

# 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 ( $\pi$ ) 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 four and evolution tree showed 3 main branches among sheep breeds in different countries, Awassi sheep showed separate branch. The results of the molecular variation AMOVA revealed 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 on human 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 Italian Muflon 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 af010406.1 was 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 ( $\pi$ ) 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.

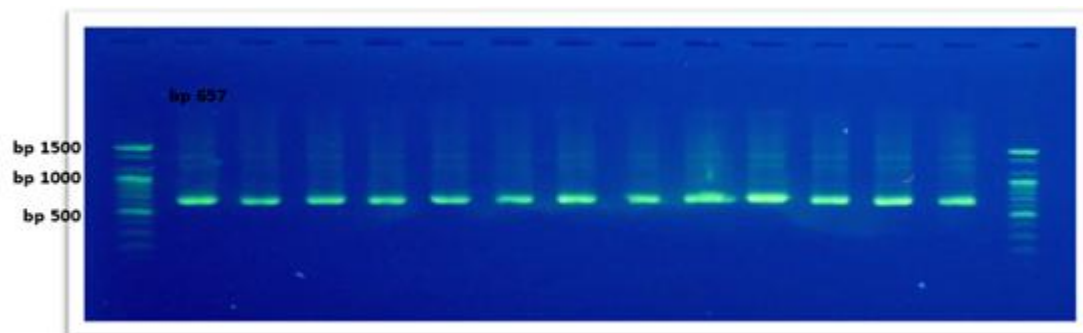


Figure 1: The amplification product of the ND5 gene on the agarose gel 2%

## 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 ( $\pi$ ) 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 ( $\pi$ )
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 haplotypes was 21, of which 13 were for the Awassi breed and 3 haplotypes for Finn, Tibetan and Merinizzata breeds, two haplotypes for 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 the Awassi, 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.

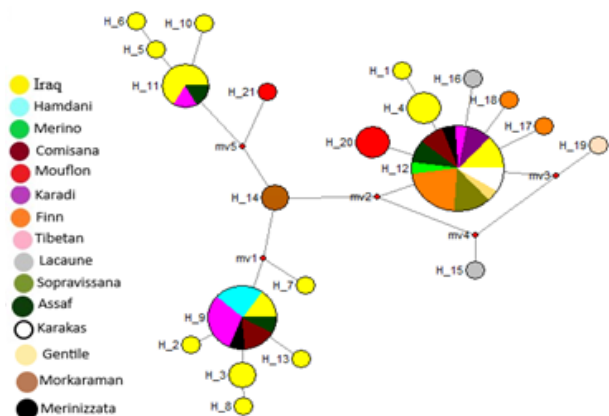
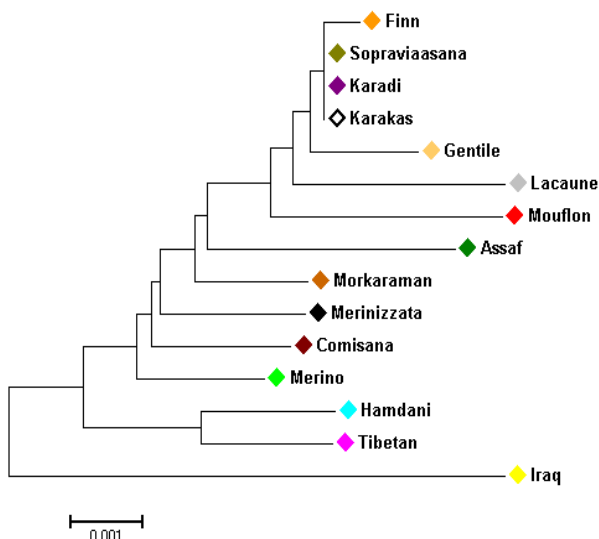


Figure 2: The haplotypes of ND5 Gene among some states

### 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 by the 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 breed or Comisana breed or Merinizzata breed or Morkaraman breed. Those branches were combined with a branch of Assaf breed and containing a Mouflon breed or Lacaune breed or Gentile breed. The whole branches were related to the branches that included the breeds Karakas, Karadi, Sopravissana and Finn.



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

### 3.4 Analysis of molecular variance AMOVA

The results of the AMOVA of ND5 gene 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 line with 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 months in 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  $\pm$  SD

Traits	Mean	Haplotype	Mean
Daily milk yield	522.99 $\pm$ 90.25	H9	524.00 $\pm$ 33.46
		H11	486.00 $\pm$ 49.50
		H12	567.60 $\pm$ 112.02
		Others*	521.37 $\pm$ 90.09
Birth weight	4.52 $\pm$ 0.70	H9	4.30 $\pm$ 0.42
		H11	4.80 $\pm$ 0.14
		H12	4.56 $\pm$ 0.36
		Others	4.51 $\pm$ 0.74
Weaning weight	17.73 $\pm$ 0.29	H9	17.80 $\pm$ 0.22 b
		H11	16.47 $\pm$ 0.17 b
		H12	19.03 $\pm$ 0.33 a
		Others	16.68 $\pm$ 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 ND5 gene on the weight of weaning and can play as a genetic marker to this trait.

#### References

- [1] Abdulrahman, Fares Younis, Khattab, Ghazi Khazali Abdullah, Ghassan Ibrahim. (1999). Genetic and phenotypic features of growth qualities in Awassi sheep. *The Journal of the cultivation of Mesopotamia*. 31 (1): 38-48.
- [2] Al-Samarai, F. R. and Al-Anbari, N. (2009). Genetic evaluation of rams for total milk yield in Iraqi Awassi sheep, *ARPN Journal of Agricultural and Biological Science*. 4: 54-57.
- [3] Anna, K. and Ewa, S. (2016). Mitochondrial control region diversity in Polish sheep breeds. *Arch. Anim. Breed.* 59: 227-233.
- [4] Anon. (2017). Annual Report, 2016-17. ICAR-Central Sheep and Wool Research Institute, Avikanagar.
- [5] Bandelt, H. j.; Forster, P. and Rohl, A. (1999). Median-Joining Networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, 16(1): 37-48.
- [6] Bottje, W. Pumford, N. R. Dirain, C. O. Iqbal, M. and Lassiter, K. (2006). Feed efficiency and mitochondria function. *Poultry Science*, 85: 8-14.
- [7] Cardinali, I.; Lancioni, H.; Giontella, A.; Cabodiferro, M.; Capomaccio, R. and Buttazzoni, S. (2016). An Overview of Ten Italian Horse breeds through Mitochondrial DNA. *PLoS ONE*. 11(4): e0153004.
- [8] Carstens, G.E. and Kerley, M.s. (2009). Biological basis for variation in energetic efficiency of beef cattle.

- Proceeding of the Beef Improvement Federation 41<sup>st</sup> Annual Research Symposium, 124-131.
- [9] Clop, A. Marcq, F. Takeda, H. Pirottin, D. Tordoir, X. Bibe, B. Bouix, J. Caiment, F. Elsen, J.M. Eychenne, F. and Larzul, C. (2006). A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nature Genetics*, 38 (7): 813-818.
  - [10] Excoffier, L. and Lischer, H. E. (2010). Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10: 564-567.
  - [11] Gholizadeh, M. and Ghafouri-Kesbi, F. (2016). Inbreeding depression in growth traits of Baluchi sheep. *Small Ruminant Research* 144: 184-190.
  - [12] Grubbs, J. K. Fricthen, A. N. Huff-Lonergan, E. Dekkers, J. C. Gabler, N. K. and Lonergan, S. M. (2015). Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs. *Journal of Animal Science*, 91 (5): 2133-2140.
  - [13] Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp.* 41: 95-98.
  - [14] Itagaki, T.; Sakaguchi, K.; Terasaki, K.; Sasaki, O.; Yoshihara, S. and Van Dung, T. (2009). Occurrence of spermic diploid and aspermic triploid forms of *Fasciola* in Vietnam and their molecular characterization based on nuclear and mitochondrial DNA. *Parasitol. Int.* 58: 81-85.
  - [15] Jafaroghli, M.; Rashidi, A.; Mokhtari, M. S. and Mirzamohammadi, E. (2013). Estimation of genetic parameters for body weight traits in Baluchi sheep. *Journal of Livestock Science and Technologies*. 1: 28-33.
  - [16] Jawasra, Khalil Ibrahim Zaal. (2000). Estimation of some of the genetic and non-genetic factors of some of the characteristics of growth in Jordanian Awasia sheep. Master thesis. college of Agriculture-University of Baghdad.
  - [17] Kumar, S.; Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-1874.
  - [18] Librado, P. and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*. 25 (11) : 1451-1452.
  - [19] Matika, O.; Van Wyk, J. B.; Erasmus, G. J. and Baker, R. L. (2003). Genetic parameter estimates in Sabi sheep. *Livestock Production Science*. 79: 17-28.
  - [20] Meadows, J. R.; Cemal, I.; Karaca, O.; Gootwine, E. and Kijas, J. W. (2007). Five ovine mitochondrial lineages identified from sheep breeds of the Near East. *Genetics*. 175: 1371-1379.
  - [21] Mera, y.; Sierra, R.; Artigas, P.; Cuervo, P.; Deis, E.; Sidoti, L.; Mas-Coma, S. and Bargues, M. D. (2009). Fascioliasis transmission by *Lymnaea neotropic* confirmed by nuclear rDNA and mtDNA sequencing in Argentina. *Vet. Parasitol.* 166: 73-79.
  - [22] Mirhoseinia, S. Z.; Zarea, J.; Hossein-Zadeha, N. G.; Khanzadeha, H.; Seidavib, A.; Laudadioc, V.; Darioc, C.; Tufarellic, V. and Selvaggi, M. (2015). Estimation of genetic parameters for body weight traits and pelt

- quality score in Iranian Karakul sheep. *Small Ruminant Research* 132: 67-71.
- [23] Moradi, M. H.; Phua, S. H.; Hedayat, N.; Khodaei-Motlagh, M. and Razmkabir, M. (2017). Haplotype and Genetic Diversity of mtDNA in Indigenous Iranian Sheep and an Insight into the History of Sheep Domestication. *J. Agr. Sci.* 19: 591- 600.
- [24] Othman, E.; Lorraine, P.; Esraa, A. B. and Marco, M. (2015). Genetic characterization of Egyptian and Italian sheep breeds using mitochondrial DNA. *Journal of Genetic Engineering and Biotechnology.* 13: 79–86.
- [25] Ozsensoy, Y. and Kurar, E. (2014). Genetic diversity of native Turkish cattle breeds: Mantel, AMOVA and bottleneck analysis. *J. Adv. Vet. Anim. Res.* 1(3) : 86-93.
- [26] Pourlis, A. F. (2011). A review of morphological characteristics relating to the production and reproduction of fat-tailed sheep breeds. *Tropical Animal Health Production.* 43: 1267-1287.
- [27] Rodriguez, M. A.; Gasca-Pineda, J.; Medellin, R. A. and Eguiarte, L. E. (2015). Analysis of Genetic diversity of Bighorn Sheep (*Ovis Canadensis*) from Mexican Population. *Journal of Mammalogy.* 96 (3): 473-480.
- [28] Semyenova, S. K.; Morozova, E. V.; Chrisanfova, G. G.; Gorokhov, V. V.; Arkhipov, I. A.; Moskvina, A. S.; Movsessyan, S. O. and Ryskov, A. P. (2006). Genetic differentiation in eastern European and western Asian populations of the liver fluke. *Fasciola hepatica*, as revealed by mitochondrial *nad1* and *cox1* genes. *J. Parasitol.* 92: 525–530.
- [29] Silva, N. M. V.; Pimenta, F. E. C.; Arandas, J. K. G.; Gomes, F. M. A.; Ferreira, E.; Del, C. I.; Fonseca, C. and Ribeiro, M. N. (2017). Polymorphism of mitochondrial DNA in the Brazilian Caninde Goat breed. *Genetics and Molecular Research.* 16(2): gmr 16029656.
- [30] SPSS(2016). *Statistical Package for Social Science, User's Guide for statistics Version 24*, Copyright IBM, SPSS Inc., USA.
- [31] Talafha, A. and Ababneh, M. (2011). Awassi sheep reproduction and milk production: Review. *Tropical Animal Health and Production.* 43: 1319-1326.
- [32] Tserenbataa, T.; Ramey, R. R.; Ryder, O. A.; Quinn, T. W. and Reading, R. P. (2004). A population genetic comparison of argali sheep (*Ovis ammon*) in Mongolia using the ND5 gene of mitochondrial DNA: implications for conservation. *Mol. Ecol.* 13:1333-1339.
- [33] Yousefi, A. R.; Kohram, H.; Shahneh, A. Z.; Nik-khah, A. and Campbell, A. W. (2012). Comparison of the meat quality and fatty acid composition of traditional fat-tailed (Chall) and tailed (Zel) Iranian sheep breeds. *Meat Science.* 92: 417-422.
- [34] Zhang, B. Chen, H. Hua, L. Zhang, C. Kang, X. Wang, X. Pan, C. Lan, X. and Lei, C. (2008). Novel SNPs of the mtDNA ND5 Gene and Their Associations with Several Growth Traits in the Nanyang Cattle Breed. *Biochem. Genet.* 46:362–368.
- [35] Zarowiecki, M. Z.; Huyse, T. and Littlewood, D. T. (2007). Making the most of mitochondrial genomes—markers for phylogeny, molecular ecology and barcodes in *Schistosoma* (Platyhelminthes: Digenea). *Int. J. Parasitol.* 37: 1401–1418.
- [36] Zulkifli, N. A. (2016). Molecular genetics of residual feed intake and mitochondrial function in cattle. PhD Thesis, School of Animal and Veterinary Sciences, The University of Adelaide, Australia, 233 pp.