ml of milk or 5g of forage were paced in clean dry crucibles, then all crucibles were placed in the oven and the temperature was adjusted to 105C for 24 hours. Then the crucibles were weighted. . The dry matter within crucibles were then placed on hot plate and were allowed to evaporate until the samples were completely charred before being transferred to a muffle furnace at a temperature of 500C. Samples were left to ash overnight. The ashed matters within crucibles were then left to cool at room temperature. A few drops of concentrated hydrochloric acid (HCl) were added to the dry ash. The samples were then dried gently on a sand bath to avoid pumping. Mineral extraction was performed by adding 10 ml of 25% HCl to the ash. The content were filtered using ashless filter paper into 25ml volumetric flask and labeled. The (ash less 41) Filter papers were washed with 1% warm HCl and the final volume made up to 25ml by adding distilled water. The digests were further diluted before analyzed. Moisture and dry matter were determined according to the method of Fertilizer and feeding stuff regulation, (1973).

2.6.2 Determination of iron (Fe) and Copper (Cu) in the milk and serum:

Iron:

The method of Harold, (1979) was used for the determination of Fe and Cu using Uincam 8625 UV /vis. Spectrophotometer.

Reagents:

1-Saturated potasium persulphate 8 g / 100 ml distilled water (D.W)

2- Sodium thiocyanate 25 g +acetone 5ml up to 100 ml D.W

3- Iron standard

4- H_2SO_4 concentrated

5- Nitric acid (concentrated)

Principles:

Some metals such as iron will form highly colored complex when react with thiocyanate ions.

Sample was digested with Conc H_2SO_4 and 60% per chloric acid. Absorbance against reagent blank (amyl alcohol) was done to the supernant after centrifugation.

Procedure:

2 ml of serum or prepared milk sample were taken in a digestion flask (kjelldahl). Then 2 ml of conc H_2SO_4 2 ml of 60% perchloric acid were added. After cooling of the flask 2 ml of nitric acid were added, then the flask was reheated until the contents were colourless. The

content was then transferred into 50 ml volumetric flask and made up to the mark with D.W.

In a centrifuge tube 0.2 ml of the solution + 9.8 ml distilled water + 0.2 ml saturated potasium persulphate +1 ml 25% sodium thiocynate + 5 ml N amyl alcohol were added .The contents were shaken gently for 2-3 minutes and centrifuged for 5 minutes at 2000 rpm. The supernat was taken and absorpance against reagent plank (amylalcohol) at 480 was recorded.

-Standard: 0.2 ml working standard and treated as the same as the sample.

Calculation:

$$\frac{T X50}{S}$$

T = Sample

S = standard

Copper:

The method of Harold (1979) was used to determine Cu using Uincam 8625 UV/vis. Spectrophotometer.

Reagents:

1- copper reagent

Zink dibenzyl- Dithio – carbamate 0.05 g

Carbon tetrachloride 1 litre

2-Cu standard

Principle:

Residues from ashing were treated with perchloric acid after appropriate dilution.

Procedure:

The test solution used for iron determination was transferred to a bottle and 5 ml of copper reagent was added to the bottle. The contents were shaken throughly until a yellow matreial appeared in the bottom of the bottle. This material was a spirate and transferred to a clean test tube using cotton plugs. Read at 435nm after a while.

50 ml of iron was taken to a digestion flask and 10 ml of 65% perchloric acid was added and boiled until dissoloved. Cooled and diluted to 100 ml with distilled water. 2 ml of stock standard in 10 ml distilled water.

Calculation

$$\frac{T \times 50}{S} = mg/100ml.$$

2.6.3 Determination of the milk and serum Magnesium (Mg):

Mg was determined by Spare, (1962) Method using Unicam 8625 UV /vis Spectrophotometer.

Reagents:

1- standard Mg Solution :

2- Polyvinyl Alcohol Reagent:

3- Titan Yellow:

4- Sodium hydroxide solution :

Principles:

Sample under test concentractions were determined, after aspirating at appropriate dilution from the constructed calibaration curve for the metal.

Procedure:

0.2 ml of serum or prepared milk sample was diluted with 2.8 ml of distilled water, for the blank 3.0 ml distilled water was used. To all tubes 0.5 ml of polyvinyl alcohol, 0.5 ml titan yellow and 1.0ml 7.5% (W/V) sodium hydroxide solution were added in the above stated order with mixing after each addition . All tubes were allowed to stand for 5 minutes before being read at 540 nm and the zero absorbence was set by the blank

Calculation:

The magnesium was calculated as follows

$$\frac{\text{Reading of unknown}}{\text{Reading of high standard}} \times 2.5 = \text{mg/100ml}$$

2.6.4 Determination of milk iodine:

The Method of Elmslie-cold weel (AOAC, 1970) was employed for the determination of iodine in milk.

Reagents:

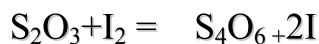
Bromine water

-phosphoric acid (85%)

-N/10 Sodium thiosulphate

Principles:

Strong reducing agents such sulphuric acid sodium thiosulphate react with iodine even in acid solution



$S_2O_3+I_2 = \quad S_4O_6+2I$

Procedure:

A sample of 10 ml milk was placed in 200-300 ml crucible. About 5g sodium carbonate, 5 ml sodium hydroxide solution (1+1), and 10 ml alcohol were added , taking care that, the entire sample was moist .The sample was dried at about 100º C to prevent spattering upon subsequent heating (30 minutes was enough).

The dish and contents were placed in furnace heated to 500ºC and kept at the temperature for 10 minutes (heating of sample at 500ºC appear to be necessary only to carbonize any soluble organic matter that would be oxdized by bromine water if not so treated).The samples were

cooled and 25ml water were added .The dishes were covered with watch glass, and boiled gently for 10 minutes. Filtration through 18cm filter paper and washing with boiling water was done , catching filterate washing in 600ml beaker (solution should total approximately 300ml).The solution was made neutral to methyle orange with 85% phosphoric acid , then 1ml excess was added.

Excess bromine water was added, and the solution was boiled gently until colourless and was then boiled further for 5 minutes. Few crystals of salicylic acid were added and the solution cooled to 20ºC .Then 1ml 85% phosphoric acid and 0.5g potasium iodide were added . Then iodine wastiterated with 0.005 N sodium thiosulphate, adding starch solution when librated iodine colour was nearly gone.

The solution was diluted to 50ml with water, 1ml of diluted acetic acid was added, and titration was carried with N/10 sodium thiosulphate, using starch mucilage as indicator.

Calculation:

Given that, each 1ml of N/10 sodium thiosulphate was equivelent to 0.01269g of iodine, the quantity of iodine equivelent to 1ml N/500 sodium thiosulphate can be estimated, and hence the quantity of iodine in the sample can be calculated.

$$\frac{\text{Sodium thiosulphate in mls X0.0129 X 10}}{2} = \text{g/100ml} = \% \text{(W/V)}$$

2.6.5Determination of plasma caeruloplasmin:

Caeruloplasmin was determined by using the method of Boyd Hovchin, (1958).

Reagents:

- 0.7%para-phenylenediamine (P P D)
- 0.02%Sodium azide

Principle:

Measurement of enzyme activity includes the camparason of rate of reaction with and without the active enzyme in the reaction mixture. Unites of enzyme are thus amount of chemical change taking place in unite time, while activity of an enzyme in a given sample is expressed as the number of unites in stated volume of the sample. Samle was buffered in acetic buffer, apropriate dilution was done. After incubation reaction stopped with sodium azide.

Pocedure:

To 1.0 ml freshly prepared 0.7% para-phenylenediamine(P PD) in acetate buffer at 37C°,0.1ml serum was added (acetate buffer, 20ml glacial acetic acid+163g sodium acetate Na C_2 H_3 O_2 .3H $_2$0. Then diluted to 1litre with distilled water –ionic strength 1.2 pH 5.2 ±0.05).

After incubation for 10 minutes at 37ºC 5.0ml of 0.02%sodium azide was added to the mixture to stop the reaction. Optical density was measured at 525nm. The optical density was detrmined within 30 minutes if water Blank is used or 5hours if colour is read against a reagent Blanck to allow for auto oxidation of the P P D.

Since no standard reading caeruloplsmin activity was determined in units /100ml from spectrophotomer directly.

2.7 Forage Samples:

2.7.1 Determination of Crude Protein (CP) and Crude Fiber (CF).

Method of analysis of the Association of Official agricultural Chemist (1965) was used to determine (CP) and (CF).

Crude fiber:

Reagents:

T. C. A. digestion mixture which was prepared as follows:

Glacial acetic acid	500 ml
Distilled water	450 ml
Nitric acid	50 ml

T.C.A 20g was dissolved in the mixture of the three acids

Principle:

The lipid free sample after ether extraction was allowed to be digested with T.C.A .digestion mixture which dissolves all the nitrogen free extract and separate it from the lipid free sample .The digested sample will contain the fiber and the ash .The ash was separated by ashing the digested sample, and the fiber content in the sample was estimated.

Procedure:

1. The residue remained in the extraction thimble after soxhlet Extraction (ether extract determination) was transferred into 750 ml wide neck conical flask.
2. T C A digestion mixture (100 ml) was added and the flask containing the residue + the digestion mixture was placed on the heating unit and the condencor attached was turned on to allow cooling water and the gas was lit.
3. The contents of the flask were allowed to boil and were boiled gently.

4. The digestion was allowed for 40 minutes and this should be measured from the moment the gas was lit.During boiling the flask was swirled occasionally.
5. After 40minutes the gas was turned off and the flask was cooled with running water.
6. The residue (sample) was filtered through weighed ashless filter Paper (42) and the filtrate was washed at least 3 times with distilled water.
7. The filtrate was placed in dry clean weighed crucible and the contents were dried overnight in the oven at 100º C then desicated, cooled and weighed.
8. The crucible +the residue were then placed in the muffle furnace and ignited at 550º C for 3 hours.
9. The ash was allowed to cool in a desicator and weighed. The loss in weight during ashing represents the weight of crude fiber in the sample.

Calculation:

Crude fiber %=

(weigh of crucible +dry residue)- (weight of Crucible +ash)

Weight of sample

Crude protein:

Reagent and chemicals:

1. Concentrated nitrogen free sulphuric acid ($H_2 so_4$) .
2. Sodium hydroxide 40% (NaOH) (prepared bby dissolving 40 g of NaOH in 100 ml distilled water).
3. Boric acid 2% (prepared by dissolving 2g of boric acid in (100 ml) distilled water.
4. Kjeldahl catalyst tabletes (sodium sulphate +the metalic mercury).
5. Indicator prepared by dissolving 200 mg of methyl red +600 mg bromocresol green and made up to litre with 95% ethanol (950 ml of 100% ethanol +50 ml distilled water).
6. N Hcl (prepared by dissolving 8.6 ml concentrated Hcl (36%) in litre of distilled water)

Principles:

The sample was digested with concentrated sulphuric acid which converts the nitrogen into ammonium sulphate when cooled with distilled water and neuteralized with sodium hydroxide which converts the nitrogen into

ionized ammonium.The sample is then distilled titerated against a week acid (0.1 N Hcl).

Procedure:

1. Oven dried sample (1g) was weighed in the Kjeldahl flask.
2. kjeldahl catalyst tablet (one tablet) was added.
3. Sulphuric acid nitrogen free (25ml) was added to the flask contents.
4. The sample was allowed to digest by placing it on the heater (digestion room) and was digested until the colour become clear (3 to 4hours). The digested sample was allowed to cool and diluted to 100 ml with distilled water in volumetric flask.
5. The diluted digested sample was well mixed and 5 ml was placed into Markham apparatus for distillation and 10 ml of 40% NaOH was added gradually.
6. The distilled sample was collected in conical flask (100 ml in volume) containing (25 ml 2% boric acid +3 drops indicator).The receiving flask was placed under the condenser of the apparatus , so that the lip of the condenser tube was above the liquid in the flask , the collection continued till the volume reached 75 ml.
7. The collected distillated sample was titerated against 0.1Hcl.

Calculation:

Titration volume × dilution factor ×

$$\frac{\text{dilution factor} \times \text{acid normality} \times 14 \times 100}{1000}$$

$$\text{Titration volume} \times \frac{100 \times 0.1 \times 14 \times 100}{5 \times 100}$$

= titeration volume × o.oo14 × 100 × 20

N% = titration volume × 2.8

Crude protein contains 16% N

$$N\% = \frac{N\% \times 100}{16} = N\% \times 6.25$$

Crude protein % = N% × 6.25

Crude protein % = titration volume × 2.8

C.P % = titration volume × 17.5

2.7.2 Determination of Cellulose, Acid Detergent Fiber (ADF) and Acid Detergent lignin (ADL).

These parameters were determined according to modification method of crampton and Maynard, (1938).

Reagent:

1- Cellulose digestion Mixture

glacial acetic acid 10ml

Conc nitric acid 112ml

De-ionized water 210 ml.

1- rectified spirit (95% ethanol)

Principle:

The sample was digested with a mixture of acetic acid and nitric acid which hydrolyses starch and protein and removes lignin by oxidation and hydrolysis. These products of hydrolysis were removed by filtration. Sugars and colouring matter were removed by ethanol. The resulting fiber was mainly cellulose.

Procedure:

I gram of sample was weighted (Ws) , 12.5 ml digestion mixture was placed in the heating block which should be stable at 140ºc. The sample were boiled rapidly tubes were removed after 20 minutes. 10 ml rectified sprit was added, the mixture was swirled and filtered thriough the a Gooch crucible, then the sides of crucibles were rinsed well with rectified sprit. The crucibles were transferred to an oven at 100ºC and dried over night,then cruciples were cooled in a desiccator and weighted (W3). Crucibles were ashed in a furnace at 500ºc for 3-4 hours and furnace was switched off, the door was opened and cruciple was allowed to cool in the furnace for at least 30 min. then cruciples were removed and allowed to cool in the dessiccator and weighed (W4).

Calculation:

$$\% \text{ cellulose} = \frac{W3 - W4 \times 100}{Ws}$$

Acid detergent fiber (ADF):

Reagents:

1- Acid detergent solution
2- Silicon anti foam
3- Acetone – reagent grade

Principle:

The ADF procedure provides a rapid method for lignocellulose determination in feedstuff. The difference between cell wall and ADF is an estimate of hemicellulose. However this difference does include

some protein attached to cell wall . The ADF is used as preparatory step for lignin determination.

The detergent removes the protein and other soluble material that would interfere with the lignin determination. The ADF residue consists of cellulose, lignin, cutin, and acid-insoluble ash (mainly selica). Treatment of with 72% H_2SO_4dissolved cellulose. Ashing of residue will determine crude lignin fraction.

Procedure:

Glass filter crucibles were weighed (W1), then 0.5 gram of sample was weighed (Ws) into long boiling tube. 25 ml of acid detegent and 1 ml anti foam were added, then Tubes were placed in an alumimium bluck heater set at 150ºC and heated for 1 hour after boiling had commenced. The tubes were shaken to reduce initial frothing. Contents of tubes were filtered through weighed filtered crucibles using vacuum. Washing with hot distilled water (90-100ºC) was done and then with acetone to remove residual colouring matter and Dekalin.

The crucible and contents were dried overnight at 100ºC and then Cooled and weighed (W3). Crucible was ashed in a muffle furnace at 550ºC for 3-4 hours then Cooled and weighed (W4).

Calculation:

$$\%ADF\ (Dry) = \frac{W3 - W1 \quad X100}{Ws}$$

$$\%ADF\ (organic) = \frac{W3 - W4 \quad X100}{Ws}$$

Acid detergent liginin:

Reagents:

Sulphurric acid 72%

Prucedure:

Crucibles were placed in an appropiate container. A sufficient acid was added to half fill the crucibles, then crucibles were kept at 20-23ºC for 3 hours, acid was filtered off and washed with hot water until free of acid. Drying add 100ºC overnight was made then cooled and weighted = W5. Ashed at 500ºC for 4-5 hours, then cooled and weighed = W6.

Calculation:

$$\%ADL = \frac{W5 - W6 \ X\ 100}{Ws}$$

2.8 Statistical Analysis:

The data were analyzed by using completely Randomized design (Gomez and Gomez, 1984).

Duncan Multiple Range Test (D M R T) was used to compare the means.

CHAPTER THREE

RESULT AND DISCUSSION

Minerals in the blood in wellfed healthy are controlled by homeostatic mechanism, which keeps the contents within the normal limits .Any variation within the reasonable limit can be influenced by dietary changes, environmental factors, (Acinsoyinue, 1982). Milk is an important source of minerals to young animals, elderly, sick people and young children .Minerals in milk are absorbed selectively from the blood. If the animal didn't receive the necessary amount of these minerals in their diet, they compensate by drawing them from the body stores of these minerals, without initially affecting milk yield and composition, (Fingerline, 1911).

3.1 Concentration of nutrients in forage samples:

The results of the analysis of forage samples eaten by camels are presented in Table A*-1 and figure (1).

The figure showed that the forage sample contains high level of acid detergent fiber and low level of fat when compared with other constituents. A decrease in fat up to the 4 to 6 months of lactation then an increase to the end of lactation in camels has been observed (Indra and Erdenebaatar, 1994; Hassan *et al*, 1987 and Belokobylenko, 1986) In the second year of lactation, milk of libyan camels had more fat compaired with the first year (Gnan and Sheriha, 1986).In Bacterian

Table (1): Some chemical constituents of forages eaten by camels in Kassala state.

Forage constitution	Level of content (%)
Protein	21.0
Moisture	12.0
Ash	17.9
Crude Fiber	24.2
Fat	9.9
Acid detergent lignin	19.0
Acid detergent fiber	64.0
Cellulose	22.4

camels, fat was the lowest when yield was the highest (Xhao, 1994;Cherepanova and Belokobylenko,1986). Lignin provides strengh and rigidity to the cell wall and growing plant because lignin generaly resistant to decay and microbial enzymes attach, it enables plants to resist various pathogenic organisms, this resistance extend to the ruminal microbial degradation, (Van Soest, 1967).

3.2 Milk minerals concentration:

The concentrations of Fe, Cu, Mg and iodine of camel's milk of groups A, B and C are presented in figure (2) and table (2).

3.2.1 Iron:

Table (2) and Fig (2) showed significant ($P<0.01$) decrease in iron level in group C when compared to the two other groups (A and B). This showed that there was significant effect of age on milk iron level. Though all animals in the present work were in early lactation. Walgren, (1932) and Plumier, (1946) have reported that the stage of lactation had no marked influence in iron contents of milk in animals.

3.2.2 Copper:

A significant ($P<0.05$) decrease in group C was demonstrated in Table (2) and Fig (2) when compared with groups A and B, the results

Table (2): Effect of age on some chemical constituents of camel milk (Means ± S.E)

Experimental groups	**Fe(%)**	**Cu (%)**	**Mg (%)**	**Iodine (%)**	**Ash (%)**
9 years (10)	0.78a	0.128a	9.0a	0.43a	**0.7a**
12 years (10)	0.72b	0.127a	8.0b	0.32a	**0.6a**
14 years (10)	0.69c	0.121b	8.0b	0.38a	**0.7a**
S.E±	**0.03**	**0.002**	**0.049**	**0.005**	**0.05**

-Means within columns followed by similar letters are not significantly different according to D M R T .
-Numbers in parenthesis indicate number of animal used.

showed decrease of Cu level with increasing age. This finding was different from that reported by Csapo and Csapo, (1984) in goats, who found Cu content was similar in all groups of age, this may be due to difference in species. All mean values in this work were substantially lower than that, reported by Ahmed, *et al*., (1977) for the Egyptian camel milk, these variations could be due the the Cu levels of feed and breed differences. Csapo and Csapo, (1984). Arinsoyinu, *et al*., (1979) and Beck (1941), reported that Cu contents in milk decreased significantly in the late lactation, while Epple and Horral, (1943) reported high Cu level in the second month of lactation in goats.

3.2.3 Magnesium:

The figure showed significant ($P<0.01$) increase of Mg level in group A when compared with groups B and C. The results showed that Mg level tend to decrease with increase of age. Hassan *et al.* (1987) stated that, Mg level showed slight increases at the begininig of lactation, followed by a gradual decrease in mid lactation and a sharp increase near the end of lactation.(Brendehaug and Alzarahamson,(1986) reported that, Mg was lower in the beginning of lactation than the end of lactation in goats.

3.2.4 Iodine:

Figure (2) and table (2) showed that there was no significant difference among the three groups, this means that there was no significant effect of age on I level, though higher levels were observed in young ages. However, radioiodine concentration in cattle milk was reduced proportionally to increase milk yield when thyroxin was administrated (Miller *et al,* 1970). Iodine concentrations in cattle milk were tended to increase with advancing lactation (Swanson, 1972).

3.2.5 Ash:

The result of the ash level in camel's milk was presented in Fig.2. There was no significant difference in the three different age groups. The mean value of the ash contents in the milk is similar to that reported by El-Bahay,(1962), less than that reported by Knoess,(1977) and El-amin,(1979) and higher than that reported by Yasin and Wahid,(1957) in camels.

The ash content of camel milk could be affected by drought conditions (Yagil and Etzon, 1980), hence it is very likely that great variations in the Ash contents of the dromedary milk could occur. Hamzawi and Hafez (1992) noted a continuous increase of ash from the early to the late lactation in camels.

3.3 Serum minerals concentration and Caerulopasmin activity:

The results of the effect of age on the serum minerals concentrations and caerulopasmin activity of lactating she-camels were presented in figure (3) and table (3).

3.3.1 Iron:

Figure (3) and table (3) showed significant ($P<0.01$) decrease in serum iron in group C when compared with groups A and B. This showed the effect of age on the iron level in camels. Many authors investigated the effect of different factors on the blood iron. Beside age Ca may reduce the absorption of iron, by interfering with indigenous metabolism or by increasing the excretion of iron, (NRC 1980). Planas and de Castor (1960) reported that significant difference in Fe of animals in the same age may be attributed to high individual variation of this parameter. Decrease in circulating erythrocytes with advancing age may affect iron level. Amna (2002) found that, lactation, pregnancy and even breed had no effect on iron level in plasma of goats. Saror (1980) and Mpofu *et al.* (1998) stated that, young animals maintained a higher Fe concentration than mature animals in cattle and gaots respectively.While Bhattacharyya *et al.* (1995) reported that, season had a remarkable influence on Fe level in goats.

3.3.2 Copper

Significant (P<0.01) decrease of Cu in group C was seen when compared with the two other groups. It was observed that age had an effective role on Cu level in the serum , this finding is in contrast to the results obtained by Tag Eldin, (1974) in goats where Cu concentration increased with age. This difference in result may be due to the nutritional factors (Underwood,1962) or may be due to difference in species. Underwood,(1962) reported that, no significant difference between pregnant and lactating goats in Cu concentration.

Csapo *et al.(*1986)) reported that Cu increased in the mid lactation and then decreased at the late lactation .

3.3.3 Caeruloplasmin activity:

Table (3) showed significant (P.0.05) increase in group A when compared with the two other groups. This mean that age has an effective role on caeruloplasmin level in camel serum. As no reports were found for caeruloplasmin in camel serum the present study cannot be assessed. There is high positive relationship between Cu and caeruloplasmin because caeruloplasmin is a constituent of a large proportion of total plasma Cu (Evans and wiederanders 1967). Camels caeruloplasmin activity was lower than other animals this may be due substrate

specificity as capacity to oxidize various substrate vary considerably, or inherently (Sravastava *et al*. 1971).

3.3.4 Magnesium

Significant difference among the three groups was observed in Table (3) and Fig (3). There was significant decrease ($P<0.05$) in group C when compaired with two other groups. The main factors involved in control of plasma Mg are absorption on the one hand and excretion and secretion on the other hand (Todd's, 1976), hence the maternal demand for foetus and milk were mostly met by dietary Mg.The mean valuesof Mg concentration mg/100ml were almost similar to that, reported by Akisoyinu,(1982) ;higher than that, reported by Vihan and Rai,(1998) and lower than that, reported by Siham (1998) in goats.

Same results on the effect of age on serum Mg level were obtained by Abead (1997). Different finding was obtained by Vihan and Rai,(1997) in goats who showed that, Mg level appear to be same the at all age groups .Akinsoyinu,(1982) reported that, Mg concentration decrease with the advance of lactation in goats .Absence of significant difference between pregnant and lactating groups in goats also repoted by Vihan and Rai,(1997) . Also Obsioma *et al* . (1994) showed that plasma Mg level of pregnant and non pregnant carbaos were the same.

Table (3): effect of age on some chemical constituents of camel serum. (Means ±S.E)

Experimental groups	Fe (mg/100 ml)	Cu (mg/100 ml)	Caeruloplasmin (unit/100ml)	Mg (mg/100 ml)
9 years (10)	5.62a	4.91a	0.03a	**2.72a**
12 years (10)	5.45a	3.99b	0.0192b	**1.97b**
14 years (10)	4.87b	2.32c	0.0153b	**1.84b**
S.E±	**0.02**	**0.017**	**0.005**	**0.8**

Means within columns followed by similar letters are not significantly different according to D M R T. ($P<0.05$)

.Numbers in parentheses indicate number of animals used.

Fig 3. Effect of age on some chemical constituents of camels' serum.

3.4 General discussion:

The decrease of levels of parameters measured in this work in the serum and milk with advancing of age may be due to the reduction in ability of absorption in aged animals. For nutritional adequancy in feeding animals, milk analysis for minerals availability is important, hence more studies in the effect of age were required to know minerals availability.

Since no data available on the effect of age on minerals in milk and serum of camels, comparison was made with available published information for other species. However, young animals are more lactating than aged ones, and known to have high level of minerals on its milk and serum (Csapo *et al* 1986). Lactating camels have specific appetite for individual minerals (Abd-Elfattah, 1982). Our results showed decrease of minerals with increasing age also the results suggest that minerals absorption might be impaired after extended advance of age.

Iodine and ash were not significantly different this may be due to the fact that the average of age for the herd studied was beyond the threshold of its effect to cause difference.

The levels obtained in the present work may be different from others due to difference of heredity and difference of feeding, climate and management. However, this has to be confirmed. More detailed

investigation of various minerals and the effect of age on their distribution in milk needed to evaluate its nutritional value.

It’s concluded that deficiencies of minerals should be prevented particularly in aged animals.

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