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Relationship between ND5 Genetic Polymorphism and Milk Production and the Growth of Lambs before Weaning of Awassi Sheep

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Abstract: ***This study was conducted at the Genetic Engineering Laboratory/College of Agriculture/University of Basrah. It aimed at the measuring the genetic polymorphism of the ND5 gene within the mitochondria of Awassi sheep and its relation to milk yield and the growth of lambs before weaning. A total of 87 sheep was used, in addition to their single lambs. Blood and milk samples were collected from ewes every 2 weeks till the age of weaning. Lambs were weighed from one day to the end of the weaning age once each fortnight. DNA was extracted from the blood and PCR technology was used to amplify ND5 factor. The Alignment sequences were lined up and aligned with the sequences of ND5 gene published within the National Biotechnology info Centre (NCBI). Haplotype and nucleotidediversities, Analysis of Molecular Variation (AMOVA) and haplotype network were established and evolution tree was constructed. ND5 gene showed thirty-seven polymorphism forms in the Awassi sheep breed. The diversity of the haplotype (HD) and the nucleotide diversity (π) of the were high (0.942, 0.01563 respectively). Total number of haplotypes (H) were 21 for different breeds of sheep used in this study, while that of Awassi was 13. The number of haplogroup was fourand evolution tree showed 3 main branches among sheep breeds in different countries, Awassi sheep showed separate branch. The results of the molecular variation AMOVArevealed that the variation within the breeds is larger than the variation between breeds (21.93% and 78.07% respectively). The overall average milk production, birth weight and weaning weight were 522.99 g, 4.52 kg, and 17 kg respectively.ND5 polymorphism has a significant effect on weaning weight, however, its effect was insignificant on milk yield and its chemical components.***

Keywords: ND5, Mitochondria, Awassi sheep, Genetic polymorphism

1. Introduction

Sheep are one of the agricultural animals that play an important role in the economies of the peoples of many countries of the world, and has been cultivated since ancient times. Sheep occupied an important place in human life because of their clear effects onhuman nutrition and daily requirements (Welham, 2006). Increasing attention has been paid to the genetic improvement of sheep, especially local breeds, for the purpose of enhancing production and preserving their genetic structures (Mohammadabadi and Sattayimokhtari, 2013). Improving the efficiency of sheep economic and biological is done by studying the genetic diversity of traits and increasing the performance of ewes in all aspects (Vajed Ebrahimi et al., 2017). Fat-tailed sheep account for 25% of the total number of sheep around the world and have the capacity to store approximately 20% of body fat (Yousefi et al., 2012). These breeds are widely spread in a number of countries, including Iraq, Iran, Syria, Jordan, Turkey, Lebanon, Palestine, Indonesia, Ethiopia, South Africa, Zimbabwe, China, Afghanistan and Pakistan (Pourlis, 2011).

Recent studies have shown that ND5 is an important source in evolutionary studies and is a good and useful marker in the study of genetic variation, evolution, and relationships between species and breeds (Semyenova et al., 2006; Zarowiecki et al., 2007; Itagaki et al., 2009; Mera et al., 2009). In sheep, there were five haplogroups that were distributed to a number of breeds. The individual group HPG A was observed in the Asian breeds, HPG B in the European breeds and HPG C, D, E in Near East sheep breeds (Meadows et al. 2007). Othman et al. (2015) found three haplogroups, HPG A, B, C in the ItalianMuflon and Sarda breeds and the Egyptian Barki, Ossimi, and Rahmani sheep breeds. Anna and Ewa (2016) found the haplogroups HPG A and B in Polish sheep breeds.

The genetic characterization of mitochondrial DNA is important in animals, including sheep.Therefore, the present study aimed at measuring the relationship between the polymorphism of ND5 gene, milk production, lamb's growth until weaning, and calculating the number of haplotypes and haplogroups within and between sheep breeds.As well as the calculation of haplotype and the AMOVA and the neutrality test in Iraqi Awassi sheep.

2. Materials and Methods

2.1 DNA extraction and gene sequencing

The study was conducted for the period from 30/11/2016 to 22/2/2017. At the laboratory of Genetic Engineering at the University of Basrah, following with the collection of data from the field up to 20/3/2018. The study included the use of 87 ewes in addition to their lambs up to the weaning at the age of 90 days. The blood samples (5ml/ewe) from the jugular vein were collected. The amount of milk production was calculated in the morning (morning milking). The amount of daily milk yield was calculated as twice the amount of morning milk production. The growth rate of the lambs was measured once every two weeks up to the age of 30 days and then monthly up to the weaning age (1, 15, 30, 60 and 90 days).

The analyses were carried out on 89 Awassi ewes. Blood from each ewe was sampled intravitally into sterile vacuum tubes containing K2EDTA (dipotassium ethylene diamine

tetra acetic acid) anticoagulant. A fragment (1053 Pb) of the mtDNA ND5 in the reference ovine mitochondrial genome Accession number NCBI af010406.1was amplified using two primers: Forward primer: F 5'-AATAGTTTATCCAGTTGGTCTTAGG -3' and Reverse primer: R 5'- AAGATTTGTTGGAGATCTCAGGTG -3 (Tserenbataa *et al*, 2004). The PCR amplifications were conducted in a 50 µl volume containing 20 ng genomic DNA, 25 µl of Master Mix, 2 µl each primer, 15 µl free water. The amplification conditions were as follows: initial denaturation at 95 C for 5 min followed by 36 cycles of denaturation at 95 C for 1 min, annealing at 52 C for 1min, and extension at 72 C for 1.5 min, and then the final extension at 72 C for 10 min. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success. The amplified products were purified with a DNA purification kit (SSufine) according to the manufacturer's instructions to remove residual primers and dNTPs. Sequencing was performed in sync ™ DNA Extraction Kit was used for DNA extraction and manufactured by the Taiwanese Geneaid company.

2.2 Data Analysis

COI sequences were aligned using the BioEdit software (Hall, 1999). Haplotype diversity (HD) and nucleotide diversity (π) were analyzed using DnaSP v5. 10 software (Librado and Rozas, 2009). Genetic distance, molecular variation (AMOVA) and neutrality test were analyzed using Arlequin 3.5.1.2 software (Excoffier and Lischer, 2010). The haplotypes network was drawn using Network 5.0.0.0 software (Bandelt et al., 1999). Neighbor-joining (NJ) tree for testing camel breed sequences and the phylogenetic tree between Aussie and other sheep breeds in the world were constructed using Megaversion 7.0 software (Kumar *et al*, 2016).

2.3 Statistical analysis

The Completely Randomized Design (CRD) was used to analyze the data of study traits. Means were compared using the General Linear Model within SPSS (2016) Statistical program Version 24. The model included haplotypes and ewe parity.

3. Results and Discussion

The results of the DNA extraction showed that the concentration was from 24.3 to 65.3 ng/µl and that the purity of 260/280 ranged from 1.68 to 2.03, which was detected by a Nanodrop device. The results of the electrophoresis on the agarose gel 2% showed the success of deoxyribonucleic acid amplification (Fig. 1), as the primer of ND5 gave a fraction of 657 bp.

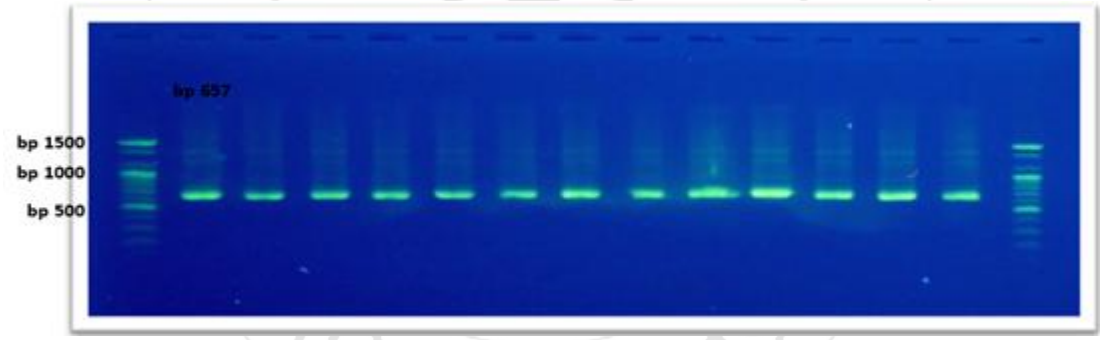


Figure 1: The amplification product of the ND5 gene on the agarose gel 2%

3.1 Genetic diversity

The results of ND5 genetic diversity showed that there were 20 total sequences and that the number of haplotypes were 13 (H) resulting in 37 genetic polymorphisms (NH) and the values of haplotype diversity (HD) and nucleotide diversity (π) were high in the Awassi sheep breed, reaching 0.942 and 0.01563 respectively (table 1).

Table 1: The genetic diversity of the Iraqi Awassi sheep breed according to the gene ND5

Gene	Number of Sequences (N)	Haplotype (H)	Number of Polymorphic (NH)	Haplotype Diversity (HD)	Nucleotide Diversity (π)
ND5	20	13	37	0.942	0.01563

3.2 Haplotype Network

The results showed that the total number of Haplotype for ND5 gene in the Iraqi Awassi breed was 13 haplotypes.When compared to other breeds of the world, the total number of haplotypeswas21, of which 13 were for the Awassi breed and 3 haplotypes for Finn, Tibetan and Merinizzata breeds, two haplotypesfor Comisana, Lacaune, Gentile, Mouflon and Assaf breeds and only one haplotype for Merino, Karadi, Morkaraman, Hamdani, Sopravissana and Karakas breeds. The H-9 haplotype pattern was shared by the breeds Awassi, Hamdani, Tibetan, Merinizzata, Comisana and Assaf. The H-11 haplotype pattern is shared by the breeds Awassi, Tibetan and Merinizzata. While the H-12 haplotype was shared by theAwassi, Karadi, Tibetan, Merinizzata, Comisana, Assaf, Merino, Finn, Sopravissana, Gentile and Karakas breeds.The haplotypes H1-H13 were found in the Awassi breed, and the haplotypes H-14 was found in the Morkaraman breed, while the haplotypes H-15 and H-16 were found in the Lacaune breed, H-17, H-18 as well as the common H-12 in the Finn breed, the haplotype H-19 and the common H-12 in The Gentile breed, the haplotype H-20 and H-21 in the Mouflon breed (Fig. 2). Some breeds did not contain any independent haplotype, the

Comisana and Assaf breed were within the common pattern of H-9 and H-12, the Tibetan and Merinizzata breed in haplotype common H-9, H-11 and H-12. While the strain was both Merino, Karadi, Sopravissana and Karakas within the common H-12 pattern. Finally, the Hamdani breed was within the common haplotype H-9.

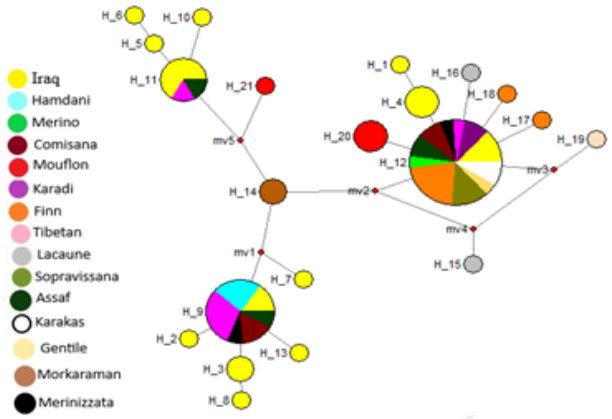
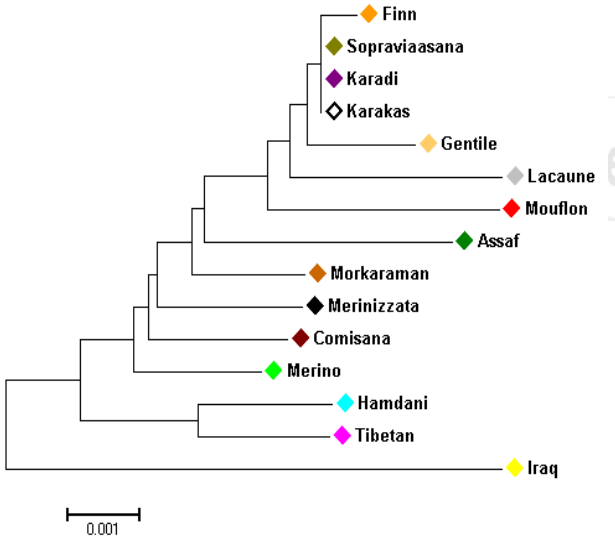


Figure 2: The haplotypes of ND5 Gene among some states

3.3 Phylogenetic tree of ND5 gene

The results of the tree of evolution of ND5 gene showed that there were three main branches. The first branch included the Iraqi Awassi breed.While the second branch divided into two branches, the first branch was presented bythe Tibetan breed and the second Hamdani breed (Fig. 3). The third main branch was divided into several sub branches, each branch consisted of either the Merino breedorComisana breedorMerinizzata breedorMorkaraman breed. Those branches were combined witha branch of Assaf breed and containing a Mouflon breedor Lacaune breedor Gentile breed. The whole branches were related to the branches that included thebreeds Karakas, Karadi, Sopravissana and Finn.



Form 3: The Phylogenetictree of ND5 gene between some sheep breeds of different countries

3.4 Analysis of molecular variance AMOVA

The results of the AMOVA of ND5gene between and within sheep breeds showed that genetic variation between breeds was 21.93% and the variation within breeds was 78.07% (table 2).This is illustrated by the fact that genetic variation within breeds is much greater than genetic variation among breeds, which may be due to the fact that the Iraqi Awassi sheep breeds have the same mother origin (Moradi *et al*., 2017). This result agreed with the findings of Rodriguez *et al*., (2015) and Moradi *et al*., (2017), who found that genetic variation within breeds is greater than genetic variation between breeds.This result agreed with some studies carried out on other animals such as goats (Silva *et al*., 2017), cows (Ozsensoy and Kurar, 2014) and horses (Cardinali *et al*., 2016).

Table 2: Analysis Molecular Variance of ND5 gene with breeds in the world

Source of variation	Degree of freedom	Sum of squares	Variance components	Variation %
Between Breeds	14	10.091	0.09390	21.93
Within Breeds	51	17.045	0.33422	78.07
Total	65	27.136	0.42812	

3.5 Milkyield, birth weight and weaning weight

The overall mean of milk production was 522.99 g/d(table, 3). This figure is in linewith the findings of Al-Samarai and Al-Anbari (2009) as they recorded 486.66 (g/day) in the Iraqi Awassi. Talafha and Ababneh (2011) showed the value of 505 (g/day).

While the overall mean of birth weight was 4.52 kg (table 3). The present finding was in agreement with other Awassi figures (Abdulrahman *et al*., 1999 and Jawasra, 2000). The overall mean weaning weight (17.73 kg) was similar to what Matika *et al*., (2003) estimated in Turkish Sabi sheep (18.5 and 16.6 kg) for both males and females, respectively.The present finding was less than many sheep breeds in the world (Jafaroghli *et al*., 2013; Mirhoseinia *et al*., 2015; Gholizadeh and Ghafouri-kesbi, 2016; Anon, 2017).

When studying the effect of ND5 gene on milk production and birth weight, no significant effect of this gene was observed on any of the studied traits (table 3).The highest production of milk (567.60 g) of ewes belongs to the twelfth haplotype but did not reach the level of significance while the lowest production of milk (486.00) for the eleventh haplotype.

As for weaning weight, it was found that there was a significant effect ($P < 0.05$) for ND5 Gene.The twelfth haplotype recorded highest weaning weight, with an average of (19.03 kg). All other haplotypes revealed similar weaning weight ranged 16.47-17.80 kg. Since this gene has significant effect on weaning weight of Awassi sheep, it can be considered as a marker to this trait. Polymorphism of the ND5 gene was shown to be associated with better body height, body length, body weight, and average daily gain at 6 monthsin the Nanyang breed, haplotype B was likely to have a positive effect on growth traits (Zhang et al, 2008). Recent studies have implicated mitochondrial function as being involved in the feed efficiency of livestock including cattle (Carstens and Kerrly, 2009), sheep (Clop et al, 2006), pigs (Grubbs et al, 2015) and poultry (Bottje et al 2006). As well as the results indicate the mitochondria may play a role

in residual feed intake in animals and that genes involve in energy metabolism may have variants that affect efficiency (Zulkifli, 2016). These results may explain the significant effect of ND5 polymorphism on lamb weaning weight in the present study.

Table 3: Effect of gene ND5 on daily milk production (gm), birth weight (kg) and weaning weight (kg), mean ± SD

Traits	Mean	Haplotype	Mean
Daily milk yield	522.99±90.25	H9	524.00±33.46
		H11	486.00±49.50
		H12	567.60±112.02
		Others*	521.37±90.09
Birth weight	4.52±0.70	H9	4.30±0.42
		H11	4.80±0.14
		H12	4.56±0.36
		Others	4.51±0.74
Weaning weight	17.73±0.29	H9	17.80±0.22 b
		H11	16.47±0.17 b
		H12	19.03±0.33 a
		Others	16.68±0.29 b

Means with different characters vary significantly at 5% (weaning weight) * other = other haplotypes

4. Conclusions

Iraqi Awassi sheep showed a high genetic diversity of the ND5 gene, demonstrating the closeness of these sheep to their domestication areas.The tree of evolution has shown the presence of Iraqi Awassi with a separate branch from other sheep breeds.Genetic variation within breed was greater than genetic variation among breeds.There was a significant effect of ND5gene on the weight of weaning and can play as a genetic marker to this trait.

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