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Association of Growth Hormone Gene Polymorphism with Body Weight performance and Some Physiological Traits of Sheep in Kerbala Governorate

THESIS

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ﴾

صدق الله العلي العظيم

البقرة / 32

DEDICATION

To :

My parents , sisters and brother who always love me unconditionally and whose good example have taught me to work hard for the things that I aspire to achieve.

My husband who is my constant source of help and encouragement. I am truly thankful for having you in my life.

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-Fatima

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Committee certification

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Summary

This study was conducted at Barakat Abul-Fadhul Station for Sheep Production in Kerbala on 63 pregnant ewes and 20 non-pregnant ewes from two local breeds (Nuimi and Awassi) to find out the association of growth hormone gene polymorphism with the body weight as well as, to report some related physiological traits. Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used to detect polymorphism of growth hormone gene to pregnant ewes to same mentioned breed by using restriction enzyme (Hae III) to determine fragment of422base pair from growth hormone gene. Lambs weights at birth and at weaning were measured and the gender was also determined. Heart Girth (HG), Body Length (BL) and Body Height (BH) to the lambs were also measured at birth and at weaning. Parathyroid, Calcitonin and Vitamin D hormones concentrations of the pregnant ewes and non-pregnant ewes were measured. Cholesterol(CH), Triglyceride(TG), High Density Lipoprotein(HDL), Low Density Lipoprotein(LDL) and Very Low Density Lipoprotein (VLDL) concentrations were also measured to pregnant and non-pregnant ewes. The molecular results revealed 3 genotypes to growth hormone gene in both breeds: AA, Aa and aa genotypes and it revealed the significant effect of genotypes on birth and weaning weights in both breeds, the results of Nuimi breeds revealed that the genotype AA was highest value (6.35±0.70) kg in birth weight and (21.50±3.50)kg in weaning weight and have a significant ($P<0.05$), while, the results of Awassi breed was reported the genotype aa had highest values in birth weight (6.82±0.68)kg and had significant effect ($p<0.05$), the value of weaning weight is(23.50±1.50)kg. Gender had significant effect on birth and weaning

weights and body measurements, the results were suggested that the male had higher values than female in birth and weaning weights as well as in body measurements in both stages: (birth and weaning)to Nuimi and Awassi sheep, as follow: the male in Nuimi breed had at birth (birth weights=6.22kg, Heart Girth=42.60cm, Body Length=35.60cm and Height=44.50cm at weaning, the value was reported as weaning weight=20.11kg, heart girth=62,30cm, length=61.30cm and height=61.55cm while, in Awassi breeds the male at birth, birth weight=6.33kg, Heart Girth=44.71cm, Length=37.50cm and Height=45.35cm whereas, at weaning the male had weaning weight=22.03kg, Heart Girth 64.92cm, length=63.07cm and height=63.85cm. Our results of studied hormones revealed major deviations in physiological traits between pregnant and non-pregnant ewes in both studied breeds, in addition to that, our results also found out a significant effect of growth hormone gene polymorphism on body weight performance in lambs of same studied breeds. The results revealed that there are high deviations between pregnant and non-pregnant ewes in both breeds in studied hormones (Parathyroid, Calcitonin and Vitamin D) in Nuimi, the levels of mentioned hormones in non-pregnant ewes were 7.11 pg/ml , 12.9pg/ml and 0.79ng/ml respectively, while in pregnant ewes in same breed were 85.53pg/ml, 75.29pg/ml and 3.81ng/ml separately, in Awassi, the levels of studied hormones (Parathyroid, Calcitonin and Vitamin D) in non-pregnant ewes were 28.37pg/ml, 12.93pg/ml and 0.93ng/ml separately, but in pregnant ewes in same breed the levels of these hormones 76.09pg/ml , 91.4pg/ml and 3.69ng/ml ,respectively.

Similarly, there are high differences between pregnant and non-pregnant ewes in both breeds Nuimi and Awassi in lipid profile (CH, TG,

HDL, LDL and VLDL). In Nuimi breed , the serum levels of mentioned lipid profile in non-pregnant ewes were (49.70, 33.50, 30.30, 12.70, 6.70) mg/dl respectively, but in pregnant ewes in same breed were (13.08, 65.66, 52.66, 65.37, 13.13) mg/dl separately, However the levels of the same lipid profile in Awassi breed in non-pregnant ewes were (43.10, 34.90, 29.20,6.9, 6.98) mg/dl, respectively, but the levels of these lipid profile in pregnant ewes in same breed were (127.51, 66.14, 52.14, 58.23, 16.27)mg/dl , respectively.

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	Abbreviations
Growth Hormone	GH
Parathyroid Hormone	PTH
Calcitonin	CT
base pair	Bp
Vitamin D	Vit.D
Polymerase chain reaction	PCR
Restriction fragment length polymorphism	RFLP
Base pair	bp
Deoxyribonucleic acid	DNA
Heart girth	HG
Body length	BL
Body height	BH
Cholesterol	Ch
Triglycerides	Tg
High density lipoprotein	HDL
Low density lipoprotein	LDL
Very low density lipoprotein	VLDL
Insulin like Growth Factor	IGF-1
Dexoynucleotide triphosphate	dNTPs
Leucine	L
Valine	V
Growth Hormone Releasing Hormone	HGRH
Single Strand Conformation polymorphism	SSCP
Messenger ribonucleic acid	mRNA
The enzyme-linked immunosorbent assay	ELASA

Chapter one
Introduction

Chapter One

Introduction

The six million sheep are considered the most important agricultural animals in Iraq through their contribution to the production of meat and milk, as well as, their contribution to the production of wool, leather and manure, (which is a natural plant fertilizer), and important because it is a suitable agricultural animal which is the main source of income for the population living in these areas, sheep now more important because of the population growth and increased demand for their products of meat and milk ***(Al-Barzinji, 2012).***

In order to continue to improve the sheep requires the modernization of methods of genetic improvement and study of genetic structures of these animals and the selection of the best ones through studying of genes that affect the characteristics of growth and production and comparison of the genotypes of sheep with the global strains (***AL-Salihi et al., 2017; Liu and Cordes, 2004)*** . Small ruminants, mainly partial breed types, play a vital function in the livelihoods of a considerable share on ethnical population within the tropics beyond socio-economic factors ***(Vajed Ebrahimi et al., 2017).*** Knowledge of genetic mutations and link to the phenotype by using " Polymerase Chain Reaction" (PCR) and " Restriction Fragment Length Polymorphism (RFLP) " . These techniques, in turn, help to study the required genes and to multiply them in vitro ***(Liu and Cordes, 2004)***. The owners of animals follow programs that increase the ability of the animal by improving its genotypes, but the time period for this is often long in animals such as sheep may be up to 4.5 year ***(Jalal et al., 2003).*** The determining of the genotypes of each animal and the detection of genetic mutations ***(AL-Salihi et al., 2017; Liu and Cordes, 2004).***

Growth hormone gene play vital role in growth process regulation as well as development in sheep ***(Farag et al., 2016).*** Growth hormone a protein hormone that activates the growth of cells and muscles by activating the amino acid intake. The most important physiological functions of this hormone is to stimulate growth in general in the body, but the main goal is muscle and bone growth (***Ayuk and Sheppared, 2006***). However, growth hormone polymorphism was considered as a genetic marker candidate for growth characteristic (***Hua et al., 2009).***

There are many researchers studied an association of polymorphism of growth hormone gene with body weight and growth such as ***Malewa et al., (2014); Abdelmoneim et al., (2016); Depison et al., (2017)*** who reported that strong relation between polymorphism of growth hormone gene and body weight and growth. However, the calcium play main role in bone mineralization and bone growth ***(Aline et al., 2008***) and the growth hormone play important role in increase absorption of calcium ***(Al-Sahookie, 2016)*** but there are other hormones important in calcium homeostasis : parathyroid, vitamin D and calcitonin(***Kurek and Adam, 2005).***

Parathyroid hormone increase the absorption of calcium from the intestines and concurrently status its reabsorption out of the skeleton (***Abd-Allah and baker, 2015***). Parathyroid hormone plays vital role in carrying the calcium from the bone, intestinal uptake of calcium from the food , renal reabsorption of calcium and through vitamin D (***Kronqvist et al., 2011).***

Vitamin D can be synthesized in the skin through exposure to bright (UV) light or can be acquired through dietary admission (***Andrew et al.,***

2012). Bone growth and bone transforming via osteoblasts and osteoclasts it's also acquired to Vitamin D (***Institute of Medicine 2010).***

Calcitonin is a main hypocalcemic hormone, recently to act on bone to inhibit osteoclastic bone resorption (***Davey and Findlay, 2013).*** Calcitonin can play as the antiresorptive agent through its function with its specific receptors, inflicting powerful inhibition of osteoclast activity, and its function as a regulator of calcium homeostasis that includes bone resorption is well documented ***(Andresen et al., 2008***).

During late pregnancy in sheep, blood serum lipids profile is characterized by way of elevated concentration level of cholesterol, triglycerides, and lipoproteins because of the limited responsiveness of goal tissues to the insulin that, as same way, multiplied mobilization of fatty acids from adipose tissue make available new sources for the fetal growth (***Piccione et al., 2009).*** The size, breeds, and age of a ewe, the delivery type, sex of lambs and support and proper nutrition status are known to importantly affect the birth way and weaning weight of lambs ***(Aliyari et al., 2012; Akta and Dogan, 2014).*** They reported up to expectation different models might be needed to predict body weight in different environmental conditions and breeds (***Younas et al., 2013).*** The aims of this study were :

1- To find out the relationship of growth hormone gene polymorphism and body performance in Nuimi and Awassi breeds.
2- To report some physiological traits in pregnant and non-pregnant ewes during pregnancy in Nuimi and Awassi breeds .

Chapter Two

Literature review

Chapter two

Literature review

2.1 Growth Hormone

Growth Hormone (GH) is an anabolic hormone synthesized and secreted via the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile way, the pattern of which performs an vital function in postnatal longitudinal boom and improvement, tissue boom, lactation, duplicate, in addition to protein, lipid and carbohydrate metabolism (***Abdel-Aziem et al., 2015).*** Growth Hormone is a peptide encoded by using a single gene (***Wickramaratne et al., 2010).*** This hormone is important for the growth (***Hua et al., 2009)***, controlling many physiological processes that consist of metabolism and growth (***Younes, 2008)***, milk manufacturing and its characteristics (***Malveiro et al., 2001; Marques et al., 2003)*** and it's miles necessary for the development of the fetus and not simplest through stimulating the formation of uterine milk however additionally stimulate the method of metabolic fetus additionally ***(Sinclair et al., 2003).***There are other essential functions of this hormone ***(Squires, 2010 ; Hua et al., 2009 ; AL.elogi , 2014***) which are :Increases protein synthesis in skeletal muscles, Increase the lipolysis and decrease the absorption of glucose from the liver, Increase absorption of calcium and phosphorus, Increase milk production by increasing nutrient absorption and increasing blood flow and Increase oxidation of free fatty acids and decrease oxidation of calcium and amino acids.

The direct physiologic results of this hormone are to stimulate protein synthesis by contributing to increase absorption of amino acids and this hormones responsible for preserving glucose within the normal values in blood ***(King, 2006)*** , as well as its impact on growing the number of ovarian follicles growing for each vesicular growth cycle ***(Gong et al.,***

1991) and its role in regulating growth balance and differentiation of cells body during embryonic improvement (***Kaiser et al., 2001).***

Indirect function of Growth Hormone it represent that growth hormone possess receptors in liver play role in stimulant synthesis of Insulin like Growth Hormone Factor-1(IGF-1) who has role in bone growth and development of growth body ***(Ramsay et al., 1995).*** The most impact s of the Growth Hormone that occurs through the stimulation of secretion ´´Insulin-like Growth Hormone Factor´´ (***Rosen and Wustler, 1996).***

Regulation of secretion of Growth Hormone via two axes: first, via the release hormone "Growth Hormone Releasing Hormone(GHRH)" which secrete from the hypothalamus, which movements to the anterior lobe of the pituitary gland to contribute to the synthesis and secretion of hormone, second, ghrelin hormone, is a peptide hormone secreted from the stomach and associated with receptors in cells somatotroph that is responsible for the secretion of Growth Hormone (***Henry et al., 2001),*** inhibition of growth hormone occurs thru somatostatin, this hormone secreted via some organs, inclusive of hypothalamus, which at once effect the inhibition of growth hormone-releasing hormone (GHRH) ***(Thomas et al., 1991)*** or by way of the negative feedback of IGF-1 hormone and the excessive levels of this hormone cause a inhibition in growth hormone concentration not best via direct effect inside the somatotrophin but also thru increase release of somatostatin from pancreas (***Wu, 2007).***

2.2 Growth Hormone Gene

Growth Hormone (GH) a single polypeptide hormone produced in anterior pituitary gland is a promising candidate gene marker for improving milk and meat manufacturing in domestic animal ***(Min et at.,***

2005). Growth Hormone genes axis possess wide physiology functions like appetite control, ageing, body composition, growth, reproduction ***(Moradian et al., 2013)***, immune responsiveness ***(Madden and Felton, 1995)***. However, growth hormone gene plays role in influence wool quality and quantity (***Beh et al., 2001; Yousefi et al., 2012).***

The structure of Growth Hormone gene in bovine consist approximately 1800 base-pair in length and contain four intervening sequences and five exons coding for a 786 long mRNA ***(Kovacs et al., 2006)***.The structure of ovine growth hormone gene is similar to other growth hormone gene but more homologous from bovine Growth Hormone gene ***(Moradian et al., 2013)***. Growth Hormone Gene in sheep found in chromosome 3 (***Allain et al., 1998)*** and the Growth Hormone gene is about 1.8 kb (***Hajihosseinlo et al., 2013).*** However, in bovine Growth Hormone gene located in chromosome 19 ***(Mohammadabadi et al., 2010)***. Ovine possess two alleles of growth hormone gene , the GH1 allele contains a single gene copy (GH1), whereas, in the GH2 allele the gene is duplicate (copies GH2-N and GH2-Z) with the two copies being located 3.5kb apart (***Valinsky et al., 1990).*** There are differences between the GH2-N and GH-Z copies have been confirmed and polymorphism have been found in ovine Growth Hormone gene coding and non-coding regions (**Ofir and Gootwine, 1997**).

2.3 Polymorphism of Growth Hormone Gene

Genetic polymorphisms in candidate genes affecting economic characters, have inspired significant studies interest because of their ability usage as a useful resource to genetic selection and to demarcate evolutionary relationships in different livestock breeds ***(Sodhi et al., 2014; Afifi et al., 2014)***. An affiliation of numerous polymorphic web site

in one of a kind candidate genes with economic trends has been extensively used and ordinary as a spreading tool in different commercially important species like cattle ***(Ge et al., 2003)***, sheep***(Al-Salihi et al., 2017 ; Afifi et al., 2014)*** and goats (***Gupta et al., 2007)***. Genetic variant at the molecular level is pervasive in all breeding programs and these editions can be a potential marker-gene aid using contemporary genetic selection applications and the development of new reproductive technology enable the concentration of acceptable genes within a breeding population ***(Croquet et al., 2006)***.

The polymorphisms of ovine Growth Hormone had been reported via making use of exceptional strategies which include restriction fragment length polymorphism (RFLP) using restriction endo-nucleases TaqI and PvuIII and EcoRI ***(Farag et al., 2016)*** as well as PCR- single strand conformation polymorphism (SSCP) approach ***(Santos et al., 2004)***. Numerous polymorphisms have been recognized within the Growth Hormone gene of goats ***(Malverio et al., 2001; Marque et al., 2003)*** however best a very few of them were exactly characterized for nucleotide changes and position inside the DNA sequence. Growth Hormone gene polymorphism was analyzed as a genetic marker candidate for growth developments in Boer goat bucks ***(Hua et al., 2009).***

2.4 Association of polymorphism of GH gene with body weight

The application of molecular genetics in identification of polymorphism in growth candidate genes that show association with specific economically relevant traits provide useful information to enhance genetic improvement program in livestock and validation of genetic markers of growth traits (***Cauveri et al., 2016).*** It has been

reported that the restriction fragment length polymorphisms (RFLPs) of GH-TaqI were associated with body weight at 7 and 13 months of age in Belgian White Blue bulls ***(Sneyers et al., 1994).*** Significant effects were found for bGH genotype on yearling weight, with positive effects associated with the LV (leucine/valine) genotype in the Canchim beef cattle ***(Pereira et al., 2005).***GH gene polymorphism was analyzed as a genetic marker candidate for growth traits in Boer goat bucks by ***Hua et al., (2009)***. Many researchers were studied the association of polymorphism of growth hormone gene with body weight and growth like ***(Malewa et al., 2014; Al-Salihi et al., 2017; Abdelmoneim et al., 2016 ; Depison et al., 2017)*** reported that strong relation between polymorphism of Growth Hormone gene and body weight and growth.

2.5 Polymerase Chain Reaction (PCR) technique

The process known as polymerase chain reaction or PCR is developed by Kary Mullis in 1983 ***(Fakruddin et al., 2013)***.The PCR is a biochemistry and molecular technique for exponentially amplifying a fragment of DNA via enzymatic replication without using a living organism ***(Pavlov et al., 2004)***. This technology can be adapted to the nature of DNA ***(Altshuler, 2006),***and for the traditional use of PCR technology, it is necessary to know the sequences of nitrogenous bases founded in the DNA region, which is to be multiplied ,in this case, the researcher must be by means of previous information produce complementary primers in the direction(5←3)Consisting of a few nitrogen bases of about 20 nitrogen bases. The main key to multiplication process is polymerase enzyme, it is resistant to heat, this enzyme read the original template of DNA in the (3←5) direction and synthesized a new complimentary template in (5→3) by using dexoynucleotide triphosphates (dNTPs) as building blocks. This technology is suitable for

the use of a number of DNA markers such as (RFLP, AFLP, RAPD, Mini satellites-SNP microsatellites ***(Piper and Bindon, 1982)*** and the steps of the PCR can be summarized in the following steps:

1-Denaturation : in this step, The DNA is separated from each other and requires a temperature of about 94 degrees Celsius.

2-Annealing : This primer is associated with one of the complementary DNA template and this step require a temperature of about 55-60 degree Celsius.

3-Extenstion : It is the last stage in this technology and the polymerase activates in the building of the complementary template of each of the original template of DNA. These steps(considered one cycle) are repeated several times in order to amplify and amplify the desired area of the gene ***(Aoyagi, 2001).***

2.6 Restriction Fragment Length Polymorphism(RFLP)

It is the first technologies used to analyze of genome and drawing the genetic maps ***(Botstien et al., 1980).*** In this way DNA is digested by restriction enzymes that recognize specific sequences and cut them at specific locations to produce binds in different sizes, and according to the nitrogenous bases included in its composition ***(Mburu and Hanotte, 2005)***. Differences appear when the pieces differ in length from each other, this proves that enzymes of digestion or restriction have cut the DNA in different locations depending on the sequence contained in one of the DNA template ***(Teneva, 2009 ; Arif et al., 2009).*** One of the advantages of RFLP technology is the production of semi-dominant markers or co-dominant markers and this allows for the distinction between homozygote genotypes and heterozygote genotypes in organisms that carry both genotypes and this giving similar and constant

results at all time, but its disadvantages are a long and expensive method of laboratory testing It also shows the presence or absence of the cut area and does not give much information about the genotypes, making many RFLP site do not give useful information and this disadvantages leaded to replacement of this technique by Microsatellites ***(Vignal et al., 2002).***The effectiveness of RFLP to illustrate genetic variation is relatively low compared to other more recent genetic markers, the removal, addition or rearrangement of the nitrogen bases in the enzymatic cutting sites is distributed in genome different species but the chances of this happening at a site under study are very rare, on the whole, the main weakness of this technology is the resultant lack of phenotyping. The alleles resulting from RPLP are condominium because both alleles can be seen in the observed genetic samples (***Liu and Cordes, 2004)***.This technology has several uses, notably the identification of genetic variation, this is done by comparing different communities or different species genetically linked, and that the difference in the number of binds and their sizes affected by genetic material is a fundamental measure of genetic variation, Therefore, this technology is important in the studies of evolution and transformation ***(Griffths et al., 1999).*** This technique also used to make genetic maps through estimation of distance between the studied genes ***(Hamadalla, 2009).***

2.7 Genetic markers

The marker is defined as a sequence of specific nucleotides used to infer a location of locus on the chromosome, the knowledge of this site helps in the inheritance of a certain characteristic close to the marker by the correlation property ***(Tenova, 2009).*** The development of Molecular Biology and Statistics opened a way and provided possibilities for identifying and using genetic variability and major genes for genetic

improvement, Molecular technologies have allowed for the presence of different or multiple genotypes among individuals in the herd in specific parts of DNA, This can be used to building genetic maps and to evaluate the difference between markers in the molecular expression of traits in families within the breeds that indicate a direct effect of these traits ***(Alsahookie, 2016).***

Molecular genetic applications in genetic improvement depend on the possibility of determining genotypes of individuals for specific genetic sites, genetic markers can be used to identify specific areas on the chromosome, which are sites of genes that affect quantitative traits ***(Davis and Denise , 1998).*** These techniques provide an chance to ensure that genes related to the studied traits can be transmitted from parents to offspring **(Akhimienmhonan, 2006)** in order for these variations to be useful in genetic applications, they must be genetically capable, that is, transmitted from parents to offspring. It is easy to distinguish them by appearance or detective of the genetic mutation by applying the molecular technologies of the genetic markers, depending on the variation of the genetic mutations, these technologies include : RFLP, RAPD, AFLP, Micro Satellite , Mini Satellite and others ***(Liu and Cordes, 2004).***

2.8 Effect of non-genetic factors on body weight in sheep

The weight of sheep influenced by numerous hereditary and non-hereditary factors ***(Kesbi et al., 2008 ; Krejcova et al., 2008; Thiruvenkadan et al., 2008; Petrovic et al., 2011; Ruzic-Muslic et al., 2011).*** The size, breeds, and age of a ewe, the delivery type, sex of lambs, and support and proper nutrition status are known to importantly affect the birth way and weaning weight of lambs ***(Aliyari et al., 2012;***

Akta and Dogan, 2014). ***Rashidi et al., (2008), Abegaz et al., (2005) and Baneh et al., (2009)*** also they mentioned lamb's sex, birth year and birth type have significant effect in breeds. A number of researchers such as ***Fisher et al., (2004) ; Babar et al., (2004) and Petrovic et al., (2009)*** they also confirmed the variations of birth weight because of numbers of factors such as year of birth, season of birth, age of dam, birth type and sex of lambs, as well as ***Momoh et al., (2013)*** reported birth and year of birth influence the estimation of breeding value. However, determination of factors that causes variation in birth weight is more important and explained the relationship of birth weight to newborn and adult health ***(Gardner et al., 2007). Siddalingamurthy, (2017)*** referred to the birth weight considered the first watched traits in life of animal which growth, production and reproduction. Determination and investigation of environmental factors that effect on traits may be have indicators to estimated genetic parameter as well breeding value to explained animal's genetic potential ***(Rashidi et al., 2008)***. According to ***Rahimi et al., (2014)*** the male sheep were heavier than females at all age of life and the male have significant effect, and these finding was confirmed by ***Ahmadi et al., (2004) ; Abegaz et al., (2005) and Rashidi et al., (2008)*** they mentioned the male body weight was more than female for all traits. Plainly, the present of a Y-chromosome and the products of sry gene activation, like androgens and Mullerian-inhibitor substance engages in sexual relations particular consequences for fetal development ***(Gardner et al., 2007).*** Contrasts among male and female sheep reflect distinction in the endocrine condition, and related differences in nutrient supplement demand between the genders as well as regulatory mechanisms controlling growth hormone sexually dimorphic ***(Ghafouri–Kesbi et al., 2016).***

2.9 Importance of the body measurements

In sheep breeding, it is well known that type traits have an important influence on sheep technique ***(Abbasi and Ghafouri-Kesb., 2011).***Measurements, As well as weight measurements, describe more completely an individual or population than do the conventional methods of weighing and evaluating ***(Salako, 2006)*** and are of value in predicting live body weight and also in judging the quantitative qualities of meat ***(Abbasi and Ghafouri-Kesbi , 2011).*** Different body estimation are of value in making a decision about the qualities of meat the quantity and also are helpful in creating of reasonable determination criteria ***(Ravimurugan et al., 2013).***These body measurements have been utilized at various times for the estimation of weights when live weights are measured alongside these parameters (**Salako et al., 2002)**. Measures of size and body form are wanted in numerous trials with sheep, including studies of growth, inheritance and ***nutrition (Janssenes and Vandepitte, 2004 ; Mandal et al., 2008).*** Body and weight estimation portray more totally an individual or population than the conventional techniques of weighing and evaluating ***(Ige et al., 2015).*** It is conformed that there is a strong relationship between the distance around an animal's chest girth and its body weight ***(Otoikhian et al., 2008).*** Alteration in body measurements and indices estimated from different combinations of conventional and non- conventional body criteria not only provide superior guide to weights but are also used as indicators of type and function in domesticated animals ***(Ravimurugan et al., 2013).***

2.10 Parathyroid Hormone

Parathyroid hormone (PTH) is only a one -chain polypeptide made up about 84 amino acids, released via the parathyroid gland, it increase the

absorption of calcium from the intestines and concurrently status its reabsorption out of the skeleton (***Abd-Allah and Baker, 2015).***Its functions, controlling phosphorus metabolism thru its re-absorption among the skeletal system as well as among stimulating the excretion of phosphates through the renal if the inorganic phosphorus excess within the body fluids ***(Kurek et al., 2005).*** Plasma calcium concentration and its ionized form were decreased, causes the accelerated secretion of PTH via the parathyroid gland, together with the durability consequential rapid rise of the elimination of calcium out of the bones then the surprising expand in the re-absorption over calcium by way of the kidneys ***(Horst et al., 1997).***

Parathyroid plays a primary role in any statuses about to lowering the calcium level in extra-cellular fluids below the accepted norms (hypocalcaemia) (***Littledike and Goff, 1987***). ***El-Hag et. al., (2012)*** reported , the parathyroid hormone has two roles: resorption of bone and production of 1.25 dihydroxyvitamin from the renal. As a results of PTH increase within the blood, additionally an influencing especial body systems in ruminants, PTH induce the metabolism of vitamin D and creates the active metabolite 1,25-(OH)2D via the kidney cells ***(Kurek et al., 2005*)**. Presumably, parathyroid hormone and active form of vitamin D have a main roles within the regulation of the serum concentration levels of Ca in pregnant as within the non-pregnant, Vitamin D is known to be increased in pregnant however makes an attempt to determine PTH concentrations levels in pregnancy have led to conflicting results ***(Frolich et al., 1991). Ardawi et al., (1997)*** reported that the parathyroid hormone was elevated from 0.26 in the first trimester to1.31pmol/l in the second trimester, and decreased to back values of the first trimester. ***EL-Hag et al., (2012***) was

working on high producing cows before the fifth birthing, and noted that changes in the level of PTH were, November 2.90ng/ml, May is 1.35ng/ml and October is 2.27 ng/ml.

2.11 Vitamin D

Vitamin D can be synthesized in the skin through exposure to bright Ultra-Violet (UV) light or can be acquired through dietary admission, However, daylight introduction is important factor influence on vitamin D status and is affected also by skin color, season of year, altitude, and in addition way of life and social practices ***(Andrew et al., 2012)***. In correlation, diet has a less influence on vitamin D status in spite of the fact that utilization of supplements can unequivocally impact status ***(Hollis et al.,2011; Prentice, 2008).***Vitamin D is essential for absorption of dietary calcium and phosphorus from the intestine, thereby adequate levels of vitamin D is essential for promoting healthy bone growth and has protective effect against several bone manifestation (***Al-Amri et al., 2017).***

Ritu Gupta (2014) reported vitamin D increases calcium absorption inside the intestine and continue to maintain serum calcium and phosphate concentrations to allow normal mineralization of bone and to prevent hypo calcemic tetany. Bone growth and bone transforming via osteoblasts and osteoclasts it's also acquired to vitamin D ***(Institute of Medicine, 2010).*** As well as, Vitamin D has another function such as modulation of cellular growth, immune function neuromuscular function, and decreasing of inflammation (***Shils and Shike, 2006 ; Norman and*** **Henry,** ***2006).*** According to ***(Andresen et al., 2008)*** nutrition vitamin D can role as anabolic agent because it has direct anabolic impact on bone and it promote the survival of osteoblasts. Vitamin Dhave also

physiological effect in reproduction ***(Luk et al., 2012).*** Pregnancy is a unique life stage due to the growing fetus effected by mom`s dietary repute ***(Karlsson, 2013).*** Vitamin D may be the parturition and first postpartum estrus in sheep ***(Abegaz et al., 2005).*** (***Lockwood et al., 2016)*** studied into labored on supplementation of Merino pregnant ewes and its lambs at birth with cholecalciferol and he find out is maternal supplementation with cholecalciferol in late pregnancy multiplied the plasma concentrations of 25(OH)D in supplemented ewes by 74 % at lambing and this doubled the plasma 25(OH)D concentrations of their lambs at the beginning.

2.12 Calcitonin

Calcitonin(CT) is a polypeptide hormone synthesized via the mammalian thyroid gland in response to hypercalcemia (***Martin, 1980).*** 32-amino acid protein hormone originate from the C cells of the thyroid gland. C cells or parafollicular cells, that are so sensitive and specifically regarded for synthesis calcitonin hormone, a hypocalcemic and hypophosphatemia hormone ***(Fernández-Santos et al., 2012).*** Subsequently the call ‘thyrocalcitonin’ or ‘calcitonin’ to suggest its starting place and function of the hormone ***(Faoura and*** **Gilloteaux, *2017).*** Calcitonin plays as the antiresorptive agent through its function with its specific receptors, inflicting powerful inhibition of osteoclast activity, and its function as a regulator of calcium homeostasis that includes bone resorption is well documented ***(Andresen et al., 2008).***

Davey et al.,(2013) suggested that the main action of calcitonin hormone and that for which there are the most complete facts is its action to inhibit osteoclast activity lead to inhibit bone resorption. Action of Calcitonin in the renal has also been shown to have an effect on the renal

dealing with of calcium via increasing renal excretion of calcium, probably via its actions to lower the tubular reabsorption of calcium ***(Findlay DM, 2006 ; Findlay* and *Sexton, 2004).*** further, calcitonin play another role in adjust serum calcium by means of promoting the renal conversion of 25-hydroxyvitamin D3 to 1,25- dihydroxyvitamin D3 (1,25(OH)2D3) with direct stimulation of the transcription of the 1a-hydroxylase gene inside the proximal tubule of the kidney (***Sexton et al., 1999).*** (***Zhong et al., 2009 ; Shinki et al., 1999)*** reported that the calcitonin, and not parathyroid hormone seems to be a important regulator of 1a-hydroxylase and serum ranges of 1,25(OH)2D3. However, (***Shappell et al., 1987)*** act on "effect of dietary and age on calcitonin and blood and milk minerals the periparturient dairy cow" and he discovered the cows not heifers fed high calcium in diets exposure to excessive hypocalcemia at calving, remained hypocalcemic for three day and had low serum calcitonin.

Garel et al., (1976) examined on "plasma immunoreactive calcitonin tiers in pregnant ewes and their lambs" proven plasma calcitonin hormone was increased within the jugular vein of pregnant ewes in the remaining 40 days of pregnancy than in control ewes. However, A calcium load is given orally (10mg/kg body weight) in newborn lambs did not change the plasma calcium concentration level but elevated the plasma calcitonin concentration.

2.13 Lipid Profile

The lipid profile involved Cholesterol(Ch), Triglyceride(Tg), High Density lipoprotein(HDL), Low density lipoprotein (LDL) and Very Low Density Lipoprotein(VLDL) ***(Phuse, 2012).*** Research on cholesterol, triglyceride, and lipoproteins in domestic animals were revealed it clear

that species variations exist, and that inside species also there are important changings(***Khoshvaghti et al., 2012)***. According to ***(Azab and* Abdel-Maksoud *, 1999 ; Tambuwal et al., 2002)*** there are important and large variations in biochemical and hematological parameters found between breeds of goat. The ordinary concentrations of serum lipids profile of some animals such as the sheep, cow, horse, camel, dog, cat pony, reindeer calf, and cheetah in various physiological situations were stated ***(Nazifi and Ghavami, 2002).***

There are a large numbers of factors effect on biochemical and hematological parameters such as Vitamins, sex, age, breed and crossbreeding, housing, reproductive situation (pregnancy and oestrus), starvation, environmental elements, strain and transportation (***Balicki et al., 2007). Swanson et al., (2004); Yokus et al., (2004)*** suggested that several elements including season, age and under nutrition also affected on biochemical parameters. Pregnancy is a unique physiological situation that effects in changing within the maternal metabolic functioning, Those changing mother and fetus to provide enough power throughout pregnancy period and ensure appropriate development of the fetus, maternal metabolism have to adapt to ensure supply the feto placental unit by amino acid, glucose, and lipids during pregnancy (***Aisling et al., 2017)*** those adaptations affected on maternal blood lipid concentrations and during pregnancy mothers may be enter normal physiological state of hyperlipidemia ***(Mazurkiewicz et al., 1994; An-Na et al.,1995).*** Changing in compositions of lipids and lipoproteins during pregnancy (***Winkler et al., 2000)*** this can lead to pass the lipids to the placenta and act on regulate the fetal lipid concentration ***(Aisling et al., 2017). Boudebza et al., (2016)*** reported the lipid during pregnancy are usually affected by involvement of maternal tissues to provide adequate energy to

the foetal growth. However, after parturition, lipid concentration back to normal concentration before pregnancy which show that this rise blood lipid should play function within the physiology of the pregnancy and development of the fetus ***(An-Na et al., 1995).***

Piccione et al., (2009) studied biochemical serum exam in ewes during pregnancy, post-parturition, lactation and dry period confirmed that is overall blood lipids showed a significant increase during pregnancy post-partum, and early lactation compared to dioestrus, at the same time as total cholesterol and triglycerides showed the opposite trend. ***(Zvonko et al., 2015)*** worked on blood lipid profile in Mediterranean sheep breed during pregnancy and he found out there is no sharp variation from the sheep's reference values except Triglyceride.

Nazifi and Ghavami, (2002) studied on serum lipid profile in Iranian fat-tailed sheep located out the concentration of cholesterol, triglyceride, high density lipoprotein and very low density lipoprotein during 7 weeks before birthing at birthing and the 7 weeks were significantly different ($p<0.05$), However, one week before parturition, level of triglycerides, cholesterol, high density lipoprotein, low density lipoprotein, and very low density lipoprotein had been higher than at different intervals.

2.13.1 Cholesterol

Cholesterol(Ch) is cyclopentanoperhydrophen anthrene, which consist of three 6-carbon rings and one 5-carbob ring ***(Khan, 2013***) . Cholesterol considered amphipathic sterol present and its found in higher animals, cholesterol a waxy lipid and involved in body tissues ***(Priya et al., 2013)***.Cholesterol plays a critical function in the building of cellular membrane, manufacturing of hormones, bile and metabolism of fats soluble vitamins and performing as an antioxidant (***Okonkwo et al.,***

2010). Cholesterol is the main substrate for placental progesterone synthesis ***(Edison et al., 2007).*** The site of synthesis of cholesterol are liver and intestine.(***Priya et al., 2013).***

Cholesterol is vital for the correct functioning of the body but possibly have a negative role when it is consumed produced within the frame in excessive quantity or produced within the frame in excessive quantity ***(Khan, 2013).***However, excessive amount of cholesterol is consumed, the particles lodge in the walls of arteries and form plaques making the arteries narrow, which lead to causes heart attacks and strokes ***(Correa, 2014)***.There are numbers of research on the effects of different phase of the reproduction cycle on biochemical parameters in domestic animal species (***Piccione et al., 2009).*** In ovine, they have been carried out, amongst others, in relation to the oestrus cycle, pregnancy and sucking periods ***(Iriadam, 2007).*** Maternal tissues are involved in providing energy for reproduction processes along out the pregnancy, which may affect biochemical values in serum, affected also by means of numerous other factors like a breed, age, malnutrition, foetal growth, or season ***(Swanson et al., 2004; Yokus et al.,2006).***

During late pregnancy in sheep, blood serum lipids profile is characterized by way of elevated concentration level of cholesterol, triglycerides, and lipoproteins because of the limited responsiveness of goal tissues to the insulin that, as same way, multiplied mobilization of fatty acids from adipose tissue make available new sources for the fetal growth ***(Piccione et al., 2009). Iriadam (2007)*** suggested the disturbances in the blood level of cholesterol content has been observed in the course of oestrus and pregnancy, as a precursor of the steroid hormones.

2.13.2 Triglyceride

Triglycerides are a kind of lipid found inside the body ***(Jessie, 2016)***. A triglyceride molecule is essentially an ester which consist of a glycerol molecule attached to three fatty acids (***Priya et al., 2013).*** The plasma lipid profile is characterized by way of low triglyceride and triglyceride-wealthy lipoprotein concentrations in sheep and other ruminants (***Mazur et al., 2009)***. However, elevated levels are associated with the prevalence of coronary heart sicknesses ***(Labreuche et al., 2009). Ma (2004)*** also confirmed the excessive levels of triglycerides in blood are also connected to coronary heart disorder. ***Smith (1975)*** reported that during late pregnancy there were increase in both cholesteryl esters and triglycerides in the liver of the sheep. ***Noble (1970)*** also confirmed plasma of pregnancy ewes contain an increase in level of triglycerides and non- esterified fatty acids.

Triglycerides in circulating blood share notably to milk fat synthesis ***(Nazife and Ghavami, 2002). Boudebza(2016)*** Look at on biochemical parameters in Ouled djellal ewes during the per- parturient stage and showed that ewes in overdue pregnancy have the higher blood cholesterol, triglyceride. ***Funda et al., (2015)*** act on blood concentrations of triglycerides and certain blood metabolites in healthy colored Angora goats throughout the per -partum period and he becomes confirmed triglycerides had been recorded at most ranges ($p< 0.05$) 2 weeks a partum, with the lowest concentrations at 3 weeks postpartum.

2.13.3 High Density Lipoprotein

The function of High density lipoprotein (HDL) in the blood is to transport cholesterol from the body tissue to the liver for elimination and to promote the manufacturing of nitric oxide ***(Bartels and* O'Donoghue,**

2011). It's been pronounced that HDL has a genetic role and as a consequence its role within the avoiding of coronary heart diseases is also guided by using the genetic make-up of a person ***(Weissglas-Volkov, 2010).*** Every other critical truth is that even though HDL tiers correlate with desirable cardiovascular fitness and particularly elevating its levels might not result in a better cardiovascular health ***(Voight et al., 2012).*** HDL is referred to as "good HDL- cholesterol" that is good for the cardiovascular system and HDL truly works to clean cholesterol from the blood (***Ma, 2004).***

In human, during pregnancy, the increase in HDL was empirical through the first trimester, but the second and third trimester values have been statistically significant ($p<0.05$) ***(Omorogiuwa and Ozor, 2015).*** High density lipoprotein-cholesterol and low density lipoprotein-cholesterol have additionally been found to be associated with the development of melancholy in pregnant (**Rabe-Jabłońska *and* Poprawska, *2000 ; Maes et al., 1997).***

2.13.4 Low Density Lipoprotein

Low density lipoprotein (LDL) plays important role to carrying and metabolism cholesterol inside the body***(Priya et al., 2013)***. LDL considered a "bad cholesterol" due to the fact plays a vital role in accumulation the cholesterol in blood vessels especially in arteries, it is known as low density as it carries little proteins and a huge quantity of fat (***Khan, 2013***). A protein referred to as apolipoprotein B-100 that carries 4536 amino acid residues is present in each LDL particle ***(Priya et al., 2013).***

The accelerated progesterone concentration contributes to the upward push in LDL ranges ***(An-Na et al., 1995)***. LDL discovered in maternal

serum at some point of pregnancy is atherogenic small and dense ***(Brizzi et al., 1999). Sy and Ak (2009***) reported the LDL found in the blood of pregnant and lambed Awassi sheep .The components of LDL are shown in the tables (2-1).

***Table (2-1) Components of LDL**

Composition	Components
450 molecules/ LDL particle	**Phosphatidylcholine**
185 molecules/LDL particle	**Sphingomyelin(SM)**
80 molecules/LDL particle	**Lysophosphatidylcholine (lyso-PC)**
10 molecules/LDL particle	**Phosphatidylethanolamine (PE)**
7 molecules/LDL particle	**Diacylglycerol (DAG)**
2 molecules/LDL particle	**Ceramide (CER)**
6 molecules/LDL particle	**α-tocopherol**

* ***Priya, et al., 2013***

2.13.5 Very Low Density Lipoprotein

Very Low Density Lipoprotein(VLDL) considered is a type of lipoprotein with the very best quantity of triglycerides and it is labeled as a kind of "bad cholesterol" because it subsequently gets transformed into LDL and reasons buildup of cholesterol at the walls of arteries ***(Shelness and* Sellers *, 2001).*** However, VLDL in pregnancy considered to altered in metabolism because of decreased lipoprotein lipase activity inside the adipose tissue and expanded activity in the placenta ,the general results of altered lipid metabolism in being pregnant are accumulation of maternal fat stores within the first half of and more induced fats mobilization in the second half of pregnancy (***Butte, 2000).*** According to ***Bartels an***

O'Donoghue, ***(2011)*** LDL-cholesterol and triglycerides are multiplied, particularly in second and third trimester.

Mazur, (2009) worked on disturbance of plasma triglyceride-rich lipoproteins and triglyceride secretion in pregnant ewes and he became discovered out the plasma and really low-density lipoprotein concentrations had been about four-fold higher in correctly-fed pregnant ewes than in non-pregnant ewes.

Chapter Three Materials and Methods

Chapter three

Materials and Methods

3.1 Field experiment

3.1.1 Experiment animals

The experiment was conducted at ″Barakat Abul-Fadhl station″ for sheep production located 10kms of south-east Kerbala governorate on the main road of Karbala _ Najaf during the period 20/9/2017 to 20/7/2018. 63 pregnant sheep were used in the experiment (36 Nuimi 27 Awassi) and 20 non-pregnant ewes (10 Nuimi and 10Awassi), all ewes aged between (2-5)years. Nuimi females were fertilized by using Nuimi males and Awassi ewes were fertilized by Awassi males.

3.1.2 Flock management

Animals were raised in semi-open barns (35% covered and 65% opened) designated for sheep production. Ear tags were used to identify sheep under study. The flock was managed according to a program that includes special feeding program for mothers during pregnancy and special nutrition for lambs after parturition as well as health and veterinary care.

3.1.3 Nutrition

The animals were fed depending on their production status and depending on the availability of feeding, as it provides green fodder or coarse feed represented by Alfa Alfa, it also provides concentrated feeding and increase quantity according to the state of animal production. There are special diets for pregnant ewes as in (table3-1) and also special diet for lambs before weaning as in (table3- 2).

Table (3-1) diet of pregnant ewes

Substances of diet	Amount of substances
Barely	**60% per ton**
Bran	**39% per ton**
Alfalfa	**0.5 to each ewe**
Coarse feed	**0.5 to each ewe**
Salt	**1% per ton**

Table (3-2) diet of lambs(15) weeks of age (weaning weight)

Substance of diet	Amount of substance
Barely	**50% per ton**
Barn	**29% per ton**
Crushed Corn	**10% per ton**
Soya	**8% per ton**
Limestone	**1% per ton**
Premix	**1% per ton**
Salt	**1% per ton**

3.1.4 health and veterinary care

1-Dipping of animals by using biothroid cypermethrin 10% to protect against external parasites.

2-Vaccation of pregnant ewes and adults animals by *clostridium* vaccine also the lambs vaccinated by this vaccine twice: two weeks after birth and again other time after one month, after this, the vaccine was repeated every 6 months to protect ewes from black leg disease.

3- Vaccination against sheep pox and foot and mouth diseases (FMD).

4-Vaccination the lambs at (3-6) age month by (Rev-1) against Brucellosis (once dose for 5 year).

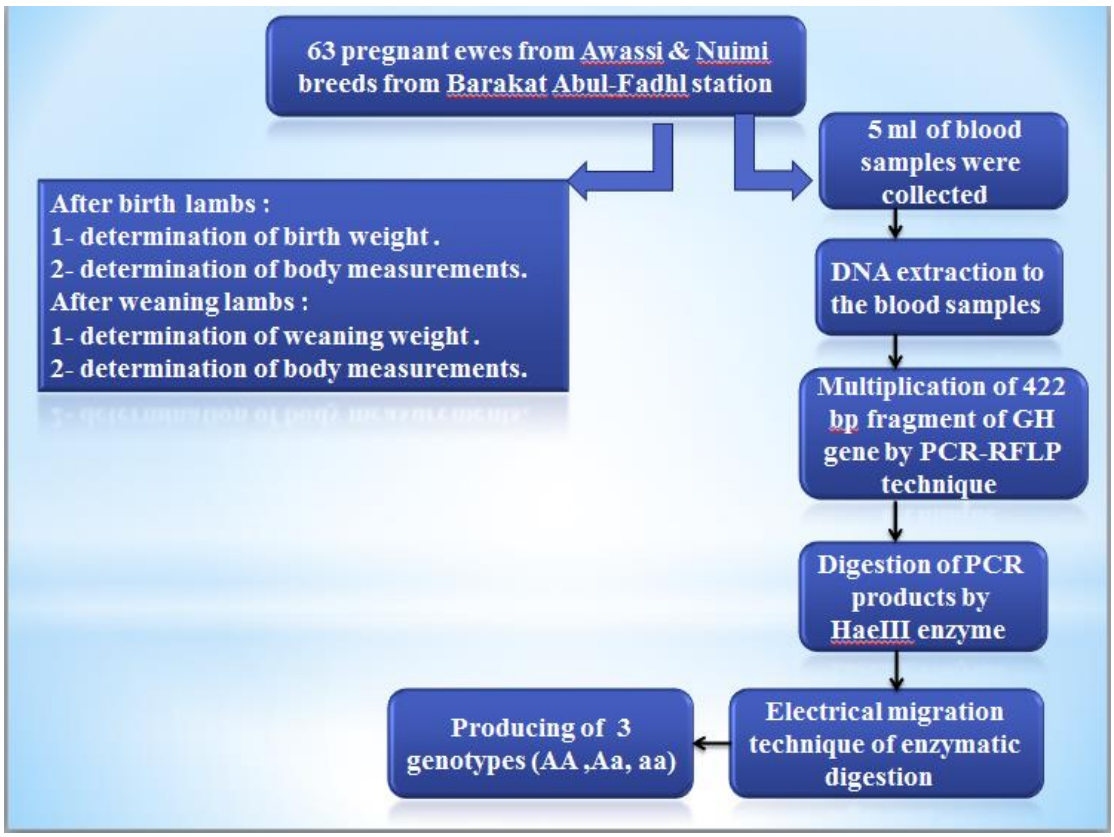
5-Drenching of sheep through mouth to protect from liver and intestine worms in March and April months.

6- Treatment of mastitis if occurred.

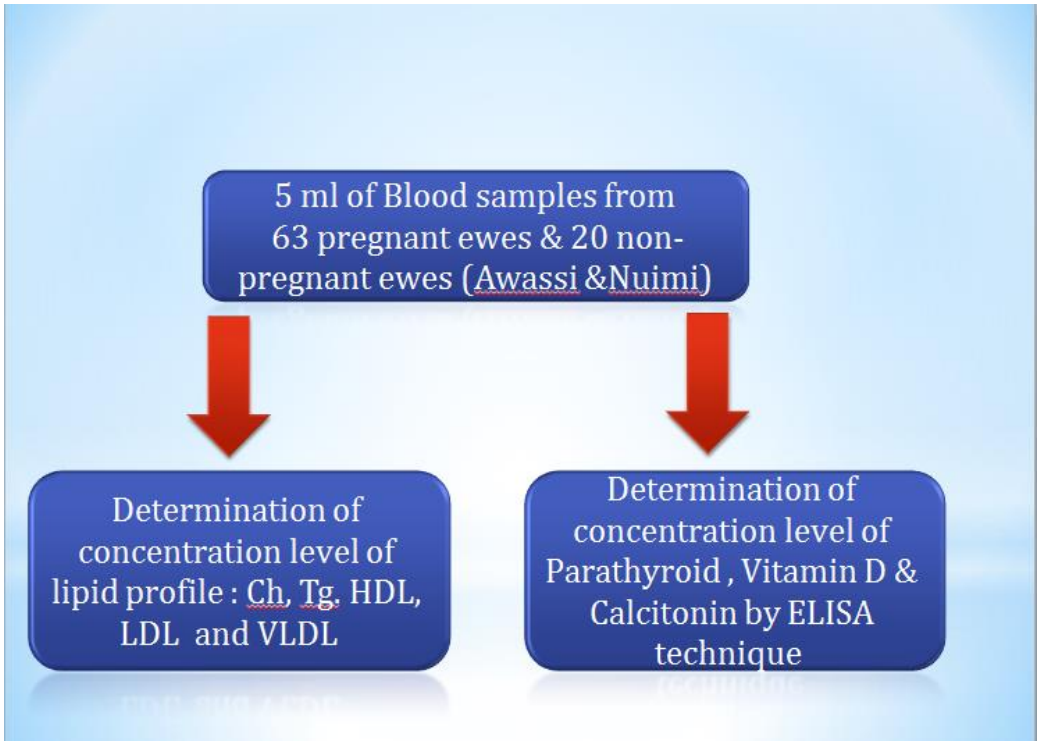
7-Yearly vaccination of animals to protect from render pest disease.

3.2 Experimental design:

A. Molecular work:



B. Physiological work:



3.3 Blood collection

Blood samples from all studied ewes were collected from the jugular vein of animals using a 10ml syringe after cleaning the jugular vein area and sterilizing the area with the alcohols. Blood samples were divided into two portions: 5ml for hormones and lipid profile analysis were put in gel tubes and 5ml for DNA extraction were put in ethylene diamine tetra acetic acid tube (EDTA tube), then numbered these tubes according to number of ewes, later on the lambs had the identical numbers of their mothers, see (appendix 1). The tubes kept frozen -4C°until the experiment were performed.

3.4 Laboratory work

Laboratory work was carried out in (The Researches Laboratory) at the College of Veterinary Medicine/ Kerbala University. A total of 63 blood samples were collected from pregnant ewes (36 Nuimi and 27 Awassi) sheep and 20 blood samples were collected from non-pregnant ewes (10 Nuimi and 10 Awassi) sheep.

3.5 Molecular laboratory work

The laboratory equipment were used as shown in the table (3-3) and chemical materials as shown in the table (3-4) for this study .

Table(3-3)equipment and devices used in the experiment

Sq.	Equipment and devices	Manufacture company and origin
1	Cool box	Uncif (Russ)
2	Laminar flow cabinet	Jeiotech (korea)
3	Water bath	Labtech(korea)
4	Centrifuge	Hereaus(Germany)
5	Microwave	LG (Kuria)
6	Electrical sensitive balance	Sartorius(Germany)
7	Polymerase Chain Reaction	Eppendroff (Germany)
8	Gel electrophoresis apparatus	Cleaver scientific (USA)
9	Electrophoresis constant power supply	Cleaver scientific (USA)
10	UV light transillminater	Cleaver scientific (USA)
12	Vortex	Dupuque(USA)
13	Automatic micropipattes	Bio basic(Canada)
14	Spectrophotometer	Shimadozoe (JAPAN)

Table (3-4) chemical materials used in experiment

Sq.	Materials	Manufacture company and origin
1	Agarose	BIO BASIC
2	10X TBE Buffer Marker	BIO BASIC
3	Bromophenol Blue	Bioneer(korea)
4	Primer	Afadna(Canada)
5	Wizard genomic DNA purification	Geneaid(korea)
6	Maxime PCR PreMix (i- Taq for 20µl rxn)	INtRON Biotechnoligy (korea)
7	Ethidium bromide stain	BIO BASIC
8	Ethanol alcohol	Scharlau(spain)
9	Dieonized water	Aquarama (Canada)
10	Restriction Enzyme (Hae III)	Bioneer (Korea)
11	DNA Ladder Marker	Dongshengbio

3.5.1 DNA extraction

DNA was extracted from the blood samples of pregnant ewes from the Awassi and Nuimi breeds using a kit supplied by (Geneaid) Korean company. See (appendix 2).

3.5.2 Determination of concentration and purity of DNA extracted

determination of DNA concentration carried out by measure of absorbeditiy of ultra-violate ray spectrum by used spectrophotometer at

260nm wavelength, where was each sample you want to estimate of DNA by using TE solution and applying the following equation :

DNA CONCENTRATION /ML= absorbidity for each ml from sample × inverse dilution(100)× 50 while the purity determination from the absorbance reading at a wavelength of 260 is obtained by reading the absorbance at a wavelength of 280 nanometers (***Maniatis et al., 1982).***

3.5.3 Electric Migration Technology

The efficiency of the extraction process was detected in obtaining the complete DNA molecule by carrying out sending the sample extracted on agarose jell (1% conc.) [dissolve 0.50 g of agarose in 50ml of diluted TBE solution (1x)] and then heated by heater for five minutes until the color is clear and let it cool down a bit and harden the gel in (the transfer basin) for the purpose of hardening .after the stiffness gels and raise comb is a template in to go to the migration to be the hole the direction of the cathode and then we add solution buffer (TBE) so that covers the jelly on high 5mm, 6ul of output DNA was mixed and 3ul of loading dye and injection mixture in the dig gels and linked polarity to power supply and set the voltage to 70 volt and current 65 ampere to 1.30 hour, then immersion jelly by (Ethidium Bromide) for 20 minutes by using UV radiation is note binds in gel ***(Maniatis et al., 1982)*** and was filming binds by using photo documentation system.

3.5.4 Polymerase Chain Reaction technique

All materials for PCR were prepared under sterile environment in sterile cabin as shown in (3-5table), it has been prepared mixture interaction PCR in(Eppendrof tube) and its size (100ul) , the size of final components 25 ul. After this, eppendrof tube was putted in PCR

equipment and the program was used in PCR technique showed in table (3- 7).

Table (3-5) size of material that using in PCR technique

Chemical material	Master Mix (ul)	Primer (ul)	DNA Matrix (ul)	Distal Water (ul)	Final size (ul)
Size	5	R-1 F-1	5	13	25

3.5.5 Migration of product reaction of polymerase reaction and electrolyte.

5ul of (DNA ladder) and 5ul of PCR were loaded into agarose gel(3%). The transfer was carried out and electric current was stabilized at 70 volts and 65 amperes for 1,5 hours and the gel was then immersed in the ethidium bromide liquid ,and note the binds by UV, then tacked picture by the mobile, as in figure (3-3).

3.5.6 Characterization of Growth Hormone Gene

Selecting the primer as shown in table (3-6) for a detection of genotypes and phenotypes to GH ***(Hau et al., 2009***

Table (3-6) Sequences nucleotides primer (GH1)

Name of gene	Sequences	
GH 1	EXON2 & EXONE 3	F: CTCTGCCTGCCCTGGACT
		R: GGAGAAGCAGAAGGCAAC

Table (3-7) Molecular detection program using PCR technology

Sq.	Steps	Temperature C°	Time	Number of Cycles
1	Start Denaturation	94	5 min	1
2	Denaturation	95	30 sec.	13 cycles and decrease the temperature about 1C° per each cycle
	Annealing	65	30 sec.	
	Extension	72	45 sec.	
3	Denaturation	95	30 sec.	35 cycles
	Annealing	52	30 sec.	
	Extension	72	45 sec.	
4	End extension	72	7 min	
5	Finish	4	Unlimited	

3.5.7Enzymatic digestion of the growth hormone gene

Preparing the samples to enzymatic digestion by Hae III enzyme from Bioneer company as shown in (table 3-8) and incubated this samples at 37C°.

Table (3-8)Compounds of enzymatic reaction

Materials using	Amount of Materials
Product PCR	**5µl**
Buffer	**3.5µl**
Restriction Enzyme (Hae III)	**1.5 µl**

3.5.8 Electric migration technique of enzymatic digestion samples.

Down loaded 10ul of products and enzymatic digestive samples and also 7ul of DNA ladder and migration extracted samples on agarose gel (2% conc.) separated by 1g of agarose diluted in 50ml of TBE solution (1x) and heating by heater for 5 minutes until get clear color, then the mixture was leaved to cool and the gel was putted in basin migrating to stiffness. After harden and comb remove and link polarity to power supply 40 volt and 45 amper for 20 minutes after this, increase the electric current to 70 volt and 65 amper for one hour, following this immersion the gel by ethidium bromide for 20 minute. At last note the binds in gel by using UV and taking picture by mobile.

3.6 Determination of weights and body measurements to the lambs.

Determination of body measurements of lambs at two stages from life: at the birth (within 24 hour) and at the weaning (15 weeks) by using A flexible tape rule was used to measure the parameters:

1- Body length : distance from point of shoulder to the point of tuber ischia.

2-Body height : distance from the base of hoof to the highest point of withers.

3- Heart Girth : body circumference around the chest just behind the elbow joint and paunch as described ***(Ravimurugan et al., 2007).***

-Birth weight and Weaning weight determinate by using balance specific to small ruminants .

3.7 Measuring the concentration of hormones

The blood serum was separated from the plasma by centrifugation for 20 minutes at the speed of 2000 r.p.m. and the serum was put in a special tube (Eppendrof tube).The concentration of studied hormones was measured by using a commercial kits from (ELabscince-China) based on the immune method ″ELISA″ and the concentrations of studied hormones was determined by comparing optical density of the samples to the standard curve by ELISA Reader device . See (appendix 3).

3.8 Determination of Serum Lipid Profile:

3.8.1 Determination of Serum Total Cholesterol mg/dl:

The determination of Total Cholesterol in serum according to the method mentioned by ***(Fossati, 1982)*** was made by kit test provided from bio Maghreb company (Morocco). See (appendix 4).

3.8.2 Determination of Triglycerides in Serum mg/dl:-

The determination of Triglycerides in serum according to method mentioned by ***(Fossati, 1982)*** was made by using kit test provided from Biomaghreb company (Morocco). See (appendix 4).

3.8.3Determination of High Density Lipoproteins (Cholesterol-HDL) mg/dl:-

Determination of HDL in serum was made by using test provided from Biosystems company (Spain). Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) in the sample precipitate with phosphtungstate and magnesium ions.

The supernatant contains High Density Lipoprotein (HDL) ***(Burstein et al., 1980).*** See (appendix 4)

3.8.4 Determination of Low Density Lipoprotein (LDL-cholesterol) mg/dl:-

The LDL-cholesterol concentration in the sample was calculated by using the following general formula:-

LDL=Cholesterol concentration – (HDL+VLDL).

3.8.5 Determination of Very Low Density Lipoproteins(VLDL-Cholesterol)mg/dl:-

The result was calculated by dividing triglycerides concentration by 5.

3.9 Statistical Analysis

It is used in the research a statistical analysis of data which was performed by using SAS (statistical analysis system – version 9.1th ed.) significant differences were compared with the Duncan Multidimensional Test **(p <0.01) , (p<0.05)** ***(Duncan, 1955).***

Allele frequency was calculated by the equations were mentioned in ***(Paputungan et al., 2012).***

Allele frequency calculated as follows:

$$X_i = \frac{(2n_{ii} + \Sigma n_{ij})}{2N}$$

Where, X_i is the A allele frequency, n_{ii} is the number of ewes with homozygote genotype, n_{ij} is the number of ewes with heterozygote genotype, N is the total number of pregnant ewes in one breed in this study.

Chapter Four
Results

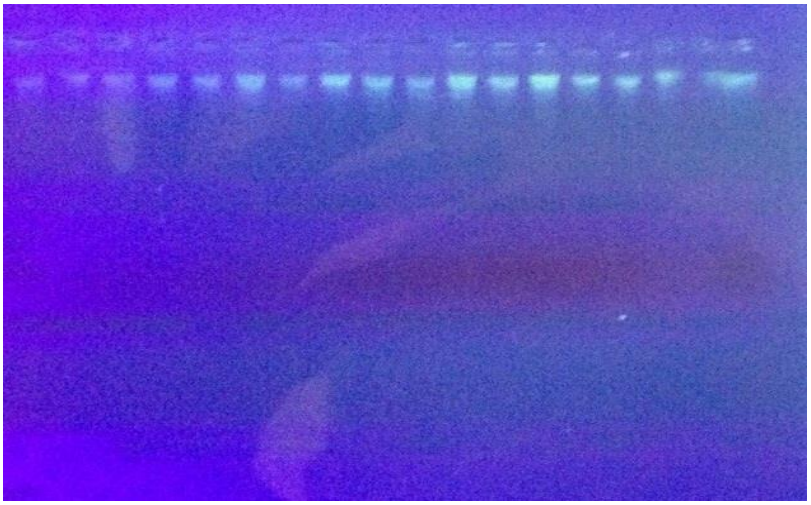
Chapter Four

Results

4.1 Polymorphism of Growth Hormone Gene

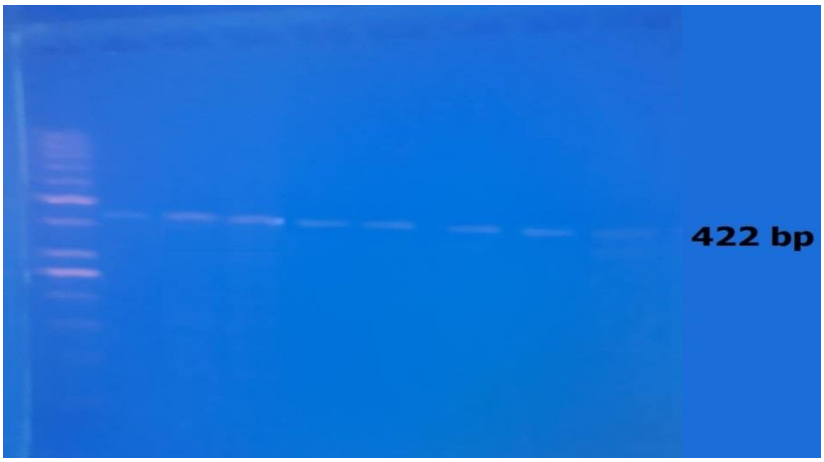
DNA was extracted at a concentration of 50-75 ng/µl as a first step for extraction of growth hormone gene with PCR technique and a total of 6µl from DNA sample and 3µl from loading dye in agarose gel (1% conc.) and 70 voltage and current 65 ampere to 1.30 hours, then immersion jelly by″ethidium bromide″ for 20 minutes by using UV radiation is note binds in gel and was filming binds by using mobile as shown in figure (4-1).

Figure(4-1) DNA extraction of blood samples to Nuimi and Awassi breeds



Growth hormone gene was amplified by using PCR technique (as described in materials and methods) and the PCR product was migrated and shown in figure (4-2) to confirm the success of extraction technique and obtaining the required fragment size 422bp.

Figure(4-2) Migration of PCR products on gel with the ladder to both breeds (Nuimi and Awassi).



4.1.1. Alleles frequency

Our results of table (4-1) in this study revealed that there are two alleles (A and a) was produced from digestion the fragment of 422 base pair from growth hormone gene (GH). Alleles frequency (A and a) in Nuimi sheep were 0.67 and 0.33 respectively, while alleles frequency in Awassi sheep were 0.685 and 0.315 respectively.

Table (4-1) alleles frequency in pregnant Nuimi ewes and pregnant Awassi ewes

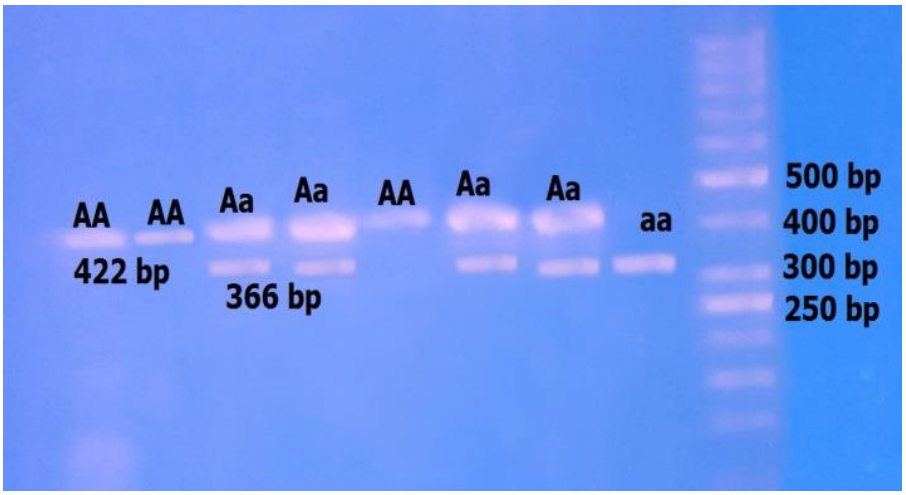
Alleles	Alleles frequency	
	Nuimi ewes	Awassi ewes
A	0.67	0.685
a	0.33	0.315

4.1.2 Gene frequency

The results of gene frequency in table (4-2) reported that three genotypes were produced by studied the fragment of 422 base pair from growth hormone gene (AA, Aa and aa) see figures (4-3) and (4-4).

The gene frequency of (AA, Aa and aa) genotypes in Nuimi breed were 0.39, 0.56 and 0.05 respectively. However, the gene frequency of (AA, Aa and aa) genotypes in Awassi breed were 0.444, 0.481 and 0.074.

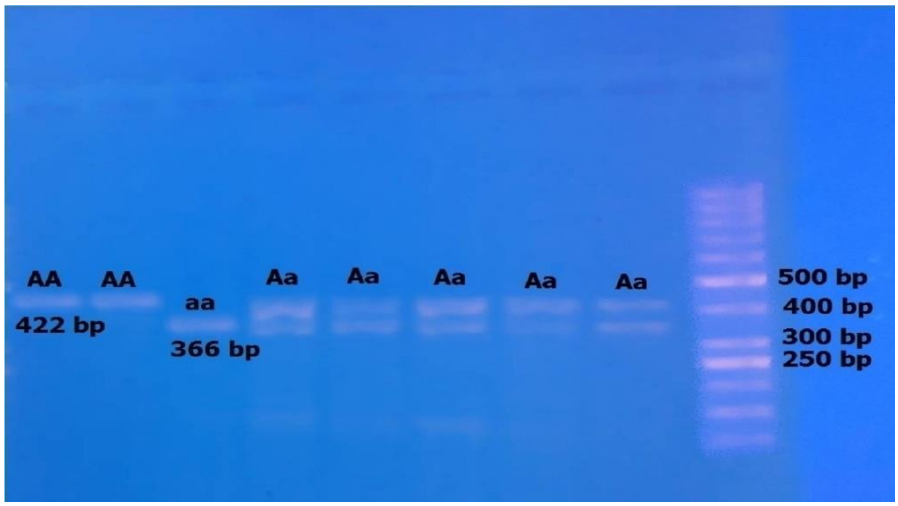
Figure (4-3) Enzymatic digestion of PCR products to Nuimi pregnant ewes



- 422 bp & 366 bp fragments were appeared .

- The fragments of 56 bp was not appeared .

Figure (4-4) Enzymatic digestion of PCR products to Awassi pregnant ewes



- 422 bp & 366 bp fragments were appeared .

- The fragments of 56 bp was not appeared.

Table (4-2) Genotypes frequency in Nuimi and Awassi pregnant ewes

Genotype	Genotype frequency	
	Awassi ewes	Niumi ewes
AA	0.444	0.39
Aa	0.481	0.56
aa	0.074	0.05

4.1.3 Polymorphism of Growth Hormone gene Vs birth and weaning weight of lambs

Table (4-3) recorded the AA genotype of Nuimi breed was excelled on (Aa and aa) genotypes in birth weight and weaning weight and AA genotype have significant effect ($p<0.05$) in weaning weights of lambs. However, the birth weights of lambs that possess AA, Aa and aa)genotypes were (6.35, 4.50 and 5.05)kg ,respectively. However, the weaning weights of lambs that possess AA, Aa and aa genotypes were (21.50, 19.09 and 20.09)kg, respectively, While, Aa genotype have lower weights in both birth weight and weaning weight.

Table(4-3) relationship of genotypes of growth hormone gene with birth and weaning weights in Nuimi sheep.

Genotype	Number of ewes	Means ± Standard Error	
		Birth weight(kg)	Weaning weight (kg)
AA	14	6.35 ± 0.70 A	21.50 ± 3.50 A
Aa	20	4.50±0.0.50 A	19.09±0.26 B
aa	2	5.05 ± 0.36 A	20.09 ± 0.44 AB
Significant level		N.S	0.05

- Different letters (A , B and AB)in column mean a significant difference p≤0.05.

The results of Awassi breed in table (4-4) revealed the lambs birth weight of AA, Aa and aa genotypes were (6.25, 4.94 and 6.82)kg ,respectively and the aa genotype has higher value in birth weight and significant effect p<0.05, while Aa genotype has lower value in birth weight. Lambs weaning weights of AA, Aa and aa genotypes were (21.79, 19.65 and 23.50)kg, respectively and aa genotype has higher

value in weaning weight and significant effect p<0.5 while Aa has lower value of weaning weight.

Table(4-4) Relationship of genotype of growth hormone gene with birth and weaning weights of Awassi sheep

Genotype	Number of ewes	Mean ± Standard Error	
		Birth weight(kg)	Weaning weight (kg)
AA	12	6.25 ± 0.23 A	21.71 ± 0.73 AB
Aa	13	4.94 ± 0.22 B	19.65 ± 0.40 B
aa	2	6.82 ± 0.68 A	23.50 ± 1.50 A
Significant level		0.05	0.05

- Different letters (A and B) in every column mean a significant difference p≤0.05.

4.2 Effect of Gender on body weight and body measurements at two stages of life: (birth and weaning).

The results of Nuimi breeds in table (4-5) recorded the birth weight in male 6.22 kg and in female 4.66kg, the male had significant effect in

birth weight p<0.02.The results of these study showed the heart girth, length and height were higher in male (42.60, 35.60 and 44.50)cm, respectively while, in female (41.00, 32.81 and41.87)cm, respectively. The male at birth had significant effect of body measurements were p<0.02 in all body measurements. However, at the weaning stage, the male also have significant effect in weaning weight p<0,04. Weaning weight was higher in male 20.11kg than in female 18.98 kg. The body measurements (heart girth, length and height) at weaning were higher in male (62.30, 61.30 and 61.55)cm than female (60.62, 59.56 and 59.37)cm, respectively.

Table (4-5) relationship of gender with weights and body measurements at birth and weaning stages of life of Nuimi lambs.

Gender	No	Mean ± Standard Error							
		At birth				At weaning (15 weeks)			
		Weight (kg)	Heart girth (cm)	Length (cm)	Height (cm)	Weight (kg)	Heart girth (cm)	Length (cm)	Height (cm)
Female	16	4.66± 0.34 B	41.00 ± 0.59 B	32.81 ± 1.07 B	41.87 ± 0.87 B	18.98 ± 0.30 B	60.62 ± 0.35 B	59.56 ± 0.53 B	59.37 ± 0.30 B
Male	20	6.22± 0.53 A	42.60 ± 0.48 A	35.60 ± 0.62 A	44.50 ± 0.69 A	20.11 ± 0.42 A	62.30 ± 0.47 A	61.30 ± 0.52 A	61.55 ± 0.55 A
Significant level		0.02	0.02	0.02	0.02	0.04	0.04	0.02	0.02

-The different letters (A and B) in every column refer to significant differences (p<0.05)

The results of Awassi breed in table (4-6) showed the male was higher in birth weight 6.33kg than female 4.94kg and the male had significant effect $p<0.04$. The male recorded higher value in body measurements (heart girth, length and height) at birth were (44.71,37.50 and 45.35)cm, respectively. The male at birth had significant effect on (heart girth, length and height) $p<0.02$, $p<0.05$, and $p<0.03$, respectively**.** The heart girth, length and height at birth in female were (41.79, 34 and 43)cm .The weaning weight in male was 22.03kg, it is higher than female 19.65kg and the male had significant effect $p<0.05$. The body measurements (heart girth, length and height) at weaning in male (64.92, 63.07 and 63.85)cm , respectively. The male had significant effect in (heart girth, length and height) $p<0.05$, 0.01 and 0,05, separately. The heart girth, length and height at weaning in female were (61.38, 60.07 and 60.23)cm.

Table(4-6)relationship of gender with body weights and body measurements at birth and weaning in Awassi lambs

Gender	No	Mean ± Standard Error							
		At birth				At weaning (15 weeks)			
		Weight (kg)	Heart girth (cm)	Length (cm)	Height (cm)	Weight (kg)	Heart girth (cm)	Length (cm)	Height (cm)
Female	13	4.94 ± 0.22 B	41.79 ± 0.96 B	34.00 ± o.57 B	43 ± 0.48 B	19.65 ± 0.40 B	61.38 ± 0.53 B	60.07 ± o.59 B	60.23 ± 0.71 B
Male	14	6.33 ± 0.23 A	44.71 ± 0.68 A	37.50 ± 1.05 A	45.35 ± 0.78 A	22.03 ± 0.66 A	64.92± 0.63 A	63.07± 0.54 A	63.85 ± 0.76 A
Significant level		0.04	0.02	0.05	0.03	0.05	0.05	0.01	0.05

-There are different letter(A and B) in every column refer to significant different($P<0.01$) , ($p\leq0.05$).

4.3 Physiological traits

4.3.1 Hormonal analysis

Our results of the studied on the concentrations levels hormones in table(4-7) revealed that there are major deviations between pregnant and non-pregnant ewes in both breeds (Nuimi and Awassi).The concentration levels of parathyroid in pregnant ewes of Nuimi sheep 85.53pg/ml and 27.11pg/ml in non- pregnant Nuimi sheep. However, the concentration levels of parathyroid hormone in pregnant Awassi sheep was 76.09pg/ml and in non-pregnant Awassi sheep was 28.37pg/ml. The results of concentration levels of calcitonin reported in pregnant Nuimi sheep was

75.29pg/ml but 12.90pg/ml in non-pregnant Nuimi sheep, the results of same hormone in pregnant Awassi sheep was 91.4pg/ml and 12.93 pg/ml in non-pregnant ewes of same breed. Moreover, the results of vitamin D recorded 3.81ng/ml in pregnant Nuimi sheep whereas, in non-pregnant sheep was 0.79ng/ml and the results of same hormone in pregnant Awassi sheep was 3.69ng/ml and in non-pregnant Awassi sheep was 0.93ng/ml. In addition to the previous mentioned results, non-significant differences were noticed in the concentration levels of hormones between Nuimi and Awassi breed

Table (4-7): The concentrations levels of hormones in pregnant and non-pregnant ewes of both breed Nuimi and Awassi

ewes	Mean ± Standard Error					
	Calcitonin pg/ml		Parathyroid pg/ml		Vitamin D ng/ml	
	non-Pregnant	Pregnant	non-Pregnant	Pregnant	non-Pregnant	Pregnant
Nuimi	a 12.90 ± 0.59 A	b 75.29 ± 16.2 A	a 27.11 ± 1.24 A	b 85.53 ± 18.2 A	a 0.79 ± 0.10 A	b 3.81 ± 0.54 A
Awassi	a 12.93 ± 0.61 A	b 91.48 ± 29.93 A	a 28.37 ± 0.89 A	b 76.09 ± 12.44 A	a 0.93 ± 0.09 A	b 3.69 ± O.29 A
Significant level	N.S	N.S	N.S	N.S	N.S	N.S

- Same letters (A) in one column mean there are no significant differences between both breeds (Nuimi and Awassi).

- Different letters (a and b) in one raw mean there are significant differences between pregnant and non-pregnant in both breeds (Nuimi and Awassi).

- Number of pregnant ewes (36 Nuimi and 27Awassi) , number of non-pregnant ewes (10 Nuimi and 10 Awassi).

4.3. 2 Lipid profile analysis

Our results in table (4-8) revealed there are large deviations in lipid profile between pregnant and non-pregnant ewes in both breeds Nuimi and Awassi . cholesterol level in non-pregnant ewes of Nuimi breed was 49.70 mg/dl but in pregnant ewes in same breed was 132.08 mg/dl, while concentration levels of cholesterol in non-pregnant Awassi ewes is 43.10mg/dl and in pregnant ewes in same breed the concentration level was 127.51mg/dl. However, the concentration level of triglyceride in non-pregnant ewes in Nuimi and Awassi breeds are33.50mg/dl, 34.90mg/dl separately, while the concentration levels of Triglyceride in pregnant ewes in Nuimi and Awassi breeds are 65.66mg/dl, 66.14mg/dl, separately. High Density Lipoprotein altered between pregnant and non-pregnant ewes within Nuimi and Awassi breeds , in Nuimi breed the level of High Density Lipoprotein in pregnant and non- pregnant ewes are 52.66mg/dl, 30.30mg/dl separately, while in Awassi breed level of High Density Lipoprotein in pregnant and non -pregnant ewes are 52.14mg/dl, 29.20mg/dl, separately. Low Density Lipoprotein level in non-pregnant Nuimi and Awaasi breeds are 12.70mg/dl, 6.92mg/dl, respectively while in pregnant ewes Nuimi and Awassi breed are 65.37mg/dl, 58.23mg/dl. At last, the levels of very low density Lipoprotein in non-pregnant Nuimi and Awassi breeds are 6.70mg/dl, 6.98mg/dl respectively, while in pregnant ewes of Nuimi and Awassi breeds are 13.13mg/dl, 16.27mg/dl, respectively.

Table (4-8): The concentrations levels of lipid profiles in pregnant and non-pregnant ewes of both breed Nuimi and Awassi

Ewes	Mean ± standard Error									
	Cholesterol (Ch) mg/dl		Triglycerides (Tg) mg/dl		High density lipoprotein (HDL) mg/dl		Low density lipoprotein (LDL) mg/dl		Very low density lipoprotein (VLDL) mg/dl	
Nuimi	non-pregnant	pregnant	non-pregnant	pregnant	non-pregnant	pregnant	non-pregnant	pregnant	Non Pregnant	pregnant
	a 49.70 ± 2.33 A	b 132.08 ± 4.02 A	a 33.50 ± 1.75 A	b 65.66 ± 1.34 A	a 30.30 ± 0.84 A	b 52.66 ± 1.48 A	a 12.70 ± 2.08 A	b 65.37 ± 3.82 A	a 6.70 ± 0.35 A	b 13.13 ± 0.26 B
Awassi	a 43.10 ± 1.73 B	b 127.51 ± 2.91 A	a 34.90 ± 1.55 A	b 66.14 ± 2.17 A	a 29.20 ± 1.36 A	b 52.14 ± 1.79 A	a 6.92 ± 1.50 B	b 58.23 ± 2.53 A	a 6.98 ± 0.31 A	b 16.27± 3.56 A
Significant level	0.03	N.S	N.S	N.S	N.S	N.S	0.03	N.S	N.S	N.S

- Different letters (A and B) in one column mean a significant difference $p<0.05$ between both breeds (Nuimi and Awassi).
- Different letters (a and b) in one raw mean a significant difference between pregnant and non-pregnant in both breeds (Nuimi and Awassi). Number of pregnant ewes(36Nuimi and 27Awassi) , number of non-pregnant ewes(10Nuimi and 10Awassi).

Chapter Five

Discussion

Chapter five

Discussion

The gene of growth hormone affect a wide variety of physiological parameters such as appetite control, growth, body organization, maturing and proliferation and additionally immune responsiveness***(Moradain et al., 2013)***.The consequences of our investigation on pregnant ewes of two local breeds, to be specific, Awassi and Nuimi recorded the distinctions in levels of hormones and lipid profile between the two neighborhood breeds. The outcomes likewise demonstrated that the quality polymorphism of growth hormone gene assumed a vital role in the change of body weight amid birth and weaning stages.

5.1 Growth Hormone Gene Polymorphism

The results of this investigation show two alleles A and a and 3 genotypes (figure 3 and 4) was found in the region 422 base pair (bp): AA genotype undigested one fragment at 422bp, Aa the digested fragment at 422bp, 366bp and 56bp and aa was two at 366 bp and 56bp this outcomes agreement with ***(Mahdi et al., 2018***) was reported for same outcomes when chipped away at Awaasi breed. ***(Othman et al., 2015)*** appeared there are two genotype GG, AG and AA genotype was absent.

5.1.1 Allele Frequency

The consequences of alleles frequency in table (4-19) announced the allele frequency of Nuimi breed A=0.67, a=0.33 and allele frequency of Awassi breed A=0.685, a=315. This outcomes concurrence with ***Kumari et al., (2014)*** who followed up on 9 breeds in India and the alleles frequency of his study are A=0.6016 , B=0.3983 and ***Malewa et al., (2014)*** who followed up on Donggala sheep allele frequency of this breed were A=0.536, B=0.464 and the values of alleles frequency of East java sheep were A=589,B=0.411.

5.1.2 Genotype Frequency

The genotypes frequency in table (4-2) in Nuimi breed AA, Aa and aa were 0.39, 0.56 and 0.05 separately, and the gene frequency in Awassi breed AA, Aa and aa were 0.444,0.481,0.074 these are results concurrence with ***Cauveri et al., (2016)*** followed up on Nilagiri sheep and the gene frequency of this breed were GG 0.48, GA 0.43 and AA 0.09 and ***Gorlov (2017)*** follow up on Salsk sheep in India and detailed (AA=57, AB=36 and BB=7) % .

5.1.3Birth weight and Weaning weight Vs Genotypes of growth hormone gene

The outcome in table (4-3) revealed the impact of genotypes on birth and weaning weights in Nuimi sheep, our outcomes recorded AA genotype has higher values in birth and weaning weights (6.35 and 21.5)kg separately, while aa genotype has 5.05 kg in birth weight and 20.09 kg in weaning weight. Nonetheless, Aa genotype has 4.50kgin birth weight and 19.09kg in weaning weight. Our consequences of the impact of genotypes on Nuimi breeds were concurrence with ***Malawi et al.,***

(2014) who acted on Donggala and East Java sheep in Jambi province and revealed that AA genotype had significantly higher in weaning weight than BB genotype both in Donggala 11.6 kg vs 9.68 kg and East Java 10.83 kg vs 9.37 kg sheep and AB Genotype did not show significant differences in weaning weight compared to AA genotypes both in Donggala and East Java sheep. ***Depison et al., (2017)*** dealt with Thin – tailed sheep in Jambi revealed that genotype +/+ have higher body weight. The outcomes in table (4-4) revealed the impact of genotypes on birth and weaning weights in Awaasi breeds, our outcomes indicated aa genotype has the higher value in birth weight 6.82 kg and 23.5 kg in weaning weight while, AA genotype has 6.25 kg in birth weight and 21.71kgin weaning weight. Anyway, the Aa genotype has lower values in birth weight 4.94kg and in weaning weight 19.65kg.These outcomes were concurrence with ***Al-Salihi et al., (2017)*** work on Awassi sheep in Iraq and his study yielded 3 genotypes AA, AG and GG, GG genotype has a higher values in birth weight 4.53kg and weaning weight 21.26 kg. While AA genotype has 3.92 kg in birth weight and 20.32 kg in weaning, However, AG genotype has lower values in birth weight 3.59 kg and weaning weight 18.26kg. Our outcomes additionally concurrence with ***Abdelmoneim et al., (2016)*** dealt with a Harri sheep and recognized of three genotypes GG, GA and AA and recorded the AA genotype has a higher value in birth weight 2.2 kg while GG genotype has 1.5 kg and GA genotype has 1.8 kg. The outcomes likewise concurrence with ***Cauveri (2016)*** work on Nilagiri sheep revealed three genotypes GG, AG and AA and Shown AA genotype had significantly $p<0.01$ in weaning weight and higher weaning weight 13.49kg.

5.2Effect of Gender on body weight and body measurements at two stages of life:(birth and weaning)

The results in table (4-5) were recorded impact of gender on weights and body measurements in Nuimi breed at two stages, birth and weaning. Our result mentioned the male has better values in weights and body measurements at delivery and weaning stages of life .

The results in table (4-6) recorded effect of gender on weights and body measurements in Awassi breed at two stages of life: delivery and weaning stage. The outcomes on this experiment detected the male has higher values in weights and body measurements at the delivery and weaning stages.

The prevailing results take a look at in both Nuimi and Awassi breeds findings in agreement with ***Nirban et al.,(2015)*** in Marwari sheep birth weight male=4.22 kg and female=4.02kg and weaning weight male =21.6kg and female=21.04kg and agreement with ***Rahimi et al., (2014)***which is delivery weight male=2.61kg and female=2.49kg and weaning weight of male=14.55kg and female=12.74kg. These results also agreement with ***Gbangboche et al., (2005)*** in birth weight male=1.95kg, female=1.91kg whilst in weaning weight male=14.01, female=13.14kg. The difference between the gender is attributed specifically because of the sexual dimorphism and anabolic effect of androgen which prompted a higher growth in males ***(Siddalingamurthy et al., 2017)*** .Our results of body measurements at birth in both breeds (Nuimi and Awassi) had been agreed with ***China Supakorn (2013)*** had been observed out the male had higher values of heart girth in birth than a female Heart Girth: male=32.92cm and female=32.63cm. Our results additionally agreement with ***Al-Azzawi, (2011)*** become stated that male

have better values in all body measurements at the birth (heart girth=29.68, body length= 31.55, height=29.32)cm while female has (heart girth=27.7, body length 30.21, body height 27.78)cm. The body measurements at weaning of Awassi and Nuimi breeds it finings in agreement with ***Afolayan (2006)*** was worked on Yankasa sheep in Nigeria and pronounced that higher values of weaning length in male is 39.67cm than female is 36.87cm and height in male is 62.80cm than female is 62.65cm however, these study in Yankasa sheep not agreement with heart girth in male 70.50cm while in female is 71.22cm.Our results also of body measurements at weaning have been settlement with ***Norozian, (2015)*** detected the male had better values in heart girth=66.61cm, length=50.16cm and height=50.48cm at the same time in female have heart girth=64.84cm, length=48.48cm and height=51.42cm. The consequences of body measurements at weaning also settlement with ***Al-Khazragi et al., (2016)*** in male, heart girth=55.24cm, height=51.06cm and length=53.44cm at the same time in the female had heart girth=53.82cm, height=50.56cm and length=52.59cm. Body measurements at weaning stage for Nuimi and Awassi breeds its agreement with ***Al-Azzawi, (2011)*** work on local goats and imported Damascus goats and found out the male had better values in body measurements at weaning heart girth=55.36cm, length=57.28cm and height=54.71cm whereas the female had heart girth=52.43cm, length=56.07cm and height=51.35cm.

5.3 Physiological traits:

Our findings on parathyroid levels in pregnant ewes of Nuimi and Awassi breeds which are presented in table (4-7) demonstrated that the concentration levels of this hormone were 85.53 pg/ml and 76.09 pg/ml, individually. These levels were higher than the ordinary typical levels

27.11pg/ml in Nuimi and 28.37 pg/ml in Awassi breed. The higher concentration level of the parathyroid hormone in the current study can be attributed to the maternal serum parathyroid level which is elevated in pregnancy, as explanation for keeping up maternal normocalcemia disregarding hypercalciuria of pregnancy and the important calcium exchange from mother to the fetus ***(Jorge, 1998)***. Our outcomes in concurrence with an results led by ***El-Tarabany (2012)*** who watched the change in levels of parathyroid hormone in early, mid and late times of pregnancy (14.05, 12.54 and 9.44) pg/ml separately. Our results are additionally steady with those readings recorded by ***Elias (1990)*** who discovered increment of parathyroid hormone in late month of pregnancy in Awassi ewes 142.6 pmol/l than non-pregnant 99.7 poml/l. In any case, ***EL.Hag (2012)*** announced that seasonal changes of level of parathyroid hormone in cow before parturition 2.90 ng/ml in November, 1.35ng/ml in May and 2.27 ng/ml in October. Our outcomes on calcitonin level in pregnant ewes of Nuimi and Awassi breeds which have been outlined in table (4-7) demonstrated that concentration levels of calcitonin in these breeds were 75.29 pg/ml and 91.48pg/ml, separately. These outcomes considered higher than the estimation of non-pregnant ewes in Nuimi breed 12.9 pg/ml and 12.93 pg/ml in Awassi breed. ***Kataria and Katariai (2006)*** who dealt with Marwari sheep and announced that the serum of calcitonin level was higher in pregnant than non-pregnant. The reason for a level of calcitonin in pregnant ewes of our experiment can be because of that the maternal calcitonin secures the skeleton of the pregnant female against inordinate demineralization, halfway by adjusting placental transport of calcium during times of sever mineralization of fetal skeleton (***Barlet, 1985)***. Concerning Vitamin D, the outcomes which have been exhibited in table (4-7) demonstrated that the concentration levels of this

hormone of pregnant ewes in the two breeds Nuimi and Awassi were 3.81ng/ml, 3.69 ng/ml separately, while the non-pregnant level of vitamin D in Nuimi breed is 0.79 ng/ml and in Awassi sheep is 0.93ng/ml. Our outcomes are steady to those revealed by ***Lockwood et al., (2016)*** who recorded that vitamin D concentration level in pregnant ewes was 29.04 ng/ml while the control ewes were 26.5 ng/ml. The argument behind the increase in the concentration level of Vitamin D may be because of that the maternal demands for vitamin D are expanded during pregnancy ***(Lucas et al., 2008). Kovacs (2008) and Lapillonne (2010)*** additionally announced that vitamin D is exchanged over the placenta to the baby which clarify the expansion in the concentration level of Vitamin D amid pregnancy .

The findings of our examination on Cholesterol (Ch) which have been appeared in table (4-8) detailed that the two breeds Nuimi and Awassi, showed that the concentration levels of pregnant ewes had 132.08 mg/dl, 127.51mg/dl separately, while the non-pregnant values of concentrations levels of Cholesterol in Nuimi breeds is 49.70mg/dl and 43.10mg/dl in Awassi breed. ***Antunovic (2011)*** noticed that expansion in levels of Cholesterol in pregnancy as non- pregnant ewes had 61.38 mg/dl while the pregnant ewes had 71.42 mg/dl. ***Boudebza (2016)*** found that ewes in late pregnancy had most elevated estimation of Cholesterol concentration than in early lactation and dry period (81, 48 and 56) mg/dl separately. ***Waziri (2010)*** detailed that Cholesterol levels was changed between non-pregnant goat and late pregnant goat (53.9 and 82.08) mg/dl separately. ***Mazur (2009***) was demonstrated the Cholesterol level of non-pregnant ewes have 49.42 mg/dl while the Cholesterol level of pregnant ewes have 54.44 mg/dl. ***Waziri (2010)*** suggested increment Cholesterol

could be a factor adding to repressing glucose synthesis or could be play role for increasing glucose take-up by the body cells.

With respect to our outcomes appeared in Table (4-8) revealed that Triglyceride concentration levels in pregnant Nuimi sheep 65.66mg/dl while, pregnant Awassi66.14 mg/dl . In any case, the non-pregnant values in Nuimi sheep 33.50mg/dl and in Awassi 34.90mg/dl. ***Antunovic (2011)*** revealed the expansion in Triglyceride level in pregnant than in non-pregnant ewes (4.50 and 3.06) mg/dl separately. ***Mazur (2009)*** noticed the increase level of Triglyceride in pregnant ewes 15.92 mg/dl than in non-pregnant ewes 7.20 mg/dl. ***Deghnouche (2013)*** reported the increment in Triglyceride levels among lactating females might be because of insulin, which play direct in fat tissue metabolism during pregnancy and its responsiveness is significantly lessened in ewes during pregnancy.

Concerning High Density Lipoprotein (HDL), results in table (4-8) revealed the HDL of pregnant ewes in Nuimi sheep 52.66 mg/dl and in Awaasi sheep 52.14mg/dl while the non-pregnant values in Nuimi 30.30mg/dl and in Awassi 29.20mg/dl. ***Zvonko et al., (2015)*** observed the concentration levels of HDL in pregnant ewes of Dubrovink breed has17.11mg/dl and 17.75mg/dl in pregnant ewes of Zeta zuja breed. Likewise ***Antunovic (2011)*** the concentration level of HDL of non-pregnant ewes 37.83mg/dl while increment in pregnant ewes 38.61mg/dl. ***Nazifi (2002)*** revealed the concentration level of HDL of pregnant Iranian Fat-followed Sheep in 15 weeks of pregnancy31.66mg/dl while in 20 weeks of pregnancy 42.08mg/dl. The reduced responsiveness of the objective tissue to insulin during late pregnancy inclines the ewes to increase lipoproteins concentration ***(Deghnouche et al., 2013).***The consequences of the present examination on Low Density Lipoprotein

(LDL) illustrated in table (4-8) demonstrate that the focus levels were 65.37 mg/dl in pregnant ewes of Nuimi while 58.23 mg/dl in pregnant ewes of Awaasi breed, however , the non-pregnant values in Nuimi 12.70mg/dl and in Awassi 6.92mg/dl. ***Zvonko et al., (2015)*** watched the levels of LDL of pregnant ewes of Dubrovnik sheep 13.15mg/dl and 14.41mg/dl in pregnant ewes of Zeta zuja sheep. ***Antunovic (2011)*** watched concentrations of LDL in non-pregnant ewes 21.23mg/dl while in pregnant ewes 28.18mg/dl. The reason of highest concentration of LDL-cholesterol in the blood of the ewes during pregnancy comparing with the non-pregnant ewes can be explained with a result of a heavier transport of the lipoproteins or lack of energy in a diet ***(Antunovic et al., 2011).***

At last, our outcomes on physiological characteristics of the studied pregnant ewes demonstrated that The concentration levels of VLDL in pregnant ewes is13.13mg/dl in Nuimi and 16.27mg/dl in Awassi breeds. while the non-pregnant levels in Nuimi is 6.70mg/dl and in Awassi breed is 6.98mg/dl. ***Ercan (2016)*** announced the concentration level of VLDL in pregnant ewes 2.50mg/dl. ***Nazifi(2002)*** represented to the level of VLDL in 15 week of pregnancy 1.93mg/dl while in late pregnancy 3.86 mg/dl. The reduced responsiveness of the target tissue to insulin during late pregnancy inclines the ewes to increase lipoproteins fixations ***(Deghnouche et al., 2013***).

Chapter Six

Conclusions & Recommendations

Chapter six

Conclusions & Recommendations

6.1 Conclusions

1-Two alleles (A and a) and three genotypes (AA, Aa, aa) of growth hormone gene were found in found in both breeds (Nuimi and Awassi).

2-Significant effect of AA genotype in Nuimi lambs was found at weaning weight while, in Awassi lambs with aa genotype have significant differences in birth and weaning weights.

3-Birth weights and weaning weights of male lambs for both breeds Nuimi and Awassi were significantly higher than that of female lambs, similarly, body measurements of Male were significantly higher those of female.

4-Significant differences of (parathyroid, calcitonin and vitamin D) hormones was found between pregnant and non-pregnant in each breed: Nuimi and Awassi .

5-There are significant differences of lipid profile between pregnant and non-pregnant in each studied breed.

6.2 Recommendations

1-Further study is required to find out the different concentrations levels of studied hormones (PTH, CT and Vit. D) in this study and lipid profile during different stages of pregnancy in both breed Nuimi and Awassi .

2-Further study is also required to determine the relationship between growth hormone gene polymorphism and some related physiological and reproductive characters.

3-Possibility of using the genetic technique for selection of sheep for production and to raise Nuimi ewes with AA genotype and Awassi ewes with aa genotype.

4-Recommended to selecting sheep with homozygote genotypes for breeding and production in both breeds was studied .

5-The possibility of weaning the lambs at the age of 15 weeks to both breeds in this study to give us a good weight and this is what depends on the station in which the study was conducted.

6-Preferably to use Awassi mutton for food consumption due to lower concentration of cholesterol in their meat in order to reduce their impact on public health.

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Appendix 1

Number of sampled ewes and their lambs (lambs were identified with similar numbers of their dams)

Number of Ear Tag in Nuimi breed	
Male	female
27*	3*
40*	4*
39*	9*
42*	13***
1*	18***
63*	22***
15*	25***
55*	29***
20*	33***
7*	36***
11*	38***
17**	43***
24**	47***
31***	50***
32***	51***
34***	61***
45***	-
48***	-
52***	-
57***	-

*means AA genotype, **means aa genotype, *** means Aa genotype.

Number of Ear Tag in Awassi breed	
Male	Female
2*	35*
10*	44*
14*	54*
21*	56***
26*	59***
28*	62***
30*	5***
60*	6***
37*	23***
41**	19***
46**	16***
49***	13***
53***	8***
58***	

*means AA genotype, ** means aa genotype, ***means Aa genotype.

Appendix 2

-DNA extraction

according to the instructions attached to it and as follows:

1-blood sample preparation: 200 microliter (ul) of whole blood was transferred to a 1.5 ml eppendrof tube. The volume to 200 ul was adjusted with PBS. 20 micro liter of proteinase K was added then by pipetting was mixed .at 60Cº for 5 minutes was incubated .

2-Cell lysis: 200microliters of CGB buffer was added then by shaking was mix vigorously .this blood sample was incubated at 60c for 5 minutes, inverting the tube every 2 minutes .

during incubation, required volume of elution buffer (200ul/sample) was transfer to a 1.5 ml eppendrof tube and was heated to 60c (for step 5 DNA Elution).

3- DNA binding: 200ul of absolute ethanol was added to the sample lysate and immediately was mixed by shaking vigorously for 10 second. GS column was placed. Centrifuged at 14-16,000 ×g for 3 minute. Following centrifugation, if the mixture did not flow through the GS column membrane , increase the centrifuge time until it passes completely. The 2ml collection tube containing the flow-through was discarded then the GS column was transferred to a new 2ml collection tube.

4- Wash: 400 ul of w1 buffer was added to the GS column. Centrifuged at 14-16,000 × g for 30 seconds then the flow-through was discarded. The GS column was placed back in the 2ml collection tube. 600 ul of wash buffer was added to the GS column back in the 2ml collection tube. Centrifuged again for 3 minutes at 14-16,000 X g dry the column matrix.

5- Elution: The dried GS column was transferred to clean 1.5ml micro-centrifuge tube and 100ul of pre-heated elution buffer was added, TE buffer or water into the center of the column matrix. let stand for at least 3 minutes to allow elution buffer. Centrifuged at 14-16000 X g for 30 seconds to elute purified DNA

Appendix 3

Steps of Procedures of (calcitonin, parathyroid and vitamin D) hormones were as following:

1- Add standard : standard wells were set. Testing sample wells were . 50µl was added to standard well
2- Add sample : blank wells were set separately .Testing sample well. Sample dilution 40µl was added to testing sample well, then testing sample 10µlwas added (sample final dilution is 5-fold), sample was added to wells and gently was mixed.
3- Add enzyme : HRP-conjugate reagent 100µlwas added to each well, except blank well.
4- Incubate: after closing plate with closure plate membrane, for 60 min was incubated . at 37Cº .
5- Configurate liquid : 20 fold wash solution diluted 20-fold with distilled water and reserve .
6- Washing: Closure plate member was uncovered, liquid was discarded, dried by swing, washing buffer was added to every well, for 30s were stilled then was drained, 5 times was repeated, dried by pat .

7- Color : Chromogen solution A 50µl and chromogen solution B were added to each well, the light preservation was evaded for 15min. at 37 C°

8- Stop the reaction : Stop solution 50µl was added to each well, stop the reaction (the blue color change to yellow color).

9- Assay : Blank well was taken as zero, absorbance was read at 450nm after adding stop solution and within 15min. Materials provided with kits of studies hormones in tables(1, 2 and 3) were as in following :

Table (1)Materials provided with kit of Calcitonin hormone

MATERIAL	96 DETERMINATIONS	STORAGE
User manual	**1**	
Closure plate membrane	**2**	
Sealed bags	**1**	
Microelisa stripplate	**1**	**2-8C°**
Standard	**0.3ml×6bottle**	**2-8C°**
HRP-Conjugate reagent	**10ml×1bottle**	**2-8C°**
Sample diluent	**6ml×1bottle**	**2-8C°**
Chromogen Solution A	**6ml×1bottle**	**2-8C°**
Chromogen Solution B	**6ml×1bottle**	**2-8C°**
Stop solution	**6ml×1bottle**	**2-8C°**
20× Wash solution	**25ml×1bottle**	**2-8C°**

-Standard concentrations was followed by :200, 100, 25, 12,5, 0 pg/ml.

Table (2)Materials provided with Parathyroid hormone kit

Material provided with kit	96 Determinations	Storage
User manual	**1**	
Closure plate membrane	**2**	
Sealed bags	**1**	
Microelisa stripplate	**1**	**2-8C°**
Standard	**0.3ml×6bottle**	**2-8C°**
HRP-Conjugate reagent	**10ml×1bottle**	**2-8C°**
Sample diluent	**6ml×1bottle**	**2-8C°**
Chromogen A	**6ml×1bottle**	**2-8C°**
Chromogen B	**6ml×1bottle**	**2-8C°**
Stop solution	**6ml×1bottle**	**2-8C°**
20×Wash solution	**25ml×1bottle**	**2-8C°**

-Standard concentration was followed by: 800,400,200,100,50,0 pg/ml.

Table(3) Materials provided with the kit of vitamin D

Materials provided with kit	96 determinations	Storage
User manual	**1**	
Closure plate membrane	**2**	
Sealed bags	**1**	
Microelisa stripplate	**1**	**2-8°C**
Standard	**0.3ml^{x}6 bottle**	**2-8°C**
HRP-Conjugate reagent	**10^{x}1 bottle**	**2-8°C**
Sample diluent	**6ml^{x} 1 bottle**	**2-8°C**
Chromogen solution A	**6ml^{x} 1 bottle**	**2-8°C**
Chromogen solution B	**6ml^{x} 1 bottle**	**2-8°C**
Stop solution	**6ml^{x} 1 bottle**	**2-8°C**
20^{x} Wash solution	**25ml^{x} 1 bottle**	**2-8°C**

Standard concentration was followed by: 20, 10, 5, 2,5, 1.25, 0ng/mL .

Appendix 4

1.Determination of Total Cholesterol mg/dl:

Steps of the procedure were as the following :

- The reagents, standard and samples were brought to the room temperature as the following (table1)

Table (1) procedure for determination total serumcholesterol

Tubes	Blank	Standard	Sample
Standard	**--**	**10µL**	**--**
Sample	**--**	**--**	**10µL**
Reagent	**1ml**	**1mL**	**1ml**

- The tubes were mixed and incubated for 5 minutes at 37 C°.
- The absorbance (A) of the samples and the standard at 500 nm was read against the blank reagent .
- Calculation was done :-

$$\text{Cholesterol mg/dl} = \frac{A_{sample}}{A_{standard}} \text{ X 200 (standard concentration)}$$

2. Determination of Serum of Triglycerides mg/dl:-

Steps of the procedure were as the following :-

The reagent , standard and samples were brought to the room temperature as following table (2).

Table (2) procedure for determination serum triglycerides

Tubes	Blank	Standard	Sample
Standard	**--**	**10µl**	**--**
Sample	**--**	**--**	**10µL**
Reagent	**1ml**	**1ml**	**1ml**

- the tubes for 5 minutes were mixed at 37C°.
- Absorbance of (A) of samples and the standard at 500nm against the blank reagent were read.
- Calculation was done as following :-

$$\text{Triglycerides mg/dl} = \frac{A_{sample}}{A_{standard}} \text{ X 200 (standard concentration)}$$

3. Determination of High Density lipoprotein(HDL-Cholesterol):-

Steps of the procedures were as the following :-

- Pipette into labeled centrifuge tubes

Table (3) sizes of sample and reagent

Sample	0.2ml
Reagent	**0.5ml**

- Mixed thoroughly and leaved stand for 10 minutes at room temperature.
- We centrifuge at minimum of 4000 rpm for 10 minutes .
- We carefully collect the supernatant .
- Reagent B was brought to room temperature.

- Pipette into labeled test tubes table(4).

Table (4) procedure for determination of serum HDL-Cholesterol

Tubes	Blank	Standard	Sample
Distilled	**50µl**	**--**	**--**
HDL-cholesterol standard	**--**	**50µl**	**--**
Sample supernatant	**--**	**--**	**50µl**
Reagent B	**1ml**	**1ml**	**1ml**

- The tubes were mixed thoroughly and were incubated for 30 minutes at room temperature
- The absorbance A of standard and sample was measured at 500nm against the blank.

Calculation was done as following :-

$$\text{HDL-cholesterol mg /dl} = \frac{A_{sample}}{A_{standard}} \text{ X 52.5 (standard concentration)}$$

الخلاصة

اجريت هذه الدراسة في محطة الكفيل لتربية الاغنام التابعة للعتبة العباسية المقدسة و التي تقع على حوالي 10 كم جنوب شرق محافظة كربلاء على طريق كربلاء – نجف ، حيث اجرينا دراستنا على نوعين من السلالات المعروفة في العراق و المنطقة العربية هما سلالتي النعيمي و العواسي لغرض دراسة التنوع الجيني لهورمون النمو و تأثيره على وزن الجسم بمرحلتين من عمر الحملان هما مرحلتي الولادة و الفطام ، و كذلك دراسة تأثير بعض الصفات الفسلجية في الأغنام حيث تم سحب عينات دم من 63 نعجة حامل من كلا السلالتين المذكورة اعلا ه (36 نعيمي و 27 عواسي) و كذلك تم سحب 20 عينة دم من نعاج غير حوامل (10 نعيمي و 10 عواسي). تم استخدام (تقنية التفاعل المتسلسل البوليميريز- تقييد اطوال القطع متعددة الاشكال PCR-RFLP) على عينات دم النعاج الحوامل لغرض معرفة التنوع الجيني لهومون النمو و علاقته بوزن الميلاد و الفطام للحملان في كلا السلالتين المذكورتين .

تم قياس اوزان الجسم للمواليد من الذكور و الاناث لكلا السلالتين بمرحلتين من العمر في مرحلة الولادة و مرحلة الفطام .و كذلك تم اخذ قياسات الجسم (محيط الصدر ، الطول و الارتفاع) للمواليد من الذكور و الاناث لكلا السلالتين . حيث تم قياس مستوى تركيز هورمون جنب الدرقية ، هورمون الكالسيتونين و فيتامين د في النعاج الحوامل و غير الحوامل لغرض مقارنة مستوى تراكيز الهورمونات المذكورة لجميع العينات المأخوذة . تم قياس مستوى تراكيز (الكولسترول ،الدهون الثلاثية، البروتينات الدهنية العالية الكثافة، البروتينات الدهنية واطئ الكثافة و البروتينات الدهنية واطئ الكثافة جدا) في النعاج الحوامل و الغير الحوامل لغرض مقارنة مستوى الدهون لجميع العينات الماخوذة.

تم الاستدلال على وجود أليلين (A,a) ناتجة من هضم القطعة B.P 422 من جين هورمون النمو بواسطة تقنية ال BCR- RFLP و ذلك باستعمال الهورمون القاطع (Hae lll) .كذلك تم الاستدلال على وجود ثلاث تراكيب وراثية (AA, Aa, aa) في كلا السلالتين .نتائج الدراسة كشفت عن تفوق التركيب الوراثي (AA) في وزن الميلاد و الفطام للمواليد النعيمي حيث كان له تأثير معنوي ($P< 0.05$) في وزن الفطام .

اما في سلالة العواسي فقد تفوق التركيب الوراثي (aa) في وزن الميلاد و الفطام للمواليد حيث كان له تأثير معنوي ($p<0.05$) في وزن الميلاد و الفطام .فقد كان وزن الميلاد للتركيب

الوراثي AA في الحملان النعيمي 6.35 كغم اما وزن الفطام فقد كان 21.50 كغم ، اما التركيب الوراثي Aa فقد احتوى على اقل وزن ميلاد و فطام لنفس السلالة المذكورة ، فقد كان وزن الفطام 4.50 كغم و 19.09 كغم في الفطام .اما التركيب الوراثي aa لنفس السلالة المذكورة في الميلاد 5.05 كغم و 20.09 كغم في الفطام .اما في سلالة العواسي فقد وجد ان وزن الميلاد للحملان ذات التركيب الوراثي AA 6.25 كغم و 21.79 كغم في الفطام ، اما الحملان ذات التركيب الوراثي Aa 4.94 كغم في الميلاد و 19.65 كغم في الفطام .اما في التركيب الوراثي aa فقد كانت اوزان الحملان 6.82 كغم في الميلاد و 23.50 كغم في الفطام .

وجد تأثير معنوي كبير للجنس على اوزان الميلاد و الفطام و كذلك قياسات الجسم في كلا المرحلتين المذكورتين ، حيث لوحظ تأثير ان الذكر اعلى قيمة في وزن الميلاد و الفطام و كذلك قياسات الجسم في المرحلتين و لكلا السلالتين .كان للذكر في سلالة النعيمي تأثير معنوي على وزن الجسم في الولادة 6.22 كغم ($p < 0.02$) و كذلك وجد له تأثير معنوي على محيط الصدر 42.60 سم ($p <0.02$) و كذلك وجد له تأثير معنوي على الطول 35.60 سم ($p <0.02$) و كذلك وجد له تأثير معنوي على الارتفاع 44.50 سم ($p <0.02$) . اما في الفطام فكان مستوى المعنوية للذكر في الوزن 20.11 سم ($p < 0.04$) اما في محيط الصدر فقد كان 62.30 سم ($p < 0.04$) و في الطول 61.30 سم ($p <0.02$) و الارتفاع 61.55 سم ($p <0.02$) .وجد ايضا في سلالة العواسي تفوق الذكور على الاناث في اوزان و قياسات الجسم في كلا المرحلتين من العمر فقد كان للذكور مستوى معنوية ($p <0.04$) في وزن الميلاد 6.33 كغم و ($p <0.05$) في وزن الفطام 22.03 كغم ، اما قياسات الجسم فكان للذكر تأثير معنوي في محيط الصدر 44.71 سم ($p <0.02$) و الطول 37.50 سم ($p <0.05$) و الارتفاع 45.35 سم ($p <0.03$) ، اما قياسات الجسم في الفطام فقد كان للذكر مستوى معنوية في محيط الصدر 64.92 سم ($p <0.04$) و الطول 63.07 سم ($p <0.02$) و الارتفاع 63.85 سم ($p <0.02$) .

نتيجة تلك الدراسة تمخضت عن وجود فروق كبيرة بتراكيز الهورمونات المذكورة آنفاً بين الحوامل و الغير حوامل من النعاج لكلا السلالتين ، حيث تركيز هورمون الجنب الدرقي في النعاج الغير حوامل 27.11pg/ml في النعيمي 28.37pg/ml اما في الحوامل فكانت التراكيز في النعيمي 85.53pg/ml و العواسي 76.09pg/ml. اما الكالسيتونين فكان مستواي التراكيز في النعاج الحوامل 75.29 pg/mlفي النعيمي و 91.48pg/ml في العواسي. اما في غير الحوامل فكانت النتائج في النعاج النعيمي 12.9pg/ml و العواسي 12.93 pg/ml .

تركيز فيتامين د كان في النعاج الحوامل في النعيمي 3.81.ng/ml اما في النعاج الحوامل في سلالة العواسي فكان 3.69ng/ml ، اما في غير الحوامل فكان في النعيمي l0.79ng/ml اما في العواسي فكان 0.93 ng/ml. تم الكشف عن وجود فروق كبيرة بتراكيز (الكوليسترول ، الدهون الثلاثيي، البروتين الدهني عالي الكثافة ، البروتين الدهني واطئ الكثافة و البروتين الدهني واطئ الكثافة جدا) بين الحوامل و الغير حوامل و لكلا السلالتين . فكان تركيز كل من (الكوليسترول 132.08، الدهون الثلاثية 66.55، البروتين الدهني عالي الكثافة 52.66، البروتين الدهني واطئ الكثافة 65.37 و البروتين الدهني واطئ الكثافة جداً 13.13) mg/dl في النعاج الحوامل النعيمية .اما في النعاج الحوامل العواسية فكان (الكوليسترول 127.51، الدهون الثلاثية 66.14، البروتين الدهني عالي الكثافة 52.14، البروتين الدهني واطئ الكثافة 58.23و البروتين الدهني واطئ الكثافة جداً16.27)mg/dl.

جمهورية العراق

وزارة التعليم العالي و البحث العلمي

جامعة كربلاء

كلية الطب البيطري

علاقة التنوع الجيني لهورمون النمو مع صفات الجسم الوزنية و بعض المعايير الفسلجية للأغنام في محافظة كربلاء

رسالة

مقدمة إلى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في علم فسلجة الحيوان

من قبل

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