Sudan University of Science and Technology

College of Graduate Studies

Association of Growth Differentiation Factor 9 *GDF9* Gene With Some Productive and Reproductive Traits of Some Sudanese Desert Sheep Ecotypes

العلاقة بين جين عامل النمو التمايُزي9 مع بعض الصفات الإنتاجية والتناسلية في بعض أنواع الضأن الصحراوي السوداني

A thesis submitted for fulfilment requirement for the degree of Doctor of Philosophy (PhD) in Meat Science and Technology

by:

Abubakr Sayed Ali Mustafa

M.Sc., (SUST, 2012)

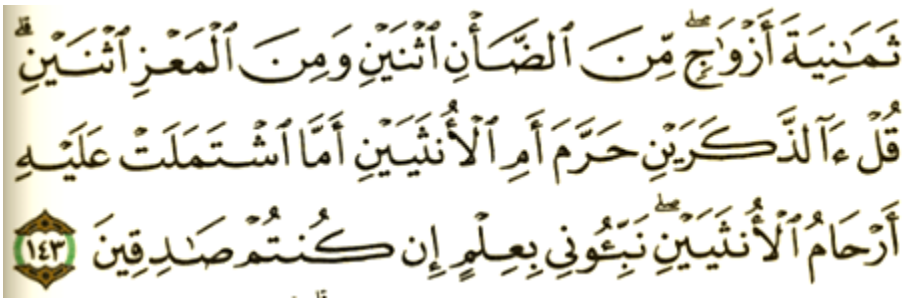
Supervisor: Prof. Dr. Mohamed Tag Eldin Ibrahim

Co-supervisor: Assoc. Prof. Maha Mubarak Mohammed

External supervisor: Prof. Dr. Gesine Leuhken

May 2017

الإستهلال


ثَمَٰنِيَةَ أَزۡوَٰجٖۖ مِّنَ ٱلضَّأۡنِ ٱثۡنَيۡنِ وَمِنَ ٱلۡمَعۡزِ ٱثۡنَيۡنِۗ
قُلۡ ءَآلذَّكَرَيۡنِ حَرَّمَ أَمِ ٱلۡأُنثَيَيۡنِ أَمَّا ٱشۡتَمَلَتۡ عَلَيۡهِ
أَرۡحَامُ ٱلۡأُنثَيَيۡنِۖ نَبِّـُٔونِي بِعِلۡمٍ إِن كُنتُمۡ صَٰدِقِينَ ١٤٣

الآية 143 سورة الأنعام

Dedication

To the soul of my father

To my dear mother

To my wife

To my kid AbdAlrahman

To my brothers and sister

To my friends and colleagues

Abubakr

Acknowledgement

First and always I express my faithful thanks to Allah for giving me health, fortune and patience to conduct this study.

I am greatly indebted and I wish to express my great thanks and gratitude to my supervisors Prof. Dr. Mohamed Tag Eldin Ibrahim and Dr. Maha Mubarak Mohammed for their guidance and close supervision through the research period. My gratefulness goes to Prof. Dr. Gesine Leuhken for her valuable advices and supervision during the staying period in her lab.

My deeply thanks extend to all staff of Institute of Animal Breeding and Genetics, Giessen-Germany.

Unique thanks to my colleague Ameer Ahmed Elobied for his available assistance and support, also thanks to my colleague Sami Salah Eldien and Abdalrahman Babikr Eltayeb for their help and assistance.

My great thanks to my students Mahmoud Mohammed Ali and Omer Adam for their valuable assistance.

Special thanks and appreciation to the German Academic Service (DAAD) for their financial support.

Finally thanks to them all who supports me during this study.

Abbreviations:-

BW=Body weight
BL=Body length
WH= Wither height
HG= Heart girth
CD=Chest depth
CW=Chest width
RL=Rump length
RW= Rump width
HL= Head length
HW= Head width
SC= Shank circumference
TC= Thigh circumference
EL= Ear length
TL= Tail length
WL= Wool length
CC= Cannon circumference

Abstract

The present study was carried out during March and May 2015 at the home-land of the studied desert sheep ecotypes, including Khartoum and Gezira states for (Ashgar and Dubasi sheep), Sinar and Blue Nile states for (Watish and Dubasi sheep) and River Nile state (Ashgar sheep). This study aims to investigate some productive and reproductive traits of some Sudanese desert sheep in different areas in the Sudan, determine the association between live weight and body measurements of Ashgar, Dubasi and Watish sheep using different mathematical models and analyze GDF9 variability and test identified variants for association with litter size among Sudanese desert sheep ecotypes.

A total of two hundred and twenty-five head of three sheep ecotypes were randomly selected [80 Ashgar (male=21, female=59), 72 Dubasi (male=22, female=50) and 73 Watish (male=23, female=50)] and according to sex [rams (n=66) and ewes (n=159)] to find out the correlation between live body weight and body measurements using different mathematical models (linear, quadratic, cubic, compound, power and S). The obtained data were tested for significance using analysis of variance ANOVA followed by least significant difference (LSD) test. Also, Independent samples T. test was used and Pearson's correlation, simple regression analysis was fitted. The live body weight and body measurements were significantly ($P<0.05$) affected by sheep ecotypes and sex except shank circumference (SC) and thigh circumference (TC) for sheep ecotype and chest depth (CD), rump width (RW), head width (HW) and thigh circumference for sheep sex. The live body weight was significantly ($P<0.01$) correlated with the majority of body measurements, the highest correlation coefficient in the studied sheep

ecotype was between the live body weight and heart girth (0.826), followed by live body weight with wither height (0.756) and body length (0.749) respectively. R^2 values of the studied ecotypes showed that heart girth was the highest association ($P<0.01$) with live body weight, followed by wither height and body length. The study concluded that sheep ecotypes and sex significantly affect body weight, Watish had the highest body weight while Dubasi had the lowest.

Twenty eight DNA samples were selected (ten from Ashgar and Dubasi and eight from Watish). For each ecotype, 50% of the samples were selected from the single lamb group and the other 50% from the more than a single lamb group these ecotypes with litter size records for at least two litters were sampled. The complete GDF9 exon 2 was sequenced in the 28 samples. An additional variant in exon 1 (c260G>A) was genotyped by restriction-length polymorphism analysis in 97 DNA samples. Differences in genotype and allele frequencies of polymorphic positions between two groups differing in litter size (only a single lamb versus more than a single lamb) were tested for significance using Fisher's exact test. GDF9 exon 2 variants c.477G>A and c.721G>A and exon 1 variant c.260G>A were found to be polymorphic in all three sheep ecotypes. Exon 2 variants c.471C>T and c.978 A>G were polymorphic in at least one ecotype. No significant associations were observed between allele and genotype frequencies of identified variants and litter size. This suggests that GDF9 variants influencing ovulation are absent in these Sudanese sheep ecotypes, and therefore cannot be used to increase litter size within this population of sheep.

ملخص الدراسة

أُجريت هذه الدراسة خلال الفترة بين مارس و مايو 2015 في مناطق تواجد الضأن الصحراوي في ولايتي الخرطوم والجزيرة للضأن الأشقر والدباسي، ولاية الجزيرة للضأن الدباسي، ولايتي سنار والنيل الأزرق للضأن الوتيش وولاية نهر النيل للضأن الأشقر. هدفت هذه الدراسة إلي التعرُّف علي بعض الصفات الإنتاجية والتناسلية لهذه الأنواع في مختلف مناطق السودان، تقدير العلاقة بين الوزن الحي و قياسات الجسم لهذه الأنواع بإستخدام معادلات رياضية مُختلفة و تحليل درجة الإختلاف في جين *GDF9* وإختبار العلاقة بين المُغايِرات المُختلفة مع حجم البطن في الثلاث أنواع من الضأن الصحراوي.

أُستخدمت إستبانة مصممة ومفصلة لجمع المعلومات من عدد مائة من مُربيي الضأن الصحراوي في مُختلف مناطق السودان بطريقة المُقابلة الشخصية. تضمنت الإستبانة المعلُومات الشخصية عن المربي، تركيب-حجم القطيع ونُظم الرعاية، الإسكان-تغذية القطيع، الصفات الإنتاجية والتناسُلية، التسويق-إستخدامات مُنتجات/مُخلفات الضأن، معايير الإستبعاد للنعاج-الكِباش والصحة العامة-معوقات إنتاج الضأن. تم تحليل البيانات المُتحصل عليها بإستخدام الجداوِل الوصفية، مربع كاي وتحليل التباين متبوعاً بإختبار أقل فرق معنوي. أظهرت النتائج أن معظم المربيين أُميُون، خريجو مرحلة الأساس أو خلاوي، كذلك أظهرت النتائج أن النعاج البالغة شكلت أعلي عددية بين مجاميع قطعان الضأن، كذلك سجلت النتائج زيادة في إنتاج الحِملان عند التغذية بإضافات غذائية في بداية فصل الخريف. أوضح مربو الضأن الصحراوي أن العمر الإنتاجي للكِباش أعلي من العمر الإنتاجي للنعاج، إضافة إلي ذلك أن الكِباش أكثر تفضيلاً في السوق يليها النعاج ثُم الحوليات. أثبتت النتائج أن التقدم في العمر هو أكثر المعايير في عزل النعاج والكباش. أيضاً من أكثر المُعوقات التي تُواجِه إنتاج الضأن هي الأمراض، قلة الماء والمرعي. أُختيرت مئتان وخمس وعشرون رأس عشوائياً من أنواع الضأن الثلاث [(80 الأشقر (ذكور=21، إناث=59)، 72 الدباسي (ذكور=22، إناث=50)، 73 الوتيش (23 ذكور، 50 إناث) وعلي أساس الجنس (الكباش=66 و النعاج=159)] لإيجاد العلاقة بين الوزن الحي للحيوان وقياسات الجسم بإستخدام مختلف المعادلات الرياضية (خطية، رباعية، تكعيبية، مركبة، الأُسية وS). أُختبرت البيانات المتحصل عليها بإستخدام تحليل التباين متبوعاً بإختبار أقل فرق معنوي(LSD). أيضاً أُستخدم إختبار ت. للعينات المُستقلة، كما تم إستخدام إرتباط بيرسون والإنحدار البسيط للمعادلات الرياضية سابقة الذكر. تأثر وزن الجسم

وقياسات الجسم معنوياً ($P<0.01$) بنوع وجنس الضأن فيما عدا محيط الساق ومحيط الفخذ لنوع الضان، عمق الصدر، عرض العجز، عرض الرأس ومحيط الفخذ بالنسبة لجنس الضأن. وُجدت علاقة معنوية ($P<0.01$) وذات إرتباط موجب بين الوزن الحي للحيوان وغالبية قياسات الجسم، وكان معامل الإرتباط أعلي قيمة (0.826) بين الوزن الحي للحيوان ومحيط الصدر متبوعاً بمعامل الإرتباط بين الوزن الحي للحيوان مع الإرتفاع عند القارب (0.756) وطول الجسم (0.749) علي التوالي. (R^2) لأنواع الضأن موضع الدراسة كانت الأعلي بين محيط الصدر والوزن الحي للحيوان ($P<0.01$) يليه الإرتفاع عند القارب وطول الجسم. خلُصت الدراسة إلي أن كلاً من نوع وجنس الضأن يُؤثران معنوياً علي الوزن الحي للحيوان، الوتيش كان الأعلي في الوزن الحي بينما الدُباسي كان الأقل وزناً.

أُختيرت ثمانيةً وعُشرين من عينات الحمض النووي (عشرٌ من الأشقر والدُباسي وثمانية من الوتيش). لكل نوع أُخذ 50% من العينات من مجموعة النعاج فردية الحِملان و50% من مجموعة النعاج التي أنتجت أكثر من حمل وذلك لأكثر من سجلي ولادة للمجموعتين. حُسب التسلسُل الكامل للجُزيئة الحيوية للمقطع 2 لجين *GDF9* في الـ28 عينة. إضافة للتوزيع الجيني للمقطع 1 عند النقطة (c260G>A) بإستخدام تقنية التقييد للقطع متعددة الأطوال (RLFP) في 97 عينة حمض نووي. تم إختبار المعنوية الإختلافات في تكرار التوزيع الجيني والأليلي في المواقع متعددة الأشكال بين المجموعتين المختلفتين في حجم الولدة (للمجموعة فردية الحملان مقابل المجموعة التي أنتجت أكثر من حمل) بإستخدام إختبار فيشر المضبوط (Fisher's exact test). المُغايرات الجينية c.477G>A و c.721G>A في المقطع 2 للـ *GDF9* والمُغايرة الجينية c.260G>A في المقطع 1 وُجدت أنها مُتعددت الأشكال في أنواع الضأن الثلاث. المُغايرات الجينية c.471C>T وc.978 A>G كانت متعددة الأشكال في نوع واحد من الضأن علي الأقل. لم يُلاحظ وجود علاقة بين التكرار الأليلي و الجيني وحجم الولدة في المُغايرات الجينية المُتعرف عليها. هذه النتائج تقترح غياب أثر مُغايرات الـ *GDF9* علي مُعدل التبويض في أنواع الضأن الثلاث، لذا لا يُمكن إستخدامها في زيادة حجم الولدة في هذا المجتمع.

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Chapter One

Introduction

Animal resources are one of the major wealth of economy backbone of several developing countries beside the agricultural products. In this context Sudan need effort to develop this section to increase the national income. In Sudan nomadic people raised most population of the sheep under an extensive system where there are bit practices of most of the modern scientific techniques, in the past decades the nomads reared their animals including cattle, sheep and goat according to the availability of pasture and water (Ockerman and Abdelrahman, 1985), but nowadays the nomads tend to rear their animals on the agricultural by-product of private schemes or that purchased from farmers to give their animals a sustainable supply of feed. Range lands in Sudan are characterized by many different plant species due to action and interaction of many factors such as soil, climate, landscape and predominant human activities. In spite of degradation resulting from overgrazing, drought, fire and desertification, they still provide 82.6% of the livestock feed (Daragge and Fadl ELMula, 1994) moreover, the productivity process of sheep faced many handicaps factors on the production in some semi-arid area in Sudan, such as, poor and low nutritive value of pastures, high ambient temperature, lack of feed and water…etc. (El-Hag *et al*., 2001).

It is important to find an economic, rapid and easy methods to predict the live body weight of animals, because determination of live weight is necessary in market and breeding process either for buyers or sheep owners, moreover, calculation of the amount of feed that meets animal requirements by computing it as function of live weight as one of important demands in farm management. Sheep eat about 4.2-5.2% as dry

matter of its live weight (El Khidir *et al.,* 1988, Atta and El Khidir, 2010), while the camel needs to eat about 2.5% dry matter of its live weight (Eltahir *et al.,* 2011). Moreover, most of animal veterinary treatments depend mainly on the unit live weight of the animal thus it is necessary to find a quick and simple method that enables the researchers to estimate the live weight of the animal. According to Sulieman *et al*., (1990) and Atta and El Khidir (2004), body length and height at wither were skeletal measurements had less variable with live weight change because they determine the growth of bone which is an early maturing tissue.

Numerous environmental conditions and human necessities are the main conditions in selection of different livestock breeds. Furthermore the genetic variety found in local livestock breeds allows farmers to enhance new characteristics in response to alterations in environment, diseases or market conditions. Lately improvements in molecular biology and statistics have opened the potentiality of categorising and using genomic differences and major genes for the genetic improvement of livestock (Hugo and Cesar, 1998).Genetic variation related with ovulation rate (OR) in sheep has been widely known and the evidences show substantial differences among breeds and in a number of cases exceptional variations within breeds/strains (Bindon *et al*., 1996). Fertility traits have a major effect on productivity and profitability in lamb meat production. Increasing fertility traits are very important and take great attention by sheep owners (Kumm, 2008). Furthermore the traits associated with fertility have low heritability in general so that the breeding enhancements should focus on phenotypic selection depending on noticeable data which are mostly inadequate, additionally studying genes related to fecundity could raise the genetic improvements in reproductive traits then it will be easier to get information of animal's

traits however the improvements of fecundity traits may take a long time to influence the profitability of sheep production (Pramod *et al*., 2013). The productivity of sheep farming mainly depends on lamb production per ewe and litter size (LS).both are vital economical traits in sheep breeding and genetics, which mainly depend on breed. Several sheep breeds show difference in LS across the world, and most produce only a single lamb per lambing, while some deliver twins or even triplets. There are a few sheep strains referring to prolific breeds, such as Australian Booroola Merino, Chinese Hu, little tailed Han sheep, and others. LS is totally reliant on OR which is under common genetic control of a multi genes (Davis *et al*., 2002, 2005 and Roy *et al*., 2011).

The objectives of this study are to:-

1- Investigate some productive and reproductive traits of some Sudanese sheep ecotypes (Ashgar, Dubasi and Watish) in different areas in the Sudan.

2- Determine the association between live weight and body measurements of some Sudanese sheep ecotypes using different mathematical models.

3- Analyse GDF9 gene variability in the Sudanese desert sheep ecotypes Ashgar, Dubasi and Watish, and to test identified variants for association with litter size.

Chapter two

2. Literature review

2.1: Sheep population and distribution

Sheep population in Sudan is about 40.6 million, representing 37.73 % of the total Sudanese livestock population which is approximately 107.6 million heads. In recent years, Sudanese sheep namely the desert type, have received great interest as an export commodity to the Arab countries (Ministry of Animal Resources, Fisheries and Ranges MARFR, 2016). Desert sheep is one of the most distributed sheep types in Sudan, it is spread across the low rainfall savannah, semi desert and desert zones. It is well adapted to arid and semiarid environments and can live in harsh conditions such as with water scarcity, poor range grasses and high ambient temperature (Mufarrih, 1991).

2.2 Classifications of Sheep

2.2.1 Classification of sheep in the world

According to the production type sheep are categorized into four groups (El-Khashab, 1997).

2.2.1.1 Meat sheep type

This breed is described by production of meat such as Oxford and Suffolk which records 100-130 kg at maturity age for males and 70-90 kg for female weigh.

2.2.1.2 Milk sheep type

This type is characterized by producing milk, e.g. Italian Lacoune breed. This breed is notable by its milking yield with average production of 211 litres in 165 days lactation period (Ibrahim, 1999).

2.2.1.3 Wool sheep type

This type is well-known by producing good quality of wool such as Merino. This breed has been adapted to Australia for nearly two centuries and it is well appropriate to generate excellent quality wool in semi-arid and arid areas (Carles, 1983).

2.2.1.4 Dual purpose sheep type

This type is considered to be resistant to environmental circumstances and characterised by low productivity compared with the other types. Caloia and Mondero is examples of this type. Both breeds are described by producing meat, milk and wool (Carles, 1983).

2.2.2: Classification of Sudanese sheep

Sheep provide meat for local consumption in addition to their share in national income through the export. Sheep are also reared for milk production. The breeds of sheep in the Sudan and South Sudan were classified into five basic types and three mixed ecotypes according to tail size (Mason and Maule, 1960), the basic types includes:

(1) Sudan desert sheep which include (Butana Gezira, Watish, Hamari, Kababish, Meidob, North River wooled, and Beja).

(2) Sudan Nilotic sheep which include (Dinka, Shilluk, Nuba mountains and Mangala).

(3) Arid upland and this is the Zaghawa sheep.

(4) Arid Equatorial sheep which is the Taposa and finally.

(5) Western African Fulani (fellata and M'Bororo), (Mcleroy, 1961).

2.2.2.1: Ashgar ecotype

Ashgar are moderately large sheep its colour ranges from light to dark brown. Generally they are found along and to the west of the White Nile, and are most common in the western part of the Gezira. In study of relationship between some body measurement and the live weight Elsheikh *et al*., (2012) found that Ashgar showed the highest values in some body measurement such as scapuloischial length (body length), wither height and heart girth as 76.1, 84.75 and 91.1cm respectively within Kabashi and Nilotic adults rams, moreover these measurements had significantly affected the live weight. Also the ewes rank the highest values litter size (1.30 lamb/ewe) through ewes of Dubasi and Watish ecotypes (Sulieman *et al*., 1990).

2.2.2.2: Dubasi ecotype

Dubasi are the model sheep of the Gezira area, particularly the northern part, and are concentrated in the villages of the *Dubaseen* tribes (hence 'Dubasi'). These sheep are similar in size to the Ashgar but their thin coat is commonly patched white and black. It is rare to find Dubasi further to the west along the White Nile. This ecotype had high records in weight at first conception and weight at first parturition as 36.8 and 40.4 kg subsequently, more over it showed the highest rank in heart girth among Ashgar and Watish ecotypes (Sulieman *et al*., 1990).

2.2.2.3: Watish ecotype

The Watish ecotype is fairly smaller than each of Ashgar and Dubasi. Three colour groups-fawn, red, and white with light spottings-have been recognise (McLeroy, 1961). Watish are hardy sheep and live under relatively high rainfall conditions between latitudes 10° and 11° N and mainly found along the Blue Nile, south of Wad Medani into the

Fung area, they are mainly owned by nomadic and semi-nomadic tribes including the Kenana, the Rufaa El Hoy and the Beni Meharib. Watish dams had the lowest weights at first conception, at first parturition and at weaning after first parturition, but their weight did not drop during the lactation period as the Ashgar and Dubasi ecotypes (Sulieman *et al.*, 1990).

2.3: Sudanese desert sheep origin

Sudan Desert sheep is the most common type in the country, Sudan Desert sheep and its hybrids comprise more than 80% of the national sheep flock. Sudan Desert sheep and its crosses are supposed to be a progeny of a sheep of Egyptian origin (*Ovis aries* var.longipes) (Devendra and Mcleroy, 1982). Also they stated that Sudan Desert sheep are spread north of latitude 12°N, extending into Eritrea and westward into Chad. Mufarrih (1991) had another assumption supposed that Desert sheep have probably an origin from cross breeding between sheep of Arab tribes that have arrived to Sudan through western boarder and the sheep of northern Fulani tribes, (*Balani* and *Ouda*), in the Lake Chad basin. This assumption was supported by the fact that Fulani sheep are long-legged and long-tailed sheep. Also, Williamson and Payne, (1965) reported that it has been forced out of Egypt by the entry of fat-tailed and cross wool sheep (Mufarrih, 1991). The similarity of management practices, environmental habitat and many body features such as the shape of the head and face, body length, coat texture, thicker tail and fuller rump between Sudan Desert sheep and Fulani sheep could support the hypotheses that said that Sudan Desert sheep might be attributed to partial inheritance from their Arab ancestors Mufarrih, (1991).

2.4: Nomenclature of Sudanese sheep

Sudan Desert sheep are held tribal or sub tribal names like Hammari, Kabashi in Kordofan, Dubasi in Central Sudan and Watish (Rufaei) along with blue Nile south of Wad Medani, and sometimes take name from it coat colour such as Shugor (Ashgar) and Bourug or Abrag (white with black or brown spots) also sheep take name of its home-land area such as Meidob found in the Meidob hills in northern Darfur (Mcleroy, 1961; Sulieman *et al*., 1990 and Mukhtar, 1985).

2.5: Factors affecting sheep production

2.5.1: Management factors

The management system has many effects on the production features of the Sudan Desert sheep El-Hag *et al*., (2001). Many researchers reported that mortality rates in breeding dams were significantly higher in nomadic one than sedentary flock, while ewes lambed under sedentary system had lower lambs birth weights than those lambed in nomadic system (3.38 vs.4.08 Kg) and lambs body weight at 30 days of age (8.05 vs. 9.42 Kg), whereas lambs weights from 60-150 days of age were not different in the two systems. In contrast, in other study, Wilson (1976) reported that death rates between sedentary and migratory flocks of Southern Darfur were not differ. The mortality rate was allmost same in both systems.

2.5.2: Nutrition

Enhancing live weight at mating had an effect on ovulation and litter size (West *et al*., 1991; Nawaz and Meyer 1991). Moreover, Njoya *et al*. (2005) noted that, protein complementary additions to ewes browsing low quality pasture improved their body weight, body condition score and reproductive performance. Also Muskasa-Mugerwa and

Lalhou-Kassi (1995) reported that sufficient nutrition is important on the reproductive trait in ewes in Ethiopia furthermore, Stephenson and Bird (1992) pointing out a valuable response in productivity of supplemented ewes eating low quality grass in Australia.

During the late gestation period pregnant ewes received feed supplementation with balanced and adequate energy and protein to support developing of embryonic and fetal growth, maintain physiological requirements of the animal, mammary gland growth, colostrum and milk production (Oeak *et al.*, 2005). Eighty percent of fetal growth arises through the last 60 days of pregnancy and it is due to 35% significant increase in nutrient requirements of the ewes (Dawson *et al.*, 1999). Thus, lamb survival is related to nutrition of ewes during late gestation (60 days) (Binns *et al.*, 2002).

The capability of nutrition during mating time to change ovulation and lambing rates of ewes in several breeds is well recognised (O'Callaghan and Boland, 1999). In a study on some British breeds, Rhind *et al.* (1989) mentioned that decreasing in ovulation rate prior mating time resulted from low animals feed intake, in addition, ova wastage rate occurs due to lower feed intake after mating time. On the other hand, Landau and Molle (1997) stated that numerous Mediterranean breeds of sheep, a short period of feed flushing before mating definitely affected ovulation. In the same issue Lassoued *et al.* (2004) reported that higher rates of feeding before and through mating time were related to improved reproductive performance in accordance with the literature reported for several sheep breeds. Lambing rates were affected by the dietary treatment. Emam and Malik, (2009) reported that the most additional feeds were cotton seed cake, groundnut hulls and sorghum grains.

2.5.3: Animal factors

2.5.3.1: Breed

Animal breed and genotype had significantly affected the birth weight, daily weight gain and 90 day weights of the animal (Cochran *et al.*, 1984 and Hassen *et al.*, 2002.), besides, Boujenane and kansari, (2002) mentioned that lamb weight and survival to 70 days differed depending on genetic composition of lamb. They also found that effects of breed were significant for fecundity, number of lambs born alive, litter size at weaning, litter weight weaning per ewe joined and lamb weight at 60 days.

2.5.3.2 Age of dam

Age of dam had significant effects on many reproductive traits such as birth weight, prolificacy, twining rate and litter size (Tauh and Baah, 1985; Ali *et al.*, 1999).In more details Al-Shorepy and Notter (1996) noted average fertility of 0.59 for third lambing and older ewes, 0.45 for second lambing ewes, 0.18 for 19 months old ewes and 0.11 for yearlings old ewes. Likewise, Boujenane (2002) reported that dam age had significant effect on birth weight and 90 days.

2.5.3.3 Type of birth

Analla *et al.*, (1998) reported that birth type had noticeable effect on birth weight and consequent live weights as 30, 60 and 90 days, so that, single lambs were heavier than twin lambs, additionally, growth rate of single lambs was faster than twins (Macit *et al.*, 2001). moreover Tuah and Baah, (1985) found that weaning weight, pre-weaning growth rate were influenced by birth type, similar findings were obtained by Cloete *et al.* (2007) in crossing Dorper ewes with Ile de France, Merino Land sheep and SA Mutton Merino rams. Also Dimsoski *et al.*, (1999) noted that

single lambs had higher daily gain than twins in the pre-weaning period. Mortality rate of single born lambs was lower than twins (Nawaz and Meyer, 1991).

2.5.3.4: Sex of lamb

Both sexes of lambs almost had the same weights at birth, 30 and 90 days of age, but it differ in late stages (El-Hag *et al*., 2001 and Hassen *et al*., 2002). These results are in contrast to Analla *et al*. (1998) and Boujenane, (2002) who found that male birth weights were heavier than those of the female and these results are applicable for 30 and 90 days. Also Cloete *et al*. (2007) mentioned that birth weight of male was higher than female lambs. Several researchers have found significant differences in body weight between male and female lambs at entirely ages (Bichard and Cooper, 1966; Gjedrem, 1967 and Mavrogenis 1996 a,b). Moreover Ali *et al*. (1999) stated that male lambs were heavier than females at birth, weaning and 6 months of age. However, (Rastogi 2001; Boujenane and Kansari 2002) noted that sex of lamb was not an important source of variation.

2.5.4: Breeding season

Lambing season significantly affected the prolificacy and twinning rate, birth weight and on consequent live weights and survival age of lambs (El-Hag *et al*., 2001; Rastogi, 2001; Hassen *et al*., 2002; Boujenane and Kansari 2002; Tuah and Baah, (1985). Lambs born in rainy season had the highest birth weight (3.83 Kg), while those born in the early dry season (3.52 Kg) were higher than those born in late dry season (3.17 kg), hence the lamb weight at 30 days of age and growth from 90-150 days were higher in lamb born in the rainy season. Moreover, El-Hag *et al*. (2001) reported that breeding season had significant effects on

desert sheep reproductive performance. The rainy season recorded higher lambing and mortality rates numbers of serviced ewes than in the late dry season.

El-Hag *et al*., (2001). Reported that the weights and mortality rate of lambs born under the nomadic system and those born during the rainy season were higher comparing to other rearing system and season. Mortality rate of lambs are an essential constituent of total flock Death (Wilson, 1976). About 30 % of mortality rate was to the age of six months, while, half of the deaths lambs happening in the first four weeks and deaths were rare during the late dry season., moreover, higher records of serviced ewes were noted in the late dry season however, higher lambing and mortality rates occurred during the rainy season(El-Hag *et al*., 2001).

In study of seasonal effects on birth weight (BWT) on prolific Assaf flock kept under intensive management, BWT of born lambs on April (4.6 kg)was significantly differs from BWT of born lambs on September (3.8 kg).BWT was inversely affected by day length among the early stage of gestation, while it was directly related with rate of changes in day length during the latter stages of gestation (Gootwine and Rozov, 2006).

2.5.5: Climatic factors

Both genetic and environmental factors and the interaction between them could effect on birth weight of lambs. Along with the environmental factors, season was also found to have an effect on birth weight with lambs born in the rainy season being smaller than spring-born lambs. Ewes pregnant in the summer season could have lower food intake, and increase heat load (Shelton and Huston, 1968) which is high during the hot season then it influences the birth weight. Furthermore, seasonal

variation in gestation length (Jenkin and Young, 2004) may also be related to seasonal variation in BWT.

2.5.6: Disease factors

Makawi, (1999) stated that infectious diseases were divided into three main groups; specific genital diseases, non-specific genital, and general infectious diseases. The main reasons of reduced productivity in sheep are the infectious reproduction diseases, and it is generally categorized into these mainly affecting the venereal tract of rams and those mainly affecting ewes causing abortion and pre-natal lamb mortality (Rahaley, 1984). Higher rate of gastro-intestinal and respiratory disease problems noted during the dry season for lactating ewes in transhumant sheep comparing to dry open, were probably a reflection of the greater nutritional stress experienced by lactating animal (Cook and Fadlalla, 1987).

2.6: Sheep breeding

2.6.1: Reproduction

The breeding season of sheep is varies for many farm animals and it occurs primarily in the fall season of the year (Robert and Thomas, 2004). The ratio of ewes to ram differs according to the management, if it is proper, ewes to ram should be 200:1 (Allison, 1975). Moreover the common ratio for tropical sheep could be 10 or 20:1 (Doney *et al.*, 1982 and Devendra and McLory, 1982)

2.6.2: Puberty

Puberty is defined as the capability of animal to be fertilized, for ewes it is the sign of the first estrus or it is the time when the estrus cycle start, and it is mating ability for rams. Puberty ranged between 5-12 months of age and is affected by breed, nutrition and lambing date

(Robert and Thomas, 2004). The average length of estrus cycles is over 16 days and the duration of estrus is 30 hours (Robert and Thomas, 2004). Estrus first signs are differ in several breeds due to different nutrition which lead to different growth rates. Younis *et al.*, (1978) found that Awassi lambs on good nutrition can reach the first estrus signs in 274 days of age, however, Rambouillet crossbred lambs in Rajasthan are about 615 days before the first display estrus (Kishore *et al.*, 1982), while in Ossimi and Barki Egyptian sheep the average puberty age was 347 days when reared on high level of nutrition, however 366 days on a low level (El-Homosi and El-Hafiz, 1982).

2.7: Prediction of live body weight

Many body measurements are important to observe the growth of the sheep and also can be used to estimate genetic association between body weight and body measurements (Mohammad *et al.*, 2012). The main method of weighing animals without balances is to revert body weight on a certain number of body dimensions. Many researchers have used body measurements to predict live body weight in several breed of sheep of Turkey (Sarti *et al.*, 2003; Atta and El Khidir, 2004; Janssens *et al.*, 2004, Riva *et al.*, 2004, Topai and Macit, 2004; Shaker and Hammam, 2008; Abdel-Moneim, 2009; Cam *et al.*, 2010 and Shehata, 2013). Several models might be used to predict body weight in different environmental conditions and breeds Enevoldsen and Kristensen (1997) and it showed positive and strongly correlated between body weights and body measurements in different ages, the correlation modules for different body measurements fluctuated between 0.506 and 0.968 Thiruvenkadan, (2005). Furthermore, Cam *et al.*, (2010) reported that fattening situations can be reflected by some measurements such as heart girth, chest depth and chest width, however, it was not reflected by height

at wither, height at rump and body length. Also Elsheikh *et al.*, (2012) conclude that live weight of the Kabashi, Ashgar and Nilotic adult rams can be estimated using heart girth with acceptable accuracy. In study of regression equation between body weight and heart girth for ewes and rams Thys and Hardouin, (1991) found high coefficient of determination as 0.88 and 0.86 for rams and ewes, respectively.

2.8: Ovulation and litter size

Ovulation differs between species depending on both genetic and environmental reasons. Mammals can be either mono or poly-ovulatory based on number of mature ovum that are released during ovulation. Ruminants normally release a single ovum per ovulation compared to pigs and rodents which have high ovulation rates (Montgomery *et al.*, 2001). The ovulation rate varies between sheep breeds, it can be one egg per ovulation in Texel and Suffolk and reaches ten eggs per ovulation in the prolific Booroola Merino breed (Hanrahan, 1984; Souza *et al.*, 2001). Alongside genetic background, other factors can affected the difference in ovulation rate among breeds as age, season and nutrition (Jansson, 2014).

Development of follicle includes a sequence of stages categorized by the number of granulosa cell layers surrounding the oocyte and based on the existence of certain hormones (Montgomery *et al.*, 2001). The development begins with the primordial phase (non-growing phase) where the oocyte is bounded by a single layer of epithelial cells then the follicle develops into the primary and secondary phase where the epithelial cells proliferate into granulosa cells and theca cells separated by the basal lamina. FSH stimulates the growth and differentiation of granulosa cells and LH affects the proliferation of theca cells which secrete androgens that are altered into estrogen. Granulosa cells are essential for ovulation meanwhile they support the oocyte and secret the

hormones estrogen and inhibin. The oocyte is also surrounded by a non-cellular material layer called the zona pellucida (Sjaastad *et al.*, 2003). The oocyte starts to arrange for ovulation after being affected by hormones (Montgomery *et al.*, 2001).The phases in follicle growth can also be divided into the pre-antral and antral stages which are gonadotropin reactive and gonadotropin reliant subsequently (Pramod *et al.*, 2013).

Litter size differs between and within sheep breeds (Davis, 2005). It is dependent on ovulation rate and is influenced by the number of fertilized oocytes. The higher ovulation rate leads to more oocytes which will be accessible for fertilization through the estrous and raise the possibility of more litters (Drouilhet *et al.*, 2013). Many studies have revealed that higher ovulation rates lead to reduced embryo survival and higher litter sizes lowers the birth weights of lambs (Fogarty, 2009). There is an obvious genetic association between litter size and ovulation rate, an incessant trait, creating indirect selection on ovulation rate more effective for making genetic improvement (Hanrahan, 1980). Litter size is optimally different between production systems and breeds. in intensive systems with dairy sheep, spring lamb production, where balanced feed forage and concentrate are available, large litters with two lambs or more are desirable, while in semi-intensive systems including tough breeds with lower milk yield, managed in high land pastures in open locations., forage may not be available, large litters are not required in those production systems (Liandrisa *et al.*, 2012). Ovulation rate is affected by several factors including genetic factors, live weight, nutrition, animal age and season. Ovulation rate rises with age and can reach a peak at 3-5 years (Evans and Maxwell, 1987).

2.9: Fecundity genes

Reproduction is a complex progression and fecundity traits e.g. ovulation rate and litter size can be heritably controlled by several genes with minor effects, and sometimes also by single gene with major effects, named fecundity (*Fec*) genes (Drouilhet *et al*., 2009).

2.9.1: Transforming growth factor β (*TGFβ*) super family

The Booroola fecundity gene (FecB) was identified in1980as the first major gene for prolificacy in sheep. Recently, several studies have showed that the ovulation rate and litter size can be genetically controlled by a set of different genes, communally named as fecundity (Fec) genes (Piper and Bindon 1982 and Davis *et al*., 1982). Three fecundity genes have identified in sheep which refer tothe *TGFβ* gene super family (Fabre *et al*., 2006), namely, bone morphogenetic protein receptor type IB (BMPRIB) or activin-like kinase 6 or FecB on chromosome 6 (Souza *et al*., 2001), growth differentiation factor 9 (*GDF9*) or *FecG* on chromosome 5 (Hanrahan *et al*., 2004) and bone morphogenetic protein15 (BMP15) or FecX on chromosome X (Galloway *et al*., 2000 and Hanrahan *et al*., 2004). Most mutations recognized to date changing ovulation rate in sheep have been in the *TGFβ* super family pathway, emphasising the key role of this family of proteins in regulating ovulation rate (Juengel *et al*., 2011). The FecB (Booroola) mutated allele is related with additive effect on ovulation rate (Souza *et al*., 2001), and is dominant for litter size (Davis *et al*., 1982). Morever, mutations of *FecG* five single nucleotide polymorphisms (SNPs) and FecX (five SNPs) are associated with increased ovulation rate in heterozygous animals and sterility in homozygous animals (Hanrahan *et al*., 2004 and Bodin *et al*., 2007).

2.9.1.1: Growth differentiation factor 9

GDF9 or *FecG* gene is located on chromosome 5 between markers BM7247 and BMS2258 (Sadighi *et al*., 2002), the gene *GDF9* spans almost 2.5 kb and contains two exons, divided by an intron of 1126 bp the exons code for a propeptide with 453 amino acids. (Bodensteiner *et al*., 1999). The *BMP15*, a close homolog of *GDF9*, is expressed in the oocyte at primary follicular stage and continues through ovulation (Dube *et al*., 1998). *GDF9* has a vital role in ovarian follicular development and ovulation rate. It has been widely studied in humans, sheep, and goats (Elvin *et al*., 1999; McNatty *et al*., 2005). The changing concentrations of *GDF9* in vivo leads to incremental changes in ovulation rate in sheep (Galloway *et al*., 2000, Juengel *et al*., 2004 and Hanrahan *et al*., 2004).

2.9.1.2: Physiology of GDF9 signalling molecules

G*DF9* plays a vital role through early folliculogenesis in female reproduction as a growth and differentiation factor secreted by oocytes in mammals (Elvin *et al*., 1999). Many studies showed that *GDF9* could regulate several key granulose cell enzymes occupied in cumulus expansion and maintenance of an optimal oocyte microenvironment during an oocyte-somatic cell interaction and synergistic action along with bone morphogenetic protein15 (BMP15), which are essential for normal ovulation, fertilization, and female reproduction (Yan *et al*., 2001 and McNatty *et al*., 2005). Expression of *GDF9* mRNA and protein were detected at all stages of ovarian follicles and luteal tissue in caprine ovary (Silva *et al*., 2004).

2.9.1.3: Genetic mutation of *GDF9* in sheep

In sheep, numerous mutations in the GDF9 coding sequence have been reported, with one single exception all of them being located in the

second exon (Table 2.1). Eight out of these 11 single nucleotide polymorphisms (SNPs) cause amino acid substitutions and some of them have an effect on OR and hence litter size. The three non-synonymous SNPs c.943C>T, c.1184C>T and c.1279A>C result in a phenotype of increased OR/LS in heterozygous ewes, and infertility linked to hypoplasia of ovary and uterus in homozygous females (Hanrahan *et al.*, 2004; Juengel *et al.*, 2013; Souza *et al.*, 2014). Infertility due to the homozygous mutant genotype was not observed for two other non synonymous SNPs, c.1111G>A (Vage *et al.*, 2013) and c.1034C>T (Silva *et al.*, 2011), which instead show an additive effect on OR and LS. As only the mature GDF9 peptide is deemed to be biological active (Paulini and Melo, 2011), mutations being located proximal to the RRHR furin protease cleavage site (proximal to amino acid position 318) are regarded as not to effect the protein function (Hanrahan *et al.*, 2004). A single non-synonymous SNP in exon 1 (c.260G>A) is located before the furin cleavage site and causes only a conservative substitution of amino acids (Arg87His) (Hanrahan *et al.*, 2004). However, this GDF9 mutation was claimed by Barzegari *et al.* (2010) to be associated with infertility (genotype AA), and at least in combination with another mutation in the gene coding for the bone morphogenic protein 15 (BMP15) with higher OR (genotype AG) in Iranian sheep. Associations of GDF9 sequence variants at positions with no obvious impact on the gene function might be due to a linkage with undetected or until now not tested causal variants. Such a linkage was recently also speculated by Albarella *et al.* (2015) for a silent G>A substitution they detected for the first time at position 750 of GDF9 in Bagnolese sheep. They observed a higher LS in sheep with the genotype GG compared to AG ($P < 0.05$). The effect of the genotype AA was not tested due to its low frequency.

Table 2.1. Published sequence variants in the coding region of ovine *GDF9*

Position in coding sequence	Variant name(s)	Amino acid change	Breed	Variant first published by
Exon 1				
c.260G>A	G1	p.Arg87His	Cambridge and Belclare	Hanrahan *et al*., (2004)
Exon 2				
c.471C>T	G2	p.Val157Val	Cambridge and Belclare	Hanrahan *et al*., (2004)
c.477G>A	G3	p.Leu159Leu	Cambridge and Belclare	Hanrahan *et al*., (2004)
c.531C>T		p.Asn177Asn	Laticauda and Bagnolese	Albarella *et al*., (2015)
c.617G>A		p.Arg206Lys	Laticauda and Bagnolese	Albarella *et al*., (2015)
c.721G>A	G4	p.Glu241Lys	Cambridge and Belclare	Hanrahan *et al*., (2004)
c.729G>T		p.Gln243His	Small Tail Han	Chu *et al.*, (2011)
c.750G>A		p.Arg250Arg	Thoka	Nicol *et al*., (2009)
c.943C>T	$FecG^{V}$	p.Arg315Cys	Brazilian Sheep	Souza *et al*., (2014)
c.953G>T		p.Arg318Ile	Laticauda and Bagnolese	Albarella *et al*., (2015)
c.978A>G	G5	p.Glu326Glu	Cambridge and Belclare	Hanrahan *et al*.,(2004)
c.994G>A	G6	p.Val332Ile	Cambridge and Belclare	Hanrahan *et al*.,(2004)
c.1034C>T	$FecG^{SI}$/$FecG^{E}$	p.Phe345Cys	Brazilian Santa Inês	Silva *et al.*, (2011)
c.1111G>A	G7	p.Val371Met	Cambridge and Belclare	Hanrahan *et a*l., (2004)
c.1184C>T	G8/$FecG^{H}$	p.Ser395Phe	Cambridge and Belclare	Hanrahan *et al*., (2004)
c.1203G>A		p.Val401Val	Laticauda and Bagnolese	Albarella *et al*., (2015)
c.1279A>C	$FecT^{T}$	p.Ser427Arg	Thoka	Nicol *et al*., (2009)
c.1358G>A		p.Arg453His	Laticauda and Bagnolese	Albarella *et al*., (2015)

2.9.2: Other fecundity genes

Several studies reported other genes not related to *TGFβ* superfamily could affect ovulation rate/litter size such as:

2.9.2.1:Lacaune gene (*FecL*)

This gene was found in The French Lacaune breed which is characterized with high prolificacy and litter size, the gene is autosomal and carried on *FecL* locus on chromosome 11, it contains two genes: insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) and

beta-1,4-N-acetyl-galactosaminyl transferase 2 (B4GALNT2),recently recent studies showed that both ovarian activity and the endocrine profiles had been affected by *FecL* locus, furthermore, many researchers have shown that the B4GALNT2 gene might be responsible for the high fecundity in Lacaune sheep. The hypotheses is held by the fact that B4GALNT2 transferase activity is localized to the granulosa cells which are important in follicular development (Sjaastad *et al.*, 2003 and Drouilhet *et al.*, 2013). The influence of the autosomal $FecL^L$ mutation on ovulation rate is additive with one copy increasing ovulation rate by about 1.5 and two copies by about 3.0.Large number of gonadotropin-dependent follicles with a diameter more than 3 mm, an access in plasma estradiol levels, and an increase in the rate of Luteinizing Hormone (LH) flow during the follicular phase, leading to a precocious LH are related with increasing OR homozygous $FecL^L/FecL^L$ ewes.

2.9.2.2: Woodland gene ($FecX2^W$)

This gene is located on the X-chromosome, hence the ewes can inherit it from either carrier parent while rams can only get it from their dam, however, the characteristics of $FecX2^W$ gene and it's mechanism by which it affects OR is unidentified to date (Feary *et al.*, 2007) but many studies have reported that the $FecX2^W$gene is not *BMPR-1B* or *BMP15* (Hanrahan *et al.*, 2004) however it has been associated to changes in the *TGFβ* superfamily pathway with alterations in manifestation levels of mRNA converting BMP15 observed in carriers the$FecX2^W$ gene when matched to wild-type individuals(Feary *et al.*, 2007). The phenotype of the mutation of $FecX2^W$gene in ovaries showing an increase in number of follicles smaller than 1 millimeter in diameter in the antral stage, moreover, Oocytes are also smaller, so that, when oocyte diameter compare to follicle diameter was scanned, the oocytes were bigger

matched to non-carriers of the mutation, This might also be found in dams carried the Booroola ($FecB^B$) and Inverdale ($FecX^I$) mutations (Feary *et al.*, 2007). The effect of the mutation is silenced if the ewe receives it from the dam (maternal inheritance) and will not give an increase in OR, while the mutation had effect on LS if the ewe inherit it from the ram (paternal inheritance) hence it is maternally etched (Davis *et al.*, 2001and Davis, 2005).

2.9.2.3:Davisdale gene (*FecD*)

In study of ovulation rate records across four progeny tests of Davisdale sheep descended from a prolific female, Juengel *et al.* (2011) found a strong evidence for a putative major autosomal gene (fecundity Davisdale *FecD*) controlling ovulation rate *FecD* gene had additive effect and increases ovulation rate by 0.4 to 0.8 in heterozygous ewes, while there is no evidence of infertility in homozygous ewes, moreover *FecD* gene does not appear to be in the *TGFβ* superfamily pathway, and not relate with mutations in *BMP15* gene, hence it is probable to follow a new pathway controlling ovulation rate.

Chapter three

3. A field study on some productive and reproductive traits of three Sudanese desert sheep (Ashgar, Dubasi and Watish) ecotype

3.1: Materials and methods

3.1.1: Study period and area

The study was carried out during March and May 2015 at the home-land of the studied desert sheep ecotypes, including Khartoum and River Nile states Ashgar, Gezira states for Dubasi, Sinar and Blue Nile states for Watish.

3.1.2: Data collection

A fitted form of detailed, structured questionnaire was used to collect information from desert sheep owners in studied area through an interview conducted over single visit (appendix 1), the questionnaire was designed to obtain information on general household information, herd structure, reproductive-productive practices (management) in the field and feeding field management practices.

3.1.3: Statistical analysis

The obtained data were summarized and analysed mainly in the form of descriptive as frequencies and percentage, Chi-square test and one way ANOVA followed by least significant difference test (LSD) were used using IBM SPSS statistics for Windows program, Version 20.0. Armonk, NY: IBM Corp.

3.2: Results

3.3: Discussion

Chapter four

4. Association between some body measurements traits and live weight of some Sudanese sheep ecotypes (Ashgar, Dubasi and Watish)

4.1: Materials and methods

4.1.1: Study area and animals

The study was carried out during March and May 2015 at the homeland of the studied sheep ecotypes, including Khartoum and River Nile states for Ashgar, Gezira state for Dubasi, Sinar and Blue Nile states for Watish. Two hundred and twenty five head of three sheep ecotypes were randomly selected from the study area [Ashgar (n=80), Dubasi (n=73) and Watish (n=72), figure 4.1] and according to sex [males (n=66) and females (n=159)].

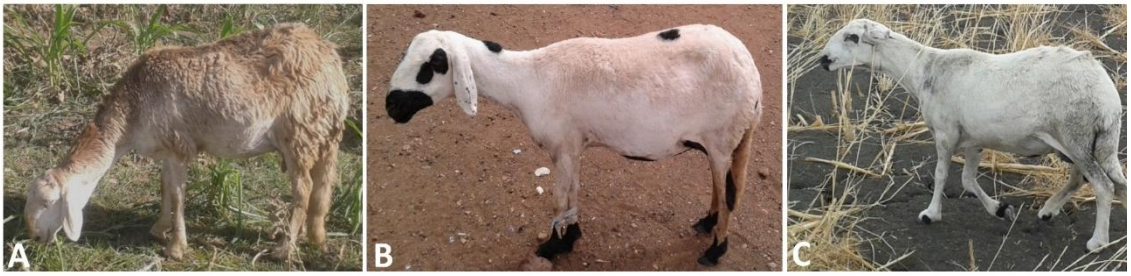

Figure 4.1 Sudanese desert sheep ecotypes A), Ashgar; B), Dubasi; C), Watish.

4.1.2: Studied body measurements

The body measurements of the three sheep ecotypes in different ages and sexes (post weaning) will be taken after animal weighing during the experimental period using metric tape according to phenotypic characterization of animal genetic resources recommended by FAO (2012), the studied body measurements as follows:

Body length: which is the distance between the dorsal tip of scapula and the tip of the ischium.

Height at wither: which is the height of the highest point of the dorsum of the animal above the scapular vertical to the ground surface at the level of the front feet.

Heart girth: which is the circumference of the chest just behind the foreleg.

Chest depth: which is the distance from the point of the couple scapular Scapular width: the distance between the spine of the two scapulars

Rump width: the distance between the two cocci.

Head length: the distance between the dorsal surface of the frontal bone to the distal end of the nasal bone

Head width: the distance between the two lateral surfaces of the temporal bones

Forelimb circumference: the circumference of the forelimb (humerous) above the elbow joint.

Hind limb circumference: the circumference of the hind limb (femur) above the knee joint.

Horn length: the distance from the base of the horn on the frontal bone to the horn tip

Ear length: the distance from the base of the ear on the parietal bone to the ear tip

Tail length: the distance from the base of the tail (last sacral vertebrate) to the tail tip

Wool length (at rump tip): the distance from the base of the hair the hair tip

Cannon circumference: the circumference of the metacarpus bone.

4.1.3: Statistical analysis

The obtained data were tested for significance using analysis of variance ANOVA followed by least significant difference (LSD) test. Also, Independent samples T. test was used and Pearson's correlation, simple regression analysis was fitted using linear, quadratic, cubic, compound, power and S mathematical models as shown below using IBM SPSS statistics for Windows program, Version 20.0. Armonk, NY: IBM Corp.

4.2: Results

4.2.1: Effect of sheep ecotype on live body weight and body measurement

With the exception of shank and thigh circumference there were significant differences ($P<0.01$) in live body weight and all body measurements among the studied sheep ecotypes (Table 4.1). Dubasi ecotype recorded the lowest values of most body measurements with exclusion of head width, shank circumference and ear length, while Watish ecotype showing the highest values of most body measurements not including rump length, thigh circumference, wool length and cannon circumference.

Table 4.1. Effect of sheep ecotype on body measurements

Measurements	Sheep ecotypes			SEM	P. value
	Ashgar (n=80)	Dubasi (n=73)	Watish (n=72)		
BW, kg	39.03[b]	36.77[c]	44.98[a]	0.61	0.000
BL, cm	68.65[b]	65.50[b]	72.24[a]	0.42	0.000
WH, cm	78.02[a]	73.44[b]	79.59[a]	0.35	0.000
HG, cm	81.98[b]	77.23[c]	86.09[a]	0.53	0.000
CD, cm	42.40[b]	38.32[c]	45.55[a]	0.32	0.000
CW, cm	17.17[b]	14.86[c]	19.92[a]	0.20	0.000
RL, cm	19.33[a]	15.04[c]	15.72[b]	0.23	0.000
RW, cm	16.60[a]	14.85[b]	19.82[a]	0.21	0.014
HL, cm	12.57[b]	11.76[c]	13.53[a]	0.15	0.000
HW, cm	8.90[c]	10.88[b]	11.01[a]	0.57	0.014
SC, cm	23.12	23.30	23.29	0.23	0.809
TC, cm	31.43	31.35	30.70	0.34	0.241
EL, cm	16.44[a]	15.76[b]	13.93[c]	0.15	0.000
TL, cm	60.65[b]	55.89[c]	67.94[a]	0.98	0.000
WL, cm	4.50[a]	4.36[b]	4.09[c]	0.09	0.004
CC, cm	7.79[a]	7.45[c]	7.49[b]	0.06	0.000

[a,b,c]: different superscript letters within the same row means significant difference at P<0.05
SEM= Standard error of mean

4.2.2: Effect of sex on live body weight and body measurement

Sex of sheep showed significant differences in live body weight and the majority of body measurements (Table 4.2) however, chest depth, rump width, head width and thigh circumference were insignificant (P>0.05). The results revealed that females were higher than males in live body weight and most body measurements.

Table 4.2. Effect of sex on body measurements of sheep ecotypes

Measurements	Sex		Overall (n=225)	P. value
	Male (n=66)	Female (n=159)		
BW, kg	39.30±0.60	41.64±0.38	40.96±0.44	0.000
BL, cm	67.89±0.41	71.43±0.26	70.41±0.32	0.000
WH, cm	76.05±0.34	77.99±0.22	77.49±0.25	0.000
HG, cm	80.98±0.51	83.65±0.33	82.22±0.38	0.011
CD, cm	42.38±0.31	41.80±0.20	42.05±0.25	0.111
CW, cm	17.69±0.13	16.95±0.20	17.52±0.17	0.002
RL, cm	17.20±0.22	16.20±0.15	16.64±0.21	0.000
RW, cm	17.10±0.20	17.07±0.13	17.09±0.17	0.895
HL, cm	12.32±0.14	12.91±0.09	12.75±0.09	0.001
HW, cm	10.71±0.56	9.82±0.36	10.03±0.30	0.174
SC, cm	24.20±0.22	22.28±0.14	22.79±0.14	0.000
TC, cm	31.20±0.33	31.11±0.21	31.13±0.19	0.814
EL, cm	15.57±0.09	15.18±0.14	15.49±0.11	0.022
TL, cm	62.68±0.95	60.31±0.61	61.10±0.60	0.037
WL, cm	4.08±0.06	4.56±0.09	4.22±0.05	0.000
CC, cm	7.69±0.06	7.47±0.04	7.53±0.03	0.002

4.2.3: Association between live body weight and body measurements of the studied sheep ecotypes

Table (4.3) showed the correlation coefficient matrix of live body weight and body measurements for the overall data of the three ecotypes of sheep, whereas table (4.4) and Appendix (15), table (4.5) and Appendix (16) and table (4.6) Appendix (17) showed the correlation coefficient matrices of live body weight and body measurement for the Ashgar-both sexes, Dubasi-both sexes and Watish-both sexes sheep ecotypes respectively. In the four tables (4.3, 4.4, 4.5 and 4.6) the animals' live body weight correlated significantly and positively ($P<0.01$) with the majority of body measurements, the highest correlation

coefficient was found between live body weight and heart girth, wither height, body length while it was moderately between live body weight and chest depth, chest width while the lowest correlation coefficient was found between shank circumference, thigh circumference, tail length and cannon circumference each other and with other body measurements. Moreover Watish ecotype showed high correlation coefficient between live body weight and body length than live body weight and wither height.

Table 4.3. The correlation matrix between different body measurements of studied sheep ecotypes (n=225)

	BW	BL	WH	HG	CD	CW	RL	RW	HL	HW	SC	TC	TL
BW	1												
BL	0.749**	1											
WH	0.756**	0.597**	1										
HG	0.826**	0.687**	0.714**	1									
CD	0.599**	0.475**	0.638**	0.732**	1								
CW	0.595**	0.607**	0.646**	0.700**	0.686**	1							
RL	0.100	0.040	0.297**	0.313**	0.320**	0.273**	1						
RW	0.478**	0.433**	0.513**	0.606**	0.669**	0.671**	0.229**	1					
HL	0.302**	0.466**	0.391**	0.456**	0.440**	0.532**	0.253**	0.402**	1				
HW	-0.070	-0.090	-0.070	-0.050	-0.020	-0.030	-0.176**	0.010	-0.060	1			
SC	0.225**	0.130	0.030	0.138*	0.100	0.020	-0.100	0.150*	-0.040	0.070	1		
TC	0.245**	0.274**	0.142*	0.172**	0.060	0.060	0.080	-0.030	0.010	-0.040	0.394**	1	
TL	0.378**	0.361**	0.415**	0.407**	0.419**	0.540**	0.120	0.434**	0.247**	0.030	0.120	-0.010	1
CC	0.080	0.060	0.149*	0.080	0.130	0.070	0.100	0.090	-0.080	0.030	0.239**	0.130	0.161*

**: correlation is significant at P<0.01, *: correlation is significant at P<0.05

Table 4.4. The correlation matrix between body measurements of Ashgar ecotype (n=80)

	BW	BL	WH	HG	CD	CW	RL	RW	HL	HW	SC	TC	TL
BW	1												
BL	0.623**	1											
WH	0.738**	0.603**	1										
HG	0.772**	0.662**	0.549**	1									
CD	0.705**	0.606**	0.557**	0.836**	1								
CW	0.516**	0.673**	0.472**	0.608**	0.531**	1							
RL	0.389**	0.621**	0.441**	0.514**	0.409**	0.520**	1						
RW	0.536**	0.504**	0.372**	0.645**	0.523**	0.456**	0.500**	1					
HL	0.135	0.473**	0.212	0.375**	0.343**	0.366**	0.652**	0.129	1				
HW	0.339**	0.143	0.313**	0.269*	0.324**	0.023	0.031	0.445**	-0.260*	1			
SC	0.190	0.034	0.084	0.130	0.275*	-0.119	-0.017	0.236*	-0.146	0.571**	1		
TC	0.165	0.267*	0.213	0.117	0.225*	0.006	0.238*	0.130	0.135	0.193	0.528**	1	
TL	0.330**	0.338**	0.356**	0.270*	0.223*	0.371**	0.160	0.193	-0.039	0.340**	0.068	-0.019	1
CC	0.102	0.115	0.139	0.061	0.187	0.036	0.004	0.232*	-0.123	0.469**	0.525**	0.154	0.237*

**: correlation is significant at P<0.01, *: correlation is significant at P<0.05

Table 4.5. The correlation matrix between body measurements of Dubasi ecotype (n=72)

	BW	BL	WH	HG	CD	CW	RL	RW	HL	HW	SC	TC	TL
BW	1												
BL	0.864**	1											
WH	0.868**	0.698**	1										
HG	0.918**	0.826**	0.784**	1									
CD	0.402**	0.306**	0.338**	0.367**	1								
CW	0.769**	0.757**	0.695**	0.691**	0.294*	1							
RL	-0.481**	-0.578**	-0.387**	-0.352**	-0.069	-.402**	1						
RW	0.316**	0.263*	0.253*	0.318**	0.234*	0.294*	0.169	1					
HL	0.391**	0.462**	0.273*	0.397**	0.071	0.535**	-0.155	0.274*	1				
HW	-0.230	-0.210	-0.129	-0.159	-0.133	-0.121	-0.068	-0.217	-0.115	1			
SC	0.233*	0.166	0.189	0.242*	0.037	0.251*	0.177	0.262*	0.131	-0.099	1		
TC	0.659**	0.629**	0.575**	0.610**	0.251*	0.506**	-0.374**	0.162	0.309**	-0.124	0.330**	1	
TL	0.051	0.22	0.102	0.004	-0.012	0.129	-0.152	0.132	0.150	-0.04	0.296*	0.222	1
CC	0.100	0.092	0.147	0.079	0.195	0.018	-0.106	-0.099	0.067	0.036	0.041	0.331**	0.192

**: correlation is significant at P<0.01, *: correlation is significant at P<0.05

Table 4.6. The correlation matrix between body measurements of Watish ecotype (n=73)

	BW	BL	HW	HG	CD	CW	RL	RW	HL	HW	SC	TC	TL
BW	1												
BL	0.780**	1											
WH	0.775**	0.504**	1										
HG	0.865**	0.671**	0.606**	1									
CD	0.695**	0.653**	0.469**	0.623**	1								
CW	0.576**	0.565**	0.320**	0.549**	0.445**	1							
RL	0.486**	0.476**	0.232*	0.504**	0.333**	0.382**	1						
RW	0.420**	0.460**	0.212	0.422**	0.370**	0.376**	0.817**	1					
HL	0.149	0.217	0.073	0.104	0.141	0.16	-0.13	-0.118	1				
HW	0.070	0.089	0.068	0.058	0.075	-0.012	0.043	0.069	0.348**	1			
SC	0.444**	0.440**	0.239*	0.432**	0.291*	0.488**	0.373**	0.392**	0.304**	0	1		
TC	0.357**	0.268*	0.22	0.422**	0.301**	0.534**	0.336**	0.294*	-0.128	-0.152	0.137	1	
TL	0.483**	0.441**	0.237*	0.429**	0.215	0.461**	0.211	0.092	0.21	-0.143	0.376**	0.251*	1
CC	0.1	0.092	0.147	0.079	0.195	0.018	-0.106	-0.099	0.067	0.036	0.041	0.331**	0.192

**: correlation is significant at P<0.01, *: correlation is significant at P<0.05

4.2.4: Regression formulas of the sheep ecotypes

The regression equations of the three sheep ecotypes were calculated to forecast the body weight from the body measurements (Table 4.7) and appendix 18, 19 and 20 for each Ashgar, Dubasi and Watish respectively. R^2 values of the regressions in the three sheep ecotypes showed that heart girth was highly associated with live body weight while, body length had the least association with live body weight. Also the results showed lowest correlation coefficients between live body weight and head length, tail length, head width in Ashgar and shank circumference in Dubasi ecotype and thigh circumference in Watish.

Table 4.7.The simple regression equations of body weight and live body measurements for the studied sheep ecotypes

Ashgar	Dubasi	Watish
$BW^{5}= 0.04\times BL^{1.61}$, $R^2= 0.431^{**}$	$BW^{3}= -288.19+7.05BL$, $R^2= 0.647^{**}$	$BW^{2}= 213.46-6.16BL+0.05BL^2$, $R^2= 0.617^{**}$
$BW^{4}= 1.82\times 1.04^{WH}$, $R^2= 0.582^{**}$	$BW^{3}= -322.43+6.70WH$, $R^2= 0.616^{**}$	$BW^{4}= 1.73\times 1.04^{WH}$, $R^2= 0.622^{**}$
$BW^{6}= e^{5.60-158.28/HG}$, $R^2= 0.637^{**}$	$BW^{3}= 7.36+0.60HG+0.013HG$, $R^2= 0.836^{**}$	$BW^{3}= -376.08+8.48HG-0.04HG^2$, $R^2= 0.888^{**}$
$BW_{6}= e^{5.53-78.24/CD}$, $R^2= 0.521^{**}$	$BW^{3}= -469.64+24.82CD-0.30CD^2$, $R^2= 0.244^{**}$	$BW^{3}= -70.48+3.86CD-0.03CD^2$, $R^2= 0.487^{**}$
$BW^{4}= 22.59\times 1.03^{RL}$, $R^2= 0.183^{**}$	$BW^{3}= -79.95+10.28CW-0.10CW^3$, $R^2= 0.639^{**}$	$BW^{2}= 85.90-6.02CW+0.20CW^2$, $R^2= 0.357^{**}$
$BW^{3}= 15.33+1.16RW+0.02RW^2$, $R^2= 0.288^{**}$	$BW^{3}= 122.56-7.62RL+0.01RL^3$, $R^2= 0.307^{**}$	$BW^{2}= 21.96+1.13RL+0.02RL^2$, $R^2= 0.237^{**}$
$BW^{2}= 147.97-18.02HL+0.74HL^2$, $R^2= 0.090^{**}$	$BW^{6}= e^{4.34-10.24/RW}$, $R^2= 0.138^{**}$	$BW^{2}= -76.15+10.60RW-0.23RW^2$, $R^2= 0.186^{**}$
$BW^{1}= 20.22+2.35HW$, $R^2= 0.115^{**}$	$BW^{6}= e^{4.38-8.61/HL}$, $R^2= 0.187^{**}$	$BW^{2}= 359.76-48.43HL+1.84HL^2$, $R^2= 0.102^{*}$
$BW^{3}= 49.08-1.75TL+0.04TL^2$, $R^2= 0.373^{**}$	$BW^{2}= 4.29+4.13HW-0.05HW^2$, $R^2= 0.327^{**}$	$BW^{2}= 195.25-14.30SC+0.33SC^2$, $R^2= 0.264^{**}$
-	$BW^{3}= -597.33+53.04SC-1.10SC^2$, $R^2= 0.149^{**}$	$BW^{1}= 20.40+0.75TC$, $R^2= 0.128^{**}$
-	$BW^{3}= -459.51-22.61TC-0.01TC^3$, $R^2= 0.574^{**}$	$BW^{3}= 120.67-2.09TL$, $R^2= 0.365^{**}$
-	$BW^{3}= -103.16+3.72TL$, $R^2= 0.191^{**}$	-

Superscript numbers represent mathematical models as 1=Linear , 2=Quadratic, 3=Cubic, 4=Compound, 5=Power and 6=S
**: significant at P<0.01, *: significant at P<0.05

4.3: Discussion

Many factors can affect the body weight of animals such as breed, sex, age, nutrition, management system and season. In the present study the variations in live body weight and body measurements are affected by ecotypes and these variations might be attributed to genetic variation and or differences in the ecological zones (Riva *et al*., 2004), moreover the results were in line with those of Elsheikh *et al*. (2012).

In most animal species normally males are heavier in live body weight than females due to differences in skeletal dimensions, hormonal system (Cloete *et al*., 2012), efficiency in feed utilization (Seideman., *et al* 1982) etc.., However, in this study, females achieved higher records in live body weight and most of body measurements. This could be due to the highest off-take and continuous demands of males in different ages for either slaughter and export while females are kept for longer time for breeding purposes.

The highest association coefficient was recorded with live body weight and heart girth, wither height, these measurements are directly associated with size and live body weight of the animal (Sarti *et al*., 2003; Riva *et al*., 2004; Afolayan *et al*., 2006; Salako 2006; Shaker and Hammam, 2008 and Cankaya *et al*., 2009). The association coefficient was moderate between live body weight and chest depth, chest width, similar results were reported by Topai and Macit (2004); Atta and Khidir (2004); Afolayan *et al,*. (2006) and Elsheikh *et al*., (2012), but shank circumference, thigh circumference, tail length and cannon circumference with other body measurements showed lower correlation coefficients, this finding was similar to those of Janssens and Vandepitte (2004); Cam *et al*., (2010). Furthermore, only Watish ecotype recorded higher correlation coefficient between body length and live body weight (0.780) than wither

height and live body weight (0.775) this result agreed with those reported by Elsheikh *et al*., (2012).

According to regression mathematical models the association of live body weight and heart girth showed the highest R^2 value this is agreed with Lawrence and Fowler (2002); Atta and El Khidir, (2004); Cam *et al*., (2010); Elsheikh *et al*., (2012) and Ali *et al*., (2014).

Chapter five

5. Growth differentiation factor 9 gene variants in Sudanese desert sheep ecotypes

This part of study was done at institute of Animal Breeding and Genetics, Giessen-Germany

5.1: Materials and methods

5.1.1: Animals and DNA samples

One hundered and fifty ewes from the three sheep ecotypes (50 for each) Ashgar, Dubasi and Watish from different regions of Sudan were selected for sampling according to their previous history of litter size (River Nile and Khartoum states for Ashgar, Gazira state for Dubasi and Sinar state for Watish). Any selected ewe must have at least two lambing records. The number of lambing records ranged from two to seven (in average 3.9 records).Ewes were divided into two groups according to their average litter size. One group comprised ewes of all three ecotypes which gave birth to single lambs in all recorded lambing (hence the average of litter size was 1.0), the other group included ewes of all three ecotypes which in average had more than a single lamb (average litter size per ewe in this group ranged from 1.5 to 3.0, the average litter size of the whole group was 2.1). Blood samples (5 mL) were drawn from the jugular vein in EDTA vacutainer tubes. The genomic DNA was extracted from white blood cells according to Montgomery and Sise, (1990).

5.1.2: Primer design

For amplification of two overlapping fragments covering the complete exon 2 of GDF9, the following pairs of primers (table 5.1) were designed using GenBank sequence AF078545.2 and the software Primer3 (Untergrasser *et al*., 2012).

Table 5.1. Primer sequences

Fragment	Sequence
Proximal primer (656 bp)	
forward	5' GGCTTGAGAATGTGGGGAGAA-3'
reverse	5'-GGGACGATCTTACACCCTCA-3'
Distal fragment (749 bp)	
forward	5'-CACAAGTGCTCAGGCTTTTC-3'
reverse	5'-CATGAGGAAGGCAGCTGTTA-3'

5.1.3: PCR amplification

For identification of sequence variants in exon.2 of GDF9, 28 samples were sequenced: 10 DNA samples each from Ashgar and Dubasi and eight from Watish. For each ecotype, 50% of the samples were selected from the single lamb group and the other 50% from the more than single lamb group and the PCR amplifications reaction were carried out in a final volume of 50 µL (table 5.2) under thermal conditions (table 5.3).

Table 5.2. The PCR amplification reaction

Reagent	µl
DNA	4
5× Colourless Go Taq Flexi buffer	10
2mM dNTPs	5
25 mM $MgCl_2$	2
Forward primer (10 pmol/ µl)	2
Reverse primer (10 pmol/ µl)	2
5 U/ µl Go Taq® Flexi Polymerase (PROMEGA, Madison, WI USA)	0.3
Distilled water	Up to 50

Table 5.3. The thermal conditions of the PCR reaction

Reaction	Temperature	Time
Initial denaturation	95°C (1 cycle)	90 sec.
Denaturation	95°C	15 sec.
Annealing	65°C (35-40 cycles)	30 sec
Extension	72°C	60 sec
Final extension	72°C (1 cycle)	5 min.

5.1.4: PCR products check

To check the quality and size of the PCR products it were visualized by staining with Midori green (NIPPON GENETICS EUROPE GmbH, Düren, Germany). following electrophoresis on 1.5% agarose gel at 170 V in 0.5 %TAE buffer for 90 minutes, then it is photographed under UV light (Biorad, Molecular Imager®).

5.1.5: PCR purification and precipitation protocol

The purification and precipitation were done using Kit from Stratec, Berlin-Germany as the follow steps:

1- Transfer the PCR product into spin filter cup and add 250 µl of binding buffer and centrifuge for 3 minutes at 12000 rpm.
2- Poured off the precipitate and centrifuge for 1 minute at 12000 rpm.
3- Transfer the filter into receiver cup and add 35-40 µl of elution buffer.
4- Incubate the cups for 1 minute in room temperature then centrifuge it at 10000 rpm for 1 minute.
5- Drop the filter and check the purified product and check its quantity. in Nanodrop 2000 spectrophotometer (VWR109 International GmbH, Erlangen, Germany) was used to check the quantity of PCR products.

5.1.6: Sequencing of GDF9 exon 2

PCR products of the two fragments were sequenced with PCR forward primer (656-bp fragment) and reverse primer (749-bp fragment), the sequencing reaction were carried out in a final volume of 10 µL (table 5.4) under thermal conditions (table 5.5) using Big Dye Terminator chemistry and the ABI 3130 Genetic Analyzer as recommended by the

manufacturer (Applied Biosystems, Foster City, CA, USA) with PCR forward primer (656-bp fragment) and reverse primer (749-bp fragment).

Table 5.4. PCR Primers sequences

Primer	Sequence
forward primer (656 bp)	5'-GGACAGAAGCACATTCTGAGG-3'
reverse primer (749 bp)	5'-CCCTTACATTGATAGATGCCACA-3'

Table 5.5. The DNA sequencing reaction

Reaction	Temperature	Time
Initial denaturation	96 °C (1 cycle)	60 sec.
Denaturation	96°C	10 sec.
Annealing	65 °C25 (cycles)	5 sec
Extension	60°C	2 min.

5.1.7: Alignment and analysis of GDF9 exon 2 sequence

Alignment and analysis of sequences from the different samples was done with the software ChromasPro version 1.33 (Technelysium Pty Ltd, Tewantin, Australia).

5.1.8: PCR-restriction fragment length polymorphism (RFLP) analysis of *GDF9* exon1 SNP c.260G>A

The SNP in exon 1 of *GDF9* (c.260G>A) was genotyped in a total of 97 ewes with litter size records (35Ashgar, 29 Dubasi and 33 Watish) by PCR-RFLP analysis using the *Hha*I restriction enzyme. Its cleavage site (GCGC) only occurs in the presence of the G allele. The amplification product size was 357 bp containing the polymorphic position c.260G>A of *GDF9* exon 1 and no additional *HhaI* cleavage site.

5.1.9: Primer design and amplification reaction of*GDF9* exon1 SNP c.260G>A

The following primers designed with Primer3 software were used forward primer 5'-TGAGGCTGAGACTTGGTCCT-3' and reverse primer 5'-ATAAAGGAGTTGGCCCTGCT- 3'. PCR amplification was carried out in a final volume of 25 µL (table 5.6) in a thermal cycler under the following conditions: initial denaturation at 95 °C for 90 sec, followed by 35 cycles consisting of denaturation at 96 °C for 15 sec, annealing at 62 °C for 30 sec, extension at 72 °C for 60 sec, and a final extension at 72 °C for 5 min.

Table 5.6. The PCR amplification reaction

Reagent	**µl**
DNA	3
5× Colourless Go Taq Flexi buffer	5
2mM dNTPs	2.5
25 mM $MgCl_2$	2
Forward primer (10 pmol/ µl)	2
Reverse primer (10 pmol/ µl)	2
5 U/ µl Go Taq® Flexi Polymerase (PROMEGA, Madison, WI USA)	0.3
Distilled water	Up to25

5.1.10: Incubation and check of the PCR product

The resulting PCR product was incubated with HhaI in 10 µL final volume as recommended by the manufacturer of the enzyme (New England Biolabs GmbH, Frankfurt am Main, Germany). Resulting DNA fragments were separated on agarose gel (2.5%) and visualized by staining with Midori green. RFLP fragment were verified by sequencing of PCR products using PCR forward primer as described before.

5.1.11: Statistical analysis

Genotype and allele frequencies were calculated for identified SNPs for all sheep and separately for each ecotype, and for the two groups of ewes with single and with more than single lamb, respectively. Differences in genotype and allele frequencies between these two groups differing in litter size were tested for significance using Fisher's exact test using IBM SPSS statistics for Windows program, Version 20.0. Armonk, NY: IBM Corp.

5.2: Results

Sequencing the complete exon 2 of the GDF9 gene in a total of 28 sheep of the Ashgar, Dubasi and Watish ecotypes revealed four polymorphic positions: c.471C>T, c.477G>A, c.721G>A and c.978A>G. Minor allele frequencies for T at position 471 and G at position 978 were very low over all sheep (0.05 and 0.02, respectively). These two SNP were monomorphic in Dubasi (c.471C>T) and Dubasi and Watish (c.978A>G) sheep, respectively. The two other SNPs in exon 2 were polymorphic in all three ecotypes. For the SNP c.477A>G, the A allele was predominant in Ashgar (0.44), whereas the G allele was the predominant allele in Dubasi and Watish (0.60 and 0.69, respectively). Genotype frequencies for all polymorphic exon 2 SNPs for all sheep, for the different ecotypes and for ewes with single and with an average of more than a single lamb are given in table 5.7 No significant differences in allele or genotype frequencies between the two groups differing in litter size were observed for any of these SNPs.

Incubation of the 357bp fragment containing the polymorphic position c.260G>A in *GDF9* exon 1 with *HhaI* restriction enzyme resulted in fragments of 222 and 135 bp for genotype GG and in fragments of 357, 222 and 135 bp for genotype AG (figure 5.1). For any

sample, only a single 357bp fragment (as expected for genotype AA) was observed after digestion with *HhaI*.

Table 5.7. Genotypes frequencies of *GDF9* exon2 SNPs in Sudanese desert sheep, ecotypes,and in ewes with single and more than single lambs

Sheep group	Sheep (n)	Genotypes of SNPs at positions c.471C>T			c.477G>A			c.721G>A			c.978A>G		
		CC	CT	TT	GG	AG	AA	GG	AG	AA	AA	AG	GG
All sheep	**28**	**0.89**	**0.11**	**0.00**	**0.33**	**0.46**	**0.21**	**0.79**	**0.14**	**0.07**	**0.96**	**0.04**	**0.00**
Ashgar	10	0.80	0.20	0.00	0.10	0.60	0.30	0.70	0.20	0.10	0.90	0.10	0.00
Dubasi	10	1.00	0.00	0.00	0.40	0.40	0.20	0.80	0.10	0.10	1.00	0.00	0.00
Watish	8	0.88	0.12	0.00	0.50	0.38	0.12	0.88	0.12	0.00	1.00	0.00	0.00
Lambing type													
Single	15	0.93	0.07	0.00	0.33	0.40	0.27	0.80	0.13	0.07	1.00	0.00	0.00
More than single	13	0.85	0.15	0.00	0.31	0.54	0.15	0.77	0.15	0.08	0.92	0.08	0.00

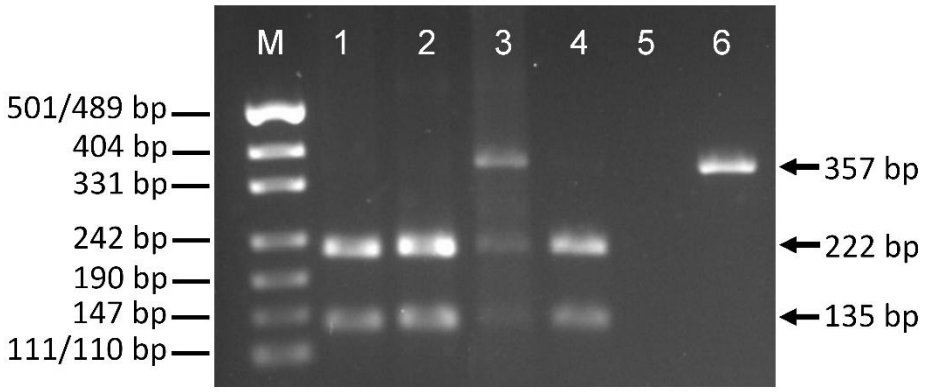

Figure 5.1. Determination of *GDF9* genotypes at position c.260G>A by RFLP analysis. M = DNA size marker PUC19 DNA/MspI (Thermo Fisher Scientific, Waltham, USA); 1 - 4 = PCR products digested with *HhaI* (1, 2, 4 = genotype GG, 3 = genotype AG); 5 = negative control; 6 = undigested PCR product.

Allele and genotype frequencies calculated for the c.260G>A variant are given in table 5.8. The frequency of the A allele was 0.10 among all genotyped sheep. In Ashgar, it was higher than in Dubasi and Watish (0.19 compared to 0.03 and 0.06, respectively), but similar to these both breeds, no sheep with the AA genotype was identified among the Ashgar sheep. Comparison of allele and genotype frequencies between ewes with only a single and with more lambs revealed no significant differences.

Table 5.8. Allele and genotype frequencies of *GDF9* exon1 SNP c.240G>A in Sudanese desert sheep ecotypes and in ewes with single/more than single lambs exon2

	Sheep	SNP c.240G>A				
	(n)	Allele frequency		Genotype frequency		
Sheep group		A	G	GG	AG	AA
All sheep	**97**	**0.10**	**0.90**	**0.80**	**0.20**	**0.00**
Ashgar	35	0.19	0.81	0.63	0.37	0.00
Dubasi	29	0.03	0.97	0.93	0.07	0.00
Watish	33	0.06	0.94	0.88	0.12	0.00
Lambing type						
Single	54	0.11	0.89	0.78	0.22	0.00
More than single	43	0.08	0.92	0.84	0.16	0.00

5.3: Discussion

In this experiment, five already known *GDF9* variants (c.471C>T, c.477G>A, c.721G>A and c.978A>G in exon 2, and c.260G>A in exon 1) were found to be polymorphic in at least one of the three Sudanese desert sheep ecotypes Ashgar, Dubasi and Watish. Only the SNP c.721G>A causes an amino acid substitution (p.Glu241Lys), which due to the change of an acidic group with a basic group is a non-conservative one. However, as for all of the identified SNPs, this variant is located proximal to the furin protease cleavage site. Therefore, it was not unexpected that no significant association between the identified variants in exon 2 of*GDF9* and litter size in Sudanese desert sheep ecotypes could be observed. Although the number of 28 sheep is very low for association testing, we refrained from genotyping the four polymorphic SNPs in exon 2 of *GDF9* in a higher number of sheep due to a complete missing of a theoretical involvement in the control of ovulation rate.

Also for the SNP c.260G>A in exon 1 of *GDF9*, no significant association was found with litter size in the sampled sheep. However, a higher frequency of the minor A allele was observed in Ashgar compared to Dubasi and Watish. From the 35 Ashgar sheep genotyped, 21 had

single lambs and 14 had more than a single lamb on average. As Ashgar sheep were observed to have a higher litter size than Dubasi and Watish (Sulieman *et al.*, 1990), it may be interesting to genotype more Ashgar sheep for this SNP and to test for association with litter size within this breed. Results from Barzegari *et al.* (2010) indicate a possible effect of this SNP on ovulation rate/litter size. However, they are also based on very few sheep and therefore should be taken with great care.

In addition to *GDF9*, the presence of other known major genes influencing ovulation rate could be tested for the desert sheep ecotypes analysed in this experiment. On the other hand, as these sheep do not show extraordinary high litter sizes (compared to some other breeds), the chance to identify such major gene variants seems to be low. By genotyping *BMPR1B*, *BMP15* and *GDF9* variants in five Tunisian sheep breeds (Barbarine, Queue Fine de L'Ouest, Noire de Thibar, Sicilo-Sarde and D'man) with litter sizes ranging from 1.14 (Queue Fine de L'Ouest) to 2.72 (D'man), Vacca *et al.* (2010) found absence of all known ovulation influencing alleles in these breeds. However, other breeds and genes are still open for research. A major gene variant increasing litter size in such a native African breed could be introduced in desert sheep ecotypes by classical inter crossing and backcrossing, and carriers of such a variant could then be identified and selected easily by genetic testing.

Chapter six

6. Overall discussion

Many factors can affect the body weight of animals such as breed, sex, age, nutrition, management system and season. In the present study the variations in live body weight and body measurements are affected by ecotypes and these variations might be attributed to genetic variation and or differences in the ecological zones (Riva *et al.*, 2004), moreover the results were in line with those of Elsheikh *et al.* (2012). Live body weight and body measurements are affected by ecotypes and these effects could be attributed to genetic variation and or differences in the ecological zones (Riva *et al.*, 2004 and Elsheikh *et al.*, 2012). Also, due to differences in skeletal dimensions, hormonal system (Cloete *et al.*, 2012), efficiency in feed utilization (Seideman., *et al* 1982) normally animal males are heavier in weight than females. In this study, females were higher in live body weight and most of body measurements. This might be due to the highest off-take and continuous demands of males in different ages for either slaughter and export. The association coefficient between live body weight and heart girth, wither height were the highest, this probably reflect that these dimensions are directly related with size and weight of the animal, this were agreed with (Sarti *et al.*, 2003; Riva *et al.*, 2004; Afolayan *et al.*, 2006; Salako, 2006; Shaker and Hammam, 2008; Cankaya *et al.*, 2009). The association coefficient between live body weight and chest depth, chest width was moderate this results were similar to Topai and Macit (2004); Atta and Khidir (2004); Afolayan *et al,*. (2006) and Elsheikh *et al.*, (2012), however shank circumference, thigh circumference, tail length and cannon circumference with other body measurements showed lower correlation coefficients Janssens and

Vandepitte (2004); Cam *et al.*, (2010). Moreover, Watish ecotype recorded higher correlation coefficient between body length and live body weight (0.780) than wither height and live body weight (0.775) this finding was agreed with that reported by Elsheikh *et al.*, (2012). Regarding regression mathematical models heart girth showed the highest R^2 value with live body weight and this is in line with Lawrence and Fowler (2002); Atta and El Khidir, (2004); Cam *et al.*, (2010); Elsheikh *et al.*, (2012) and Ali *et al.*, (2014).

Five already known *GDF9* variants (c.471C>T, c.477G>A, c.721G>A and c.978A>G in exon 2, and c.260G>A in exon 1) in this study were found to be polymorphic in one of the three Sudanese desert sheep ecotypes Ashgar, Dubasi and Watish. Just the SNP c.721G>A makes an amino acid substitution (p.Glu241Lys), which due to the change of an acidic group with a basic group is a non-conservative one. However, as for all of the identified SNPs, this variant is located closely to the furin protease cleavage location. Hence, it was revealed that no significant association between the identified variants in exon 2 of *GDF9* and litter size in Sudanese desert sheep ecotypes could be observed. Although the number of 28 sheep is very low for association testing, we refrained from genotyping the four polymorphic SNPs in exon 2 of *GDF9* in a higher number of sheep because of a complete missing of a theoretical involvement in the control of ovulation rate. No significant association was found with litter size in the sampled sheep in the SNP c.260G>A in exon 1 of *GDF9*. But, A allele had the highest frequency of the minor observed in Ashgar compared to Dubasi and Watish. From the 35 Ashgar sheep genotyped, 40% (14) had more than a single lamb and (60%) 21 had single lambs on average, this finding was in line with Sulieman *et al.*, (1990) who observed that Ashgar sheep were highest in litter size than Dubasi and Watish, it may be interesting to genotype more

Ashgar sheep for this SNP and to test for association with litter size within this breed. Barzegari *et al*. (2010) indicated a probable effect of this SNP on ovulation rate/litter size. However, they had also used very few sheep and therefore should be taken with great care. In addition to *GDF9*, the presence of other known major genes affecting ovulation rate could be tested for the desert sheep ecotypes analysed in this experiment. Alongside, as these sheep do not show extraordinary high litter sizes (compared to some other breeds), the probability to identify such major gene variants seems to be low. Vacca *et al*. (2010) found absence of all known ovulation influencing alleles by genotyping *BMPR1B*, *BMP15* and *GDF9* variants in five Tunisian sheep breeds. However, other breeds and genes are still open for research.

Conclusion and recommendations

The study concludes that:-

- Semi sedentary system could be suitable for desert sheep due to lack of ranges and rain fluctuation.
- Providing concentrates at the beginning of wet summer might make flushing for ewes and increase litter size.
- most constrains facing desert sheep production were diseases, lack of water and lack of feed.
- Heart girth might be the best measure for prediction of live body weight in Ashgar, Dubasi and Watish sheep ecotypes.
- Five positions in *GDF9* gene were found to be polymorphic in at least one of the Sudanese desert sheep ecotypes Ashgar, Dubasi and Watish.
- c.260G>A in exon 1 variant had a higher frequency of the A allele in the more prolific Ashgar sheep compared with the less prolific Dubasi and Watish sheep.
- No significant associations of these *GDF9* variants with litter size were observed.

The study recommended that:-

- More consideration and care should be given to sheep owners and their animals to enhance sheep production conditions (range management, diseases awareness and increase the productivity).
- Further studies and research on fecundity genes should be done in different Sudanese sheep ecotypes.

References

Appendices

Appendix 1

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire about some productive and reproductive in Sudanese sheep

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Date / /20 Respondents No.: ……

(1) Personal information:

1.Owner's name: ……

2.Location: …… State: ……

3.Age: ……

4.Occuption?

1- Animals breeder ( ) 2- Farmer ( ) 3- Govt. sector ( ) 4- Private sector ( )

5. Experience in rearing animals (year)

6.Educational level:

1- Illiterate ( ) 2- Khalwa or Basic ( ) 3- Secondary ( ) 4- University ( ) 5- Post graduate ( )

(2) Herd formation:

1- What kind of animals you are reared?

Sheep	Goat	Cattle	Camel	Donkeys	Horses	Poultry

1- What types of sheep you are reared?

- Ashgar ( ) - Dubasi ( ) - Watish ( ) - Others ( )

2- Total Numbers of?

1- Rams ( ) 2- Yearlings ( ) 3- Ewes ( ) 4- Lambs ( )

3- What is the production age/ year for? Ewe ( ). Ram ( ).

(3) Managment systems and flock feeding:

1- Managment systems:

1. Intensive system ( ). 2. Semi- intensive system ( ). 3. Extensive system ( ).

2- Feeding management:

1/ what kind of feed that your animals eat?

1-Natural range (   )   2-Agricultural residues (   )   3- Additional feeds (   )
4- Others: ……………………………………………………………………………………………………..

2/ Which kind of concentrates you provided to your animals? ……………………………………………..

3/If you aren't provided concentrates what is reason? ………………………………………………………………

……..

4/ When did you provide the concentrates? 1- Drought (   ). 2- Travelling (   ). 3- Both of them (   ).

4-Other ……….

5/ If your animals depend on the natural range, what are the preferable plants that it eat?

1- ……………………………………………….. 5-……………………………………………….
2- ……………………………………………….. 6-……………………………………………….
3- ……………………………………………….. 7-……………………………………………….
4- ……………………………………………….. 8-……………………………………………….

6/ Does the range is improved? 1- Yes (   ). 2- No (   ).

7/ The methods of range improvement if present?

1-Sowing seeds of preferable plants (   )   2- Awareness by animals density in the range (   )
3-Extention campaigns on keeping range (   ) 4-Other ………………………………………….

(3) Housing system:

1- Type of housing:

1.Open enclosure or space (   ) 2. Open side shade (   ) 3. Tethering around homestead (   )

2- Kind of material used in build the houses:

1.Local building materials (   ) 2.Different building materials (   ) 3.No using of any materials (   )

(4) Productive and reproductive traits:

1. Male/Female ratio (   )   2. Age at 1st lambing/days (   )
3. Birth wt. (   )   4. Weaning wt. (   )   5. Puberty wt. (   )
6.Gestation period/days (   )   7.Age at weaning/days (   )   8. Age at puberty/days (   )
9.Average milk production/lb (   )   10. Does the milk for sells? Yes (   ). No (   )
11. How much is the lb of milk/SDG (   )
12. Have you got ewes lambed twice a year? Yes (   ). No (   )
13. Number of ewes produced single (   )   14. Number of ewes produced twins (   )
15. Number of ewes produced triplets or more (   )   16. Number of lambs/year (   )
17. In which months of the year abound in lambs ……………………………………………………....
18. The criteria in ewe selection?
Size and feature (   )   Body color (   )   Lambs growth and surviving (   )
Twining rate   (   )   Good motherhood (   )   Early maturing age (   )
The criteria in ram selection?
Feature (   )   Body color (   )   Growth (   )   Horn (   )
Sexual capacity (   )   Early maturing age (   )   Pedigree (   )   Stamina and adaptability (   )

(5) Information about the ram:

1. Have you got more than one ram? Yes ( ). No ( ). Number of ram = ……..……..…..
2. Source of ram outside the flock ( ) inside the flock ( )
3. If it from the flock, What kind of it birth? 1. Single ( ) Twin or more ( )
4. Is it produce twin or more? Yes ( ). No ( ).
5. Is there more than one ewe produce twin from it? Yes ( ). No ( ).
6. Are you pay the ram to other adjacent flock? Yes ( ). No ( ).
7. Does you allow the ram to mate his? 1. Mother ( ) 2. Sister ( ) 3. Daughter ( )

(6) Marketing and uses of animal products/by-products:

1- Price of these products/by-products (SDG)

1/ Lamb ( ) 2/ Yearlings ( ) 3/ Ram ( ) 4/ Ewe ( )
5/ Kg of mutton ( ) 6/ Visceral ( ) 7/Liver and heart ( ) 8/ Skin ( )

2- Uses of skins
1/ Local use ( ) 2/ For sell ( ) 3/ No use ( )

3- Uses of skins
1/ Local use ( ) 2/ For sell ( ) 3/ No use ( )

4- How can you treated manure? ……..……..……..……..……..……..……..……..
……..……..……..……..……..……..……..……..……..……..……..……....

(7) Culling and exclusion:

1/What is the number of animals?
1. Sold ( ) 2.Purchased ( ) 3. Dead/lost ( )

2/Does you cull ewes from the flock? Yes ( ). No ( ).

3/ What are the reasons?
1.Disease ( ) 2.Over age ( ) 3. Sterility ( ) 4. General weakness ( )
5.Less of production ( ) 6. Good motherhood ( ) 7.Others ( )

4/Does you cull ram from the flock? Yes ( ). No ( ).

5/ What are the reasons?
1.Disease ( ) 2.Over age ( ) 3. General weakness ( ) 4. Weakness in sexual capacity ( )
5.Less of production ( ) 6.Weak or malformation of horn ( ) 7. Others ……....

6/ What is the culling age for? 1. Ewe: ……..……... 2. Ram: ……..……..…….

7/ The culled animals are: 1.Sold ( ) 2.Slaughter ( ) 3. Replacement ( ) 4. Others ( )

(8) Flock public health:

1/ Public diseases:

Mature animals diseases	**Lambs disease**
1-……..……..……..……..……	1-……..……..……..……..……
2-……..……..……..……..……	2-……..……..……..……..……
3-……..……..……..……..……	3-……..……..……..……..……
4-……..……..……..……..……	4-……..……..……..……..……
5-……..……..……..……..……	5-……..……..……..……..……

2/ Curing and protection from diseases

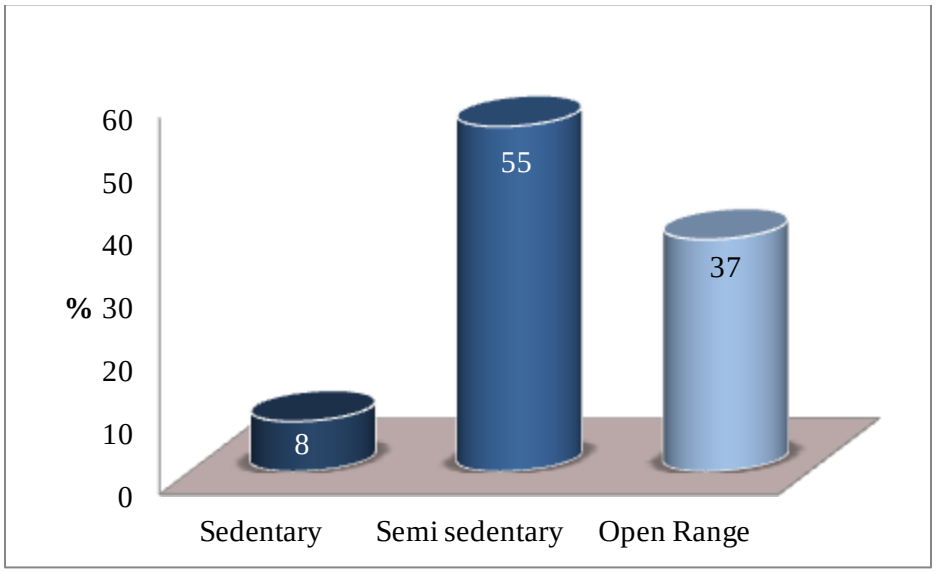
1.Curing by:
1/Drugs ( ) 2/Drugs + vaccines ( ) 3/ Local remedies ( )
2. What is most infected ages?
1/Lambs less than 5 months ( ) 2/Lambs more than 5 months ( ) 3/Mature males ( )
4/Mature females ( )
3. What is most mortality ages?
1/Lambs less than 5 months ( ) 2/Lambs more than 5 months ( ) 3/Mature males ( )
4/Mature females ( )
4. Did you receive any vaccination services? Yes ( ). No ( ).
5. When did you receive the vaccination services?
1. In outbreak ( ) 2. Any time in the year ( ) 3. Others ( )
6. What is the source of drugs and vaccines?
1/Governmental ( ) 2/Non governmental organization ( ) 3/Private veterinary sector ( )
4/ others ( )

(9) Production constrains:

1- Diseases	( )	5- Predators	( )
2- Lack of feed and range	( )	6- Lack of extension services	( )
3- Lack of water	( )	7- Drouht and rain fluctuated	( )
4-Lack of labor	( )	8-Lack of security	( )

Appendix 2: Occupation of sheep owners

Occupy	n	%
Animal breeder	93	93.0
Farmer	6	6.0
Employee	1	1.0
Total	100	100.0



Appendix 3: Rearing system of sheep owners

Appendix 4: Association between kind of material and housing type used in the study area

Housing type	Kind of materials				Overall	
	No Materials		Different Materials			
	n	%	n	%	n	%
Open spaces	46	100.0	30	55.6	76	76.0
Open shelters	0	0.00	8	14.8	8	8.0
Barns	0	0.00	16	26.9	16	16.0
Total	46	100.0	54	100.0	100	100.0

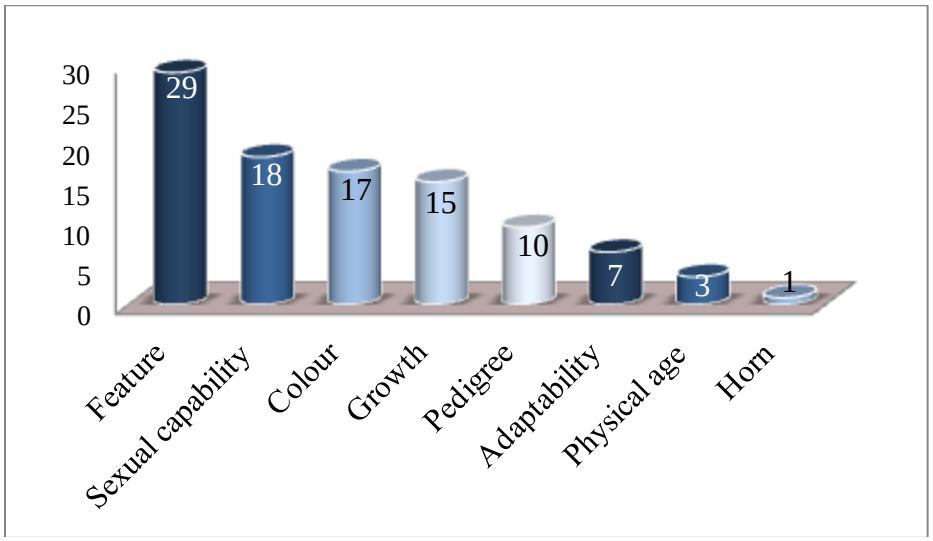
$\chi^2 = 26.901$, $P<0.01$

Appendix 5: Sheep feeding system in the study area

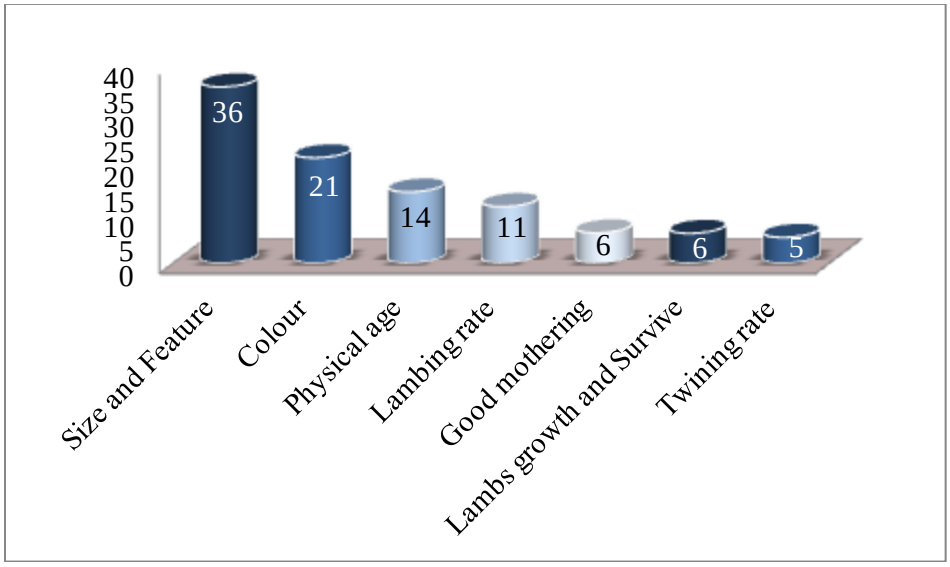
Type of nutrition	n	%
Natural range	17	17.0
Agricultural residues	31	31.0
Additive feeds	2	2.0
Natural range and agricultural residues	50	50.0
Total	100	100.0

Appendix 6: Preferable plants (ranks) by sheep in the study area

Plants	Mean	%	
Tabar	6.86	22.27	*Ipomea cardosepala*
Hantoot	6.10	19.81	*Ipomea cordofanum*
Dafari	2.55	8.28	*Cotalaria senegalensis*
Lblb	1.90	6.17	
Sharaya	1.57	5.10	*Indigofera arenaria*
Umlbain	1.17	3.80	*Euphorbia spp.*
Fakha	1.11	3.60	*Achryanthes aspera*
Hemla	1.08	3.51	*Aristida adscensionis*
Khadra	0.98	3.18	*Corchorns spp.*
Raihan	0.96	3.12	*Ocimum basilicum L.*
Rabah	0.92	2.99	*Tragus berteroianus*
Difra	0.75	2.44	*Echinochloa colona*
Molaita	0.70	2.27	*Launaea cornuta*
Gabash	0.65	2.11	*Guiera sengeglensis*
Abuareeda	0.56	1.82	
Draisa	0.56	1.82	*Tribulus terrestris*
Siha	0.38	1.23	*Blepharis edulisi*
Adar	0.38	1.23	*Sorghum halepense*
Gbain	0.34	1.10	*Solanum incanum*
Damblab	0.32	1.04	*Schima ischaemoides*
Um smaima	0.28	0.91	*Aristida pallida*
Naal	0.26	0.84	*Cymbapogon nervatus*
Rabaa	0.20	0.65	*Gisekia pharnacoides*
Umrgiga	0.13	0.42	*Hibiscus esculentus*
Soreeb	0.09	0.29	*Sesbania pachycarpa*
Total		100.00	



Appendix 7: Selection criteria (ranks) of rams favoured by sheep owners



Appendix 8: Selection criteria (ranks) of ewes favored by sheep owners

Appendix 9: Uses of leather		
Leather uses	n	%
Local use	18	18.0
Sold	70	70.0
No use	12	12.0
Total	100	100.0

Appendix 10: Faeces uses and treatments in the studied area

Type of Treatment	Faeces uses				Overall	
	Local use		No use			
	n	%	n	%	n	%
No use	0	0	84	100	84	84
Burning	1	6.25	0	0	1	1
Fertilizer	15	92.75	0	0	13	13
Total	16	100.0	84	100.0	100	100.0

$\chi^2 = 200$, $P<0.01$

Appendix 11: Culling age of the studied sheep ecotypes

Culling age (year)	Sheep ecotype			P. value
	Ashgar	Dubasi	Watish	
Ewe culling age	7.54±0.69	7.64±0.71	7.26±0.45	0.053
Ram culling age	8.14±1.15	8.29±1.06	7.85±0.72	0.203

Appendix 12: Common adults diseases (ranks) found in the study area

Disease	Mean	%
Tick borne diseases	7.33	33.83
Botulism	6.37	29.4
Pneumonia	3.79	17.49
Worms	2.39	11.02
PPR	1.79	8.26
Total		100.00

Appendix 13: Common lambs diseases (ranks) found in the study area

Disease	Mean	%
Diarrhea	7.39	32.56
Pneumonia	6.15	27.09
Mouth infection (*Gulakh*)	4.98	19.53
Tick borne diseases	3.2	16.1
Botulism	0.99	4.72
Total		100.00

Appendix 14: Time of receiving vaccine and vaccine source in the studied area

Time	n	%	Vaccine source	n	%
At outbreak	88	88.0	Governmental	12	12.0
Any time in the year	12	12.0	Private	88	88.0
Total	100	100.0	Total	100	100.0

Appendix 15: The correlation matrix between body measurements in different sexes of Ashgar ecotype, male (n=21) and female (n=59) ecotypes

		BW	BL	WH	HG	CD	CW	RL	RW	HL	HW	SC	TC	TL
BL	Male	0.944**	1											
	Female	0.230	1											
WH	Male	0.771**	0.745**	1										
	Female	0.658**	0.383**	1										
HG	Male	0.942**	0.922**	0.732**	1									
	Female	0.572**	0.095	0.268*	1									
CD	Male	0.944**	0.930**	0.753**	0.963**	1								
	Female	0.505**	0.232	0.376**	0.717**	1								
CW	Male	0.854**	0.806**	0.604**	0.884**	0.920**	1							
	Female	0.126	0.400**	0.214	0.127	0.166	1							
RL	Male	0.902**	0.895**	0.671**	0.858**	0.923**	0.852**	1						
	Female	-0.177	0.176	0.143	-0.148	-0.149	0.071	1						
RW	Male	0.900**	0.894**	0.675**	0.887**	0.931**	0.950**	0.931**	1					
	Female	0.225	0.024	0.112	0.310*	0.127	0.003	0	1					
HL	Male	0.794**	0.888**	0.671**	0.804**	0.837**	0.773**	0.860**	0.882**	1				
	Female	-0.357**	0.077	-0.147	-0.116	-0.001	-0.016	0.482**	-0.419**	1				
HW	Male	0.589**	0.632**	0.685**	0.583**	0.579**	0.491*	0.611**	0.554**	0.607**	1			
	Female	0.506**	0.119	0.423**	0.406**	0.373**	0.080	-0.158	0.535**	-0.510**	1			
SC	Male	0.739**	0.594**	0.432	0.754**	0.727**	0.843**	0.719**	0.779**	0.533*	0.483*	1		
	Female	0.472**	0.292*	0.417**	0.360**	0.465**	-0.050	-0.034	0.200	-0.107	0.478**	1		
TC	Male	0.507*	0.297	0.329	0.523*	0.482*	0.416	0.344	0.315	0.107	0.302	0.653**	1	
	Female	0.116	0.421**	0.264*	-0.032	0.178	-0.075	0.297*	0.073	0.210	0.114	0.557**	1	
TL	Male	0.704**	0.738**	0.785**	0.715**	0.691**	0.590**	0.542*	0.572**	0.604**	0.748**	0.400	0.352	1
	Female	0.259*	0.268*	0.278*	0.149	0.099	0.373**	0.037	0.084	-0.203	0.323*	0.081	-0.074	1
CC	Male	0.385	0.432	0.438*	0.355	0.400	0.544*	0.375	0.552**	0.565**	0.537*	0.443*	0.024	0.575**
	Female	0.316*	0.378**	0.376**	0.273*	0.332*	0.214	0.046	0.163	-0.240	0.252	0.271*	0.154	0.262*

For this table and subsequent tables, **: correlation is significant at P<0.01, *: correlation is significant at P<0.05

Appendix 16: The correlation between body measurements in different sexes of Dubasi ecotype, male (n=22) and female (n=50)

		BW	BL	WH	HG	CD	CW	RL	RW	HL	HW	SC	TC	TL
BL	Male	0.652**	1											
	Female	0.269	1											
WH	Male	0.821**	0.515*	1										
	Female	0.820**	0.284*	1										
HG	Male	0.838**	0.739**	0.643**	1									
	Female	0.831**	0.195	0.680**	1									
CD	Male	0.728**	0.582**	0.768**	0.623**	1								
	Female	0.051	-0.253	-0.024	-0.056	1								
CW	Male	0.715**	0.535*	0.841**	0.557**	0.514*	1							
	Female	0.042	0.08	0.144	0.021	-0.079	1							
RL	Male	0.731**	0.414	0.456*	0.653**	0.565**	0.418	1						
	Female	-0.187	-0.116	-0.043	-0.101	-0.071	0.290*	1						
RW	Male	0.774**	0.779**	0.625**	0.672**	0.709**	0.643**	0.739**	1					
	Female	0.028	-0.201	0.013	0	0.009	0.044	0.311*	1					
HL	Male	0.403	0.554**	0.405	0.462*	0.283	0.640**	0.432*	0.641**	1				
	Female	-0.16	-0.088	-0.189	-0.173	0-.204	0.193	0.055	0.041	1				
HW	Male	-0.316	-0.282	-0.118	-0.133	-0.170	-0.099	-0.284	-0.422	-0.213	1			
	Female	-0.187	-0.029	-0.172	-0.186	-0.245	0.058	0.092	0.127	0.755**	1			
SC	Male	0.798**	0.438*	0.678**	0.575**	0.640**	0.546**	0.397	0.497*	0.129	-0.154	1		
	Female	-0.104	0.205	-0.082	-0.029	-0.387**	0.194	0.043	0.137	0.168	0.249	1		
TC	Male	0.554**	0.433*	0.521*	0.478*	0.481*	0.262	0.39	0.506*	0.065	-0.11	0.645**	1	
	Female	0.191	0.158	0.195	0.122	-0.136	0.036	-0.381**	-0.205	0.162	0.09	0.083	1	
TL	Male	0.044	0.504*	0.18	-0.035	0.249	0.158	-0.316	0.181	-0.001	-0.058	0.326	0.388	1
	Female	0.014	0.014	0.022	-0.029	-0.324*	0.146	0.119	0.111	0.375**	0.286*	0.256	-0.045	1
CC	Male	0.264	0.489*	0.361	0.23	0.209	0.23	-0.129	0.267	0.111	0.037	0.437*	0.698**	0.653**
	Female	0.198	0.05	0.151	0.088	0.207	-0.048	-0.268	-0.208	0.07	0.229	-0.185	0.295*	-0.144

Appendix 17: The correlation between body measurements in different sexes of Watish ecotype, male (n=23) and female (n=50)

		BW	BL	HW	HG	CD	CW	RL	RW	HL	HW	SC	TC	TL
BL	Male	0.275	1											
	Female	0.386**	1											
WH	Male	0.871**	0.023	1										
	Female	0.893**	.427**	1										
HG	Male	0.716**	0.307	.483*	1									
	Female	0.813**	0.223	.758**	1									
CD	Male	0.094	0.338	0.02	0.162	1								
	Female	0.460**	0.224	.435**	0.480**	1								
CW	Male	0.265	-0.164	0.141	0.320	0.156	1							
	Female	-0.007	0.184	0.061	0.019	-0.061	1							
RL	Male	0.302	0.305	0.054	0.485*	0.09	0.271	1						
	Female	0.155	0.165	0.05	0.194	-0.007	0.078	1						
RW	Male	0.113	0.185	-0.157	0.286	0.282	0.346	0.550**	1					
	Female	0.174	0.264	0.128	0.186	0.069	0.106	0.841**	1					
HL	Male	-0.025	0.417*	-0.14	-0.061	0.193	-0.299	0.072	-0.04	1				
	Female	-0.014	-0.017	0.062	-0.053	-0.104	0.191	-0.386**	-0.303*	1				
HW	Male	-0.032	-0.046	-0.045	-0.057	0.099	-0.32	-0.101	-0.036	0.411	1			
	Female	0.058	0.098	0.079	0.117	0.012	0.003	0.049	0.076	0.313*	1	1		
SC	Male	0.266	0.319	0.039	0.29	0.079	0.511*	0.435*	0.485*	0.367	-0.134	1		
	Female	-0.028	0.034	0.077	-0.146	-0.152	0.178	0.096	0.125	0.155	0.026	1		
TC	Male	0.219	-0.024	-0.01	0.538**	0.232	0.600**	0.551**	0.563**	-0.325	-0.269	0.29	1	
	Female	0.264	0.13	0.178	0.208	0.127	0.457**	0.149	0.075	-0.12	-0.269	0.29	1	
TL	Male	0.163	0.115	0.063	0.353	0.089	0.425*	0.232	0.359	-0.056	-0.139	-0.176	0.547**	1
	Female	0.024	-0.003	0.011	-0.046	-0.373**	0.144	-0.094	-0.243	0.177	-0.213	0.243	0.089	1
CC	Male	0.161	-0.346	0.428*	0.063	-0.039	-0.031	-0.021	-0.066	-0.439*	-0.234	0.188	-0.011	-0.09
	Female	0.275	0.06	-0.093	0.001	-0.041	0.134	0.094	0.117	-0.299*	0.067	-0.052	0.009	0.048

Appendix 18: Multiple regression analysis of live body weight on body length, heart girth and other measurements of Ashgare cotype

variables	Intercept	B1	B2	B3	B4	B5	B6	R^2	R^2 change
Male									
BL	-40.98	1.17						0.890	0.000
BL+HG	-39.79	0.62	0.44					0.925	+0.035
BL+HG+CD	-44.42	0.53	0.27	0.59				0.929	+0.004
BL+HG+CD+RL	-34.79	0.43	0.37	0.11	0.47			0.934	+0.005
BL+HG+CD+RL+RW	-34.59	0.43	0.37	0.10	0.46	0.03	-	0.934	0.000
Female									
WH	-64.86	1.35						0.433	0.010
WH+HG	-105.26	1.11	0.70					0.602	+0.169
WH+HG+CD	-105.48	1.12	0.71	-0.03				0.602	0.000
WH+HG+CD+SC	-101.41	1.04	0.70	-0.13	0.35			0.614	+0.012
WH+HG+CD+SC+CC	-101.14	1.05	0.70	-0.13	0.35	-0.15	-	0.614	0.000
Overall									
HG	-36.30	0.93						0.597	+0.141
HG+WH	-86.36	0.63	0.95					0.738	+0.007
HG+WH+BL	-86.34	0.63	0.94	0.01				0.738	0.000
HG+WH+BL+RW	-85.96	0.59	0.95	0	0.18			0.739	+0.001
HG+WH+BL+RW+CD	-86.99	0.60	0.95	0.03	0.19	-0.11		0.740	+0.001
HG+WH+BL+RW+CD+SC	-89.53	0.59	0.94	0.02	0.10	-0.03	0.20	0.746	+0.006

Appendix 19: Multiple regression analysis of live body weight on body length, heart girth and other measurements of Dubasi ecotype

variables	Intercept	B1	B2	B3	B4	B5	B6	R^2	R^2 change
Male									
HG	-34.47	0.89						0.702	0.000
HG+WH	-69.29	0.56	0.83					0.837	+0.136
HG+WH+BL	-69.58	0.54	0.83	0.04				0.838	0.000
HG+WH+BL+CD	-69.43	0.53	0.75	0.02	0.18			0.840	+0.002
HG+WH+BL+CD+SC	-66.23	0.45	0.54	0.05	-0.03	1.04	-	0.894	+0.054
Female									
HG	-48.22	1.12						0.691	0.000
HG+WH	-47.95	0.69	0.46					0.811	+0.121
HG+WH+BL	-51.20	0.69	0.45	0.06				0.813	+0.001
HG+WH+BL+CC	-54.19	0.69	0.44	0.06	0.52			0.820	+0.007
HG+WH+BL+CC+TC	-54.72	0.69	0.44	0.05	0.50	0.03	-	0.820	0.000
Overall									
HG	-63.75	1.31						0.843	0.000
HG+WH	-80.41	0.88	0.68					0.900	+0.057
HG+WH+BL	-74.69	0.59	0.62	0.31				0.924	+0.024
HG+WH+BL+CW	-73.37	0.59	0.57	0.26	0.34			0.926	+0.002
HG+WH+BL+CW+TC	-75.90	0.60	0.55	0.24	0.35	0.21		0.928	+0.002
HG+WH+BL+CW+TC+RL	-70.19	0.63	0.53	0.17	0.37	0.20	-0.22	0.931	+0.003

Appendix 20: Multiple regression analysis of live body weight on body length, heart girth and other measurements of Watish ecotype

variables	Intercept	B1	B2	B3	B4	B5	B6	R^2	R^2 change
Male									
WH	-64.06	1.41						0.759	0.000
WH +HG	-61.38	1.11	0.24					0.873	+0.114
WH +HG+BL	-87.52	1.15	0.20	0.35				0.896	+0.023
WH +HG+BL+CW	-97.35	1.16	0.17	0.42	0.30			0.905	+0.009
WH +HG+BL+CW+RL	-97.65	1.18	0.16	0.39	0.27	0.18	-	0.908	+0.003
Female									
WH	-44.97	1.08						0.797	0.000
WH +HG	-66.52	0.79	0.54					0.840	+0.043
WH +HG+CD	-66.21	0.78	0.52	0.04				0.841	+0.001
WH +HG+CD+BL	-69.70	0.75	0.54	0.03	0.06			0.843	+0.001
WH +HG+CD+BL+TC	-69.47	0.75	0.52	0.03	0.05	0.09	-	0.849	+0.006
Overall									
HG	-39.83	0.98						0.749	0.000
HG+WH	-87.90	0.71	0.90					0.848	+0.099
HG+WH+BL	-104.02	0.51	0.79	0.57				0.896	+0.049
HG+WH+BL+CD	-101.49	0.48	0.77	0.49	0.18			0.902	+0.006
HG+WH+BL+CD+CW	-100.39	0.45	0.79	0.44	0.17	0.20		0.905	+0.003
HG+WH+BL+CD+CW+SC	-101.02	0.44	0.79	0.43	0.18	0.17	0.10	0.906	+0.001