

Polymorphisms of the prolactin gene in Iraqi ducks and its association with body weight

Sala Mahdi Alsudany, Huda Falih Saad, Bashar F. Zaqeer and Jaafar M. Owaid
University of Basrah, College of Agriculture
salah.mohsen@uobasrah.edu.iq

Abstract

A study was conducted to explore polymorphism for prolactin gene exon 1. The aim is to investigate how polymorphisms and haplotypes influence the body weight of Iraq ducks. The nucleotide sequence of the PRL gene in ducks was aligned with the GenBank LC565025 sequence using the ClustalW program in Bioedit software. Three polymorphisms (SNP) were observed in Iraq Ducks. (LC565025: g.40 G >T, LC565025 g.41 C >T, LC565025: g.96 A >T, LC565025: g.103 C >A, LC565025: g.168 G >T, LC565025: g.211 G >T, LC565025: g.222 T >A, LC565025Z: g.273 T >C), and the Haplotype was obtained using the program DNAsp 5.10. And select Four haplotypes, namely haplotype1 (ttaaggtc), haplotype 2 (ttacggtc), haplotype 3 (gcacttac), and haplotype 4 (gctcttac), have been identified in exon 1 of the prolactin gene in Iraqi ducks. According to the presented data, the presence of polymorphism (SNP) and haplotypes of the prolactin gene exon1 did not have a significant effect on the average weights of Iraqi ducks.

Keywords: polymorphism, prolactin gene, haplotype, Ducks

Introduction

Breeding duck comes in the second division after poultry in terms of importance as a source of meat and eggs (Shubber, H. W. K., 2006). Ducks are primarily raised in small rural farms to produce eggs and meat, which have a high nutritional value for humans (Nevien M. et al, 2020). Southern Iraq is home to waterfowl, including ducks, that are raised for meat and egg production (Moussa. R. K., et al 2010). Approximately 7% of all birds documented in Iraq are wild ducks. (Mohammad K., 2016). Identifying important genes that are associated with different economic traits can offer promising prospects for selection programs in the future (Nandedkar et al., 2016). By identifying candidate genes associated with quantitative traits, modern molecular genetic techniques have increased duck productivity, resulting in improvements in productive traits and the enhancement of breeding programs (Basumatary et al., 2019). Although traditional breeding techniques involving selection and cross-breeding have enhanced duck productivity and resulted in novel hybrids, the progress using this method has been slow (Asiamah et al., 2019). The discovery of polymorphisms in genes associated with economic traits can offer valuable insights for the progression of genetic improvement programs (Yurnalis et al, 2019). The PRL gene belongs to the family of growth hormone genes and is predominantly produced in the anterior pituitary gland of all vertebrates. (Wang C, et al, 2011, Jiang, R.S. et al 2011 and Wang, C. et al 2011). In ducks, the prolactin gene comprises of five exon regions that are interspersed with four introns, and it encodes 229 amino acids (Kansaku, N et al, 2005, Au, W.L. and Leung, F.C, 2002).

Materials and Methods

Thirty four Iraqi domestic duck breeds were included in this study, and blood samples were collected from their feet veins. These samples were obtained from local markets and people in Basra, Iraq, and were stored at low temperatures for DNA extraction. The Scientific Committee of the Department of Animal Production at the University of Basrah approved this study.

Blood samples were collected and DNA was extracted using a kit. The extracted DNA samples were then stored at -20°C until used for PCR amplification. The PRL gene sequence of ducks from Genbank was used to design primers for amplification. The Primer3 program was used for primer design, and the primers were F: 5'-ATCCGCCACATGGACAACAT-3 and R: 5'-GACCACCGAGTTGCAGATGA-3. PCR was performed on a 30 µL reaction mixture, which included 15 µL PCR Master mix, 1 µL of each primer, 2 µL of DNA, and 11 µL of nuclease-free water. The PCR temperature profiles consisted of an initial denaturation at 95 °C for 5

minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 63 °C for 30 seconds, and extension at 72 °C for 45 seconds. The final extension was performed at 72 °C for 5 minutes. Electrophoresis of the PCR products was performed on a 1% (w/v) agarose gel in 1x TAE buffer solution, along with a 100 bp DNA tag, at a constant voltage of 90 volts for 30 minutes. The products were visualized by UV light after staining with ethidium bromide.

Once the desired gene sequence was obtained, it was aligned with the GenBank: LC565025 nucleotide sequence of the duck nucleotide sequence in the PRL gene using the ClustalW program in the Bioedit software. (Program DNAsp 5.10) was used to get the Haplotype.

Results and discussion

In this study, exon 1 of the prolactin gene in Iraqi Ducks has amplified the discovery of eight polymorphisms (SNP) was made through sequence alignment. (Figure 1). And these (SNP) are (LC565025: g.40 G >T, LC565025 g.41 C >T, LC565025: g.96 A >T, LC565025: g.103 C >A, LC565025: g.168 G >T, LC565025: g.211 G >T, LC565025: g.222 T >A, LC565025Z: g.273 T >C) As in (Table 1). Found (Nevien M. et al, 2020) Several single nucleotide polymorphisms (SNP) were in exon 1 of the prolactin gene within the sequences of four Egyptian duck breeds. Also, a study (Cui Wang. et al, 2011) showed that 12 SNPs were detected in the exon 2, exon 4, and exon 5 in six Chinese native duck breeds. In this study., None of the eight polymorphisms of the prolactin gene exhibited any impact on the body weight of Iraqi ducks As found (Artur Mazurowski et al ., 2016) there were no significant differences ($P > 0.05$) observed in the body weight and body measurements of Mulard ducks due to PRL gene polymorphisms at 3, 5, 7, 10, and 12 weeks of age.

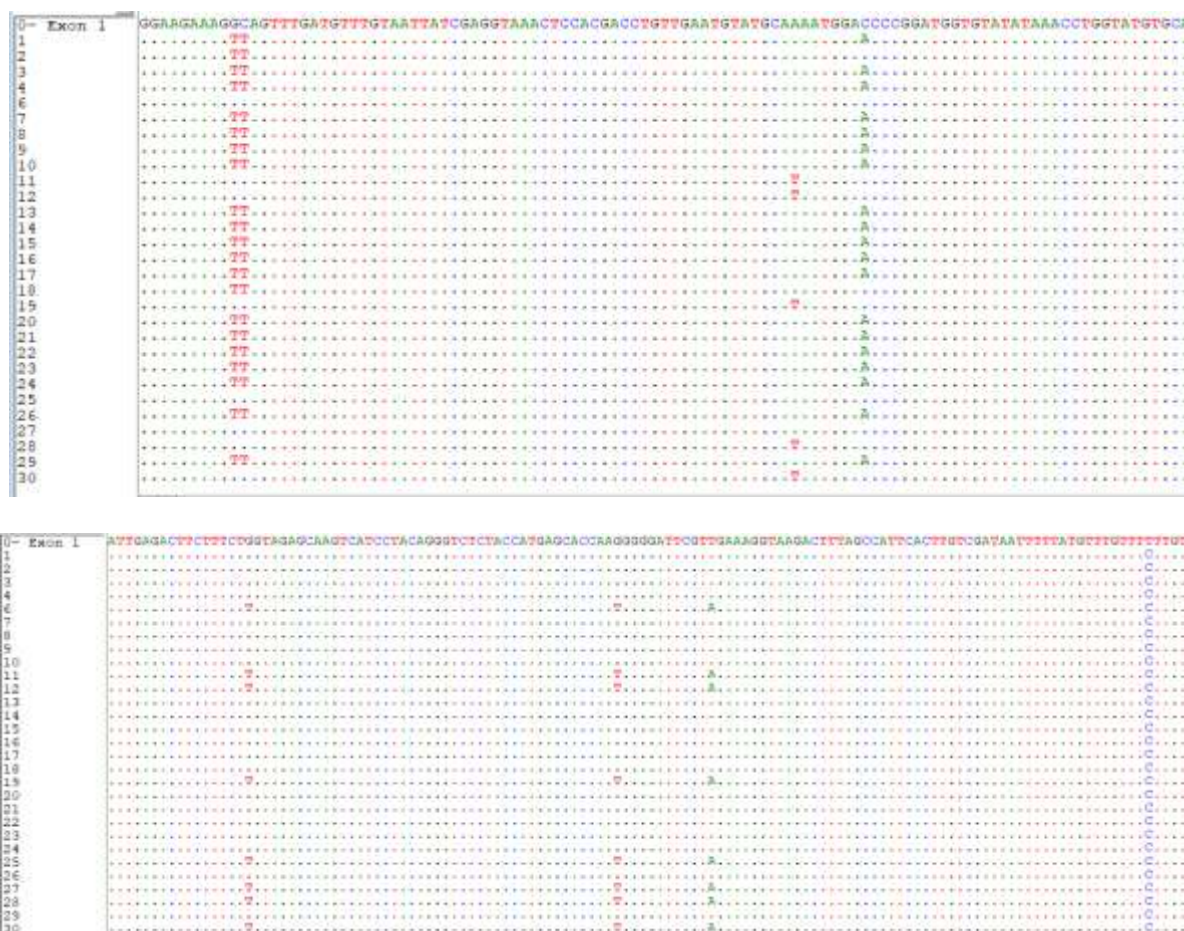


Fig. 1. Comparative alignment of conceptualized nucleotide sequence of PRL gene in Iraqi Ducks with NCBI reference sequence LC565025

Table (1) represents the location and type of SNP of Exon1 from prl gene

Position	Nucleotide substitution	No
LC565025:g.40 G >T	G >T	1
LC565025 g.41 C >T	C >T	2
LC565025:g.96 A >T	A >T	3
LC565025:g.103 C >A	C >A	4
LC565025:g.168 G >T	G >T	5
LC565025:g.211 G >T	G >T	6
LC565025:g.222 T >A	T >A	7
LC565025Z:g.273 T >C	T >C	8

The DNAsp 5.10 Program enabled the identification of four haplotypes from exon 1 of the prolactin gene in Iraqi ducks: haplotype1 (ttaaggtc), haplotype 2 (ttacggtc), haplotype 3 (gcacttac), and haplotype 4 (gctcttac). The distribution of these haplotypes is shown in Table (2), with haplotype (1) being present in the most significant number of ducks 21 Duck, followed by haplotype (4) 6 Duck, haplotype (2) 4 Duck, and haplotype (3) 3 Duck. The data presented in Table No. (3) suggests that the average weights of Iraqi ducks were not significantly affected by the presence of multiple haplotypes of the prolactin gene exon1. At week 14, ducks carrying haplotypes 1, 2, 3, and 4 had an average body weight of (1277, 1273, 1280, and 1267), respectively. At the age of 20 weeks, their respective body weights were (1322, 1308, 1319, and 1316). This suggests that there is no association between the prolactin gene haplotype and body weight in Iraqi ducks. The study (Sartika et al., 2017) conducted on Indonesian ducks revealed that certain haplotypes of the prolactin gene were linked to higher body weight at 12 weeks of age. There could be several reasons why there was no association observed between the haplotype and polymorphisms (SNP) of the prolactin gene and duck weight in the study. One possible explanation is that the effect of the prolactin gene on duck weight is minimal, and other genes or environmental factors may have a greater impact. Additionally, the sample size used in the study may not have been large enough to detect significant differences in weight between different haplotypes. It is also possible that the study did not account for all relevant variables that may affect duck weight, such as nutrition, housing conditions, and management practices.

Table (2) represents the haplotype and haplotypes distribution of Exon 1 from prl gene

Haplotypes distribution	Haplotypes	Number of Haplotypes	No
21	ttaaggtc	Hap 1	1
4	ttacggtc	Hap 2	2
3	gcacttac	Hap 3	3
6	gctcttac	Hap 4	4

Table (3) average body weight corresponding to each haplotype

average body weight\gm		Number of Haplotypes	No
week 20	week 14		
1322	1277	Hap 1	1
1308	1273	Hap 2	2
1319	1280	Hap 3	3
1316	1267	Hap 4	4

Reference

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