

**Sudan University of Science and Technology
College of Graduate Studies**

**An Investigation on the Chemo-genetics Characteristics of
Roselle Seed (*Hibiscus sabdariffa*) as an alternative source of
protein influencing fattening performance of Sudanese
desert Sheep**

**دراسة الخصائص الكيمو- جينية لبذور الكركدي ومصدراً بروتينياً بديلاً
مؤثراً علي أداء تسمين الضان الصحراوي السوداني**

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in Animal Production (Animal Nutrition)**

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الإستهلال

بسم الله الرحمن الرحيم

((و الأنعام خلقها لكم فيها دفاء ومنها تأكلون))

الآية (5) سورة النحل

((وان لكم في الأنعام لعبرة نسقيكم مما

في بطونه من بين فرث ودم لبنا خالصا

سائغا للشاربين (66)))

سورة النحل

Dedication

To the spirit of my late father and mother.

Still attending in every moment.

To my wife Dr. Aziza Hussein Bakheit.

To all my brothers especially Khassan.

To Sawsan, Khada and Zaryat

To my great family sad faragalla

To my Friends and all colleagues.

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Table of Abbreviations:

Abbreviation	Item	Page
MAR	Ministry of Animal Resource	1
MARF	Ministry of Animal Resource and Fishers	1
DNA	Deoxyribonucleic acid	2
3D	Three dimension structure	3
GNC	Groundnut cake	4
CSC	Cottonseed cake	4
SFC	Sunflower cake	4
CRS	Crushed roselle seed	4
MT	Metric tons	12
<i>S.E</i>	<i>Staphylococcal enterotoxin (SE)</i>	19
FDA	Food and drugs administrations	20
ICMSE	International corporation of microbiological specialist	20
VT	Verocytotoxins	21
EPEC	Enteropathogenic <i>Escherichia coli</i>	21
EAEC	Enterotoxigenic <i>Escherichia coli</i>	21
DGEC	Diarrhoeagenic <i>Escherichia coli</i>	21
UPEC	Uropathogenic <i>Escherichia coli</i>	21

NMEC	Neonatal meningitis <i>Escherichia coli</i>	21
VFA	Volatile fatty acid	23
CP	Crude protein	24
DM	Dry matter	24
UDP	Undegradable protein	25
SCP	Soluble crude protein	27
NPN	Non protein nitrogen	27
RUP	Ruminal Undegradable protein	27
RMP	Ruminal microbial protein	27
MP	Metabolizable protein	27
pH	Hydrogen ion concentration	29
RE	Rumen ecology	30
GP	Gas production	32
NCBI	National center of biotechnology information	38
FCR	Feed conversion ratio	41
FAO	Food and agricultural organization	45
(HCN)	Hydrocyanogenic acid	50
PA	Pyrrolizidine alkaloids	50
DPPH	Di phenyl 1-2 picryl hydroxyl	51
PG	Propyl Gallate	51
DMSO	Di- Methyl- Sulphoxide	51

EE	Ether extract	61
MAPRI	Medicinal and aromatic plant institute	65
NCR	National research center	65
CFC	Colony forming unit	66
PE	Petroleum ether	68
KoH	Potassium hydroxide	71
UV	Ultra violet	71
NDF	Neutral detergent fiber	71
ADF	Acid detergent fiber	72
ACTAB	Acetyl- trithyl – ammonium bromide	72
ADL	Acid detergent lignin	73
RSP	Roselle seed protein	74
PG	Propyl gallate	75
PCR	Polymerase chain reaction	76
DMSO	Di- methyl Sulphoxide	76
SPSS	Statistical package for social science	82
LSD	Least significant difference	82
NFE	Nitrogen free extract	84
AL	Amino acids length	95
RSA	Radical scavenging activities	119

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Abstract

Roselle seed (*Hibiscus sabdariffa*) known in Sudan as Karkadeh is important annual crop, which grows successfully in the tropics and sub-tropics, mainly for producing of calyxes. The present study was conducted at El Huda National Sheep Research Station to evaluate Roselle seed (*Hibiscus sabdariffa*) as an alternative protein source used as ruminants feed. Four levels of crushed roselle seeds were used (0%, 10%, 20% and 30%), which were incorporated in four Iso-caloric Iso-nitrogenous diets (A, B, C and D respectively). Twenty- four male Sudanese desert lambs, ecotype A *Shugar*, were selected according to their ages (5 – 6 month) and average live body weight (21.77 kg). The lambs were assigned to four treatments (6 animals each). The animals in each treatment were subdivided in two groups, of three animals. The animals were fed on the experimental rations for (42) days and (10) days for adaptation period, (2kg / head / day). The daily weight gain was 200, 200, 220 and 240 gm and dry matter intake were 1.69 , 1.65 , 1.65 and 1.61 kg / week for treatments A, B, C and D respectively. No significant differences at ($P>0.05$) among the different treatments were seen on feed intake, feed conversion ratios and final body weight gain respectively.

However economic evaluation study of four experimental rations showed highly significant differences ($P<0.05$) in total benefit cost of ingredient (303 - 296 - 274 and - 252) SDG per 1kg for diet A, B, C and D respectively. Diet (C and D) recorded lowest ingredient total, benefit cost, while diet (A and B) recorded highest ingredient total benefit cost.

From *In Sacco* experiment degradability result of the present study of nylon bags technique through Cannula were used to determine dry matter and crude protein disappearance % showed a highly significant difference ($P<0.05$) among four plant protein sources (74.55 – 41.17), (76.87 –

61.88), (78.54 – 62.86) and (88.22 – 77.39) for dry matter and crude protein disappearance % at 72 incubation time /hrs of CSC, CRS, SFC and GNC respectively. The effective degradability of above plant protein source at out flow rate K= 0.02 were found (63.88 - 29.30), (63.31 – 34.61), (67.59 – 43.60) and (74.50 – 57.71) of dry matter and crude protein effective degradability for CSC, CRS, SFC and GNC respectively.

From *In vitro* experiment digestibility used microbial digestion by fresh rumen liquor and pepsin enzyme to determine gas production volume between four plant protein source showed highly significance different among experimental four protein source CSC singed a lowest gas production volume (61.64) dcm , followed by CRS (65.26) dcm , SFC (67.8) dcm while GNC singed greater gas production (72.30) dcm respectively.

In Silico bioinformatics Technology were used in present study and it was found that *Hibiscus sabdariffa* is similar to that species of *Hibiscus altissima* because they have same mutation in position number (237 – 258) have Lysine and Glycine amino acid for both. While they are different in position number (252) *sabdariffa* has Tyrosine while *altissima* has R in position number (242). *Hibiscus cannabinus* and *Hibiscus acetosella* different from those in all position. Also the Bioinformatics program determines amino acid concentration and molecular weight respectively between four *Hibiscus* species and signed (116 – 13469), (116 – 13530), (116 – 13516) and (118 – 13598) for amino acids and molecular weight of *Hibiscus sabdariffa*, *cannabinus*, *acetosella* and *altissima* respectively.

The biological test of the present study including Anti-oxidant, Anti-fungal, Anti-microbial and Phytochemical test revealed that Roselle seed has some chemical compound related with nutrition characteristics

through this study roselle seed were recorded Antioxidant (22 - 26 %) , (56 – 67%) for undigested and digested roselle seed by enzyme respectively.

Moreover roselle seed found to possess high anti-microbial activities were recorded (20 – 19 – 20 – 20 mm) for antimicrobial against (*Escherichia Coli*, *Pseudomonas arginosa*, *Staphelo areious* and *Bacillus subtillus*) respectively.

Anti-fungal were signed (22 – 19mm) for antifungal against *Candida albicans* and *Aspergillus niger* respectively.

Phytochemical compound showed that roselle had positive test for (Sterol, Tritreptine, Alkaloids, Cumarins and Cyanogenic) compounds this Results showed increase in the protein quality and nutritive value of roselle seed. Roselle gave negative result in test of (Saponins, Tannin, Anthraquinone glycoside and Flavonoids). This result showed a reduced nutritive value of the protein .Tannin and Saponins are classified as anti-nutritional factors.

ملخص البحث

نبات الكركدي يعرف في السودان كمحصول نقدي هام يزرع بصورة وأسعة في المناطق المدارية وشبه المدارية لإنتاج الثمار والبتللات بصورة أساسية. أجريت هذه الدراسة بمركز أبحاث الهدى للضأن لتقييم بذور الكركدي كمصدر بروتيني بديل ، والمستخدم في تغذية المجترات الصغيرة . حيث أستخدمت بذور الكركدي المجروشة بأربعة مستويات مختلفة (0 % ، 10 % ، 20 % و 30 %) ، في أربعة علائق متساوية في الطاقة والنايتروجين (أ ، ب ، ج ، د علي التوالي) . تم اختيار عدد (24) رأس من الضأن الصحراوي (الأشقر) إستناداً علي العمر (5 - 6) أشهر . ومتوسط الوزن (21.77) كجم . قسمت الحملان لأربعة معاملات (6) حيوان لكل معاملة ومن ثم قسمت الحيوانات في كل معاملة الي مجموعتين (تكرارات) بكل تكرار عدد (3) حيوانات ، غُذيت هذه الحيوانات علي علائق هذه التجربة لمدة (42) يوماً وفترة التأقلم (10) أيام بواقع (2 كجم / للرأس / اليوم) . الوزن اليومي المكتسب سجل (200 ، 200،220 و 240 جرام) والمادة الجافة المأكولة (1.61، 1.65 ، 1.65، 1.69 كجم /اليوم) للمعاملات (أ ، ب ، ج و د علي التوالي). أظهرت الدراسة عدم وجود فروق معنوية ($P>0.05$) بين المعاملات لكل من الوزن اليومي المكتسب والمادة الجافة المأكولة ومعدل التحول الغذائي (FCR) بينما سجل التقييم الإقتصادي لتكلفة العلائق فرقاً معنوياً كبيراً ($P<0.05$) في الإستفادة للتكلفة الكلية لمدخلات العلف والتي سجلت (2.74 – 2.96 – 3.03 و 2.52) لسعر الكيلوجرام جنيه سوداني ، للمعاملات أ ، ب ، ج و د علي التوالي. حيث كانت أقل العلائق تكلفة هي المعاملة (ج ود) التي أدخلت فيها بذور الكركدي المجروشة بنسبة (20% ، 30%) علي التوالي . بينما لا توجد فروق معنوية بين المعاملتان (أ وب) والتي أدخلت فيها بذور الكركدي المجروشة بنسبة (0 % ، 10%) علي التوالي . أظهرت نتائج الدراسة أنه من الممكن إستخدام بذور الكركدي المجروشة كمصدر بروتيني بديل لأمبازات الحبوب الزيتية الأخرى ، في علائق المجترات حتي مستوي (30%) حيث أعطت نتائج جيدة ومرضية في تسمين وأداء الحملان وقللت من تكلفة الإنتاج .

النتائج المتحصل عليها في هذه الدراسة من خلال تجربة تقانة أكياس النايلون عبر فتحة الكرش الجراحية والمستخدمه لتحديد معدل تكسر وإختفاء المادة الجافة والبروتين الخام في الكرش ، أظهرت وجود فروق معنوية عالية ($P<0.05$) بين مصادر البروتينات المختلفة والتي سجلت (41.17 – 74.55) ، (61.88 – 76.78) ، (62.86 – 78.54) و (88.22 – 77.39) لنسبة معدل تكسر وإختفاء المادة الجافة والبروتين الخام في الكرش عند ساعات

التحضير (72 ساعة) لأمبار بذرة القطن ، وبذور الكركدي المجروش ، أمبار بذرة زهرة الشمس و أمبار بذرة الفول السوداني علي التوالي .

بينما سجلت نتائج تأثير التكسر ومعدل التدفق في الكرش عند ك = 0.02 (63.88 - 29.30) ، (34.61 - 63.31) ، (43.60 - 67.59) و (47.50 - 57.71) لتأثير التكسر للمادة الجافة والبروتين الخام لأمبار بذرة القطن ، بذور الكركدي المجروشة ، أمبار بذرة عباد الشمس وأمبار بذرة الفول السوداني علي التوالي .

من خلال تجربة الهضم الميكروبي والإنزيمي في المختبر باستخدام سائل الكرش وإنزيم الببسين لتحديد حجم الغاز المنتج لمصادر البروتين المختلفة والتي سجلت إختلافات معنوية كبيرة ، حيث سجل أمبار القطن أقل كمية في حجم الغاز المنتج (61.64) ديسمتر ، يليه بذور الكركدي المجروشة (65.26) ديسمتر ، وأمبار زهرة عباد الشمس (67.81) ديسمتر بينما سجل أمبار الفول السوداني أعلى كمية للغاز المنتج (72.30) ديسمتر علي التوالي . عند ساعات التحضير 96 ساعة.

أُستخدمت تقانة المعلومات الإحيائية المحوسبة (Bioinformatics) في هذه الدراسة والتي وجدت تشابه بين صنف الكركدي من نوع (*Hibiscus sabdariffa*) و (*Hibiscus altissima*) وذلك تبعاً للطفرات المتطابقة في طول السلسلة الأمينية عند الطفرتين في الموقع رقم (237 - 258) والتي تحتويان علي الحمض الأميني اللايسين والجلالسين لكل منهما ، بينما يختلفان في طفرة الموقع رقم (252) و (242) . (*Hibiscus sabdariffa*) يحتوي علي الحمض الأميني التايروسين في الطفرة رقم (252) ، بينما يحتوي (*Hibiscus altissima*) علي الحمض الأميني R في الطفرة رقم (242) ، يختلفان كل من (*Hibiscus cannabinus*) و (*Hibiscus acetosella*) عن بعضهما وعن النوعين الآخرين في كل مواقع الطفرات أيضاً حدد البرنامج تركيز الأحماض الأمينية والأوزان الجزيئية بين أنواع الكركدي الأربعة والتي سجلت (116 - 13469) ، (116 - 13530) ، (116 - 13516) و (118 - 13598) للأحماض الأمنية و الوزن الجزيئي لأصناف الكركدي الأربعة: (*Hibiscus sabdariffa*) ، (*Hibiscus altissima*) ، (*Hibiscus cannabinus*) و (*acetosella*) علي التوالي .

شمل إختبار المركبات الحيوية في هذه الدراسة علي مضادات الأكسدة ، ومضادات الفطريات ومضادات المكروبات البكتيرية و التحليل الكيمائي للنبات. والذي أظهر أن بذور الكركدي تحتوي علي بعض الخصائص والتي سجلت مضادات الأكسدة (22 - 26) ، (65 - 67) لبذور الكركدي المجروشة والمهضومة بإنزيم الببسين وحمض البنكرياتين علي التوالي .

علاوةً على ذلك وجد أن بذور الكركدي تحتوي على أنشطة مضادات البكتريا ضد الانواع التالية:

(*E.coli, Pseudomonas argons, Bacillus subtillus and Staphelo arieous*)
مضادات الفطريات سجلت (19 – 22) (*Aspergillus niger and Candida albicans*).
أظهر إختبار التحليل الكيميائي للمركبات النباتية نتيجة إيجابية لكل المركبات التالية:
(Sterol, Alkaloid, Tritreptine, Cumarins and Cyanogenic).
هذه المركبات تزيد من جودة وقيمة بروتين الكركدي . بينما سجلت بذور الكركدي نتائج سالبة
في مركبات (Anthraquinone glycoside, Flavonoids, Tannin and Saponins)
وهذه المركبات تقلل من قيمة البروتين الغذائية بانها تصنف من العوامل غير الغذائية .

CHAPTER ONE

INTRODUCTION

Sudan livestock contributes about 22 % in total national income, and 38% of agricultural export in recent years the export of sheep is relatively increased. (M.A.R.F,2008). Sudan has one of the largest populations in the continent, which is estimated at about (140.5), of which 41.84, 52.24, 43 and 4.4 million head of cattle, sheep, goats and camels respectively. MARF (2008) showed that in the year 1991, live meat production was 1.473 million tones and the export was 8829 tons. Most of this quantity was mutton, which equals to 60%of total meat export. Nevertheless, in the same period 1.62 million live sheep were exported. In the year 2000 – 2001 for example, sheep exports has contributed with 261.34 million \$ to the national exchange earning at an annual off take rate of 21.778 million head (M.A.R., 2002). Ali, (2003) mentioned that in spite of the economic importance most sheep are still raised under nomadic conditions with traditional methods of management and natural grazing.

Roselle seeds hve many names in different parts of the world. In most places, it is either known as red sorrel or roselle (Qi *et al*, 2005). roselle seeds is a species of hibiscus related to the tropical old world. It is believed that roselle originated from Africa, where it was largely planted. It can grow annually and attain a height between 2 and 2.5 m (Chandramohan and Marimuthu, 2011).

Roselle Seed (*Hibiscus Sabdariffa*) family malvacea is known commonly as Karkadeh. Jamaica sorrel, India sorrel, Guinea sorrel, sour and Greens land jelly plant. (Mahadeven and Shivali, 2009; Morton 1987). Flowers and calyces from *Hibiscus sabdariffa* L. can be used for making tea or beverages. People in Jamaica and other islands make sorrel drink from flowers of *H. sabdariffa* (roselle). Roselle leaves and calyces can also be used as vegetables. Another species, *H. cannabinus* L.(Kenaf), is intensively used for making rope and paper products *Hibiscus* species also produce small seeds which can be potentially used for producing edible oils or feeding livestock. In Sudan locally called

(Karkadeh), is an important annual crop grown in tropical climate (Copley, 1975). The plant is originated in tropical Africa (Mc Clean, 1973). In Sudan the plant is mainly grown under rain fed condition in the western part of the country. Two red cultivars are grown in Sudan; El Fashir and El Rahad varieties. The plant is 0.5 to 2 meters high. The color of calyx plays an important role in determining the quality of the crop. (El Naim and Ahmed, 2010).The crop is produced in traditional grown conditions by small farmers, depending on rain fed and natural soil fertility without using chemical fertilizers or insecticides. A portion of crop produced is used locally; however the large portion of it is exported. It has many industrials and domestic uses. In Sudan dried calyx is soaked in water to prepare a colorful cold drink, traditionally, the product has been used for medicinal purposes importance in the manufacture of many small industries, e.g. cosmetics, sweets, jams, and jellies substitute for tea and also used as medicine, especially with the problems related to the respiratory system. The roselle seeds have a good potentiality as a new vegetable oil and protein (Al Wandawi *et al.*, 1984). Few studies were conducted (Salih and Abdel Wahab, 1990; Mohammed and Idris, 1991; Darran, 2007; Eltoum 1992; Bakheit 1993; Beshir; 1996 and Agib, 1999) on the feeding value of roselle seeds meal on broiler, layer and sheep. The results of these studies indicated the high potentiality of Karkadeh seed meal as animal feed specially in fattening animals and improve the meat characteristics (McDonald *et al*, 2002).

Roselle seeds production in the Sudan has been increasing during the last year (150 lb/ hectares) and the total area cultivated is estimated as 8334 hectares. Sudan is the world largest exporter of Karkadeh, Sudan export of calyx have risen from 454 tons in 1960 to about 952.000 tons in 1995 and 1200.000 tons in 2002 (FAO, 2002). The increased area of roselle raised production from 454 tons in the 1960s to 26,000 tons in the 1999 / 2000 (El Awad, 2001). Roselle seeds production in Sudan facing many problems resulted in unstable total production, such as the fluctuation of rain fall and the high cost of labor for harvesting, which

amount about half of the total cost of production. Many researchers have been published on the ecology, taxonomy, genetic, cytology, chemotaxonomy, physiology, seed germination and economic uses of family *Malvaceae* such as (El Rjoob *et al*, 2009). in ecology; in taxonomy Tate *et al*, (2005), in chemotaxonomy Blunden *et al*, (2001) and in genetic researches Baum *et al*, (2004) studied the pollen.

Bioinformatics can be defined as one branch of molecular biology, also its computer science devoted to collecting organization Deoxy ribo nucleic acid DNA and protein sequence, structure and function. The computed parameter includes Chimera tools version 1.9 Software online was used for present study to evaluate characterization the tertiary model of the roselle protein analyzed molecular weight, amino acids concentration; extinction coefficient estimated half -life, instability index. Position variation (mutation) and 3D structure of protein. (Huang, 2014).

Research Problem:

Unknown of stable and unstable protein quality using in animal feed, also most source of protein are expensive.

Research hypothesis:

Roselle seeds it's a cheaper protein source than other oilseed cakes available in Sudanese market and low uses till now in animal feeding.

Research Objectives:

General objectives:

1 -To study the chemo-genetic characterization of roselle seeds as, used in fattening performance of Sudanese desert lambs.

Specific Objectives of study are:

1- To examine *in vitro* digestibility of roselle seeds using enzymatic digestion and picric acid to study the gas production volume / decimeter.

- 2- To determination of *in sacco* degradability of experimental plant protein sources for evaluates the disappearance percentage of dry matter and crude protein residual.
- 3- To determine the biological test of roselle seeds including anti-oxidant, Phytochemical, anti-microbial and anti-fungal activities.
- 4- To assess the effect the use different levels of roselle seeds on fattening Sudanese desert sheep.
- 5- To determine neutral detergent fiber, acid detergent fiber of four plant protein sources Sunflower cake (SFC), Groundnut cake (GNC) and Cottonseed cake (CSC) and crushed roselle seed (CRS).
- 6- To investigate roselle seeds are potential alternative protein sources for fattening Sudanese desert sheep.
- 7- To evaluate the chemical composition and Minerals of roselle seeds (*Hibiscus Sabdariffa l*).
- 8- To determine economical evaluation study of (*Hibiscus sabdariffa l*) on feed formulation.

CHAPTER TWO

LITERATURE REVIEW

2 Ruminants:

2.1 Ruminants livestock in Sudan:

Ruminants play an important role in the livelihood of farmers in the developing world, providing milk and meat animal traction, manure for crop production, cash income from sales of their products and satisfy net of capital assets to face risk and misfortune in harsh environment (Qrskov, 1993). Livestock production systems in Africa are influenced by the annual rainfall and its effect on main vegetation characteristics adequate feeding is usually regarded as the major factor limiting sheep production particularly in the tropics (Wilson, 1991). In north Kordofan, El Hag, (2001) conducted that the production characteristics of Sudan desert sheep reflects the seasonal nutritional status and husbandry system. This author found no significant difference between sedentary and nomadic flock in the number of ewes served, conception and lambing or abortion rate.

2.1.1 Ecological habitat of Sudan desert sheep:

Sudan desert sheep are strictly confined to the semi-arid climatic zone. Their homeland is roughly bound in the south by latitude 12° N, although this southern border has recently retreated further south due to the southward advance of the desert. The western border is marked by range of rocky hills from Jabel Marra in the two Zaghawa plateau in the north. To the east the area extended to the Red Sea hills, to the North. It fades away with an undulating border in the Nubian Desert (Suleiman and Eisawi, 1984).

2.1.2 General Taxonomy of sheep:

The sheep (*Ovis*) are ungulate animals which belong to the phylum Chordata order family Bovidae, subtribe Caprinae and species *Ovis*, (Mc Leroy 1961). The Sudan sheep had been classified by, Mc Leroy 1961). The classification was based on ancestry, route of entrance, morphological features and ecological distribution, histological all Sudan sheep belong to two species A/ *Ovis longipes* B/ *Ovis platyura*.

2.1.3 Ecological habitat of Sudanese desert sheep study area:

2.1.3.1 Kordofan region:

The total population of the region according to 1993 census is 3.1 million out of this number the urban communities account for 13%, the nomads for 24% and the rural settlers for 63% , the soil of region vary from sandy types at the north to the fertile light and cracking calyx at the south. Both sandy and calyx Pedit plains occupy the traditional productive areas constituting above 65% of the total available lands. Saline soils are confined to the mountainous areas, while loamy and alluvial deposits are limited to the wadi bottoms. The region has a wide range of climatic zones which range from north to south as follows (Farah, 2006).

- a- Desert types with rainfall below 100mm.
- b- Semi-desert, with the rainfall varying between 100-225mm.
- c- Arid zone, with annual rainfall 225- 400mm.
- d- Semi-arid zone, with annual rainfall range from 400-700mm.
- e- Dry wind zone, with annual rainfall ranging between 600-800mm.

The basement complex is the oldest and the most extensive rock unit covering more than 50% of the total area of region. The Nubian sand stone formation occupies some 25% of the total area. The Um Rawaba sediments fill the major trough of Bara and Bagara basins, the source of water in the main region is itemized as surface water and ground water. The main annual rainfall in Kordofan ranges from 50 to 850mm / year, with availability ranging from

18% in the north to 100% in the south. This corresponds to a total amount of rain water of some $150 \times 10^6 \text{ m}^3$ / annum. This water falls in three months between July and September. Kordofan suffers from water deficit of some 650 in the south to 1400 mm/ annum in the north (Farah, 2006).

The economy of Kordofan region is predominantly depending on agricultural production, the leading from agriculture is rain fed crop farming with traditional livestock grazing practiced through nomadic systems or as village based activity coming next in importance, the two economic are in many instances found to exist as integrated forms of production.

There are two types of production systems under which staple food crops such as sorghum, millet and cash crops as sesame, groundnut, water melon and roselle are produced. The two systems are traditional farming system which is widely practiced in the region and it is completely dependent on family labor and mechanized farming system is practiced in the southern part of the region. Mechanized crop farming is relatively stable agricultural enterprise compared to the traditional system production. Horticultural crops are also produced on a limited scale for the local market with some surplus marketed outside the region. All crops produced in the region are annually attacked by pests such as desert locus, grass hopper, rats and bird.

Kordofan region is considered to be one of the most important livestock areas in the Sudan. All traditional livestock types are found in Kordofan. The livestock census of (1976 – 1977) showed that there were 15%, 10%, 21% and 36% of the nation cattle, sheep, goats and camels respectively in Kordofan region. Based on this and giving especial account for the considerable decline in livestock population during the drought of 1984 –1989, the Administration of Animal Resources of Kordofan in Elobied, figure out that for the year 1992/1993, there were 3.3 million heads of cattle, 4.1 million heads of sheep, 3.4 million heads of goats and 1.1 million heads of camels in Kordofan (Farah, 2006). The total wood land area in Kordofan is estimated at 30.7 million

feddans. The total area of the preserved forests is 483, 876 feddans and the last figure constitutes about 15.7% of the entire wood land in the region and 0.5% of the total area of the region, of these about 8% is plantation forests. The entire wood land constitutes only 34% of the region and the reserved forest in the region makes 0.6% (Farah, 2006).

2.1.3.2 Gezira region:

Gezira (*Aljazeera*) is the well- populated area suitable for agriculture. The area was the southern end of Nubia and little known about its ancient history and only limited archaeological work has been conducted in this area. It was part of the Kingdom of Alodia for several centuries and with that states collapse in the early sixteenth century became the center of Funj Kingdom of Sennar, the region has benefited from the Gezira – Scheme a program to foster cotton farming begun in 1925. At the time of Sennar Dam and numerous irrigation canals were built. Al Gezira became the Sudan's major agriculture region with more than 2.5 million acres (10,000km²) under cultivation. The initial development project was semi-private, but the government nationalized it in 1950. Cotton production increased in the 1970s but the 1990s increased wheat production has supplanted a third of land formerly seeded with cotton (Wikipedia, 2014).

2.1.3.3 Butana plain region:

The area is composed of mountainous ranges interesting the plain to the western and southern borders. Its crossed by many seasonal rives namely, Atbara, Seitite, Ba, Salam Gash and Rahad Rivers. Small temporary seasonal valleys do run through these plains during the rainy season. However, the amount of the water and persistence of reserves during the summer dry season depend on the quantity of rainfall during the wet season. In the Butana, a tropical continental climate prevails raging from a sub- equatorial condition with rain in the south to desert climate in the north. Most of rains are in the form of showers or thunderstorms (Hussien, 2012 and Darosa and Agab, 2013).

The rainfall in Butana region is highly variable from one year to other. Are ranges between 600mm/ year in the southeast to less than 100mm/ year in the northeast? As always in the semi arid regions, rainfalls are the most important climatic factor which supports the growth of the vegetation for their animals. The annual main temperature ranges from 32° C during the day to 16° C at night in January (winter) and from 46° C to 27° C at night in May – June (summer). Two vegetation zones are existing in the area, namely semi- desert Acacia shrub and short grasslands of the North Central Sudan and secondly, the low woodland savaging of central Sudan. The vegetation of Butana is constantly as a result of annual rainfall, accidental fire outbreaks and expansion of agriculture and grazing (Saint *et al*, 1992).

The Butana area is inhabited by transhumant camel owning tribes in its northern part while the southern part is populated by sheep breeders whom are agro-pastoralists practice mainly mechanized rain-fed agricultural activities for production of sorghum and sesame grains besides considerable livestock raising activities (Darosa and Agab, 2013).

The area of the central Butana is characterized by arid and semi-arid climate. The rainy season extend from July to September. The average annual rainfall ranges between 150 – 400mm, and the average annual temperature in summer is around 40° C (March – October) and 25° C in winter (November – February). Grass cover the plains during the rainy season and the few succeeding months. It decreases in intensity from south to north. Acacia trees such as *A. mellifera* (ketir), *A. nubica* (laout) constitute the main shrub and are mainly confined to the channels (Hussien, 2012).

According to the 1993 census in Sudan, the total population in area (including Butana and East Nile province), reaches 1.500.000, while 25.000 inhabitants live in the Butana area. The inhabitants are mainly members of the Shukria tribe. Sudan sheep population is about 48.136.000 as estimated by (MAR, 2002), Northern Kordofan, Western Kordofan, Western Darfur, Northern Darfur, Gezira

and Khartoum State are produce about 8.4%, 7.7%, 7.50, 7.38, 5.5 and 4.5 percentages respectively of Sudanese desert sheep production and also exported sheep meat during the period (MAR, 2002) as 705000 tons. In 2007 (000 heads) the productivity of sheep was estimated by MARF to be Northern State, Kordofan States, Darfur States, Eastern State and Khartoum State are produce about 1955, 10049, 11194, 3374, 11062, 431 head of sheep respectively. And about 5571 tons of as mutton and 1.7 million heads of live sheep were exported in 2004 (MARF, 2007).

Rain –fed agriculture is the second major occupation of the population of Butana area. The only quarrying activity is the production of marble blocks which are cut and polished in Khartoum, or the production of lime by burning Marble to be used as wall paint) Darosa and Agab 2013, Hussien, 2012) stated that, the soil cover of central this region is founded mainly of sticky dark Clays of montmorillonitic composition, which readily Swell when wet, and shrink forming wide and deep cracks when they dry up. Depending on the topography (Hussien, 2012) classified the clayey Soil into three distinct types of follows:

1. High land soil: Locally Known as Dahar is brown to gray with a hard Crust on the top, mainly mixed with gravel and rock series, known as Azaza.
2. Low land soil: heavy dark clays, locally known as Mahwa.
3. The Deltaic soil: rather heavy soils, containing a large silty protein form most of the alluvial fans at the mouth of the wadies.

2.2 The sheep:

Sudan desert sheep are reared strictly within the semi-desert belt of the Sudan, in association with the camel. Sudan is well endowed with livestock resources. In (MARF, 2007) the total Sudanese livestock population was estimated at 139 million heads, comprising 41, 51, 43, and 4 million heads of cattle, sheep, goats and camels respectively. In addition, it provides the country with foreign currencies; as mutton and live animals are exported annually. About 5571 tons of mutton and 1.7 million heads of live sheep were

exported in 2004 (MARF, 2007). The average annual growth rate of the livestock sector was estimated at 3.1% (Faki *et al*, 2009). They owned exclusively by the nomadic tribes in the region. Because of their nomadic existence their origin has been difficult to trace (Mufarrih, 1991). (Wilson, 1981) mentioned that, This Arab type of sheep, presumably owned by this tribes, with its woolly coat and short legs, could not have endured the stress of intensive solar radiation and prolonged migration in search of grazing and water of sudden attacks by the enemy. (Ellamin and Suleiman, 1983) added, to obtain an animal which would satisfy this requirements, while retaining the describable characteristics of their original sheep, this Arab tribes might have decided to cross-breed their sheep with other types which possessed the required traits. (Mufarrih, 1991) added that, Sudan desert sheep, however, possess a thicker tail and fuller rump. These valuable characteristics might be attributed to partial inheritance from their Asian ancestors. Sheep is classified in sub-family caprinae, family bovidae and all domesticated sheep are including in the genus *ovisaries*. (Wiilliamson and Payne, 1965) mentioned that there are four major species of wild sheep.

1. Themoufflon (*Ovis. musion*).
2. Theurial (*O.Orientalis*).
3. The Argali (*O. ammon*)
4. The bighorn (*Canadensis*).

2.2.1 Sudan desert sheep husbandry methods:

The vast –majority of Sudan desert sheep exist under migratory range conditions while a few small flocks exist under a semi-residential system.

The pattern of management adapted in the whole region is essentially the same (Mufarrih, 1991).

The usual size of flock in traditional desert sheep rangelands is 250-500 ewes. It has been realized, however, that large flocks create herding difficulties and lessen the lambing rate. The time of grazing varies between seasons. In dry

seasons most of grazing is done at night. The herders are aware of the benefit of night grazing in lessening water requirement and avoiding the stress of the solar heat.

They firmly believe in a local saying which states that “the ewes are like a rabbit. When is grazing at night lies shade drying the day, it will produce twins and triple lambs. It has been widely recognized that exposure to high ambient Temperature reduces fertility of rams. (Mufarrih, 1991 and Suleiman and El Tahir, 1984).

In rainy season the availability of the drinking - water and succulent grazing enables sheep to ingest their daily requirement in a few hours. Because of mild temperature and frequent cold, the sheep will continue to graze and lie down in the open air until late in the afternoon. Rainy season grazing is restricted to period from 09:00 hours to about 16:00 hours when the plants are without dew.

Disease such as Foot-rot is known to result from grazing at night or early morning while the grasses are cold and damp. Salt is supplied in sufficient amounts for free-choice nibbling once or twice a week in winter and during the rainy season. During the hot and dry season salting is reduced to a minimum to avoid increase water requirement. Sometimes salt is dissolved in drinking water so that each individual animal takes in an adequate amount while drinking (Suleiman and El Tahir, 1984).

2.2.2 Sheep production in Africa:

Tropical Africa contains one – sixth of the world sheep production. On average, there is one sheep on every 10 hectare, and there are 1.1 head of sheep per person employed in the agricultural sector (Wilson, 1991). Total meat production from African sheep is estimated at 934.000 metric tons (MT) FAO, (1995). Sudan contributed with 74.000 (MT)., which is equivalent 7.9% of African total meat production.

2.2.2.1 Sudan desert sheep production performance:

Under tropical environmental conditions, sheep are raised primarily for meat, although milk is also important. The value of breeding ewe is determined by the quantity and quality of lamb or mutton produced and the length of its productive life (Suleiman and El Tahir, 1984).

Field –collected data on the lambing rate of Sudan desert sheep indicate wide Differences between localities presumably attributable to climatic, nutritional and management factors. Personally acquired information on migratory groups in the western Kordofan and Eastern Darfur areas indicated 150 -170 percent lambing rate (Tothill, 1984 Wilson,1981) reported the lambing rate for nomadic flock of Sudan Desert sheep in Southern Darfur province to be 146 percent.

Wide differences in lambing rates also exist among individual flocks under semi-residential system maintained in irrigated areas (Mufarrih, 1991). Under a residential system, (Suleiman and Eisawi, 1984).report an over lambing rate of 119 percent and rate of 125 percent for the *Shugor* variety alone. This subnormal rate can probably be attributed to the low nutritional level experienced by the sheep for a considerable portion of the year.

(El Hag *et al*,2001) reported that, the nutritional limitation, low nutritive value of the range, high ambient temperature, scarcity of feed and water are have great effect on the production of the sheep in semi- arid area of Kordofan state as compared to that in temperate region.

2.2.2.2 Classification of Sudan desert sheep:

Medani, (1996) stated that, Sudan desert sheep have been classified based on physical features and ecological distribution. Four main local groups have been identified: Sudan Desert, Sudan Nilotic, Sudan Arid Upland and Sudan Equatorial Upland. Fused ecotype groups, resulting from non- systemic crossbreeding at the boundaries of the eco-zones of the pure types, have also been recognized.

Medani, (1996) classified Sudan desert sheep under these main ecotypes: Gezira and Butana sheep this group (includes *Shugor*, *Watish* and *Dubasi*); *Kabashi* sheep; *Medoub* sheep; *Albega* sheep; Northern Nilotic sheep and Western African sheep or Sudan Arid Upland this group includes: *Fulani* and *Zaggawa* sheep.

Sudan desert sheep and their crosses makeup about 80% of the sheep found in Sudan and mainly predominate north of 12 N°, they are raised mainly under high dry land farming conditions for meat production (Idris *et al*, 2011).

The vast majority of Sudan desert sheep existing under range conditions while few small flocks are under semi-residential system. The pattern of management adopted in the whole region is essentially the same (Mufarrih, 1991).

2.3. Tribal subtypes of Sudanese desert sheep:

2.3.1. Shugor

Shugor are moderately large sheep ranging in color from light to dark brown they have occasional patches of wool under the hair. They are found mainly along and to the west of west of the White Nile, and are most common in the western part of the Gezira, where they graze on cotton residues and other agricultural by-products. Their migratory movements are longer than those of the Dubasi and it is not uncommon to find Shugor flocks deep in the Gezira area (Medani, 1996; Mufarrih 1991; Gumaa and Gaili, 1983).

2.3.2 Dubasi

Dubasi are prototype sheep of the Gezira area, especially the northern part and are concentrated in the villages of the Dubaseen tribes (hence Dubasi). These sheep are similar in size to the Shugor but their thin coat is usually parti-colored white and black. The distribution of the black patches on the skin varies among regions and breeders. Some breeders select sheep with saddled backs with black plates or patches. It is rare to find Dubasi farther to be west along the White Nile (Mufarrih 1991 and Medani, 1996).

2.3.3 Watish

The Watish subtype is somewhat smaller and stockier than either the Shugor or the Dubasi. Three color groups – fawn, red, and white with light spotting – have been recognize (Mc Leroy, 1961). Watish are hardy sheep and live under relatively high rainfall conditions between latitude 10° and 11° and mainly along the Blue Nile, south of Wad Medani into the Fung area. They are mainly owned by nomadic and semi- nomadic tribes, including Kenana

Rufaa El Hoy and BeniMehariba (Medani, 1996; Mufarrih, 1991; Hassan and Mukhtar, 1970).

2.3.4 Kabashi

It is classified into tribal subtypes Kabashi and Shanabli in the north and east Kordofan state (Mukhtar 1985 and El Hag *et al.*, 2001). Kabashi is regarded as the best of the desert sheep in the Sudan. This type is characterized by the producing milk, meat and skin. Mature male weight is 90 Kg but female is 70 Kg and its dressing percentage 56.4%. The dominant color is white or red ewes produce milk at rate of 2-3 Kg per day and with lactation period of 4months (Babiker and Abdel Hamid, 1988; Medani 1996).

2.3.5 Hamari sheep

This type is raised by Hamari tribes in Kordofan State and is considered as a prototype of export. The average weight of this type about 26 Kg yielding a 30 Kg carcass and dressing percentage of 56.4%. Mature male are 75-85 cm at the shoulder and have long pendulous ears. The color is brown or gray. This type produces meat, milk and skin (Medani, 1996; Babiker and Abdel Hamid, 1988).

2.4 Function of Sheep:

In livestock sector, sheep play a remarkable role in livelihoods of Sudanese people, providing food, income and enriching the land with animal manure. In addition, it provides the country with foreign currencies; as mutton and live animals are exported annually. About 5571 tons of mutton and 1.7 million heads of live sheep were exported in 2004 (MARF, 2007).

2.5 Socio-economic important of sheep:

Sheep and goats play an important role in food production in development countries. However most of Islamic countries and social occasional, particularly Korfani (Byrum). In many cases the sheep contribute to the Sustenance of landless, small and marginal farmers because of the lower capital cost and their easy management (Tolera, 1998).

2.6 Advantages of sheep rising:

Sudan is well endowed with livestock resources. In 2007 the total Sudanese livestock population was estimated at 139 million heads, comprising 41, 51, 43, and 4 million heads of cattle, sheep, goats and camel, respectively. The average annual growth rate of livestock sector estimated at 3.1% Faki *et al*, (2008) In addition provides the country with foreign currencies; as mutton and 1.7 million heads of live sheep were exported in 2004 (MARF, 2007).

Sheep have many advantages over some other classes of livestock. They produce two main different products in a year, wool and lambs, bringing in rather quick return, sheep also can improve fertility of many farms. An individual sheep can add 0.05 - 0.7 tones of manure to the soil every year, which contains twice the nitrogen and potassium in cattle, manure (Singh, 1969). This author also mentioned that sheep have the ability to produce prime carcasses on roughage alone, and reported that sheep are especially well adapted for many areas unable to produce grain profitably, and degraded environment owing to more selective grazing.

Church, (1991) conducted that sheep do not require expensive building and equipments, and they are known to have higher fecundity and early puberty.

2.7 Sheep management:

Sheep are favored for their economic efficiency in converting available feed into has product that have higher consumer demands, namely, wool, milk and meat. Production system of this demand may differ in deferent regions of the

world restricting the generalization about sheep production and nutrition (Church, 1991).

2.7.1 Sheep nutrition:

Nutrition plays a major role in the overall productivity, health and well-being of the sheep flock. Because feed costs account for approximately two-thirds of the total costs of production on most sheep farms, it's important that products consider nutrition management a top priority. Nutrition requirement of sheep vary with different in age, body weight, and stage of production (Umberger, 2009). During the grazing season, sheep are able to meet their nutrient requirements from the pasture and a salt and minerals supplement. Hay is provided to the flock when forages are limited, and grain may be added to the diet at the certain stages of production when additional nutrient supplementation is required (El Hag *et al*, 2001).

In spite of the importance of sheep they are still reared under nomadic condition with traditional methods of the management and national grazing.

Many socio- economic factors affected of nomadic flock including natural pasture (Tothill, 1984). El Hag *et al*, (2001) reported that, the specific problems regarding nutrition under range land condition is that of feed shortage and nutrient deficiencies. This situation is critical during the dry season which extended from November – June. Really farmers provide their animals with different supplements during the critical period of feed shortage. Supplements used are mainly oilseed cakes and cereal grains. The most critical period of grazing sheep in the semi desert zone of Sudan is from February to June, when the ambient temperature becomes hot and range grazing is scanty and depleted of nutrients and vitamins. Shortage feed in this season is the main factor, that effect of sheep production in the range land of Kordofan, taking into account that natural pasture by product are poor in their quality and most the range exposed to the over grazing, especially near the water resources. Seasonal

nutritional status and husbandry affect sheep production characteristics (El Hag *et al*, 2001; and Suleiman and Eisawi, 1984).

In the central and eastern regions, where vast areas of traditional grazing land have been converted into crop farms, many nomadic families have adapted themselves to residential or semi-residential existence. Their sheep spend the dry season within or around the cropping areas sustain themselves on crops residues. However these residues are only able to sustain this large amount of livestock for about three to five weeks. The animal are then forced out so that the land can be prepared for the next cultivation. (Mufarrih, 1991; Tothill, 1984).

2.8 Rumen digestion:

Ruminants so named because they ruminate (chew the cud) have a stomach that consist of a non- secretory fore-stomach and a secretory stomach compartment (the abomasums).The fore-stomach consist of three compartments (the reticulum, the rumen and Omasum), the abomasums, like the stomach of the simple stomached animals is largely medium. The food of ruminants forages and roughages, consist mainly of β - linked polysaccharides such as cellulose, which cannot be broken down by mammalian digestive enzyme. Ruminants have therefore evolved a special system of digestion that involved fermentation of food prior to exposure to their own digestive enzymes The reticulum-rumen provides a continuous culture system for anaerobic bacteria, protozoa and fungi, Protozoa are present in much smaller numbers (10^6 per ml of rumen content) than bacteria numbers ($10^9 - 10^{10}$). Over 200 species of bacteria have been identified; most are non-spore forming anaerobic bacteria. Ingested food is digested through the action of various microorganisms (bacteria, protozoa and fungi). In the reticulum.(Mc Donald *et al*, 2002a).

2.8.1 Rumen Bacteria:

The bacteria number is ($10^9 - 10^{10}$) per ml of rumen content. Over 200 species have been identified; most are non-spore forming anaerobic. The activities of some species of bacteria may vary from one strain of that species to another. The total number of bacteria and the relative population of individual species vary with the diet, example diet rich in concentrate foods promote high total counts and encourage the proliferation of lacto bacilli (Garry, 2005).

2.8.2 Clostridium botulinum:

Clostridium botulinum is the causative agent in four types of botulinum: a,b,c and f cause food borne, infant, wound and those classified as undetermined. The type of Clostridium botulinum and food products involved in various foods borne the botulinum toxin can cause a severe flaccid paralysis disease in human and other animals.

The type of C. botulinum outbreak are discussed in this review most food borne botulism outbreaks result from consumption of home – processed or home - canned foods; relatively few are caused by commercial products. Various physical and chemical treatments that can be used in foods either to destroy Clostridium botulinum spores or control their outgrowth and toxin production are presented and concerns about potential food borne outbreak from new generation food are discussed (Peck, 2009; Lindstrom and Korkeala, 2006).

2.8.3 Staphylococcus aureus:

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans. *S. aureus* is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal enterotoxin (SE) and is

responsible for almost all staphylococcal food poisoning (Montville and Matthews 2008; FDA, 2012).

S. intermedius, a *Staphylococcus* species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning (Le Loir *et al*, 2003).

The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (w_a), pH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains (Stewart, 2003).

The temperature range for growth of *S. aureus* is 7 – 48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. Growth of *S. aureus* occurs over the pH range of 4.0 – 10.0, with an optimum of 6 – 7 (ICMSF 1996; Stewart 2003). *S. aureus* is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart, 2003).

2.8.4 *Bacillus subtilis*:

The admission of *Bacillus subtilis* to piglet feed has several pathways in which it may improve production parameters. *B. subtilis* consumes oxygen in the gut tract and additionally it produces certain enzymes like subtilisin and catalase. This results in a positive environment for beneficial bacteria such as Lactobacilli. Lactobacilli colonize the gut mucous membranes and block adhesion sites for pathogens, a mechanism known as competitive exclusion. Besides, Lactobacilli produce lactic acid, which reduces the amount of pathogens such as Salmonella, E. coli, Campylobacter and Clostridium (Hosoi *et al*. 2000). Numerous trial results are available on the reduction of these pathogens by *B. subtilis* (Fritts *et al*, 2000, Ragione and Woodward 2003). An interesting observation is that not only a reduced number of infected animals

were reported; also the amount of pathogenic bacteria in the faeces of the positive animals was reduced (Maruta *et al.* 1996).

2.8.5 *Escherichia coli*:

Ruminant animals, in particular cattle are the main reservoir for VTEC. These toxins are synonymously either called verocytotoxins (VT). Beef has historically been most linked to VTEC infections however a wide variety of other sources have been implicated in infection, including unpasteurised milk and fruit juice, sprouts, lettuce, spinach, cantaloupe, cheese, mushrooms, sprouts and salami. Waterborne transmission occurs through swimming in contaminated lakes, pools, or drinking untreated water. Direct contact with animal faecal material through recreational activities and person to person contact are also sources of infection. (Johan *et al.*, 2006). Certain isolates of *Escherichia coli* have been implicated in a wide range of diseases that affect either animals or humans worldwide. To date, eight pathovars and their mechanisms of disease have been extensively studied. These pathovars can be broadly classified as either diarrhoeagenic *E. coli* or extraintestinal *E. coli* (ExPEC)1 pathovars — enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC; including *Shigella*), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) — are diarrhoeagenic, and two pathovars — uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) — are the most common ExPEC isolates. Other pathovars have been identified, but their mechanisms of pathogenesis are not as well defined (Kaper *et al.*, 2004).

2.8.6 *Pseudomonas aeruginosa*:

Pseudomonas aeruginosa is a motile, gram negative, oxidase positive bacteria, grows well on MacConkey agar media. It is a rod shaped bacteria occurring singly or in short chains, microscopically, margins of the colonies are having hook like projections. The organism is a strict aerobe, also grows readily

on other common bacteriological media. The bacteria usually produces a water soluble green pigment composed of fluorescent and pyocyanin with a characteristics fruity odor. The organism is ubiquitous, often associated with soil, water and humid environment. *P. aeruginosa* is the most common pseudomonad causing infections in animals and human. It can be highly virulent causing 50 - 100% mortality in experimentally inoculated 4-week-old chickens. *P. aeruginosa* is include a significant economic loss to the farm by causing high mortality of newly hatched chickens and death of embryo at later stage. (JVMAH, 2010).

2.9 *Aspergillus niger*:

Aspergillus section Flavi includes *A. flavus*, *A. parasiticus* and *A. nomius* species that produce aflatoxins, potent carcinogenic compounds of concern in food safety. These toxic secondary metabolites are group of structurally related difuranocoumarins. Aflatoxins a detected as natural contaminants in foods are named B1 B2, G1 and G2 based on their fluorescence under UV, light (Blue or Green) and relative chromatographic mobility. Aflatoxin B1 is the most potent natural hepatocarcinogen known and is usually the major aflatoxin produced by toxigenic strains. Aflatoxins can be produced by several species belonging to this section of the *Aspergillus* genus but the most important aflatoxin producers occurring naturally in food commodities are *A. flavus*, *A. parasiticus* and, to a lesser extent, *A. nomius* (Astoreca *et al.*, 2011).

2.10 *Candida albicans*:

Candida albicans is a common fungal pathogen of humans and animals that colonizes the skin and mucosal surfaces of most healthy individuals. Until recently, little was known about the mechanisms by which mucosal antifungal defences tolerate colonizing *C. albicans* yet react strongly when hyphae of the same microorganism attempt to invade tissue. In this Review, we describe the properties of yeast cells and hyphae that are relevant to the interaction with the host, and the immunological mechanisms that differentially recognize

colonizing versus invading *C. albicans*. (Gow *et al*, 2013) It also causes a variety of infections that range from mucosal candidiasis to life-threatening disseminated candidiasis (Dixon *et al.*, 1996; O'Dwyer *et al.*, 2007; Terrier *et al.*, 2007).

2.11. Protein digestion in rumen:

Protein is fermented to V.F.A methane, carbon dioxide and ammonia (end product), peptide and amino acids are intermediates and used by rumen micro-organisms to synthesis microbial cell. Ammonia is either absorbed dietary across the rumen wall or passes out of the rumen with fluid phase of digest or is incorporated in to the abomasums and duodenum and is digested by enzyme hydrolysis (Kempton *et al*, 2008).

Nutrient inputs are first exposed to fermentative digestion by ruminal microorganism. The end product of microbial fermentation becomes available as energy volatile fatty acid (VFA) and protein (microbial cells) for the animal tissues metabolism (Pen, 2007).

2.11.1 Microbial population and requirement:

Increased levels of rumen protozoa type following *Saccharomyces cerevisiae* ingestion were also reported. This beneficial effects on the proportion of different protozoa type in rumen, leading to positive effects on cellulose digestibility and to systemic metabolic consequence (characterized by increasing of serum total protein, urea and calcium concentration and decrease of triglyceride concentrations) have been recently evidenced in rams (Galip, 2006). On the other hand (Newbold *et al*, 1996) mentioned that the ability of the different strains of *Saccharomyces cerevisiae* to stimulate the viable count of bacteria in the rumen appears to be related to their ability to remove oxygen from rumen fluids. The yeast content of high carboxylic acid, particularly malic acid has been shown to be the possible cause of stimulation *in vitro* (Nisbet and Martin, 1990). But *in vivo* it does not appear to cause the most important effects on yeast (Newbold *et al* 1996). *In vitro* and *in vivo* studies showed that yeast

and yeast cultures stimulate growth of rumen cellulolytic bacteria (Callaway and Martin, 1997) and outflow rate of microbial nitrogen post ruminal all that were noted to improve digestibility of dry matter (DM), crude protein (CP) and hemicelluloses (Wiedmeier *et al.*, 1987; Wohlt *et al.*., 1991; Erasmus *et al.*, 1992; Hariset *et al.*, 1992; Wallace and Newbold 1992; Wohlt *et al.*, 1998; El-Waziry *et al.*, 2000; Kholif *et al.*, 2005; kholif and Khorshed, 2006; El Waziry and Ibrahim2007). Some strain of *S. cerevisiae* has been shown to favor the establishment of fibrocystic bacteria in the digestive tract of genetically related lambs, accelerated microbial activities in the rumen. The microbial population of rumen is depends on the particular ecological balance that tends to prevail in the rumen. Digestion of complex carbohydrates depends on the type of micro-organism found in the rumen, which are two affected by the p^H of the rumen. (Van Soest, 1993). A key of the digestive processes in ruminant is the production of microbial cell and synthesis of microbial protein. If this synthesis is for any reason inefficient, food protein will be wasted (Mc Donald *et al.*, 1996) found that there was an increase microbial protein synthesis in the rumen of sheep in diets with the casein as nitrogen source compared to urea based diets. (Lenge *et al.*, 1983).

Smith, (1981) stated that there is complex interaction between the microbial ecosystem in the rumen and diets. Microbial protein is a good biological value

2.11.2 Extent of degradation of dietary protein in the rumen:

The effective degradation of dietary protein in vivo may be calculated from the amount of feed remaining at any time and the rate of microbial degradation of that material. Equation for this calculated value have been published by Orskov and Mc Donald; 1979 Miller; 1980 and Mc Donald, 1981). Although any one feedstuff contains a number of protein types, each of which may have characteristic rate of degradation, a sufficiently dose description of the rate of disappearance of (N) from synthetic fiber bags can be a achieved by the assuming. The first is the soluble component, which is rapidly washed out of the

forming the proportion (a). Orskov and Mc Donald, (1979) found that the second fraction is insoluble but potentially degradable and soluble by the micro-organisms according to the first order kinetics, forming the proportion (b) in the equation. The effective degradability (p) can then be calculated from the simple expression

$P = a + (b \cdot c / (c + k_1))$ (Orskov and Mc Donald 1979) where (c) is the degradation rate the (b) fraction and (k₁) the outflow rate of small particles.

With the relatively low protein fibrous feeds, they may be a lag phase before the (b) fraction begins to disappear, and this can be allowed for by use of and a proportion (1-(a + b)) is insoluble and undegradable.

2.11.3 Undegradable by-pass protein:-

Feeding levels, proportion of roughage to concentrate, particle size of the basal feed, environmental temperature, feeding frequency and stage of production are to alter the rumen liquor turnover rate (NRC, 1984). One way of overcoming this effectively is to feed a diet containing protein that cannot be degraded in rumen but which can be subsequently digested. Protein supplements of animal origin, such as meat and bone, feather and fishmeal contain a substantial component of undegraded dietary protein (UDP), but plant- dried supplements are generally quite degradable.

The 30% limit is to prevent substantial depression of digested energy of the basal diet, McDonald, (1981) found that fishmeal supplementation significantly enhanced the degradability of the structural carbohydrate with both untreated and ammonia treated barley straw. The improvement in the straw digestibility due to the ammonia treatment and fishmeal supplementation was additive.

2.11.4 Relationship between crude protein and dry matter:-

The degradation of protein is complicated by secretion of urea into the rumen via epithelial, absorption of ammonia and other nitrogenous components by rumen epithelium and recycling of bacteria and protozoa protein in the rumen. However calculation assumed that all dietary protein (crude protein)

leaves the rumen as either microbial protein undigested feed (Gihad and Elbadawy, 1980). The rate of extent degradation of dietary protein in rumen affected by outflow of small particles from the rumen has been shown to result in a significant decrease of dry matter (D.M) and crude portion (C.P) degradability (Qrskov, 1983).

2.12 Types of protein:

The different types of protein can be given as follows according to (Mohammed, 2008).

2.12.1 Fibrous protein:

Are insoluble animal proteins which are resistant to this group contain collagens (the main protein of connective tissue s).

2.12.2 Globular protein:

This includes all enzymes, antigens and hormones. It divided to albumins, which are water soluble and heat congealable they found in milk, eggs, blood and many plants, globulins, lacto globulin, limestone which are basic protein in cell. They are also associated with Deoxyribonucleic acid (DNA).

2.12.3 Conjugated protein:

Such as glycoprotein, which is conjugated proteins with one or more, they are components of the mucous secretions, which act as lubricants in many parts of body.

With lipid such as lecithin and cholesterol chromo- protein contain pigment like hemoglobin, Cytochrome and flavor- proteins Neuclo- protein are high molecular weight compounds.

2.12.4 Crude protein (CP):

Crude protein measures total nitrogen requirement by the Ruminates
The fodder nitrogen present as protein and the method used to measure crude protein (C.P).

2.12.5 Soluble protein (SCP):

Is the crude protein which is soluble in buffer solution, water or a rumen fluid, chemically as protein soluble in borate phosphate buffer (pH 0.9). Soluble Crude protein (SCP) contains all the non-protein nitrogen and some true protein. It is degraded by rumen microbes rapidly.

2.12.6 Non-protein nitrogen (NPN):

It is nitrogenous compounds, which do not have the complex structure of the true protein. It includes ammonia, small peptides, and free amino acids; (NPN) is rapidly converted to ammonia in the rumen. (Ikhmioyal *et al*, 2005).

2.12.7 Ruminant undegradable protein (RUP):

It is the dietary protein, which is not degraded by rumen microbes or it is dietary protein that escaping protein from microbial attack in the rumen. RUP is estimated by the *in vivo*, *in vitro* and *in situ* methods. The *in situ* method is the common method to estimate RUP. (Ikhmioyal *et al*, 2005).

2.12.8 Ruminant microbial protein (RMP):

It refers to the protein of protein synthesized by rumen microbes. Rumen microbe's utilization ammonia and peptides to synthesize microbial protein 80% of the microbial protein are digestible by animal. (Ikhmioyal *et al*, 2005).

2.12.9 Metabolizable protein (MP):

(MP) it is the net quantity of true protein (microbial protein) or amino acids (feed protein) absorbed in small intestine.

2.12.10 Protein digestion in small intestine:

Digestion of protein in the abomasums and small intestine in ruminants is similar to that in monogastric animals, so the digestion of protein in abomasums is carried out mainly by pepsin in very acidic environment about pH Optimal activity of trypsin, chymotrypsin and carboxypeptidase occur in middle jejunum, peak activity of exo-peptidases occurs in mid ileum (Mc Donald, *et al.*, 2002a).

2.12.11 Degradation of cell wall by microorganisms:

The microorganisms, to avoid carry by digestion flow; they need to adhere to the particulate matter. The microorganisms use cell wall polysaccharides existing in food particles that enter the rumen.

The fact, the epicuticular waxes and cuticle, which prevent the rumen micro flora from attaching to its epicuticular, may protect wall or the surface of particles, entering the rumen.

Digestion in the ruminant depending on the activities of microorganisms, energy (ATP), nitrogen (amino acids, peptides and ammonia), minerals and medium p^H , range of 6 – 6.5 (Chalmers, 1960).

2.13. Volatile fatty acid (VFA):

Volatile fatty acid is (acetic acid, propionic acid, and butyric acid) depending on the rumen contents pH, diet kind and time intervals between feeding and sample taken (Shriver *et al*, 1986).

Mendoza (1993) proved interdependency of certain VFA level and protozoa number; he observed significantly lower levels of butyric, Iso valeric, acid and valeric acids in sheep.

2.13.1 Dry matter degradability:

Although water is useful substance to the animal, so is the presence in the feed acts as diluents. Therefore, most feeds are analyzed and the data presented on dry matter basis, with information shown on the moisture content after drying in microwave ovens or in an ordinary oven, using temperatures of 60 C° to 105C°, which require 24 hours, or more depending on the nature of the product. Variation in the nutrient quality was reported to be as result of the genetic diversity and crop age at time of harvest (Abdelmomein *et al*; 1990).

2.13.2 Rumen ammonia:

Ammonia (NH₃) is the major end product of protein, peptide, amino acids (Wallace, 1996), amides (Abu Akkada and Blackburn, 1963), urea (Bendall, 2001), nitrates and some other non-protein nitrogen (NPN) compounds.

Ammonia exists as free (NH_3) at high p^{H} but as the ammonia ion (NH_4) at the lower p^{H} , because these tissue membranes are permeable to lipid soluble NH_3 form but impermeable to the charged NH_4 form, more ammonia is absorbed at high p^{H} than at low pH (Hogan, 1961; Bloomfield *et al*, 1963). This significance of this is that a large NH_3 pool can be maintained in the rumen for microbial protein synthesis.

Ammonia is an essential nutrient in growth of bacterial protein (Moon *et al*, 2010). Many species of rumen microorganisms use NH_3 as the nitrogen sources for synthesis of their nitrogenous constituents (Khan *et al*, 2011).

Ammonia assimilation by rumen microbes depends on rumen p^{H} (Veth and Kolver, 2001) and rumen ammonia concentration (Mehrez *et al*, 1977). An increased NH_3 concentration is accompanied by increased degradable nitrogen losses in the urine (Oldham, 1983). Ruminant NH_3 production is cyclic and reaches a maximum 1 to 2 hours after feeding (Leibholz, 1969, Agle *et al* 2010).

The NH_3 absorbed from digestive tract is very efficiently converted by the urea and should therefore give a response in the concentration of the blood urea (Ciszuk, 1973). A relation between rumen NH_3 and blood urea, after administration of the various proteins, was first established by Lewis (1951) and confirmed by the other (Tagriet *et al*, 1964); Abu Akkada and El Shazly, 1965). The rumen NH_3 often varies widely throughout the day depending on the feeding regime and feed quality.

This variation appears to be partly reflected in blood urea content but variation delays and with less marked values, i.e. the blood urea concentration reflected only the high peaks in rumen NH_3 (Ciszuk, 1973).

2.13.3 Rumen pH:

Rumen pH is a good indicator for rumen health and function. The normal rumen pH has been considered to be 5.5 – 5.6 in sheep (Owens *et al*, 1998). Rumen pH varies in regular manner depending on the nature of diet and

on the time that it is measured after ingestion (Orskov, 1986 Owens *et al*, 1998). Under physiochemical circumstances, the primary indication of an animal suffering from acidosis is a reduced rumen pH as the consequent result of rapid fermentation of carbohydrates (Krehbel *et al.*, 1995; Nocek, 1997; Lower values of rumen pH impair microbial function. In particularly protein synthesis process (Krajcarski *et al.*, 2002). Furthermore low rumen pH is accompanied by reduced fiber digestibility in beef cattle (Koenig *et al.*, 2003). Moreover, a reduction in feed particle size tends to cause a reduction in pH (Cheng and Kironak, 1973). On the other hand, rumen pH decreases following a meal and rises again by the next feeding (Bevans *et al.*, 2005; Rotger *et al* 2006).

2.13.4 Rumen Ecology (RE):

The ideal environment maintaining stable microbial population in the rumen of the adult ruminant animals, is the warm (39 - 42°C) anaerobic, chemical reducing environment, rich in organic matter, pH of rumen content is approximately 5.5 – 6.5.

2.14 Protein Degradability:

Evaluation the quality of protein presented to the small intestine for absorption is the sum of the microbial cell protein and feed protein that has escaped or by-pass ruminal digestion (Garry, 2005). In the other study, rumen degradation is the variable and its value depends on the competition of unfermented feed passing to the Omasum and abomasums (Van Soest, 1993). Rumen degradation of proteins depends of the nature of feed. Food proteins are hydrolyzed by the rumen micro-organism in to peptides and amino acids or ammonia, volatile fatty acids, carbon dioxide and other materials.

Rumen degradation is variable; its value depends on the competition between rates of digestion and passage. This competition potentially digestible substrate in the proportion of unfermented feed passing to the Omasum and abomasums (Van Soest *et al*, 1982). There are many systems to measure protein bypass or escape in the rumen. These include measurement of solubility in various

solvent, loss of protein or accumulation of ammonia or amino acid in vitro, and loss of protein upon incubation with various proteolytic enzymes.

In vivo measurement and animal response are the only applicable method, which directly determine the proportion of the by – pass. Solubility plus the in situ measurement may correlate with by pass. Values when adjusted by comparison with same reference protein (Zinn and Owens, 1986). Degradation of protein and amino acid is by bacterial (usually surface), protease and peptidase (Blachbum 1968. Perston and Leng then degraded (Nolan and Leng, 1983).

Rumen degradation of protein from dietary feed ingredient is the one of the most important factors influencing intestinal amino acids supply to the ruminates. Proteolysis determines the availability of ammonia nitrogen, amino acids, and branched – chain volatile fatty acids, which influence microbial growth rates in the rumen synthesis but also the quality and quantity of undegraded dietary protein that reach the duodenum The rate of extent of degradation of dietary protein in the rumen is affected by the outflow of small particles from the rumen. Increasing the outflow rate of the small particles from the rumen has been showing to result in a significant decrease of dry matter DM and crude protein CP degradation of protein supplements (Orskov, 1983). Effective DM and CP degradability of Soybean and fish meals at 0.05 and 0.08 per hour outflow rate were similar for Chios sheep and Damascus goats (Hadjipnayiotou, 1985).

2.14.1 Protein Deficiency:

In feral ruminant species the diet has surplus protein at times, particularly during lush growth in the spring (or raining season), it is adequate for some part of the year, and borderline to deficient during winter month or dry season. Probably less fluctuation occurs with domestic stock, but it is a well-documented fact that protein may often be deficient in many situations, although the deficiency may often be complicated with deficient of other

nutrients such as some of the minerals elements and available energy. One of the first effects of protein deficiency is a reduced feed consumption by ruminant animals. Studies with the sheep show that low protein diets will result in reduced wool production (Egan, and Kellawy 1971). An interaction between protein and energy levels has also been shown by Walker and Norton *et al.*, (1970).

2.14.2 The gas production (GP):

Many attempts to develop rapid, simple reliable and cheap *in vitro* methods which can be used to screen a large number of raw materials and predict their digestibility and metabolism energy contents. Menke *et al*, (1979) developed the *in vitro* gas production technique to evaluate the nutritive values of feedstuff. *In vitro* gas production is a method that detects a small different a feedstuff and allowed more frequent sampling than *in vitro* digestibility (DePeter *et al*, 2003). The gas production during fermentation can be used as indicator measure of dry matter digestibility. The gas production in 24h from incubation of 200mg food dry matter can be used together with the concentration of crude protein and ash to estimate metabolizable energy and organic matter digestibility (Menke *et al*, 1979). Gas production is associated with volatile fatty acid production following fermentation of substrate so the more fermentation of substrate the grater the gas production (Blummel and Orskov, 1993).

2.14.3 Digestion of ruminal microbial protein (RMP):

It is refer to portion of protein synthesized by the rumen microbes. Rumen microbes utilize ammonia amino acid and peptides to synthesize microbial protein. 80 % of microbial protein is digestible by the animal. (Ikhimioyal *et al*, 2005).

In the other hand reported by (Abdelrahman, 2011) found a new method for estimate the RUP by *Insilco* technique and determine protein stability according to the molecular weight and amino acids concentration.

2.14.4 Non – Protein nitrogen (NPN) utilization:

It is nitrogenous compounds, which do not have complex structure of a true protein. It includes ammonia, small peptide, free amino acids, amines and amides. NPN is rapidly converted to ammonia in the rumen. (Ikhimioyal *et al*, 2005).

Urea is readily hydrolyzed in the rumen and the ammonia may be used for microbial protein synthesis or it may be absorbed across the rumen wall and may be re-synthesized into urea and excreted in urine. Some authors have suggested that if the rate of hydrolysis were slower or if urea was ingested at periodic intervals, efficiency of N utilization would be increased. However, no differences were observed in N balance or protein N between sheep infused with equal amounts of urea twice a day or continuously (Streeter *et al*, (1973). reported a greater N retention in lambs in which urea was infused during nine or 3 hours period following each of two daily feeding compared to continuous infusion. Acetohydroxamic acid was observed by (Moore *et al*, 1968, Streeter *et al*1973). Research with older dairy cattle showed that urea is utilized but the response, compared to conventional protein supplement, was variable. Age of animals fed urea may be a factor as a result cattle older than 6 months achieved daily gain of one kg on corn silage and urea, while younger calves should need Alfalfa to support a high rate of gain (Thomas *et al.*, 1975).

Response to feeding urea to cattle fed high roughage rations has not been consistent. Supplementing mature grass (6% CP) with molasses- urea improved daily gain (Chiou and Jordan, 1973).

Apparently, urea- molasses supplements are beneficial to digestibility of low protein roughage (White *et al*, 1973). Increase in the crude fiber and energy digestibility when urea was added at level of 1-3 to rations composed of rice straw and molasses. Supplementing protein hay with 3% urea and molasses increased dry mater intake by 28 and 65% as compared to hay alone (Ernst *et al*, 1975).

Oltjen *et al.*, (1963) obtained similar performance from lambs supplemented with urea or soybean meal while Amos *et al.*, (1970) found that corn gluten meal improved gain in lambs as compared to urea. With high straw rations, performance tends to be less with urea than soybean protein (Bhattacharya and Khan, 1973). In beef cattle nutrition there is trend toward using greater amount of urea. However pointed out by Owens *et al.*, (1973). It appears that some amino acids may be limiting for growth in certain diets. Methionine and soybean meal as supplement. Sulfur containing amino acids were the most limiting for animals fed urea containing diets.

There have been a number of growth studies in which virtually the entire dietary N was supplied. When NPN completely replaces protein in purified diets, rate of growth and feed efficiency are generally lower than when soybean protein is used (Oltjen, *et al.*, 1962); Tillman and Sidhu, (1969) reported data showing that feed depression with high urea level was due to a physiological effect. Depression in feed consumption occurred when urea level exceeded 2% of the ration but feed consumption was depressed more urea was administered directly into the rumen.

2.14.5 Protein–Energy relationships:

Black and Griffiths, (1975) observed that N balance was independent of energy intake when N intake was less than the amount required. Rather, it was linearly related to absorbed N and metabolic weight. When N intake was in excess of the amount required, N balance increased linearly with ME intake at a rate that decreased with increasing live weight. N requirement / M.J decreased with increased body weight. In calf efficiency of transformation of dietary protein to tissue protein increases with increased energy concentration (Jahn *et al.*, 1976). In pregnant ewes retention increased as energy although apparent digestibility of N was not affected (Guda *et al.*, 1975). In non – lactation animals as increase in energy intake normally leads to the improvement in N balance. In

the lactating cow the daily milk production and utilization of digestible N for milk production are closely related to ME intake (Brater *et al*, 1969).

Digestibility of both crude protein and energy were depressed in the low protein diet. NE was lower for low protein diet, but efficiency of energy utilization on the low protein diet was primarily caused by lower digestibility (More and Tyrrel, 1977).

2.15 Protein:

Proteins are complex organic compounds of high molecular weight, they contain carbon, hydrogen, nitrogen and sulphuric addition some protein contains phosphorus, iron, zinc and copper. Proteins are found in all living cells and each species has its own specific protein and single organism has many different proteins (Mc Donald, 1981).

Proteins are made up of amino acids, which are produced when protein are hydrolyzed by enzymes, acids or alkalis, amino acids are characterized by having a basic of nitrogenous group an amino group-NH₂ and acidic carboxyl group COOH. The general formula of amino acid R-C-H. Protein are built up from amino acids by linkage between the α carboxyl of amino acid and α amino group of another acid, this linkage is known as the peptide linkage proteins are classified into three main group according to their shape, solubility and chemical composition, the first one is fibrous protein, b- Globular protein, c- conjugated protein (Mohammed, *et al* 2002).

Some older reports in the literature have demonstrated marked differences in animal gain or nitrogen retention when relatively pure sources of protein have been fed to ruminants (Oltjen *et al.*, 1962). Of the major factor affecting protein digestibility is the amount of protein consumed per day; this is illustrated in the work by (Egan and Kellaway, (1971). The results of this study on apparent protein digestibility showed that there is a good linear response between nitrogen intake and apparent digestibility. One of the reasons is that metabolic

fecal nitrogen represents a smaller amount of the nitrogen excreted in feces as dietary consumption increase. In addition soluble and digestible protein in a given plant material generally increases as nitrogen content increases. Relationship between percentage of the ingested nitrogen intake and urine nitrogen (Egan and Kellaway, 1971) showed that urine nitrogen also increases as intake increases. At low levels of nitrogen intake the urinary nitrogen out is high because of endogenous nitrogen components in urine, at high levels of intake, nitrogen is utilized at lower efficiency. Similar data on the effect of amount of nitrogen consumed on efficiency of utilization when growing calves were fed complete ration of soybean meals was illustrated by (Neville *et al.*, 1977). Not that digestibility of protein increased with increasing consumption. Urinary nitrogen production, as a percentage of intake is strongly dependent upon level of nitrogen intake e.g. at high levels of intake, utilized with lower efficiency and urinary nitrogen output as a percentage of nitrogen intake is at a minimum (Egan and Kellaway, 1971). Similar data on the amount of nitrogen consumed on efficiency of nitrogen consumed on efficiency of utilization was recorded by Neville *et al.*, (1977) when growing calves were fed complete rations in which majority of the added protein was supplied by Cottonseeds, Sunflower, and Groundnuts cakes.

2.15.1 Oilseed cakes:

2.15.2 Dietary protein sources:

Oilseeds such as Cottonseeds, Soybean, Sesame, Sunflower, and Groundnuts, are annual plants (O, Brien *et al*, 2002). There are the largest source of vegetable oil even through most oil –bearing tree fruits provides the highest oil yield e.g. Olive, Coconut and plant tree (Gunston, 2002).

Oilseeds cakes (20.46) % are also used in animal nutrition. Because of their high protein content, their seeds contain energy for the sprouting embryo mainly as oil, compared with cereal, which contains the energy inform of starch (Lucas, 2000). Most oilseeds residues are of tropical origin, they include Groundnut,

Cottonseed, Sesame and Sunflower seed cakes, some seeds like castor not suitable for animal nutrition, because it source of fiber, phosphorus, magnesium, vitamins E, niacin and foliate, also contain phytoestrogenics (Goldberg, 2003). The husk of groundnut cake, cottonseed and sunflower seed are rich in fiber, have a low digestibility in processing, decorticated removal by cracking and ridding decrease crude fiber and raise their nutritive value (Mc Donald, *et al*, 2002).

2.15.3 Groundnuts cake (*Arachis hypogea*):

Groundnut and sesame cake have been widely used in poultry ration because they are less fibrous, and contain higher crude protein than cotton seed cake. Groundnut cakes have been widely used in the ruminant and poultry ration because they are less fibrous and contain higher crude protein than cotton seed cakes, Pollott *et al.*, (1978). Observed that male and female of Sudan desert lambs fed on concentrate ration containing 40% groundnut cakes achieved growth rates of 150 and 106 mg/day respectively. In the other study the chemicals treatments of groundnuts cake decreased rate of ruminal degradation of both dry matter and crude protein as well effective degradability. (Lynch *et al*, 1987). Found the best protein protection upon using 70% ethanol for SBM. Treatment with the spraying by 0.5N NaOH or 0.5 HCL exhibited the lowest protein protection compared with the other treatment procedure. This result agrees with the result of the (Waltz and Loerch, 1986) who found that spraying with 2.5 or 5% acetic acid or propionic acid reduced the rate of nitrogen disappearance at low rate than other treatment (Mohammed, (2008) studied the effect of chemicals and physical treatment on degradation characteristics of groundnuts (*Arachis hypogea*). She treated it with 0.3% formaldehyde (smoking and air drying). Results showed that the treatment was effective in protecting GNC protein from rumen degradability at all intervals of Ruminal incubation and in reducing effective degradability. (Abdelrahman, 2011) was evaluate the characterization of some Sudanese oilseed cakes and using

Software technique and reported that Cottonseed cake are stable protein, while Groundnut cake are unstable protein in this analysis using bioinformatics method analyzed data base by using (Protparam program tools) to determine molecular weight and amino acids concentration. Also protein structure collecting organizing DNA and protein sequence, structure and function. Protparam program tools which allow computation of various physical and chemical parameters for given protein stored in the Swiss- port, Or for use entered sequence. The computed parameter includes molecular weight, amino acids composition, and extinction coefficient estimated half-life and instability index. (WWW. NCBI. Protparam program 2010).

The results of bioinformatics analysis for GNC, CSC and SFC. Are recorded (23834-207), (17306.5-159) and (16135.5-141) of GNC, CSC and respectively SFC for molecular weight and amino acids concentration respectively. The result indicated that the GNC include high level in (Alanine, Glutamine, Lysine, and Valine). And low in (Sistine, Histamine, Arginine and Tryptophan). CSC recorded medium molecular weight and amino acids concentration high in (Arginine, Glycine, and leuthine) and low in (Tryptophan, Methionine, lysine and Threonine). While the sunflower cake high in (Valine, Glycine, and low in Tyrosine, and Alanine. But sunflower cake is differ than cottonseed cake and Groundnut cake because has absent of amino acids Tryptophan. The result of software technique classified Groundnut cake as unstable protein while Cottonseed cake and sunflower cake classified as stable protein according to their molecular weight and amino acids concentration (Abdelrahman, 2011).

2.15.4 Sunflower cake (*Heliuthus annuus*):

Sunflower meal is processed by the hydraulic pressure or solvent extraction, it content a high fiber up to 420(g\ kg Dm). When it processed by hydraulic pressure, but solvent extracted meals contain 220g crud fiber and 430g crud protein (per kg\ Dm). sunflower meals has a short shelf life because it has high

contain of polyunsaturated fatty acid which leads to rancidity development, sunflower meal are useful source of protein (McDonald *et al.*, (2002a). El-Wasabi, (1994) evaluated sunflower seeds cake comparison to cotton seeds cake in ration for sheep and revealed no differences in live weight gain, carcass characteristics and yield of wholesale cuts. El Khidir *et al.*, (1995) and El Wasabi (1994) have concluded that Balanites kernel cake and sunflower cake were equal to cotton seeds cake as a protein source in ration for sheep.

2.15.5 Cottonseed cakes (*Gossypium barbadense*):

Cotton seed cakes have been widely used in fattening rations as a protein source. It supplies the protein need of the animals; improve the average daily gain and possibly the feed conversion. The magnitude of improvement in these parameter depend on the amount of cotton seed cakes use in the ration and the length of the period use by investigators Hassan and Mukhtar, (1970) reported an average daily gain of 150 gm and a feed conversion ratio of 7.26 when Sudan desert sheep were fed diets, which contained cotton seed cake as a sole sources of protein. Other investigators reported an average daily gain of 237 gm/ day and a feed conversion ratio of 5.4% when Gezira weaned lambs were fed a ration containing 25% cotton seed cake (Suleiman,1999). In another experiment, Osman, (1985) demonstrated that feeding a ration containing 35 Cotton seed cakes to crossbreed sheep resulted in an average daily gain of 170 gm / day. However, 38% dietary Cotton seed cake inclusion in fattening ration of desert lambs (*Shugor* male lambs), resulted in 162.6gm daily live weight gain, 1.07 Kg daily feed intake and 6.71 Kg feed / Kg gain (Ahmed and Suleiman, 1988). Balanites Kernel cake (20%) was compared with Cotton seed cake (30%) in feeding two groups of lambs that were slaughtered at about 40 Kg live weight (El Khidir *et al.*, 1983). No significant differences were detected between two groups of lambs in feed lot performance and carcass characteristics. El- Khidir *et al.* (1983) reported that feeding lambs on 30% Cotton seed cake dietary inclusion resulted in 192 gm average daily gain.

However, El Khidir *et al* , (1988) investigated that post weaning performance of lambs fed a diet containing 10% Cotton seed cake inclusion, and reported slaughter weight of 40 kg and daily live weight gain of 157gm. This difference of performance was attributed to difference in ration composition, age and breed. Cotton seeds contribute 4% to the world vegetable oil production but its production is linked to the demand for cotton fiber (Bruisma, 2003). It has a good quality protein but has a low content of the Cystein, Methionine and lysine. Cotton seeds contain from 0.3 to 20 (g\ kg D) of gossypol (yellow pigment), which is a poly phenolic aldehyde is an antioxidant and polymerization inhibitor; it is toxic to simple stomached animals.

2.16 Utilization of other oil seeds in sheep production:

In Sudan the by-product of vegetable oil industry such as the cakes of Cottonseed cake, Sunflower cake, Groundnut cake and Sesame cakes represent valuable source of nutrients to farm animal. These cakes are rich in protein, which amount to about 26% in cotton seed cakes, 39.8% in groundnut cakes and 43.8% in Sesame cakes (NRC, 1984).

2.16.1 Utilization of Roselle seeds:

2.16.2 Utilization of roselle seed meal in poultry rations:

Nutrient content of roselle seed meal were comparable to those of groundnut meal (Mohammed and Idris, 1991). Both contained reasonable levels of crude proteins, high enough to allow the inclusion of these meals in broiler diets as protein sources. Also the same authors have attributed the poor performance of chicks fed on roselle quality of the meals incorporated in the diets. However broiler receiving roselle seed and groundnut meals diets gained at equal rate in spite of the depressed feed intake observed in the former diet. The reduced feed intake had been associated with roselle seed meal acid taste.

Mohammed and Idris, (1991) reported that roselle seed cake could be used up to 30% of the diet without exhibiting any deleterious effect on broiler performance. However Salih and Abdel Wahab, (1990) provided evidence that roselle seed meal can be replace groundnut and sesame meals without significant reduction in weight gain, feed intake, feed conversion ratio (FCR), mortality and dressing percentage of broilers.

Eltoum, (1992) concluded that feeding graded levels of roselle seed (0,7.5, 15 and 22.5%) to broiler chicks showed a liner reduction in feed intake whereas mortality rate, body weight at four weeks of age, weight gain and ratio were not significantly affected by the level of seed tested as full-fat untreated seed. In another experiment, he observed that soaking Roselle seed for 24 hours had no significant effect on body weight at four weeks of age, weight gain and feed intake but it reduced mortality rate. In the other hand, crushing and socking of Roselle seed for 24 hours reduced body weight at four weeks of age, weight gain and feed intake and it had no effect on effect mortality rate. The author suggested that untreated Roselle seed could be included up to the level 22.5% (Bakheit, 1993) revealed a liner increase in feed intake when fed at the levels 5,10,15, or 20% on layer performance.

The finding of Bakheit, (1993) also revealed a liner increase in rate of lay (hen – day), and body weight, which he attributed to an increase in feed consumption. He concluded that the increase in rate of lay might indicate that the protein quality of Roselle seed meal supported satisfactory laying rate. The author also reported that Roselle seed meal to levels tested has no detectable effect on feed conversion ratio (FCR), mortality rate, shape index, albumen height and color, thus he concluded that Roselle meal had no deleterious effect on layers livability.

2.16.3 Utilization of Roselle seed meal in lambs rations:

Beshir, (1996) conducted a study to evaluate the nutritional value of Roselle seeds and its use in feeding lambs. She used three graded levels of seeds (0, 10, and 20%) being added to Iso-nitrogenous Iso-caloric rations. Her resulted showed that there is a significant liner increase in feed intake with the increase seed level in the diet. She, however, concluded that the protein and energy values of roselle seeds were enough to allow its inclusion as protein and energy source on ruminant diet.

2.17 Agronomical aspect of Roselle seed:

2.17.1 Origin, botanical description and ecology:

Sudan is considered as being a rich country in forage. The available total green forages to animals in Sudan are about 3.78 million ton. It includes Sorghum, Wheat, Cotton, Sunflower, Groundnut, Alfalfa and forage Sorghum (M.A.R 2001- 2002).

Roselle seed (*Hibiscus sabdariffa* L.) known in the different countries by common names include roselle, razelle, red sorelle, Jamaican sorelle Indian sorelle, Guinea sorelle, Queensland jelly, (Mahadeven and Shavali, 2009). In English speaking countries is known as rosella, natal roselle and rosella, (Kays, 2011).

Roselle (*Hibiscus sabdariffa* L.) Known in Sudan, as Karkadeh an important cash crop and a source of income for small farmers in western Sudan especially in North Kordufan state. Roselle is a bush – like annual crop; grow up to two meters high, its cultivated for the dark red calyx surrounding the fruit. The plant is tolerant to many types of soil and need 2-6 months to harvest (Gohl, 1981).

The crop is growing mainly by traditional farming methods, exclusively under rain fed condition (El Naim *et al*, 2010). Rosella is important annual cash crop, which growing successfully in the tropic and sub- tropics. The plant belongs to

the general order Malvels, family Malvacea and tribe *Hibiscuses*. The species name, *Sabdariffa* is of Turkish origin (Mc lean, 1973). The world best Roselle come from Sudan, but the quality is low, and poor processing hampers quality. Mexico, Egypt, Senegal, Tanzania, Mali and Jamaica are also important but production is mostly used domestically. (Mohamed *et al*, 2002).

2.17.2 Cultivation of Roselle seed (*Hibiscus sabdariffa*):

The best condition of its growth is considerably low temperature and short day length with rainfall 18-20 inches spread over 3 to 4 months Copley (1975) reported that the plant flowers best, in eleven –hour days. The crop has an overall growing during period of 2-6 months (Mc lean, 1973). Soil preparation should be deep, (about 20 cm).

The seed rate is 11-22 kg / hectare depending upon the soil .weeding during the first month is important, probably native from India to Malaysia, Roselle was introduce to the other parts of the world such as west Indies, central American and Africa (Purseglove, 1968; Morton, 1987) where it best grown in tropical and sub- tropical regions. The plant tolerates warmer and more humid climate with night time temperature not below 21°C, it require 13 hours of the sun light during the first months of growth. (Duke, 1983). Roselle is a versatile plant similar to the coconut tree (Quezone, 2005). Roselle can be found in almost all warm countries such as India, Saudi Arabia, and Malaysia Indonesia, Thailand, Philippines, Vietnam, Egypt and Mexico Quezone, (2005). Origin of Roselle is uncertain; while others believe that it home country is India, (Mat, *et al*,1985) and Saudi Arabia (Abu Tarboush and Ahmed, 1997). A part from nutritional and health importance, Roselle play an important role in income generation and subsistence among rural farmers in developing countries (Ahmed *et al*, 1993). The crop is cultivated for its leaves, seeds, and calyces used as vegetables Wange *et al*, (2000). And for medicinal and health purposes

(D, Heureux and Badrie, 2004). Nutritionally young leaves of *Hibiscus sabdariffa* contain nutrients such as phosphorus, calcium, magnesium, and potassium (Atta *et al*, 2002). The calyces of Roselle are utilized in producing drinks, jellies, sauces, chutney, wines, preservative, calyces drink which has received industrial attention internationally. It is the readily available and inexpensive source of vitamin C. Roselle is also considered to be one of the most famous folk medicinal plants due to its colored calyces which are used for pharmaceutical and cosmetics industries (Ibrahim and Hussein, 2006).

2.17.3 Uses of Roselle and economic importance of Roselle seed (*Hibiscus sabdariffa*):

The economic importance of roselle lies in many uses of its different plant parts, the stem bark that is used as fiber source is suitable for sacking and can substitute jute in Nigeria (Barker, 1970). In Angola the leaves are used as emollient where their acidity makes them good anti scorbatic and as a soothing cough remedy (Watt and Bryer, 1962). The red calyx is boiled with sugar to make jams (Khider, 1997). (Mat and Abdaziz, 1985); Emmy, (2008). Dried roselle is used for making tea, jelly marmalade, Ice – cream, sherbatets and other desserts. Calyx is used in western India to color and flavor rum. In Sudan roselle seeds are used as animal feed particularly as supplement for depleted and emaciated animal during the dry season. Recently in Sudan, the crop is used in the food industry for other purposes, e.g jams, and jellies as well as coloring material for other foods (Hassan, 2005). Roselle Cakes used for the preparation of an herbal drink, cold and warm beverages and jellies (Rao, 1996; Abu Tarbush *et al*, 1997, Tasi, 1996). Calyces are also used in West India as color and flavor ingredient in rum. Food applications, the flower fleshy fruit are used in pharmaceutical industry to relieve symptoms of bronchitis; cough, diarrhoea, ceylon mouth and many other diseases Faraji and Tarkhani, 1999). Furthermore other studies have reported that it is good in reducing hypertension

rate. (Adegunloy *et al*, 1996; Onyenekwe *et al*, 1999). Calyces from the Roselle flower are processed to produce juice and vitamin B1 vitamin B2 and vitamin B complex.

2.17.4 Roselle productions in Sudan:

Sudan is the world largest exporter of Karkadeh, Sudan exports of calyx have raised from 454 tons in 2002 (FAO, 2002). The area under production varies from about 4167 to 12500 hectares (FAO, 1995) average calyx yield is estimated to be about 95- 120Lb / .04 hectares.

2.17.5 Raw materials and additives used and their handling

Roselle seeds (*Hibiscus sabdariffa*) are cultivated mainly for its calyx. The traditional processing activities of the calyx are for the production and particularly of drinks / beverages. The drink is made from an extract obtained by aqueous extraction from a solid – solvent ratio. The extraction operation is carried out at temperature between 25 and 100c. After filtration, sugar and other ingredients. Such as other fruit juices, flavor ants and fruit pieces

(Pineapple, strawberry and ginger) may be added. The conception of this drink is widespread in Africa and Asia in Senegal, where it is very popular its conception is highest during the month of Ramadan. In Mali and Burkina Faso, the drink is called "da Bilenni" in Egypt it is known as the drink of the pharaohs. In Sudan the name is " tee Karkadeh" this beverage is consumed cold or hot depending on the season. (Cisse *et al*, 2009). The description and processing methods in Sudan fully developed fleshed (calyx). Is peeled off from the fruit by hand and dried naturally under shade to give the dry (calyx), which is consumable product. The plant is 0.5 to 2 meters in high. The color of calyx plays an important role in determining the quality of the crop. (El Naim and Ahmed, 2010).

2.17.6 Roselle seed chemical composition:

Roselle seed are a secondary product of the crop (*Hibiscus sabdariffa*) and it is rich sources of oil and protein. A search in the literature has shown that there is a good potential of seed as available source of addible oil for human conception and protein supplement for animals.

Mukhtar, (2007) reported that roselle seed contained 8.2% moisture, 91.8% dry matter, 17.43% ether extract, 21.35% crude protein 11.98% crude fiber , 5.34% ash, 43.9% carbohydrates, 388mg/100 calcium, 503mg/100g phosphors and 12.04MJ/Kg calculated metabolizable energy (ME).

AL Wandawi *et al*, (1984) reported that roselle contained 16.3% crude fiber, 56.64% total carbohydrates and 5.19% ash content. EL Nour, (1991) reported that roselle seed conception of AL Rahad variety was 4.73% moisture, 5.44% ash, 13.71% fat and 13.67% crude fiber.

Recently AL- Adawy and Khalil, (1994) reported that roselle seed contained 2.05% - 2.60 % non-protein nitrogen, 5.80% – 6. 89% total ash, 21.60% - 23.26% total lipids, 1.23% - 4.12% crude fiber, 35.12% - 36.37% total carbohydrates and 9.25% - 11.66% moisture.

Ahmed and Nour (1993) consider the protein of roselle seeds as a good source of sulphur containing amino acid (3.63g/16 gN) with Threonine being first limiting amino acid. Roselle seed protein contained a good amount of lysine (3.84g/16gN) half of which was present in available from and the protein efficiency ratio was 1.63. Recently EL Adway and Khalil, (1994) evaluated the chemical of Egyptian roselle seed cultivars and found that the whole seed contained 21.02 – 30.11% protein.

They also found Glutamic, aspartic and Arginine acids were major amino acids and values of 21.30 - 2178% - 10.91% and 10.13 – 10.55% of total amino acids respectively. The limiting amino acids were found to be Valine, Isoleucine, and

tryptophan while total sulphur – containing amino acids were not limiting. They concluded that the lysine content of roselle seed ranged from 5.37 to 5.56 g/16g N similar to that of FAO reference protein so it could be used as a supplement food mixture for poor lysine source.

2.17.7 Roselle seed minerals:

AL- Wandawi *et al*, (1984) reported that the element K, Na, Mg and Ca were the major mineral element in the roselle seeds. While Fe, Zn, Se and Ni were present in abundant amounts in relation to quantities needed in the diet. They conclude that the mineral composition (*Hibiscus sabdariffa*) seed as mg/100 g on dry weigh basis were copper < 1, Rubidium <1, chromium 1, strontium 1.6, Nickel 3.5, zinc 6.8, Iron 8.2, Magnesium 580, calcium 300, Na 740, and K1600. The values reported by EL- Adawy and Khalil, (1994) for the elements Ca, Mg, Mn, Cu and Fe were in good agreement with those reported by AL- Wandawi *et al*, (1984) while the values for Na (590 – 680) and K (1300 – 1390) were slightly higher.

Gohl, (1981) found that roselle leaves in India were rich in Ca (1.31%) and potassium (0.28%).

2:17.8 Roselle seed phytochemical compound and anti-nutritional factors:

Despite the rich nutritional composition of roselle seed, there are reports of the presences of a number of anti-nutritional (toxic) factors. However, there has been conflicting result as per the presence concentration of these factors probably due to varietal differences. The most commonly reported toxic factor in roselle seed are total phenols and tannins (Abu EL Gasim *et al.*, 2008; Kwari *et al*, 2011) and Phytic acid Kwari *et al*, (2011). Glycosides such as delphinidin-3monoglucosides and delphinidin (Morton, 1987; Ojkoh *et al*, 2006) and cyanogenic glycosides (Aletor, 1993) have also been reported in roselle seed. Recently, Mukhtar, (2007) reported that roselle contains traces of gossypol, a

phenolic compound which causes undesirable physiological effect in poultry. Abu- Tarbush and Ahmed, 1996, Abu-Tarbush *et al*, (1997) and Hansawasdi and Kawaba, (2000) reported low levels of tannin, amylase inhibitors, protease inhibitors, Phytic acid and gossypol in roselle seed. There are few reports on the processing of roselle seed with the view to reducing the toxic factors and improve its feeding value.

Abu EL Gasim *et al*, (2008) reported a significant reduction of phenolic compounds in the soaked, cooked or sprouted sorrel seed compared to the raw, but the Phytic acid content was not affected by processing. In a similar study, Kwari *et al*, (2011) observed significant reduction of the total phenolic and condensed tannin of the seed by the soaking, sprouting or fermentation. These processing methods, however, had both beneficial and adverse changes in the profile of the certain nutrients. In their study, Abu EL Gasim *et al*, (2008) observed that the reduction of Phenolic by the soaking, cooking or sprouting were accompanied by significant increase in protein, fat and crude fiber contents while the ash and soluble carbohydrate contents were reduced. The authors also reported an increase in the sulphur amino acids (Methionine, Cysteine and Threonine) and decrease in lysine contents of the soaked compared to the raw seed. In contrast, Kwari *et al*, (2011) observed that soaking reduced the methionine but had no adverse effect on the lysine contents of roselle seed. Abu EL Gasim *et al*, (2008) soaked the seed in the sodium azide solution for 12 hours.

2.17.8.1 Gossypol

Gossypol, the phenolic compound found in cottonseed and known to cause undesirable physiological effects in non- ruminants (Pons, 1977), was found only as traces in Roselle seed (AL- Wandawi *et al*, 1984 and EL- Nour 1991).

2.17.8.2 Tannins:

Tannins are a complex group of polyphenolic compounds that plants have evolved to deter herbivory (Foley *et al.*, 1999). Tannins are commonly classified as plant secondary compounds (as are oxalates, terpenes, saponins, etc.) and generally have negative effects on animal production. Originally, the term “tannin” consist of high molecular weight which contains a large number of phenolic hydroxylic groups to enable it to form effective cross-links with proteins (Swain, 1979).Tannins are ubiquitous in nature, and are widely found in feedstuffs, forages, fodders, and agroindustrial wastes. Dietary tannins have been shown to decrease intake, growth, and caused damage to the gastrointestinal tract in mammals (Hervas *et al.*, 2003). EL-Adawy and Khalil (1994) reported low level (1.13 – 1.37%) of tannins in roselle seed compared with faba beans (1.57%), which has been reported by Ziena *et al*, (1991). Many factors including change in weather, ambient temperature, rainfall and soil nitrogen content can alter the tannin contents in oak leaves and acorns and so may affect the risk of toxicosis (Yeruham *et al*, 1998).

2:17.8.3 Phytic acids:

EL-Adawy and Khalil, (1984) reported that Phytic acid analysis revealed that the levels was 0.92 – 1.18%, which is considerably lower than the reported for beans (EL-Adawy and Khalil, 1984) and higher than that reported for faba beans (Ziena *et al.*, 1991).

2.17.8.4 Hemagglutinin activities:

EL-Adawy and Khalil, (1984) reported the absence of Hemagglutinin activity in roselle seed; such activity has been found in some legumes such as beans, believed to exert anon- seductive adverse effect on the absorption of nutrient from the intestinal tract.

2.17.8.5 Cyanogenic glycoside:

Many species of the family Gramineae are capable, under circumstances, of giving rise to poisoning in animals. These plants contain hydrocyanogenic acid (HCN). The HCN content is highest during the period of active growth. (Knight and Walter, 2001) example is *Sorghum halepense*.

2.17.8.6 Alkaloids:

Alkaloids are estimated present in 15 – 20% of all vascular plants. Their bitter test largely precludes toxicosis in grazing animals. There are at least 10 groups of alkaloids categorized by chemical structure. Toxicosis is usually recorded

When animals have no choice but eat the plants, and on very poor or overgrazed pasture, or when they contaminate other feedstuffs such as hay. Pyrrolizidine alkaloids (PA) are the most important of alkaloids groups in the animal's health in most countries. (Knight and Walker, 2001).

2.17.8.7 Saponin:

Solanum Spp (Solanaceae). All part of the plant are dangerous but the saponins are concentrated particularly in the berries. The toxicity varies enormously with soil, climate and season (Knight and Walker, 2001).

2.18 Antioxidant activities:

To produce antioxidant peptide from Roselle seed protein RSP, enzymatic hydrolysis was performed using enzymes (pepsin followed by pancreatin). Whole Roselle seeds were ground and defatted with hexane, following a small-scale hexane extraction method described by Tzeng *et al*, (1990) The defatted roselle meal samples were vacuum-packed and stored at – 20°C prior to protein extraction as described above. The crude protein content of RSP was determined by Kjeldahl analysis according to the AOAC, (2000) method in order to calculate the amount of sample required for the hydrolysis

Process, based on enzyme/protein ratio. The RSP samples were divided into three groups (each containing 20 g and 400 ml of water) and have been hydrolyzed in 500 ml reactor with temperature and pH control devices. Sample 1 was hydrolyzed by pepsin for 30 min followed by pancreatin for 1 h and sample 2 was hydrolyzed by pepsin for 1 h followed by pancreatin for 1 h. Finally, sample 3 was hydrolyzed; using pepsin for 1 h followed by pancreatin for 2 h. Conditions were constantly monitored and maintained throughout the process. Upon completion of the hydrolysis, the enzymes were deactivated by heating in a boiling water bath for 10 min. The reaction mixtures were then filtered and the *Hydrolysates* were collected. The protein *Hydrolysates* obtained were freeze-dried and stored at -20°C for subsequent analysis. The degree of hydrolysis (DH) was determined by measuring the nitrogen content soluble in 10% trichloro acetic acid as discussed by Kim *et al*, (2001).

2.18.1 Scavenging effect of DPPH and radical:

Di-phenyl-1-2 picryl hydroxyl DPPH is a relatively stable organic radical characterized by a typical deep purple color and a maximum absorbance in the range at 515–520 nm. DPPH radical scavenging activity test system can be used for the primary characterization of the scavenging potential of compounds. Nagai *et al*, (2002) and Zhu *et al*, (2005). Therefore, the antioxidant activities of different *Hydrolysates* were evaluated by the DPPH free radical scavenging activity test and iron chelating system; all the *Hydrolysates* resulting from different hydrolysis processing were capable of scavenging DPPH radicals.

The radical system used for the antioxidant activity evaluation may influence the experimental protein hence two or more radical systems are required to investigate the radical-scavenging capacities of a selected antioxidant according to Yu *et al*, (2002).

Therefore removal of hydroxyl radicals is probably one of the most effective defense mechanisms through which living body defends its self against various diseases. Admittedly, the hydroxyl radical possesses the strongest chemical

activity among the active oxygen species, and easily reacts with biomolecules such as amino acids, proteins, and DNA (Cacciuttolo *et al.*, 1993). The antioxidant activity of *Hydrolysates* from many kinds of food proteins has been studied in recent years. Peng *et al.*, (2009), reported that whey protein *Hydrolysates* and its peptide fractions showed antioxidant properties against hydroxyl radical similar to the results of this study.

2.19 Antimicrobial antifungal and antiparasitic action of roselle seed:

Oil extracted from seeds of (*Hibiscus sabdariffa*) has been shown to have an *in vitro* inhibitory effect on *Bacillus anthracis* and *Staphylococcus albus*, but not *Proteus vulgaris* and *Pseudomonas aeruginosa* (Gangrade *et al.*, 1979). An ethanol extract of the dried leaves of the plant has been shown to reduce aflatoxin formation (El-Shayeb and Mabrook, 1984), and to have an *in vitro* inhibitory effect against some fungi that include *Aspergillus fumigatus*, *Rhizopus nigricans* and *Trichophyton mentagrophytes* (Guerin and Reveillere, 1984). An ethanol extract of the dried leaves was found to be ineffective against *Lumbricus terrestris* (Boum *et al.*, 1985). An aqueous extract of dried sepals of *H. sabdariffa* (100 ppm) was active against *Schistosoma mansoni*. However, an aqueous extract of dried seeds (10 000 ppm) was inactive against this trematode (Elsheikh *et al.*, 1990).

2.20 Polmrse chain reactin (PCR):

The QuickExtract™ Seed DNA Extraction Solution is a fast, simple and inexpensive method for preparing genomic DNA for PCR amplification from under 10 mg of ground plant seed. After grinding the seed sample, the subsequent DNA extraction requires only heat treatment to lyse the ground seed and release DNA for PCR amplification. The solution has been used to successfully extract PCR-amplifiable DNA from maize, rice and other ground plant seeds. Single-copy gene targets were amplified successfully.

2.20.1 Bioinformatics (in *selico technology*):

Bioinformatics defined as a branch of molecular biology or computer science collecting, organizing (DNA) protein sequence through software technique (Protparam Program) to determine molecular weight, amino acids concentration and estimated half-life (NCBI, 2010 and NCBI,2013). Because large quantities of crops (especially groundnut, sunflower, and cottonseed and sesame seed) are present in Sudan, it is needed to study their rumen degradability characteristics due to possible usage of them as an alternative animal protein. Molecular weight and amino acids concentrations recorded (23834 – 207), (17306.5-159) and (16135.5-141) for SFC, GNC and CSC respectively. The result indicated that the SFC had a higher molecular weight includes higher percentages of alanine, glutamine, leucine and arginine, while it had lower percentages of valine, lysine, threonine, serine and phenylalanine amino acids. The CSC recorded medium molecular weight and amino acids concentrations and signed sub-optimal percentages of threonine, glutamic acid, serine, methionine and leucine, but was low in cysteine, histidine, glutamine, arginine and tryptophan. The GNC had protein of high level in valine, glutamine, leucine, arginine and serine, low in lysine, isoleucine and tyrosine and tryptophan did not detected for GNC. The result of software technique classified groundnut cake as unstable protein while cottonseed cake and sunflower cake classified as stable proteins according to the molecular weight and amino acids concentration which was proved by degradability trial. (Turki and Actham, 2011).

2.22 Sheep feedlot performance:

2.22.1 Daily feed intake:

Animal feed required during the day is one of the methods of assessing feed intake which affected by some factors, for example breed, sex, age, season, level of physiological status of animal, and animal health. Beshir,(1996). Study

the effect feeding different level of roselle seeds of sheep performance and reported that significant difference ($p < 0.05$) among the treatment on daily feed intake (1.14, 1.16 and 1.35). Hassan, (2005) reported no significant difference ($p > 0.05$) in daily feed intake for desert lamb (1.67, 1.64 and 1.60). When he fed three levels (0%, 15% and 25) of crushed roselle seeds.

2.22.2 Factors affecting live weight gain:

The values of an animal for meat could not be measured from its live weight, or even its carcass weight, without considerations of age, sex, breed and state fatness factors (Hammond, 1960), indicated that the commonest measure of growth is the increase in weight as well as in feedlot performance. Growth rate is obtained calculating the absolute gain per unit time.

2.22.3 Plan of nutrition and feed intake:

Nutrition is the most important factor affecting growth ration especially in early life. The plan of nutrition determines the shape of the growth curve and this in turn determines the proportions and composition of the body, growth rate of lambs is considerably by affected by the amount of fiber in the diet; high protein with low fiber produces high rate of gain while in low protein and high fiber diet the stomach becomes full and the appetite satisfied and does not supply so enough nutrients needed for high growth (Hammond, 1960).

Although fast rate of growth caused by high plane of nutrition can lead to an earlier onset of the fattening phase of growth, the nature of the diet is also an important growth regulating factor, thus as protein energy ratio is increased the faster growing animal may become leaner (Campbell *et al*, 1982).

2.22.4 Age and sex:

Hamdan, (2006) studied the effect of feeding different protein sources (cotton seed cake and groundnut cake) on the performance of young and mature male of desert shugor sheep, who found that there were significance ($p < 0.05$) between mature and young animals and two type of cake in daily weight gain (146 and 91g) for both young and mature which fed with cotton cake) and (129

and 96 g) for both young and mature which fed with groundnut cake) for animals assigned for cotton seed cake and groundnut cake respectively.

El Hassan, (1994), reported an average daily gain of (217, 204, 216, 236, 230 and 241 g/day for ecotype Shugor, Dubasi, Watish, (Watish \times Dubasi), (Shugar \times Dubasi) and (Shugor \times Watish) respectively.

2.22.5 Feed conversion ratio (F.C.R):

Efficiency of production in farm animals is defined as production per unit of feed eaten by animals. Charay *et al*, (1992) studied the sheep production in humid tropics of Africa and showed efficiency with ranged between 7 – 12 depending on various factors related to the animals, age, sex and condition in addition to the extent to ratio balanced.

F.C.R values 7.2 and 7.6 were reported by (Gaili and Elnaim, 1992) on Nagdi lambs (4 months) fed chopped berseem and barley 3:1 or chopped berseem and dibs (date by products) plus 2% urea respectively. Thus the type of diet and its composition affect F.C.R.

However (Arabi, 1995 and Beshir, 1996) reported F.C.R of 8.25 and 7.34 respectively for desert sheep. Hamdan, (2006) studied the effect of feeding different protein sources (Cotton seed cake and groundnut cake) on the performance of young and mature male of desert Shugor sheep, who demonstrated that highly significance ($p < 0.001$) between mature and young animals and two type of cake in feed conversion ratio (9.33, and 9.03 for both young and mature which fed with cotton seed cake) while (10.40 and 12.82 for both young and mature fed groundnut cake) for animal recorded cotton seed cake and groundnut cake respectively.

CHAPTER THREE

MATERIAL AND METHOD

3. Experiment (1)

3.1 *In Sacco* degradability study:

3.1.2 Experiment location:

The experiment was conducted at the farm of Veterinary Medicine College, Sudan University of Science and Technology located in Kuku area, during the period (April – May 2013) to investigate the degradability of roselle seed (*Hibiscus sabdariffa*) and some oilseed cakes in the rumen. Three castrated bulls from the local breed Kenana at age 3-3.5 years were fitted with rumen cannula as described by Brown *et al*, (1968). Bulls fed diet to fill the maintenance requirements.

3.1.3 Samples collection and preparation:

Samples of Sunflower cake (SFC), Groundnut cake (GNC), and Cottonseed cake (CSC).were bought from local market at Kuku. Crushed roselle seed (CRS) collected from Abu Gaude area western of Kordufan state. The capsules containing the seeds were collected after removing its calyxes. The seeds were separated from calyxes by handling method, then removing any foreign matter or dirt. Dried seeds were prepared by direct sun – drying until constant weight% (3.51 ± 0.131) moisture content (Figure1, 2, 3 and 4). Finally the seeds were crushed into fine particle size ($20\mu\text{ m}$) and kept at room temperature until it was used.



Figure (1) roselle seed calyx of Al Rahad Varity



Figure (2) roselle seed calyx of Al Rahad Varity



Figure (3) roselle seed peeled off from fruit by hand



Figure (4) traditional drying of roselle seed under Sun

3.1.4 Degradability technique:

The method of the nylon bags technique was described and used by many researchers to determine the degradability values of each type of the feed stuff (Orskov,1977).The cannula was fitted into the rumen of the experimental animals. The cannula was a plastic tube 12 cm long and 4 cm in diameter, locally made from polythene with a flange made in one end to prevent it from coming outside the rumen. Bags were prepared from polythene length 10.5 cm, width 6.5 cm and weight 2.5 -3 g. The empty bags were individually weighed and their weight was recorded. Five gram from the cakes of sample and crushed roselle seed were placed in bag and bag tied with nylon ribbon, and introduced into plastic tube of 45.5 length, and 0.8 cm diameter above the fistula level to ease the movement of the bag inside the rumen. The bags (3 bags / animal / period) were incubated in the rumen for different period. (3, 6, 9, 12, 24, 36, 48 and 72) hrs. The bags were removed at the end of each incubation period; they were then washed under tap water and dried in oven at 70°covernight, then taken out, cooled in dessicator and weighed. Dry matter of residues in the bag was calculated in percentage of dry matter loss as following equation:-

$$\frac{\text{W. of (DM) sample incubated} - \text{W. of (DM) residue after incubation}}{\text{W. of (DM) sample incubated}} \times 100$$

Where:

W: Weight

DM: Dry matter

The dry matter disappearance at zero time (soluble fraction), was estimated as the washing loss of samples weighed in to the nylon bag, then rinsed under tap water. Residual samples after incubation for every period were separately mixed, pooled and made ready for analysis.

While crude protein of residues in the bag was calculated in percentage of crude protein loss as follows:-

$$\frac{\text{C.P of sample incubated} - \text{C.P of residues incubated}}{\text{C.P of samples incubated}} \times 100$$

The degradation kinetic of incubated cake described by the curve Linear regression of dry matter or crude protein loss from the bags, with time (Orskov and McDonald, 1979).

$$P = a + b (1 - e^{-ct}) \dots\dots\dots (1)$$

t = incubation time

a = intercept at time zero represent soluble and completely substrate that rapidly was washed out of the bags.

b= the difference between the intercepts (a) and the asymptote. Represent soluble but potentially degradable substrate, which is degraded by micro-organisms.

C = rate constant of (b) fraction

Equation (1) provides curve constants than can be then used in conjunction with predicted other rates for specified diet to estimate effective degradability of sample.

$$\text{Effective degradability} = \frac{a + b}{C + k}$$

Where a / b / c are constants as defined in equation (1). k = rumen small particle outflow rate. Then a graph was plotted by fitted values of dry matter disappearance % or crude % against time of incubation in hours to form a curve.

3.1.5 Chemical analysis (proximate analysis):

The chemical composition and proximate analysis was carried out at Faculty of Animal Production University of Khartoum. Crude protein (C.P) %, ether extract (E.E) %, Moisture% and ash % According to proximate analysis methods of forage, Crude protein % was determined by determination of nitrogen using Kjeldhal method, then multiplying the resulted nitrogen value according to (AOAC, 2003). Crude fiber content was determined by acid / alkali digestion method of (Southgate, 1976).

3.1.5.1 Moisture determination:

Five gram of well-mixed sample from each experimental nutrient was placed in clean pre-heated crucible of known weight, and then was put in oven of 105°C over night. After that the crucible was taken and placed in dessicator (drying apparatus) for 15 minutes, weighed and the loss of weight represent moisture content according to (Zaklouta *et al*, 2011).

$$\text{Moisture \%} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

Wet weight

$$\text{Moisture \%} = \frac{W1 - W2}{W1} \times 100$$

W1 = weight of sample + crucible (before drying)

W2 = weight of sample + crucible (after drying)

Ws = weight of sample

3.1.5.2 Dry matter determination:

Dry matter content is percentage of the remaining material after drying (removing moisture). According to Zaklouta *et al*, (2011).

Calculation:

$$100 - \text{Moisture}\% = \text{DM}\%$$

3.1.5.3 Determination of ash:

Five gram of sample was put in crucible of known weight then placed in muffle furnace at 600°C for five hours. Then removed out from furnace when sample color became grey. Placed in dessicator to cool for half an hours, weighed and remaining material represented the ash% (Zaklouta *et al*, 2011). According to following equation.

$$\text{Calculation:} \quad \text{Ash \%} = \frac{W_1 - W_2}{W_s} \times 100$$

Where:

W1 = weight of sample + crucible before burning

W2 = weight of sample + crucible after burning

Ws = weight of sample before burning

3.1.5.4 Determination of Crude Protein

Determination of total nitrogen (crude protein) is conducted using the Kjeldahl method. The sample is digested in sulfuric acid using K₂SO₄/ CuSO₄/TiO₄ as a catalyst. N is converted into NH₃, then distilled trapped in boric acid and titrated with H₂SO₄.

3.1.5.4.1 Crude Protein Determination Procedure:

- Add 1g of dry sample in digestion tubes (250 ml)
- Consider blank tube
- Consider standard sample of known nitrogen contents
- Add half a tablet of catalyst

- Add 13 ml of concentrated sulfuric acid (H₂SO₄)
- Insert rack with 20 tubes, including blank and standard sample in digestion block heater under fume hood, and install exhaust manifold connected to water aspirator.
- Keep in digester at 420 °C until liquid becomes transparent
- Remove rack with exhaust manifold from digester and allow to cool to room temperature under fume hood
- Remove exhaust manifold and transfer tubes separately to distillation unit
- Automatic distillation: 65 ml dist. water + 35 ml of 40% sodium hydroxide solution
- Collect condensed liquid in Erlenmeyer flask with 10 ml indicator solution
- Titrate liquid with 0.1142 N sulfuric acid until color turns purple.

3.1.5.4.2 Crude protein calculations:

$$\%N = [1.4007 \times (V_a - V_b) \times N] / W$$

V_a: volume of acid used for sample titration V_b: volume of acid used for the blank

N: Normality of acid W: sample weight in grams

1.4007: conversion factor milliequivalent weight of nitrogen and N percent

Calculation: Percent Crude Protein (CP) % CP = %N × F

F = 6.25 for all forages F = 5.70 for wheat grains F = 6.38 for milk

3.1.5.5 Degradability trials:

Three fistulated bulls from local breed (Kenana) at age of 3- 3.5 years were used. They were fitted rumen cannula described by (Brown *et al* 1968). The nylon bags technique was used in this study to determine the effective degradability and disappearance % of dry matter and crude protein, the bags were incubated in the rumen at different incubation period for (3, 6, 9, 12, 24,

36, 48, and 72hrs).The bags were removed at the end of each incubation period, washed under tap water dried in oven (70°C) overnight and cooled in dessicator and weighed immediately, to determine dry matter and crude protein disappearance % .Proximate analysis was used to determine the chemical composition of experimental oilseed cakes and crushed roselle seed according to (AOAC,2003). Samples from fresh residuals were taken regularly for dry matter and crude protein calculation. Fit curve program was used to determine the disappearance of crude protein and dry matter, and for evaluation of effective degradability of dry matter and crude protein.

3.2 Experiment (2):

3.2.1 *In vitro* digestibility of different plant protein sources:

3.2.2. Rumen liquor preparation:

Rumen fluid was obtained from fistulated bulls of experimental animal, and then samples of rumen liquor were taken before morning. Feeding using a thermos container prepared (39°C). Then filtered through four layers of muslin and squeezed by hand, kept at (39°C) and flushed with Co₂ before use to remove all oxygen from flask, the preparation of rumen liquor – buffer mixture solution at ratio of 1:2 g was made according to (Menke and Steingass,1988). Buffer solution which considered of 474ml distilled water, 237ml main element solution, 237ml buffer solution, 0.12 ml trace elements solution, and 50ml freshly reduction solution was mixed and stirred under Co₂ until use.

3.2.3 Samples Preparation for Gas Production technique:

The gas production during fermentation can be used as indicator measure of dry matter digestibility. (Depeters and Aseltim, 2003).*In vitro* gas production method (Close and Menke, (1986) was used in the present study for samples of Sunflower cake (SFC), Cottonseed cake (CSC), Groundnut cake (GNC) and Crushed roselle seed (CRS) at different incubation period. Roselle seeds were

crushed to pass through 1mm screen, and about 230 mg air dry material was placed into the bottom of a glass syringe. Samples of (SFC), (CSC), (GNC) and (CRS) were incubated in tri-replicates at different incubation period for (3, 6, 12, 24, 48, 72, and 96) hours. Rumen liquor collected from fistulated bulls fed on a roughage diet, homogenized, stirred 40ml of rumen liquor medium mixture was pumped with pipette into each syringe glass (artificial rumen). Samples of (SFC), (CSC), (GNC) and (CRS), were calibrated in a glass syringe in different incubation periods basically by the procedure of (Menke *et al*, 1979; and Steingass and Menke, 1988) at (39°C ±0.5°C) with holes to hold the syringes put upright down. Incubator were started in the morning, thus the second readings were done 3 hours later. The syringes were shaken manually by hand carefully during every time of incubation period. Reading was recorded at different time intervals post infusion. All readings were taken quickly to avoid a change in temperature. The gas production was compared with standard concentration samples and hay which had been used by (Steingass and Menke, 1986). Calculation of gas production, volume results were estimated by fit curve exile program (Chen, 1997). Gas production is associated with volatile fatty acid production following fermentation of substrate so the more fermentation of substrate the greater the gas production (Blummel and Orskov, 1993).

3.3 Experiment (3):

3.3.1 Biological test anti-microbial and fungal procedure:

3.3.2 Plant materials:

A sample of crushed roselle seed (CRS) was used for anti-microbial activities. (CRS) was analyzed at Medicinal and Aromatic plants Research Institute (MAPRI) at Sudan National Research Center (NCR). Each of the coarsely powdered plant material (50g) was exhaustively extracted for 20 hours with chloroform in Soxhlet apparatus. The chloroform extract was filtered and

evaporated under reduced pressure using Rota-vap. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with methanol. The ethanolic extract 80% conc. was filtered and evaporated under reduced pressure again using Rota-vap.

Each residue was weighed and the yield percentage was determined. The chloroform residue (2 g) was dissolved or suspended in a mixture containing methanol: petroleum ether (2:1) to a final volume 20ml (con. 100 mg/ml). The methanol residue (2g) was dissolved in methanol 20 ml (con. 100mg/ml), and kept in refrigerator until used.

For aqueous extract (100 g of each plant sample) was soaked with 500 ml hot water for 4 hours then filtered with Whatt man filter paper. Extracts kept in Deep freezer for 48 hours, then induced in freeze dryer till completely dried. The residue was weighed and the yield percentage was determined. The aqueous residue (2g) was dissolved in sterile distilled water 20 ml (con. 100mg/ml), and kept in refrigerator until used.

3.3.3 Preparation of the test organisms:

3.3.3.1Preparation of bacterial suspensions:

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37° C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 - 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then

incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

3.3.3.2 Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

3.3.3.3 *In vitro* testing of extracts:

3.3.3.4 Testing for antibacterial Activity:

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

One ml of the standardized bacterial stock suspension $10^8 - 10^9$ C.F.U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 °C. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes.

The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each of the oils dilutions in 80% methanol using automatic microtitre pipette, and allowed to diffuse at

room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 18 hours.

Two replicates were carried out for each extract against each of the bacterial test. After incubation the diameters of the resultant growth inhibition zones were measured, averages and the mean values were tabulated.

3.3.3.5 Testing for antifungal activity:

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25 °C for two days for the *Candida albicans* and three days for *Aspergillus niger*.

3.3.4. Phytochemical screening:

Phytochemical screening for the active constituents was carried out using the methods described by (Martinez and Valencia, 1999), (Sofowara,1993), (Harborne, 1984).

The materials used in the screening were the same as those of the biological screening. The screening was carried out for the samples, which gave high antibacterial activity.

3.3.4.1 Preparation of the extract:

Ten gram of the powder plant protein source of Sunflower cake (SFC), Groundnut cake (GNC), Cottonseed cake (CSC) and Crushed roselle seed (CRS). Kernel of above samples was refluxed with 100ml of 80% of ethanol four 4hours. The cool solution was filtered80%ethanolwas passed through the volume of the filtrate to 100ml. This prepared extract (PE) was used for the various tests (Harborne, 1992).

3.3.4.2 Determination of Sterols and Triterpenses:

In this test 10 ml of the petroleum ether (P.E) was evaporated to dryness on a water path and the cooled residue was stirred several times with PE to remove

most of the coloring materials. The residue was then extracted with 20ml chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5ml portion of the chloroform solution was mixed with 0.5ml of acetic anhydride followed by 2drops of conc. Sulphuric acid The gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or Triterpenses (pink to purple) in the sample. (Sofowara, 1993)

3.3.4.3 Determination of Alkaloids:

Test of Alkaloids take 7.5 ml of the P.E was evaporated to dryness on a water bath. 5 %ml of $2NHCl$ was added and stirred while heating on the water bath for 10minutes, cooled filtered and divided into two test tubes. To one test tube few drops of *Mayer's reagent* was added while to the other tube few drops of *Valser's reagent* was added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids (Harborne, 1973).

3.3.4.4 Determination of Flavonoids:

Test of flavonoids take 17.5 ml of the *petroleum ether PE* was evaporated to dryness on water bath, cooled and the residue was defatted by several extractions with *petroleum ether* and the defatted residue was dissolved in 30ml of 80% ethanol and filtered. The filtrate was used for following tests:

A/ to 3ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution was in *methanol* was added. Formation of a yellow color indicated the presence of Flavonoids. Flavones or and chalcone.

B/ to 3ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of Flavonoids compounds (flavones or flavonenes) chalcone and or flavonols.

C/ 2ml of the filtrate 0.5ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavonenes were present in the plant sample (Harborne, 1984).

3.3.4.5 Determination of Tannins:

For this test 7 ml quantity of the PE was evaporated to dryness on water bath. The residue was extracted several times with n-hexane and filtered. The insoluble residue was stirred with 10ml of hot saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10ml with more *saline solution*. 5ml of this solution was treated with few drops of *gelatin saltre agent*. Formation of immediately precipitate was taken as evidence for the presence of tannin in the plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins (Sofowara, 1993).

3.3.4.6 Determination of Saponins:

One gram of the original dried powder plant material was placed in a clean test tube. 10 ml of *distilled water* was added and the tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of (honeycomb) the appearance of honeycomb, which persisted for least an hour, was taken as evidence for presence of Saponins (Martinez and Valencia, 1999)

3.3.4.7 Determination of Cyanogenic glycoside:

Three gram of the powdered plant sample was placed in Erlenmeyer flask and sufficient water was added to moisten the sample, followed by 1ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split cork which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to

various shades of red was taken as an indication of the presence of Cyanogenic glycoside (Harborne, 1984).

3.3.4.8 Determination of Anthraquinone glycoside:

One gram of the powdered plant sample was boiled with 10 ml of 0.5 N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10ml of benzene. 5ml of the benzene esolution was shaken with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of Anthraquinone was indicated if the alkaline layer was found to have assumed pink or red color (Harborne, 1992).

3.3.4.9 Determination of Cumarins:

Three gram of the original powdered plant sample boiled with 20ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of Cumarins was indicated if the spot have been found to be adsorbed the UV light (Martinez and Valencia, 1999).

3.3.5 Detergent Fiber Analyses:

3.3.5.1 Determination of Neutral Detergent Fiber (NDF)

Important fractions of feed are fibers that affect the digestibility. They consist of cellulose, hemicelluloses and polysaccharides that are bound to protein and phenols, especially to lignin. Detergent fiber is the residue of plant cells after fractionation using detergent solutions for the volatilization of protein and starch According to (Zaklouta *et al*, 2011). Insoluble fiber in feed is determined as neutral detergent fiber (NDF).Neutral detergent solution recovers its main components cellulose, hemicelluloses and lignin.

3.3.5.2 Neutral Detergent Fiber technique:

Firstly add 0.5 –1 g of experimental samples of (SFC), (CSC), (GNC) and (CRS) to 600 ml Berzelius beaker. Adding 100 ml of neutral detergent solution

and 0.5 g sodium sulfite, (Na₂SO₃) to the crucible with sample above. Then transfer to oven and boiled for one hour in refluxing apparatus. Rinses materials in crucible with acetone repeatedly until drained liquid is to be clear. Drying the samples at 105 °C overnight, then cooling at room temperature in dessicator NDF calculation according to following equation.

3.3.5.3 NDF calculations:

$$\text{NDF \%} = \frac{W_o - W_t}{W_s} \times 100$$

Cell soluble material = 100 – N%

$$\text{NDFash\%} = \frac{W_s - W_t}{W_o - W_t} \times 100$$

Ws: weight of sample.

Wt: weight oven – dry glass crucible.

Wo: weight of sample and crucible.

Wa: weight of crucible and residues.

3.3.5.4 Determination of Acid Detergent Fiber (ADF):

Acid detergent fiber is determined as the residue remaining after adding an acidified solution. It is the NDF without the hemicelluloses. Cell soluble, hemicelluloses and soluble minerals dissolve. Acetyl triethyl-ammonium bromide (ACTAB) separates proteins from the remaining cellulose and lignin, and minerals (ash). The acid detergents solution recovers cellulose and lignin. While ADF determination is a preparation step for lignin determination. According to (Zaklouta *et al*, 2011).

3.3.5.5 Acid Detergent Fiber technique:

One or half gram of experimental cakes (GNC), (CSC), (SFC) and (CRS) were taken. All samples were transferred to the beaker with 100 ml of acid detergent Solution. The samples above were boiled for one hour on refluxing apparatus. Rinse crucibles with approximately 50 ml hot water four times until all traces are removed. Then added acetone repeatedly until drained liquid is to

be cleared, Samples of cakes and crushed roselle seed were dried at 105 °C overnight. Cool at room temperature in dessicator, ash residues for three hours at 550 °C was burned. According to (Zaklouta *et al*, 2011).

3.3.5.6 ADF Calculations

$$\text{ADF \%} = \frac{W_o - W_t}{W_s} \times 100$$

ADF expressed as organic matter:

$$\text{ADF ash \%} = \frac{W_a - W_t}{W_o - W_t} \times 100$$

Where:

Ws: weight of sample.

Wt: weight oven – dry glass crucible.

Wo: weight of sample and crucible.

Wa: weight of crucible and residues.

3.3.5.7 Determination of Acid Detergent Lignin (ADL)

Lignin is the indigestible non-carbohydrate component of forages. Residues from ADF determination are treated with sulfuric acid. Lignin represents the indigestible NDF fraction. According to (Zaklouta *et al*, 2011).

3.3.5.8 Acid Detergent Lignin technique:

Transfer crucibles with residues samples of (GNC) (CSC), (SFC) and (CRS) from ADF to a flat container or tray cover contents crucible with cooled at 72% sulfuric acid (H₂SO₄). Fill crucible and samples about half full with sulfuric acid. Filter under vacuum and Rinses twice with 400 ml hot water. Dried the samples overnight at 105 °C Cooled in dessicator and weighed immediately. Then burned ash under 550 °C for three hours to determine the lignin content according to (Zaklouta *et al*, 2011).

3.3.5.9 ADL Calculations

$$\text{ADL \%} = \frac{W_a - W_o}{W_s} \times 100$$

Where:

Ws: weight of sample.

Wt: weight oven – dry glass crucible.

Wo: weight of sample and crucible.

Wa: weight of crucible and residues

3.4 Experiment (4):**3.4.1 Determination anti-oxidant activity of plant extracts:**

In the present study, Roselle seed protein (RSP) was hydrolyzed with pepsin followed by pancreatic at different time in order to determine the more suitable hydrolysis time for production of antioxidant peptides. In quantitative work on protein hydrolysis it is necessary to have a measurement for the extent of hydrolytic degradation. It should be evident that the number of peptide bonds cleaved during the reaction is the parameter that most closely reflects the catalytic action of proteases. (Alder,1986) The Degradation hydrolysis DH is generally used as a parameter for monitoring proteolysis and is the most widely used indicator for comparison among different protein *Hydrolysates*.

The 80% ethanolic extracts and fractions of the plants material were diluted to concentrations of 500, 250, 125, and 62.5µg / ml. respectively in dimethyl sulphoxide (DMSO). Propyl gallate (P.G) Ascorbic acid and Ethylene diamine tetra acetic acid (EDTA).Were used as comparative anti oxidants against crushed roselle seed.

3.4.2 DPPH radical scavenging assay:

The di-phenile picryl hydrazine (DPPH) radical scavenging was determined according to the method of (Shimada *et al*, 1992) with some modification. Antioxidant of the crude extracts was tested using (DPPH) photometric assay according to the method of Farzan *et al.*, (2005).With same modifications. DPPH is a stable free radical than can accept an electron or hydrogen radical to become stable diamagnetic molecule for anti- oxidant against crushed roselle

The radical scavenging potential of extracts and their fractions is determined by measuring the decrease in absorbance due to DPPH at 517 nm, representing the formation of its reduced form. DPPH, which is yellow in color.

The reaction mixture containing 10 μ l of test sample (500 μ g/ml DMSO) and 90 μ l of stable free radical DPPH (300 μ M) in ethanol was taken in 96 well micro plate and incubated at 37° C for 30 min. After incubation, decrease in absorbance was measured at 517 nm using Multiskan spectrum. Percentage radical scavenging activity was determined by comparison with a DMSO control group. IC₅₀ values represent concentration of extract to scavenge 50 % of DPPH radicals was calculated. Propyl gallate PG and Ascorbic Acid were used as positive control.

3.4.3 Iron chelating antioxidant assay:

The iron is an extremely reactive metal and will catalyze oxidative changes in lipids, proteins and other cellular compounds. The chelating activity was measured against Fe²⁺ and reported as Ethylene diamine tetra acetic acid EDTA equivalents to test for roselle seed as anti-oxidant activities. Ferrozine can quantitatively form complexes with Fe²⁺ iron. In the present age of chelating agents. The complex formation is disrupted resulting to a decrease in red color of the complex. Measurement of color decline there for makes possible the estimation of metal chelating activity of the coexisting chelator.

The iron chelating ability was determined according to the modified method of (Kexue *et al.*, 2006).

The Fe²⁺ were monitored by the measuring the formation of ferrous ion-Ferrozine complex. The plant extracts in concentrations 500, 250, 125, and 62.5 μ M /ml respectively. Samples were mixed with 35 μ l FeSO₄ (0.0625 μ M), reaction initiated by adding Ferrozine (60 μ l at concentration 5 mM). The mixture was shaken and left at room temperature for 10 min.

The absorbance was measured at 562nm. EDTA was used as stander and DMSO as control. All tests and analysis were run in triplicate.

3.4.4 Total minerals of experimental plant protein sources:

Minerals determined in samples extract prepared by the drying ash method as described by Pearson (1981). Phosphorus was determined by the ammonium molybdate / ammonium, van date method of (Chapman and Pratt 1986). Calcium and Magnesium was determined by titration method of (Chapman and Pralt, 1961). Sodium and potassium was determined according to the AOAC (2003) using flam photometer (corning EEL).

Minerals in the samples were extracted by the method described by (El Maki *et al*, 2007). One gram of the sample was extracted using 10ml of 0.03 N HCL with shaking at 37°C for 3 hours then, the extract was filtered and the clear supernatant was at 100°C, incinerated at 550°C for 4 hours. Thereafter, the samples were cooled and 5ml of HCL were added and heated gently on a sand bath for 10 minutes. After cooling samples were diluted to 100 ml. Extractability each element was calculated as a percentage of the total amount the element.

3.5 Experiment (5):

3.5.1 PCR and amino acid concentration of roselle seed (*Hibiscus sabdariffa*):

Polymerase chain reaction technique (PCR) using in this study. Roselle seed collected from Abu Gaude area western of Kordofan state. prepare 10g of roselle seed, seed coat crushing with liquid nitrogen easy to extract DNA from inside of nuclic, adding buffer solution and protease (k) 1-2 drops in spin Colum containing silica easy to catching the DNA, vortexing sample three minute, putting in water path 37°C then sample putting in agrose gel and conducting with electro power to determine DNA band figure (5).

3.5.1.1 QuickExtract™ Seed DNA Extraction Solution:

1. Grind or crush seed samples into small pieces and weigh out
2. Place ground seed or seed fragments into a 500-µl tube or a well of a 96-well plate, add 100 µl of QuickExtract Seed DNA Extraction Solution, and mix by vortexing the sample.
3. Heat the samples at 65°C for 6 minutes then at 98°C for 2 minutes.
4. Place the samples on ice. Use 1 µl of sample as template for PCR (25-50-µl reaction volumes).

3.5.2 Bioinformatics *in silico* analysis of (*Hydrolysates*) protein sequence of roselle seed:

New technique of bioinformatics through software was used in present study to collect data base from National Center of Biotechnology Information (NCBI, 2013) online. The sequence of different four species of Roselle seed (*Hydrolysates*) protein for (*Hibiscus sabdariffa*, *Hibiscus cannibns*, *Hibiscus acetosella* and *Hibiscus altissima*). Was viewed by computational program. Sequence of the beta – lactamase gene was searched from sequence similarity using nucleotide BLAST, according to (Atschul *et al* 1997). Highly similar sequences were retrieved from National Center of Biotechnology Information (NCBI, 2013) subjected to multiple sequence alignment using Bio Edit Software, according to (Hall, 1999). In gene mark version 4.25 gene sequences were translated in to amino acid sequence, according to (John and Mark, 2001). Protein sequences similarity were searched BLAST highly similarity sequence were achieved from NCBI and subjected multiple sequence alignment and evolutionary analysis using Bio Edit Software among different species of roselle seed.

Phylogenetic tree of TME beta lactamase gene and their evolutionary different species of above samples and study relationship of those obtained from database were done online by Clustal W2 (Mc William and Uludag, 2013).

The secondary structures of predicted amino acids were carried out by phyre 2 (Kelley, 2009).

Chimera 2013 version 1.9 was used to predict the tertiary model of the protein for different species of roselle seed from NCBI (Huang *et al*, 2014).

The physiochemical properties of the different roselle seed sequences it was follow by mutations through according to (An e-Science 2010). While the Expasy program online study more identification and characterization of the different *Hydrolysates* Genes of *Hibiscus sabdariffa* to determine instability index.

3.5.3 DNA characterization of roselle seed (*Hibiscus sabdariffa*):

3.5.3.1 BLAST Analysis:

The nucleotides sequences of four experimental Roselle seed species are (*Hibiscus sabdariffa*, *Hibiscus cannibns*, *Hibiscus acetosella* and *Hibiscus altissima*) *Hydrolysates* Genes was searched for sequences similarity using nucleotide BLAST program. According to (Atschul *et al*, 1997).

3.5.3.2 Molecular Weight and Amino Acid Concentration:

Highly similar sequences were retrieved from National Center of Biotechnology Information (NCBI, 2013) and subjected to multiple sequence alignment using Bio Edit according to (Sofowara and Hall, 1999). In Gene Marks version 4.25 the gene sequences of four species of Roselle seed (*Hibiscus sabdariffa*, *Hibiscus cannibns*, *Hibiscus acetosella* and *Hibiscus altissima*) *Hydrolysates* Genes were translated into amino acid sequence according to (John *et al*, 2001). Protein sequences similarity was searched with BLASTP.

3.5.3.3 Multiple Sequence Alignment:

Highly similar sequences of experimental *Hydrolysates* gene of four Roselle seed species are (*Hibiscus sabdariffa*, *Hibiscus cannibns*, *Hibiscus acetosella*

and *Hibiscus altissima*) were achieved from (NCBI, 2013) and subjected to multiple sequence alignment and evolutionary analysis using Bio Edit program. Phylogenetic tree of four experimental of *Hydrolysates* genes were used also to evolutionary relationship with those obtained from database was done online by using Clustal W2 source according to (Mc Willian *et al*, 2013).

3.5.3.4 DNA Similarity and Mutation:

Chimera 2013 version 1.9 according to (Sofowara, 1993) was used in the present study to predict the tertiary model of four experimental Roselle seed species are (*Hibiscus sabdariffa*, *Hibiscus cannibns*, *Hibiscus acetosella* and *Hibiscus altissima*) among different *Hydrolysates* Genes protein according to (Huang, 2014). Mutation and position variation of different *Hydrolysates* genes analysis was done online by using source of (An – Science, 2010), and Expasy program according to (Gasteiger *et al*, 2005).

3.6 Experiment (6):

3.6.1 Feedlot performance:

3.6.2 Location of study:

This experiment was carried out from (15 April to 15 June 2015) of El-Huda national sheep research station lies at approximately 14° - 15° Latitude and 32° - 50°E Longitude, at an Altitude of about 250 m, about 90 km north – west of Wad Madani and about 150km South of Khartoum. To investigate the Roselle Seed (*Hibiscus sabdariffa*) as alternative source of protein for fattening performance of Sudanese desert sheep.

3.6.3 Animals experimental feeds

Four Iso-caloric and Iso-nitrogenous diet treatment were formulated. Inclusion of Roselle seed with different level was (0, 10, 20, and 30%). Signed

as group A, B, C and D respectively. The experimental diets composition was shown in table (1).

3.6.4 Experimental animals:

Twenty-four male lambs of Sudan desert sheep ecotype Shugar, breed were used in the present study. The animals were selected according to their age (5 – 6 months) and their average body weight of (21.4 kg). They were vaccinated against hemorrhagic septicemia, anthrax, and foot and mouth disease and sheep box. Also drenched with Albendazol 25% concentration and Ivermectine were given for treatment internal and external parasites. Experimental animals were given prophylaxes doses of oxy tetracycline as preventive dose. They were ear tagged, wool shearing and fed for 10 days adaptation period.

3.6.5 Animals housing:

The experimental animals were housed in semi open housemen closed building and covered with zinc sheet. Each house contains (6) animal of each groups, and then each group divided in (2) replicates of three animals each instead of three replicate due to lack of facilities. Animals provided with clean water and feed troughs.

3.6.6 Experimental procedure:

Immediately after the adaptation period the experimental animals were individually weighed by using small ruminant's balance (0 – 50 kg capacity) and the initial weight was recorded. Then they were randomly divided into four groups (6 animals in each groups), with similar average body weight, then each group was randomly sub divided in two groups of three animals each (replicates) with approximately similar average body weight of (21.77 ± 0.3) kg. The eight replicates were randomly assigned to consider initial weight to the four experimental groups.

Table (1) Ration formulation of experimental diet procedure:

Ingredient	A 0% CRS	B 10% CRS	C 20% CRS	D 30% CRS
Crushed Roselle seed	0	10	20	30
Groundnut Cake	25	19	14	9
Sorghum grain	40	40	37	31
Molasses	16	15	10	8
Groundnut hulls	17	14	17	20
NaCl₂	1	1	1	1
Limestone	1	1	1	1
Total	100	100	100	100
Calculated CF%	16.95	17.43	17.56	17.87
Calculated ME/MJ	10.87	11.09	11.13	11.22

3.6.7 Feeding management:

The rations were mechanically mixed and molasses was added with water 50Kg weekly then let to drying by air and packed in labeled sacks (A, B, C, and D). To avoid the fermentation and rancidity of the diet, the rations were given to the lambs daily 2Kg per head, every morning at 8:00 am and the refusal part was collected in the next morning at 7:00 am, weighed and subtracted from the daily offered amount to calculate the actual feed intake and registered immediately. Roughage (groundnut hay) was available *ad libitum*, green fodder (*Clitoris*) was also offered once a week at rate of 1kg / head to avoid vitamin (A) deficiency. Clean water and salt licks were available throughout period, which extended for 42 days.

3.6.8 Experimental measurements:

Initial body weight (kg) measurement at the beginning of the experiment after that weight gain was taken every week. Also body temperature, respiratory rate, heart rate, plus rate, were taken. While urine and feces residual samples were taken from experimental animals to determine N balance and crude protein digestibility.

3.6.9 Crude protein in feces:

To determine crude protein digestibility feed intake/ kg/ day was and protein content was determined and recorded. Fecal samples from each experimental sheep were collected freshly of mooring and dried under sun shed till constant weight and transferred to lab for subsequent analysis to determine crude protein digestibility. Calculated of crude protein in feces was analyzed as N% .According to (Zaklouta, *et al* 2011).

3.6.10 Nitrogen balance in urine and feces:

Nitrogen in urine included nitrogen synthesized from non protein nitrogen (NPN). The (NPN) was determined through equation: $N \text{ intake} = (N \text{ in feces} + N \text{ in urine}) = N \text{ retained}$. Samples of urine were collected from sheep penis at morning before drinking water, using external urine collector, and then adding two drops of sulphuric acid concentration for urine to stop fermentation while the feces samples collected via external sac dried under sun till constant weight. The analysis was performed according to (Zaklouta *et al*, 2011).

3.6.11 Statistical analysis (SPSS):

The data obtained were analyzed for significant difference ($p < 0.05$) according to analysis of variance one way (ANOVA) using the SPSS program, version 16. Significant difference of means was compared using the least significant difference LSD test according to (Gomez and Gomez, 1984). While degradability were calculated at out flow rate 0.02, 0.05 and 0.08 respectively.

CHAPTER FOUR

RESULTS

4:1 Chemical composition of experimental cakes and crushed roselle seed:

The Chemical composition of the four plant protein sources are Sunflower cake (SFC), Cotton seed cake (CSC), crushed Roselle seed (CRS) and Groundnut cake (GNC), shown in table (2). Results showed that there are highly significant differences ($P < 0.01$) among the different protein sources in protein %, GNC has higher value of protein content (42.33%) followed by CRC (27.38%) and SFC (26.91%) C.P while the lowest ($P < 0.01$) content was signed by CSC (25.16%) C.P %. In contrast GNC has highest significance difference ($P < 0.01$) C.F (9.21%). Also a significant difference between the other sources was observed of (17.25%, 18.09%, and 22.26%) C.P % for SFC, CRS, and CSC, respectively.

4:2 Proximate analysis % of roughages (groundnut hay):

Table (3) revealed chemical analysis of roughages diet offered to the experimental animals. Average of groundnut hay in dry matter and crude fiber were recorded highest value (92.15 %) and (22.66%) while the lowest value found in ether extract (2.26%), crude protein (7.82%) and ash (10.44%).

4:3 Proximate analysis % of four experimental diet groups:

Table (4) showed chemical composition of the experimental diets the protein % ranged between (18.17% to 16.95%) for all diets while the total crude fiber recorded the lowest value of (14.88%) by diet (D) and highest value of (15.40%) by diet (C). Ash content recorded between (11.42% to 12.42%) for all experimental diet.

4:1 Table (2) chemical composition of experimental cakes and crushed roselle seed:

INGREDIENT	SFC	CSC	CRS	GNC	±SE	Sign
D.M%	97.34 ^b	97.91 ^a	96.49 ^c	96.33 ^c	0.14	**
C.P%	26.91 ^b	25.16 ^c	27.38 ^b	42.33 ^a	0.04	**
C.F%	17.25 ^c	22.62 ^a	18.09 ^b	9.21 ^d	0.09	**
E.E%	10.94 ^a	8.10 ^b	5.73 ^d	7.30 ^c	0.17	**
N.F.E%	36.99 ^b	35.20 ^c	39.18 ^a	32.35 ^d	0.13	**
ASH%	5.25 ^d	5.82 ^b	6.11 ^a	5.14 ^c	0.05	**
Moisture%	2.66 ^d	3.09 ^c	3.51 ^{bc}	3.67 ^{ab}	0.35	**

Means bearing different superscripts within rows are significantly (P<0.05) different

** : Highly significant difference at (P< 0.01).

NFE: Nitrogen free extract.

CRS: Crushed Roselle seed.

GNC: groundnut cake.

SFC: sunflower cake.

CSC: cottonseed cake

Table (3) the proximate analysis % of roughages (groundnut hay):

Ingredient	Offered
Dry matter %	92.15
Crude protein %	7.82
Crude fiber %	22.66
Ether extract %	2.26
Ash %	10.44

Table (4) proximate analysis% of four experimental diet groups:

Groups	DM%	CP%	CF%	EE%	Ash%	NFE%	Dig%
Diet(A)	93.45	18.17	15.23	3.15	11.53	45.38	80.89
Diet(B)	92.96	16.95	14.98	4.09	12.42	44.54	81.16
Diet(C)	93.83	17.82	15.40	3.75	11.69	45.22	81.39
Diet(D)	95.22	17.93	14.88	4.02	11.93	46.48	83.24

4.4 Dry matter disappearance % of experimental plant protein sources:

Table (5) include rumen dry matter disappearance percentage% using nylon bags technique of four plant protein sources. In different incubation period for (0, 3, 6, 9, 12, 24, 36, 48, and 72hrs) respectively. Groundnut cake recorded a highly $p(<0.01)$ degradable value ($P<0.01$) (88.22%) for rumen dry matter disappearance % at 72 hrs incubation period. While CSC had the lowest degradable value (74.55%) for rumen dry matter disappearance % at 72 incubation period.

4.5 Dry matter effective degradability % of experimental plant protein sources:

Table (6) shows *in Sacco* dry matter effective degradability of the four experimental plant protein sources. Using *in sacco* nylon bags technique. The measures include (a, b and c) degradability, potential degradability and effective degradability in different level $K = (0.02, 0.05 \text{ and } 0.08)$ respectively. Groundnut cake recorded the highest significant ($P<0.01$) ($93.18 \% \pm 2.93 - 60.68 \% \pm 1.74$) for dry matter potential degradability and effective degradability respectively. While CSC recorded the lowest value ($80.70 \% \pm 0.91 - 51.71 \% \pm 1.77$) for potential degradability and effective degradability respectively.

4.6 Crude protein disappearance % of experimental plant protein sources:

Table (7) demonstrated *in Sacco* crude protein disappearance % of the four experimental plant protein sources, at different incubation period for (0, 3, 6, 9, 12, 24, 36, 48, and 72hrs). (GNC) revealed the highest ($P<0.01$) ($77.39 \% \pm 1.53$) for crude protein disappearance % at 72 hrs incubation period. While (CSC) was record the lowest significant value ($41.17 \% \pm 1.59$) of crude protein disappearance % at 72 incubation period.

4.7 Crude protein effective degradability % of experimental plant protein sources:

Table (8) revealed *in Sacco* crude protein effective degradability % of the experimental protein sources. Groundnut cake had a highest ($P < 0.01$) for potential degradability and crude protein effective degradability of $(82.75\% \pm 1.05 - 42.60\% \pm 1.88)$ respectively. While CSC recorded the lowest degradable value $(61.70\% \pm 0.93 - 18.90\% \pm 1.44)$ for potential degradability and crude protein effective degradability respectively.

4.8 *In vitro* degradability % of experimental plant protein sources:

Table (9) include *in vitro* degradability % of the four experimental plant protein sources. GNC shows the highly significant difference ($P < 0.01$) of $(28.47\% \pm 0.19)$ in *in vitro* degradability. While CSC was found the lowest significant value of $(12.78\% \pm 0.19)$ in *in vitro* degradability.

Table (5) Dry Matter Disappearance % of experimental plant protein Sources:

Time/hours	GNC±SD	SFC±SD	CRS±SD	CSC±SD	Sig
0	34.89 ^a ±1.27	34.22 ^{ba} ±1.36	33.00 ^b ±1.22	32.44 ^c ±1.51	**
3	35.56 ^a ±1.67	34.78 ^{ab} ±1.30	34.00 ^b ±1.00	33.11 ^c ±1.17	**
6	36.22 ^a ±1.92	35.61 ^a ±1.11	35.11 ^a ±1.05	34.00 ^{ba} ±0.71	**
9	51.11 ^a ±2.47	47.22 ^b ±1.20	44.33 ^c ±2.12	38.33 ^d ±1.22	**
12	55.00 ^a ±2.10	51.11 ^b ±1.76	47.44 ^c ±0.70	41.56 ^d ±1.23	**
24	84.11 ^a ±1.70	75.41 ^b ±2.04	73.11 ^c ±1.46	71.00 ^d ±0.70	**
36	86.33 ^a ±2.5	77.34 ^b ±2.20	74.22 ^c ±2.96	73.55 ^d ±1.34	**
48	87.78 ^a ±1.39	78.22 ^b ±1.30	75.87 ^c ±2.03	74.43 ^d ±3.01	**
72	88.22 ^a ±0.83	78.54 ^b ±1.47	76.87 ^c ±2.03	74.55 ^d ±1.04	**

Means bearing different superscripts within rows are significantly (P<0.05) different GNC: Groundnut cake SFC: Sunflower cake CRS: Crush Roselle seed CSC: Cottonseed cake **: Highly significance difference at (P<0.01)

Table (6) dry matter effective degradability % of experimental plant protein sources:

Fitted value	GNC±SD	SFC±SD	CRS±SD	CSC±SD	Sig
a	27.20±0.96	27.95±0.88	27.31±1.36	25.91±0.63	-
b	65.37±2.09	55.46±1.94	53.68±1.03	55.43±1.07	-
c	0.53±0.00	0.51±0.00	0.05±0.01	0.43±0.00	-
a+b	92.57±2.93	83.41±1.51	80.99±1.26	81.34±1.33	-
Pd	93.18 ^A ±2.93	84.03 ^{AB} ±3.94	81.11 ^C ±1.65	80.70 ^C ±0.91	**
Ed (0.02)	74.50±2.70 ^A	67.59±3.94 ^{AB}	63.31±1.65 ^B	63.88±0.91 ^C	**
Ed (0.05)	60.68±1.74 ^A	55.77±0.82 ^{AB}	54.04±1.77 ^B	51.71±0.92 ^B	**
Ed (0.08)	53.11±1.35 ^A	49.63±0.60 ^{AB}	47.98±1.71 ^B	46.45±3.16 ^C	**

Means bearing different superscripts within rows are significantly (P<0.05) different

GNC:Groundnut cake.

SD: stander divisions.

a: washing loss.

SFC: Sunflower cake

CSC: Cottonseed cake.

CRS: Crush Roselle seed

****:** Significant difference at ($P < 0.001$)

.NS: Not significant difference at ($P > 0.05$).

b: water insoluble nutrient which is potentially degradable by micro-organism Degradation rate of b/hours.

Pd: potentially degradability. **SD:** Stander division. **C:** degradation rate of b/hours.

Ed: effective degradability at the rumen fraction out flow rate (0.02, 0.05, and 0.080).

Table (7) Crude protein disappearance % of experimental plant protein sources:

Time /hours	GNC\pmSD%	SFC\pmSD%	CRS\pmSD%	CSC\pmSD%	Sig
0	19.07 ^a \pm 1.13	11.90 ^b \pm 1.25	12.03 ^b \pm 2.01	9.01 ^c \pm 1.06	**
3	19.78 ^a \pm 1.13	11.41 ^b \pm 1.05	12.55 ^b \pm 1.51	9.60 ^c \pm 1.32	**
6	24.34 ^a \pm 1.09	15.35 ^b \pm 1.85	16.55 ^b \pm 1.60	11.60 ^c \pm 1.17	**
9	32.70 ^a \pm 1.14	18.95 ^b \pm 1.22	20.53 ^b \pm 1.90	13.69 ^c \pm 1.00	**
12	43.22 ^a \pm 2.03	22.49 ^b \pm 1.08	24.26 ^b \pm 1.82	15.56 ^c \pm 1.33	**
24	53.32 ^a \pm 1.55	34.40 ^b \pm 1.03	36.44 ^c \pm 1.77	22.54 ^d \pm 1.75	**
36	62.38 ^a \pm 1.54	44.10 ^b \pm 1.08	45.62 ^b \pm 1.99	28.33 ^d \pm 1.17	**
48	74.74 ^a \pm 1.54	51.80 ^b \pm 1.30	52.47 ^b \pm 1.32	33.39 ^d \pm 1.01	**
72	77.39 ^a \pm 1.53	62.86 ^b \pm 1.94	61.88 ^b \pm 1.74	41.17 ^d \pm 1.59	**

Means bearing different superscripts within rows are significantly ($P < 0.05$) different

****:** Highly significant difference at ($P < 0.01$).

SFC: Sunflower cake. **CSC:** Cottonseed cake **CRS:** Crush Roselle seed

GNC: Groundnut cake. **SD:** Stander division.

Table (8) Crude protein effective degradability % of experimental plant protein sources:

Fitted value	GNC	SFC	CRS	CSC	Significant
A	12.11±0.90	7.23±0.89	8.01±0.67	7.40±0.63	-
B	70.20±2.08	70.00±1.92	66.04±1.66	54.10±1.07	-
C	0.41±0.01	0.19±0.01	0.02±0.02	0.14±0.00	-
a+b	82.72±2.93	77.42±1.53	74.07±1.45	61.64±1.33	-
Pd	82.75 ^A ±1.05	77.65 ^B ±1.43	74.44 ^B ±1.42	61.70 ^C ±0.93	**
Ed (0.02)	57.71 ^A ±2.07	43.60 ^B ±2.01	34.61 ^B ±1.90	29.30 ^C ±0.92	**
Ed (0.05)	42.60 ^A ±1.88	27.70 ^B ±1.78	29.10 ^B ±1.70	18.90 ^C ±1.44	**
Ed (0.08)	34.90 ^A ±1.35	21.50 ^B ±1.15	23.01 ^B ±1.09	15.22 ^C ±1.01	**

Table (9) *In vitro* degradability % of experimental plant protein sources:

Ingredient	SFC	CSC	CRS	GNC	±SE
A	0.64 ^c	2.19 ^a	1.77 ^{ab}	1.28 ^{bc}	0.23
B	27.96 ^a	10.59 ^d	16.78 ^c	27.19 ^b	0.90
C	0.14 ^d	0.66 ^a	0.23 ^c	0.33 ^b	0.50
A+B	28.60 ^a	12.78 ^c	18.55 ^d	28.47 ^a	0.19

Means bearing different superscripts within rows are significantly (P<0.05) different

a-b means within the same raw followed by different superscripts are significantly (P< 0.05) different.

A: washing loss.

SE: Stander error of means.

B: water insoluble nutrients which is potentially degradable by microorganisms.

C: degradation rate of b/hours.

4.9 *In vitro* microbial crude protein digestibility% (gas production) / dcm:

Table (10) shows *in vitro* microbial digestibility (gas production) % of the different experimental protein sources are Groundnut cake (GNC), Sunflower cake (SFC), Crushed roselle seed (CRS) and Cottonseed cake (CSC) at different incubation time, for (3, 6, 12, 24, 48, 72 and 96 hrs). Groundnut cake revealed the highest ($P<0.01$) *in vitro* microbial crude protein digestibility% value (72.30%) at 96hrs incubation period. While SFC, CRS and CSC were recorded significantly ($P<0.01$) value of (67.81%, 65.25% and 61.64%) respectively for *in vitro* microbial crude protein digestibility % (gas production) at the same incubation period.

Table (10) *In vitro* microbial digestibility gas production/dc of experiential plant protein sources:

INCB\TIME	SFC	CSC	CRS	GNC	±SE	Sig
0	2.15 ^b	3.75 ^a	2.75 ^b	3.60 ^a	0.19	**
3	3.2 ^c	5.2 ^a	4.16 ^b	4.26 ^b	0.18	**
6	5.1 ^d	10.65 ^a	6.20 ^c	7.15 ^b	0.15	**
12	7.05 ^d	11.64 ^b	8.15 ^c	16.25 ^a	0.17	**
24	15.15 ^b	12.10 ^d	13.65 ^c	21.20 ^a	0.15	**
48	19.65 ^b	13.15 ^d	15.15 ^c	26.15 ^a	0.15	**
72	20.60 ^b	14.60 ^d	16.70 ^c	28.70 ^a	0.17	**
96	67.81 ^b	61.64 ^d	65.26 ^c	72.30 ^a	0.14	**
Dig%	67.81 ^b	61.64 ^d	65.26 ^c	72.30 ^a	0.14	**

Means bearing different superscripts within rows are significantly ($P<0.05$) different

4.10 Antioxidant activities of roselle seed (*Hibiscus sabdariffa*):

Table (11) revealed antioxidant analysis of undigested and digested crushed Roselle seed (*Hibiscus sabdariffa*). The result shows that digested roselle seed revealed highly level of antioxidant activities than undigested roselle for both test Di Phenyl Picryl Hydroxyl, (DPPH) and (Iron Chelating) activities.

4.11 Macro minerals of roselle seed (*Hibiscus sabdariffa*):

Table (12) shows the macro element profile of experimental plant protein sources. Results revealed a highly significant differences ($P < 0.01$) among the experimental protein sources in main minerals. SFC has a highly significance difference ($P < 0.01$) in Ca content of (0.375%) followed by CRS, CSC of (0.320 and .250%) respectively. GNC has the lowest ($P < 0.01$) in Ca content of (0.210%). CRS content highest significance difference ($P < 0.01$) level of Phosphorus (0.532%) while GNC recorded the lowest significance difference ($P < 0.01$) value of Phosphorus of (0.036%). Generally the highest significance difference ($P < 0.01$) in ash content signed by CRS OF (6.11%) followed by CSC of (5.82%) while SFC and GNC had a lowest significance difference ($P < 0.01$) in ash content (5.25% and 5.14% respectively).

Table (11) Antioxidant activities % of roselle seed:

No	Samples	DPPH \pm SD	Iron Chelating \pm SD
1	Crushed (<i>H.s</i>) undigested %	(22 \pm 0.06)	(26 \pm 0.03)
2	Crushed (<i>H.s</i>) digested %	(56 \pm 0.03)	(67 \pm 0.05)
3	(PG/ADTA) and (DMSO) %	(86 \pm 0.02)	(86 \pm 0.02)

DPPH: Di Phenyl Picryl Hydroxyl. **PG:** Propyl Gallate.

DMSO: Di Methyl Sulphoxide. *Hs:* *Hibiscus sabdariffa*.

Table (12) Macro minerals% of experimental plant protein sources:

MINERALS	SFC	CSC	CRS	GNC	\pm SE	Sig
Ca%	0.370 ^a	0.250 ^b	0.320 ^b	0.210 ^c	0.09	**
Na%	0.050 ^b	0.085 ^a	0.033 ^c	0.028 ^d	0.09	**
Mg%	0.360 ^a	0.260 ^a	0.305 ^b	0.180 ^c	0.06	**
P%	0.491 ^c	0.527 ^b	0.532 ^a	0.036 ^d	0.01	**
K%	0.886 ^d	1.240 ^b	1.330 ^a	0.984 ^c	0.07	**
Ash%	5.25 ^d	5.82 ^b	6.11 ^a	5.14 ^c	0.05	**
Mc%	1.770 ^d	2.545 ^b	2.720 ^a	0.233 ^d	0.01	**

Means bearing different superscripts within rows are significantly (P<0.05) different

Ca: Calcium **P:** Phosphorus.

Na: Sodium **K:** Potassium.

Mg: Magnesium **Mc:** Moisture content.

****:** Highly significant difference at (P<0.01).

4.12 PCR and amino acids concentration/mol of different roselle seed species:

Electrophoresis bands of roselle seeds extracted protein have been studied in multi accessions, the distribution of protein bands in the different accessions consist of (10) bands found in figure (4). Table (13) shows distribution of amino acid concentration of different species of Roselle seed. Results revealed large variations among different species amino acid concentration and recorded (99.08 %, 99.03 %, 98.02% and 97.89%) of total amino acid concentration% for (*Hibiscus sabdariffa*, *Hibiscus altissima*, *Hibiscus acetosella* and *Hibiscus cannabinus*) respectively. Also (*Hibiscus sabdariffa* and *Hibiscus altissima*) were recorded highest amount of essential amino acid value of (6.03, 5.93%) / mol–(5.17, 5.06%) / mol for glycine and lysine. In contrast *Hibiscus cannabinus* and *Hibiscus acetosella* were revealed highest value of (3.59, 2.59%) / mol of Methionine than above sample. PCR (Polymerase Chain Reaction) technique were revealed different length of (116, 116, 116 and 118) of DNA length (*Hibiscus sabdariffa*, *Hibiscus cannabinus*, *Hibiscus acetosella* and *Hibiscus altissima*,) respectively.

4.13 Bioinformatics *in silico* analysis of different roselle seed species:

Table (14) stated bioinformatics (*In silico* Information) of the four different species of Roselle seed are (*Hibiscus sabdariffa*, *Hibiscus cannibns*, *Hibiscus acetosella* and *Hibiscus altissima*) respectively. The results found that the above species were stable protein, and similar in number of mutation, but they were differed in position variations. The Phylogenetic of above species classified that (*Hibiscus cannibns*) was the first origin specie source followed by (*Hibiscus sabdariffa* and *Hibiscus altissima*) which are the second of origin. While (*Hibiscus acetosella*) represented the last origin source.

Table (13) PCR and amino acids concentration % / mol of different species of roselle seed:

No.	Amino acids	R.S % sabdariffa	No. A.L	R.S % cannibns	No. A.L	R.S% altissima	No. L.A	R.S% Acetosella	No. A.L
1	Alanine	9.48	11	3.62	10	1.17	12	9.48	11
2	Cystine	2.59	7	2.29	3	2.54	3	2.59	3
3	Asparagine	6.03	7	3.03	7	5.93	7	6.03	7
4	Glutamine	6.03	7	6.90	8	5.93	7	4.03	7
5	Phenilalanin	2.59	3	2.59	3	2.52	3	2.59	3
6	Glycine	6.03	7	3.17	6	5.93	7	5.16	6
7	Histidine	0.86	1	5.46	1	6.03	2	0.86	1
8	Isoleucine	3.45	4	5.45	4	3.39	4	3.45	4
9	Lysine	5.17	6	4.31	5	5.06	6	4.31	5
10	Leucine	6.03	7	5.17	6	4.93	7	5.17	6
11	Methionine	2.59	3	3.59	3	2.54	3	2.59	3
12	Asn	6.03	7	5.17	6	5.65	7	6.90	8
13	Proline	6.00	8	3.40	8	7.78	8	6.90	8
14	Gin	1.72	2	6.72	2	2.54	3	1.72	2
15	Arginie	5.17	6	3.03	7	5.93	7	6.06	7
16	Serine	3.45	4	9.03	7	4.24	5	5.17	6
17	Therionine	5.17	6	4.31	5	4.24	5	5.17	6
18	Valine	6.90	8	2.26	9	7.78	8	6.03	7
19	Tryptophan	5.17	6	8.57	6	4.24	5	5.17	6
20	Tyrocine	8.62	10	7.82	10	5.63	9	8.62	10
21	Total	99.08	116	97.89	116	99.03	118	98.02	116

A.L: Amino acid length

PCR: Polymerase chain reaction

Table (14) Bioinformatics or *in selico* analysis of different species of roselle seed:

Item	<i>Hibiscus sabdariffa</i>	<i>Hibiscus cannabinus</i>	<i>Hibiscus acetosella</i>	<i>Hibiscus altissima</i>
Amino acid concentration	116 Length	116 Length	116 Length	118 Length
Molecular weight	13469.52 Daltons	13530.51 Daltons	13516.44 Daltons	13598.64 Daltons
Instability index	Stable protein	Stable protein	Stable protein	Stable protein
Number of Mutations	3 Mutation	4 Mutation	4 Mutation	3 Mutation
Position of mutation	(238 - 258 and 252)	(177 - 178 - 233 and 237)	(212 – 228 – 236 and 237)	(238 - 258 and 242)
Phylogenetic classification	Second Source	First source origin	Thread source	Second source

4.14 Antimicrobial activities/mm of roselle seed (*Hibiscus sabdariffa*):

Anti-microbial activities of Roselle seed (*Hibiscus sabdariffa*) was shown in table (15). The results revealed that Roselle seed had highly activities concentration against anti-microbial for (*Escherichia Coli*,). While it had moderate activities concentration against anti- microbial for (*Staphylo coccus auerus*, *Psodomonus arginosa* and *Bacillus substillus*,) respectively.

4.15 Antifungal activities of roselle seed (*Hibiscus sabdariffa*):

Table (16) revealed anti-fungal test of Roselle seed (*Hibiscus sabdariffa*) against two type of fungal. Results of this study revealed that roselle seed are highly activities of anti-fungal against (*Candida albicans*). However moderate activities of anti-fungal against (*Aspergillus niger*).

4.16 Phytochemical screening of specific compound of roselle seed (*Hibiscus sabdariffa*):

Table (17) shows phytochemical screening for active test of (*Hibiscus sabdariffa*) in phytochemical specific compounds. The chemical compounds of (*Hibiscus sabdariffa*) that had negative test of those compounds (Tanine, Saponins, Anthraquinone glycosides and Flavonoids). While recorded positive results in (Alkaloids, Sterol, Coumarins, Tritreptine and Cyanogenic) for moderate, high, high, low and moderate concentration respectively.

4.17 Neutral detergent fibers (NDF) % of experimental cakes and crushed roselle seed (*Hibiscus sabdariffa*):

Table (18) includes neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) content of protein sources. NDF is the common measure of fiber use for animal feed analysis, but also does not a unique class of compound. (NDF) is the most structural in the plant e.g lignin, cellulose and hemicelluloses. CSC was recorded the highest significant difference ($P<0.01$) in (NDF, ADF, ADL, cellulose and hemicelluloses) of (60.17, 30.07, 16.32, 30.35 and 13.75) respectively. While GNC recorded the highly significant difference ($P<0.01$) in ADL % of (17.12%).

Table (15) Biological test of Anti-microbial Activities / mm of Roselle seed (*Hibiscus sabdariffa*):

Samples	Bacterial Species	Result
1	<i>Escherichia. Coli</i>	(21)mm
2	<i>Staphylo coccus arrius</i>	(19)mm
3	<i>Psodomonus arginosa</i>	(17)mm
4	<i>Bacillus subtillus</i>	(15)mm

mm: diameter zone for reading activities against anti-microbial around media growth.

From 10mm – 14 mm extract weak activities against bacteria.

From 15mm – 19 mm extract moderate activities against bacteria.

From 20 mm – above extract highly activities against bacteria.

Table (16) Biological test of Anti-Fungal Activities / mm of Roselle seed (*Hibiscus sabdariffa*):

Samples	Bacterial Species	Result
1	<i>Candida albicans</i>	(22) mm
2	<i>Aspergillus niger</i>	(19) mm

mm: Diameter zone reading for anti-fungal activities around media growth.

From 10 mm– 14 mm extract weak activities against fungal.

From 15mm – 19 mm extract moderate activities against fungal.

From 20 mm – Above extract highly activities against fungal.

Table (17) phytochemical screening of specific compounds of roselle seed (*Hibiscus sabdariffa*):

No	Chemical compound test	Result
1	Saponins	(_)
2	Tannin	(_)
3	Sterol	(+ +)
4	Tritreptine	(+ + +)
5	Alkaloids	(+ + +)
6	Cumarins	(+)
7	Anthraquinone glycosides	(_)
8	Cyanogenic	(+ +)
9	Flavonoids	(_)

(+) Low antioxidant concentration. (+ +) Moderate antioxidant concentration.

(+ + +) High antioxidant concentration. (—) Negative values of antioxidant concentration.

Table (18) Neutral Detergent fiber % analysis of experimental plant protein sources:

INGREDIENT	SFC	CSC	CRS	GNC	±SE	Sig
NDF	51.19 ^c	60.17 ^a	37.50 ^d	52.20 ^b	0.17	**
ADF	25.40 ^b	30.07 ^a	19.90 ^c	25.12 ^b	0.49	**
ADL	16.36 ^b	16.32 ^a	15.32 ^c	17.12 ^a	0.15	**
CELLULOSE	25.85 ^b	30.35 ^a	17.62 ^c	26.05 ^b	0.21	**
HMICELLULOSE	9.06 ^b	13.75 ^a	4.42 ^c	9.46 ^b	0.30	**
LIGNIN	16.35 ^b	16.32 ^b	15.32 ^c	17.12 ^a	0.14	**

Means bearing different superscripts within rows are significantly (P<0.05) different

NDF: Neutral detergent fiber.

ADF: Acid detergent fiber.

ADL: Acid detergent lignin.

SFC: sunflower cake.

CSC: Cottonseed cake.

CRS: Crush roselle seed.

GNC: Groundnut cake.

4.18 Body weight and daily body weight gain / kg of experimental animal groups:

Table (19) demonstrated initial body weight and final body weight/kg of the experimental animal groups fed roselle seed for (42) days for group (A, B, C, and D) respectively. Results revealed that initial live body weight did not show significant different ($P>0.05$) among experimental animal groups (21.47 ± 0.35 - 21.78 ± 0.45 - 21.83 ± 0.34 and 21.99 ± 0.33 kg) for group A, B, C and D respectively. In contrast there is a significant difference within animal groups in initial and final body weight gain. However did not show significant different ($P>0.05$) among animal groups (29.83 kg ± 1.32 – 30.33 kg ± 1.64 , 31.66 kg ± 1.14 – and 33.00 kg ± 0.98), in final body weight for group A, B, C and D respectively.

4.19 Nitrogen balance% in feces and urine of experimental animals groups:

Table (20) includes crude protein content of four experimental animal groups (A, B, C and D) respectively. Diet (C) recorded the highest significance difference ($P<0.01$) for amount of nitrogen in the feces, while diet (D) showed the highest significance difference ($P<0.01$) value in amount of nitrogen in urine and retained.

4.20 Feces proximate analysis % of experimental animal groups:

Table (21) shows proximate analysis of crude protein in feces of the four experimental animal groups. The results recorded highly significance ($P<0.01$) among experimental groups. Animals in group (D) exerted higher C.P% in feces of (22.59%) followed by group (A) (21.57%). Group (C) recorded (20.36%) finally group (B) had (19.57%) crude protein in feces.

4.21 Overall feedlot performance of roselle seed fed different levels in lambs fattening:

Overall feedlot performance values of experimental lambs fed Roselle seed for (42) days shown in table (22) fed graded level of (CRS) in the diet (0%, 10%, 20% and 30%) for group (A, B, C and D) respectively. Daily feed intake showed no significant differences ($P>0.05$) among the experimental lambs (1.60 ± 1.03 , 1.64 ± 0.97 , 1.65 ± 1.18 and 1.60 ± 1.05) kg dry matter intake for group A, B, C and D respectively. Average total live weight gain and daily weight gain was recorded (8.36 ± 0.65 - 8.46 ± 1.22 - 9.38 ± 0.45 and 11.01 ± 1.22) kg, and (0.200 ± 1.13 – 0.200 ± 1.098 – 0.220 ± 1.09 and 0.240 ± 0.95 g) for group A, B, C and D respectively. While the average feed conversion ratio (FCR) was showed highly significant differences ($P<0.01$) among the experimental animal groups (8.36 ± 0.19 – 8.55 ± 0.32 – 7.16 ± 0.48 and 6.42 ± 0.65) for group A, B, C and D respectively.

Nitrogen retained of experimental animal groups was signed a highly significant differences ($P<0.05$) (35.64 ± 0.02 – 33.52 ± 0.02 – 31.49 ± 0.02 and 31.19 ± 0.02) for group D, C, B and A respectively.

While the economical evaluation study revealed highly significant differences ($P<0.05$) between total cost / SDG ingredient of diet which was found (3.03 ± 0.33 – 2.96 ± 0.34 – 2.74 ± 0.87 and 2.52 ± 0.23) for total price cost /SDG of 1kg DM of experimental diet.

Table (19) Body weight and final body weight gain of experimental animal groups:

Group s	Initial weight \pm S D	Week1 \pm S D	Week2 \pm S D	Week3 \pm S D	Week4 \pm S D	Week5 \pm S D	Week6 \pm S D	Si g
A 0% CRS	21.47 \pm 0.3 5	22.57 \pm 0.37	24.15 \pm 1.20	25.50 \pm 0.70	26.33 \pm 0.94	28.97 \pm 0.94	29.83 \pm 0.71	*
B 10% CRS	21.78 \pm 0.4 5	22.69 \pm 0.38	23.80 \pm 0.70	25.74 \pm 0.21	27.17 \pm 1.18	28.33 \pm 0.23	30.33 \pm 1.41	*
C 20% CRS	21.83 \pm 0.3 4	22.95 \pm 0.24	24.93 \pm 1.22	26.88 \pm 0.28	27.94 \pm 0.45	28.89 \pm 0.46	31.66 \pm 0.23	**
D 30% CRS	21.99 \pm 0.3 3	22.76 \pm 0.26	25.13 \pm 1.83	27.50 \pm 0.95	28.84 \pm 0.87	30.60 \pm 1.37	33.00 \pm 0.23	**
Sign	NS	NS	NS	NS	NS	NS	NS	-

Means bearing different superscripts within rows are significantly ($P < 0.05$) different

NS: not significant difference $p > 0.05$. **: highly significant $p < 0.01$

*: significant $p < 0.05$

Table (20) Crude Protein content of experimental diet groups:

Groups	N-intake%	N-in feces%	N-in urine%	N-retained%
A	35.58 ^d	16.44 ^d	14.67 ^b	31.19 ^d
B	38.50 ^a	18.12 ^b	13.38 ^d	31.49 ^c
C	37.64 ^b	18.97 ^a	14.22 ^c	33.52 ^b
D	37.40 ^c	17.10 ^c	18.42 ^a	35.19 ^a
SE	0.03	0.01	0.02	0.02
Sig	**	**	**	**

Means bearing different superscripts within rows are significantly ($P < 0.05$) different

** : highly significant different at ($P < 0.01$)

N: nitrogen.

SE: stander error

Table (21) Feces proximate analysis of experimental animal groups:

Groups	DM%	CP%	CF%	EE%	Ash%	NFE%
A	89.33 ^b	21.57 ^b	19.40 ^c	2.03 ^a	3.09 ^d	43.24 ^a
B	84.45 ^d	19.57 ^d	20.21 ^b	1.65 ^c	5.51 ^c	37.53 ^c
C	84.71 ^c	20.36 ^c	17.62 ^c	1.54 ^d	6.66 ^b	38.54 ^b
D	89.57 ^a	22.59 ^a	21.51 ^a	1.90 ^b	7.83 ^a	35.75 ^d
SE	0.03	0.3	0.03	0.02	0.02	0.02
Sig	**	**	**	**	**	**

Means bearing different superscripts within rows are significantly (P<0.05) different

******: highly significant different at (P < 0.01)

SE: stander error.

DM: dry matter.

CP: crude protein.

CF: crude fiber.

EE: ether extract.

NFE: nitrogen free extract.

Table (22) Overall feed lot performance of experimental lambs:

Item	Group A	Group B	Group C	Group D	SE	LSD
Number of Animals	6	6	6	6	–	–
Feed lot period / day	42	42	42	42	–	–
Ave. Initial body weight (Kg)	21.47	21.78	21.83	21.99	0.64	NS
Ave. final body weight (Kg)	29.83	30.33	31.66	33.00	0.22	NS
Total live weight gain (Kg)	8.36	8.55	9.83	11.01	0.95	NS
Daily wt. gain (g/head/day)	200	200	220	240	0.58	NS
feed intake (Kg/head/day)	1.69	1.65	1.65	1.61	0.87	NS
Feedconversion efficiency (Kg DM/Kg gain)	8.36 ^a	8.55 ^b	7.16 ^c	6.42 ^d	0.06	**
N intake	35.58 ^d	38.50 ^a	37.40 ^c	37.64 ^b	0.03	**
N retained	31.19 ^d	31.49 ^c	32.04 ^b	35.52 ^a	0.02	**
Cost of 1 Kg DM. (SDG)	3.03 ^a	2.96 ^b	2.74 ^c	2.52 ^d	0.06	**

Means bearing different superscripts within rows are significantly ($P < 0.05$) different

NS: No significant difference $P (> 0.05)$.

** : Highly significant difference $P (< 0.001)$.

SE: Stander error of means.

SDG: Sudanese geneth.

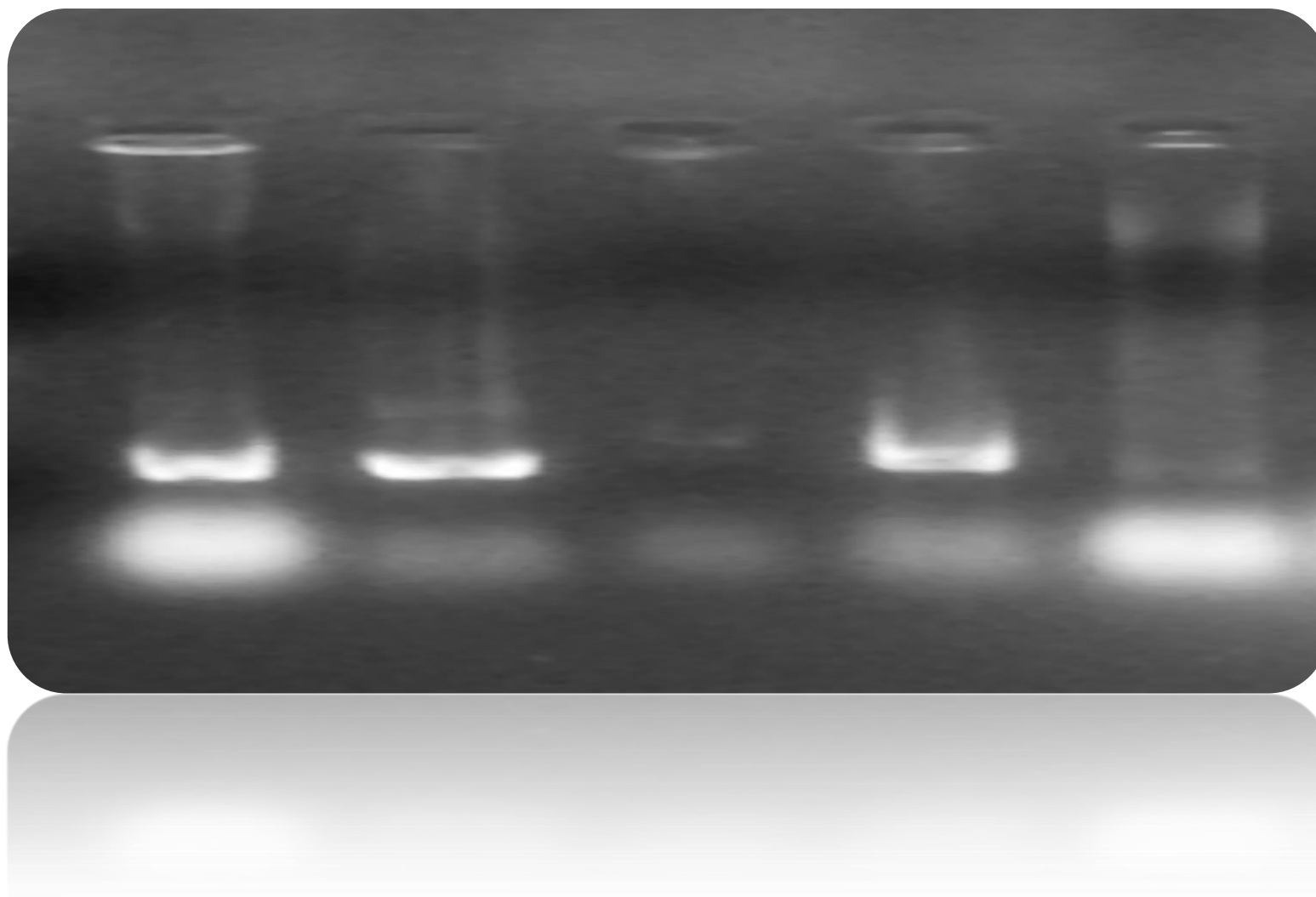


Figure (5) DNA band of (*Hibiscus sabdariffa*) El Rahad variety

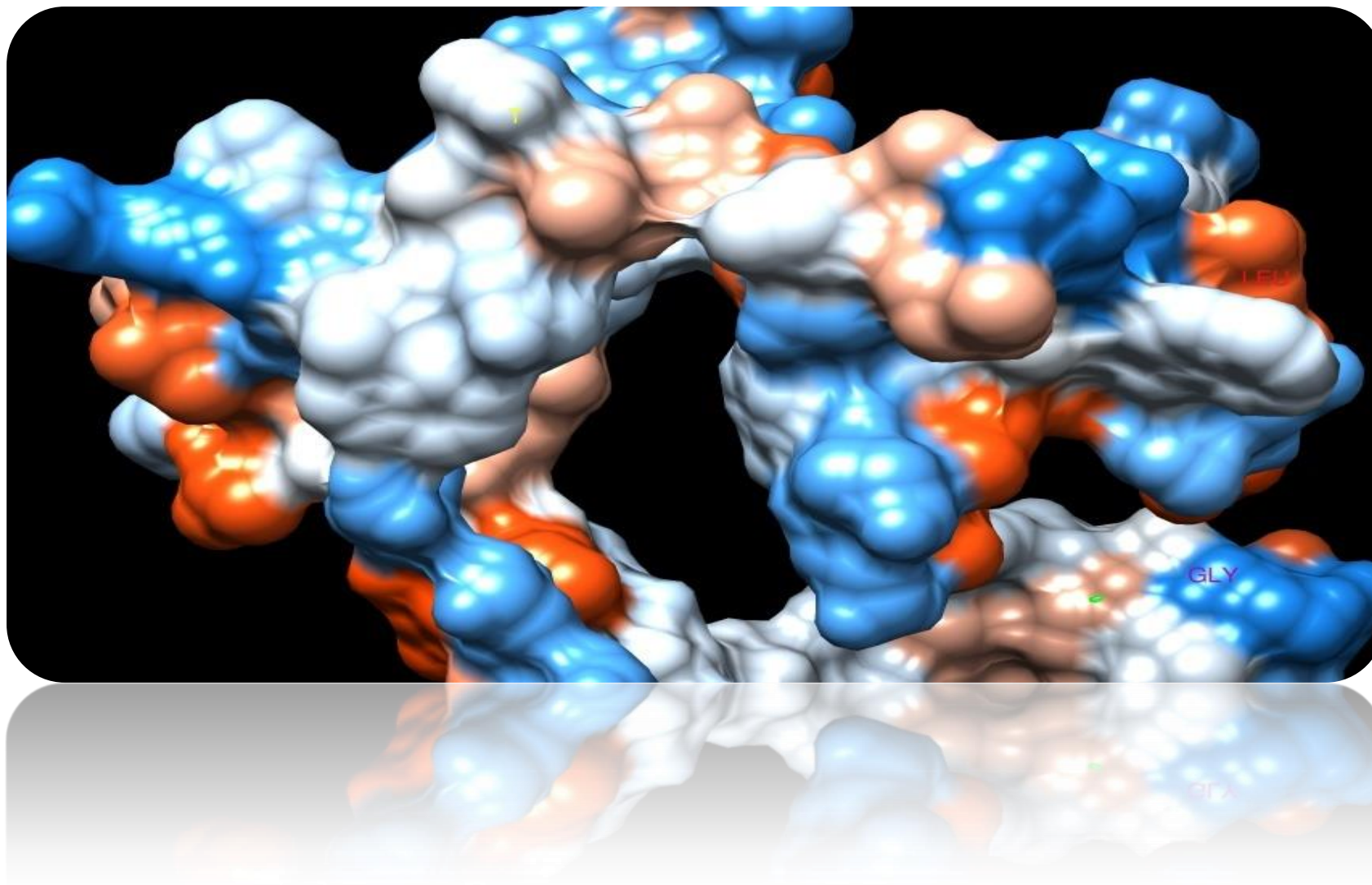


Figure (6) (*Hibiscus sabdariffa*)3D Protein Structure Mode

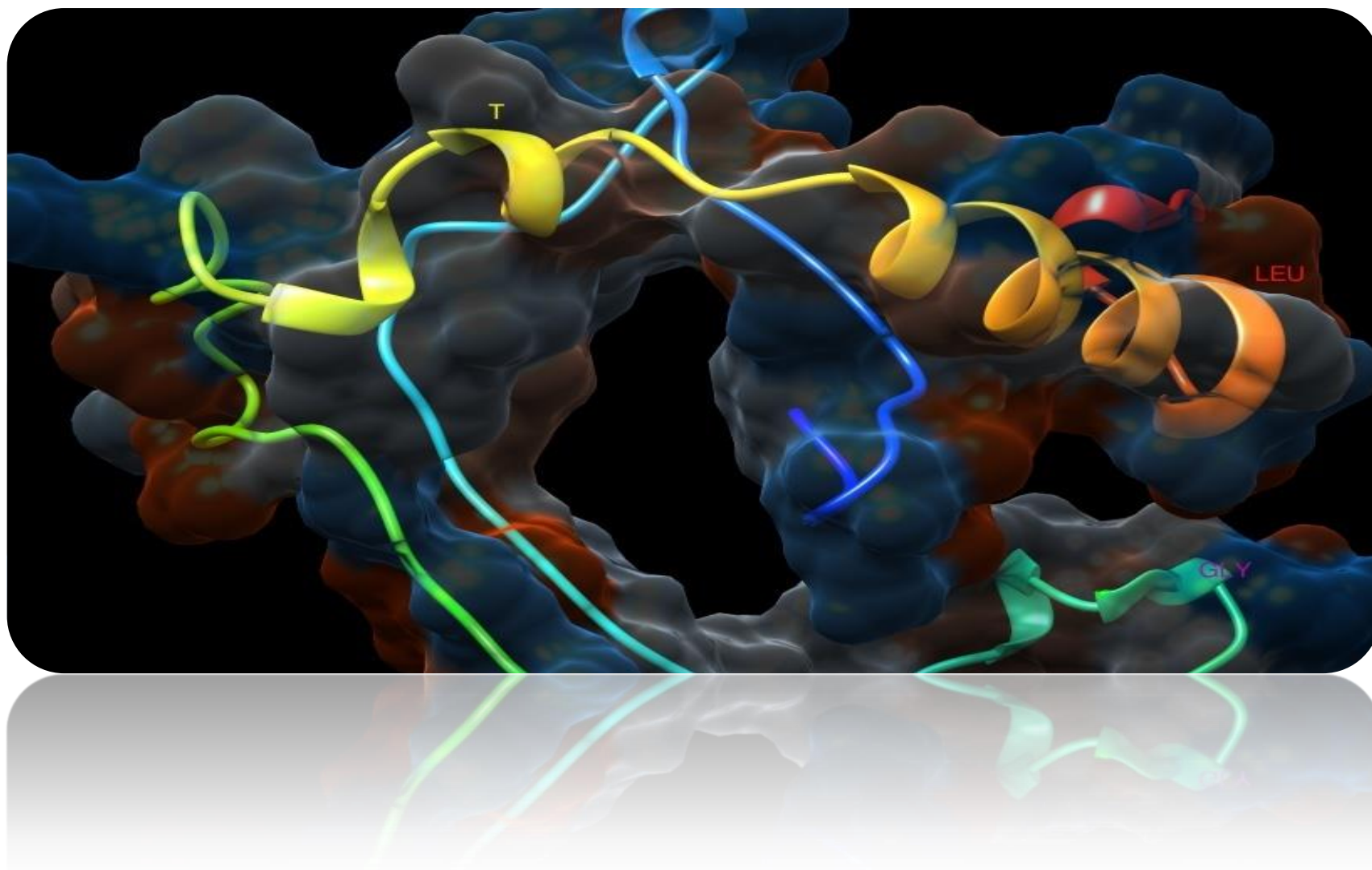


Figure (7) (*Hibiscus abdariffa*) Transparent 3D Structure Model

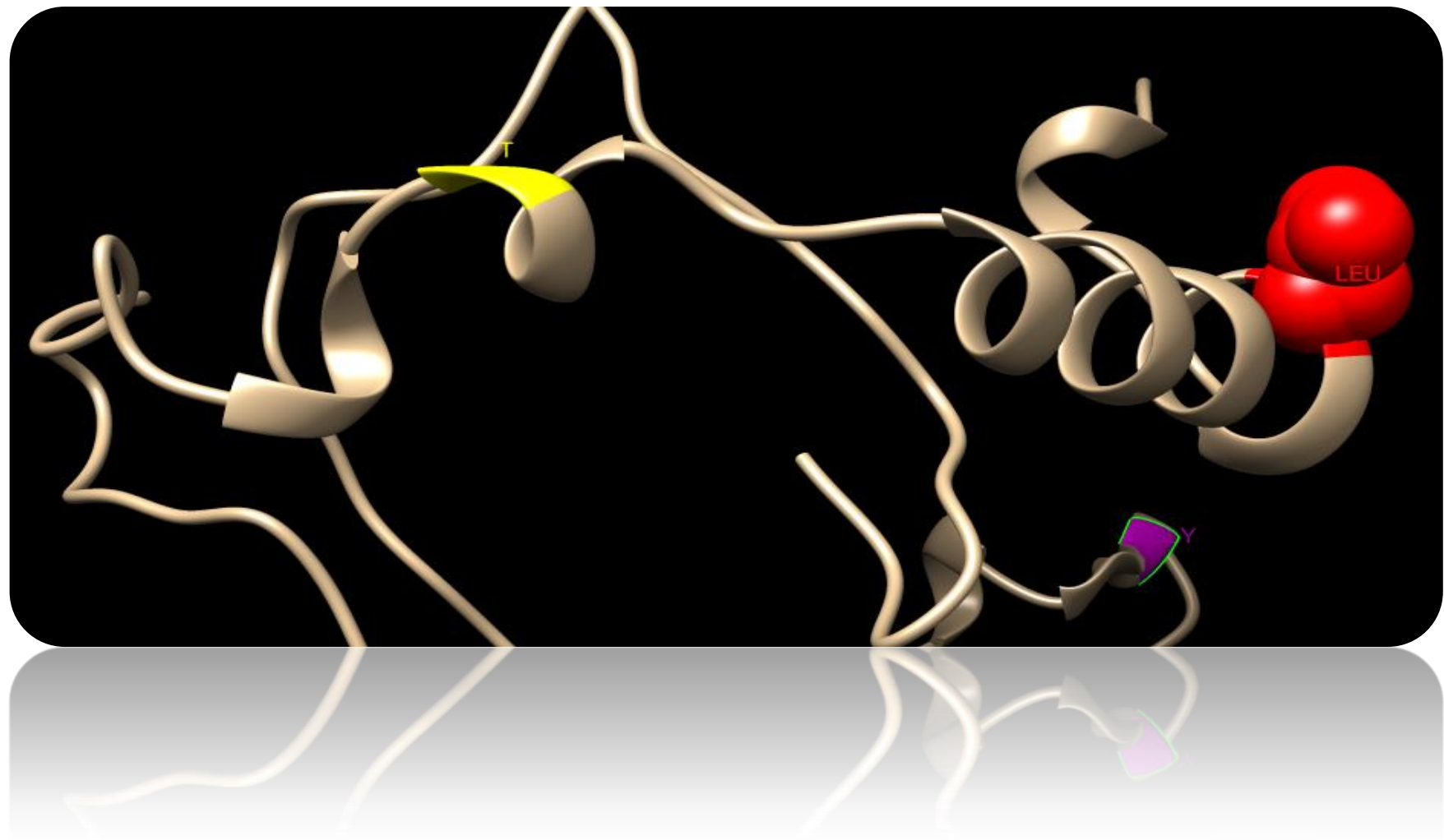


Figure (8) (*Hibiscus sabdariffa*) Carton model 3D Structure

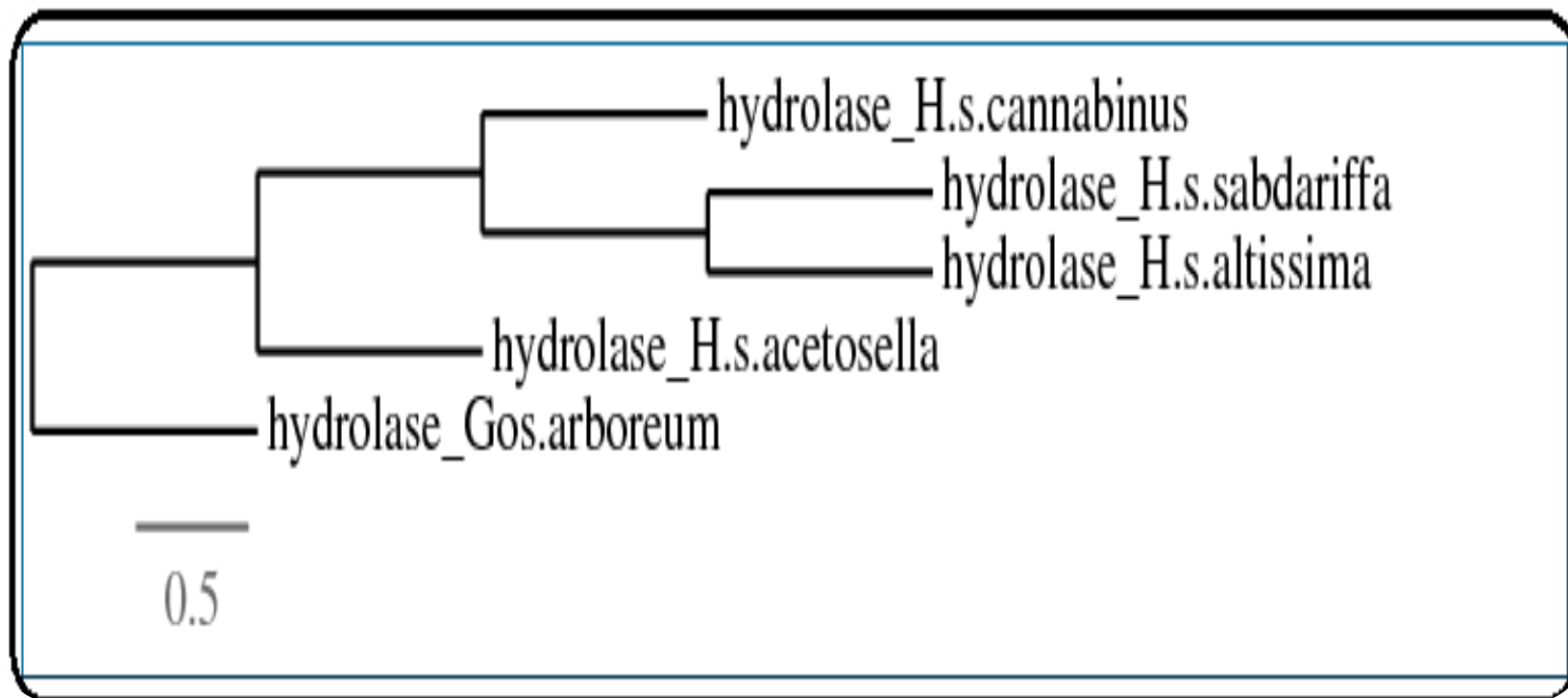


Figure (9) Phylogenetic of four (*Hibiscus*) species and *Gos.Arboareum*

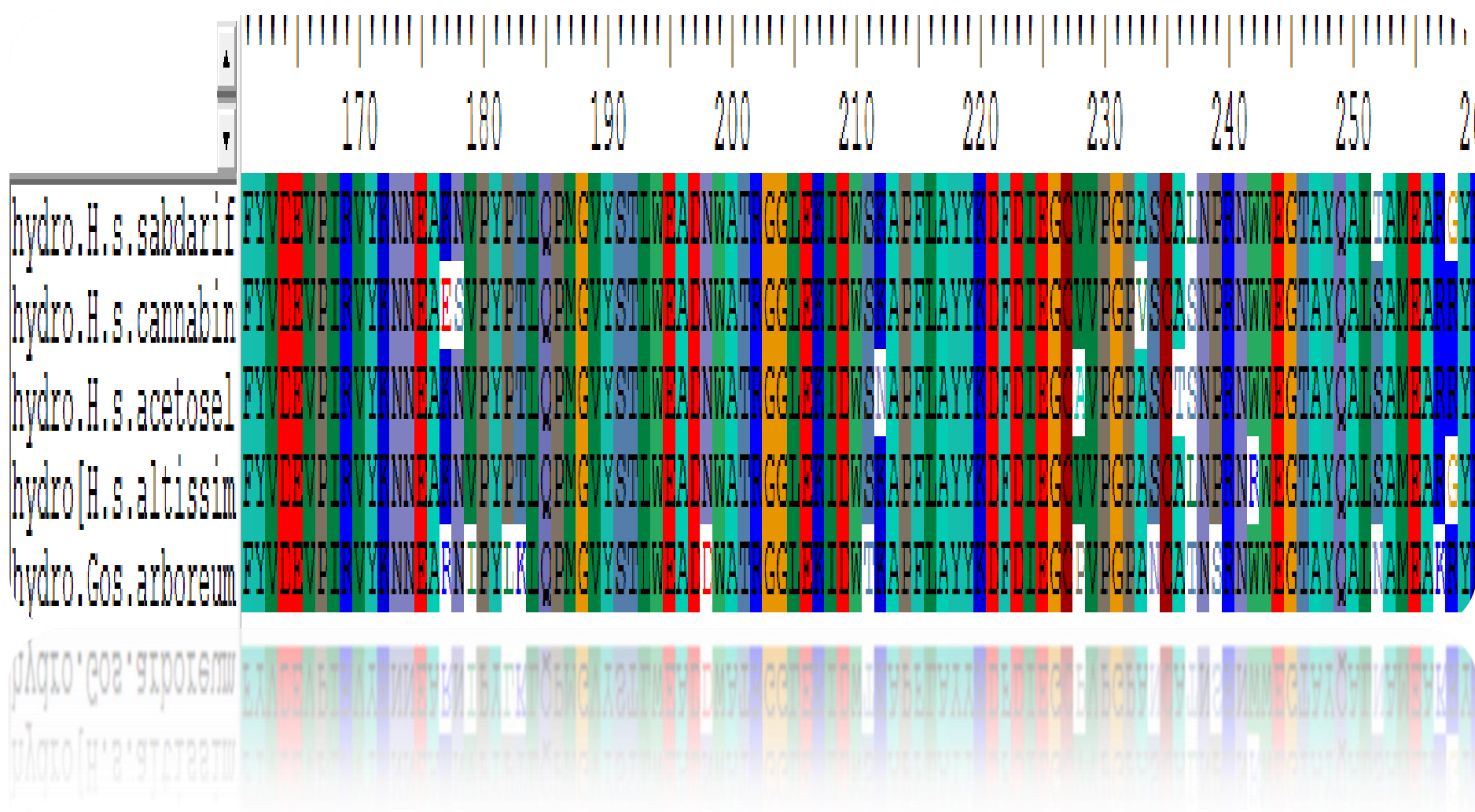


Figure (10) different mutation of four (*Hibiscus*) species and *Gos. Arboreum*

CHAPTER FIVE

Discussion

5:1 Chemical composition of experimental plant protein cakes and crushed roselle seed:

The results of the chemical composition of four plant protein sources Sunflower cake (SFC), Cottonseed cake (CSC), Crushed roselle seed (CRS) and Groundnut cake (GNC) are shown in table (2) dry matter and crude protein were (97.34 – 26.91)%, (96.91 – 25.16)%, (96.49 – 27.38)% and (96.33 – 42.33)% respectively. These results were in range of those obtained by (Turki and Abdelrahman, 2011) who mentioned that the chemical composition and characteristic of dry matter and crude protein of GNC, SFC and CSC were (96.4 – 40), (94.6 – 25.3) % and (95.7 – 27.5)% respectively. Moreover they determined that GNC are unstable protein, while SFC and CSC are stable proteins according to the high molecular weight and amino acid concentration of cakes. This result was not including roselle seed. On the other hand investigation by (Mabrouk, 2002) who reported that chemical composition of SFC, CSC and GNC, dry matter and crude protein was (94.6 – 26.9)%, (95.6 – 24.3)% and (95.4 – 24) % respectively. While the chemical composition of Roselle seeds in the present study recorded (96.49 – 27.38 – 18.09 and 11.98) % of dry matter, crude protein, crude fiber, ether extract and ash respectively. This result agree that obtained by (Mukhtar, 2007) who reported that chemical composition of roselle seed recorded (91.8 – 21.35 – 11.98 and 5.34) % for dry matter, crude protein, crude fiber and ash respectively. Abdelrahman *et al*, (2016), El Adway and Khalil, (1994) also evaluated the chemical composition of roselle seed and found that the whole seeds contained (21.02 – 30.11) % crude protein. This amount of crude protein % is in line with the present study and support breeders to use roselle seed as alternative protein source in animal feeds.

5:2 Proximate analyses % of experimental rations and roughages (groundnut hay):

Chemical analysis of four experimental diet ingredients and roughages were found in table (3) and (4) formulated according to fitween program, this experiment includes four treatments with four different levels of roselle seeds (0, 10, 20, and 30%) for groups A, B, C and D respectively. The ratio was Iso-caloric and Iso- nitrogenous. Diet (D and C) was found the best ratio reflected the higher body weight gain performance in feedlot experiment, followed by diet (B and A) were recorded the lowest body weight gain. This result in line with that obtained by (Hassan, 2010), used roselle seeds in defferent levels (0%, 15% and 30%) and reported (92.52- 91.24%), (22.88-20.74%), (22.33-23.26%), (7.30-5.68%) and (12.50-10.80%) for DM, C.F,C.P, E.E and ash respectively.

5:3 Degradability trails:

5:3:1 Dry matter disappearance% of experimental plant protein sources:

Significant differences ($P < 0.05$) were obtained among the oilseed cakes for dry matter and crude protein degradability in term of feed samples and incubation time table (5) figure (11). In the present study (GNC) had highly significant differences ($P < 0.01$) for dry matter disappearance percentage after 72hrs which had $(88.22 \pm 0.83) \%$, $(78.54 \pm 1.47) \%$, $(76.87 \pm 2.03) \%$ and $(74.55 \pm 1.04) \%$ for GNC, SFC, CRS and CSC respectively. Thesis results are in agreement with those obtained by (Adnan *et al*, 2010; Turki and Actham , 2011) who had investigated degradability and characterization of some oilseed cakes using nylon bags technique. They report that groundnut cake had the highest dry matter disappearance percentage% and signed $(94.40, 80.20, 78.20) \%$ for GNC, CSC, and SFC respectively, at incubation time 48hrs. This result not include CRS, while in the present study (*Hibiscus sabdariffa*) was recorded medium dry matter disappearance% and signed $(76.87 \pm 2.03) \%$ at incubation

time 72hrs. Because high cell wall of roselle seed reduce micro-organisms attack, also they are many factors may be affected degradability of DM and CP in the rumen. Variation in the degradation values may could be referred to the season, samples washing procedure of nylon bags, animal digestive system performance...etc. (Nocek , **1997**).

5:3:2 Dry Matter effective degradability% of experimental plant protein sources:

Table (6) showed highly significant difference ($P<0.01$) on dry matter effective degradability % GNC was recorded highest degradability in the rumen followed by CRS and SFC while CSC recorded the lowest degradation rate compared to other cakes and were signed (92.57 ± 3.93 , 83.41 ± 1.26 , 81.43 ± 1.33 and 80.99 ± 1.51)% for GNC, CRS, CSC and SFC respectively. Potential degradability (Pd) of CRS at outflow rate $K= 0.02$ were recorded lowest Pd (63.31 , 67.59 , 63.88 and 74.50) % for CRS, SFC, CSC and GNC respectively. This result in line of that obtained by (Abdelrahman, **2011**) who found that the dry matter effective degradability % of GNC, CSC and SFC were recorded ($88.6\pm1.43 - 80.5\pm1.45$ and 74.6 ± 1.82) % respectively. Degradability of organic dry matter in rumen refer to the activities of micro-organisms, because ruminates therefore evolved a special system of digestion that involved fermentation of feed to produce volatile fatty acid (VFA). In the present study low degradability of crushed roselle seed referred to many factors such as strong wall cell, biological characteristic of anti-microbial, antifungal activities and high molecular weight. Results of the present study showed variation in degradation values may be referred to seasons, sample washing procedures and animal digestive performance (Niekerk and Gasey, **1988**).

5:3:3 Crude protein disappearance % of experimental plant protein sources:

Crude protein disappearance % of experimental samples found in table (7) and figure (12). Cottonseed cake signed the lowest crude protein disappearance % followed by CRS and SFC they signed medium crude protein disappearance %, respectively at incubation period 72hrs. GNC was recorded a highest crude protein disappearance % of (77.74 % \pm 1.53), compared to the others those oilseed cakes at incubation period 72hrs, finding in this result was similar to that obtained by (Abdelrahman, 2011) who recorded that GNC had the highest degradable crude protein value of disappearance % in the rumen and were signed (97.68, 88.64 and 78.31) for GNC, CSC and SFC respectively at incubation period 48hrs. Roselle seed in present experiment reflected medium degradation values, this amount of protein provide animal by protected protein from micro-organisms attack. Because the end product of microbial fermentation becomes available as energy volatile fatty acid (VFA) (Pen, 2007).The reason of variation between these studies and others may refer to samples, experimental animal and environmental condition.

5:3:4 Crude protein effective degradability% of experimental plant protein sources:

Crude protein effective degradability from the rumen is shown in table (8) the result demonstrated that the different oilseed cakes affected crude protein degradability at $k = 0.02$ were signed a significant difference ($P < 0.01$) observed among experimental oilseed were (57.71% \pm 2.07, 43.60 % \pm 2.01, 34.61 % \pm 1.90 and 29.30 % \pm 0.92) for GNC, SFC, CRS and CSC respectively. While *in vitro* degradability found in table (9) demonstrated that CSC, recorded the lowest *in vitro* degradability % followed by CRS, GNC and SFC were (12.78 %, 18.55%, 28.47% and 28.60%) respectively. This result disagrees with result observed by (Abdelrahman, 2011) who had investigated the crude protein

effective degradability of some Sudanese oilseed cakes they recorded crude protein effective degradability values of (92.9%, 90.01% and 78.6%) for GNC, CSC and SFC respectively. Also (Turki and Actham, **2011**) reported that characterization of GNC, CSC and SFC were (16135.5 Dalton-141%), (13706.5 Dalton – 159%) and (23834.6 Dalton – 207%) respectively. for molecular weight and amino acid concentration. These results classified that SFC and CSC are stable protein while GNC are un- stable protein according to molecular weight and amino acid sequence of cakes. This result agree with that obtained by (Mohammed, **2008**) who found that GNC degradable protein in rumen recorded (88.98 % , 82.24 % , 87.21 % and 71.02 %) for GNC treated GNC by HCL control, HCL air, HCL spray and HCL heat respectively. Results of different treated GNC reduce the degradation rate in rumen but at the same time effect at protein solubility in small intestine.

5:4 *In vitro* microbial digestibility (gas production) volume / ml of experimental cakes:

Table (10) and figure (13) shows the gas the production volume / ml at different incubation times. In the present study gas production volume showed increase *in vitro* digestibility of different experimental plant protein sources as was reflected by greater gas production volume. GNC (*Arachis hypogeal*) was recorded highest ($P<0.01$) gas production volume / ml at different incubated time, from initial (6)hrs GNC started increase in gas production volume / ml and signed (3.60 – 4.26 – 7.15 – 16.25 – 21.20 – 26.15 and 28.70 /ml) for 3, 6, 12, 24, 48, 72 and 96 hrs respectively. While both of SFC (*Heliuth annuus*) and Crush roselle seeds CRS (*Hibiscus sabdariffa*) were recorded minimum ($P<0.01$) gas production volume /ml and signed (3.2 – 4. 20 /ml), (5.10 -6. 20 /ml), (7.05 -8.15 / ml), (15.15 – 13.65 / ml), (19.65 -15.16 / ml) and (20.60 – 16.70 ml) for SFC and CRS at 3, 6, 12, 24, 48, 72 and 96hrs incubation times respectively. However CSC (*Gossypium barbadanse*), recorded the lowest

($P < 0.01$) gas production volume / ml at different incubated time started from 24 up to 96 hrs and recorded (11.64 – 12.10 – 13.15 – and 14.40 / ml) for gas production volume / ml. This result agree with that obtained by Idris *et al*, (2012), who was investigated gas production of some Sudanese grasses and found that the gas production volume were recorded (23.8 ± 1.13 – 22.2 ± 1.10 / ml), (37.1 ± 1.17 – 29.4 ± 1.19 / ml), (43.1 ± 1.10 – 30.9 ± 1.20 / ml), (48.5 ± 0.98 – 30.5 ± 1.00 / ml) and (51.3 ± 1.51 – 37.6 ± 1.34 / ml) of Groundnut cake in comparison to roselle seed for (4, 8, 12, 24 and 48 hrs) respectively. This result agree with those by Idris *et al*, (2012) was mentioned that hemicelluloses and cellulose content of roselle seed might explain the lower organic dry matter digestibility. In the present study the lignin and tannin reduce micro – organism's activities this result is similar to that reported by Ahmed and El Hag, (2004) and khazal *et al.*, (1993), who recorded that anti- nutritional factors of Tannin decrease the digestibility of dry matter. High content of cell wall structure may restrict micro-organism activities. (Carvalho *et al*, 2005). The gas production volume in this study was lowered, and decreases the degradability of dry matter and crude protein. The gas production volume of different plant protein sources at different time intervals showed that there are relationship between degradability *in vivo* and gas production (carbon dioxide and methane) *In vitro* digestibility, when the feeding stuff is incubated with rumen liquor for 96 hours. It can be used for estimation of digestibility of organic matter and metabolizable energy of grass and protein (Steingass and Menke 1988; and Steingass 1988). In this study the gas production of some plant protein source at different time intervals recorded to lower level compared to the study carried by Ahmed and El Hag, (2004). The variation between two studies may be due to many factors, such as oilseed species, varieties, season and rain fed, all these factors may affect the plant components. The gas production results of oil seeds and Roselle seed are in agreement with (Kubuga and Darko, 1993; and Blummel and Orskov, 1993).

5:5 Antioxidant activities % of Roselle seed:

The radical scavenging activities (RSA) of *Hibiscus sabdariffa* at concentration of 500µ/m shown in table (11). There are many published reports on the constituents of different plant parts of *Hibiscus sabdariffa*, which have been summarized briefly by Ross, (2003). Two antioxidant tests against (DPPH) Di-phenyl picryl-hydrazyl \pm SD and Iron chelating activities \pm SD as indicators were used to examine digested roselle seed by pepsin and picric acid, and undigested roselle seed. From present study results of undigested roselle seed recorded low antioxidant activities value (22 ± 0.06 %, 26 ± 0.02 %) for tow test control (DPPH) Di-phenyl picryl-hydrazyl and Iron chealting activities respectively. In contrast *in vitro* protein extracted from roselle seed after digestion by pepsin and picric acid was showed high antioxidant result and recorded (56.33 % ± 0.03 - 67 ± 0.05 %) for DPPH and iron chelating activities respectively. While control Propyl Gallate and DMSO were recorded the highest antioxidant activities value (86 ± 0.02 %) for both above test. This result in line of result investigated by Eunkyung *et al*, (2013) who found that roselle seed showed high antioxidant in extract of ethanol 80% and concentration of scavenging 500 showed (74.59 ± 0.57 % - 46.11 ± 0.63) for roselle extracted by water and ethanol 80% respectively against DPPH test. In present study results showed that highly activity against DPPH free radical and iron chelating test of roselle digested by enzyme than crushed roselle seed untreated by enzyme and picric acid. This result similar to that obtained by (Guo *et al*, 2001), who found that digested of *Hibiscus sabdariffa* seeds by pepsin and picric acid for (1hr) have shown greater antioxidant activity ranged (44.01 % , 70.47 % and 76.66 %) for roselle Albumin, roselle Globulin and roselle Glutamine for DPPH and Irion chelating activities respectively. On the other hand Norhaizan, (2010) who reported that roselle seed extracts were found to have the highest antioxidant activity and strongest radical-scavenging activity of all plants tested. This study

suggested that roselle seeds have the potential to be used as food / feed antioxidants. Eunkyung *et al*, (2013) reported that roselle is capable of providing a source of minerals, natural antioxidant, as well as enhancing mineral availability from other food sources through the low pH of the extracts. This result agreed the present study which may draw attention that roselle seed can be used as natural antioxidant, and preservative protein in diet. Both results served as base data for nutritionists and traditional medical practitioners who may be looking for an alternative source of good source of protein and minerals for some mineral deficiency diseases. Another study observed by Badreldin *et al*, (2005) agreed present study in antioxidant activities who stated that pharmacological actions of the calyx and seeds of roselle seed extract include strong *in vitro* and *in vivo* antioxidant activity. In rats and rabbits, the extract showed antihypercholesterolaemic, and antipyretic action.

5:6 Macro minerals % of experimental plant protein sources:

The minerals contents in the different plant protein sources tested are shown in table (12) the result indicates that the SFC, CSC were recorded highest significant difference ($P < 0.01$) in Calcium, Sodium and Magnesium contents. This aids the active transport of nutrient substance in animal cell, followed by Roselle seed while GNC recorded the lowest ($P < 0.01$) in Calcium content of ($0.210 \% \pm 0.09$) minerals content, this result may reduced the nutritive value of the protein. On the other hand investigated by Abu El Gasim *et al*, (2008) who reported that minerals content of raw Karkade was ($0.064 \% - 0.129 \% - 0.121 \% - 0.549 \%$ and 1.48%) % of Ca, Na, Mg, P, and K respectively. Also he mentioned that roselle seed consider a good source of potassium and phosphors content from raw seed. This result agrees the present study which found that roselle seed had rich amount in K and P content ($1.330 \% - 0.532 \%$). While other trace element recorded ($0.320 \% - 0.033 \% -$ and 0.305%) of Ca, Na, and Mg, respectively. El Wandawi *et al*, (1984) reported that the

element of K, Na, Mg and Ca are the major element of the roselle seed. While Gohl, (1981) agreed present study who found that roselle seed in India were rich in Ca (1.31%) and P(0.28%)The variation between present studies in other minerals content refers to sample and soil variety.

5:7 PCR and Bioinformatics:

5.7.1 PCR and amino acids concentration of different *Hibiscus* species:

PCR Polymerase chain reaction result found in plate (2). Study identification and genetic variation among different species of roselle seed and revealed that theirs view similarity among local species *Hibiscus sabdariffa* varieties bands and others from NCBI (National Center of Biotechnology Information). Plate (15), and figure (5, 6 and 7) respectively. Mordent technique of PCR can be used for improvement of *Hibiscus* cultivars in crops production, this result of electrophoresis it similar to that result obtained by Yaye *et al*, (2004), used direct genetics transformation to improve *Hibiscus* cultivars in Senegal.

Results of amino acids concentration / mol % in the present study found in table (13). Figure (17, 18, 19 and 20 respectively). Study compare different concentration of essential amino acids among four *Hibiscus* species, and recorded high of Glycine, and Lysine value (6.03, 5.17, 2.59 – 5.93, 5.06, 2.54) / mol %for *Hibiscus sabdariffa*, and *Hibiscus altissima* than other species. However *Hibiscus cannabinus* and *Hibiscus acetosella* were signed highest value of (3.59 - 2.59) of Methionine than above sample.

5.7.2 Bioinformatics (*Insilco*) test of different *Hibiscus* species:

Bioinformatics or *Insilco* analysis shown in table (14). It uses *Hydrolysates* protein sequence from NCBI (National Centre of Biotechnology Information) and analyzes the four different species of *Hibiscus* (*hydrolysates*) protein sequence to study their characteristics of *Hibiscus* varieties.

Sequence from NCBI was inserted in chimera program 2013 according to (Huang, 2014). Results of *Hydrolysates* protein signed a large variation among different roselle seed species in molecular weight (13469 – 13530 – 13516 – and 13598) Daltons for *Hibiscus sabdariffa*, *Hibiscus cannabinus*, *Hibiscus acetosella*, and *Hibiscus altissima* respectively. All *Hibiscus* species sequence was equal in DNA length and content (116) length for all experiment samples except (*Hibiscus altissima*) content (118) in DNA length. Moreover the Phylogenetic figure (8) and multiple sequence alignment Figure (9) pointed that the roselle seeds (*Hibiscus sabdariffa*) species were similar to that of (*Hibiscus altissima*) in position number (238 – 258) obtained lysine and Glycine amino acid respectively. (*Hibiscus sabdariffa*) different in position number (252) has tyrosine while (*Hibiscus altissima*) has arginine in position number (242). While (*Hibiscus cannabinus*) species has mutation in position number (177- 178- 233 and 237) for methionine, spartic acid, valine and Serine respectively. Finally (*Hibiscus acetosella*) singed different mutations at position number (212- 228- 236 and 237) for niacne, alanine, tyrosine and Serine respectively Figure.

In the present study using Chimera program version (1.9), according to (Huang, 2014). Investigated that roselle *Hibiscus sabdariffa* recorded (13469.52 Dalton - 116%) for high molecular weight, and rich in essential amino acid concentration%, Figure (17) compared to other species *Hibiscus* Figure (18,19 and 20) respectively. Result of bioinformatics classified roselle is stable protein, and justify that the long chain of amino acid and high molecular weight

protect the protein of roselle from micro-organisms attack, and increase by - pass protein to small intestine this characteristics provide roselle seed as good protein in nutritive value, also roselle seed can used as natural preservative protein in diet.

Till now there view study in molecular characterization used to evaluate the protein characterization of roselle seed. Isozymes polymorphisms are used effectively to assess genetic relationships among individuals, populations and Closely related species of roselle seed, (Soltis and Soltis, 1989; Murphy *et al*, 1996 and (Mustafa *et al*, 2005). All those study the applications of isozymes polymorphism are still important for population genetic studies and in addressing infra-specific relationships. So far roselle seed requires more investigation on molecular characterization and bioinformatics in order to produce calyx with good quality of cash crop, while seeds consider by - product can be used for alternative source of protein in animal feed. Biochemical evidences such as seed storage protein electrophoresis and isozyme polymorphisms are convenient evidences for assessing genetic relationships (El-Din *et al*, 2004) and (Simova *et al*, 2006).

5:8 Biological tests of roselle seed:

5.8.1 Antimicrobial and antifungal activities / mm of roselle seed (*Hibiscus sabdariffa*):

The biological test anti-microbial of the (*Hibiscus sabdariffa*) shown in table (15) and plate (3) revealed that roselle seed recorded highly activities concentrations against antimicrobial values (21 – 19 – 17 – 15) mm for (*Escherichia Coli*, *Staphylo coccus arreous*, *Psodomonus arginosa*, and *Bacillus subtillus*) respectively. This result agree with that observed by (Eunkyung *et al*, 2013) who mentioned that roselle seed has highly action against (*Escherichia Coli*, *Psodomonus arginosa*, *Staphylo arreous* and *Bacillus subtillus*) . Anti-fungal activities of roselle seed shown in table (16) and plate

(4) they recorded values (22 – 19) mm highly action against (*Candida albicans* and *Aspergillus niger*) respectively. These characteristics provide roselle seeds preservative protein in diet and growth promotion of sheep due to the highly action activities against bacteria and fungi.

Present results agreed those obtained by Gangrade *et al*, (1979), in anti-microbial action including (*Bacillus anthraces*, *Staphylo coccus albus* and *Pseudomonas arginosa*) respectively. (El Shayeb and Mabrook, 1984) mentioned that oil extracted from seeds of *H. sabdariffa* has been shown to have an *in vitro* inhibitory effect against microbial and anti aflatoxin formation that result agree the present study. On the other hand found that roselle seed had highly activities against anti-fungal *in vitro* inhibitory effect against some fungi included *Aspergillus fumigatus*, *Rhizopus nigricans* and *Trichophyton mentagrophytes* (Guerin and Reveillere, 1984). Also (Atta *et al*, 2002) found that nutritionally young leaves of (*Hibiscus sabdariffa*) contented nutrients such as phosphorus, calcium, magnesium, and potassium the calyces of roselle are utilized in producing drinks, jellies, sauces, chutney, wines, calyces drink which has received industrial attention internationally.

5:8:2 Phytochemical analyses of specific compounds of Roselle seed:

Phytochemical test of Roselle seed (*Hibiscus sabdariffa*) shown in table (17). The result of the test recorded a positive in some compounds were signed (low, moderate, moderate, highly and highly) concentrations for (Cumarins, Sterol, Cyanogenic and Alkaloids and Tritreptine) respectively. Sterol compounds are major compound play an important role to produce estrogen hormones. Phyto estrogenic are estrogenic compounds found in plant affected the Central Nervous System (CNS). Hassan, 2010 agreed present study, and found that roselle seeds as good source of estrogenic factors enhancing growth and carcass characteristic of Sudanese desert sheep. While the negative values concentration compounds were recorded for (Saponins, Tanine, Anthraquinone

glycoside and Flavonoids) respectively. This result agree that investigated by (El Adawy and Khalil, **1994**) who reported that roselle seed have low Tannine concentration (1.13 – 1.37%) (Ziena *et al*, **1991**) demonstrated that Tanine and Saponins are represent anti-nutritional factors the roselle seeds are showing a negative result of this compound that might be increased the nutritive value of the protein.

5:8:3 Neutral detergent fibers (NDF) % of experimental plant protein sources:

Table (18), Showed neutral detergent fiber of four plant protein sources. Neutral detergent fiber represent component of cellulose, hemicelluloses and lignin. Results of present study pointed that CSC, was recorded highest level in neutral detergent fiber (30.35 – 13.75) % for both cellulose and hemicelluloses respectively. SFC and GNC were reflected minimum cellulose and hemicelluloses content (25.85 – 9.06), (9.46 – 17.12) for sunflower cakes and Groundnut cakes respectively. CSC recorded highest value in (ADF) and (ADL) followed by GNC. While CRS were recorded (37.50 – 19.90 and 15.32) For (NDF, ADF and ADL) respectively. This result is greater than that observed by (Idris *et al*, **2012**) who investigated neutral detergent fiber of roselle seeds in diet and reported values (20.46 – 12.98 and 3.95)% for (NDF, ADF and ADL) respectively. Variation between two studies may be due to many factors, such as species of roselle, season and rain fed and soil all these factors may affect the plant components.

5.9. Body weight and body weight gain / (kg) / week / head of experimental lambs:

The final body weight (kg) and body weight gain for experimental animal groups (A, B, C and D) shown in table (19). The results pointed that there is no significant differences ($P>0.05$) among experimental animal groups for above

parameter of experimental animals which received roselle seed in different level (0, 10, 20, and 30)% for group A, B, C and D respectively.

In this study, initial live body weight was (21.47 ± 0.35) , (21.78 ± 0.45) , (21.83 ± 0.34) and (21.99 ± 0.03) kg of group (A, B, C and D) respectively. While final body weight (kg) in were showed no significance difference ($P > 0.05$) among experimental groups $(29.67 \pm 0.071 \text{ kg})$, $(30.33 \pm 1.64 \text{ kg})$, $(31.66 \pm 0.23 \text{ kg})$ and $(33.00 \pm 0.23 \text{ kg})$ kg for final body weight of group (A, B, C and D) respectively. In present study daily weight gain increased during the experiment period gradually. Animal received (CRS) showed positive and best value of crushed roselle seed maybe related to high nutritive value of Roselle seed in diet. In this study daily weight gain for experimental groups were (0.20, 0.20, 0.22 and 0.24 kg) for group A, B, C and D respectively. This result in line with result obtained by (Hassan *et al*, 2012) who reported that the daily weight gain were (0.19, 0.20 and 0.20kg), also they mentioned that the highest levels of crushed roselle seed meal resulted in high daily gain and feed conversion rate.

Also present result agree with that observed by (Hassan, 2010) who investigated the estrogenic effect of crushed roselle seed on performance and carcass characteristics of Sudanese desert sheep fed graded level of roselle seed 0, and 30% found that daily weight gain were $(188.67 \pm 14 - 219 \pm 7.0 \text{ kg})$. So many researchers agree this study weight gain increased as increased level of crushed roselle seed. Mohammed, (2006), Bashir, (1996) reported similar results that agree with present results, they found that the rate of gain increase with the level of the crushed roselle seed increase.

5.9.1 Nitrogen balance % of feces and urine % of experimental animal groups:

Nitrogen balance in urine shown in table (20). Nitrogen intake were recorded (35.58 – 38.50 – 37.64 and 37.40) %. For group A, B, C and D respectively. The result recorded a highly significant different in nitrogen balance at ($P < 0.05$) among experimental group. Nitrogen amount in urine and feces among animals

groups were signed (14.76 – 16.44), (13.38 – 18.12) (14.22 – 18.97) and (18.42 – 17.10) % for group A, B, C, and D respectively. Lowest nitrogen exertion found in group D recorded (2.21%) followed by (4.12, 4.39 and 7.01%) of nitrogen exertion for group (C, A and B) respectively. Thus the amount of nitrogen retained were recorded (31.19 – 31.49 – 33.19 and 35.52) % for groups A, B, C and D respectively. This result explain N- amount intake that to similar N returned in feces and urine, this justify that all experimental animals in equilibrium health condition.

5.9.2 Proximate analysis % feces of experimental animal groups:

The crude protein digestibility of feces of experimental animal groups is shown in table (21). The result of this study showed highly significant ($P < 0.05$) increased in the amount of N in feces and the values were (31.19, 31.49, 33.52 and 35.19) % for group A, B, C and D respectively.

5.9.3 Overall feedlot performance of experimental animal groups:

5.9.3.1 Daily feed intake / kg / day of experimental animal groups:

The plane of nutrition and nutritive value of ingredients are the major factor affecting the daily feed intake. The ruminant animals eat until they reach satisfy, while monogastric animals eat to satisfaction their energy requirements. The amount required to satisfy a certain nutrition requirements decreased. In this study the feed intake showed no significant difference ($P > 0.05$) found in table (22) figure (14) was (1.69, 1.64, 1.65 and 1.60) Kg per for group (A, B, C and D) respectively. These results highest with the result obtained by (Mousa, 2011) in Awassi lamb (1.123 – 1.186 kg), Beshir, and Babiker (2009) who reported (1.14 -1.35 kg). Mentioned that feed intake increase an increase level of roselle seed. Beshir, (1996), Hassan, (2005) and Darran, (2007) who found that feed intake decreased with an increasing of roselle seed meal. While Mohammed and Idris, (1991) found a drop in feed intake in poultry, as the level of (CRS) meal increased, and they attribute that to acid test, and high fat content of roselle seed meal. The differences in daily feed intake; in different studies maybe attributed

to the system of fattening, seasons, animals kinds, age of animal and type of breed.

5.9.3.2 Total body weight gain / kg / week of experimental animal groups:

The total body weight gain for (42) days was shown in table (22) figure (15). The results pointed that (8.36, 8.55, 9.83 and 11.01) kg. For experimental group (A, B, C and D) respectively. This finding is different of that report obtained by (Mousa, 2011) in Awassi lamb for (90) days (13.48, 15.65) kg. While the present study was higher result obtained by (Beshir *et al*, 2009) for Sudan desert sheep for (45) days (5.30, 8.59) kg. In the other hand observed by (Hassan, *et al*, 2010) found that the total weight gain of Sudanese desert sheep fed graded level Karkadeh 0 and 30% were (11.27 ± 0.89 – 13.12 ± 0.41). These results similar with that results investigated by (Beshir and Babiker, 2009) for Sudanese desert sheep (9.84 – 11.49) kg. In the present study total weight gain not significantly different ($P > 0.05$) among experimental treatment groups. It increase at level of Karkadeh the maximum gain attained by group D, followed by group C , B and last group A. all those researcher found that roselle seed as good protein source and effecting on fattening performance of Sudanese desert sheep. Thus the present study may draw attention to believe of some people that roselle meal has some chemical characteristic, antioxidant, antimicrobial and anti-fungal effect and this true concerning to results of the present study and conceder roselle seed protein as preservative protein in the diet. While the view variation between present study and other may due to animal age, experimental period, season, type of animals and breed.

5.9.3.3 Feed conversion ratio (F.C.R) and economical evaluation study of roselle seed using grade levels in experimental lambs fattening:

Economical evaluation study and feed conversion (FCR) ratio shown in table (22). Feed conversion efficiency (FCE) of ration is an important factor reflecting the nutritive value of the feed and efficiency of utilization of the animals. (FCE) of this study showed highly significant difference ($P < 0.01$)

among the different dietary treatments, were signed (8.38 ± 0.02 – 8.55 ± 0.05 – 7.55 ± 0.03 and 6.42 ± 0.12) for diet A, B, C and D respectively. This result agreed with that observed by (Hassan, *et al*, **2012**), who found that the FCR were (11.20 ± 1.06 – 9.24 ± 0.35) for Sudanese desert lambs fed deferent graded levels of roselle seed. Variation between two studies may due to animal age, type of breed and digestive system performance.

Economical evaluation cost of 1kg dry matter / SDG in the present study shows highly significant differences ($P < 0.01$) in total cost / SDG of 1kg dry matter ingredient were recorded (3.03, 2.96, 2.74, and 2.52) of diet (A, B, C and D) respectively. According to the result of (F.C.R) lamb of group (D and C) was reflected a cheaper diet ingredients which received 20 and 30% roselle seed and found the lowest price cost / SDG of 1kg dry matter compared to other treatment (A and B) which received 0, 10 % of roselle seed respectively. FCR of the present study support the economical cost SDG and found that diet (D and C) recorded the best FCR values (6.42 ± 0.03 – 7.16 ± 0.03) for diet (D and C) than (8.55 ± 0.03 – 8.36) for diet (B and A) respectively. This result agrees with that obtained by (Hassan *et al*, **2012**). Who study effect of roselle seed fed graded level 0, 15 and 25% of diet A, B and C respectively in feeding sheep. FCR were recorded (8.50, 8.70 and 8.60 for diet A, B and C respectively. However economical evaluation total cost / SDG of ingredients was found high than present study and recorded (4.00, 3.70 and 3.60 for diet A, B and C respectively.

Present results agree with those of (Mousa, **2011**, Beshir, **1996**). They reported a similar trend of F.C.E, increase as the level of roselle seed meal increased. However, the age or the weight of the animal may affect the feed conversion efficiency, as the requirements of growth are differ from that of the fattening.

Chapter Six

Conclusion and Recommendations

6.1 Conclusion:

- ❖ The use of crushed roselle seeds showed a positive effect on fattening performance of Sudanese desert sheep and cheaper protein source used in feedlot ration.
- ❖ By using an *In vitro* digestibility test for roselle seeds (Pepsin and picric acid) the resulting Protein (*Hydrolysates*) showed a positive effect as an antioxidant. DPPH and Iron (Fe^{++}) chelating activities were used.
- ❖ Phytochemical tests showed high positive effect in compounds (Sterol, Tritreptine, Cyanogenic glycoside, Coumarins and Alkaloids) and raising the nutritive value of roselle seeds.
- ❖ Anti-microbial activities of roselle seeds including (*Escherichia coli*, *Psodomonus arginosa*, *Bacillus subtilus* and *Staphelo coccus arieaus*) showed highly activities against these bacteria.
- ❖ Anti-fungal of roselle seeds also revealed highly activities against *Candida albicans* and *Aspergillus niger* this gives roselle seeds preservative protein in diet.
- ❖ Bioinformatics or *In Selico* technology found that different species of roselle seeds rich protein in essential amino acid.
- ❖ Crushed roselle seeds had no deleterious effect on ruminants' performance, thus roselle seeds maybe used up to 30% in lambs feeding with satisfactory result.
- ❖ Chemical composition showed that roselle seed protein rich in minerals (Na, K, Mg, Ca and P).
- ❖ Finally Use roselle seeds as natural growth promotions additives and in animal feed.

6.2 Recommendations:

- ❖ Study of the characteristics of lamb carcass fed different levels of roselle seeds should be further investigated.
- ❖ The controversy that roselle seeds have an anti-estrogenic effect requires further study on this regard.
- ❖ The use of roselle seeds as preservative protein in diet needs further researches investigation to prove this regard.
- ❖ Use of PCR and bioinformatics technique in animal nutrition that leads the researchers to open new line in nutritional genomic.

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Appendixes

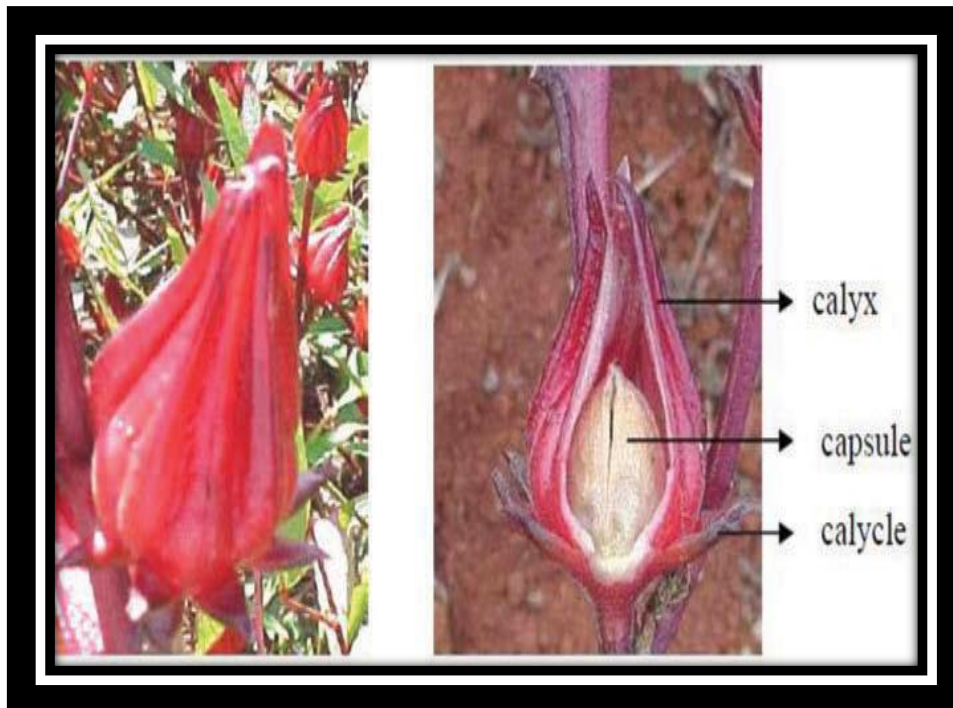


Plate (1) Roselle seed in side of capsule

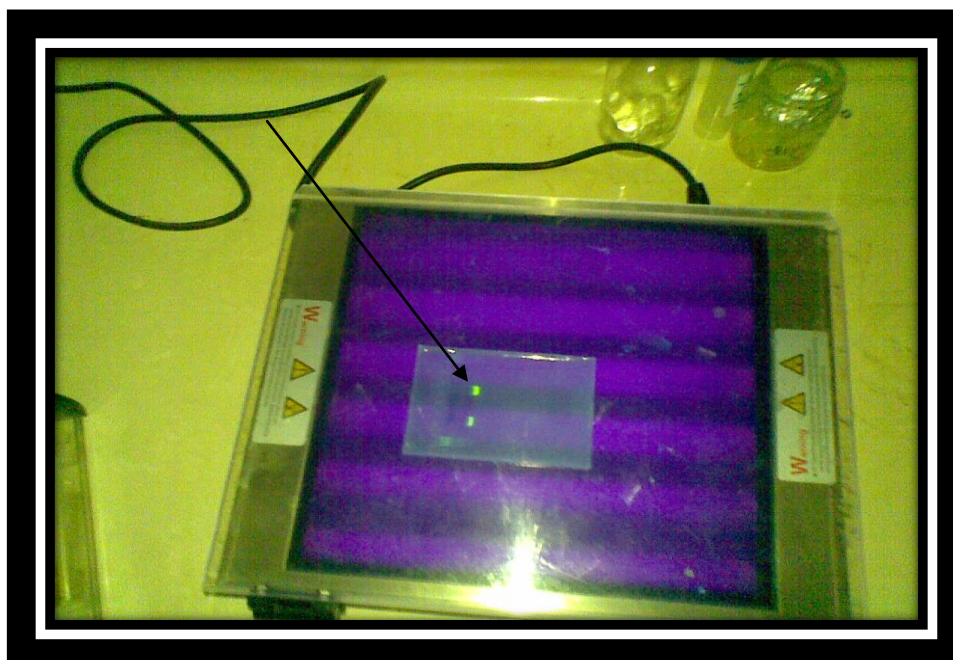


Plate (2) DNA leader bands and DNA of roselle seed

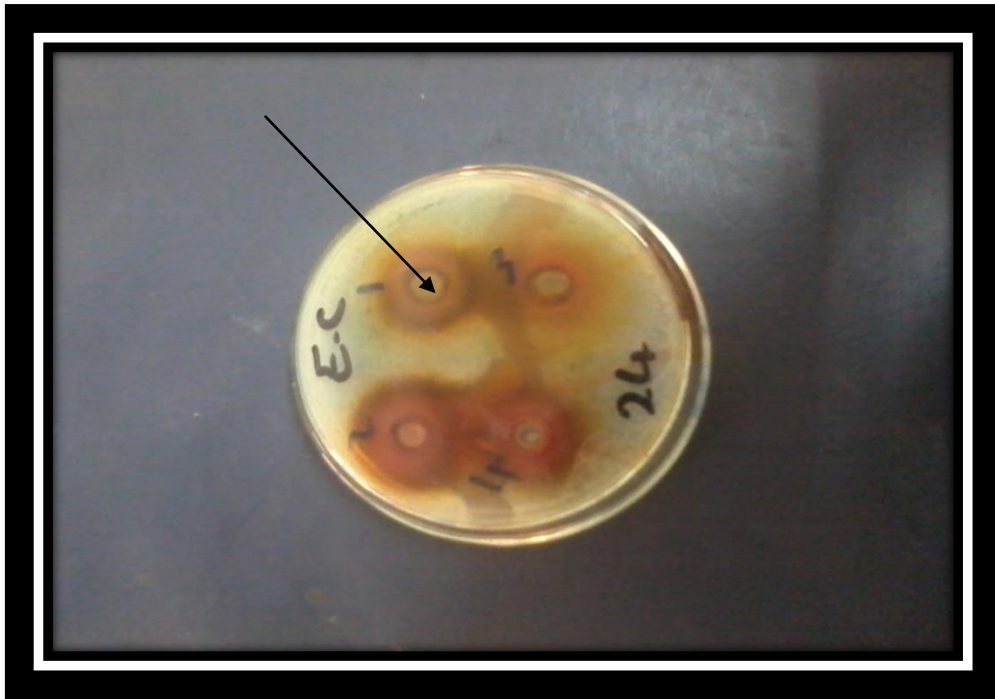


Plate (3) Roselle seed Anti-microbial Activities

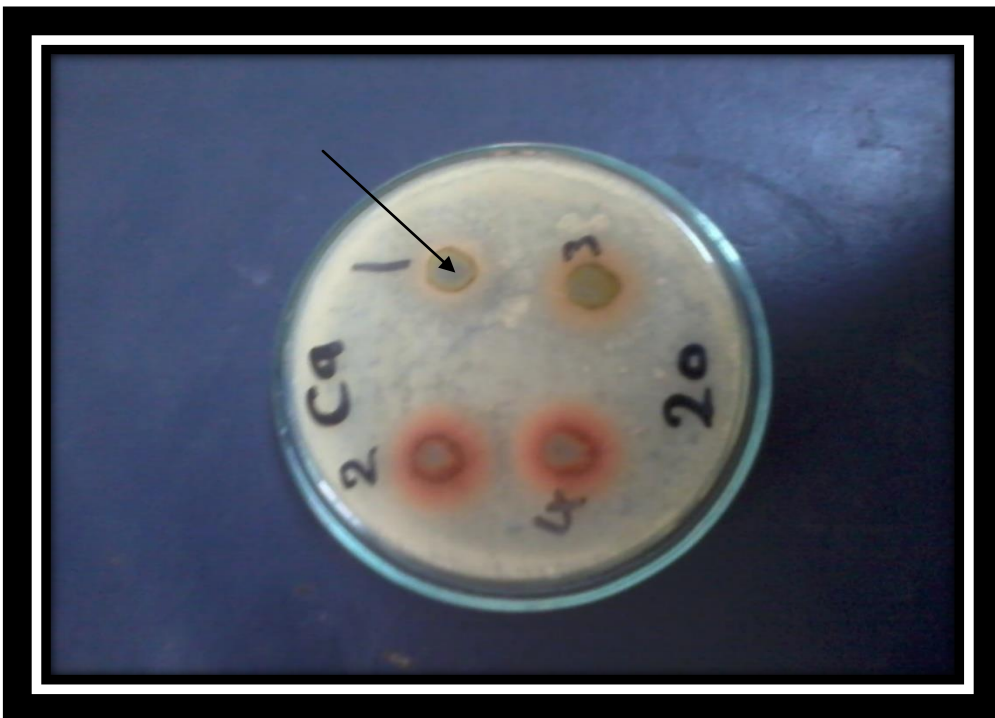


Plate (4) Roselle seed anti-fungal Activities

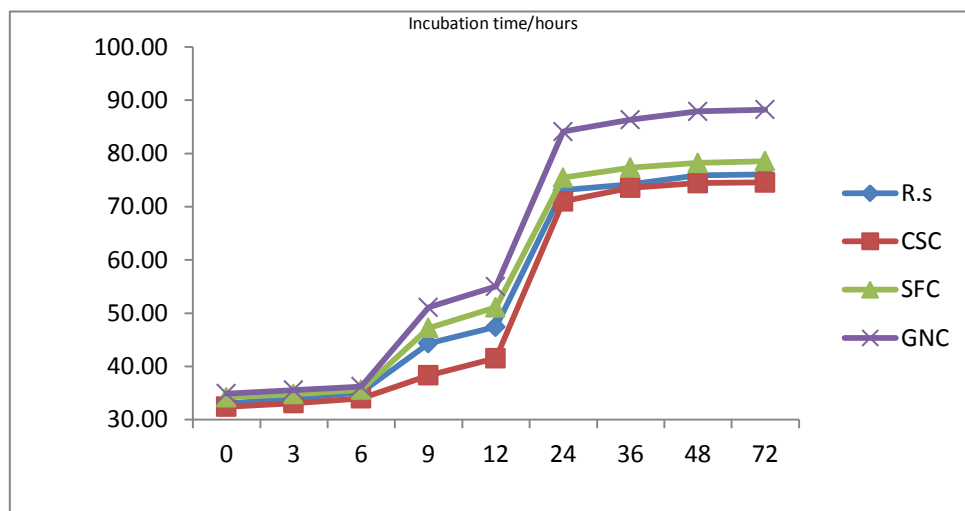


Figure (11) Dry matter disappearance % Curve

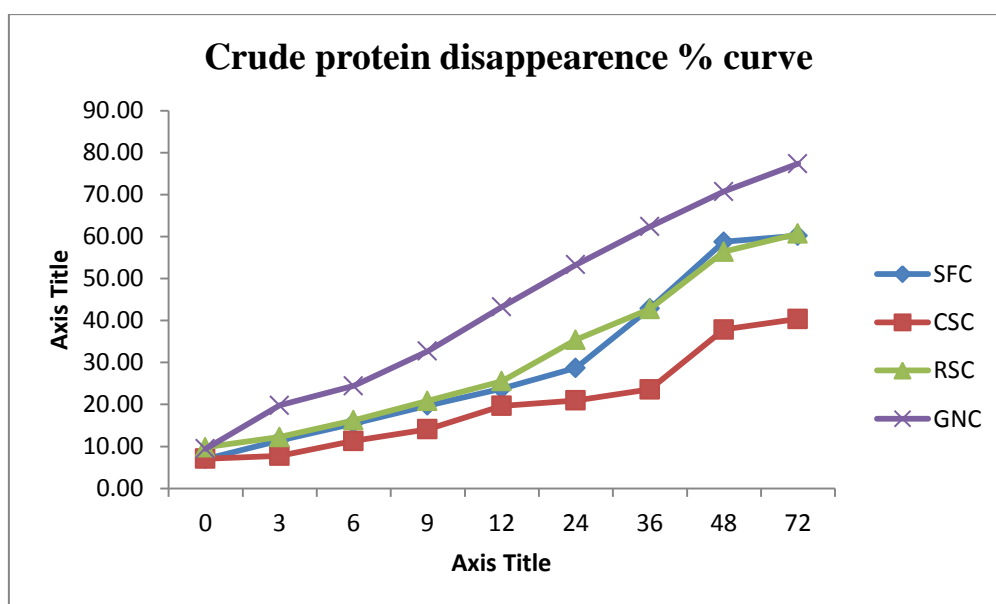


Figure (12) Crude protein disappearance % curve

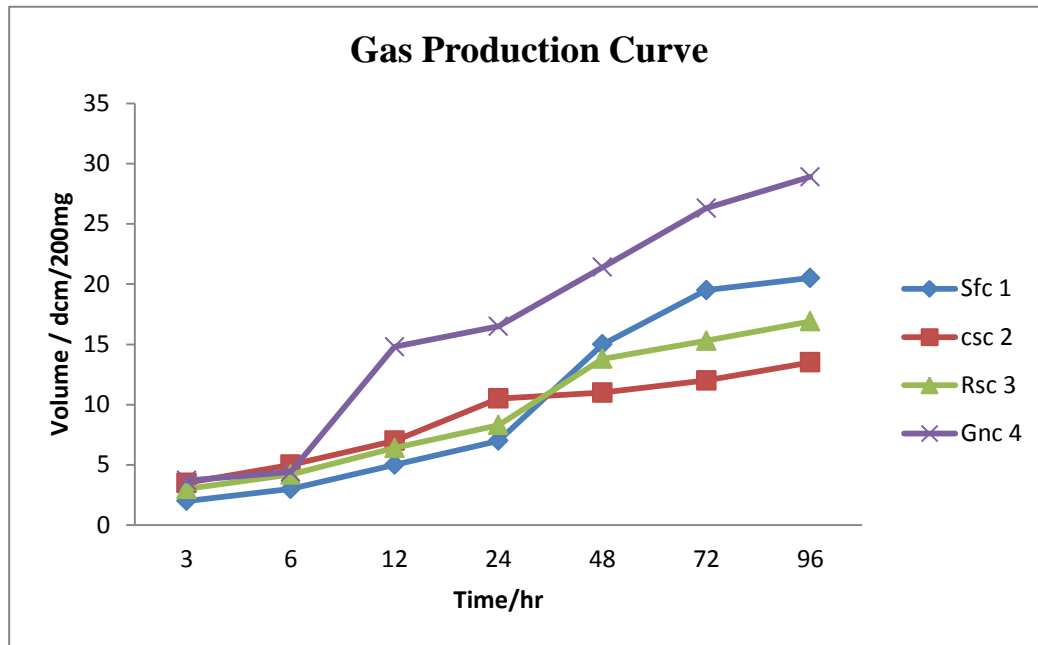


Figure (13) Gas production curve

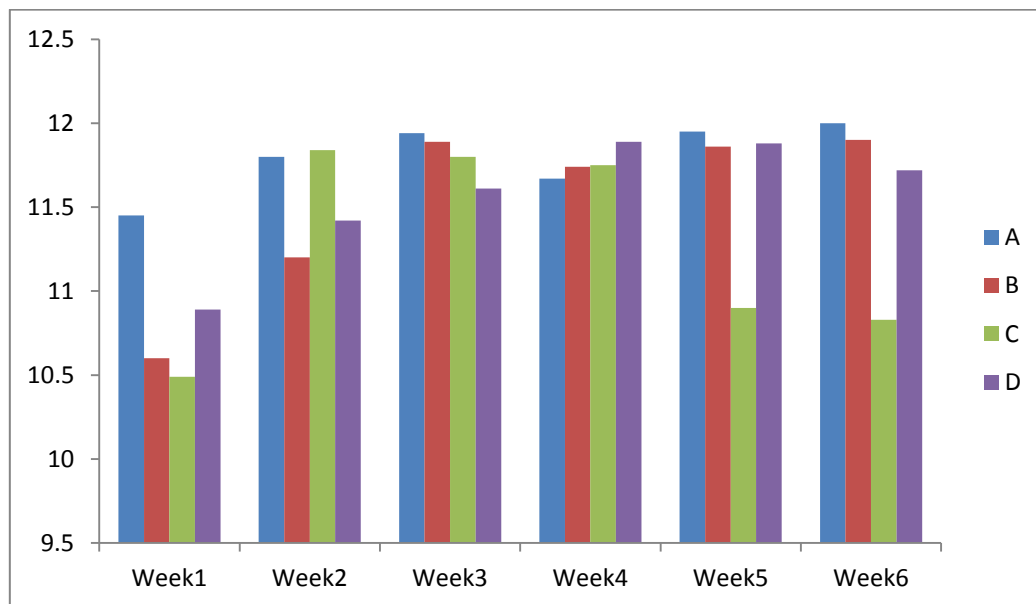


Figure (14) Feed intake (kg)/week/head of experimental lambs

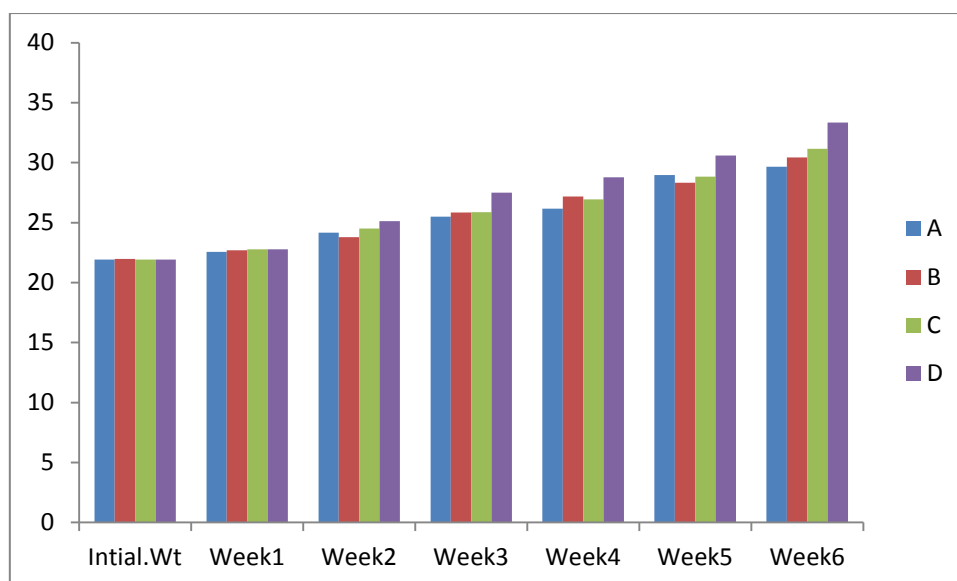


Figure (15) Weight gain (kg)/week/head of experimental lambs

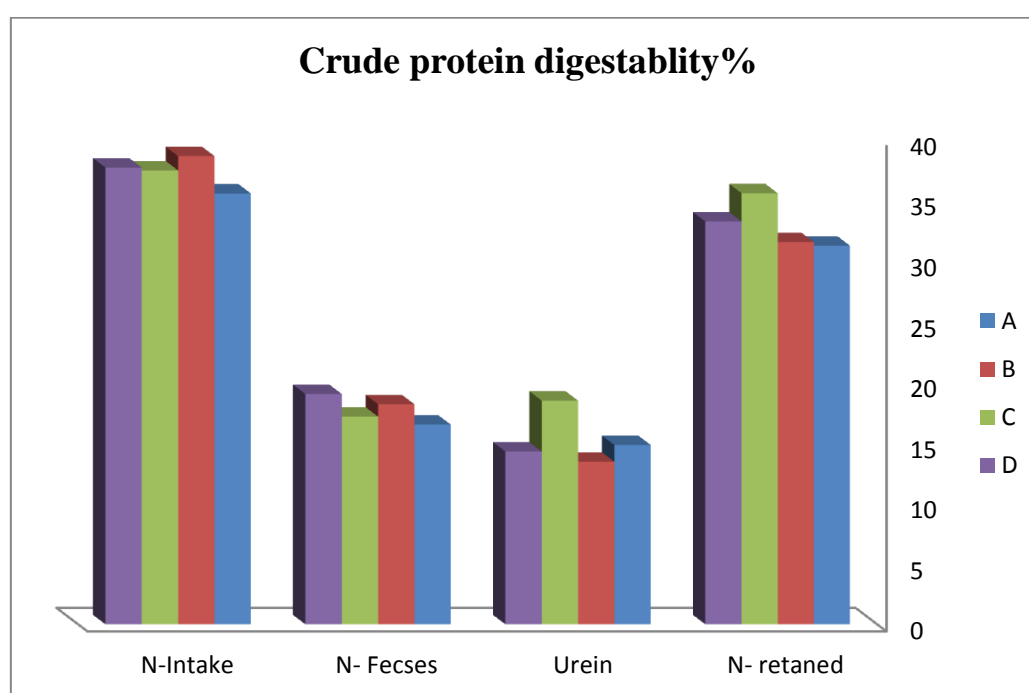


Figure (16) Crude protein digestibility %

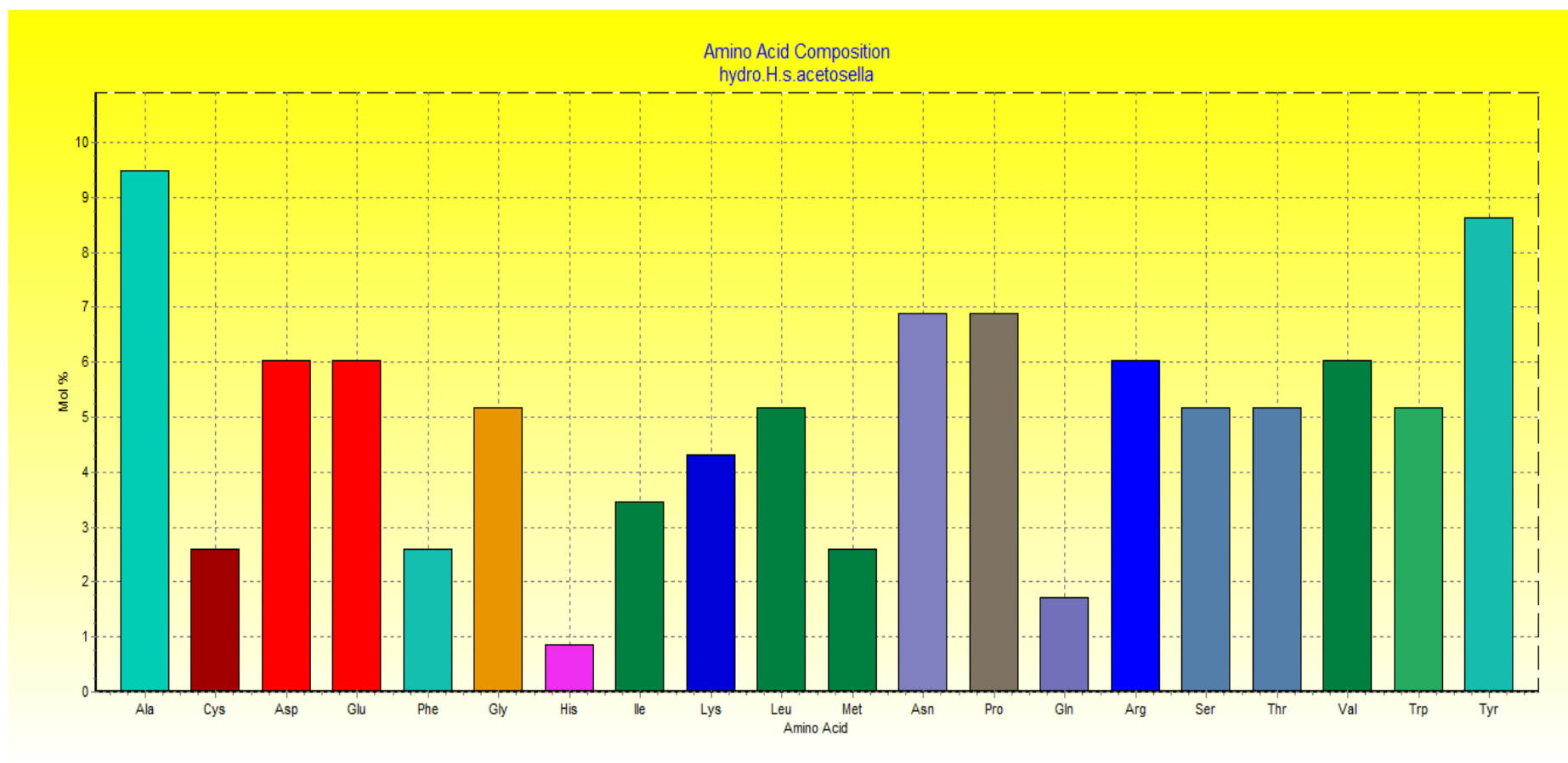


Figure (17) Amino acids composition of *Hydrolasates* protein of (*Hibiscus acetosella*)

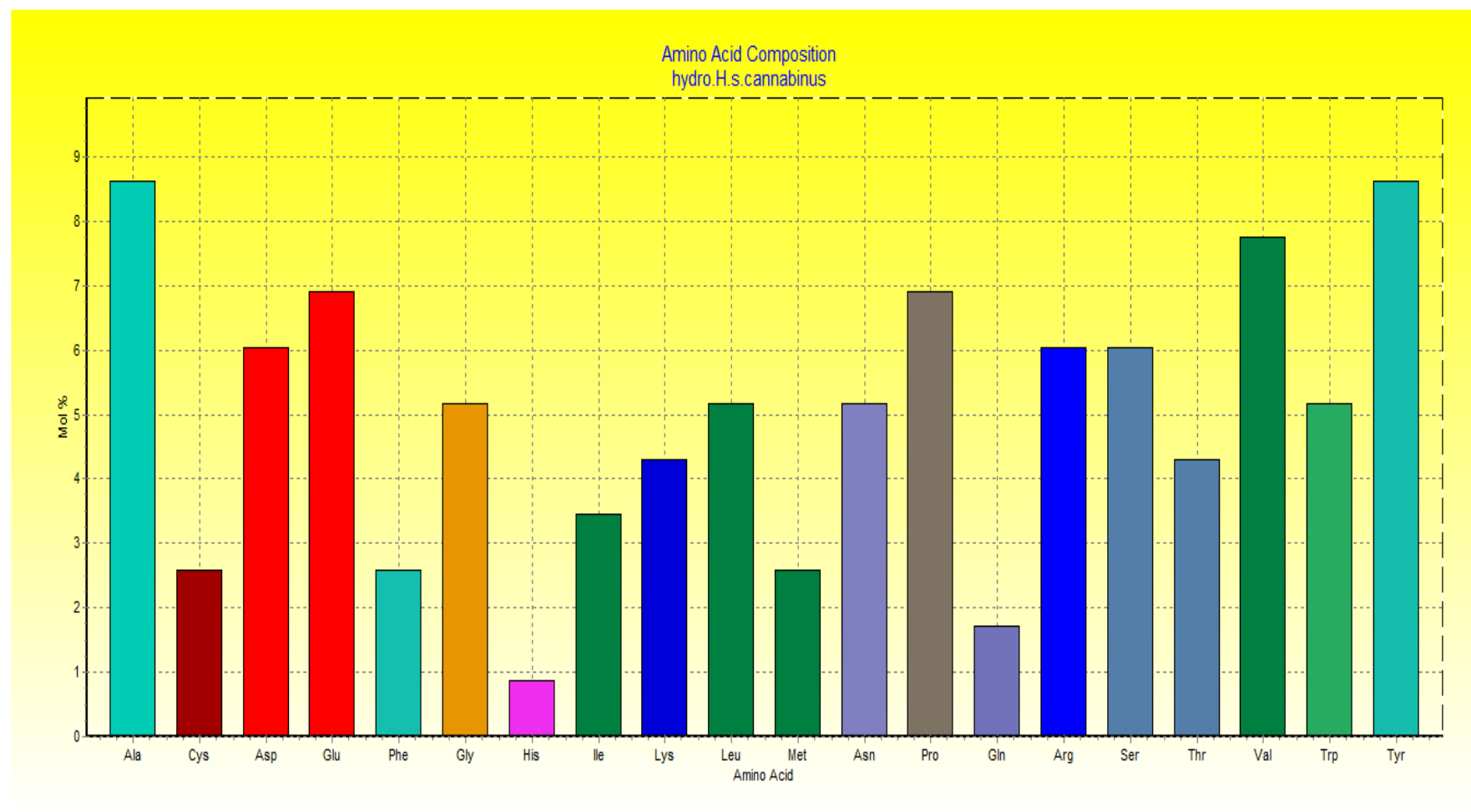


Figure (18) Amino acids composition of *Hydrolasates* protein of (*Hibiscus cannabinus*)

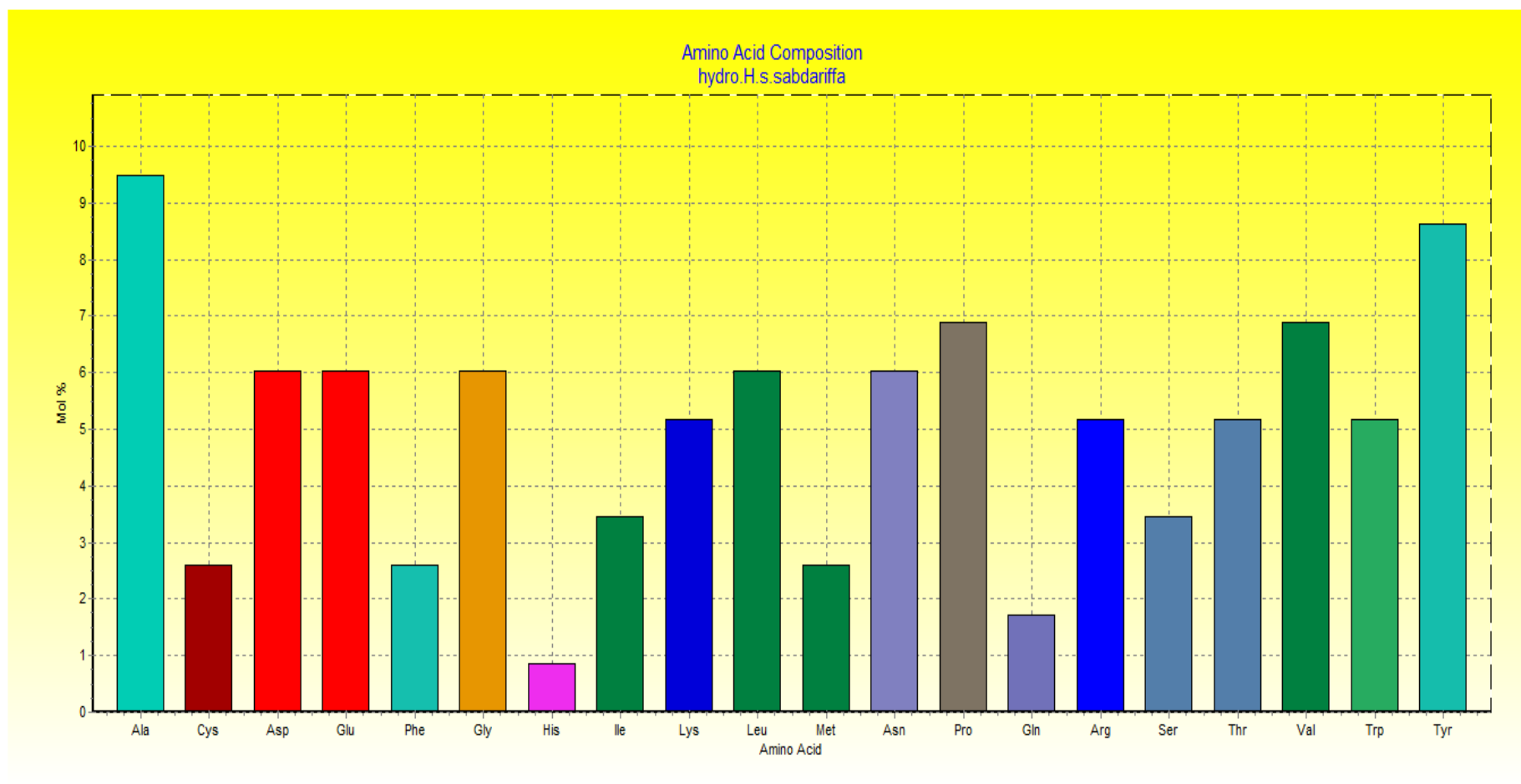


Figure (19) Amino acids composition of *Hydrolasates* protein of (*Hibiscus sabdariffa*)

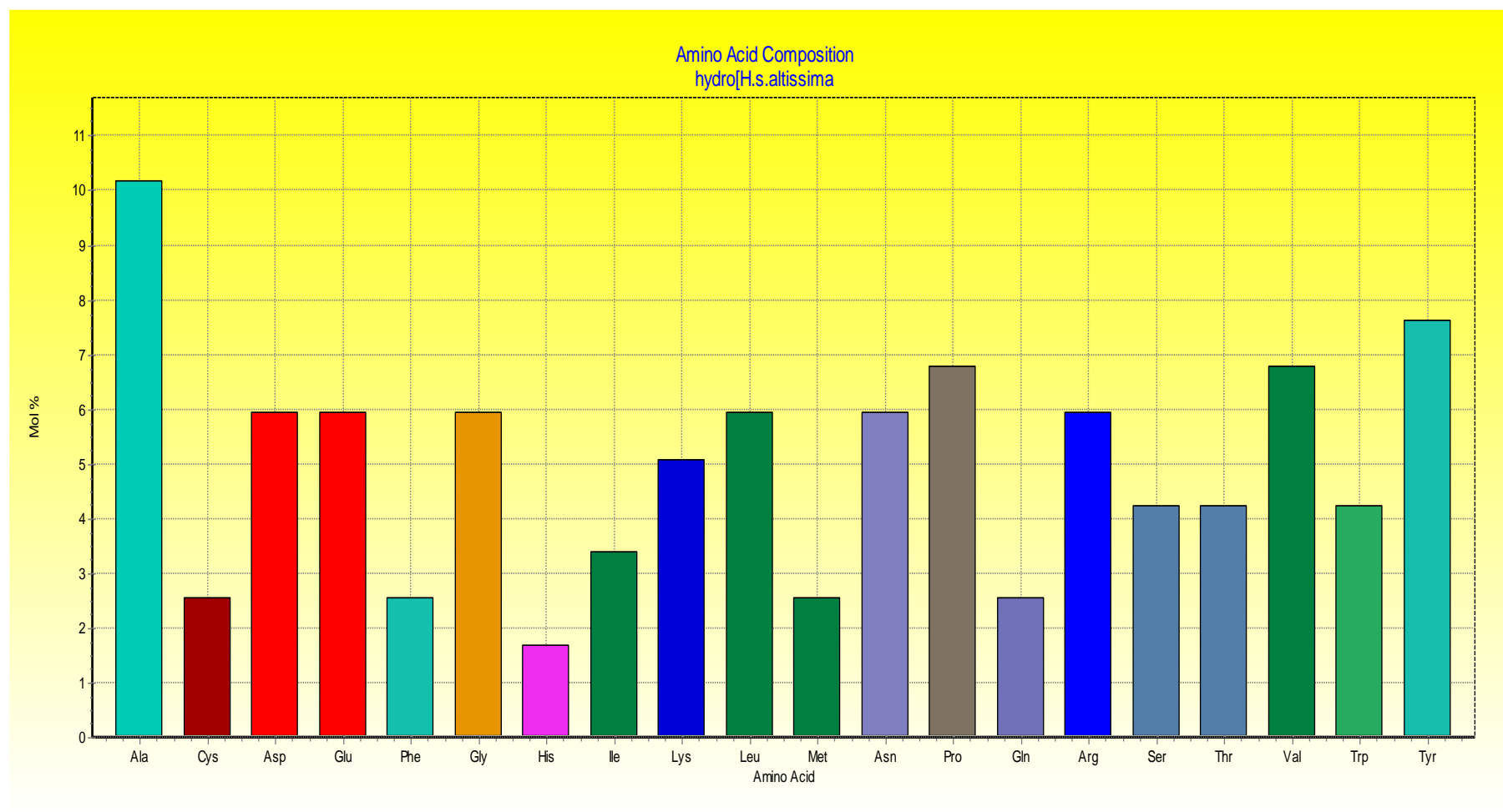


Figure (20) Amino acids composition of *Hydrolasates* protein of (*Hibiscus altissima*)



Plate (5) Removal antioxidant sample of roselle seed from multiskan spectrum



Palte (6) Enzymatic and micobial digestibility of different plant protein sources in waterpath