Study of mtDNA COI Gene Polymorphism and Relationship With Some Productive Traits in Awassi Sheep

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Abstract

This study was carried out in two locations (the field part), first in the Ruminants Researches Station (20 km west of Baghdad), and the second in the Aldawar research station located in Anbar governorate (Up to 35 km west of Ramadi city), which included 87 animals from Awassi sheep. As for the laboratory part, it took place in two locations, the first in the Animal Breeding and Improvement Laboratory of the Ruminant Research Department, and the second in the Scientific Progress Laboratory/Baghdad. This study aimed to investigate the relationship between polymorphism of mtDNA-COI gene and growth traits, milk yield, and lactation period in Awassi sheep. DNA was extracted from the blood and the polymerase Chain Reaction (PCR) technique was used to amplify the COI gene. DNA sequencing analysis. The results showed that there are 6 SNPs in a COI gene, there polymorphism for this gene also appeared (TT, CC). The 1st site (5566), CC polymorphism achieved the highest percentages (39.09) compared with TT polymorphism, with an allelic frequency of 0.57. While it appeared in the 2nd, 3rd, 4th, and 6th sites the polymorphism TT and CC with percentages (79.31 and 20.69)%, (58.42 and 41.38)%, (20.69 and 79.31), (79.31 and 20.69)% and with the allele frequency (0.79 and 0.21), (0.59 and 0.41), (0.21 and 0.79), (0.79 and 0.21) respectively.

There were significant differences (P≤0.05) of COI polymorphism in birth weight, weaning weight and gain from birth to weaning as the TT recorded the highest average for both traits (3.82, 25.39 and 21.57 kg respectively). The mean of daily milk yield for TT polymorphism was 330.21 gm/day, which significant (P≤0.05) the CC polymorphism, no significant between COI polymorphism and lactation period.

It was possible to conclude from this study the possibility of COI gene polymorphism in the development of genetic improvement strategies and breeding programs that achieved the best productive performance sheep.

Keywords : Awassi sheep, mtDNA COI gene polymorphism, Performance.

1.Introduction

The Awassi breed is widespread across the eastern Mediterranean, and it is the main breed in Iraq [1]. The number of sheep raised in Iraq is nearly 7 million, and it is considered one of the most important farm animals, addition, it is represent more than half of the number of sheep breeds in Iraq [2, 3], and it is also an important source of livelihood and food security for most of the rural population, especially in developing countries [4]. Awassi sheep are a major source of meat production and a secondary source of milk and wool in Iraq, the meat produced from sheep is of main quality and desirable by the Iraqi consumer compared to the meat of other animals [5]. Awassi sheep have been imported to more than 30 countries, due to its superiority in meat and milk production, and has been considered as an international trans-boundary breed by FAO [6]. Geographical areas and years affect the productivity of Awassi sheep, and many other factors may affect in birth and weaning weights and growth rate in lambs, as well as polymorphism [7]. COI is one of the important coding genes in the mitochondrial genome, as it is found between pairs of nucleotides that begin with the 5904 and 7444 base pair of the mitochondrial genome [8]. It has been used in the detection of processed meat products of various types due to their effect on human health [9, 10]. Due to the lack of studies on the relationship between the polymorphism of the COI gene with growth traits and milk production in Awassi sheep, so the study aimed to investigate the extent of the relationship between this gene and the traits of birth weight, weaning weight, growth between them, daily milk yield and lactation period in Awassi sheep.
2. Materials and Methods

2.1. Animal experiment

A total of 87 samples from Awassi sheep were collected from 15 September 2019 to 2 October 2020 from two locations: The first, in the ruminant research station (58 samples) of the State Board for Agricultural Researches of the Ministry of Agriculture / Abu Ghraib (20 km west of Baghdad) and the second: in the Al-Dawar research station (29 samples) located in Anbar governorate (about 35 km west of Ramadi).

2.2. Sample collection

Blood samples were collected from the Awassi sheep breed by drawing blood from the jugular vein in the neck (5 ml / animal) using a 10 ml vacuum tube containing an anticoagulant (Tripotassium Ethylene Diamine Tetra Acetic Acid- K₃EDTA) after the vein area was cleaned and sterilized with ethyl alcohol (70%), and the samples were kept refrigerated until the DNA extraction process was performed. The early morning milk production of each ewe in the flock was measured by recording the production once every 15 days, for a period of three months with a graduated cylinder prepared for this purpose (as the lambs were isolated from their mothers in the evening and then milked in the morning), the daily milk production was calculated by multiplying the average daily milk production by the length of the milk season.

2.3. DNA extraction

The DNA was extracted in the Animal Breeding and Improvement Laboratory of the Ruminant Research Department, Agricultural Research Department, Ministry of Science and Technology, using an extraction kit supplied by the Illumina Company, USA, according to the recommended instructions.

2.4. Primers selection

Primers were selected to perform molecular analysis and to find out the polymorphisms and mutations in the COI gene. COI gene was amplified using two primers: Forward primer : F 5' - CAGAGTTTGAAGCTGCT - 3' and Reverse primer : R 5' - AGCTGACGTGAAGTAAGC - 3' [11]. The PCR amplifications were conducted in a 20 μl volume containing 2 μl of genomic DNA template, 10 μl of GoTaq Green Master Mix, 1 μl each primer, 6 μl Nuclease-Free Water. The amplification conditions were as follows: initial denaturation at 95° C for 5 min. followed by 30 cycles of denaturation at 95° C for 30 sec., annealing at 55° C for 30 sec., and extension at 72° C for 30 sec., and then the final extension at 72° C for 7 min., later, hold temperature at 10° C for 10 min.

2.5. Statistical analysis

The data was analyzed by used Statistical Analysis System [12] to study of mtDNA COI gene polymorphism according to the mathematical model, significant differences was compared by used Least square means method.

$$Y_{ijklmn} = \mu + C_i + A_j + S_k + T_l + O_m + e_{ijklmn}$$

$Y_{ijklmn}$: Observed value, $\mu$: Overall means, $C_i$: Effect of COI gene polymorphism (TT, CC), $A_j$: Effect of age of dam (2, 3, and 4 year), $S_k$: Effect of sex of lamb (male, female), $T_l$: Effect of type of birth (single, twin), $O_m$: Effect of month of birth (October, November and December), $e_{ijklmn}$: Random error which distributed normally with mean = 0 and variation $\sigma^2$e.

Chi-square-$\chi^2$ test were used to compare between the percentages of COI gene polymorphism.

3. Results and Discussion

The concentration and purity of the DNA was measured after extraction by a Nanodrop, as the concentration was from 18.4 to 1130.8 ng/μl and the degree of purity (wavelength range between 260/280 nm) ranged from 1.74 to 2.15 nm. The results of electrophoresis on a 1.5% agarose gel showed successful DNA amplification (Figure 1), yielding a piece of COI gene consisting of 1052 base pair.
3.1. Analysis of mtDNA COI sequencing

The results of the genetic sequence analysis showed that there are 6 SNPs occurring in the COI gene in the Awassi sheep (Table 1) and in several sites (Figure 2) along the gene (The genetic sites: 5566, 5764, 5785, 5851, 5902, 5980), the results also showed that there are two polymorphism in the Awassi flock (TT and CC), and two dominants allele (T, C) accept 4th site (5851) which only the A allele appeared (point mutation, T to A).

Table 1. Numbers and percentages of the distribution of polymorphisms and allele frequency of mtDNA COI gene for all SNPs.

| Site of SNP | Polymorphism | No. | Percentage (%) | P-value | Allele | Frequency |
|-------------|--------------|-----|----------------|---------|--------|-----------|
| 5566        | CC           | 53  | 60.92          | 0.0084 **| C      | 0.61      |
|             | TT           | 34  | 39.08          |         | T      | 0.39      |
| 5764        | TT           | 68  | 78.16          | 0.0001 **| T      | 0.78      |
|             | CC           | 19  | 21.84          |         | C      | 0.22      |
| 5785        | CC           | 53  | 60.92          | 0.0084 **| C      | 0.61      |
|             | TT           | 34  | 39.08          |         | T      | 0.39      |
| 5851        | CC           | 68  | 78.16          | 0.0001 **| C      | 0.78      |
|             | AA           | 19  | 21.84          |         | A      | 0.22      |
| 5902        | TT           | 68  | 78.16          | 0.0001 **| T      | 0.78      |
|             | CC           | 19  | 21.84          |         | C      | 0.22      |
| 5980        | TT           | 68  | 78.16          | 0.0001 **| T      | 0.78      |
|             | CC           | 19  | 21.84          |         | C      | 0.22      |

** (P<0.01)
The CC polymorphism in the 1st SNP site showed the highest percentage (60.92%) of the TT polymorphism (P≤0.01) and the highest of allele frequency (0.61) comparative T allele (0.39), while the TT polymorphism for the 2nd site was higher (P≤0.01) than the CC polymorphism by 78.16%, the allele frequency of the T allele was higher than the C allele by 0.78 (Table 1). As for the other sites (3rd, 4th, 5th, and 6th), the dominant polymorphism were CC (60.92%), CC (78.16%), TT (78.16%), and TT (78.16%), and the dominant allele frequencies for the same sites were C (0.61), C (0.78), T (0.78), and T (0.78) respectively. The presence of the substitution mutation that occurred in the 4th site, which replaced the T base with A base, led to a slight deviation in the mitochondrial work and pathway of the respiratory chain or subunits of tRNA or rRNA [13].

3.2. Effect of mtDNA COI gene Polymorphism on growth traits

The results showed that there were significant differences between the polymorphism of the mtDNA COI gene in the weaning weight, as the CC polymorphism in 3rd site recorded highest average (P≤0.01) of the TT for this trait, as it reached 25.14 kg (Table 2). The overall average of weaning weight was 24.29 kg, which was close to findings by [14, 15], and higher than findings by [16] in their study on Awassi sheep to find out the effect of polymorphism of the COI gene and its relationship to weaning lambs, which amounted to 17.73 kg, and this result higher than that found by [17] in the Turkish Sabi sheep, as well as higher than findings by [18] in the Turkish Awassi sheep (16.80 kg) and a study [19]. The TT polymorphism also outperformed (P≤0.05) the CC polymorphism of the 1st SNP site for the same trait, reaching 25.10 kg, while the CC polymorphism was significantly (P≤0.05) in the 2nd, 5th and 6th SNPs sites, it reached 25.21 kg for the same sites, as for the 4th site, the AA polymorphism was significantly (P≤0.05) for weaning weight, which reached 25.21 kg. There was no significant effect of the polymorphism of all SNPs sites on birth weight.

The average birth weight in this study is close to what they found [20], and is lower than that of [16] was reached 4.52 kg, and [21], while the weaning weight in this study was higher than findings [17] and [18] in Turkish Awassi sheep. The results also indicated that there was a significant effect of polymorphism on the gain trait from birth to weaning, as the highest average was 21.17 kg due to the effect (P≤0.05) of the TT polymorphism for 1st site, also, the CC polymorphisms were significant for 2nd, 3rd, 5th and 6th SNPs sites in gain trait (21.79, 21.42, 21.79, 21.79 kg respectively).

The effect of some polymorphism for several SNPs sites can cause some physiological changes in mitochondria, as some point mutations appeared in some sites of this gene (replacement of T base by the A base) that would cause a change in the enzyme complexes of oxidative phosphorylation [22], as a number of mutations in the mitochondrial genome cause a number of diseases, and those mutations associated with a wide range of clinical diseases have been identified since 1988 [23, 24].
Table 2. Effect of polymorphism of mtDNA COI gene for all SNPs sites on Gain traits.

| Site of SNP | Polymorphism | No. | Birth weight (kg) | Weaning weight (kg) | Gain (kg) |
|-------------|--------------|-----|------------------|--------------------|----------|
|             |              |     | Mean±SE | Sig.       | Mean±SE | Sig. | Mean±SE | Sig. |
| 5566        | CC           | 53  | 3.68 ± 0.11 | NS             | 23.35 ± 0.59 | *    | 19.90 ± 0.62 | *    |
|             | TT           | 34  | 3.63 ± 0.16 | **             | 25.10 ± 0.61 | 21.17 ± 0.62 |
| 5764        | TT           | 68  | 3.62 ± 0.11 | NS             | 23.71 ± 0.50 | 19.92 ± 0.54 |
|             | CC           | 19  | 3.79 ± 0.16 | NS             | 25.21 ± 0.88 | 21.79 ± 0.71 |
| 5785        | CC           | 53  | 3.69 ± 0.11 | ***            | 25.14 ± 0.50 | 21.42 ± 0.48 |
|             | TT           | 34  | 3.61 ± 0.14 | NS             | 22.32 ± 0.73 | 18.84 ± 0.82 |
| 5851        | CC           | 68  | 3.62 ± 0.11 | NS             | 23.71 ± 0.50 | 19.92 ± 0.54 |
|             | AA           | 19  | 3.79 ± 0.16 | NS             | 25.21 ± 0.71 | 21.79 ± 0.71 |
| 5902        | TT           | 68  | 3.62 ± 0.11 | NS             | 23.71 ± 0.50 | 19.92 ± 0.54 |
|             | CC           | 19  | 3.79 ± 0.16 | NS             | 25.21 ± 0.88 | 21.79 ± 0.71 |
| 5980        | TT           | 68  | 3.62 ± 0.11 | NS             | 23.71 ± 0.50 | 19.92 ± 0.54 |
|             | CC           | 19  | 3.79 ± 0.16 | NS             | 25.21 ± 0.71 | 21.79 ± 0.71 |

*(P≤0.05), ** (P≤0.01), NS (No significant)

3.3. Effect of mtDNA COI gene Polymorphism on milk production

The overall mean of daily milk yield for TT polymorphism in 1st site was 366.04 gm/day, which significant (P≤0.01), likewise for the 2nd SNP site, the TT polymorphism was significantly (P≤0.01), reaching 338.30 g/day, and for the 3rd site, CC polymorphism was significantly (P≤0.05), which It reached 326.48 g/day. The CC polymorphism was significant (P≤0.01) in the 4th site in the daily milk production, which was 338.30 g/day.

As for the 5th and 6th sites, the TT polymorphism was highly significant, as the overall mean of the two polymorphism were 338.30 g/day. As this result was less than findings of [25] as they recorded 486.66 gm/day in Iraqi Awassi sheep, [26] estimated of mean was 506 gm/day and 505 gm/day by [27]. No significant between 2nd, 3rd SNP site polymorphism of COI gene and Lactation period (table 3).

The results also showed that there was a significant (P≤0.01) of TT polymorphism for the 1st, 5th and 6th SNPs sites for lactation period, which amounted to 126.63, 128.19 and 128.19 days, respectively (table 3), with contrast to the result reached by [16] in the absence of a significant effect for the genetic polymorphism of the COI gene (site 5980) and the the lactation period in Awassi sheep. The CC polymorphism in 4th SNP site was significant (P≤0.05) in lactation period, which reached to 128.19 days, while no significant for 2nd and 3rd SNPs sites in the lactation period.
### Table 3. Effect of polymorphism of mtDNA COI gene for all SNPs sites on milk production.

| Site of SNP | Polymorphism | No. | Daily milk production (gm/day) Mean±SE | Sig. | Lactation period (day) Mean±SE | Sig. |
|-------------|--------------|-----|----------------------------------------|------|---------------------------------|------|
|             |              |     | **                              **    |      |                                 |      |
| 5566        | TT           | 34  | 366.04 ± 20.11                      | **   | 126.63 ± 9.42                    | *    |
|             | CC           | 53  | 296.07 ± 14.52                      |      | 107.84 ± 9.05                    |      |
| 5764        | TT           | 68  | 338.30 ± 13.92                      | **   | 120.71 ± 10.57                   |      |
|             | CC           | 19  | **                              **    |      | 119.42 ± 10.81                   | NS   |
| 5785        | TT           | 34  | 313.33 ± 18.42                      |      | 114.57 ± 12.53                   |      |
|             | CC           | 68  | **                              **    |      | 128.19 ± 10.37                   |      |
| 5851        | AA           | 19  | 267.13 ± 22.58                      | **   | 106.78 ± 11.63                   |      |
|             | TT           | 68  | **                              **    |      | 128.19 ± 10.37                   |      |
| 5902        | CC           | 19  | 267.13 ± 22.58                      | **   | 106.78 ± 11.63                   |      |
| 5980        | TT           | 68  | **                              **    |      | 128.19 ± 10.37                   |      |
|             | CC           | 19  | **                              **    |      | 106.78 ± 11.63                   |      |

*(P≤0.05), ** (P≤0.01), NS (No significant)

### Conclusion

Since this polymorphism of COI gene has significant effect on growth and production traits in Awassi sheep, it can be considered as a genetic marker to this traits, the reasons for this may be due to the fact that many studies have indicated the role of mitochondria and feed intake in improving feeding efficiency in ruminants, and the mitochondrial genes involved in energy metabolism may have variants that affect efficiency.

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