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Analysis of Selected Milk Traits in Palestine Cattle in Relative to Morphology and Genetic Polymorphism

By

Zyiad Mushref Abu Khaizaran

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The undersigned hereby certify that they have read and recommend to the Faculty of Scientific Research and Higher Studies at the Palestine Polytechnic University and the Faculty of Science at Bethlehem University for acceptance a thesis entitled:

Analysis of Selected Milk Traits in Palestine Cattle in Relative to Morphology and Genetic Polymorphism

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Zyiad Mushref Abu Khaizaran

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ABSTRACT

Genes encoding milk proteins and hormones are superior linkage analysis and Quantitative Trait Loci (QTL). This is because of their biological desired quantitative traits that play key roles in milk production. In this research, two aspects of milk production were analyzed. The first aspect involved genetic analysis of three key genes directly related to milk production: prolactin *(PRL*), bovine kappa-casein *(K-CN)*, and the pituitary-specific transcription factor (*PIT-1)*. The second aspect emphasized on morphology evaluation, i.e. the body scoring conditions of the dairy cows in addition to other non-genetic factors that affect milk production, such as nutrition, farm location, and milk yield.

The Genomic DNA was extracted from 144 healthy cattle (101-Holstein-Friesian, 18-Hybrid cattle, and 25-Local cattle), and used as a template for the amplification for *PRL*, *K-CN*, and *PIT-1* genes based on available GenBank sequences from previous work. To monitor single nucleotide polymorphisms in the selected cattle, the amplified fragments of *PRL* (294-bp), *K-CN* (530-bp) and *PIT-1* (451-bp) were digested with(*RsaI*, *HindIII*, and *HinfI*), respectively.

Polymorphism of the prolactin gene in Palestinian cattle was analyzed in this study as a candidate gene responsible for variation and genetic trends in milk yield and composition trait. It was found that the allelic substitution (AG, GG) play role in milk production with the AG allele of *PRL* being more favorable for milk production compared to the GG allele which was less favorable for milk production. Genetic variants of the bovine *K-CN* gene play role in milk production with the AA allele possessing more positive effect than the BB and AB alleles which were less favorable for milk production. Similarly, the allelic substitution of the *PIT-1* gene affected milk production with the AA allele exercising more positive effect followed by the AB and

BB alleles which were less favorable for milk production, respectively. Analysis of body scoring conditions revealed its significant involvement in milk production, whereas milk yield appeared to be more significant in the second and third milking year. Nutrition and dairy farm location have also been found to affect milk production in the tested Friesian breeds. Among the three studied breeds (Frisian, hybrid, and local), results show that the Frisian breed possesses the higher overall milk production in Palestine compared to the other two breeds.

ملخص الدراسة

علاقة الجينات المسؤولة عن إنتاجيّة الحليب كما ,ونوعا بالشكل الخارجي في الأبقار في فلسطيني

يشغل الاهتمام بإنتاجية الأبقار للحليب (كما و نوعا) بال الباحثين و المستثمرين في تربية الأبقار،وخصوصا الهولندية (الفريزيان) ؛حيث إنها تشكل النسبة الأكبر عالمياً (وفي فلسطين على وجه الخصوص).

وفي فلسطين توجد مزارع نموذجية لها خبرة كبيرة في مجال تربية الأبقار(من ناحية المكننة، والإدارة) خاصة الحلوب منها (مما يجعلها تضاهي الكثير من الدول المجاورة) وتلبي هذه المزارع جزءاً لا بأس به من احتياجات السوق الفلسطيني من منتجات الحليب و مشتقاته. علما أنّ الإنتاج الفلسطيني من الألبان لا يلبي حاجات السوق المحلي الآخذة في الازدياد، ولوجود هذا النقص في إنتاجية الأبقار الفلسطينية (إنتاجية الحليب) بما فيها الأبقار الهولندية أجري الكثير من الدراسات و الأبحاث لتحسين إنتاجية الحليب ، وخاصة في مجال علم الجينات.

نظرا للتقدم في علم الجينات وتحديد المورّثات المسؤولة عن إنتاجية الحليب (كماً ونوعاً) وللعلاقة الوطيدة بين الإنتاجية والشكل الخارجي والجينات المُحسَّنة المسؤولة عن إنتاج الحليب. فقد هدف هذا البحث إلى معرفة طبيعة هذه العلاقة من الناحية الإحصائية ومدى صدق الأدوات المستخدمة وفعاليتها في القياس لتحديد المورّثات بأرقام إحصائية.

لقد تم جميع العينات من المزارع الفلسطينية على ثلاث مراحل :

الأولى - تسجيل المعلومات المتعلقة بإنتاجية الحليب سنويا (٣٠٥ يوماً) وذلك من خلال قاعدة البيانات الموجودة من الحالبات الإلكترونية وكذلك موسم الحليب (عدد سنوات الحليب) وكمية التغذية ونوعيتها في اليوم الواحد.

الثانية - تحديد الشكل الخارجي للأبقار من خلال إعطاء قيمة عددية لكل جزء من الهيكل العظمي. مثل الردف، منطقة الحوض، الخطاف، الخاصرة، الضلوع .

الثالثة - تحديد ثلاثة جينات ذات علاقة بإنتاجية الحليب واختيارها، وهى:١. جين الحليب (*PRL*) . ٢. جين الكازيين (*K-CN*) . ٣. و كذلك جين عامل النسخ الخاص بالغدة النخامية (*PIT-1*).

تم استخراج الحمض النووي (DNA) من (١٤٤) بقرة خالية من الأمراض تضم (١٠١) من الماشية الفريزيان، و (١٨) من الماشية المهجنة و (٢٥) من الماشية المحلية.

وباستخدام التحليل الإحصائي- تحليل التباين (ANOVA) تبيّن أن جين الحليب (PRL) يؤثّر بقيمة إحصائية في إنتاجية الحليب، وأن المورّثات التي تحمل الصبغة الوراثية (AG) أفضل بكثير من الناحية الإنتاجية من نظيرتها التي تحمل المورّثات (GG)، كما أن جين الكازيين (K-CN) يلعب دوراً إحصائياً في الإنتاجية والجودة على حدّ سواء، حيث كانت المورّثات التي تحمل (AB) أفضل من المورّثات التي تحمل (BB) من الناحية الإنتاجية، في حين أنّ المورّثات التي تحمل (AA) أفضل بكثير من المورّثات السابقة (BBوAB). بالإضافة إلى ظهور قيمة إحصائية عالية ذات اثر كبير، ومهم في الإنتاجية لجين عامل النسخ الخاص بالغدة النخامية (*PIT-1*) ؛ حيث كانت المورّثات التي تحمل(AB) أفضل من المورّثات التي تحمل (BB) من الناحية الإنتاجية في حين أنّ المورّثات التي تحمل(AA) أفضل بكثير من المورّثات السابقة (BBوAB). وكانت القيمة الإحصائية في معدلات إنتاج الحليب للموسم الثاني والثالث أفضل من المواسم الأخرى، (تتراجع الإنتاجية ،والشكل الخارجي مع مرور الزمن)، مع الأخذ بعين الاعتبار أنّ كميات التغذية ونوعيتها تلعب دوراً كبيراً من الناحية الإحصائية الإنتاجية.

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LIST OF ABBREVIATION

Name	Abbreviation
ANOVA	Analysis Of Variance
BCS	Body Condition Scoring
bp	base pair
dH2O	Distilled water
DNA	Deoxyribonucleic acid
DnaSP	DNA Sequence Polymorphism
Dntp	Deoxyribo nucleotide Triphosphate
EDTA	Ethylene diamine tetra acetic acid
EtOH	Ethanol
EtBr	Ethidium bromide
J	Jenein
j	Jericho
H	Hebron
h	Hebron
K3EDTA	Potassium EDTA
KHCO3	Potassium hydrogen carbonate or Potassium bicarbonate
MgCl2	Magnesium Chloride
mg	Miligram
ml	Mililiter

M	Molar
mM	Milimolar
MVSP	Multi Variate Statistical Package
NaAc	Sodium Acetate
N	Nablus
ng	Nanogram
NCBI	National Center for Biotechnology Information
NH4CL	Ammonium chloride
PCR	Polymerase Chain Reaction
PRL:	Prolactin gene
pp:	Page
pmol:	Picomoles
Rpm:	Rotations per minute
RFLP	Restriction fragment length polymorphism
SDS:	Sodium dodecyl sulfate Tymine
PIT-I	The pituitary-specific transcription factor gene
TAE Buffer:	Tris Acetate EDTA Buffer
T	Tubas
Tt	Tumoun

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	studied in 144 dairy cows. The observed and expected heterozygosities are also included.	

CHAPTER 1.0

1.0 INTRODUCTION

1.1 Cattle domestication:

Cattle belong to the Bovidae family, which appeared in the Miocene approximately 20 million years ago with over 800 cattle types recognized worldwide. Cattle are considered the most significant domesticated economic animal (Loftus et al. 1994). Since their domestication, Cattle, through the use of their meat and milk, have contributed significantly into the human daily nutrition. Several archaeological records for the domestication of wild forms of cattle (*Bos primigenius*) indicated that the process of cattle domestication occurred independently likely at 2-3 areas in the old world (Bradley et al. 1998; Götherström et al. 2005). Two major types were derived from the *Bos primigenius*, taurine (humpless, *Bos taurus*) and the zebu (humped, *Bos indicus*), which were named as separate species. However, due to complete interfertility between these species, they are more often considered as subspecies (Loftus et al. 1994).

Both taurine and zebu were probably domesticated and kept around for easy access to food, including milk, and meat, and for their use as load-bearers and plows. Remains of taurine cattle were found somewhere in the Fertile Crescent and aged about 8,000 years ago and in archaeological sites of northeastern Asia (China, Mongolia and Korea) aged about 5000 years (David et al. 1997).

The domesticated *Bos indicus*, which is also called zebu, were adapted to hot climates and were the first to be introduced into Africa some 4000 years ago. *Bos taurus* started to become widespread about 700 AD with the Arabic migrations into North and East Africa. The *Bos taurus*, which is the typical cattle of Europe, north-eastern

Asia, and parts of Africa (David et al. 1997), adapted to cooler climates (Bradley et al. 1998). Evidence for the domestication of humped cattle, *B.indicus* has been discovered at the site of Mehrgahr in the Indus Valley of Pakistan about 7000 years ago (Bradley et al. 1998).

A third possible domestication event had likely happened in Africa, specifically found at Capeletti, Algeria, about 6500 BP. The *Bos* remains were also found in Egypt at Al Nabta Playa and Bir Kiseiba as long ago as 9000 years, and they might be domesticated. If these remains were indeed domesticated, then they represent the first event of domesticating cattle (Loftus et al. 1994).

The individuals of the species of pre-domesticated ones werewith skeletal structure bigger in size than the domestic ones. This means, domestication must have been harboring the highest level of genetic diversity (Nichols et al. 2001). It can be anticipated that the diversity in cattle breeds was reduced due to migration in the direction away from the domestication center by the founders (Nichols et al. 2001).

Domesticated cattle are the major component of pastoral economics in the world. They are in the central of human culture and represent one of the most important domesticated animals (Loftus et al. 1994). Cattle provide the bulk of the animal protein in the nutrition of many world societies. In addition to milk, cattle contribute other important commodities including meat, hides, traction, and dung. The evolution and domestication of cattle have been always contentious research particularly in determining the relationship between the two main types of cattle the humped zebu and humpless taurine. Due to the importance of cattle, the morphological and genetic differences observed between the two subspecies are still an active area of research and speculations (David et al. 1997).

1.2. Cattle in modern agriculture

The Holstein-Friesian breed comprises the largest dairy qualities cattle since over 2000 years (Holstein Association USA). This breed originated in Northern Holland and Friesland, from which it received its nickname. It represented the regional cattle of the two tribes Batavii and Frisii, who settled in the coastal Rhine region around 2000 years ago. The Friesian breed is characterized of the animals large size and the sharply defined black and white spotted markings. The black and white spots resulted from crossing of two breeds, the black cows and white cows of the Batavians and Friesians (British Friesian Breeder Club). The Dutch breeders apparently bred and oversaw the development of the breed with the aim of raising animals that could best feed on grass, the area's most abundant resource. Over the centuries, these animals evolved genetically into an efficient and high producing black and white dairy cows known as the Holstein-Friesian (British Friesian Breeder Club). Due to the economic value of this breed, the Holstein-Friesian attracted investors and animal breeders from all over the world starting in the mid 1970s in many countries like United States, Britain, Europe, Asia, parts of Africa and by the Israelis who started a large scale Holstein-Friesian improvement program (Jasiorowski. 1988) in the northern parts of the occupied Holy Land. Breeds from different sources were brought in with the aim of improving locally available cattle. Sometimes, imported Dutch bulls were owned and operated cooperatively (Mason. 1996). The concerted effort for genetic improvement on a broader scale began with the onset of artificial insemination in the mid 1940s (Mason. 1996). The impressive number of Holstein-Friesians imported from North America during the 1950s and the strict observance of nation-wide breeding plans contributed decisively to the establishment of the“Israeli-Holstein Breed" (Mason. 1996).

In the early 1920s Israeli settlers in Palestine started modern dairy cattle improvement of Friesian bulls imported from the Netherlands and Germany to upgrade the indigenous dairy cows of the Damascene and Baladi breeds existed at that time in Palestine. In 1947, ten Holstein bulls were imported from Canada and their sons were heavily used through artificial insemination. From 1950 through 1962, Holstein bulls and cows were imported from the United States and since1963 nearly all Israeli dairy cows have been mated to bulls bred locally (Mason. 1996). The Israeli-Holstein cow was developed as a result of a series of crosses, by first taking a Damascus cow and breeding it with an imported Dutch bull creating an F1 cross (50%). The offspring was then bred with a different imported Dutch bull, creating an R2 cross (75%). The R2 cross, when mated with an Israeli-Dutch bull, created an R2 cross (87.5%) which was bred with other Israeli-Dutch bulls producing later generations of the cross with higher percentages. These crosses were then bred with the Holstein-Friesian bulls which resulted in the typical "Israeli-Holstein cow" (Mason. 1996). Since then, the Israelis are pioneering an advanced agriculture technology targeting the improvement of the Holstein-Friesian cattle breed.

The occupied Palestine is solely dependent in its livestock and diary production and development on the Israeli government. There are several agreements that allow Palestinians to import the Israeli Holstein and Dutch Holstein-Friesian bulls for breeding and insemination from Israeli cattle breeding centers. Palestine cattle are used mainly for milk production and meat and there is an estimation of 33,925 head of cattle in Palestine as of 2010 (Palestinian Central Bureau of Statistics). There are several cattle farms in Palestine, particularly in Hebron area applying international standards for milk production, including a comprehensive database for milk associated traits. Palestine mainly has two major populations of cattle; the Holstein-

Friesian and the indigenous Baladi and a third subpopulation comes from the hybridization of the two types (Holstein-Friesian and Balad). Although Palestine has established the "Palestinian Centre for Livestock Improvement" in 2001, unfortunately, there is no efficient cattle breeding program and data on improvement of cattle breeds are usually limited and fragmented. Most cattle growers prefer to use the Dutch Holstein-Friesian bulls for breeding in addition to artificial insemination.

1.3. Quantitative traits of bovine milk production

Molecular genetic markers are widely used for the characterization of milk production traits in dairy cattle. Markers are also used for the detection of genetically inherited diseases and for the determination of the evolution of the desired breeds and thus can be utilized to improve livestock populations (Kolbehdari et al. 2009).

The quantitative trait locus (QTL) analysis in particular is quite helpful in bridging the gap between genes and the phenotypic traits that result from them. QTL is a statistical method that links two types of information, the phenotypic data (trait measurements) and genotypic data (usually molecular markers) in an attempt to explain the genetic basis of variation in complex traits (Bjorn et al. 2010). This allows researchers to link certain traits with specific regions of chromosome to identify the number, action, interaction, and precise location of these regions (Cecelia. 2008).

As a result of the recent advances achieved in genomics and molecular biology techniques and the completion of bovine genome sequence, whole cattle genomes can be screened for QTL using molecular maps to locate traits that can affect, for example, milk production (Kolbehdari et al. 2009). This screening has proven critical for the identification of important traits that can provide linkages of phonotypic data with the genetic polymorphism of three genes associated with milk production in Cattle. Three

genetic markers; the prolactin (*PRL*) gene, bovine kappa-casein (*K-CN*) gene, and the pituitary-specific transcription factor (*PIT-1*) gene are used to determine the quality of cow milk production and can affect the economic value of the cattle (Cecelia. 2008; Bjorn et al. 2010).

1.3.1 Prolactin (*PRL*) gene

Genetic markers that play key role in milk production are considered excellent candidates for QTL analysis. There are several milk protein/hormone genes identified with specific linkages affecting important traits in milk production in terms of yield and quality (He et al. 2006; Kolbehdari et al. 2009; Mehmannavaz et al. 2009; Javed et al. 2011; Olenski et al. 2012; Othman et al. 2012). Among several hormones that regulate lactation and reproduction, prolactin (PRL) is a pleiotropic polypeptide hormone that is synthesized in and secreted from primarily the lactotrophic cells of the anterior pituitary gland in bovines and in other vertebrates (Freeman et al. 2000). This hormone family includes placental lactogen and growth hormone (Ladani et al. 2003; Freeman et al. 2005). Prolactin is a polypeptide hormone that plays a major role in the growth and development of the mammary gland (mammogenesis), synthesis (lactogenesis), and maintenance of milk secretion (galactopoiesis) (Wang. 1994). Due to its important role in determining milk yield and quality, PRL is considered an excellent trait locus. Quantitative characteristics and single nucleotide polymorphisms (SNPs) occurring within the prolactin gene have been suggested to influence the chemical composition of milk or at least be an effective DNA marker of a sub-region of dairy cattle genome (He et al. 2006; Kolbehdari et al. 2009; Alfonso et al. 2012). This makes prolactin a favorite marker and an instrumental genetic tool in research targeting enhancement and development of dairy cattle with high milk production

qualities (Alfonso et al. 2012). Milk production is considered a complex phenomenon at which several genetic and hormonal factors interact, such as insulin, thyroxin and most importantly, the prolactin gene (Alfonso et al. 2012). The Prolactin gene was identified and mapped to chromosome 23 in bovine and the whole sequence of this chromosome is available at NCBI (AC_000180.1) (Hallerman et al. 1988). The *Bos taurus* prolactin precursor (*PRL*) gene complete sequence is 9388 bp long (GenBank, Accession No: AF426315) composed of five exons and four introns (Brym et al. 2005). Mature prolactin encodes 199 amino acids (Camper et al. 1984; Mehmannavaz et al. 2009). Several studies have screened the genetic polymorphism of bovine prolactin gene and reported more than 20 SNPs within *PRL* structure gene sequence (He et al. 2006; Halabian et al. 2008; Mehmannavaz et al. 2009). Despite that most of the identified SNPs were either silent mutations and/or located within introns, one important SNP involves the whole exon four and part of introns three and four that can be recognized by *Rsa1* endonuclease digestion due to polymorphic transition of G into A at position 8398 (Brym et al. 2005). This SNP has become a popular genetic marker tool commonly used for genetic characterization and identification of possible linkage associations between *PRL* gene and milk performance traits (Chung et al. 1996; Dybus. 2002; Khatami et al. 2005; He et al. 2006; Othman et al. 2011).

1.3.2 kappa-casein (*K-CN*) gene

Casein is a milk protein secreted by mammary gland cells (Aleandri et al. 1990). It constitutes about 78-82% of bovine milk, and is divided into four main groups: αS1casien, αS2 casein, β-casein, and κ-casein (Azevedo et al. 2008; Darshan et al. 2008; Abbas et al. 2011). The beta-lactoglobulin (B-lg) and kappa-casein (K-CN) are considered two of the most important milk proteins due to their crucial role in milk

quality, coagulation process in cheese, butter and the formation, stabilization, and aggregation of the casein micelles (Vătăúescu et al. 2000; Abbas et al. 2011). Nevertheless, K-CN possesses specific quality roles in milk more than B-lg and constitutes approximately 12% of the casein. But K-CN can play an important role in marker assisted selection of milk trait, because it was linked, and inherited as cluster of alleles (Azevedo et al. 2008). The *K-CN* locus has been shown different genome variations strongly associate with differences in milk composition and processing properties that affect dairy products (Riaz et al. 2008).

The genetic variations of *K-CN* gene give it more important roles as a protein milk marker, which can increase the frequency of favorable and decrease the frequency of unfavorable alleles within the population (Galila et al. 2008). The bovine *K-CN* is located on chromosome 6q31 with an overall length of approximately 13 kb. The*K-CN* gene contains 5 exons and four introns with most of the coding sequence of the mature K-CN protein located in the fourth exon (Ferretti et al. 1990). The point mutation in exon four of kappa-casein (*CSN3*) gene results in two allelic variations; A and B (Ferretti et al. 1990). Although nine variants have been described in*K-CN* gene; A, B, C, E, F, G, H, I, and A1 polymorphisms, the frequent alleles are the A and B variants (Prinzenberg et al. 1999). The A and B variants occur in amino acids located relatively close to several glycosylation sites like amino acids in position 136 and 148 aa of primary structure. In this variation, threonine is replaced by isoleucine in position 136 aa, whereas aspartic acid is replaced by alanine in position 148 aa for A and B, respectively (Antonio et al. 2005; Azevedo et al. 2008).

1.3.3 The pituitary-specific transcription factor (*PIT-1*) gene

Pituitary specific transcription factor (*PIT-1*) gene has been identified as a regulator of the expression of the growth hormone (GH) and prolactin (*PRL*) gene in the anterior pituitary (Herr et al. 1988; Rosenfeld et al. 1991). The *PIT-1*gene is known by different names; pituitary-specific positive transcription factor 1, growth hormone factor 1, pituitary growth factor, POU domain class 1 and transcription factor 1 (Tuggle et al. 1996; Maria and Cataldo. 2011).

The PIT-1 (official nomenclature–POU1F1) is a member of the POU-family and it is named so because the first 3 members identified were PIT-1 and OCT-1 (MIM 164175) in mammals and Unc-86 of *Caenorhabditis elegans* (Herr et al. 1988), which were transcription factors that regulate mammalian development (Herr et al. 1988; Ligang et al. 2012).

The development and function of mammary gland is mainly controlled by the growth hormone and prolactin, which are secreted in the anterior pituitary gland and whose synthesis, and regulated influence of pituitary factor 1 (PIT-1 or POUIF1 (Carsai et al. 2012). Its expression is needed for normal differentiation, development, and survival of the three adenhypohysis cell types (thyrotrophs, somatotrophs and lactotrophs) (Pan et al. 2008). Hence, *POU1F1* mutations may result in different expressions of GH, PRL, TSH, and *POU1FI* gene itself (Pan et al. 2008; Carsai et al. 2012). In mammals, *POU1F1* mutations have been found to be associated with mice Snell dwarf and Jackson dwarf mutants and also result in human dwarfism (Li et al. 2006; Pfaffle et al. 1992). The *POU1F1* gene was studied in many domestic animals including cattle and is located on chromosome bands 1q21-q22 (Woollard et al. 1994), and in porcine was marked to 13q46. The genetic variations of *POU1F1* gene in cattle and porcine are considered associated with important economic traits including

production performance (Yu et al. 1995; Renaville et al. 1997; Stančeková et al. 1999; Sun et al. 2002; Zhao et al. 2004). This is supported by the QTL analysis which revealed that the region surrounding *POU1F1* on chromosome 1q21-q22 affects cattle production (Woollard et al. 1994), a direct indication of *POU1F1* gene potential consideration in growth trait analysis (Pan et al. 2008; Maria and Cataldo. 2011).

The *PIT-1* cDNA was sequenced and made available to the public in 1988 (Woollard et al. 1994). Studies on *PIT-1* cDNA sequence sub-localized the gene to the centromeric region of the bovine chromosome1 located midway between TGLA57 and RM95 (AC_000158.1, 35008949..35024718, complement) (Dybus. 2002). The *PIT-1* protein is approximately 33 kilodalton with two functional domains; the POU-specific and POU-hemeo. Both are needed for high DNA binding affinity to *GH* and *PRL* gene promoters (Herr et al. 1988; Rosenfeld et al. 1991). The *PIT-1* is activated in part by the N-terminal trans-activation domain which is rich in hydroxylated amino acid residues (Dybus el al. 2004). It was reported that the inhibition of PIT-I synthesis has resulted in decreased GH and PRL expression and the proliferation of somatotropic and lactropic cell lines (Dybus et al. 2004; Ligang et al. 2012). Similar to *PRL* and *K-CN*, several polymorphisms were identified in the cattle *PIT-1* locus (Dybus. 2002). The first polymorphism is on exon 6 characterized by a substitution of an adenine with a guanine (A207G) located in the *HinfI* restriction site. This SNP is used to characterize the A and B alleles, respectively (Renaville et al. 1997). The second polymorphism is located on exon 3 (cytosine/adenine (C577A), whereas the third consisted of several polymorphisms identified in *PIT-1* locus: one located in exon 2, two located in intron 3, one in intron 4 and one in intron 5 (Renaville et al. 1997). Several studies have suggested that the *PIT-1* polymorphisms play a key role in milk yield and, to a lesser extent, in determining the fat percentage in dairy cattle

(Dybus et al. 2004; Arash et al. 2005). The A allele of *PIT-1*, however, was found to be superior for milk and protein yield compared to fat percentage in dairy cattle (Renaville et al. 1997; Dybus et al. 2004).

1.4. Cattle body conditions scoring and milk production

Waltner et al (1993) defined the body conditions score (BCS) as a quick, noninvasive, inexpensive, yet somewhat subjective means of estimating fat stores in dairy cows independent of the animal's frame size and body white (BW) (Waltner et al. 1993). BCS has long been recognized by many researchers (Wildman et al. 1982; Butler and Smith, 1989; Domecq et al. 1997) as a valuable tool in predicting the productive and reproductive performance of many domesticated animals. BCS main practical advantage lies in its ability to allow the farmer to monitor and manage the nutritional and health status of high producing cattle during their productive cycle (Berry et al. 2002). The milk production of cattle correlates with their BCS, which is a widely recommended method of evaluating the nutritional management of dairy cattle (Aeberhard et al. 2001).

There are two systems for the evaluation of the body scoring; the first is by giving each bone a mark with a final 100% score. The second system is body condition scoring that provides information on the relative obesity of a cow through the use of nine-point scale system (Appendix 1, and 2 -Body Condition Scoring System (BCS) for Cattle and data information of farmer and production). For example, a cow with a body score of 1 is considered extremely thin and weak while a cow with body score of 9 is considered extremely fat. This scoring system provides information on the relationships between body condition scores and rebreeding efficiency of dairy cattle and is considered a powerful management tool to cattle breeder and growers. Further,

it can allow breeders to evaluate the nutritional program by evaluating cow condition at strategic times of the year to reduce the feed and hay needs to a minimum (Richards et al. 1986).

1.5. Other factors that affect milk production

1.5.1. Nutrition

Cattle feed contains some nutritional elements that are needed in large amounts, while others are needed in small amounts depending on the stage of animal growth and lactation period, etc. In general, feed contains five main types of nutrients where each type of nutrients plays a different role in building the animal's body (Aseltine and Schingoethe. 1998). These nutrients are; carbohydrates, proteins, vitamins, minerals and water, needed for the animal to be healthy and productive (Aseltine and Schingoethe. 1998). Nutrients rich with carbohydrates furnish and provide most of the energy needed for the animal's movement and heat, etc. Proteins are considered the building blocks of the body needed to build muscles, enrich blood, internal organs, skin, and many other parts of the body. Vitamins are needed, however, in smaller amounts for the animal's body. Although vitamins are needed in small amounts, they are still necessary for the normal functioning of the animal's body, such as for general metabolism and for bone and teeth growth and maintenance (Aseltine and Schingoethe. 1998).

High quality silage contributes greatly to supplying the energy needed, starch and forage neutral detergent fiber (the most common measure of fiber used for animal feed analysis) needed for high producing dairy cows, whereas grains and by-product supplements all result in generating good revenues for dairy producers (Luiz and Randy. 2012). Most feeds that are used for ruminant feeding usually

contain all the minerals the animal needs except salt (sodium, chlorine, calcium and phosphorus). Water is the last nutrient, which is not usually thought of as a nutrient, but it is extremely important since water makes up about 3/4 of the animal's weight. It is used to carry other nutrients into and through the body and to carry wastes out of the body and also used in chemical reactions and many other functions of the body (Aseltine and Schingoethe. 1998).

1.5.2. Climate

Milk production is the end result of a series of complex physiological processes incident, and recurrent factors in dairy cattle. Some kinds of genes interact and play a role in determining production. In addition, internal and external environmental factor influence milk production of dairy cattle. Environmental factors consist of elements of feed, management and climate (Lia and Willyan. 2012). Climate directly and indirectly affects livestock production. It has several components, such as temperature, rainfall, and humidity (Piotr and Sabina. 2012). One of other most important challenges in modern barns is microclimate in cattle farms, such as sufficient air temperature, humidity, air flow velocity, low pollution (with dust particles and microorganisms) and low content of gases within the farm. Those factors contribute to the proper development and maintenance of cattle welfare, which influence milk production in a significant way (Piotr and Sabina. 2012). High and low temperatures affect the physiological state of cattle that can cause a decrease in milk production (Piotr and Sabina. 2012). Cattle are, however, able to adapt well to changeable temperature conditions. The temperature scope from -0.5 to +20°C has little effect on milk production. The critical maximum temperature for cattle is assumed to be at the level of 25-26°C (West et al. 2003) or 24-27°C (Broucek et al. 2009). Different values are attributed to the fact that operative temperature for cattle are influenced by a

number of factors, such as pregnancy, milk production, air movement around the animal, relative air humidity, and the degree of acclimatization (Broucek et al. 2009). The interrelation between air temperature and humidity is important from the point of view of animal welfare and cattle production profitability (Piotr and Sabina. 2012). Low temperature accompanied with high humidity can be unfavorable for milk production. When air temperature is low, cattle emit more heat to the environment and at the same time increase heat production and consume more feed in order to compensate body energy losses (Romaniuk et al. 2005). On the other hand, when the cattle are overheated, high humidity may lead to infections in respiratory tract or udder. High temperature and low relative air humidity may dehydrate mucous membranes, thus increasing vulnerability to viruses and bacteria (Romaniuk et al. 2005).

1.6. Analysis of allele frequencies

Clearly, allele frequencies can change over time within a single population and frequently differ between populations (Excoffier et al. 2006). Factors affecting allele frequencies include genetic isolation, migration (gene flow), mutation, natural selection, artificial selection and chance (Excoffier et al. 2006). There are a number of ways in which genes can flow into the gene pool of a breed from other sources. Undetected accidental crosses between breeds have undoubtedly caused some changes in the allele frequencies of many breeds (Excoffier et al. 2006). Population geneticists study frequencies of genotypes and alleles within populations rather than the ratios of phenotypes that Mendelian geneticists use. By comparing these frequencies with those predicted by null models that assume no evolutionary mechanisms occurring within populations, they draw conclusions relative to the evolutionary forces in operation (Excoffier et al. 2006). In a constant environment,

genes will continue to sort similarly for generations upon generations. The observation of this constancy led the two researchers, G. Hardy and W. Weinberg, to express an important relationship in evolution and put forward a law that describes this relationship, which is now bearing their names (Excoffier et al. 2006). The Hardy-Weinberg Equilibrium theory serves as the basic null model for population genetics and has been used to study the genetic populations of many agriculturally important livestock animals, including cattle (Excoffier et al. 2006).

In the present study, we used three genetic markers; Prolactin (PRL), Kappa-casein (K-CN), and Pituitary-specific transcription factor (PIT-1), which are related to milk production. Hence, the genotypes of three genetic markers; PRL (AG, GG), K-CN (AA, AB, BB), and PIT-1 (AA, AB, BB) are economically important in cattle milk production. In Palestine, there are no molecular technology methods currently used to evaluate Palestinian cattle in dairy farms relative to milk production traits. Farmers are solely dependent on either, the information that accompanies the purchased animal/semen from Israeli sellers, for example, and/or the morphology evaluation of the animal (phenotypic data). Cattle farms are considered large investments in Palestine and there is an increasing demand for milk products. To improve the selection procedures for dairy cow performance, this study has been carried out. Both genotypic and phenotypic analysis of a representative sample of Palestine cattle have been studied in relative to milk production. This study will allow us to determine what breed should be selected for better milk production in dairy farms and also introduce this technology in Palestine dairy industries. Dairy farms that participated in this study will be made aware of the results.

CHAPTER 2.0

2.0 PROBLEM STATEMENT AND OBJECTIVES

Dairy products are essential components in food industries and individual nutrition in Palestine. Cattle provide the majority of milk used in dairy industries. There are several standard cattle farms in Palestine, mostly in the Hebron area, and there is an increasing demand on milk in Palestine to meet the growing needs of the society for dairy products. The annual milk yield per cow, however, is lower than that of the Israeli cattle and neighboring countries, suggesting that Palestinians are either using less valuable nutritional feed and/or that milking cows are not genetically favorable for high milk production. The overall objective of this study is to determine the genetic disposition of milking cattle in representative Palestinian farms genetically forward with production for high milk yield and provide data that will help in improving cattle breeding and farming styles in Palestine.

2.1. Specific objectives:

- To study and identify polymorphisms in three cattle breeds used in Palestine in relation the genetic markers (*Prolactin, Kappa-casein* and *Pituitary-specific transcription factor*) that are involved in milk production.
- To provide morphological evaluation of milking production through body scoring systems in relation to milk yield.
- To study the effect of nutrition and temperature on milk production in representative cattle farms by comparing with literature review.
- To introduce the technology of genotypic analysis and enhance the awareness among the dairy industries and cattle growers in Palestine.

CHAPTER 3.0

3.0 MATERIALS AND METHODES

3.1 Blood sample collection

In the present study, three healthy native Palestine cattle breeds were examined; Frisian, Hybrid, and Local baladi. Their distribution is as follows: black-white Friesian cattle selected from the south-north, and the middle of the West Bank; local cattle (baladi) cattle chosen from the south-north of the Jordan valley; and hybrid cattle selected from the south-north of the Jordan valley in addition to other cities in the north of the West Bank. The total blood samples used in this study were 144 taken from native cattle breeds used for genetic analysis. Collection of samples depended on an experimental design that was completely randomized (CRD) for the three groups (Friesian, baladi and hybrid) that were distributed across the West Bank (Figure 3.1). Permission to get blood samples was obtained from individual farm in response to distributed relevant form (Appendix 1). The cattle selected with different age; (3-4) years old for the Friesian cattle, Hybrid and Baladi of birth from (3-10) years old, because most Hybrid and Baladi cattle in Palestine are old and it was difficult to find dairy cattle that were 3-4 years like in Friesian. In total, 101 blood samples from black-white Friesian cattle collected from the cities of Jenin, Tubas, Tumon, Nablus and Hebron, 18 blood samples from hybrid cattle selected from a village and the cities of Jenin and Tubas, and 25 blood samples were collected from local cattle from villages around Jericho and Jenin.

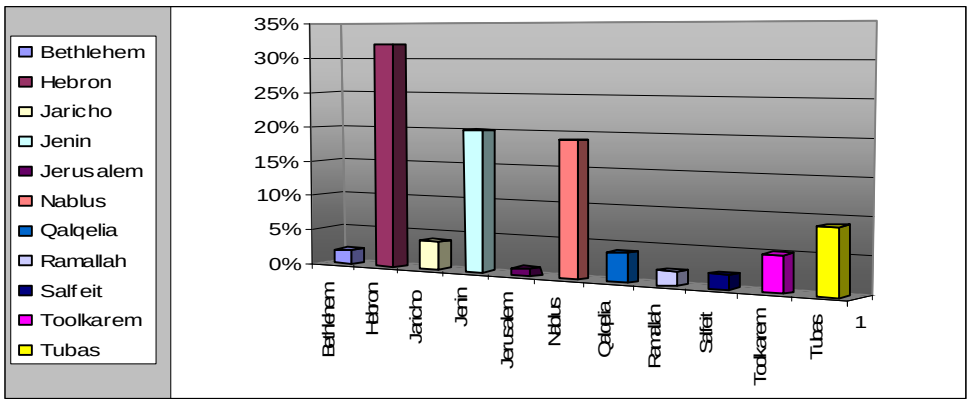

Figure 3.1 The percentage of Frisian cattle distribution in Palestine. As shown, Hebron got the highest percentage (32%) of Friesian cattle followed by Jenin (20%). In total, there are approximately 24,000 Frisian cattle in Palestine as of 2011 (Palestinian Agriculture Statistic 2011).

All samples were collected by myself (Zyiad Abu Khaizaran) and a staff member (veterinarian) from the hosting farm. In a 5 ml EDTA tubes, blood was collected from jugular and tail veins. Samples were stored at +4 °C during transportation and then placed at +4 °C until used for DNA extraction. DNA extracted from the blood samples (i.e., the 144 collections) used for genotypic analysis of the three milk performance enhancer genes, Prolactin (*PRL*), Kappa-casein (*K-CN*), and the Pituitary-specific transcription factor (*PIT-1*) as described in Table 3.1.

Table 3.1 Information on cattle blood samples used in this study. The number of samples used for each study, breed information and the genotypes analyzed are shown. The three genotypes for each locus (*PRL*, *K-CN*, *PIT-1*) were analyzed in lactating dairy cows for the three different breeds.

Breeds	Total samples collected	*PRL*	*K-CN*	*PIT-1*
Friesian	101	101	101	101
Hybrid	18	18	18	18
Local	25	25	25	25

3.2 DNA extraction

DNA isolation from buffy coat, the white layer of white blood cells (WBCs) after centrifugation, which is located in the middle layer between the plasma- supernatant and red blood cells (RBCs) pellet. It was done by using the EZ-DNA Isolation Reagent method from Biological Industries (Cat No 20-60050). Blood samples were collected in 5 ml tubes containing EDTA to prevent coagulation and then centrifuged at 5000 rpm for 10 minutes at 4°C to precipitate white blood cells (buffy coat). After the separation of 300 µl buffy coat in 2 ml Eppendorf tubes, 800 µl 2 X (RBC) lysis buffer were added. The RBC lysis buffer was prepared by adding 7.7 g NH_4CL and 0.1g $KHCO_3$ in 1 Liter of distilled water (DW). Tubes were mixed by inversion and incubated for 10 minutes at 37 °C (water bath) before centrifugation at 1300 rpm for 30 sec for the first wash. The pellet was then resuspended in 800 µl 2 X RBC lysis buffer by vortexing and the pellet was collected by centrifugation at 1300 rpm for 30 sec as a second wash. To lyse the white cells, 1ml EZ-DNA Isolation Reagent was added to the pellet, resuspended before incubation for 5 minutes at RT. Following incubation, 1 ml of absolute (99.9%) ethanol was added and the mixture mixed gently by inversion. The DNA was collected by centrifugation at 1300 rpm for 30 sec and washed twice by 70% ethanol. Following air drying, the precipitated DNA was dissolved in 50 µl TEB buffer and the quality of DNA was verified on agarose gel electrophoresis and the remaining DNA was stored at -20°C.

3. 3 Primer design for the amplification of the *PRL*, *K-CN* and *PIT-1* Genes

Primers used in the present study (Sigma Aldrich) were designed according to available cattle gene sequences, which show high degree of nucleotide sequence

conservation between the cattle. We used the BLAST N program to ensure the specificity of forward and reverse primers for the three genes (*PRL*, *K-CN*, *PIT-1*) studied. All primers showed 100% specificity of forward and reverse primers for the three genes (*PRL*, *K-CN*, *PIT-1*). The information in relative to PCR primers and restriction enzyme analyses used in the present study are available in (Table 3.2).

Table 3.2 Primer sequences and restriction enzymes used in this study.

Gene	Primer sequence	Annealing temperature ºC	Restriction enzyme used	References
PRL	F- CCA AAT CCA CTG AAT TAT GCT T R- ACA GAA ATC ACC TCT CTC ATT CA	58	*RsaI*	Brym et al. 2005
K-CN	F -ATA GCC AAA TAT ATC CCA ATT CAG T R- TTT ATT AAT AAG TCC ATG AAT CTT G	57	*HindIII*	Denicourtet et al. 1990
PIT-1	F -AAA CCA TCA TCT CCC TTC TT R- AAT GTA CAA TGT GCC TTC TGA G	56	*HinfI*	Renaville et al. 1997

3.4 PCR amplification of *PRL*, *K-CN* and *PIT-1* genes

For *PRL* gene, a 294-bp fragment was amplified using specific primers as described above (Table 3.2). For *K-CN*, a 530-bp fragment was amplified and a 451-bp for *PIT-1* using appropriate primers as described (Table 3.2). The PCR reaction contained 1 X buffer, 25 mM $MgCl_2$, 1 µM dNTPs, 1 unit Taq DNA Polymerase, 0.5 µM primers (forward and reverse) and approximately 50 ng of template genomic DNA in a 25 ul reaction. The PCR conditions used for the amplification of the*PRL*, *K-CN*, and *PIT-1* genes were as follows: initial denaturation of 2 min at 94 ºC,

followed by 36 cycles of 30 sec denaturation at 94ºC, 30 sec annealing at 56 ºC, 45 sec extension at 72 ºC and a final extension of 10 min at 72ºC.

The presence of PCR products were analyzed on 1.5% agarose gel electrophoresis. A 5 µl from each PCR reaction were loaded on the gel and a 1500- bp ladder was used to determine the fragment size. The gel was visualized using UV fluorescence and photographed by digital camera. To verify the sequence of amplified fragment for all three genes, the PCR products were amplified, purified and sequenced using the Heredity Lab in Bethlehem University. Sequence identify results are included in (Appendix 3- result of sequences and PCR amplified gel). Result of the three genes; *PRL, K-CN and PIT-1* were blasted against available GenBank sequences and checked by Blast n version 4 program to generate alignments for SNP identification.

3.5 Enzyme digestion and detection of genotypes

The PCR amplified 294-bp of *PRL* product was digested by *Rsa1* restriction enzyme in a digestion reaction consisting of 10 µl PCR product, 2 µl 10 x buffer, 2 µl enzyme in a final volume of 32 µl. For the *K-CN* and *PIT-1* genes, the amplified PCR products of 530-bp and 451-bp were digested by *HindIII* and *HinfI* restriction enzyme, respectively, as described for *PRL* above.

The digestion reactions for the three enzymes were incubation for 16 hours at 37 ºC in a thermocycler to control temperature. Following incubation, the digestion mixture was loaded on 3% agarose gel and visualized under UV as described above. The sizes of the resulted fragments were measured for each digestion.

3.6 Analyses of body conditions scoring and feed

All three native Palestine cattle breeds were examined for two sets of information; genotypic and phenotypic analysis in relative to milk yield. The milk production was obtained from farm databases that were updated by average daily, monthly and yearly milk yield for each individual cow. The Phenotypic data were obtained through morphology evaluation of dairy cows primarily focusing on body scoring conditions, such as rump/pelvic area including tail head, hooks, Thurl, loin, short ribs, spine and visible bone (Figure 3.2). Each bone was given a mark with a final 100% score. A map for each score on the body of the cattle is provided (Figure 3.2). The quantity of nutrition (Kg provided daily per cow) and quality of feed (type of nutrition – concentrate and ray or silage) was also determined from the databases.

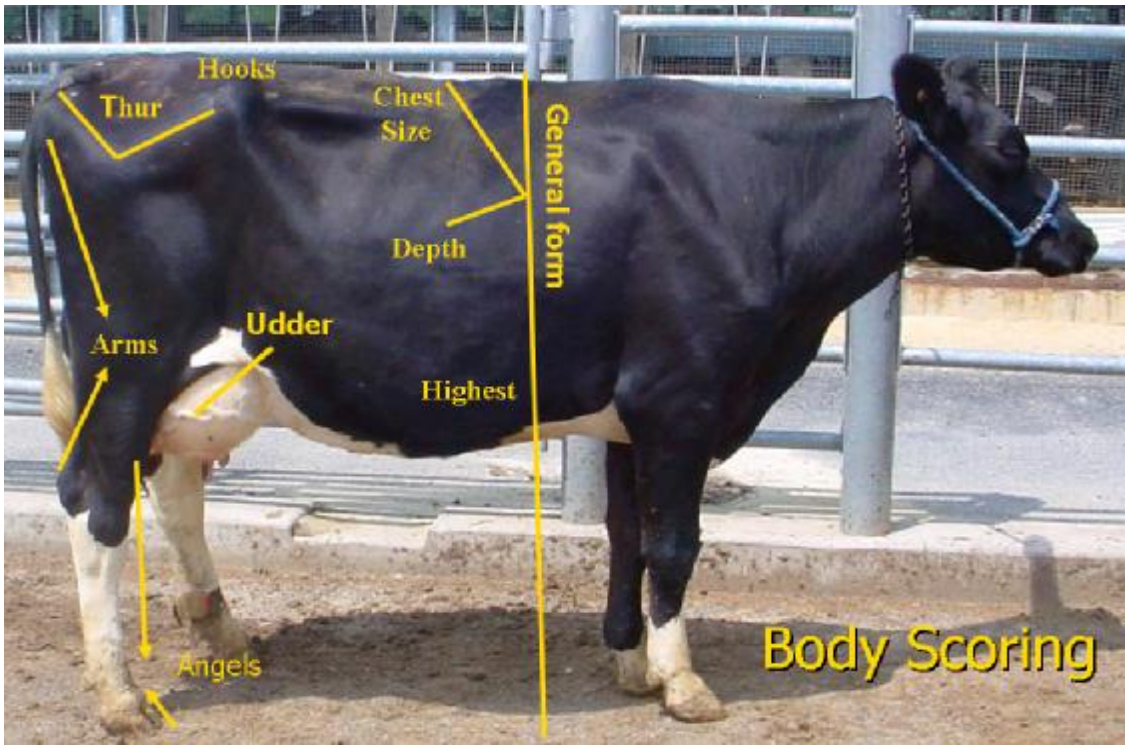

Figure 3.2 Dairy cow morphology and organs involved in the determination of cattle body scoring. This system provides percentages for each character and thus allows the evaluation of phenotype efficiency of the animal. (Photographed in a tubas farm).

3.7 Analysis of allele frequencies

Allele frequency is a measure of the relative frequency of an allele of a genetic locus in population and presents the genetic diversity of a species population. In the present study, there were 2 alleles for *PRL* (G and A), *K-CN* (A and B), and *PIT-1* (A and B). The frequencies of each allele were calculated for the three studied populations; Friesian, Hybrid and the Local cattle.

Allele frequencies were calculated according to the formula given below

$$\text{Frequency of an allele (p)} = \frac{(2 \times \text{number of homozygote}) + (\text{number of heterozygote}}{2 \times \text{total number of individuals (N)}}$$

Individuals exhibiting Heterozygosity, which presents the measurement of genetic variation in a population, was calculated with formula given below;

$$\text{Observed Heterozygosity} = \frac{\text{Number of homozygotes (sample)}}{\text{Number of homozygotes (N) (Population)}}$$

The presence of Hardy-Weinberg equilibrium in the three breeds for *PRL* ،*K-CN* and *PIT-1* genes was checked by calculating the expected genotype frequencies in comparison to the observed ones. All Hardy-Weinberg requirements were assumed to be true when calculating the expected genotype frequencies, which were calculated as follows:

$p^2 + 2pq + q^2 = 1$, and $p + q = 1$

p = frequency of the dominant allele in the population

q=frequency of the recessive allele in the population

p2 =percentage of homozygous dominant individuals

q2 =percentage of homozygous recessive individuals

2pq = percentage of heterozygous individuals

q^2 and p^2 Expected frequency of homozygote =

Expected frequency of heterozygotes (expected heterozygosity) = 2 p q

$$\text{Number of homozygotes} = \frac{(2\text{x number of homozygote}) + (\text{number of heterozygote})}{2 \text{ x total number of individuals (N)}}$$

$$\text{Observed Heterozygosity} = \frac{\text{Number of homozygotes}}{\text{Number of homozygotes (N)}}$$

3.8 Statistical analysis using Multi Variate Statistical Package-MVSP and ANOVA

MVSP is Microsoft Windows of A Multi Variate Statistical Package that performs a variety of functions and cluster analyses. It provides an inexpensive yet easy means of analyzing data in fields ranging from ecology and geology to sociology and market research (Kovach. 1988). In this study, the MVSP version 3.21 analysis was used to analyze data and to generate 2D and 3D graphs for the three populations.

In this study we used one way ANOVA analysis for Friesian breed by comparing the milk production with city, body scoring, nutrition, yield of milk, and*PRL*, *K-CN*, *PIT-1*, and gene frequencies.

CHAPTER 4.0

4.0 RESULTS AND DISCUSSION

There are three cattle breeds, Friesian, local and hybrid that exist in Palestine and used mainly for milk production and meat. Dairy industries purchase milk from cattle farms and to a lesser extent from individual farmers who grow small numbers of cattle in their farms. The south of Palestine, mainly in Hebron area, is considered the center of dairy industries with more than 17 standard farms that employ some type of management including a database for their cattle. Cows are milked 2-3 times per day on most farms; with most farms are milking their cows 3 times per day because there is an estimated 10% increased in milk production that can be obtained by milking the cows 3 times per day.

In Palestine, milk is taken either row or processed into a variety of dairy products; the most common in Palestine are the cheese, yoghurt labanih and Jamid, which is a dried salty processed milk very common in the West Bank. There are different preferences for milk in terms of yield, protein and fat contents, but in Palestine, the milk yield was always the major selection criteria favored by dairy cow owners and breeders. Before this study, however, the selection for high yielding dairy cows is based either on the farmer's observation of individual cow yield and body morphology or on the information received from the sellers on the grown dairy cow. During the course of this study, we have not found farms that would employ or take the genotypic variations of the dairy cows into consideration in relative to milk yield. This is likely because of the lack of awareness among cattle growers to the role and importance of these genetic variations in milk production. Most cattle farms in

Palestine are owned and managed by the same family members who own the farm and these farms rarely employ full-time veterinarians and animal breeders in their farms.

Tracking the genes in cattle which are known to be associated with the properties of the milk can identify the status of the breed in relative to milk property under consideration. Farmers can then manage the breed based on the genetic variations that can be used to select specific criteria in relative to demands in the dairy industries. Since most of the traits in relation to milk properties are not controlled by a single gene, this study has considered three different genes related to milk traits, the*PRL*, *K-CN* and *PIT-1* that are known to be associated with milk yield in dairy cattle (Bradley et al. 1998). Furthermore, while focusing on the selection of one locus in these genes (*PRL- 294bp, K-CN-530 pb, PIT-1*-451*bp*), the possible interrelations between these genes has been analyzed. Variables analyzed in this study were; milk yield, nutrition, location, and body scoring which all appear to play a role in milk production.

4.1. PCR amplification of milk genes:

High quality genomic DNA was used as a template for the amplification of the three milk genes; *PRL, K-CN,* and *PIT-1* using appropriate primers and PCR reaction as shown in Materials and Methods (Table 3.2). The PCR products for the three genes were checked on 1.5% Agarose gel and photographed under UV light. All three amplicons were of expected correct sizes: 294 bp for *PRL*, 530 bp for *K-CN* bp, and 451 for *PIT-1* (Figure 4. 1). The amplicons of the three genes (*PRL, K-CN, PIT-1*) presented in Figure 4.1 are derived from genomic DNA templates isolated from cows 1-10 chosen from Jenin city (J1-J10). Other amplifications using genomic DNA collected from cattle in other areas are included in (Appendix 3). Sequence result showed that 99.9 % of three genes; *PRL, K-CN* and *PIT-1* are correct when comparing

their accession number from NCBI data base. The PCR (Figure 4.1) and sequence results (Appendix 4) clearly indicate that the correct target genes were investigated in this study.

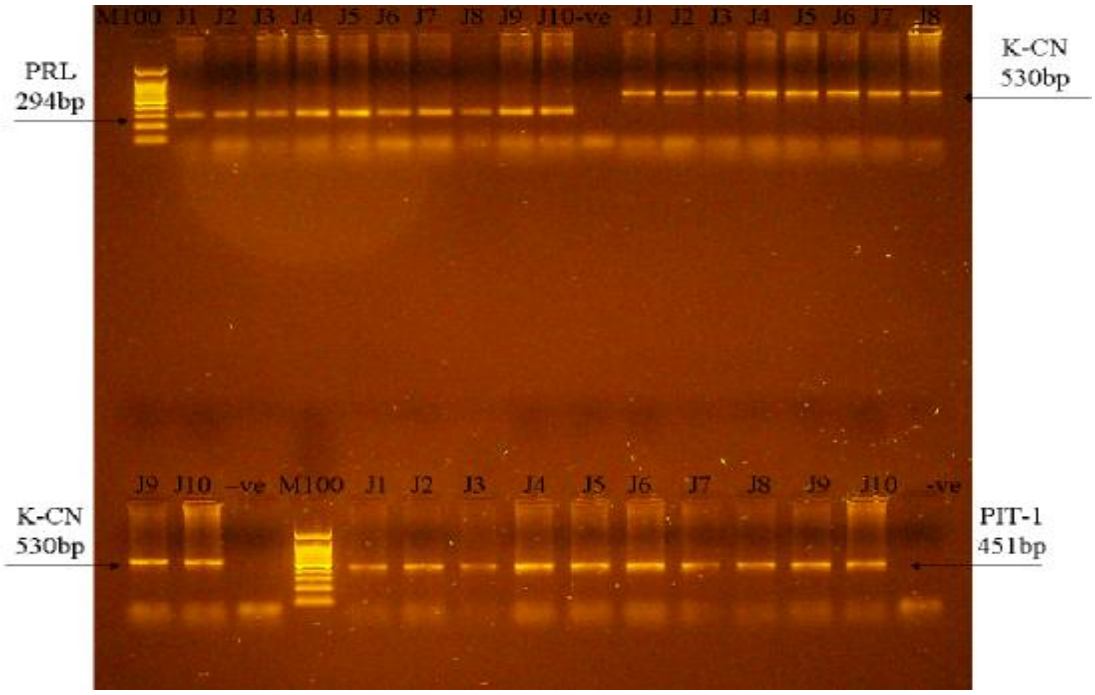

Figure 4.1. A representative PCR amplification of three milk genes investigated in Palestine cattle in this study. The PCR products of the three milk genes (*PRL, K -CN, and PIT-1*) were amplified using appropriate primers and genomic DNA template from blood samples collected from cows from Jenin area (J1-J10). For the results of other collected samples please see (Appendix 3). Bands as seen on a 1.5% agarose gel stained with EtBr are of the expected sizes for *PRL* ٢٩٤- bp (top left ،(*PIT-I* ٤٥١- bp (bottom right), and *K-CN* ٥٣٠- bp (top right and bottom left). A negative control (-ve) lacking genomic DNA template was run for each gene as shown. Lanes labeled with the M100 are the 100 bp DNA ladder.

4.2. Genotypic analysis of three milk genes; *PRL, K-CN* and *PIT-1*:

The quantity and quality of DNA are quite important for a successful RFLP analysis. Following quantification of DNA on a spectrophotometer, the quality of bands as appeared on agarose gels was fundamental in deciding which DNA to be used for further RFLP analysis. As described above, the three genes (*PRL, K-CN*, and *PIT-1*)

were amplified using appropriate primers and samples of the PCR products were loaded on 1.5 % Agarose gel for quality checkup.

Genotyping of PCR products of *PRL*, *K-CN* and *PIT-1* genes were done by using PCR-RFLP method. The genotype for each individual cattle for each studied locus and final results including the body scoring and milk production are provided in (Appendix 4). Below are the results of the genotypic analyses of the three genes from DNA selected from Hebron samples (H1-H31).

4.2.1 *PRL* gene

The *PRL* gene was amplified using PCR amplification procedures as mentioned in the previous chapter (Chapter 3.0). The quality of amplification was determined on agarose gel as described above. After amplification, PCR products were digested with *Rsa1* restriction enzyme at 37 °C in a reaction incubated overnight on a thermocycler for temperature control before the reaction mixture was loaded on a 3% agarose gel. Since the *PRL* amplified region was a small amplicon of only 294 bp, the digested fragments of 162 and132 bp appeared close to each other on the gel (Figure 4.2), but it was possible to see the resulted two alleles, GG and AG (Figure 4.2). Genotypic analysis of the *PRL* gene showed that two alleles were presented for this gene, the homozygous (GG) genotypes appeared as undigested one band of 294 bp and the heterozygous (AG) genotypes appeared as two digested bands of 163 and 132 bp in addition to the undigested 294 bp G allele (Figure 4.2).

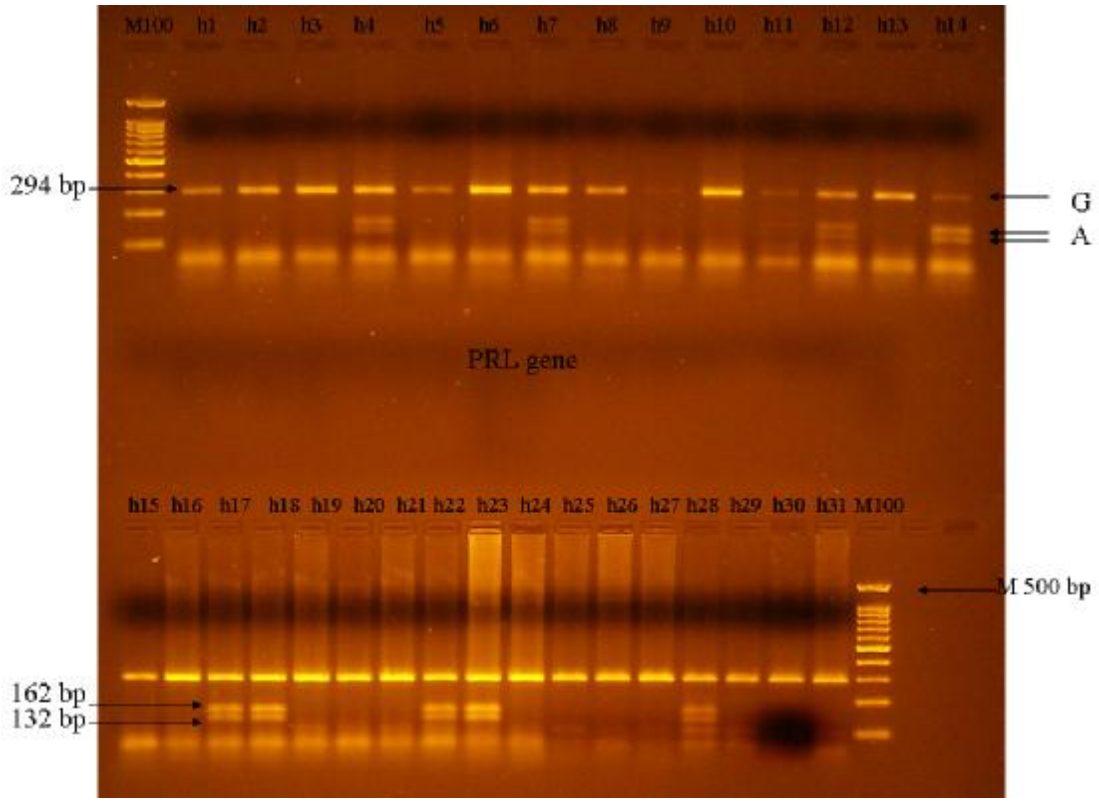

Figure 4.2. The genotypic analysis of *PRL* gene in Palestinian cattle. PCR products amplified from genomic DNA collected from blood samples extracted from Hebron city cattle (h1- h31) and digested with *Rsa1* before loading on a 3% agarose gel as described above. Alleles revealed by the genotypic analysis were the GG and AG. The homozygous (GG) genotypes appeared as undigested one band of 294 bp, whereas the heterozygous (AG) genotypes appeared as digested two bands (163 -132 bp) and one undigested G allele. Lanes labeled (h4,h7,h1-,h14,h17,h18,h22,h23,h28) were all AG heterzygous genotype, while the lanes lablled (h1-h3,h5,h6,h8-h11,h13,h15,h16,h19-h21,h24-h27,h29-h31) were all homozygous GG genotype.

Polymorphism of prolactin gene was analyzed as a candidate gene responsible for variation and genetic trends in milk yield and composition. The SNP of G into A has become a popular genetic marker tool commonly used for genetic characterization and identification of possible linkage associations between PRL gene and milk performance traits (He et al. 2006). ANOVA analysis revealed that the allelic substitution (AG, GG) effect was significant for milk production with p-value = 0.00643 and α (0.001**). The GG allele was unfavorable for milk production with average mean less that 9000 L per 305 day, whereas the AG allele was shown to be more favorable for milk production with average mean of more than 11000 L (Figure 4.3).

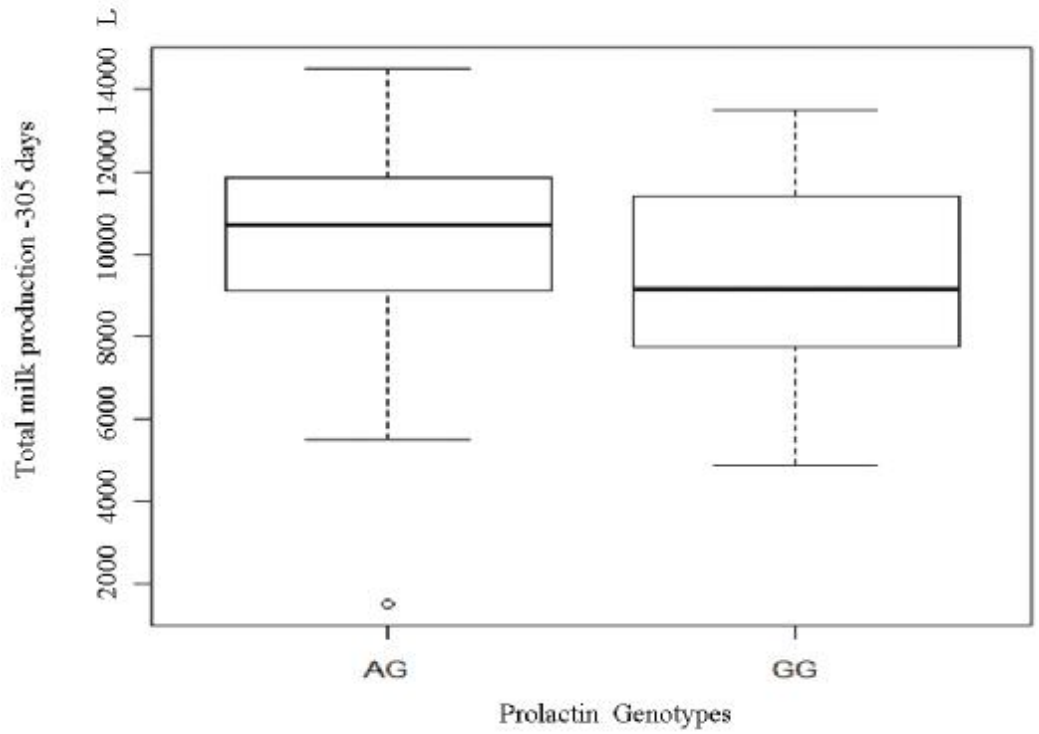

Figure 4.3. Analysis of *PRL* allelic substitution effect on milk production. As shown, the AG allele was more favorable for milk production with an average mean of more than 11000 L compared to the less favorable GG allele with an average mean of less than 9000 L milk produced per 305 days. The *PRL* allelic substitution (AG, GG) effect is significant for milk production with p-value = 0.00643 and α = 0.001 ** as analyzed by ANOVA on 101 blood samples.

Because the *PRL* gene is considered a genetic marker for production traits in dairy cattle (Masoud et al. 2007), the gene has been cloned and characterized in many other animal species (Li et al. 2006) and genetic screening for polymorphisms in bovine prolactin gene identified more than 20 SNPs within the gene sequence (He et al. 2006; Halabian et al. 2008; Mehmannavaz et al. 2009). Most of the identified SNPs were, however, either silent mutations and/or are located within introns. The SNP that was used in this study is the most popular genetic marker tool and is commonly used for genetic characterization and identification of possible linkage associations between the *PRL* gene and milk performance traits (Chung et al. 1996; Dybus. 2002; Khatami

et al. 2005; He et al. 2006). Association of *RsaI/PRL* variants with milk related traits was confirmed in different studies on several cattle breeds such as Jersey cows (e.g., Brym et al. 2005) and Russian Red Pied cows (e.g., Alipanah et al. 2007). The *PRL* allelic variations were analyzed also in Iranian Holstein bulls (Mehmannavaz et al. 2009). The frequencies reported for A and G alleles were 0.069 and 0.931, respectively. The allelic substitution effect was significant for milk and protein yield ($p < 0.05$) where the G allele was unfavorable for milk and protein yield (Mehmannavaz et al. 2009).

The present results show that all 101 tested Holstein-Friesian for allele frequencies of A and G were 0.28 and 0.71, respectively (Table 4. 4), thus different from frequencies reported in Brym et al. (2005) study, who reported (0.11 and 0.88 for A and G, respectively) for black-white cows and 0.70 and 0.29 for A and G, respectively, in Jersey cows. Black and White cows with genotype AG showed the highest milk yield, while cows with genotype GG showed the highest fat content. The high frequencies of G allele were reported in other cattle breed including the Brown Swiss (0.61) and Holstein breed (0.95) (Chrenek et al. 1998). The differences in genotypes are likely due to long-term artificial inseminations and selection towards high milk production and quality.

4.2.2 *K-CN* gene

The *K-CN* gene was amplified using PCR amplification procedures as mentioned in the previous chapter 3.0. The quality of amplification was determined on agarose gel as described above. After amplification, PCR products were digested with *HindIII* restriction enzyme at 37 °C in a reaction incubated overnight on a thermocycler for temperature control before the reaction mixture was loaded on a 3% agarose gel. The

K-CN amplified region showed a 530 bp amplicons on gel (Figure 4.1). Digestion analysis of the *K-CN* gene revealed three genotypes (Figure 4.4): the homozygous AA as undigested band of 530 bp, the heterozygous AB genotype as three bands one of 530 bp and two digested bands of 370 and 160 bp for the B allele. The third was the homozygous BB genotype consisted of two bands, one undigested 530 bp and one digested shorter band of 160 bp (Figure 4.4).

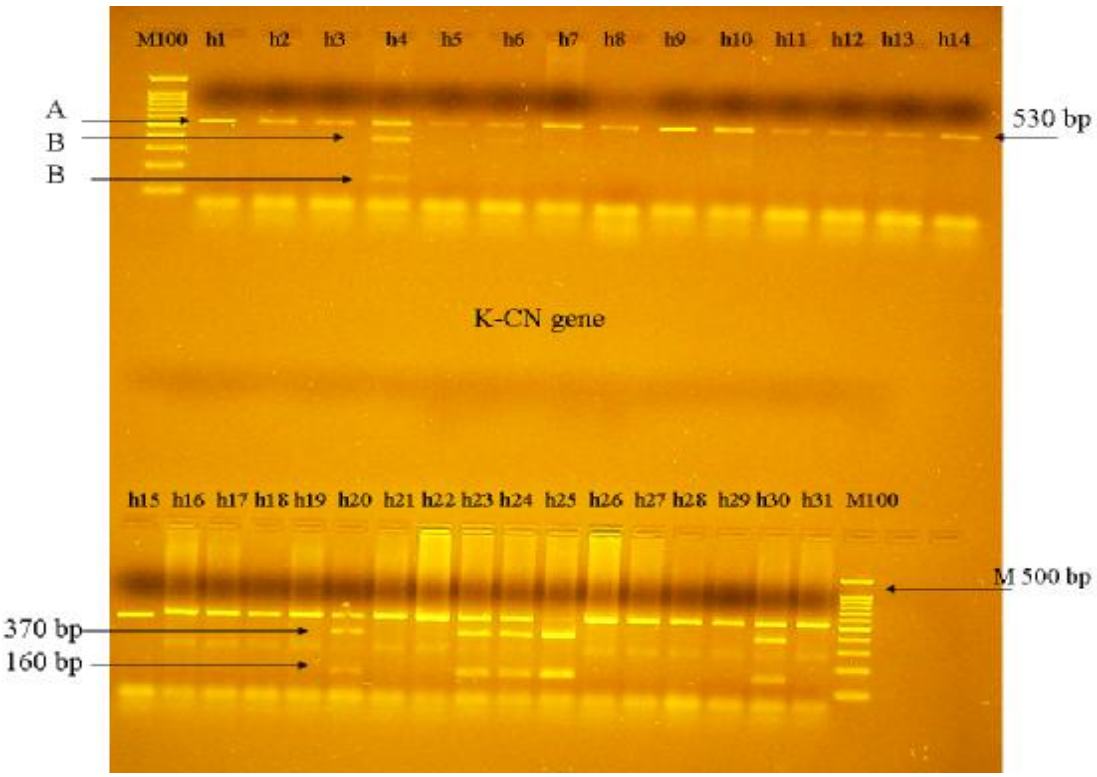

Figure 4.4. The genotypic analysis of *K-CN* gene in Palestinian cattle. PCR products amplified from genomic DNA collected from blood samples extracted from Hebron city cattle (h1- h31) and digested with *HindIII* before loading on a 3% agarose gel as described above. Alleles revealed by the genotypic analysis were the AA, AB and the BB. The homozygous (AA) genotype appeared as undigested one band of 530 bp, whereas the heterozygous (AB) genotype appeared as one undigested 530 bp fragment and two digested bands of 370 and 160 bp. The BB restriction bands were 370 and 160 bp. Lanes labeled (h1-h3,h5,h7-h19,h21,h22, h26-h29,h31) are AA, whereas (h4,h6,h20,h23,h24,h30) are AB genotype. The BB genotype appeared in lane (h25).

Polymorphism of *K-CN* gene was analyzed using ANOVA. *K-CN* genetic variations (e.g., AA, AB, BB alleles) are strongly associated with differences in milk composition, processing properties, and thus affecting dairy products. The SNP of *K-*

CN has become a popular genetic marker tool commonly used for genetic characterization and identification of possible linkage associations between*K-CN* gene and milk performance traits. ANOVA analysis revealed that the allelic substitution (AA, AB, BB) effect was significant for milk production with p-value = 0.04071 and α (0.01 *). The AA and BB alleles were favorable for milk production, whereas the AB allele was less favorable for milk production (Figure 4.5).

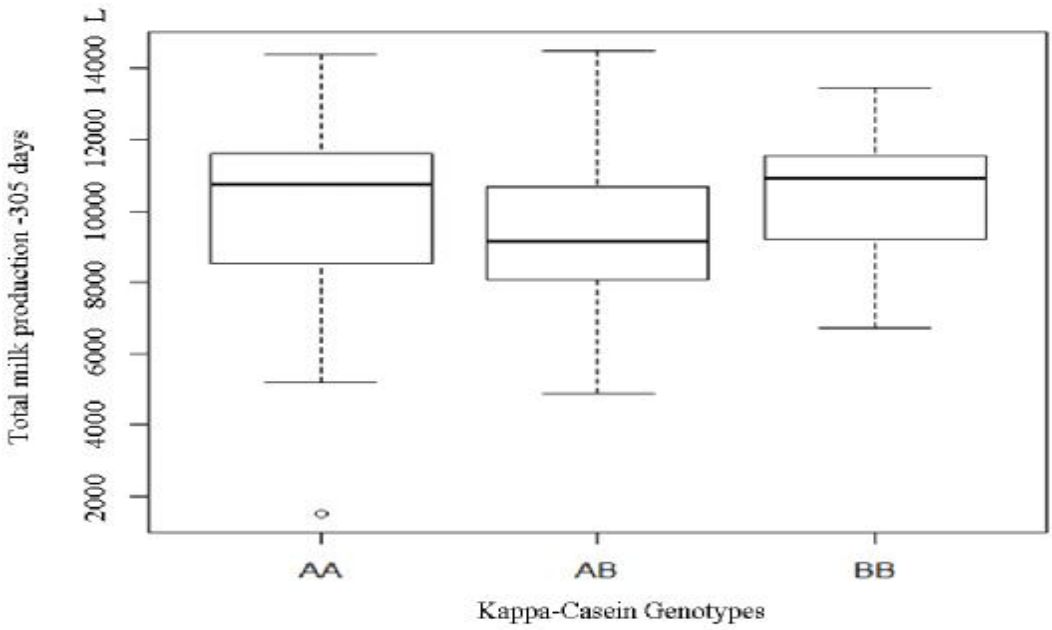

Figure 4.5. Analysis of *K-CN* allelic substitution effect on milk production. As shown, the AA and BB alleles were more favorable for milk production with an average mean of more than 11000 L compared to the less favorable AB allele with an average mean of less than 9000 L milk produced per 305 days. The *K-CN* allelic substitutions (AA, AB, BB) effect is significant on milk production with p-value = 0.04071 and *α* = 0.01 * as analyzed by ANOVA on 101 blood samples.

The -Lg and K-CN are considered two of the most important milk proteins due to their crucial role in milk quality, coagulation process in cheese and butter, and the formation, stabilization, and aggregation of the casein micelles (Văţăúescu et al. 2000; Abbas et al. 2011). Nevertheless, *K-CN* gene possesses specific quality roles in milk more than -Lg and constitutes approximately 12% of the casein in milk. But it can

play an important role in marker assisted selection of milk trait, because it was linked and inherited as a cluster (Azevedo et al. 2008). The *K-CN* locus has been shown in different genome variations strongly associated with differences seen in milk composition and processing properties that affect dairy products (Riaz et al. 2008). Genetic variants of bovine *K-CN* gene are associated with protein content of milk and have influenced rennet clotting time, firmness and cheese yield of milk with a superiority of milk from cows with K-CN/BB compared to K-CN/AA genotype as shown in previous studies (Marziali and Ng-Kwai-Hang. 1986). In the present study, both AA and BB alleles had approximately similar mean average of milk production that was higher than the AB allele (Figure 4.5). The allele frequencies were 0.80 and 0.19 for A and B, respectively. The association of *HindIII\K-CN* variants with milk related traits was confirmed in other studies carried out on several breeds (Denicourt et al. 1990). The allelic variants of the *K-CN* gene in Sahiwal and Tharparkar cattle breeds were analyzed (Rachagani and Gupta. 2008). The *K-CN/BB* genotype had more influence on the milk, fat, and protein yield in the Sahiwal cattle. According to Marziali and Ng-Kwai-Hang (Rachagani and Gupta. 2008), cheese production can be increased by 10 percent if milk is from a cow of the *K-CN/BB* genotype when compared to *K-CN/AA* genotype. Therefore, it has been proposed to increase the frequency of *K-CN/BB* genotype in breeding programs preferring sires with the *K-CN/BB* genotype. The effect of *K-CN* polymorphism on milk performance traits was also studied in Holstein-Friesian heifer cows (Beata et al. 2008). However, in contrast to studies that suggested the association between *K-CN/BB* genotype and high milk yield (e.g., Rachagani and Gupta. 2008), the authors reported that the *K-CN/AA* genotype characterized by the highest milk, fat and protein yield, whereas *K-CN/BB* genotype showed the lowest fat and protein contents in their milk (Beata et al. 2008).

This is in agreement with the current study and that of Curi et al (2005) (Curi et al. 2005), where the association between *K-CN/AA* genotype and high milk production was observed. It also points to the involvement of other factors in milk production besides the K-CN.

4.2.3 *PIT-I* gene

The *PIT-1* gene was amplified using PCR amplification procedures as mentioned in the previous chapter 3.0. The quality of amplification was determined on agarose gel as described above. After amplification, PCR products were digested with *HinfI* restriction enzyme at 37 °C in a reaction mixture incubated overnight on a thermocycler for temperature control before it was loaded on a 3% agarose gel. The *PIT-1* amplified region showed a 451 bp amplicons on gel (Figure 4.1). Digestion analysis of the *PIT-1* gene revealed three genotypes (Figure 4.6): the homozygous AA as undigested band of 451 bp, the heterozygous AB genotype as three bands; one undigested of 451 bp and two digested bands that were close to each other with sizes of 207 and 244 bp. The third was the homozygous BB genotype consisted of two digested, but shorter bands of 207 and 244 bp (Figure 4.6).

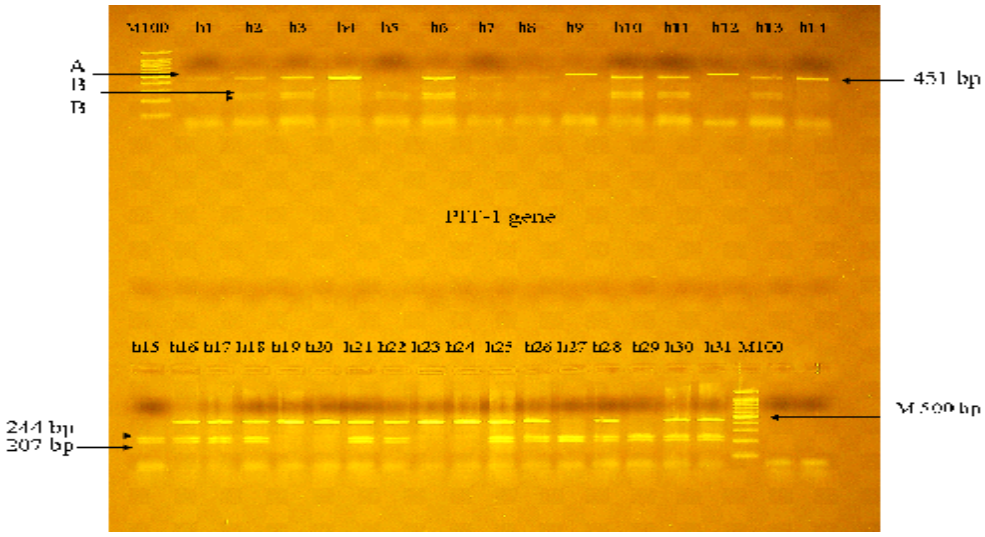

Figure 4.6. The genotypic analysis of *PIT-1* gene in Palestinian cattle. PCR products amplified from genomic DNA collected from blood samples extracted from Hebron city cattle (h1- h31) and digested with HinfI before loading on a 3% agarose gel as described above. Alleles revealed by the genotypic analysis were the AA, AB and the BB. The homozygous (AA) genotypes appeared as one undigested band of 451 bp, whereas the heterozygous (AB) genotypes appeared as one undigested 451 bp fragment and two digested bands of 207 and 244 bp. The BB restriction bands appeared as 244 and 207 bp fragments. Lanes labeled (h1,h9,h12,h14,h19,h20,h23,-h24) are the homozygous AA, whereas the heterzygous AB are in lanes (h2,h3,h6-h8,h10,h11,h13,h16h18,h21,h22,h25,h26, h28,h30, h31) and for the BB genotype, lanes (h5, h15,h27,h29).

The *PIT-1* genetic variations (e.g., AA, AB, BB alleles) are strongly associated with differences in milk yield and animal growth. The SNP of *PIT-1* has become a popular genetic marker tool commonly used for genetic characterization and identification of possible linkage associations between *PIT-1* gene and milk performance traits. ANOVA analysis revealed that the allelic substitution (AA, AB, BB) effect was significant for milk production with p-value = 0.274e-05 and $\alpha = 0$ ***. The AA was the most favorable allele for milk production with an average milk production of more than 11000 L compared to the intermediate AB (less than 9000 L) and the lowest favorable BB alleles with an average milk production of less than 8000 L per 305 days (Figure 4.7).

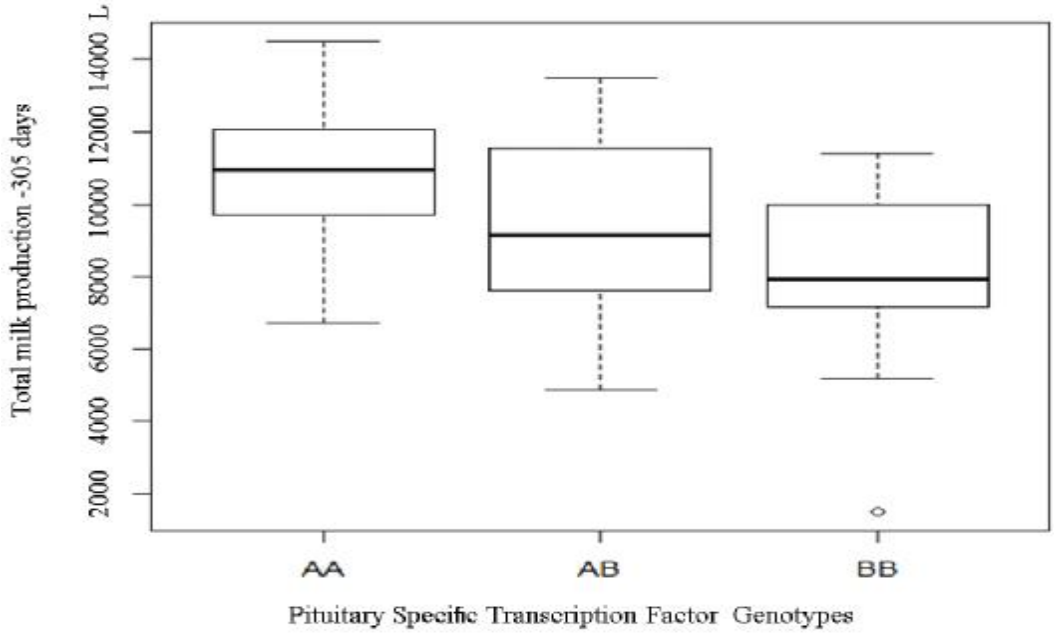

Figure 4.7. Analysis of *PIT-1* allelic substitution effect on milk production. As shown, the AA allele was the most favorable for milk production with an average mean of more than 11000 L per 305 days compared to the intermediate AB allele with an average mean milk production of less than 9000 L and the least favorable BB allele of an average mean of milk production of less than 8000 L per 305 days. The *PIT-1* allelic substitutions (AA, AB, BB) effect is significant on milk production with p-value = 2.274e-05 and α = 0.0 * as analyzed by ANOVA on 101 Friesian blood samples.**

In cattle, *PIT-1* was found in several studies to be associated with body weight and average daily gains (Renaville et al. 1997; Carrijo et al. 2008) and milk production traits (Renaville et al. 1997; De Mattos et al. 2004; Xue et al. 2006). Other studies, however, could not verified the association between *PIT-1* and production traits (Di Stasio et al. 2002; Zwierzchowski et al. 2001; Dybus et al. 2004; Zhao et al. 2004; Maria and Cataldo. 2011).

There are several polymorphic cattle *PIT-1* loci identified (Zwierzchowski et al. 2001). In Holstein breed, it was shown that A allele, characterized in site from exon 6 of *PIT-1* gene, has significant positive effect on production traits in cattle (Carsai et al. 2012). These polymorphisms have been shown to play a key role in milk yield and, to a lesser extent, in determining the fat percentage in dairy cattle (Dybus. 2002; Arash et al. 2005). The A allele of *PIT-1* was also found to be superior for milk and protein yield, but inferior for fat percentage in dairy cattle (Renaville et al. 1997; Dybus. 2002). In this study, the *PIT-1* gene polymorphism was shown to play a significant role in milk production, with the *PIT-1/AA* genotype being more important for milk production than the *PIT-1/AB and PIT-1/BB* genotype respectively (Figure 4.7).

The Polymorphism within bovine *PIT-1* gene effect on production traits was also reported in several studies (Woollard et al. 1994; Renaville et al. 1997) where the A

allele seemed to be linked to higher milk yield and more protein yield but less fat percentage. Furthermore, Hori-Oshima and Barreras-Serrano (2003) studied the *PIT-1* gene polymorphism in Baja California Holstein cattle and found that the *PIT-1/AA* genotype possessed a significant effect on milk yield (Hori-Oshima and Barreras-Serrano. 2003) similar to what was reported by Renaville et al (1997) and Viorica (2007), where the A allele was found to be superior for milk and protein yields and inferior for fat percentage in Romanian Simmental cattle (Renaville et al. 1997; Viorica et al. 2007). Allele frequencies in present study for A and B were 0.68 and 0.31, respectively. In studies on Canadian Holstein bulls, the frequency of B allele was found to be 0.79 (Sabour et al. 1996) and 0.812 in Italian Holstein Friesian bulls (Renaville et al. 1997). This is slightly higher than the B allele frequencies reported in Polish Black and White cattle, which were very similar in three studies, 0.75 (Klauzinska et al. 2000), 0.74 (Oprzadek et al. 2003) and 0.757 (Dybus et al. 2004).

4. 3 Relevance of body scoring to milk yield in Palestinian cattle

Cows are usually milked twice per day, but because milk production can increase up to 10%, most farms in Palestine milk cows 3 times per day and many dairy farms are beginning to do so. Milk yield is an average milk production per day that is recorded by computer systems at the milking parlors. In this study, the cows that were selected for study were also measured for body conditions scoring in relative to recorded milk production. These data are quite important to determine if there is a relationship between milk yield and body scoring. There is no available similar study in Palestine to compare results with, and therefore this is a unique investigation that considers two important parameters, genotypes and body scoring in relative to milk yield. The information in relative to farms, milk production and body scoring for the cattle

studied in this project are reported in (Appendix 4). Representative data on individual cows taken from Nablus city cattle: N1-N10 are shown in Table 4.3.

Table 4.3. Information on milk production related data from 10 dairy cow. Information collected from Nablus city dairy cows that include the serial number of the cattle, date of birth, milk yield, morning and evening milking, average milk production per day, month and 305 days.

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# Serial	Age Of Cattle	Milk Yield Lactation	Product AM Milk	PM Milk Product	Milk Total Daily	Average Monthly	Average milk Yearly	Amount of Fodder	Hay Kg	Product per year 305
N1	3	2	14	14	28	25	22	10 Kg	15 Kg	6710 L\Year
N2	3	2	17	15	32	28	25	"	"	7625 L\Year
N3	4	3	25	25	50	40	30	"	"	9150 L\Year
N4	3	2	13	12	25	20	18	"	"	5490 L\Year
N5	3	2	17	15	32	28	25	"	"	7625 L\Year
N6	3	2	14	14	28	24	20	"	"	6100 L\Year
N7	4	3	5	5	10	9	5	"	"	1525 L\Year
N8	3	2	14	14	28	24	22	"	"	6710 L\Year
N9	4	3	20	20	40	34	28	"	"	8540 L\Year
N10	4	3	15	13	28	25	22	"	"	6710 L\Year

4.4 Allele frequencies, heterozygosities and Hardy-Weinberg equilibrium

Allele frequency is a measure of the relative frequency of an allele of a genetic locus in a selected population and can provide us with information on the genetic diversity of the Palestinian dairy cattle. In the present study, the existence of two alleles for the three milk genes was verified in Palestinian cattle, with two alleles for the *PRL* (G and A), two for *K-CN* (A and B), and two for *PIT-1*(A and B). The frequency for each

allele was calculated for the three studied populations (Friesian, hybrid, and the local baladi cattle) (Table 4.4).

For the *PRL* gene, two genotypes (GG, AA) were identified and the highest frequency was (0.9444) for G allele detected in the hybrid breeds (Table 4.4). On the other hand, when Frisian breeds were compared with the other two breeds, the highest frequency was found in the G allele of the local breeds (0.8200) and lowest in the Frisian breeds (0.7128). The Frisian breeds possessed the highest frequency (0.2871) for the A allele and the highest expected heterozygosity (0.4092) when compared with local and hybrid breeds for the A allele (0.1800), (0.0556) and expected heterozygosity of (0.2952) and (0.1050), respectively**.**

For the *K-CN* gene, the frequency of A allele was higher than the B allele in all of the breeds studied (local, Friesian and hybrid- 0.8400, 0.8019, 0.7500, respectively). The Hybrid breed possessed the highest expected heterozygosity number of (0.3750) when compared with the Friesian (0.3175) and with the Local breeds (0.2688).

For the *PIT-1* gene, the A allele was higher than the B allele in the three breeds. Friesian breed possessed the highest number in allele frequency (0.6831) compared to the other two populations, the Hybrid (0.3333) and the Local (0.2200) breeds. Friesian breeds, however, possessed lower expected heterozygosity (0.4328**)** when compared with the Hybrid breeds (0.4439), but the highest expected heterozygosity (0.3432) when compared with the Local breeds. Information obtained from the allele frequencies of the three genes in Friesian, hybrid and local cattle can be used as a tool to select the sperms of bulls to achieve improvements and enhance the selection of high milk yield dairy cows. As shown in the results, the Friesian breed represents the highest yielding cattle and is primary selected breed in dairy farms.

Table 4.4. Allele frequencies and genotypes for the three population cattle breeds studied in 144 dairy cows. The observed and expected heterozygosities are also included.

Loci	Population	N	Allele Frequencies		Genotypes			Expected Hetrozygosity	Observed Hetrozygosity
					Observed Number				
			G	A	GG	AG			
PRL	Frisian	101	0.7128	0.2871	43	58		0.4092	0.4257
	Hybrid	18	0.9444	0.0556	16	2		0.1050	0.8888
	Local	25	0.8200	0.1800	16	9		0.2952	0.6400
			A	B	AA	AB	BB		
K-CN	Frisian	101	0.8019	0.1980	66	30	5	0.3175	0.7029
	Hybrid	18	0.7500	0.2500	10	7	1	0.3750	0.6111
	Local	25	0.8400	0.1600	19	4	2	0.2688	0.8400
			A	B	AA	AB	BB		
PIT-1	Frisian	101	0.6831	0.3168	52	34	15	0.4328	0.6633
	Hybrid	18	0.3333	0.6666	3	6	9	0.4439	0.6666
	Local	25	0.2200	0.7800	1	9	15	0.3432	0.6400

4.5 A Multi Variate Statistical Package- MVSP

The MVSP 3.21 version has been used to analyze data obtained from the three populations, Friesian, hybrid and local breeds through the generation of 2D and 3D graphs that make the illustration of specific parameters, such as body scoring data, in relation to milk yield possible and easier to interpret. The MVSP is commonly used to describe a set of numerical techniques in which the main purpose is to divide the objects of study into discrete groups. Analyzed groups are based on the characteristics of the objects and clusters that share some sort of significant correlations (Kovach. 1988). Cluster analysis is used in many scientific disciplines and a wide variety of techniques have been developed to suit different types of approaches. The most

commonly used ones are the agglomerative hierarchical methods. Hierarchical methods arrange the clusters into a hierarchy, so that the relationships between the different groups are apparent. The results of this type of analysis are generally presented in a multi-dimensional diagram called a dendrogram. The dendrogram is produced by starting with all the objects to be clustered separated, then it successively combines the most similar objects and/or clusters until all are in a single, hierarchical group (Kovach and Batten. 1994).

The Frisian breeds were used in clustering analysis because Frisian cattle are more represented in dairy farms and provide better milk production. Cluster analysis was used to assess the relationship of milk production and yield with body scoring conditions using 2D and 3D dendrogram diagrams. When milk yield (per year) was compared to body scoring in Frisian breeds it revealed that the best body scoring effect on milk yield was in the second and third years of milk yield (Figure 4.8). Following the third year, there is no major effect of increased body score on the yield of milk.

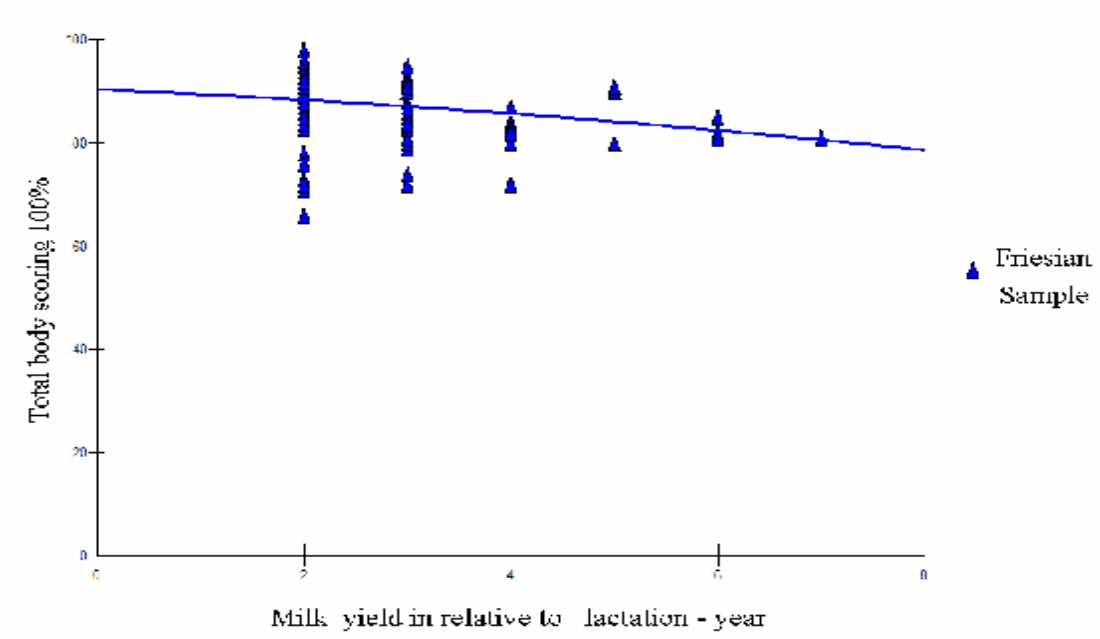


Figure 4.8. A 2D dendrogram showing the correlation between milk yield and body scoring in Frisian dairy breeds. The yield of milk in relative to yearly lactation is plotted on the X-axis and the total body scoring conditions in (100%) on the Y-axis. The dendrogram shows that the blue line decreases by years, which means that the yield of milk decreases following the third year independently of increased body scoring. The 3.21 version of MVSP was used for analysis in this study.

In fact, when the milk production peaks and the energy requirements exceed intake, the cattle enter into a negative energy balance (NEB), because they mobilize their lipid reserves, getting thinner and lose their BCS (Aeberhard et al. 2001; Coffey et al. 2002; Agenäs et al. 2003). On the other hand, not all cattle reduce their BCS equally, because the high genetic merit dairy cattle have a higher predisposition for mobilization of body fat reserves to cover milk production demands (Veerkamp. 1998; Pryce et al. 2002). This was demonstrated in cattle selected for higher milk yield (Berry et al. 2003). The cattle had lower BCS during lactation and their BCS changes were higher after calving than cattle with lower genetic merit (Buckley et al. 2000; Horan et al. 2005). Thus, mobilization of body fat reserves and milk production are considered closely related (Pryce et al. 2002). These findings were confirmed by a study by Gallo et al (1996) who observed a higher and more prolonged BCS loss in cows with higher milk yield. Therefore, BCS and milk yield are in a negative correlation (Veerkamp and Brotherstone. 1997) and high yielding dairy cattle generally have a lower BCS (Pryce et al. 2002). Clustering analysis was used to construct a 3D dendrogram to compare milk yield (lactation period started –years) with milk production per 305 days per lactation, and then identify the distances between them to detect the nearest and farthest neighbors. This analysis allows us to determine milk production in relative to milk yield. By using the nearest distance between one group and another, the distance is taken between their two closest points,

whereas the farthest neighbor takes the distance between the two farthest points between the two groups. These methods are also known as single linkage and complete linkage, respectively (Kovach and Batten. 1994). Analysis revealed that there is a relationship between milk yield and milk production (Figure 4.6) as shown by the distance between them. The best milk production appeared to be in the second yield as most of the samples clustered above 12000 L-305 day with nearest distance of 0.1 in all yield. The third yield showed better milk production and nearest neighbor in second yield (0.3) if compared with the farthest neighbor of the fourth and sixth yield (>0.5). On the other hand, most samples of the third yield clustered between 8000 and 12000 L per 305 days with over than 9000 and 8000 L for the fourth and fifth yield, respectively.

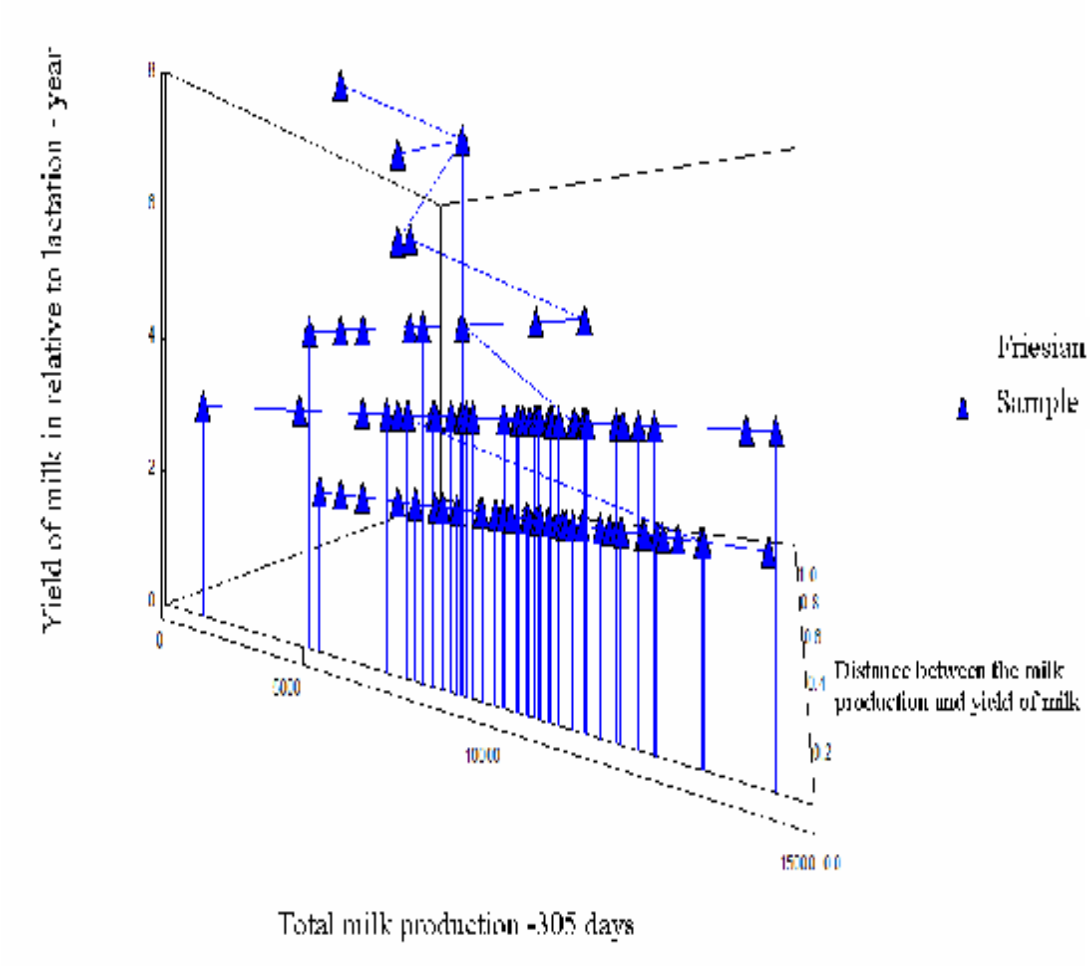


Figure 4.9. A 3D dendrogram showing the correlation between milk yield and milk production in Frisian dairy breeds. The milk production is plotted on the X-axis and the yield of milk is on the Y-axis, whereas the distance of nearest and farthest neighbors on the Z-axis. The dendrogram shows that milk yield and milk production are related as it appeared from the distance between them. The best milk production happened in the second yield as most samples clustered above 12000 L per 305 days a year with nearest distance of 0.1 in all yields. The third yield showed better milk production and nearest neighbor in second yield (0.3) if compared with the farthest neighbor of the fourth and sixth yield (>0.5). On the other hand, most samples of the third yield clustered between 8000 and 12000 L per 305 days with over than 9000 and 8000 L for the fourth and fifth yield, respectively. The 3.21 version of MVSP was used for analysis in this study.

For the three breeds investigated in this study, the Friesian, hybrid and the local baladi, a 3D clustering analysis dendrogram was constructed to compare between the three populations in relative to body scoring conditions, total milk production and the yield of milk (Figure 4.10). Results showed that the highest body scoring and milk production were in the Friesian breed (70% > 95%) and (8000 L >14000 L), respectively. The hybrid breed is the second highest in milk production and body scoring (2500 L > 5000L) and (60% > 70%), respectively, whereas the local breed is the lowest in milk production and in body scoring among the three populations with (800 L > 1500 L) and (50% > 60%), respectively. Thus, the Friesian cattle are the best for investment in dairy cow farms in Palestine.

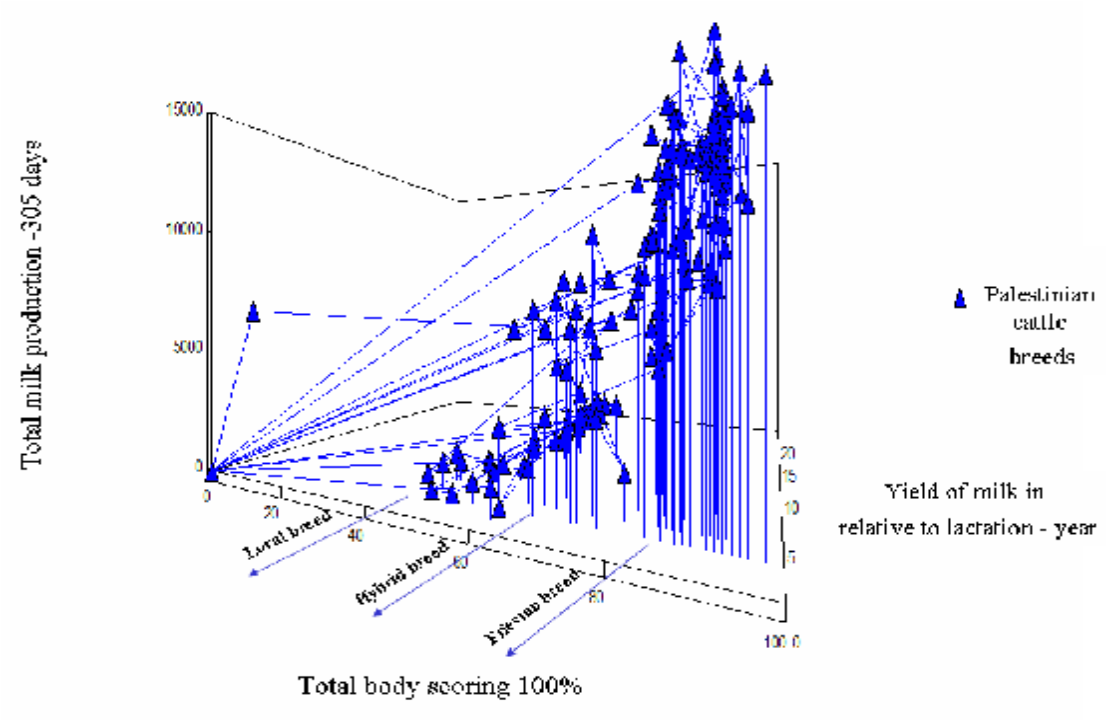


Figure 4.10. A 3D dendrogram showing the correlation between milk yield and milk production in Frisian, hybrid and local breeds. The total body scoring is plotted on the X-axis and the total milk production is on the Y-axis, whereas the yield of milk is plotted on the Z-axis. The dendrogram shows that the highest body scoring and milk production are in the Friesian breed (70% > 95%) and (8000 L >14000 L), respectively. The hybrid breed is the second highest in milk production and body scoring (2500 L > 5000L) and (60% > 70%), respectively. Local breed is the lowest in milk production and in body scoring among the three populations (800 L > 1500 L) and (50% > 60%), respectively.

4.6 Cattle Nutrition Analysis

The quantity and quality of nutrients are expected to play a critical role in milk production, i.e., Kg of nutrition provided daily and type of nutrition, e.g., hay or silage. In north cities of Palestine, nutrition consists mostly of hay like in Jenin, Tubas, Nablus cattle farms, and most of the farmers had little experience in using silage in feed and also have a limited background on the variability of nutrition and the effect this may have on milk production. Cattle farms in south Palestine, however, prefer silage nutrition particularly in Hebron area. Analysis indicated that the nutrition play a

significant role on milk production with a p-value = 0.00381, andα 0.001‘** (Figure 4.11). The relationship between the feed type and quantity in Kg per day with milk production was studied and analyzed using ANOVA on data obtained from Palestinian farms in the north and south, Jenin, Tubas, Tumon, Nablus and Hebron. Results showed that the tested Frisian dairy cattle provided highest milk production when received nutrition approximately 45 kg per day. Results showed that silage nutrition is better than hay and other feed nutrition. Since some samples showed equal milk production at different amounts of feed provided and also was independent of the type of nutrition, it is possible that the cow genotype is interfering in the milk production**.**

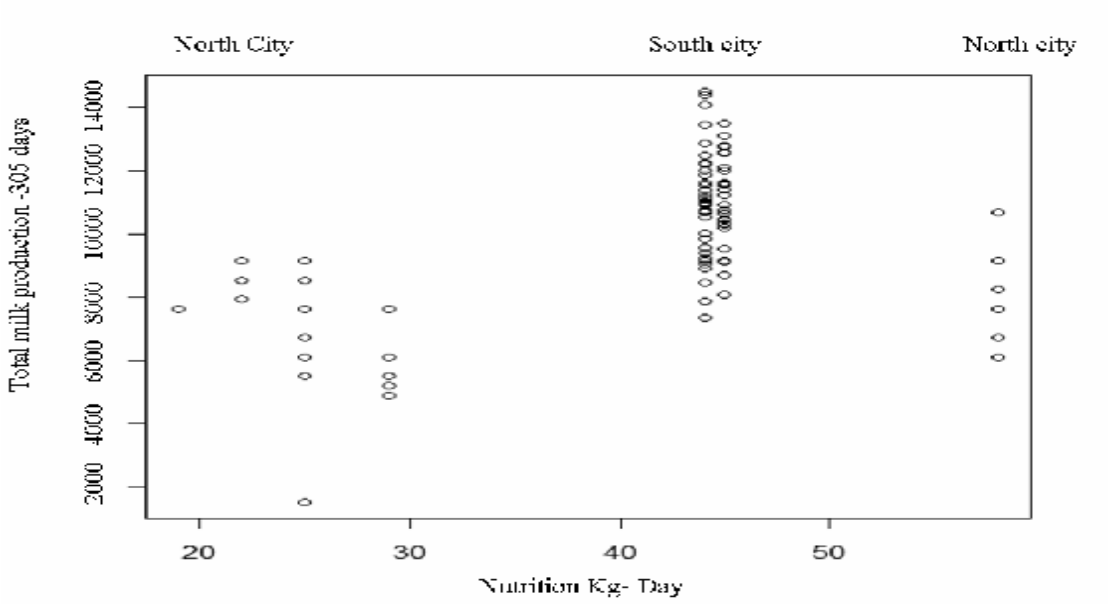


Figure 4.11. Analysis of the effect of nutrition on milk production. Data obtained from Friesian dairy cows from the cities of Jenin, Tubas, Tumon Nablus, and Hebron, and were analyzed using ANOVA and plotted with the nutrition in kg on the X-axis and the total milk production per 305 days on the Y-axis. All analysis results clustered between 40-50 kg nutrition per day showed the highest milk production and were for cows in Hebron area, which were fed on silage. Dairy cows from the north cities showed the lower milk production and were fed 30 > 50 kg of hay and feed nutrition.

4.7 Farm city analysis

Samples were collected from the north and south dairy farms, where large number of the Friesian breed is distributed. Here, the relationship between the location from where data were collected (i.e., Jenin, Tubas, Tumon, Nablus and Hebron) and the total milk production was analyzed. The highest milk production per dairy cow was in the city of Hebron followed by Jenin, Tubas, Nablus and Tumon, respectively (Figure 4.12). Results reported above, e.g., Figure 4.11, showed that dairy cattle from Hebron gave the highest milk production and where better fed in terms of nutrient quality. This may due in part to the large investment this industry receives in Hebron and also the experience gained in farming dairy cattle. Dairy industry in Hebron area has more than 20 years of experience in raising cattle and administering farms and most farms use standard procedures that affect all aspects of the farm, in terms of animal feed, farm maintenance, milking practices and data collection. Dairy industries in the north, however, are less standardized; usually exist in fragmented family-owned small farms of approximately 15 cows administered mostly by same family members who lack enough experience in dairy cattle management. In addition, hay and feed are popular nutrition used in the north in less than 30 kg per day, which has proven to be less favorable for milk production than silage (Figure 4.11). Analysis to detect these effects in relative to general practices followed in dairy farms in the south and north of Palestine have shown that dairy cattle growers in Hebron area follow farm procedures that favor milk production in comparison to all other farms studied in the North (Figure 4.12). Analysis using ANOVA showed a significant role played by the location of the farm on milk production with p-value = 1.306e-08 and α 0 '***' (Figure 4.9).

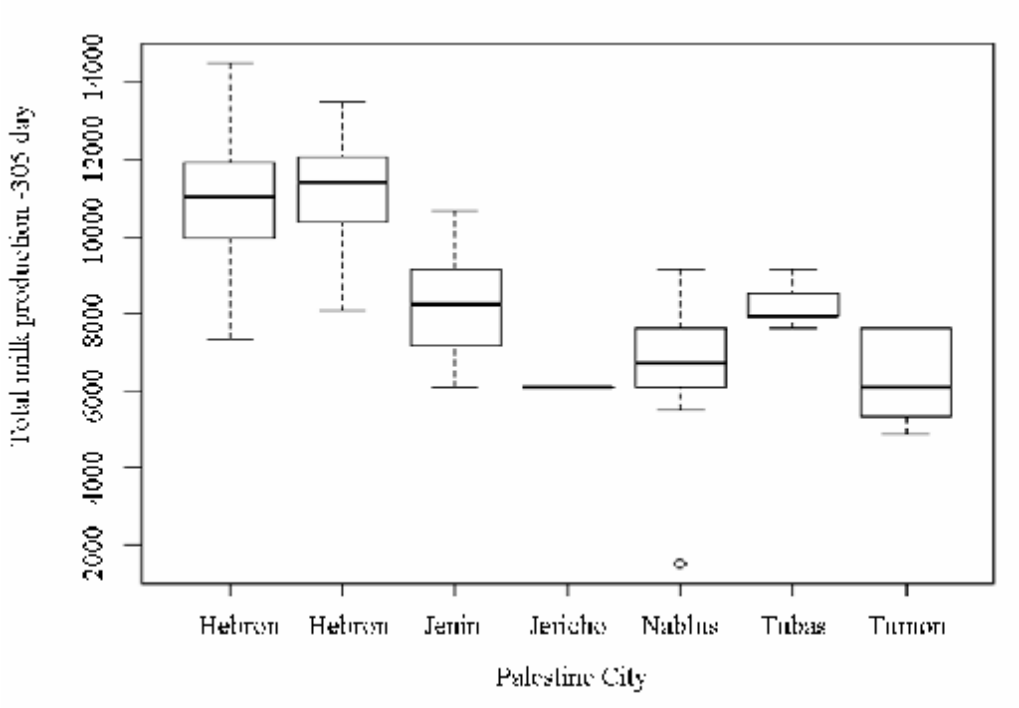


Figure 4.12. Analysis of the effect of farm location on milk production. Data obtained from Friesian dairy cows from the cities of Hebron Jenin, Nablus, Tubas and Tumon and were analyzed using ANOVA and plotted with the name of the city on the X-axis and the total milk production per 305 days on the Y-axis. The highest milk production per dairy cow was in the city of Hebron followed by Jenin, Tubas, Nablus and Tumon, respectively.

In order to check if the temperature play a role in the production of milk by the dairy cows, average maximum and minimum temperatures of Hebron and Jenin were compared the milk production in the same breed for each city to exclude genetic variation, focusing on the Friesian breeds. The two cities were chosen because climate is considerably different between the two cities and their surrounding areas (Figure 4.13). Climate directly and indirectly affects livestock production through several components including temperature, rainfall and humidity(Lia and Willyan. 2012). This is likely due to the sensitivity of dairy cows to excessive temperature and

humidity (Yoram et al. 2012). For example, increasing air temperature, temperature-humidity index and rising rectal temperature above critical thresholds can cause a decreased dry matter intake and milk yield and thus a reduction in the efficiency of milk yield (West. 2003). Also, Holstein milk production decreased with climate change (Yoram et al. 2012) and animals suffered thermal stress showed decreased milk production (Piotr and Sabina. 2012). It is then possible that, in addition to nutrition quantity and quality, the climate plays an effect on the difference in milk production observed between the south and north of Palestine, favoring Hebron area.

Figure 4.13. The average monthly temperature reported in 2011 for two Palestinian cities, Hebron in the south and Jenin in the north. The graph shows the maximum (orange line) and minimum (blue line) average monthly temperatures for both areas. Although the cities are less than 300 km away from each other, yet there is an average of 9 C degrees difference between the two cities.

CHAPTER 5.0

5.0 OVERALL CONCLUSION AND FUTURE WORK

In the present study, the economically important three cattle genes (*PRL, K-CN, PIT-1*) for three cattle breeds (Frisian , Hybrid and local-baladi) have been used as a molecular tool for a representative genetic analysis of cattle in Palestinian. To identify the optimal breed for the highest milk production, an RFLP based molecular genotyping was established in our laboratory for livestock improvement to select the favorable milk production trait. Search for high milk yield breeds has also include analyses on cow body condition scoring, type of feed used in farms and location effects of farms in relative to milk yield. It was concluded that the Palestinian native (Local/Baladi) breed was of a low milk yield compared to the other Hybrid and Frisian cattle used in dairy farms. Frisian was the best cattle breed for milk production, though genetic analysis revealed significant variations within the same breed in relative to yearly milk yield in 305 days. These variations were likely due to the nutritional value of the provided feed and temperature, favoring silage over hay and moderate temperature over high ones. Body conditions' scoring was also found to play a role in milk production, favoring Friesian, then Hybrid and baladi cattle, respectively.

In most developing countries, biotechnology applications relevant to livestock research are still considered rarely used in cattle farms and most cattle selection is dependent on body morphology. In Palestine, animal owners are generally resource-poor and mostly are small-scale operators who own little or no land and few animals and are not aware of recent advances in biotechnology that would help them selecting

the appropriate breeds for milk production. It is therefore recommended that some national strategy being applied to increase the awareness of farm owners and operators by conducting relevant and periodical workshops to expose cattle farmers to relevant biotechnology applications and help them transferring this technology into application in their farms in Palestine. Livestock is becoming increasingly important to economic growth in Palestine and the application of biotechnology will be likely dictated by commercial considerations and socio-economic goals. Using technology to support livestock production is an integral part of viable agriculture in multi-enterprise systems. Livestock are part of a fragile ecosystem and a rich source of animal biodiversity, as local species and breeds possess genes and traits that may be of interest (e.g., in adapting to local environment) to breeders to establish a Palestinian fit dairy cow. Molecular markers are increasingly used to identify and select the particular genes that lead to these desirable traits and it is now possible to select superior germplasm and disseminate it using artificial insemination, embryo transfer and other assisted reproductive technologies. These technologies should be actively used in genetic improvement of livestock in Palestine through the enhancement of the national Palestinian livestock improvement center role in applying and transferring the latest biotechnology tools to farmers through partnerships with universities like PPU and the Ministry of Agriculture to develop a national breeding program for cattle and livestock in general.

Reference

1. Abbas, D, Asghar, A ,and Behnam, M. (2011). African Journalof Agricultural Research Vol. 6(19). 4467-4470.
2. Aeberhard, K , et al. (2001). Journal of Veterinary Medicine Series A, 48, 97–110.
3. Agenäs, S , Burstedt, E , and Holtenius, K. (2003). Journal of Dairy Science, 86, 870–882.
4. Aleandri , R , et al. (1990). Journal of Dairy Science: 73: 241-255.
5. Alfonso, E, et al. (2012). African Journal of Biotechnology Vol.11(29), pp. 7338-7343.
6. Antonio, R , et al. (2005). Genetics and Molecular Biology, 28, 2, 237-241.
7. Arash, J, et al. (2005). Iranian Journal of Biotechnology , Vol. 3, N 2.
8. Aseltine & Schingoethe (1998) in Kellems & Church (1998), NRC(2001), and Jurgens (2002). Animal Nutrition Handbook. Section 15: Dairy Cattle Nutrition and Feeding Page 392.
9. Alipanah, M , Kalashnikova, L, and Rodionov, G. (2007). Iranian Journal of Biotechnology. 5, 158–161
10. Azevedo, A , et al. (2008). Genetics and Molecular Research 7 (3): 623-630.
11. Beata, S , Wojciech, N, and Ewa ,W.(2008) Cent. Eur. Agriculture . 9, 641–644.
12. Berry , P, et al. (2003). Journal of Dairy Science: 86, 2193–2204.
13. Berry, P, et al. (2002). Journal of Dairy Science: 85:2030–2039.
14. Bjorn, K , et al. (2010). BMC Genomics 2010, 11:158.
15. Bradley, D, et al. (1998). Evolutionary Anthropology 6(3):79-86.
16. Brouček, J, et al. (2009). Slovak Journal of Animal Science 42(4), 167-173.

17. Brym, P, Kaminski, S, and Wojcik, E. (2005). Journal of Applied Genetics. 45. 179-185.
18. Buckley, F, et al. (2000). Journal of Dairy Science, 83, 1878–1886.
19. Butler, W, and Smith, R. (1989). Journal of Dairy Science: 72:767–783.
20. Camper, S, et al. (1984) .DNA 3:237-249.
21. Carsai, T, et al. (2012). Animal Science and Biotechnologies, 45 (1).
22. Carrijo, S , Alencar ,M ,and Toral , F. (2008). Science Agriculture. 65: 116-121.
23. Cecelia , M .(2008). Nature Education 1(1).
24. Chung, E , Rhim , T ,and Han, S. (1996). Korean Journal of Dairy Science 38: 321-336.
25. Chrenek, P, et al. (1998) Journal of Animal Science .43, 53–55.
26. Coffey, M , Simm, G, and Brotherstone, S. (2002). Journal of Dairy Science, 85, 2669–2678.
27. Curi, R, et al. (2005). Genet. Mol. Biol. 262–266.
28. Darshan, R , et al. (2008). Science Asia 34: 435–439.
29. David, G, et al. (1997). Genetics 146 1071-1086.
30. Denicourt. D, Sabour M, and McAllister. A. (1990). Anim. Genet. 21, 215–216.
31. De Mattos, K, et al. (2004). Brazilian Journal of Agricultural Research, 39 (2004), 147-150.
32. Domecq, J, et al. (1997. Journal of Dairy Science: 80:113–120.
33. Di Stasio , L ,Sartore ,S and Albera , A. (2002). Animal. Genet. 33: 61-64.
34. Dybus, A, et al. (2004). Arch.Tierz. Dummerstorf. 47 . 557–563.
35. Dybus, A . (2002). Animal Science Papers and Reports. 20: 203-212.

36. Excoffier, L , Laval, G, and Schneider, S .(2006). Arlequin ver 3.1 An Integrated Software Package for Population Genetics Data Analysis.
37. Ferretti, L , Leone, P, and Sgaramella , V .(1990). Nucleic Acids Res 18, 6829–33.
38. Freeman, A , et al. (2005) . Animal Genetics. 37: 1-9.
39. Freeman, A , et al. (2000). Physiological Reviews. 80.
40. Galila. A, et al. (2008). A PCR-RFL . Arab J. Biotech., Vol. 11, No. (1) Jan.
41. Gallo, L, et al. (1996). Journal of Dairy Science; 79, 1009–1015.
42. GenBank database [http://www.ncbi.nlm.nih.gov/.
43. Götherström, et al.(2005) . Proceedings of the Royal Society B 272(1579)-:2345-2350.
44. Halabian, R , et al. (2008). Biotechnology 7(1).118-123.
45. Hallerman , E , et al. (1988). Anim. Genet. 19: 123-131.
46. He, et a . (2006). Asian-Australasian Journal of animal Sciences 19: 1384-1389.
47. Herr, W, et al. (1988). Genes & Dev. 2: 1513.
48. Horan .B, et al. (2005). Journal of Dairy Science: 88, 1231–1243.
49. Hori-Oshima, S , and Barreras-Serrano, A . (2003). Journal of animal Sciences :54 .252–254
50. http://holsteinusa.com/holstein_breed/breedhistory.html
51. http://www.britishfriesian.co.uk/content/history.shtml
52. Jasiorowski, H . (1988). Proceedings of II World Buffalo Congress. New Delhi, India. Vol II .Part I, pp. 285-294.
53. Javed, R, et al. (2011) . International Journal of Livestock Production Vol. 2(6), pp. 79-83.

54. Khatami, S , et al. (2005). J. Genet. 41,167–173.

55. Klauzinska, M , et al. (2000). Animal Sciences Pap. Rep. 18 . 107–116.

56. Kolbehdari , et al. (2009). Jnim. Breed. Genet 126:216-227.

57. Kovach, W, and Batten, D. (1994). In: Traverse, A. (ed.), Sedimentation of Organic Particles. Cambridge University Press. p.391-407.

58. Kovach, W. (1988). The Paleontological Society Special Publication, 3:72-104.

59. Ladani, D, et al. (2003). Buffalo Jourrnal . 2 : 237-242.

60. Li, J, et al.(.2006). Asian-Aust. Journal of animal Sciences. Vol 19, No. 4 : 459-462.

61. Lia, B ,and Willyan, D. (2012). Lucrări Ştiinţifice -Seria Zootehnie, vol. 58.

62. Ligang, T , et al.(2012). African Journal of Biotechnology Vol. 11(42), pp. 9906-9910.

63. Loftus, R, et al.(1994). Proc. Natl. Acad. Sci. U.S.A. 91, 2757–2761.

64. Luiz, F , and Randy, S.(2012). Tri-State Dairy Nutrition Conference. April 24 and 25, 2012.

65. Maria, S , and Cataldo, D. (2011). African Journal of Biotechnology Vol. 10(55), pp. 11360-11364.

66. Masoud, A , Lobov, K , and Genadi, R .(2007). Iranian Journal of Biotechnology , Vol. 5, No. 3.

67. Mason, I.L. (1996). A World Dictionary of Livestock Breeds, Types and Varieties. Fourth Edition. C.A.B International. 273 pp.

68. Marziali, A , and Ng-Kwai-Hang, K. (1986) . Journal of Dairy Science: 69, 2533–2542.

69. Mehmannavaz, Y, et al. (2009). African Journal of Biotechnology Vol. 8 (19), pp. 4797-4801.

70. Nichols, R , Bruford, M , and Groombridge, J. (2001). Mol. Ecol. 10:593–602.
71. Oleński . K , et al. (2012). Animal Science Papers and Reports vol. 30. no. 1. 5-12
72. Oprzadek, J, et al. (2003) . Animal Sciences. Pap. Rep. 21,135–145.
73. Othman, E, et al. (2011). Journal of Genetic Engineering and Biotechnology 9, 97–102.
74. Pan, C.Y, et al. (2008). Czech Journal of Dairy Science: 53: 523–527.
75. Pfaffle, R , et al.(1992). Science 257(5073): 1118-1121.
76. Piotr, H , and Sabina. A .(2012) . Animal Science Papers and Reports vol. 2012. 30 .4, 363-372.
77. Prinzenberg .E , Krause I, and Erhardt. G .(1999). Anim. Biotechnol. 10: 49-62.
78. Pryce . J, et al.(2002). Journal of Dairy Science,: 85, 1590–1595.
79. Rachagani, S , and Gupta , I.(2008) .Genet. Mol. Biol. 31 , 893–897.
80. Renaville . R , et al.(1997). Journal of Dairy Science :80 (1997) 3431–3438.
81. Riaz .M, et al.(2008) Pakistan Vet. J. 28 (2008) 103–106.
82. Richards , et al. (1986). Journal of animal Sciences : 62:300.
83. Romaniuk, W, et al. (2005). Projekt Bliźniaczy PHARE, Standardy dla Gospodarstw Rolnych. Warszawa: Instytut Budownictwa, Mechanizacji i Elektryfikacji Rolnictwa; Duńskie Służby Doradztwa Rolniczego.
84. Rosenfeld, M , et al (1991). Genes and Development, 5, 897-90
85. Tuggle ,C, and Trenkle, A .(1996). Domest. Anim. Endocrinol., 13(1): 1-33.
86. Sabour, M , et al.(1996) . Journal of animal Sciences .79. 1050–1056.
87. Stančeková , K , et al.(1999). Animal Genetics, 30, 313–315.
88. Sun, H , et al.(2002). Animal Reproduction Science, 69, 223–237.

89. Vătăúescu, B, et al.(2000).University of Bucharest, Faculty of Biology, Molecular Biology Center, Bucharest.pp1-4.

90. Veerkamp, R. (1998). Journal of Dairy Science : 81, 1109–1119.

91. Veerkamp, R, and Brotherstone, S. (1997) . Animal Science, 64, 385–392.

92. Viorica, A, et al (2007). Biotehnologii 40, 59–64.

93. Waltner, S, McNamara, J, and Hillers, J .(1993). Journal of Dairy Science 76:3410–3419.

94 .Wang, C , et al.(1994). Genet. Sel. Evol., 26, 91–115.

95.West , J . (.2003). American Dairy Science Association 86:2131–2144.

96.Wildman, E, et al.(1982). Journal of Dairy Science : 65:495-501.

97.Woollard, J, et al.(1994). Journal of Dairy Science :72 (1994) 3267..

98. Xue, K, et al.(2006). Acta Genetica Sinica, 33, 901–907.

99. Yu T.P, et al.(1995). Journal of Animal Science, 73, 1282–1288.

100. Yoram, B , et al.(2012). University of Washington, Seattle WA 98195-2802.

101. Zwierzchowski , L , et al.(2001). Animal Science . Pap. Rep. 19: 65-78.

102. Zhao, Q, Davis, M , and Hines, H. (2004). Journal of Animal Science, 82, 2229–2233.

Appendix 1-(A)

Data collection form distributed to farms. Forms included information as shown, to request permission for blood samples, milk production , and body scoring .

أخي المزارع يهدف هذا البحث إلي معرفة الجينات المسؤولة عن إنتاج الحليب كما، ونوعيا في الأبقار الهولندية ،حيث أن متوسط إنتاجية هذه الأبقار في فلسطين تتراوح بين (٥٠٠٠- ٨٠٠٠) لتر سنويا،وتعد هذه النسبة قليله بالمقارنة مع الدول المجاورة والتي تتجاوز ١١٠٠٠ لتر .علما بأن هذا القطاع (تربية الأبقار)،قطاع استثماريا مهما في فلسطين، وللأسف لا توجد هناك طريقة علمية مستخدمة للكشف عن هذه الجينات في الأبقار،وان الطريقة السائدة تعتمد على الشكل الخارجي للأبقار ولا تعتبر هذه الطريقة كافية لمعرفة الجينات.وفي هذا البحث سيتم مسح جيني للأبقار من مزارع نموذجية في فلسطين ومن جميع المحافظات، وسيتم الربط بين الجينات التي تحملها هذه الأبقار ،والشكل الخارجي لها ،وكذلك معدل إنتاج الحليب.

Cattle Survey Samples Sheet -Palestine Polytechnic University \ المحافظة :محافظة أريحا\محافظة جنين

Submitters Name: قدري عليان درا غمة	Telephone # ٠٥٩٨- ١٢٨٦٦٣	Palestine Polytechnic University
Contact number: ٠٥٩٨ -١٢٨٦٦٣	Phone #	Southern Part Of West Bank
Address: عين المالح\أريحا	Fax #.....	unit Biotechnology Research
Zyiad _abukhaizaran :collection by Sample	Email :.....	Dr. Fawzi Al-Razem
٠٥٦٩٦٧٢٧٩٩	No of samples : 5 samples (local)	EXT. 145 -١٩٢١-٢٢٣-٢-٩٧٢+:# Telephone

Name Submitters: عصام محمود أبو الهيجى	Telephone # ٠٥٩٨٥٦٩٩٣٣	Palestine Polytechnic University
Contact number: ٠٥٩٨ -٥٦٩٩٣٣	Phone #	Southern Part Of West Bank
Address؛الياموناجنين\	Fax #.....	unit Biotechnology Research
Zyiad _abukhaizaran :collection by Sample	Email :.....	Dr. Fawzi Al-Razem
٠٥٦٩٦٧٢٧٩٩	No of samples :4 samples (hybrid)	EXT. 145 -١٩٢١-٢٢٣-٢-٩٧٢+:# Telephone

Appendix 1-A- Continued

اسم الموقع : مشترك (أريحا \جنين)

ملكية المزرعة للسيد :قدري درا غمة \عصام أبو الهيجى

تاريخ : ١٢-٧-٢٠١٢

رقم البقرة	الرقم التسلسلي	موسم الحليب	رقم موسم الحليب	كمية الحليب مساءا	كمية الحليب صباحا	معدل الإنتاج اليومي	معدل الإنتاج الشهري	معدل الإنتاج السنوي	نوع وكمية العلف	نوع وكمية العلائق	كمية الحليب سنويا
1 L	j1	5	4	2	3	5	4	3	2kg	5kg	915 L\Year
2 L	j1	5	4	2	2.5	4.5	3.5	3.5	"	"	1067 L\Year
3 L	j3	4	3	2	3	5	3	3	"	"	915 L\Year
4 L	j4	5	4	2	4	6	4	2.5	"	"	762.5 L\Year
5 L	j5	3	2	2	3	5	3	2.5		"	762.5 L\Year
1008	j6	5	4	8	18	26	24	20	10kg	15kg	6100 L\Year
1 B	j7	4	3	8	8	16	15	13	"	"	3965 L\Year
2 B	j8	3	2	7	7	14	10	9	"	"	2745 L\Year
3 B	j9	3	2	8	7	15	12	11	"	"	3355 L\Year

Appendix 1-A- Continued

المحافظة :محافظة أريحا\محافظة جنين

الموقع :عين المالح\اليامون \ تاريخ التقييم : ١٢-٧-٢٠١٢

رقم البقرة	الرقم التسلسلي	تاريخ الميلاد	عدد الولادات	الإطراف		الحدبة		الهيكل العام				الضرع					التقليم العام				
				زاوية الظلف ٥%	رؤيا جانبية ٥%	ميل الحدبة ٥%	عرض الحدبة ٥%	الزوايا ١٠%	العمق ١٠%	سعة الصدر ١٠%	الارتفاع ١٠%	اربطه أمامية ١٠%	أربطة خلفية ١٠%	فاصل مركزي ١٠%	الحلمات		مظهر عام ٥٠%	أربطة الضرع ٢٠	الحدبة ١٠%	الضرع ٢٠%	التقييم النهائي ١٠٠%
															حجم ٥%	تماثل ٥%					
1L	j1	5	4	2	2	2	2	6	6	6	6	6	5	6	2	2	28	11	4	10	53
2L	2	5	4	2	2	2	2	6	5	5	6	5	5	5	2	2	22	10	4	10	46
3L	j3	4	3	2	2	3	2	6	6	5	5	5	5	5	2	2	22	10	5	9	46
4L	j4	5	4	2	2	3	3	6	5	6	6	5	5	5	2	2	23	10	5	9	47
5L	j5	3	2	2	2	2	3	5	6	5	6	5	5	5	2	2	22	10	5	9	46
1008	j6	4	3	3	3	3	3	8	8	8	8	8	8	7	3	3	38	15	6	13	72
1B	j7	5	4	3	3	2	3	8	8	8	8	8	8	8	3	3	32	12	4	13	72
2B	j8	2	1	4	4	2	2	6	6	6	6	6	6	7	2	3	32	12	4	12	60
3B	j9	4	3	3	4	4	4	8	8	7	8	8	8	8	3	3	37	16	8	15	76

Appendix 1- B

أخي المزارع يهدف هذا البحث إلي معرفة الجينات المسؤولية عن إنتاج الحليب كما، ونوعيا في الأبقار الهولندية ،حيث أن متوسط إنتاجية هذه الأبقار في فلسطين تتراوح بين (٥٠٠٠- ٨٠٠٠) لتر سنويا،وتعد هذه النسبة قليله بالمقارنة مع الدول المجاورة والتي تتجاوز ١١٠٠٠ لتر .علما بأن هذا القطاع (تربية الأبقار) ،قطاع استثماريا مهما في فلسطين، وللأسف لا توجد هناك طريقة علمية مستخدمة للكشف عن هذه الجينات في الأبقار،وان الطريقة السائدة تعتمد على الشكل الخارجي للأبقار ولا تعتبر هذه الطريقة كافية لمعرفة الجينات.وفي هذا البحث سيتم مسح جيني للأبقار من مزارع نموذجية في فلسطين ومن جميع المحافظات، وسيتم الربط بين الجينات التي تحملها هذه الأبقار ،والشكل الخارجي لها ،وكذلك معدل إنتاج الحليب

Cattle Survey Samples Sheet -Palestine Polytechnic University

المحافظة :محافظة جنين

Submitters Name: سالم محمد سليم أبو عبيد	**Telephone # ٠٥٦٨- ١٨١٥١٨**	**Palestine Polytechnic University**
Contact number:٠٥٦٨ -١٨٥٠١٨	**Phone # .....**	**Southern Part Of West Bank**
Address: كفر قود \ جنين	**Fax #.....**	**unit Biotechnology Research**
Zyiad _abukhaizaran :collection by Sample	**Email :.....**	**Dr. Fawzi Al-Razem**
٠٥٦٩٦٧٢٧٩٩ # Phone	**No of samples : 3 samples (Hybrid)**	**EXT. 145 -١٩٢١-٢٢٣-٢-٩٧٢+:# Telephone**

Submitters Name: يوسف توفيق يوسف زيود	**Telephone # ٠٥٩٨٦٠٢١٠٢**	**Palestine Polytechnic University**
Contact number:٠٥٩٨ -٦٠٢١٠٢	**Phone # .....**	**Southern Part Of West Bank**
Address: السيلة الحارثية \جنين	**Fax #.....**	**unit Biotechnology Research**
Zyiad _abukhaizaran :collection by Sample	**Email :.....**	**Dr. Fawzi Al-Razem**
٠٥٦٩٦٧٢٧٩٩ # Phone	**No of samples :7 samples (Friesian)**	**EXT. 145 -١٩٢١-٢٢٣-٢-٩٧٢+:# Telephone**

Appendix 1- B Continued

اسم الموقع :مشترك (كفرقود\ السيلة الحارثية- جنين)

ملكية المزرعة للسيد :مشترك(سالم أبو عبيد\ يوسف زيود)

رقم البقرة	الرقم التسلسلي	موسم الحليب	رقم موسم الحليب	كمية الحليب مساءا	كمية الحليب صباحا	معدل الإنتاج اليومي	معدل الإنتاج الشهري	معدل الإنتاج السنوي	نوع وكمية العلف	نوع وكمية العلائق	كمية الحليب سنويا
215	J1	7	6	20	25	45	40	30	20KG	35KG	9150 L \ Year
227	J2	5	4	25	25	50	45	35	"	"	10675 L \ Year
701	J3	5	4	15	15	30	30	22	"	"	6710 L \ Year
206	J4	6	5	20	25	45	35	25	"	"	7625 L \ Year
1	J5	4	3	20	20	40	35	27	"	"	8235 L \ Year
886	J6	6	5	25	20	45	40	30	"	"	9150 L \ Year
174	J7	7	6	15	15	30	26	20	"	"	6100 L \ Year
1b	J8	8	7	8	8	16	15	14	8kg	15kg	4270 L \ Year
2b	J9	10	9	14	14	28	24	22	"	"	6716 L \ Year
3b	J10	4	3	9	8	17	16	15	"	"	4575 L \ Year

Appendix 1- B Continued

المحافظة :محافظة: محافظة جنين

الموقع :السيلة الحارثية \كفركود \ تاريخ التقييم : ١٥-٧- ٢٠١٢

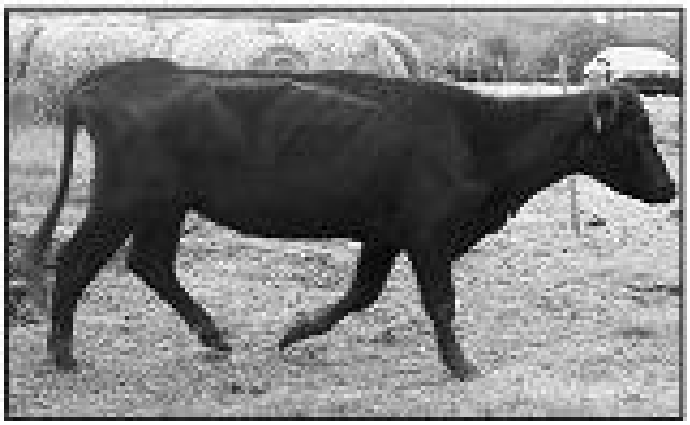
رقم البقرة	الرقم التسلسلي	تاريخ الميلاد	عدد الولادات	الأطراف		الحدبة		الهيكل العام				الضرع					التقييم العام				
				زاوية الظلف ٥%	رؤيا جانبية ٥%	ميل الحدبة ٥%	عرض الحدبة ٥%	الزوايا ١٠%	العمق ١٠%	سعة الصدر ١٠%	الارتفاع ١٠%	اربطه أمامية ١٠%	أربطة خلفية ١٠%	فاصل مركزي ١٠%	الحلمات		مظهر عام ٥٠%	أربطة الضرع ٢٠	الحدبة ١٠%	الضرع ٢٠%	التقييم النهائي ١٠٠%
															حجم ٥%	تماثل ٥%					
215	J1	7	6	4	4	3	4	9	10	10	10	8	8	8	4	3	47	16	7	15	85
227	J2	5	4	3	4	4	3	10	10	10	10	8	8	7	4	3	47	16	7	14	84
701	J3	5	4	3	3	3	3	9	10	10	10	8	8	8	4	3	45	16	6	15	83
206	J4	6	5	3	3	3	3	9	10	10	10	7	8	7	2	3	45	15	7	14	82
1	J5	5	4	4	3	4	4	10	10	10	10	8	8	8	4	3	48	16	8	14	87
886	J6	6	5	3	4	3	3	9	10	10	10	8	7	7	4	3	45	15	6	14	81
174	J7	7	6	3	3	3	3	7	10	10	10	7	7	8	3	3	45	14	6	15	81
1b	J8	8	7	2	2	2	2	7	7	8	7	6	6	5	3	2	33	12	4	10	59
2b	J9	10	9	3	2	2	2	8	8	7	9	7	7	7	3	3	37	14	4	13	68
3b	J10	4	3	3	3	2	2	9	7	7	8	7	7	7	3	3	37	14	4	13	68

Appendix 2

Information relevant to body condition scoring System (BCS) for Beef Cattle

Thin

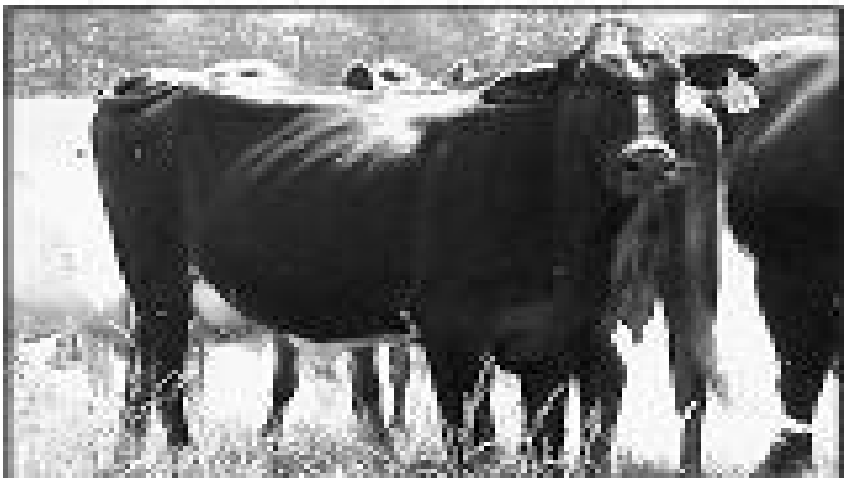
Score 1 (equivalent to 60- 75% in our study). Emaciated –The cow is severely emaciated and physically weak with all ribs and bone structure easily visible. Cattle in this score are extremely rare and are usually inflicted with a disease and/or parasitism.



Score 2 (equivalent to 75- 85 % in our study) Poor – Cow still appears somewhat emaciated but tail-head and ribs are less prominent. Individual spinous processes are still rather sharp to the touch, but some tissue cover over dorsal portion of ribs.

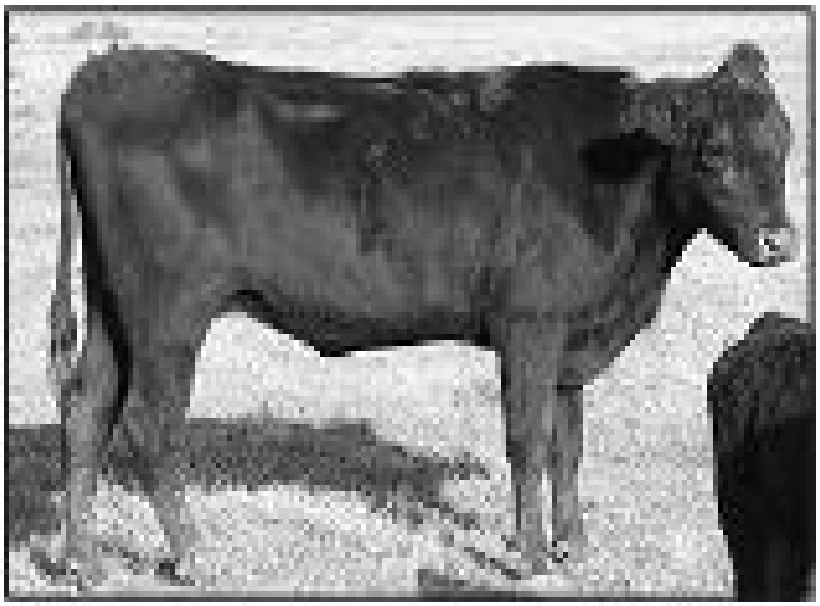
Appendix 2 – continued

Score 3. (equivalent to 80-90% in our study).Thin – Ribs are still individually identifiable but not quite as sharp to the touch. There is obvious palpable fat along spine and over tail-head with some tissue cover over dorsal portion of ribs.



Borderline

Score 4. (equivalent to 90-95% in our study) Borderline – Individual ribs are no longer visually obvious. The spinous processes can be identified individually on palpation but feel rounded rather than sharp. Some fat cover over ribs, transverse processes, and hip bones.



Appendix 2 – continued

Optimum/moderate

Score 5. (equivalent to 60-70% in our study) Moderate – Cow has generally good overall appearance. On palpation, fat cover over ribs feels spongy and areas on either side of tail-head now have palpable fat cover.

Score 6. (equivalent to 55-65% in our study). High moderate – Firm pressure now needs to be applied to feel spinous processes. A high degree of fat is palpable over ribs and around tail-head.

Appendix 2 – continued

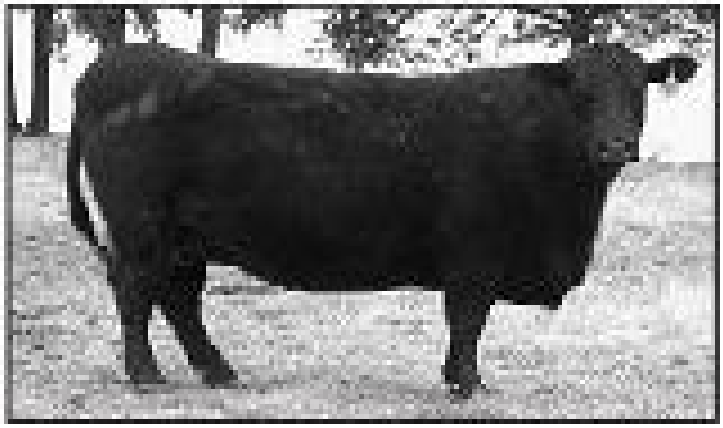
Fat

Score 7. (equivalent to 50-60% in our study). Good – Cow appears fleshy and obviously carries considerable fat. Very spongy fat cover over ribs and around tail-head. In fact, “rounds” or “pones” beginning to be obvious. Some fat around vulva and in crotch.

Score 8. (equivalent to 50-55% in our study). Fat – Cow very fleshy and over-conditioned. Spinous processes almost impossible to palpate. Cow has large fat deposits over ribs and around tail-head, and below vulva.“Rounds” or “pones” are obvious.

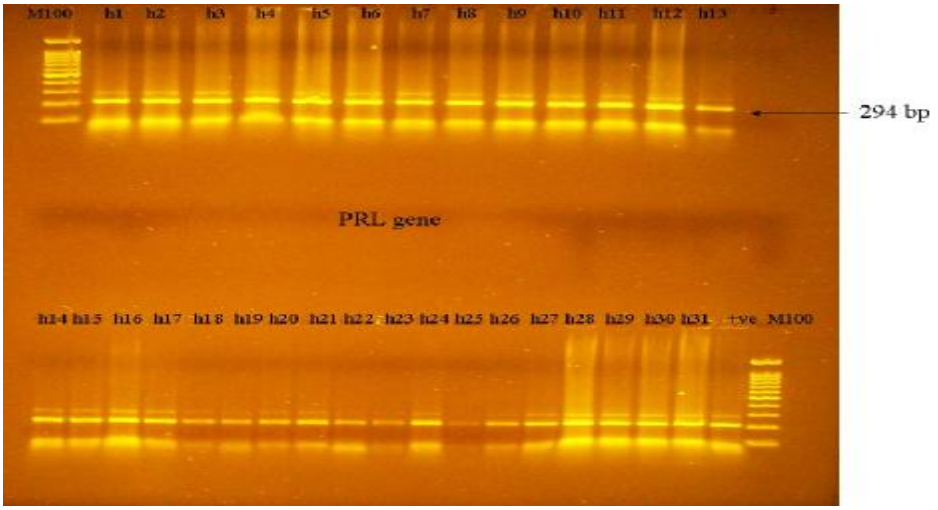
Appendix 2 – continued

Score 9. (equivalent lower 50% in our study) Extremely fat – Cow obviously extremely wasty and patchy and looks blocky. Tail-head and hips buried in fatty tissue and “rounds” or “pones” of fat are protruding. Bone structure is no longer visible and barely palpable. Animal’s mobility might even be impaired by large fatty deposits.

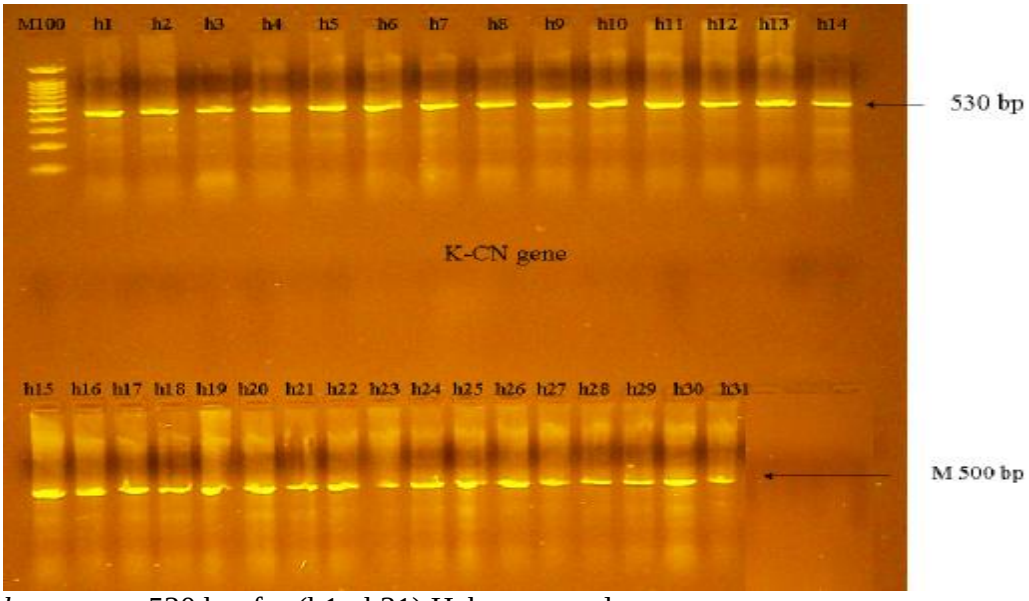


Appendix 3

PCR amplification result of three milk gene. Bands as seen on a 1.5% agarose gel stained with EtBr are of correct expected sizes for PRL ٢٩٤- bp, PIT-I ٤٥١- bp, and K-CN ٥٣٠- bp. A negative control (-ve) lacking genomic DNA template was run for each gene as shown. Lanes labeled with the M100 are the 100 bp DNA ladder. The figure below for Hebron city for three genes *(PRL, K-CN , and PIT-1)* respectively before used restriction enzyme.

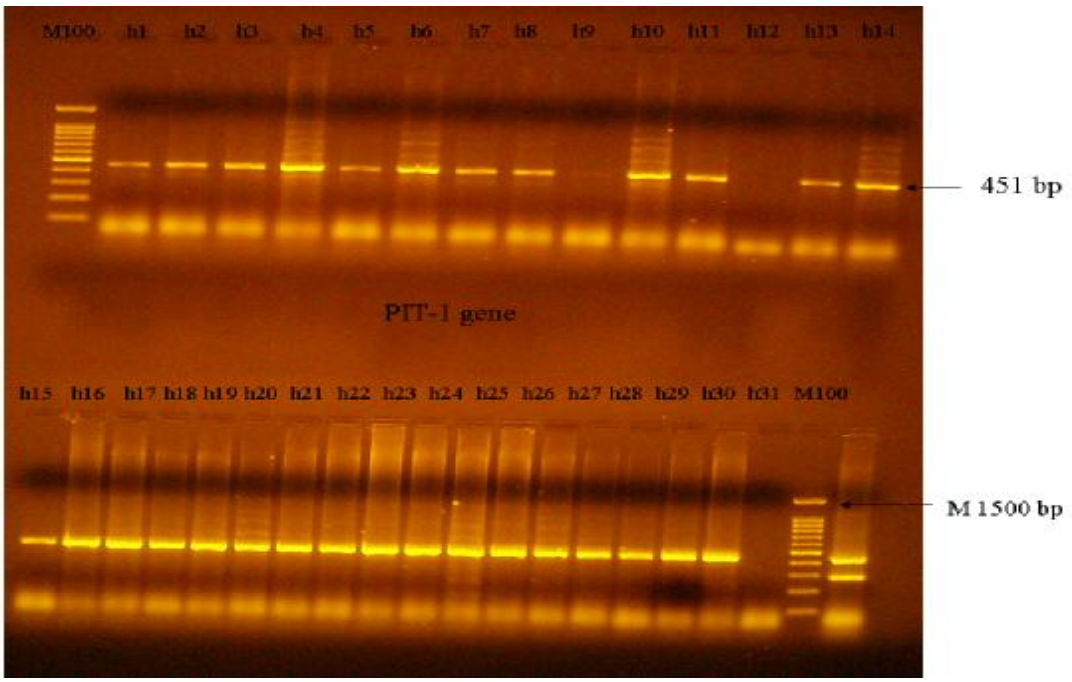


PRL- gene-294 bp for (h1 –h31) Hebron samples.



k-cn- gene-530 bp for (h1 –h31) Hebron samples.

Appendix 3- continued



Pit- 1gene-451 bp for (h1 –h31) Hebron samples.

Result of sequence and Blast n for three gene (*PRL, K-CN , AND PIT-1*) for Frisian ,

and Baladi breeds .

K-CN--h2-F

CGGTGGTTGAAAAAGGTATCCCTAGTTATGGACTCAATTACTACCAACAG
AAACCAGTTGCACTAATTAATAATCAATTTCTGCCATACCCATATTATGCA
AAGCCAGCTGCAGTTAGGTCACCTGCCCAAATTCTTCAATGGCAAGTTTTG
TCAAATACTGTGCCTGCCAAGTCCTGCCAAGCCCAGCCAACTACCATGGC
ACGTCACCCACACCCACATTTATCATTTATGGCCATTCCACCAAAGAAAA
ATCAGGATAAAACAGAAATCCCTACCATCAATACCATTGCTAGTGGTGAG
CCTACAAGTACACCTACCACCGAAGCAGTAGAGAGCACTGTAGCTACTCT
AGAAGATTCTCCAGAAGTTATTGAGAGCCCACCTGAGATCAACACAGTCC
AAGTTACTTCAACTGCAGTCTAAAAACTCTAAGGAGACATCAAAGAAGAC
AACGCAGGTAAATAAGCAAAATGAATAACAGCCAAGATTCATGGACTTAT
TAAATAAAA

Appendix 3- continued

K-CN-N7-F

AGTGGTGGAAGGTTTTCCTAGTTATGGACTCAATTACTACCAACAGAAAC
CAGTTGCACTAATTAATAATCAATTTCTGCCATACCCATATTATGCAAAGC
CAGCTGCAGTTAGGTCACCTGCCCAAATTCTTCAATGGCAAGTTTTGTCAA
ATACTGTGCCTGCCAAGTCCTGCCAAGCCCAGCCAACTACCATGGCACGT
CACCCACACCCACATTTATCATTTATGGCCATTCCACCAAAGAAAAATCA
GGATAAAACAGAAATCCCTACCATCAATACCATTGCTAGTGGTGAGCCTA
CAAGTACACCTACCACCGAAGCAGTAGAGAGCACTGTAGCTACTCTAGAA
GATTCTCCAGAAGTTATTGAGAGCCCACCTGAGATCAACACAGTCCAAGT
TACTTCAACTGCAGTCTAAAAACTCTAAGGAGACATCAAAGAAGACAACG
CAGGTAAATAAGCAAAATGAATAACAGCCAAGATTCATGGACTTATTAAA
TAAAA

Appendix 3- continued

PRL-H2 –F

TATTGGAAAATGTTTCTTGTGGTTGTTCGCATGAAGTCCTTATGAGCTTGA
TTCTTGGGTTGCTGCGCTCCTGGAATGACCCTCTCTATCACCTAGTCACCG
AGGTGCGGGGTATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATA
GAGATTGAGGAAGAAAACAAACGACTTCTGGAAGGCATGGAGATGATAT
TTGGCCAGGTGAGCAGCTTCATGAAAGCTTCCTTGCTATTTTCATGAATGA
GAGAGGTGATTTCTGT

PRL-H4-F

TTGTAAACCCCCCCAGAACCAACGATATGCATAGTTTCTTGTGGTTGTTCG
CATGAAGTCCTTATGAGCTTGATTCTTGGGTTGCTGCGCTCCTGGAATGAC
CCTCTCTATCACCTAGTCACCGAGGTGCGGGGTATGAAAGGAGCCCCAGA
TGCTATCCTATCGAGGGCCATAGAGATTGAGGAAGAAAACAAACGACTTC
TGGAAGGCATGGAGATGATATTTGGCCAGGTGAGCAGCTTCATGAAAGCT
TCCTTGCTATTTTCATGAATGAGAGAGGTGTTTTTCTGAA

PRL-H5-F

TATGAGAATGTTTCTTGTGGTTGTTCGCATGAAGTCCTTATGAGCTTGATT
CTTGGGTTGCTGCGCTCCTGGAATGACCCTCTCTATCACCTAGTCACCGAG
GTGCGGGGTATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATAGA
GATTGAGGAAGAAAACAAACGACTTCTGGAAGGCATGGAGATGATATTTG
GCCAGGTGAGCAGCTTCATGAAAGCTTCCTTGCTATTTTCATGAATGAGAG
AGGTGTTTTCTGTA

PRL-H6-F

TATGAGAATGTTTCTTGTGGTTGTTCGCATGAAGTCCTTATGAGCTTGATT
CTTGGGTTGCTGCGCTCCTGGAATGACCCTCTCTATCACCTAGTCACCGAG
GTGCGGGGTATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATAGA
GATTGAGGAAGAAAACAAACGACTTCTGGAAGGCATGGAGATGATATTTG
GCCAGGTGAGCAGCTTCATGAAAGCTTCCTTGCTATTTTCATGAATGAGAG
AGGTGTTTTCTGTA

P-J1-F

TGTTACAAAAACAAAAAACGATTATTAAGAAAAGTTCTTGGGGGTGGTCC
CCTGGAAATCCTTTAGAACTTGGTTTTTGGGTTGGTGGGCTTCTGGGAGGC
CCTTTTTATTAACCAATTAACCGGGGGCCGGGGATGAAAGGGGCCCCCAA
TGCTTTTCTTACCAGGGCCCTTAAAAATGGAGAAAAAAACCAACCAATTT

Appendix 3- continued

TGGAAAGGCTGGAAAAGAAATTTGGCCCGGGGAACCACTTTATTAAAACT
TCCTTTGCTATTTTATTGATGAAAAAAGGAATTTTTCGGAA

PRL-J2-F

AAACGAAATAGGTTTTTGGGGGTGGTGAACATGGAAATCCTTAAGAACTT
GGTTTTTGGGTTGGTGGGCTTCTGGGATGGACCTTTTTATTAACCAATTAA
CCGGGGGCCGGGGATGAAAGGGGCCCCCAATGCTTTTCTTACCGGGGCCC
TTAAAAATGGAGAAAAAAACCAACCAATTTTGGAAAGGCTGGAAAAGAA
TTTTGGCCCGGGGAACCACCCTATGAAAACTTTCTTTGTTTTTTTATGAAT
GAAAAAAGGGAATTCTTGA

PRL-J3-F

TTTATTGAGTTTGTTTCCTGTGGTTGTTCGCATGAAGTCCTTATGAGCTTGA
TTCTTGGGTTGCTGCGCTCCTGGAATGACCCTCTGTATCACCTAGTCACCG
AGGTACGGGGTATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATA
GAGATTGAGGAAGAAAACAAACGACTTCTGGAAGGCATGGAGATGATAT
TTGGCCAGGTGAGCAGCCTCATGAAAGCTTCCTTGCTATTCTCATGAATGA
GAGAGGTGATTTTCTGTA

PRL-J4-F

TTCAATGGGGAATAGTGTATCACTGTGGTGTGTTCAGCATGAGTCCTTATG
AGCTTGATTCTCTGGGTTGCTGCGCTCCTGGAATGACCCTCTGTATCACCT
AGTCACCGAGGTGCGGGGTATGAAAGGAGCCCCAGATGCTATCCTATCGA
GGGCCATAGAGATTGAGGAAGAAAACAAACGACTTCTGGAAGGCATGGA
GATGATATTTGGCCAGGTGAGCAGCCTCATGAAAGCTTCCTTGCTATTCTC
ATGAATGAGAGAGGTGATTTCTGTA

PRL-J5-F

GAGTAAAAACACAAAAAACAATTGAAATGGTTCTTGGGGGTGGTCCGATG
GAATCCTTAAGAACTTGGTTTTTGGGGTGGTGGGCTTCTGGGAGGCCCTTT
TTTTTACCTTATTAACCGGGGGCCGGGGATGAAAGGGGCCCCCAAATCTTT
CCTTACCAAGGCCCTTAAAAATGGAGAAAAAAACCAACCAATTTTGGAAA
GGCTGGAAAAGAATTTTGGCCCGGGGAACCACCTTATTAAAACTTTCTTTG
TTTTTTTATTAATGAAAAAAGGAATTTTTTGGAA

PRL-J7-F

GGTTCAAACACCAAAAAAATAAATAGATAAGTTTTGGGGGTGGTCCCTGG
AGCCTTTAGAACTTGGTTTTTGGGTTTGTGGCCTTCTGGGAGGCCCTTTTTT

Appendix 3- continued

TTAACTTATTAACCGGGGGCCGGGGATGAAAGGGACCCCCAAATCCTTTC
TTACCGGGGCCCTTAAAAATGGAGAAAAAAACCAACCAATTTTGGAAAGG
CTGGAAAAGAATTTTGGCCCGGGGAACCACTTTATTAAAACTTTCTTTGTT
TTTTTATTAATGAAAAAAGGGTTTTTCTGAAA

PIT-1-N7-F

ATACAAACAAAAAAAGATATATAAATTGGTTTTTGGGGTTGGTCCCATGG
AATCCTTTAGAACTTGGTTTTTGGGTTGGTGGGCTTCTGGGAGGCCCTTTT
TTTTAACTAATTAACCGGGGGCCGGGGATGAAAGGGGCCCCCAATGCTTT
TCTTTTCGGGGCCCTTAAAAATGGAGAAAAAAACCAACCAATTTTGGAAA
GGATGGAAAAGAATTTTGGCCCGGGGAACCACCTTATTAAAACTTTCTTT
GTTTTTTTATGAATGAAAAAAGGGATTTTTTTGGAAA

PIT-1-H7-F

TAAATTCCCACTCCCATATTGCTGCTAAGTCGCCCTGGAGTAGACACTTTG
GAGAACAGAATAAGCCTTCCTCTCAGGAGAATCCTGCGGATGGCTGAAGA
ACTAAACCTGGAGAAAGAAGTGGTGAGGGTTTGGTTTTGTAACCGAAGGC
AGAGAGAAAAACGGGTGAAGACAAGCCTnAATCAGAGTTTATTTACTATT
TCTAAGGAGCATCTCGAATGCAGATAGGCTCTCCTATTGTGTAATAGCGA
GTGTTTCTACTTTTCATTCCTTTCTCTTCTCCAGCCAAAATAGAAATTAGTT
ATTTGGTTAGCTTCAAAAAATCACATCAGTAATTTTTGCAGAAGTGTTTCT
TTTCTACTTTAAAAATAAATACAATTTAAATTATGTTGATGAATTATTCTC
AGAAGGCACATTGACCCATTA

PIT-1-J3-F

TCCTCCCCCCCTCCCCAATATTGCTGCTAAGAGCCCTGGTATAGTACACTT
TGGAGCAACAGAATAAGCCTTCCTCTCAGGAGATCCTGCGGATGGCTGAA
GAACTAAACCTGGAGAAAGAAGTGGTGAGGGTTTGGTTTTGTAACCGAAG
GCAGAGAGAAAAACGGGTGAAGACAAGCCTGAATCAGAGTTTATTTACTA
TTTCTAAGGAGCATCTCGAATGCAGATAGGCTCTCCTATTGTGTAATAGCG
AGTGTTTCTACTTTTCATTCCTTTCTCTTCTCCAGCCAAAATAGAAATTAGT
TATTTGGTTAGCTTCAAAAAATCACATCAGTAATTTTTGCAGAAGTGTTTC
TTTTCTACTTTAAAAATAAATACAATTTAAATTATGTTGATGAATTATTCTC
AGAAGGCACATTGTAACCATTTA

PIT-1-N7-F

TTCCTTTCCCTCACTCCCAATATTGCTGCTAAGCCGCCCTGGTATTAGACA
CTTTGGAGAACAGAATAAGCCTTCCTCTCAGGAGATCCTGCGGATGGCTG
AAGAACTAAACCTGGAGAAAGAAGTGGTGAGGGTTTGGTTTTGTAACCGA
AGGCAGAGAGAAAAACGGGTGAAGACAAGCCTnAATCAGAGTTTATTTAC

Appendix 3- continued

TATTTCTAAGGAGCATCTCGAATGCAGATAGGCTCTCCTATTGTGTAATAG
CGAGTGTTTCTACTTTTCATTCCTTTCTCTTCTCCAGCCAAAATAGAAATTA
GTTATTTGGTTAGCTTCAAAAAATCACATCAGTAATTTTTGCAGAAGTGTT
TCTTTTCTACTTTAAAAATAAATACAATTTAAATTATGTTGATGAATTATTC
TCAGAAGGCACATTGTAACATTA

Appendix 4

Results in relative to Friesian cattle include the body scoring , milk production ,milk yield , and three genes *(PRL,K-CN, and PIT-1)* genotypes

City	Cattle number	Serial Number	PRL-gene number of bands\size of bands	PRL-gene Genotypes\homo-hetrozygose	K-CN-gene number of bands\size of bands	K-CN-gene Genotypes\homo-hetrozygose	PIT-1-gene - number of bands\size of bands	PIT-1-gene Genotypes\homo-hetrozygose	milk Yield	Total milk production 305 Days	Total body scoring 100%	Nutrition kg
Tubas	6149	T2	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	8540	83	22 kg
Nablus	136	N1	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	2 bands \ 370-160 b.p	BB \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	6710	73	25 kg
Nablus	620	N2	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	1 band \ 451 b.p	AA \ Homo zygose	2	7625	78	25 kg

Nablus	887	N4	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	5490	76	25 kg
Nablus	502	N5	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	2	7625	71	25 kg
Nablus	718	N6	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	6100	72	25 kg
Nablus	1	N8	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	6710	66	25 kg
Tumoon	1	Tt 2	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	5490	86	29 kg

Hebron	2729	H 1	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	8063	90	45 kg
Hebron	2245	H 8	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	10931	89	45 kg
Hebron	2438	H 12	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	10180	86	45 kg
Hebron	2657	H 13	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	8708	84	45 kg
Hebron	2165	H 14	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	12121	87	45 kg
Hebron	2247	H 16	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	2	10754	86	45 kg

Hebron	2361	H 17	3 bands \ 294-162-132 b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	2	11238	91	45 kg
Hebron	2229	H 18	3 bands \ 294-162-132 b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	2	12788	93	45 kg
Hebron	2163	H 19	1 band \ 294 b.p	GG \ Homozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	12551	93	45 kg
Hebron	281	H 20	1 band \ 294 b.p	GG \ Homozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	2	12594	86	45 kg
Hebron	221	H 26	1 band \ 294 b.p	GG \ Homozygose	1 band \ 530 b.p	AA \ Homozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	2	12004	92	45 kg
Hebron	2684	H 27	1 band \ 294 b.p	GG \ Homozygose	1 band \ 530 b.p	AA \ Homozygose	2 bands \ 244-207 b.p	BB \ Homozygose	2	9540	96	45 kg

Hebron	7600	H 28	3 bands \ 294-162-132 b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	2	13113	95	45 kg
Hebron	2397	H 30	1 band \ 294 b.p	GG \ Homozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	2	10485	92	45 kg
Hebron	2149	H 31	1 band \ 294 b.p	GG \ Homozygose	1 band \ 530 b.p	AA \ Homozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	2	13497	92	45 kg
hebron	9084	h 4	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	11533	92	44 kg
hebron	1025	h 6	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	2	11368	88	44 kg
hebron	9129	h 7	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	11096	93	44 kg
hebron	9228	h 8	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	2	11181	93	44 kg

hebron	7252	h 9	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	10029	85	44 kg
hebron	239	h 11	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homo zygose	2	9035	92	44 kg
hebron	263	h 13	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	12883	98	44 kg
hebron	98	h 14	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	11238	88	44 kg
hebron	448	h 15	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	12008	96	44 kg
hebron	1019	h 17	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	10704	93	44 kg
hebron	251	h 18	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homo zygose	2	11850	92	44 kg
hebron	920	h 20	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	12013	93	44 kg
hebron	96	h 23	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	2	14402	92	44 kg

hebron	1091	h 24	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	2	9581	85	44 kg
hebron	9183	h 33	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	12212	94	44 kg
hebron	9129	h 34	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	11096	90	44 kg
hebron	140	h 35	3 bands \ 294-??b.p	AG \ Hetrozygose	2 bands \ 370-160 b.p	BB \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	10917	91	44 kg
hebron	246	h 37	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	10531	91	44 kg
hebron	236	h 38	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	9861	93	44 kg
hebron	214	h 40	3 bands \ 294-??b.p	AG \ Hetrozygose	2 bands \ 370-160 b.p	BB \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	2	13459	92	44 kg
Tubas	530	T5	1 band \ 294 b.p	GG \ Homozygose	1 band \ 530 b.p	AA \ Homozygose	2 bands \ 244-207 b.p	BB \ Homozygose	3	7625	72	19 kg

Nablus	652	N3	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	9150	74	25 kg
Nablus	369	N7	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	3	1525	79	25 kg
Nablus	2400	N9	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	3	8540	86	25 kg
Nablus	3929	N10	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	3	6710	80	25 kg
Tumoon	888	Tt 4	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	3	7625	88	29 kg
Tumoon	976	Tt 7	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	4880	84	29 kg

Hebron	391	H 2	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	11411	92	45 kg
Hebron	7708	H 3	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	11582	90	45 kg
Hebron	2586	H 4	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	1 band \ 451 b.p	AA \ Homo zygose	3	9122	88	45 kg
Hebron	2108	H 5	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	3	11411	87	45 kg
Hebron	2412	H 6	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	11624	85	45 kg
Hebron	2888	H 7	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	3	12121	83	45 kg

Hebron	2366	H 9	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	3	12750	86	45 kg
Hebron	2384	H 11	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	11556	86	45 kg
Hebron	2373	H 15	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	3	10432	85	45 kg
Hebron	33	H 21	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	10931	93	45 kg
Hebron	2347	H 22	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	Ą \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	10310	93	45 kg
Hebron	2078	H 23	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	1 band \ 451 b.p	AA \ Homo zygose	3	10335	92	45 kg

Hebron	2680	H 24	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	1 band \ 451 b.p	AA \ Homo zygose	3	9150	93	45 kg
Hebron	2384	H 25	1 band \ 294 b.p	GG \ Homo zygose	2 bands \ 370-160 b.p	BB \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	3	11556	93	45 kg
Hebron	2378	H 29	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	3	10651	93	45 kg
hebron	17	h 1	3 bands \ 294-??b.p	AG \ Hetroz ygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	1 band \ 451 b.p	AA \ Homo zygose	3	10675	81	44 kg
hebron	225	h 2	3 bands \ 294-??b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	3	10741	86	44 kg
hebron	183	h 3	3 bands \ 294-??b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	1 band \ 451 b.p	AA \ Homo zygose	3	11610	85	44 kg
hebron	218	h 5	3 bands \ 294-??b.p	AG \ Hetroz ygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	1 band \ 451 b.p	AA \ Homo zygose	3	12492	87	44 kg

hebron	186	h 10	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	3	9144	93	44 kg
hebron	78	h 12	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	12238	93	44 kg
hebron	1711	h 16	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	3	7877	91	44 kg
hebron	182	h 19	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	8902	87	44 kg
hebron	121	h 21	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	3	14496	87	44 kg
hebron	507	h 22	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	3	8467	93	44 kg
hebron	7800	h 25	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	10959	84	44 kg
hebron	670	h 26	3 bands \ 294-??b.p	AG \ Hetrozygose	2 bands \ 370-160 b.p	BB \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	9238	92	44 kg

hebron	30	h 27	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	3	10034	95	44 kg
hebron	158	h 28	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	9383	90	44 kg
hebron	46	h 29	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	14099	92	44 kg
hebron	63	h 30	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	10551	92	44 kg
hebron	36	h 31	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	3	11110	90	44 kg
hebron	994	h 32	3 bands \ 294-??b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	1 band \ 451 b.p	AA \ Homozygose	3	7347	92	44 kg
hebron	3	h 36	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	10985	92	44 kg
hebron	76	h 39	3 bands \ 294-??b.p	AG \ Hetrozygose	1 band \ 530 b.p	AA \ Homozygose	1 band \ 451 b.p	AA \ Homozygose	3	11376	91	44 kg

Tubas	2987	T1	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	4	9150	82	22 kg
Tubas	5123	T4	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	4	7930	80	22 kg
jerico	1008	j 6	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	4	6100	72	25 kg
Jenin	227	J 2	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	2 bands \ 244-207 b.p	BB \ Homo zygose	4	10675	84	58 kg
Jenin	701	J 3	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	4	6710	83	58 kg
Jenin	1	J 5	1 band \ 294 b.p	GG \ Homo zygose	3 bands \ 530-370-160 b.p	AB \ Hetroz ygose	2 bands \ 244-207 b.p	BB \ Homo zygose	4	8235	87	58 kg

Tumoon	7496	Tt 5	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	2 bands \ 244-207 b.p	BB \ Homo zygose	4	5185	82	29 kg
Tumoon	8366	Tt 6	3 bands \ 294-162-132 b.p	AG \ Hetroz ygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	4	6100	82	29 kg
Hebron	3028	H 10	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	4	11580	87	45 kg
Tubas	12	T3	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	5	7930	80	22 kg
Tumoon	84	Tt 1	1 band \ 294 b.p	GG \ Homo zygose	1 band \ 530 b.p	AA \ Homo zygose	3 bands \ 451-244-207 b,p	AB \ Hetroz ygose	5	7625	90	29 kg

Tumoon	1294	Tt 3	3 bands \ 294-162-132 b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	5	7625	91	29 kg
Jenin	215	J 1	1 band \ 294 b.p	GG \ Homozygose	1 band \ 530 b.p	AA \ Homozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	6	9150	85	58 kg
Jenin	206	J 4	1 band \ 294 b.p	GG \ Homozygose	1 band \ 530 b.p	AA \ Homozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	6	7625	82	58 kg
Jenin	886	J 6	1 band \ 294 b.p	GG \ Homozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	6	9150	81	58 kg
Jenin	174	J 7	3 bands \ 294-162-132 b.p	AG \ Hetrozygose	3 bands \ 530-370-160 b.p	AB \ Hetrozygose	3 bands \ 451-244-207 b,p	AB \ Hetrozygose	7	6100	81	58 kg