**Sudan University of Science and Technology**

**College of Graduate Studies**

# Biochemical Analysis of Synovial Fluid and Serum in Sudanese Cattle

# التحليل الكيميائي الحيوي للسائل الزليلي ومصل الدم في الأبقار السودانية

A thesis submitted in Fulfillment of the Requirements for The Master Degree M.V.M. In Biochemistry

By

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# Dedication

I dedicated this work

To the soul of my father

To my lovely mother

To my lonely sister Afnan

To my brothers Ahmed and Abubaker

## ACKNOWEDGEMENTS

First of all, praise be to Allah, a lot of praise good and blessed as should his majesty and good authority, praise be to Allah, who gave me strength and health to finish this work.

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## List of abbreviations

| | |
|---|---|
| **ALP** | Alkaline Phosphatase |
| **ALT** | Alanine aminotransferase |
| **ANOVA** | Analysis of Variance |
| **ATP** | Adenosine tri phosphate |
| **Ca** | Calcium |
| **CI** | Confidence Interval |
| **Da** | Dalton |
| **DF** | Dilution Factor |
| **DIJ** | Distal Interphalangeal Joint |
| **DSB** | Distal Sesamoid Bursa |
| **FAO** | Food and Agriculture Organization |
| **G** | Gauge |
| **g/dl** | Gram per Deciliter |
| **HCENR** | Higher Council for Environment and Natural Resources |
| **K** | Potassium |
| **Kg** | Kilogram |
| **LDH** | Lactate Dehydrogenase |
| **LSD** | Least Significant Difference |
| **Mg** | Magnesium |
| **mg/dl** | Miligram per Deciliter |
| **min** | Minute |
| **ml** | Mililiter |
| **mmol/L** | Milimole per Liter |
| **MOAR** | Ministry of Animal Resources |
| **Na** | Sodium |
| **Na F** | Sodium Fluoride |
| **NADH** | Nicotinamide Adenine Dinucleotide Hydrogen |

| | |
|---|---|
| **nm** | Nanometer |
| **P** | Phosphorus |
| **P.M** | Post Meridiem |
| **ppm** | Part per Million |
| **rpm** | Revolution per Minute |
| **SPSS** | Statistical Package of Social Science |
| **TP** | Total Protein |
| **U/L** | Unit per Liter |
| **UV** | Ultra Violet |

# ABSTRACT

This study was conducted to establish reference values for some biochemical parameters of synovial fluid in Sudanese cattle and to compare them with the corresponding values in serum, also to establish the correlation and regression among serum and synovial fluid parameters. The bulls were selected from the herd of the Animal Production Research Centre- Khartoum North- Hilat kuku, from January 2018 to October 2019. Eleven blood samples and twenty two synovial fluid samples were collected from carpal and tarsal joints of clinically healthy Baggara bulls. The serum, carpal and tarsal synovial fluids concentrations of Total Protein (TP), Albumin, Globulin, Glucose, Alanine amino transferase (ALT), Calcium (Ca), Phosphorus (P) and Magnesium (Mg) were analysed using a spectrophotometer (UV mini-1240-Japan), while Sodium (Na) and potassium (K) concentrations were analysed by flame photometer (PFP7-UK). The data were analysed statistically by ANOVA and LSD using SPSS programme (version 16). The glucose concentration of carpal joint was significantly (P= 0.024) lower than that of the tarsal joint and serum (27.43 ± 2.5 mg/dl versus 38.29 ± 2.9 mg/dl and 40.56 ± 4.5 mg/dl), while the other metabolites were significantly lower (P= 0.000) than serum. There is no significant difference between carpal and tarsal synovial fluid metabolites. The sodium concentration of tarsal joint was significantly (P= 0.004) lower than that of serum and carpal joint (153.04 ± 6.83 mmol/L versus 176.26 ± 1.99 mmol/L and 163.94 ± 3.09 mmol/L), the calcium concentration of tarsal joint showed lowest significant value (P= 0.000) against serum and carpal joint (7.88 ± 0.24 mg/dl versus 11.81 ± 0.64 mg/dl and 9.49 ± 0.31 mg/dl), while the phosphorus concentration of tarsal joint was significantly (P= 0.001) higher than that of serum and carpal joint (5.42 ± 0.07 mg/dl versus 4.98 ± 0.09 mg/dl and 5.14 ± 0.06 mg/dl). Potassium and magnesium did not vary among serum, carpal or tarsal joints.

The positive correlation was found between serum protein and globulin (0.894). Also, In synovial fluid, there are positive correlations between total protein, albumin, globulin and ALT (0.693, 0.933 and 0.787), respectively and between globulin, albumin and ALT (0.444 and 0.816), respectively. In minerals, positive correlations were obtained between serum sodium and carpal joint sodium (0.745), carpal joint sodium and tarsal joint calcium (0.751) and between tarsal joint potassium and tarsal joint sodium (0.674). The negative correlations were found between serum calcium and carpal joint magnesium (-0.631) and between potassium in tarsal joint and phosphorus in tarsal joint (-0.829). Also, the simple linear equations of serum and synovial fluid metabolites were established. In conclusion, the obtained data will be useful to differentiate between normal and abnormal joints of cattle.

# ملخص الاطروحة

أجريت هذه الدراسة للحصول علي قيم مرجعية لبعض المؤشرات البيوكيميائية للسائل الزليلي في الأبقار السودانية ومقارنة هذه النتائج مع القيم المناظرة لها في مصل الدم، ايضا للحصول على الإرتباط والإعتماد لمؤشرات مصل الدم والسائل الزليلي. تم إختيار الثيران من قطيع يتبع لمركز بحوث الإنتاج الحيواني- شمال الخرطوم- حلة كوكو، في الفترة مابين شهر يناير من العام 2018م إلى شهرأكتوبر من العام 2019م. إحدى عشرة عينة دم وإثنان وعشرون عينة سائل زليلي تم جمعها من مفصل الرسغ ومفصل العرقوب من ثيران سلالة البقارة الصحيحة إكلنيكيا. تراكيز البروتين الكلي لمصل الدم والسائل الزليلي لمفصل الرسغ ومفصل العرقوب، الالبومين، القلوبيولين، الجلكوز، وناقل الأمين (ALT)، الكالسيوم، الفسفور والمغنسيوم تم تحليلها بإستخدام جهاز مقياس الطيف الضوئي (UV mini- 1240-Japan)، بينما تم تحليل الصوديوم والبوتاسيوم بإستخدام جهاز مطياف اللهب (PFP7-UK). تم تحليل البيانات إحصائيا بإختباري تحليل التباين وأقل إختلاف معنوي وذلك بإستخدام برنامج التحليل الإحصائيSPSS (النسخة 16). كان تركيز الجلكوز في مفصل الرسغ أقل معنويا (قيمة إحتمالية = 0.024) من نظيره في مفصل العرقوب ومصل الدم (27.43 ± 2.5 ملي جرام/ ديسيلتر مقابل 38.29 ± 2.9 ملي جرام/ ديسيلتر و40.56 ± 4.5 ملي جرام/ ديسيلتر)، بينما المستقلبات الأخرى كانت اقل معنويا (قيمة إحتمالية = 0.000) مقارنة بمصل الدم. لا يوجد إختلاف معنوي بين مستقلبات السائل الزليلي الموجودة في مفصل الرسغ ومفصل العرقوب. كان تركيز الصوديوم في مفصل العرقوب أقل معنويا (قيمة إحتمالية= 0.004) مقارنة مع نظيره في مصل الدم ومفصل الرسغ (153.04 ± 6.83 ملي مول/ لتر مقابل 176.26 ± 1.99 ملي مول/ لتر و 163.94 ± 3.09 ملي مول/ لتر)، وكان تركيز الكالسيوم في مفصل العرقوب ذو قيمة اقل معنويا (قيمة إحتمالية = 0.000) مقارنة مع نظيره في مصل الدم ومفصل الرسغ (7.88 ± 0.24 ملي جرام/ ديسيلتر مقابل 11.81 ± 0.64 ملي جرام/ديسيلتر و 9.49 ± 0.31 ملي جرام/ ديسيلتر)، بينما كان تركيز الفسفور في مفصل العرقوب ذو قيمة أعلى معنويا (قيمة إحتمالية= 0.001) مقارنة مع نظيره في مصل الدم ومفصل الرسغ (5.42 ± 0.07 ملي جرام/ ديسيلتر مقابل 4.98 ± 0.09 ملي جرام/ ديسيلتر و 5.14 ± 0.06 ملي جرام/ديسيلتر). لمْ يُظهر البوتاسيوم والمغنسيوم إختلاف بين مصل الدم، مفصل الرسغ أو مفصل العرقوب. تم إيجاد إرتباط موجب بين البروتين الكلي والقلوبيولين (0.894) في مصل الدم. ايضا وجدت إرتباطات موجبة في السائل الزليلي بين البروتين الكلي، الألبومين، القلوبيولين وناقل الأمين (0.933،0.693 و 0.787)، كل علي

حده، وبين القلوبيولين و الألبومين و ناقل الأمين (0.444 و 0.816)، كل على حده. تم الحصول على إرتباطات موجبة للمعادن بين الصوديوم في مصل الدم ومفصل الرسغ (0.745) وبين الصوديوم في مفصل الرسغ و الكالسيوم في مفصل العرقوب (0.751) وبين البوتاسيوم والصوديوم في مفصل العرقوب (0.674). وجدت إرتباطات سالبة بين الكالسيوم في مصل الدم والمغنسيوم في مفصل الرسغ (-0.631) وبين البوتاسيوم والفسفور في مفصل العرقوب (-0.829) أيضا تم الحصول على المعادلات الخطية البسيطة لمستقلبات مصل الدم والسائل الزليلي. ختاما هذه البيانات المتحصل عليها ستكون ذات فائدة للتفريق بين المفاصل الصحيحة وغير الصحيحة للأبقار السودانية.

# CHAPTER ONE

# INTRODUCTION AND LITERATURE REVIEW

# CHAPTER ONE

# INTRODUCTION

Sudan is characterized by the diversity of its ecosystem and animal wealth such as ruminants, equine, poultry. Cattle in Sudan belongs to *Bos indicus* (zebu cattle) Which has an ability to tolerate high temperature of the tropical zone. The main breeds of cattle are baggara, butana and kenana (HCENR, 2014).

The fluids in the body are divided into two main divisions: intracellular fluid (that lying inside the cell) which represents two third of the total body water and the extracellular fluid (Kaneko, Harvey and Bruss, 2008). The Extracellular fluid represents one third of the total body water (Barrett, Barman, Boitano and Brooks, 2010). It contains many fluids such as trans-cellular fluid (Kaneko *et al*., 2008), to which synovial fluid belongs (Coles, 1986). The distribution of water between intracellular and extracellular fluid is governed by their contents of potassium and sodium ions, respectively (Kaneko *et al*., 2008).

Synovial fluid is plasma ultra filtrate, the difference only is the addition of hyaluronic acid to synovial fluid by synovial membrane (Swenson and Reece, 1993), it is a viscous, transparent, colourless to light yellow fluid (Altintas, Karagul, Fidanci, Uysal, Besalti, Pekcan, Aypak, Ciftci, Bilgihan and Hanedan, 2010 and AbdEllah, Ali and Semieka, 2012) which contains in addition to hyaluronic acid (mucin), leucocytes, electrolytes and proteins (Latimer, 2011). Synovial fluid plays an important role in reducing friction between articular cartilages due to the presence of the hyaluronic acid and lubricin and may act as a nutrient for them (Swenson and Reece, 1993). Thus withdrawl of synovial fluid and physical, chemical and cytological analysis is

considered as a diagnostic value in diseases (Latimer, 2011). Also it is used as the treatment for lameness in equine (Rulcker and Lindholm, 1981).

The prevalence of lameness in cattle was 21% (Solano, Barkema, Pajor, Mason, LeBlanc, Zaffino Heyerhoff, Nash, Haley, Vasseur, Pellerin, Rushen, de Passille and Orsel, 2015). In tropics, the information about normal synovial fluid in different joints are scarce. The joint disorders affect the animal health and production and lead to loss of animals, if not diagnosed and treated properly.

## Objectives of Study

### General Objective

Comparison between some biochemical constituents in synovial fluid (carpal and tarsal joints) and serum in normal local Sudanese cattle breed.

### Specific objectives

- To measure total protein, albumin, globulin, glucose, Alanine aminotransferase, sodium, potassium, calcium, phosphorus and magnesium in serum and synovial fluid.
- To compare between carpal and tarsal synovial fluids on one hand and compare them with serum on the other hand.
- To obtain the correlation between total protein , albumin , globulin , glucose, Alanine aminotransferase (ALT) and between sodium, potassium, calcium, phosphorus and magnesium in serum and synovial fluid.
- To establish simple linear equations of serum and synovial fluid parameters.

# LITERATURE REVIEW

## 1.1. Cattle

Cattle belong to Bovidae family. They concentrate in the natural grass, wet rain forest and agricultural areas (Payne and Wilson, 2013). Their primary domestication was before 8000 Bp in Western Asia, which led to change the behavior of man from hunting cattle to be a farmer (Payne and Wilson, 2013) and shift of diets towards the animal products (FAO, 2015). The beef meat contains the highest percentage of protein and fat in comparison with camel and goat meat, it also contains non protein nitrogen substances (Alamin, Ahmed and Ahmed, 2014) and macro and micro minerals which, their percentage differ from breed to breed (Domaradzki, Florek, Staszowska and Litwinczuk, 2016).

### 1.1.2. Cattle population in Sudan

Sudan is the second African country after Ethiopia, which posses largest animal wealth (FAO and AGAL, 2005), the total number of live stock population in all states of Sudan is **104.911.762** from which total cattle population is estimated to be about 30 millions (MOAR, 2012). In the tropical area, the most abundant breeds are *Bostarsus* and *Bosindicus* cattle, which are used for milk, meat and for work, also they are important in social status for cattle owners as they represent the sign of wealth and respectability (Payne and Wilson, 2013).

The types of Zebu cattle in Sudan are: Eastern Sudanese cattle, White Nile cattle, Nuba Mountain cattle, and Northern kordfan cattle, the main types are Central Sudanese dairy breed cattle (Kenana and Butana) and Western Baggara beef producing cattle (HCENR, 2014). The baggara cattle are

inhabitants of savanna region between the Nile and western border of the Sudan, they have many skin colours (the most dominant colour is the white with red and black marks) also they have large dewlap. As humped zebu cattle, the hump in baggara cattle is cervio thoracic hump (Payne and Wilson, 2013). The mean age of baggara cow at first calving range from 51.76 - 51.97 months and the average daily milk production in extensive and semi extensive production system is 1.882 ± 0.430 and 2.188 ± 0.542 kg, respectively (Bashir and Elzubeir, 2013).

## 1.2. Blood

Blood is the fluid, that contains red blood cells (erythrocytes), white blood cells (leucocytes), platelets and plasma. It has many functions, which depend on its components such as transportation of nutrients and oxygen to the different cells and taking the waste products away from them. Also, it represents the defense line against foreign invaders. Moreover, it has a role in regulating the body temperature (Frandson, Wilke and Fails, 2009).

## 1.3. Body fluids

Water is the essential life element for all living organisms, from which body fluids represent 60% of animal body weight depending on the amount of fat in animal body. Body fluids are composed of Intracellular fluid and Extra cellular fluid, which contains: plasma, interstitial fluid and trans-cellular fluid (Kaneko *et al*., 2008).The transcellular fluid is the fluid that is found in body cavities like digestive tract and intra ocular fluids (Reece, Erickson, Goff and Uemura, 2015), it contains important fluids such as Synovial fluid and Cerebrospinal fluid (Coles, 1986).

### 1.3.1. Extra cellular fluid

Extracellular fluid is the fluid, that is found outside the cell, and is considerd as internal milieu (Reece *et al*., 2015). It's volume is affected by sodium ion and vasopressin hormone (Barrett *et al*., 2010).

#### 1.3.1.1. Plasma

Plasma is the suspension of red blood cells, white blood cells and platelets. It is colourless or slight yellowish in pet animals and darker in cattle and horses, it represents 55-70% of the blood (Reece *et al*., 2015). Approximately, 92% of plasma is water, the remainder is proteins, glucose, non protein nitrogen compounds and minerals. On the other hand, serum is the plasma minus fibrinogen and other coagulation factors (Swenson and Reece, 1993). In serum all metabolites were analyzed in different animal species (Radostits, Gay Hinchcliff and Constable, 2007).

##### 1.3.1.1.1. Minerals

Minerals are important inorganic elements for animals growth and reproduction. According to the quantity that animals need, the minerals are divided into macro and micro minerals. The requirement of macro minerals is in large quantity compared to that of micro minerals (Reece *et al*., 2015). Macro minerals have important roles in the body, they maintain acid base balance, osmotic pressure, membrane electrical potential and nerve transmission. Also it represents the main constituents of bone and body fluids (Reece *et al*., 2015). On the other hand the trace minerals act as enzyme cofactors (zinc, selenium and manganese) (Berg, Tymoczko and Stryer, 2012), also it is the part of some hormones (Reece *et al*., 2015).

#### 1.3.1.1.1.1. Sodium

Sodium is the major extra cellular cation. It has an important role in keeping osmotic pressure, acid base balance, also it has a role in nerve impulses transmission and involves in absorption of monosaccharides and amino acids (Reece *et al*., 2015). In bulls, the serum sodium level is affected by season (Cerutti, Scaglione, Arfuso, Rizzo and Piccione, 2018).

#### 1.3.1.1.1.2. Potassium

Potassium is an important cation inside the cell, it is involved in control of resting membrane potential and acid base balance. Also it is important for growth and insulin secretion (Reece *et al*., 2015). In bulls, the potassium concentration is influenced by seasonal changes (Cerutti *et al*., 2018). Also, potassium concentration is influenced by the physiological status in cows, which showed highest level in pregnany against dry and lactation period (Kulcu and yur, 2003).

#### 1.3.1.1.1.3. Calcium

Calcium is one of the important macro minerals, it acts as blood clotting factor, important constituent in milk and bone, transmit nerve impulses and has a role in contraction of muscles (skeletal and cardiac muscles). Approximately 98% of calcium is found in the skeleton and 2% located in extra cellular fluid. 45 - 50% of calcium in plasma is in ionized form (Reece *et al*., 2015). In cattle, the serum calcium concentration is not influenced by season, breed, sex, parity, range land type and physiological status of animals (Mapiye, Chimonyo, Dzama and Marufu, 2010), however, in bulls it changes according to season (Cerutti *et al*., 2018).

#### 1.3.1.1.1.4. Phosphorus

Phosphorus is an essential element of bone second to calcium, it is involved in acid base system. Approximately 1/3 of phosphate in blood is inorganic, the remainder combines with organic molecules such as phospholipids, phosphoproteins and ATP (the most important molecule in metabolic pathways) (Reece *et al*., 2015). The serum phosphorus concentration in cattle is not affected by gender, season and reproductive status (Cerutti *et al*., 2018).

#### 1.3.1.1.1.5. Magnesium

Magnesium is the most important cation that found intracellulary, it is involved in necessary enzymatic reactions as a co factor of many key enzymes of glycolysis, fatty acid oxidation (acyl coA synthetase) and Tricarboxylic acid cycle (succinyl coA synthetase). The extra cellular magnesium is important for nerve conduction and normal bone formation (Reece *et al*., 2015). The serum magnesium level in cattle is influenced by season (Mapiye *et al*., 2010; Cerutti *et al*., 2018) and reproductive status, which showed higher concentration in post partum period against pre partum period (Jagos, Dvorak and Bouda, 1981).

### 1.3.1.2. Lymph

Lymph is the tissue space fluid, that is lying in lymphatic vessels, it is clear, colour less fluid, that is similar to plasma , from which it originated. However, the lymph drained from intestine has milky colour due to the presence of fat (Frandson *et al*., 2009). It contains few protein, red cells, glucose, non protein nitrogenous compounds and many lymphocytes. At last, lymph is drained to blood after filtration by lymph nodes (Frandson *et al*., 2009).

### 1.3.1.3. Synovial fluid

Synovial fluid is the dialysate of plasma to which hyaluronic acid is added, its synthesis takes place in joint cavity by synovial membrane (Swenson and Reece, 1993). Analysis of synovial fluid include: physical examination (quantity, viscosity, turbidity, specific gravity and clot formation) (Coles, 1986), chemical examination such as protein determination, glucose concentration against serum glucose, electrolytes, mucin clot test and enzymes assay (Coles, 1986 and Latimer, 2011) and cytological ones that include total number of cells (leukocytes and erythrocytes) and percentage of polymorpho nuclear cells to mononuclear one (Coles, 1986).

The smaller molecules (less than 10.000Da) are simply diffused and the larger ones are prevented from passing through capillaries into synovial fluid (Swenson and Reece, 1993).

#### 1.3.1.3.1. Chemical analysis of synovial fluid

Chemical analysis was done in cattle, camels, horses, donkeys, buffaloes, sheep and rodents. It is considered as a tool to determine the time of death in animals (Yahia and Abd El-Hakiem, 2014).

##### 1.3.1.3.1.1. protein

Proteins are organic compounds, which consist of amino acids. Their synthesis take place in liver and immune system (Kaneko *et al*., 2008). They act as catalysts (enzymes), transporters, buffers, maintaining osmotic pressure, taking place in defense mechanism and represents the major constituents of the cell (Kaneko *et al*., 2008).

The normal synovial fluid contains few proteins in comparison with serum (Liberg, Magnusson and Schougaard, 1977) because the larger

molecules are prevented from passing through the capillaries into synovial fluid (Swenson and Reece, 1993). In cattle, there are 37 proteins identified in the synovial fluid, these proteins are immunoglobulins and acute phase proteins. Regarding the sex, $\alpha_1$-antytripsin is the protein, that is identified in the synovial fluid of heifers against steers (Di filippo, Lannes, Meireles, Nogueira and Quirino, 2019).

Normal Protein concentration was obtained in cattle (El-Amrousi, Soliman and Youssef, 1966; Altintas *et al*., 2010 and AbdEllah *et al*., 2012; Nazifi, Ghandomani and Parizi, 2012), camels (Nazifi, Rezakhani and Gheisari, 1998; Bani Ismail and Al-Rukibat, 2006; Al-Rukibat, Al-Zghoul and Bani Ismail, 2006a; Al-Rukibat, Bani Ismail and Al-Zghoul, 2006b; Al-Rukibat and Bani Ismail, 2014 and Najizadeh, Pourjafa, Chalmeh, Badiei, Nazifi and Naghib, 2014), sheep (Ameri and Gharib, 2005), buffaloes (Baniadam and Razi Jalali, 2005), alpacas, llamas (Waguespack, Belknap, Spano, Wenzel and Pugh, 2002), horses (Vanpelt, 1967) and rodents (Brombini, Rahal, Bergamini, Lopes, Santos and Schimming, 2017).

In comparison between cattle, buffaloes, donkeys and camels, donkeys showed the highest value of total protein and globulin, where camels revealed highest value of albumin against other animals and cattle showed higher globulin level against camels (AbdEllah *et al*., 2012).

**1.3.1.3.1.2. Glucose**

Glucose represents the main source of energy for all biological processes in mammalian cell (kaneko *et al*., 2008). Chemically, It is a monosaccharide sugar that contains six carbon atoms and aldehyde group (Murray, Bender, Botham, Kennelly, Rodwell and Weil, 2009). In normal synovial joint, it enters the joint faster than the other small molecules (Swenson and Reece, 1993).

In cattle, glucose concentration was obtained in distal interphalangeal joint, distal sesamoid bursa (Nazifi *et al*., 2012), metacarpophalangeal joint (AbdEllah *et al*., 2012), carpal joint of calves (Altintas *et al*., 2010) and tibiotarsal joint of bulls and calves ( El-Amrousi *et al*., 1966). In other animals such as camels, Al-Rukibat and BaniIsmail (2014) established reference values for glucose concentration in carpal, tarsal and fetlock joints. Moreover, glucose level was obtained in metacarpophalangeal joint (AbdEllah *et al*., 2012) and elbow joints in normal camels (Nazifi *et al*., 1998). Furthermore, other studies revealed glucose concentration in sheep, buffaloes, donkeys and rodents (Ameri and Gharib, 2005; Baniadam and Razi Jalali, 2005; AbdEllah *et al*., 2012 and Brombini *et al*., 2017).

**1.3.1.3.1.3. Enzymes**

Enzymes are biological polymers (mainly proteins),which are responsible for catalysis of chemical reactions (Murray *et al*., 2009). The enzyme concentration in plasma is proportional to that of cells (Kaneko *et al*., 2008).

The enzymes activity was significantly lower in synovial fluid against that of serum, except alkaline phosphatase, which is the same in both fluids (Al-Rukibat and BaniIsmail, 2014). In female buffaloes the Alanine amino transferase (ALT) and Lactate dehydrogenase (LDH) were significantly higher than that in males (Baniadam and Razi Jalali, 2005).

In a comparative study between farm animals, cattle showed highest significant value of Alkaline phosphatase (ALP) against buffaloes, camels and donkeys (AbdEllah *et al*., 2012).

#### 1.3.1.3.1.4. Minerals

Macro and micro elements were detected in synovial fluids of different animals. In cattle, the concentration of chloride, calcium and inorganic phosphorus were obtained in synovial fluids of bulls and calves. The chloride concentration in calves was lower than that of bulls, while calcium and inorganic phosphorus concentrations were higher than that in bulls (El-Amrousi *et al*., 1966).

In camels, the macro elements were detected in synovial fluid of adult (Nazifi *et al*., 1998) and young camels (Al-Rukibat and Bani Ismail, 2014). On the other hand, micro elements (zinc, iron, copper and selenium) were obtained in synovial fluids of adult dromedary camels (Chalmeh, Badiei, Pourjafar and Nazifi, 2016).

In a comparative study between cattle, camels, buffaloes and donkeys, the chloride concentration was obtained, cattle revealed highest level of chloride concentration in synovial fluid against other animals (AbdEllah *et al*., 2012).

## 1.4. Joint Structure

### 1.4.1. Synovial joint

The articular surface, cartilage, cavity, joint capsule and ligaments are the main structures of synovial joint. The surface of articulation is composed of compact bone layers which cover by hyaline cartilage (articular cartilage), this lead to the presence of space between the articular surfaces of bones (articular cavity), that is lined by the joint capsule (Frandson *et al*., 2009). The joint capsule consists of two layers, the synovial membrane and the superficial capsular layer, these layers extend from the tip of the articular cartilage,

however, they do not cover it. The articulating bones ligate together by ligaments, which consist of connective tissue bands (Frandson *et al*., 2009).

#### 1.4.1.1. Carpal joint

Carpal joint is a complex joint, which consists of small bones, that oriented in two rows. In the proximal row, there are three bones: radial, intermediate and ulnar bones (from medial to later aspect). The distal row is composed of four carpal bones. Also in the lateral aspect, there is the accessory carpal bone, that is directed caudally. In horses, the first carpal bone, if present is the small bone. However, the ruminants first carpal bone is not present and there is a fusion between the second and third carpal bones (Frandson *et al.,* 2009).

#### 1.4.1.2. Tarsal joint

Tarsal joint consists of many bones in proximal, central and distal rows. The proximal row, consists of the talus and calcaneus bones. In the distal row, there are four tarsal bones. In horses, there is one central bone in the central row, in the distal row; the first and second tarsal bones are fused, the movement between the tarsal bones in horses is limited. In the ruminants, the second and third tarsal bones are fused, also the fusion occurred between the central and $4^{th}$ tarsal bones. The movement of tarsal joint in sheep and ox is hinge movement (Frandson *et al*., 2009).

### 1.5. Joint disorders

In abnormal joints, the physical, chemical and cytological properties of synovial fluid were affected. In arthritic joints the synovial fluid has cloudy to purulent appearance (Altintas *et al*., 2010), also acute phase proteins, inflammatory cytokines (Najizadeh *et al*., 2014) and nucleated cell counts (Nazifi *et al*., 2012) have showed high level in abnormal joints. On the other

hand, the glucose level and enzyme activity are low in abnormal joints. In comparison between infectious and non infectious arthritis of cattle, the total protein concentration, total nucleated cell count and specific gravity have higher significant values in infectious against non infectious arthritis (Rohde, Anderson, Desrochers, ST-Jean, Hull and Rings, 2000).

# CHAPTER TWO

# MATERIALS and METHODS

# CHAPTER TWO

# MATERIALS AND METHODS

## 2.1. Experimental animals

In this study, a total number of eleven apparently clinically healthy local Sudanese Baggara bulls were used in this study. They aged (28-49) months. The animals weights were (220-370) Kg (Avery balance-England). The bulls were selected from the herd of the Animal production research centre at Khartoum North (Hillat kuku). They were adapted for two months at the research centre under supervision of cattle fattening research department and then slaughtered for human consumption. The study was performed from January 2018 to October 2019.

## 2.2. Housing and management

The bulls were kept in sheds close to the slaughter house at animal production research centre. The sheds were provided with adequate ventilation. The housing system was provided with appropriate facilities for feeding and watering. The nutritional regimen comprised roughages and concentrate. The local prepared concentrate, Molasses based diet (Molasses, Wheat bran, groundnut cake, Urea and Common salt) were fed daily, early in the morning. The bulls were also supplemented with roughages (Sorghum straw) at 12.00 P.M. The animals were screened for routine clinical examination to ensure that they are healthy. Throughout the study period, the animals were allowed free access to tab water.

## 2.3. Biochemical measurements

### 2.3.1. Collection of blood sample

Six mls of Blood were collected from the jugular vein of bulls using 10 ml disposable syringes. Immediately, 2.5 ml of blood was transferred to clean dry test tubes containing sodium fluoride (Na F) as anticoagulant for glucose test. The rest of the blood was allowed to stay for 2 hours at room temperature and then centrifuged at 3000 r.p.m for 10 min to separate serum. The separated plasma was used for glucose determination. Haemolysed free serum samples were harvested into clean vials and immediately frozen at - 20 C° for subsequent analysis.

### 2.3.2. Collection of synovial fluid (Arthrocentesis)

The synovial fluid of carpal and tarsal joints was collected immediately after slaughtering of the animals. It was collected under aseptical condition, using 18G needle according to Chauhan and Agarwal (2006).

### 2.3.3. Spectroscopy

Spectroscopy is one of the major techniques in analytic chemistry (Drees and Wu, 2010), which depends on the interaction that happens between electromagnetic waves and molecules in the sample (Boyer, 2000). The Beer and Lambert law on which the spectrophotometer works, describes the direct dependency of material absorption on the concentration of that material (Boyer, 2000).

### 2.3.4. Flame photometry

The flame photometry is the technique that is used to measure the concentration of alkali metals (sodium, potassium, lithium) by measuring the light emitted by excited atoms. The sample spirated by the flame photometer, then the flame breaks the chemical bonds to give atoms that excite and move to high electronic state. When the atoms return to their ground state, they emit light, that is focused and pass through specific monochromator. The monochromator of sodium, potassium and lithium are at 589nm, 767nm, 671nm, respectively. Then the light is detected by the photodetector, that converts the light to electrical signal, which is equal to the concentration of atoms (Bishop, Duben-Engelkirk and Fody, 2000)

### 2.3.5. Metabolites

#### 2.3.5.1. Glucose

The glucose concentration was determined by colorimetric method using a commercial kit (Biosystems-Spain). This method was adopted by Trinder (1969). The enzymatic oxidation occurs in the presence of glucose oxidase to form hydrogen peroxide which reacts with phenol and 4- amino-antipyrine to form a coloured complex. The optical densities were measured at a wave length of 500nm, using a spectrophotometer (UV mini-1240-Japan).

$$\text{Glucose} + 1/2\ O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconate} + H_2O_2$$

$$2H_2O_2 + 4 - \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

### 2.3.5.2. Total protein

The total protein concentration was determined by a colorimetric method (Burtis, Ashwood and Bruns, 2005), using a kit (Biosystems-Spain). The method is based on that the protein in the sample reacts with copper ion to give a coloured complex. The optical densities were measured at a wave length of 545nm, using a spectrophotometer (UV mini-1240-Japan).

### 2.3.5.3. Albumin

The albumin concentration was measured according to the colorimetric method (Doumas, Watson and Biggs, 1971), using a kit (Biosystems- Spain). The albumin reacts with bromocresol green in acidic medium, forming a coloured complex, which is measured at 630 nm, using a spectrophotometer (UV mini-1240-Japan).

### 2.3.5.4. Globulin

The globulin concentration was obtained by subtraction of total protein concentration from albumin concentration.

### 2.3.5.5. Alanine aminotransferase (ALT)

Serum ALT activity was determined by the UV enzymatic method (IFCC, 2010) using commercial kit (Biosystems-Spain). ALT catalyzes the transfer of amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of nicotinamide adenine dinucleotide (NADH). The ALT catalytic concentration was determined from the decreasing rate of (NADH) at 340nm, using a spectrophotometer (UV mini-1240-Japan).

L-Alanine + 2-oxoglutarate $\xrightarrow{ALT}$ Pyruvate + glutamate

Pyruvate + NADH + $H^+$ $\xrightarrow{LDH}$ Lactate + $NAD^+$

### 2.3.6. Minerals

#### 2.3.6.1. Sodium

The sodium concentration was measured according to the calibration curve method (Ahluwalia, 2015). The stock standard of sodium is 1000 ppm. 100ppm of sodium standard was prepared from the stock standard in 500 ml deionized water, then serial of sodium standards (0, 2, 4, 6, 8, 10 ) were prepared from 100ppm of sodium standard. The emission of the serial standards and the diluting samples were obtained using flame photometer (PFP7-UK). The sodium concentration was calculated according to the following equation:

$Y = 5.4429x + 2.2857 * DF$ ($R^2 = 0.9934$)

Y : Emission  x :  Sodium concentration as ppm.

#### 2.3.6.2. Potassium

The potassium concentration was determined by a calibration curve method (Ahluwalia, 2015). 100ppm of potassium standard was prepared from the stock standard (1000 ppm) in 500ml deionized water. six of potassium standards (0, 2, 4, 6, 8, 10) were prepared from potassium standard (100 ppm), then the emission of these standards and diluting samples were detected by flame photometer (PFP7-UK). The potassium concentrations were obtained according to the following equation:

$Y = 5.3143x + 1.4286 * DF$ ($R^2 = 0.9954$)

Y : Emission  x :  Potassium concentration as ppm.

#### 2.3.6.3. Calcium

The calcium concentration was measured according to the colorimetric method (Burtis *et al*., 2005), using a kit (BioSystems-Spain). The calcium reacts with o-cresolphthalein complexone, forming a coloured complex, which is measured at 560 nm, using a spectrophotometer ( Elico-India).

#### 2.3.6.4. Phosphorus

The phosphorus concentration was determined by a colorimetric method (Burtis *et al*., 2005), using a kit (BioSystems-Spain). The method is based on that the phosphorus in the sample reacts with molybdate in acidic medium to give a phosphomolybdate complex. The optical densities were measured at a wave length of 340 nm, using a spectrophotometer (Elico-India).

#### 2.3.6.5. Magnesium

The concentration of magnesium was measured according to the colorimetric method (Burtis *et al*.,2005), using a kit (BioSystems-Spain). The magnesium reacts with xylidyl blue in alkaline medium, forming a coloured complex, which is measured at 520nm, using a spectrophotometer (Elico-India).

## 2.4. Statistical analysis

The generated data was analysed by ANOVA and the separation of treatments mean using LSD. The correlations between the studied parameters were done and when a significant correlation was obtained, simple linear regression was done according to the following model

$y = a + b x$. The statistical analysis was done using SPSS programme (version 16).

# CHAPTER THREE

# RESULTS

# CHAPTER THREE

# RESULTS

This study aimed to assess the metabolites and minerals of serum and synovial fluid (carpal and tarsal joints) in beef cattle.

## 3.1 Metabolites

### 3.1.1. Total Protein

The concentration of total protein (TP) in serum was 7.03 ± 0.22 g/dl, while the TP mean concentrations of carpal and tarsal joints were 0.87 ± 0.14 g/dl and 0.57 ± 0.09 g/dl, respectively. This represented a highly significant difference (P= 0.000) of serum versus synovial fluid total protein, where as the difference between carpal and tarsal joint was not significant (Table 3.1). The percentage of total protein in carpal and tarsal joints (12.4% and 8.11%) was obtained, respectively.

### 3.1.2. Albumin

The mean values of serum, carpal and tarsal albumin were obtained (2.50 ± 0.12 g/dl 0.37 ± 0.05 g/dl and 0.27 ± 0.03 g/dl), respectively. The serum albumin concentration was significantly (P= 0.000) higher than the carpal and tarsal one (Table 3.1).

### 3.1.3. Globulin

The serum mean concentration of globulin was 4.52 ± 0.26 g/dl, which is highly significant (P= 0.000) than synovial fluids globulin values (0.50 ± 0.11g/dl and 0.26 ± 0.07g/dl) (Table 3.1).

### 3.1.4. Glucose

In this study, the glucose concentration in serum was measured (40.56 ± 4.5 mg/dl). Furthermore, the glucose levels of carpal and tarsal joint were obtained (27.43 ± 2.5 mg/dl and 38.29 ± 2.9 mg/dl), respectively. This indicated significant difference (P= 0.024) between serum and carpal joint but no significant difference was observed between serum and tarsal synovial fluid (Table 3.2).

### 3.1.5. Alanine amino transferase

The highly significant difference (P= 0.000) of Alanine amino transferase (ALT) in serum (15.77 ± 1.13 U/L) against ALT concentration of synovial fluids was obtained (8.94 ± 1.77 U/L and 5.45 ±0.96 U/L). On the other hand, no significant difference was observed between the two synovial fluids (Table 3.2).

## 3.2. Minerals

### 3.2.1. Sodium

The mean values of serum, carpal and tarsal sodium were obtained (176.26 ± 1.99 mmol/L, 163.94 ± 3.09 mmol/L and 153.04 ± 6.83 mmol/L), respectively. The serum sodium concentration was significantly (P= 0.004) higher than sodium tarsal joint, while there were no significant difference between sodium in serum and in carpal joint and between sodium in carpal and in tarsal joints (Table 3.3).

### 3.2.2 Potassium

The concentration of potassium in serum was 4.31 ± 0.07 mmol/L, while the potassium mean concentration of carpal and tarsal joints were 3.80 ± 0.17 mmol/L and 4.31 ± 0.25 mmol/L, respectively. There was no

significant difference (P= 0.090) between serum, carpal and tarsal joints in potassium (Table 3.3).

### 3.2.3. Calcium

In this study, the calcium concentration in serum was measured (11.81 ± 0.64 mg/dl). Also the calcium level of carpal and tarsal joints were obtained (9.49 ± 0.31 mg/dl and 7.88 ± 0.24 mg/dl), respectively. There was significant difference (P= 0.000) between serum, carpal and tarsal joints calcium (Table 3.3).

### 3.2.4. Phosphorus

The mean values of serum, carpal and tarsal phosphorus were obtained (4.98 ± 0.09 mg/dl, 5.14 ± 0.06 mg/dl and 5.42 ± 0.07 mg/dl), respectively. There was significant difference (P= 0.001) between serum and tarsal joint phosphorus and carpal and tarsal joint phosphorus, but no significant difference was observed between serum and carpal joint phosphorus (Table 3.3).

### 3.2.5 Magnesium

The serum magnesium concentration was obtained (1.92 ± 0.11 mg/dl), moreover, the carpal magnesium level (2.33 ± 0.19 mg/dl) and tarsal magnesium level (2.28 ± 0.25 mg/dl) were measured. The difference between serum, carpal and tarsal joints magnesium was not significant (P= 0.275) (Table 3.3).

**Table 3.1 Mean concentration (g/dl) of protein, albumin and globulin of serum and synovial fluids**

| Mean ± SE (95% CI - Lower and Upper bound) | | | | |
|---|---|---|---|---|
| | Serum | Carpal joint | Tarsal joint | P value |
| Protein | $7.03 \pm 0.22^{a}$ (6.53 - 7.52) | $0.87 \pm 0.14^{b}$ (0.56 - 1.18) | $0.57 \pm 0.09^{b}$ (0.36 - 0.79) | 0.000 |
| Albumin | $2.50 \pm 0.12^{a}$ (2.24 - 2.77) | $0.37 \pm 0.05^{b}$ (0.25 - 0.48) | $0.27 \pm 0.03^{b}$ (0.21 - 0.33) | 0.000 |
| Globulin | $4.52 \pm 0.26^{a}$ (3.9 - 5.1) | $0.50 \pm 0.11^{b}$ (0.25 - 0.75) | $0.26 \pm 0.07^{b}$ (0.10 - 0.42) | 0.000 |

a , b: means within the same row followed by different superscripts are significantly ($p<0.05$) different.

**Table 3.2 Mean concentration of glucose and ALT of serum and synovial fluids.**

| Mean ± SE (95% CI - Lower and Upper bound) | | | | |
|---|---|---|---|---|
| | Serum | Carpal joint | Tarsal joint | P value |
| Glucose (mg/dl) | $40.56 \pm 4.5^{a}$ (30.5 - 50.6) | $27.43 \pm 2.5^{b}$ (21.8 - 33.04) | $38.29 \pm 2.9^{a}$ (31.8 - 44.8) | 0.024 |
| Alt (U/L) | $15.77 \pm 1.13^{a}$ (13.3 - 18.3) | $8.94 \pm 1.77^{b}$ (4.99 - 12.89) | $5.45 \pm 0.96^{b}$ (3.32 - 7.58) | 0.000 |

a ,b: means within the same row followed by different superscripts are significantly ($p<0.05$)different.

**Table 3.3 Mean concentration of sodium, potassium, calcium, phosphorus and magnesium in serum and synovial fluids**

| Mean ± SE<br>( 95% CI - Lower and Upper bound) | | | | |
|---|---|---|---|---|
| Parameters | Serum | Carpal joint | Tarsal joint | P value |
| Sodium (mmol/L) | 176.26 ± 1.99 [a]<br>(171.81 - 180.70) | 163.94 ± 3.09 [ab]<br>(157.24 - 170.64) | 153.04 ± 6.83 [b]<br>(137.83 - 168.25) | 0.004 |
| Potassium (mmol/L) | 4.31 ± 0.07 [a]<br>(4.15 - 4.47) | 3.80 ± 0.17 [a]<br>(3.41 - 4.19) | 4.31 ± 0.25 [a]<br>(3.74 - 4.87) | 0.090 |
| Calcium (mg/dl) | 11.81 ± 0.64 [a]<br>(10.38 - 13.24) | 9.49 ± 0.31 [b]<br>(8.80 - 10.19) | 7.88 ± 0.24 [c]<br>(7.35 - 8.41) | 0.000 |
| Phosphorus (mg/dl) | 4.98 ± 0.09 [a]<br>(4.78 - 5.18) | 5.14 ± 0.06 [a]<br>(5.01 - 5.28) | 5.42 ± 0.07 [b]<br>(5.27 - 5.58) | 0.001 |
| Magnesium (mg/dl) | 1.92 ± 0.11 [a]<br>(1.67 - 2.17) | 2.33 ± 0.19 [a]<br>(1.91 - 2.75) | 2.28 ± 0.25 [a]<br>(1.72 - 2.84) | 0.275 |

a ,b ,c: means within the same row followed by different superscripts are significantly ($p<0.05$) different.

## 3.3. Correlations

High correlation between serum protein and globulin was observed (0.894). Also, the significant correlation of synovial fluid total protein with albumin, globulin, ALT were obtained (0.693, 0.933 and 0.787), respectively, the results revealed highest significant correlation between synovial fluid total protein and globulin. Furthermore, the correlation of synovial fluid globulin with albumin and ALT were observed (0.444 and 0.816), respectively, with highly significant correlation of globulin with ALT (Table 3.4). In minerals, highly significant positive correlation was obtained between serum sodium and carpal joint sodium (0.745) and between carpal joint sodium and tarsal joint calcium (0.751). Also the potassium in tarsal joint correlated positively with sodium in tarsal joint (0.674). The negative correlation was observed between serum calcium and carpal joint magnesium (-0.631) and between tarsal joint potassium and tarsal joint phosphorus (-0.829) (Table 3.5).

## 3.4. Regression

The linear regression equations of serum and synovial fluid metabolites were designed. The association between serum globulin and protein was obtained ($r^2$= 0.80), while in synovial fluid the association of globulin with protein was observed ($r^2$= 0.87). This results indicated the association of synovial fluid protein with globulin is higher than in serum (Table 3.6).

**Table 3.4 Correlations between some serum (lower triangle) and synovial fluid (upper triangle) metabolites.**

| Metabolites | T. protein | Albumin | Globulin | Glucose | ALT |
|---|---|---|---|---|---|
| T. protein | | **0.693**** | **0.933**** | 0.136 | **0.787**** |
| Albumin | - 0.024- | | **0.444*** | - 0.126- | 0.374 |
| Globulin | **0.894**** | - 0.467- | | 0.155 | **0.816**** |
| Glucose | - 0.117- | 0.287 | - 0.219- | | 0.086 |
| ALT | - 0.007- | - 0.126- | 0.056 | 0.376 | |

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

**Table 3.5 Correlations between some serum and synovial fluid minerals**

| Parameters | Serum Na | Serum K | Serum Ca | Serum P | Serum Mg | Carpal Na | Carpal K | Carpal Ca | Carpal P | Carpal Mg | Tarsal Na | Tarsal K | Tarsal Ca | Tarsal P | Tarsal Mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum Na | | | | | | | | | | | | | | | |
| Serum K | -0.131- | | | | | | | | | | | | | | |
| Serum Ca | -0.106- | -0.127- | | | | | | | | | | | | | |
| Serum P | -0.255- | -0.380- | -0.085- | | | | | | | | | | | | |
| Serum Mg | -0.053- | -0.269- | -0.105- | 0.100 | | | | | | | | | | | |
| Carpal Na | **0.745**** | -0.116- | 0.096 | -0.290- | -0.483- | | | | | | | | | | |
| Carpal K | 0.531 | 0.109 | -0.079- | -0.460- | -0.015- | 0.468 | | | | | | | | | |
| Carpal Ca | 0.462 | -0.133- | 0.160 | -0.418- | 0.573 | 0.087 | 0.050 | | | | | | | | |
| Carpal P | 0.302 | 0.472 | 0.000 | 0.031 | -0.305- | 0.411 | 0.326 | -0.043- | | | | | | | |
| Carpal Mg | 0.229 | 0.033 | **-0.631*** | -0.219- | -0.315- | 0.382 | 0.469 | -0.248- | 0.292 | | | | | | |
| Tarsal Na | 0.045 | -0.070- | -0.305- | 0.270 | -0.047- | -0.082- | 0.341 | -0.414- | 0.022 | 0.206 | | | | | |
| Tarsal K | 0.500 | 0.184 | -0.532- | -0.183- | -0.043- | 0.172 | 0.463 | -0.022- | 0.026 | 0.365 | **0.674*** | | | | |
| Tarsal Ca | 0.422 | 0.119 | -0.219- | -0.469- | -0.264- | **0.751**** | 0.514 | -0.038- | 0.234 | 0.541 | -0.064- | 0.244 | | | |
| Tarsal P | -0.492- | 0.000 | **0.628*** | 0.100 | 0.115 | -0.322- | -0.310- | 0.103 | 0.120 | -0.351- | -0.590- | **-0.829**** | -0.425- | | |
| Tarsal Mg | -0.136- | 0.139 | 0.113 | -0.239- | 0.254 | -0.412- | 0.085 | 0.377 | 0.099 | -0.090- | 0.206 | 0.026 | -0.523- | 0.212 | |

**Correlation is significant at the 0.01 level.
*Correlation is significant at the 0.05 level.

**Table 3.6 Regression equations of some serum and synovial fluid metabolites.**

| Serum | Synovial fluids |
|---|---|
| Globulin (g/dl) = - 2.887 + 1.054T. protein(g/dl) ($r^2$= 0.80) | Globulin (g/dl) = -0.155 + 0.742T. protein(g/dl) ($r^2$= 0.87) |

# CHAPTER FOUR

# DISCUSSION

# CHAPTER FOUR

# DISCUSSION

In the present study, the mean concentration of total protein, albumin, globulin, glucose, Alanine amino transferase (ALT), sodium (Na), potassium (K), calcium (Ca), phosphorus (P) and magnesium (Mg) were analysed in serum, carpal and tarsal synovial fluid. The serum metabolites and minerals of the present work lie within the normal range of cattle (Radostits *et al*., 2007) with the exception of sodium concentration, which showed higher value against corresponding value in healthy cattle; this refers to the effect of season on sodium concentration (Cerutti *et al*., 2018).

In the present work, the total protein percentage in carpal and tarsal joints was 12.4% and 8.11%, respectively. In cattle, the protein concentration in synovial fluid is less than 2g/100 ml (Chauhan and Agarwal, 2006). This finding is in close agreement with our results of carpal (0.87 ± 0.14 g/dl) and tarsal (0.57 ± 0.09 g/dl) total protein concentration. Similar findings were obtained in carpal joint of buffalo (0.89 ± 0.058 g/dl) (Baniadam and Razi Jalali, 2005), which belongs to the same family of cattle. On the contrary, high protein value was obtained in radiocarpal joint of alpacas and llamas (2.54 g/dl) (Waguespack *et al*., 2002), sheep (2.31 ± 0.55 g/dl) (Ameri and Gharib, 2005) and camels (2 ± 1 g/dl) (Al-Rukibat *et al*., 2006a), this may be due to different animal species used in the previous studies.

The albumin concentration in metacarpophalangeal joint of cattle was obtained (0.45 ± 0.17 g/dl) (AbdEllah *et al*., 2012), which accords with the range of this work, with regard to the carpal and tarsal joints, these

observations concluded that there is no difference in albumin concentration between different joints of cattle.

The minimum value of globulin mean concentration (1.80 ± 1.18 g/dl) in metacarpophalangeal joint of cattle (AbdEllah *et al*., 2012) is in accordance with the carpal and tarsal synovial fluid range of the present work. According to the results obtained in the current study, no significant difference between plasma glucose and tarsal synovial fluid glucose was seen (P= 0.024).

Similar findings were observed between serum and stifle joint of cattle (S. A. Omer, 2019. Personal. Communication). In the present work, the glucose concentration range of carpal joint is (21.8 - 33.04 mg/dl). This is similar to the glucose concentration obtained by Nazifi *et al* (2012) in DIJ of cattle (32.55 ± 0.52 mg/dl). In the current work, the significant differences observed between plasma glucose and carpal synovial fluid on one hand and between tarsal and carpal synovial fluid on the other hand, may be due to limitation of carpal joint movement against tarsal joint movement.

The maximum value of the ALT mean concentration (3.97 ± 1.09 unites/L) (Nazifi *et al*., 2012) in DSB of cattle is in accordance with the range of the present work.

Sodium concentration in the elbow joint of adult camel (119.63 ± 17.03 mmol/L) obtained by Nazifi *et al*., 1998, is less than the sodium concentration range of both carpal and tarsal synovial fluids of this work (157.24 – 170.64 mmol/L and 137.83 – 168.25 mmol/L, respectively). This may be due to the difference of extracellular volume in different animal species. Also, the mean of sodium concentration obtained in carpal, tarsal and fetlock synovial fluid of young camels (152.4 ± 16.63 mmol/L)

(Al-Rukibat and BaniIsmail, 2014), lies within the range of sodium in tarsal synovial fluid obtained in this current work (137.83 − 168.25 mmol/L).

According to results obtained in the current study, no significant difference was observed between serum sodium concentration and carpal synovial fluid sodium concentration, however, sodium level in serum was higher than synovial fluid (Nazifi *et al*., 1998; Al-Rukibat and BaniIsmail, 2014), which disagreed with the results of the current study of carpal synovial fluid. This may be due to limitation of carpal joint movement against tarsal joint movement; the influx of sodium and glucose into cells decrease and the sodium concentration in serum will be similar to as carpal synovial fluid. In this study, no significant difference between carpal and tarsal synovial fluid sodium concentration was seen, which accords with the study obtained by Al-Rukibat and BaniIsmail (2014).

The potassium concentration was measured in synovial fluid of camel (3.93 ± 0.58 mmol/L) (Nazifi *et al*., 1998), was found to be in accordance with the range of the carpal and tarsal synovial fluids of this study (3.14 – 4.19 mmol/L and 3.74 – 4.87 mmol/L, respectively). Furthermore, this present work showed non significant difference in potassium concentration between serum, carpal and tarsal synovial fluids (P= 0.090). In contrast, the potassium concentration showed significant value in serum against synovial fluid (Nazifi *et al*.,1998; S.A. Omer, 2019.Personal.Communication). The reason beyond this may be due to the movement of electrolytes between different body fluids which is governed by the Gibbs-Donnan theory (Coles, 1986). The Gibbs-Donnan theory is concerned with the diffusible and non diffusible ions, when there is non diffusible ions, the diffusible ions distribute, so that at equilibrium status, their concentration is equal. The non significant difference between potassium concentration in carpal and tarsal

synovial fluids in this work agreed with the findings of Al-Rukibat and BaniIsmail (2014).

Calcium concentration obtained in synovial fluid of camel (4.5 ± 7.72 mg/dl) (Al-Rukibat and BaniIsmail, 2014), which is less than the present work of carpal and tarsal synovial fluids, respectively (9.49 ± 0.31 mg/dl and 7.88 ± 0.24 mg/dl). Also, El-Amrousi (1966) analysed the calcium concentration in synovial fluid of bulls (9.3 ± 0.18 mg/dl) which is in accordance with the range of carpal synovial fluid of this work (8.80 - 10.19 mg/dl). The serum calcium concentration in this study showed higher value than carpal and tarsal synovial fluids, which agreed with Nazifi *et al* (1998) and Al-Rukibat and BaniIsmail (2014). There is a significant difference between calcium level in carpal and tarsal synovial fluids (P= 0.000) in this current study, which contradicted the findings of Al-Rukibat and BaniIsmail (2014). This might be refer to the Gibbs-Donnan theory, which controls the movement of electrolytes (Coles, 1986).

The maximum value of phosphorus concentration in synovial fluid of young camel was obtained (5 ± 5.01 mg/dl) (Al-Rukibat and BaniIsmail, 2014), which lies within the range of carpal synovial fluid of the current study (5.01 – 5.28 mg/dl). Also, the lower limit of phosphorus concentration in tibiotarsal joint of bulls (El-Amrousi *et al*., 1966) lies within the carpal synovial fluid range of this work. No significant difference was observed between serum and carpal synovial fluid phosphorus concentration in this study, this is similar to the finding obtained by Al-Rukibat and BaniIsmail (2014). Also, the present work revealed significant difference in phosphorus concentration (P= 0.001) between carpal and tarsal synovial fluids, which disagrees with the previous study, that revealed, no significant difference in phosphorus concentration between carpal, tarsal and fetlock joints

(Al-Rukibat and BaniIsmail, 2014). This may refer to excessive movement of tarsal joint, which needs ATP as the source of energy.

The magnesium concentration in this study was measured in carpal and tarsal synovial fluids, respectively (2.33 ± 0.19 mg/dl and 2.28 ± 0.25 mg/dl). Also, the magnesium level was measured in camel synovial fluid (1.4 ± 0.47 mg/dl) (Al-Rukibat and BaniIsmail, 2014), which is less than the corresponding value in this current work. The significant difference between serum and synovial fluid of magnesium concentration was observed in camel (Nazifi *et al*., 1998; Al-Rukibat and BaniIsmail, 2014), which disagrees with the results of the current work. The differences of magnesium concentration between this study and the previous studies, might be due to the fact of diffusion of the electrolytes is under the control of Gibbs-Donnan theory (Coles, 1986). In this study, the magnesium concentration showed no significant difference between carpal synovial fluid and tarsal synovial fluid (P= 0.275). Similar findings were obtained in carpal, tarsal and fetlock joints of camel (Al-Rukibat and BaniIsmail, 2014).

In this current work, positive correlations were found between T.protein, albumin, globulin and ALT, between albumin and globulin and between globulin and ALT of synovial fluid; this may be due to the fact that all the correlated metabolites are proteins. Also, the correlation between serum sodium and carpal sodium, serum calcium and carpal magnesium, serum calcium and tarsal phosphorus, carpal sodium and tarsal calcium, tarsal sodium and potassium and the correlation between tarsal potassium and phosphorus were obtained in this work. The correlations of synovial fluid (metabolites and minerals) and the subsequent linear regression equations of synovial fluid metabolites were established for the first time, thus no comparable results were found.

## Conclusion

- The reference values for total protein, albumin, globulin, glucose, Alanine amino transferase, sodium, potassium, calcium, phosphorus and magnesium were established in synovial fluid of Sudanese cattle.
- There is significant difference between serum and the corresponding synovial fluid metabolites with the exception of tarsal synovial fluid glucose which is parallel to that of serum.
- There is no significant difference between serum and synovial fluid minerals with the exception of sodium, phosphorus and calcium.
- The correlation and subsequent linear regression equations of synovial fluid and serum metabolites and minerals were established.

## Recommendations

- Comparison between synovial fluid and other body fluids should be done.
- Simple linear equations of serum and synovial fluid metabolites should be used to obtain the concentration of metabolites from others.

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# Publications

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# Comparative Study between Synovial Fluid and Serum Metabolites in Sudanese Cattle

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## Abstract

This study was conducted to establish Normal values for Total protein (TP),Albumin (Alb), Globulin (Gl), Glucose(G) and Alanine aminotransferase (ALT) from synovial fluids in Baggara Sudanese cattle , compared with the corresponding values in serum and to investigate the correlation and regression among serum and synovial fluid of these metabolites. Eleven blood samples and twenty two synovial fluid samples were collected from carpal and tarsal joints of clinically healthy Baggara bulls, during the period from January to March 2018. The colorometric methods were used to determine the concentration of these metabolites using commercial kits which were analysed using Spectrophotometer (UV mini-1240-Japan). The data was analysed by ANOVA and LSD using SPSS programme (version 16). The glucose concentration of carpal joint was significantly (p=0.024) lower than that of the tarsal joint and serum, while the other metabolites were significantly lower (p=0.000) than serum. The correlation (between total protein, albumin, globulin, and ALT, between albumin and globulin and between globulin and ALT ) and the subsequent simple linear equations of synovial fluid metabolites were established. In conclusion, data obtained will be useful as diagnostic tool to differentiate between normal and abnormal joints of cattle which will help in the diagnosis of joint disorders and diseases. The study recommends that, other studies of synovial fluid should be made in large number of cattle and comparison between synovial fluid metabolites and other body fluid metabolites such as cerebrospinal fluid should be done.

**Keywords**: Total protein, Glucose, ALT, Carpal joint, Tarsal joint.

## Introduction

Synovial fluid is the dialysate of plasma, to which hyaluronic acid is added, its synthesis takes place in joint cavity by synovial membrane (Swenson and Reece,1993). It is considered one of the important transcellular fluid (Coles,1986). It is viscous, transparent, colourless to light yellow fluid (Altintas *et al*., 2010 ; AbdEllah *et al*., 2012), which contains in addition to hyaluronic acid, proteins, glucose, electrolytes, enzymes, cells (Coles,1986 ; Latimer, 2011) and antioxidant vitamins (Chalmeh *et al*., 2016). Determination of physical, chemical and cytological characteristics of synovial fluid are considered as diagnostic valuable tools in diseases (Latimer, 2011). Several studies in camels (Al-Rukibat *et al*., 2006), horses , donkeys , buffaloes (AbdEllah *et al*., 2012) ,sheep (Ameri and Gharib, 2005) and rodents (Brombini *et al*., 2017) analysed the synovial fluid in one joint, but there is scarce data in cattle concerning the synovial fluid analysis in different joints .

This study was designed to determine normal values of some metabolites in synovial fluid (carpal and tarsal joint) and serum in normal local Sudanese Baggara cattle breed.

## Materials and Methods

### Animals

Eleven apparently clinically healthy local Sudanese Baggara bulls were used in this study, they were selected from the herd of the Animal Production Research Centre - Khartoum north –Hillat kuku. They aged (28-49) months and their weights were ranged from 220 to 370 kg.

### Collection of blood

Six mls of blood were collected from the jugular vein using 10 ml disposable syringe. Immediately, 2.5 ml of blood was transferred to clean dry test tubes containing sodium fluoride (Na F) as anticoagulant for glucose test. The separated plasma was used for glucose determination. The rest of the blood was allowed to stay for 2hrs at room temperature and then centrifuged at 3000 r.p.m for 10 minutes to separate serum. Haemolysed free serum samples were harvested into clean vials and immediately frozen at -20C° for subsequent analysis.

### Collection of synovial fluid (Arthrocentesis)

The synovial fluid of carpal and tarsal joints was collected immediately after the animals were slaughtered. It was collected under a septical condition, using 18G needle according to Chauhan and Agrawal ,(2006).

### Biochemical analysis

Colorimetric methods were adopted for determining total protein, albumin, glucose concentration and activity of Alanine amino transferase using commercial kits (Biosystems-Spain), while globulin is obtained by subtraction of total protein from albumin. The analysis was done using spectrophotometer (UV mini -1240-Japan).

### Statistical analysis

The generated data was analysed by ANOVA and means were separated using LSD. The correlations between the studied parameters were done and when a significant correlation was obtained simple linear regression was done according to the following model $y = a + b x$ . The statistical analysis was done using SPSS programme (version 16).

## Results

### Metabolites

The mean values of serum, carpal and tarsal joint of total protein (TP), albumin, globulin (Table1). Glucose in plasma, carpal and tarsal joints and alanine aminotransferase (ALT) in serum, carpal and tarsal joints were

displayed in Table 2. Serum showed significantly (p=0.000) higher concentration of TP, albumin, globulin and ALT compared to carpal and tarsal joints, while no significant variation was found between the two joints. Carpal joint synovial fluid showed significantly (p= 0.024) lower concentration of glucose than tarsal joint and serum, while no significant variation was observed between serum and tarsal joint with regard to glucose.

## Correlation

A highly correlation between serum protein and globulin was observed. Also significant correlation among synovial fluid metabolites (between total protein, albumin, globulin and ALT, between albumin and globulin and between globulin and ALT) was obtained (Table 3).

## Regression

The linear regression equations of serum and synovial fluid metabolites were designed. The association between serum and synovial fluid metabolites were observed .The results indicated that, the association of synovial fluid protein with globulin is higher than that of serum (Table 4).

**Table (1) Normal values (g/dl) of protein, albumin and globulin of serum and synovial fluids**

| Mean ± SE | | | | |
|---|---|---|---|---|
| Metabolites | Serum | Carpal joint | Tarsal joint | Sign. |
| T. protein | $7.03 \pm 0.22^{a}$ (6.53 - 7.52) | $0.87 \pm 0.14^{b}$ (0.56 - 1.18) | $0.57 \pm 0.09^{b}$ (0.36 - 0.79) | .000 |
| Albumin | $2.50 \pm 0.12^{a}$ (2.24 - 2.77) | $0.37 \pm 0.05^{b}$ (0.25 - 0.48) | $0.27 \pm 0.03^{b}$ (0.21 - 0.33) | .000 |
| Globulin | $4.52 \pm 0.26^{a}$ (3.9 - 5.1) | $0.50 \pm 0.11^{b}$ (0.25 - 0.75) | $0.26 \pm 0.07^{b}$ (0.10 - 0.42) | .000 |

**Table (2) Normal values of Plasma glucose and ALT of serum and synovial fluids.**

| Mean ± SE | | | | |
|---|---|---|---|---|
| Metabolites | serum | Carpal joint | Tarsal joint | Sign. |
| Plasma glucose (mg/dl) | $40.56 \pm 4.5^{a}$ (30.5 - 50.6) | $27.43 \pm 2.5^{b}$ (21.8 - 33.04) | $38.29 \pm 2.9^{a}$ (31.8 - 44.8) | .024 |
| ALT (U/L) | $15.77 \pm 1.13^{a}$ (13.3 - 18.3) | $8.94 \pm 1.77^{b}$ (4.99 - 12.89) | $5.45 \pm 0.96^{b}$ (3.32 - 7.58) | .000 |

a ,b: means within the same row followed by different superscripts are significantly ($p<0.05$)different.

**Table 3 Correlations between some serum (lower triangle) and synovial fluid (upper triangle) metabolites.**

| Metabolites | T. protein | Albumin | Globulin | Glucose | ALT |
|---|---|---|---|---|---|
| T. protein | | 0.693** | 0.933** | 0.136 | 0.787** |
| Albumin | -0.024- | | 0.444* | -0.126- | 0.374 |
| Globulin | 0.894** | -0.467- | | 0.155 | 0.816** |
| Glucose | -0.117- | 0.287 | -0.219- | | 0.086 |
| ALT | -0.007- | -0.126- | 0.056 | 0.376 | |

** correlation is significant at the 0.01 level.
* correlation is significant at the 0.05 level.

**Table 4 Regression equations of some serum and synovial fluid metabolites.**

| **Serum** | **Synovial fluids** |
|---|---|
| Globulin (g/dl) = - 2.887 + 1.054 T. protein(g/dl) ($r^2$ = 0.80) | Albumin (g/dl) = 0.154 + 0.237 T. protein(g/dl) ($r^2$ = 0.48) |
| | Globulin (g/dl) = -0.155 + 0.742 T. protein(g/dl) ($r^2$ = 0.87) |
| | ALT (U/L) = 0.399 + 9.422 T. protein(g/dl) ($r^2$ = 0.62) |
| | ALT (U/L) = 2.520 + 12.279 globulin(g/dl) ($r^2$ = 0.67) |
| | Globulin (g/dl) = 0.047 + 1.046 albumin(g/dl) ($r^2$ = 0.20) |

a , b: means within the same row followed by different superscripts are significantly ($p<0.05$) different

## Disscusion

In this study, the mean concentration of total protein, albumin, globulin, plasma glucose and Alanine amino transferase (ALT) were analyzed in serum, carpal and tarsal synovial fluid. The serum metabolites of the present work lie within the normal range of cattle (Radostits *et al*.,2007). The total protein percentage in carpal joint and tarsal joint were **12.4%** , **8.11%** respectively, to that of serum. Also in cattle, the protein concentration was obtained in synovial fluid, which is less than 2g/100 ml (Chauhan and Agarwal, 2006). This finding is in close agreement with our results of carpal (0.87± 0.14 g/dl) and tarsal (0.57 ± 0.09 g/dl) total protein concentration. Similar findings were obtained in carpal joint of buffalo (0.89±0.058 g/dl) (Baniadam and Razi Jalali, 2005), which belongs to the same family of cattle.

The albumin concentration in metacar-pophalangeal joint of cattle was obtained (0.45 ± 0.17 g/dl) (AbdEllah et al., 2012) which accords with the range of this work with regard to the carpal and tarsal joints, these observations concluded that there is no difference in albumin concentration between different joints of cattle.

According to results obtained in the current study, no significant difference between plasma glucose and tarsal synovial fluid glucose. Similar findings were observed between serums and stifle joint of cattle (S.A.Omer, April.14.2019. Personal. Communication.). In the present work, the glucose concentration range of carpal joint is (21.8 - 33.04 mg/dl). The glucose concentration obtained by Nazifi *et al* .(2012) in DIJ of cattle (32.55 ± 0.52 mg/dl) lie within our range . In the current work, the significant differences observed between plasma glucose and carpal synovial fluid on one hand and between tarsal and carpal synovial fluid on the other hand, may be due to limitation of carpal joint movement against tarsal joint movement.

The maximum value of the ALT mean concentration (3.97 ± 1.09 unites/L) (Nazifi *et al*., 2012) in DSB of cattle is in accordance with the range of the present work.

The minimum value of globulin mean concentration (1.80±1.18 g/dl) in metacarpophalangeal joint of cattle (AbdEllah et al., 2012) is in accordance with the carpal and tarsal joint range of the present work. Also the accordance of protein concentration in the carpal joint of buffaloes (Baniadam and RaziJalali, 2005), with our study lead to agreement of globulin concentration of this work with ours.

In this study, the positive correlations were made between T. protein, albumin globulin and ALT, between albumin and globulin and between globulin and ALT of synovial fluid; this may be due to the fact that all the correlated metabolites are proteins. The subsequent linear regression equations were designed for the first time, thus no comparable results were found.

## Conclusion

Normal values of synovial fluid in carpal and tarsal joints were determined. The total protein percentage of carpal and tarsal joint against serum was obtained .Also the correlation and linear regression equations among serum and synovial fluid metabolites were established. These equations will be useful in estimation the concentration of one metabolite from other. All these information will help in diagnosis of various joint disorders and diseases.

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# دراسة مُقارنة بين مستقلبات السائل الزليلي ومصل الدم في الماشية السودانية

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**المستخلص**

أجريت هذه الدراسة للحصول على القيم الطبيعية للبروتين الكلي، الالبومين، القلوبيولين،الجلكوز وناقل الامين (ALT) من السائل الزليلي لأبقار البقارة السودانية، مقارنة هذه النتائج مع القيم المناظرة لها في مصل الدم، وللكشف عن الإرتباط والإعتماد لهذه المستقلبات في مصل الدم والسائل الزليلي. إحدى عشرة عينة دم وإحدى وعشرون عينة سائل زليلي تم جمعها من مفصلي الرسغ والعرقوب لثيران البقارة الصحيحة إكلينيكيا، في الفترة مابين شهري يناير ومارس من العام 2018 . تم إستخدام طرق القياس اللوني لتحديد تركيز هذه المستقلبات بإستخدام محاليل تجارية تم تحليلها بجهاز مقياس الطيف الضوئي(UV mini-1240-Japan) . تم تحليل البيانات بإختباري تحليل التباين واقل إختلاف معنوي باستخدام برنامج التحليل الإحصائيSPSS (النسخة 16) . تركيز الجلكوز في مفصل الرسغ كان أقل معنويا( قيمة إحتمالية =0.024 ) مقارنة مع مفصل العرقوب ومصل الدم، وكانت المستقلبات الاخرى اقل معنويا ( قيمة إحتمالية = 0.000) من مصل الدم . تم الحصول على الإرتباط ( بين البروتين الكلي ، الألبومين، القلوبيولين و ALT ، بين الألبومين والقلوبيولين وما بين القلوبيولين و ALT) و المعادلات الخطية البسيطة الملحقة له لمستقلبات السائل الزليلي. خلصت الدراسة إلي أن هذه البيانات المتحصل عليها ستكون ذات فائدة للتفريق بين المفاصل الطبيعية وغير الطبيعية للماشية السودانية والتي ستساعد في تشخيص إضطرابات وأمراض المفاصل،وتوصي الدراسة بإجراء دراسات اخري للسائل الزليلي في عدد كبير من الأبقار، ومقارنة مستقلبات السائل الزليلي مع مستقلبات سوائل الجسم الأخرى كالسائل النخاعي الشوكي.