



## Polymorphism of the Prolactin Gene in Egyptian Duck Breeds

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### ABSTRACT

In avian, the prolactin hormone triggers and regulates ovarian follicle development. This study aims to detect the Prolactin (*PRL*) gene polymorphisms (exons 1 and 5) in four Egyptian duck breeds, namely Campbell, Moulard, Muscovy, and Pekin using PCR-RFLP technique and sequence analysis. It also investigated the association of this gene with egg production, egg weight, and body weight. The present results revealed that *PRL* gene exon 1 and part of intron 1 showed two alleles A and B (polymorphic) in each of Campbell and Moulard, however, Muscovy and Pekin had only one allele (monomorphic). The allele A was more dominant with frequencies of 0.70, 0.60, and 1.00, compared to the allele B (0.30, 0.40, and 0.00) for Campbell, Moulard, and Muscovy, respectively. For Pekin, the allele B only appeared with the frequency of 1.0. Ducks with the high frequency of allele A were superior at egg weight, compared to others. Furthermore, for *PRL* gene exon 5, there were two alleles G and C (polymorphic) in Campbell, Moulard, and Muscovy, however, Pekin had only one allele (monomorphic). The allele G was more dominant (0.15, 0.74, 0.0, and 0.84) than the allele C (0.85, 0.26, 1.0, and 0.15) for Campbell, Moulard, Pekin, and Muscovy, respectively. Ducks having a high frequency of allele C were superior at egg production. Furthermore, there were many single nucleotide polymorphisms (SNPs) in the sequences in all breeds. The utmost ones exist at the restriction sites of *Xba*I enzyme for the amplified fragment, in the promotor, exon 1 and intron 1 (T378C in intron 1), and *Dra*I enzyme for that in exon 5 (A5871G in exon 5).

**Keywords:** Duck, Genetic polymorphism, Genotyping, Prolactin gene

### INTRODUCTION

Meat and eggs of ducks have a high nutritional value for humans. Ducks are foremost raised in small rural farms to produce eggs and meat to improve economic livelihood. Ducks have great economic importance to many countries (Narhari, 2009). The Pekin duck (*Anas platyrhynchos domestica*), Muscovy duck (*Cairina moschata*), and Moulard ducks (crossing between Muscovy and Pekin duck), are the main duck species that are used globally to produce meat of ducks (Baeza, 2006). The Muscovy duck (*Cairina moschata*) meat has a distinct taste, with low calories, so it has global economic importance (Veeramani et al., 2016). The Muscovy and Moulard are characterized by lower fatness and higher meat production as compared by Pekin ducks (Wawro et al., 2004). The Campbell duck (*Anas platyrhynchos*) is a high egg-production breed. Moreover, the nutritional value of duck eggs is higher than chicken ones, in its mineral salts, vitamins, and amino acids (Sadar et al., 2014).

Traditional breeding approaches of selection and crossbreeding have improved the productivity of duck and devised new hybrids, while improvement using this method was slow (Asiamah et al., 2019). Duck productivity was increased using modern methods of molecular genetics, by identifying candidate genes associated with quantitative traits to improve productive traits and enhance breeding programs (Basumatary et al., 2019). One of the utmost serious problems in farming ducks is the low productivity and quality of production. Therefore, their genetic improvement aims to increase productivity, improve product quality and elevate economic value (Chang et al., 2012). Genetic improvement programs using traditional and modern methods for meat-type ducks have successfully enhanced their productive performance. In Pekin ducks, the genetically improved type for higher meat production reached to 3.2 kg at seven weeks, while unimproved duck production reached to 1.7 kg at 11 weeks of age (Zeng et al., 2016).

Prolactin hormone gene (*PRL*) is a single-chain polypeptide belongs to the family of growth hormone genes, and synthesized by the anterior pituitary gland of poultry (Wang *et al.*, 2011). The size of duck prolactin gene is 1000 bp in size and consists of five exons and four introns encoding 229 amino acids. As the chicken *PRL* gene was cloned and sequenced (Watahiki *et al.*, 1989), a bulk of studies have targeting this gene polymorphism (Xu *et al.*, 2015; Shamsalddini *et al.*, 2016; Li *et al.*, 2017). In recent years, polymorphisms in the *PRL* gene have been associated with egg production in hens (Sarvestani *et al.*, 2013; Kulibaba, 2015; Tempfli *et al.*, 2015; Mohamed *et al.*, 2017; Yadav *et al.*, 2018; Bai *et al.*, 2019; Basumatary *et al.*, 2019).

Identifying single nucleotide polymorphisms (SNPs) in candidate genes and their association with egg laying traits is an important technique used to enhance the avian genetics (Feng *et al.*, 2018). Therefore, the present study used four Egyptian duck breeds; Moulard, Campbell, Pekin and Muscovy ducks to screen polymorphism of the duck *PRL* gene. Identifying of such SNPs can be employed as genetic markers for selection of genetically superior ducks to increase productivity.

## MATERIALS AND METHODS

### Ethical approval

Permission for collecting the samples used in this work was received from the management of El-Nubaria farm, owned by National Research Centre that was included in this study. The samples were collected per standard sample collection procedures without any harm to animals.

### Animals

The present study used a total of 60 healthy ducks, of four breeds; Campbell, Moulard, Muscovy and Pekin, raised in El-Nubaria experimental farm, owned by the National Research Centre-Giza-Egypt. 15 blood samples (3mL) were aseptically collected from each breed, using EDTA (0.5 M) as an anticoagulant.

### DNA Extraction

Genomic DNA was isolated from whole blood of ducks using salting out method (Shokrzadeh and Mohammadpour, 2018), with minor modifications. Briefly, blood (3 ml) was mixed with cell lysis buffer (640 mM sucrose, 10 mM MgCl<sub>2</sub>, 20 mM Tris-HCl, 2% Triton 100X) and placed on ice for 30 min then centrifuged at 5000 rpm for 15 min at 4°C and the supernatant was

discarded. Nuclei lysis buffer (10 mM Tris-base, 400 mM NaCl, 2 mM EDTA) was added to the pellet and mixed with 20% Sodium Dodecyl Sulfate (SDS) and proteinase K then placed in a water bath at 55°C for two hours. NaCl (saturated) was added and centrifuged to separate a clear upper layer containing DNA. Only 30 µl of the clear layer was transferred to a tube containing absolute ethanol to fish the DNA and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA) at 37°C for 2 hours. Purity and concentration of DNA were measured using Nano Drop™ 1000 Spectrophotometer (Thermo Fisher Scientific, USA). Extracted DNA was stored in -20°C till usage.

### Polymerase Chain Reaction (PCR)

The primers used were F: 5'-AAATTCCTCTCACAG TTACA-3'; R: 5'-GATGCAGAGACAAGTTTCACC-3' for *PRL* (intron 1) to produce a product of 417 bp and F: 5'-TGCAAACCATAAAAGAAAAGA-3'; R: 5'-CAATGAAAAGTGGCAAAGCAA-3' for *PRL* (exon 5) to produce a product of 400 bp. PCR amplification was carried out in a 25 µl reaction volume, including 2 µl genomic DNA (50 ng), 2.5 µl 10X buffer, 2.5 mM of deoxyribonucleotide triphosphates (dNTPs), 2.5 µl of each primer (10 pmol), and 0.5 µl *Taq* polymerase (Thermo-Fisher Scientific, USA). The PCR conditions were; 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 30 sec at the annealing temperature 54°C, 2 min at 72°C and a final extension of 7 min at 72°C.

### Restriction Fragment Length Polymorphism (RFLP)

Digestion of the PCR products was performed overnight at 37°C in a final volume of 30 µl with specific restriction enzymes (Thermo Fisher Scientific, USA); *Xba* I (5'T↓CTAGA-3') for *PRL* (Intron 1), and *Dra*I (5'TTTAAA↓3') for *PRL* (Exon 5). The RFLP reaction mixture composed of 10 µL of PCR products, 1U of restriction enzymes, 2 µl of buffer and 18 sterile H<sub>2</sub>O. The digested products were run in 2.5% agarose gel and different genotypes were visualized under U.V. transilluminator and photographed using Gel documentation system.

### DNA Sequencing and Analysis

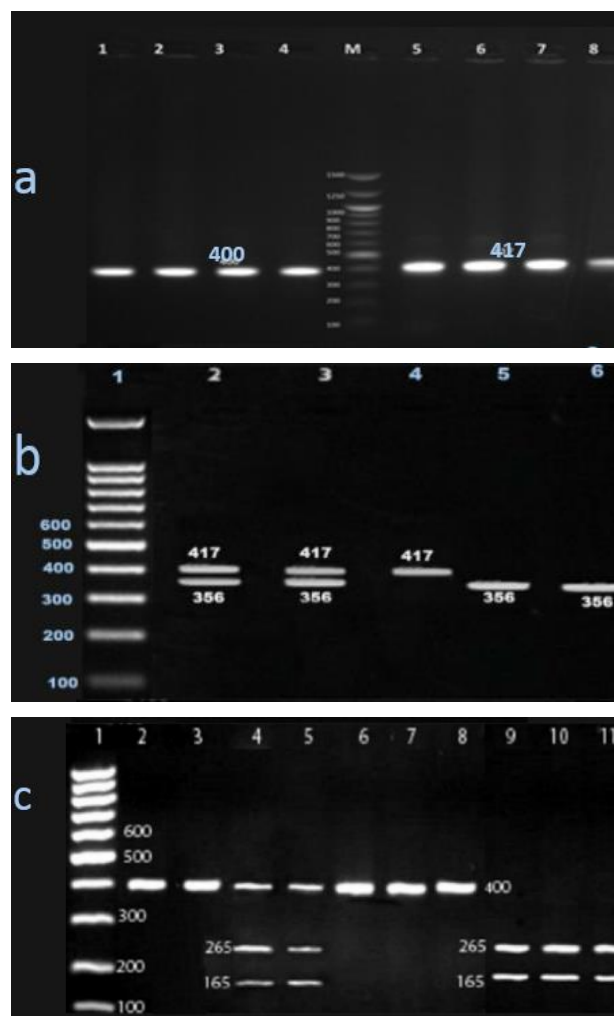
The PCR products were purified using Gene JET Gel Extraction Kit (Thermo-Fisher Scientific, USA) according to the manufacture instructions. The purified PCR products were sequenced using an automated sequencing service (Macrogen, South Korea). Sequence analysis was

performed via program BLAST/NCBI (National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov/>). Sequence alignment were accomplished by Clustal Omega version (1.2.4) (<https://www.ebi.ac.uk/Tools/msa/clustalo>), as described by (Larkin et al., 2007). The phylogenetic trees between the studied breeds, and other avian were done, using unweighted pair group method with arithmetic mean (UPGMA). To detect chromosomal localization of prolactin gene (exons 1 and 5) from Egyptian duck breeds in the duck genome, *Anas platyrhynchos* isolate PK-2015, IASCAAS\_PekingDuck\_PBH1.5, was used from NCBI.

## RESULTS

In the current study, the DNA fragment (417 bp), covering the *PRL* gene promoter (220 bp), exon 1 (28 bp) and part of intron 1 (169 bp), was successfully PCR amplified, as well as the other fragment (400 bp), covers the coding region of *PRL* gene exon 5 (192 bp), and part of intron 4 (137 bp, Figure 1a).

The *Xba*I/RFLP for *PRL* (the promoter, exon 1 and part of intron 1) revealed three fragments; non-cut fragment (417 bp) or homozygous genotype (AA), cut fragments (356 bp and 61 bp) or homozygotes (BB) [61 bp band is not visible because it is too short], and the combined fragments (417 bp, 356 bp and 61 bp) or heterozygotes (AB Figure 1b). As illustrated in Table 1, both Campbell and Moulard breeds are polymorphic resulted two alleles (A and B). However, Muscovy (A) and Pekin (B) were monomorphic resulted one allele. The allele A frequency was much higher than allele B in the four breeds. The results revealed that the four investigated duck breeds, *PRL* gene exon 1 and part of intron 1, had two alleles (A and B) and three genotypes AA, AB, BB, ducks with higher frequency of allele A superior at egg weight, at 10-weeks-old body weight compared to other duck breeds (Table 1).



**Figure 1a.** Electrophoresis of the PCR products of prolactin exons 1 and 5 on agarose gel. Gel was stained with ethidium bromide, Lane 1-4: prolactin (exon5), Lanes 5-8: prolactin (exon1), Lane M: 100-bp DNA ladder. **Figure 1b.** Electrophoresis of the digestion products of *Xba*I on agarose gel. Gel was stained with ethidium bromide, Lanes 2 and 3: AB genotype, Lane 4: AA genotype, Lanes 5 and 6: BB genotype, Lane 1: 100-bp ladder. **Figure 1c.** Electrophoresis of the digestion products of *Dra*I on agarose gel. Gel was stained with ethidium bromide, Lane 2, 6, 7, and 8: AA genotype, Lanes 4 and 5: AB genotype, Lanes 9, 10 and 11: BB genotype, Lane 1: 100-bp DNA Ladder.

**Table 1.** Relation between the four Egyptian duck breeds (Campbell, Moulard, Pekin and Muscovy) and body weight, egg number per year and egg weight, allelic and genotypic frequencies

Breed	BW10	Eggs number /year	Egg weight	Allelic Frequency				Genotypic Frequency					
				Exon 1		Exon 5		Exon 1			Exon 5		
				A	B	G	C	AA	AB	BB	GG	CG	CC
<b>Campbell</b>	1306.57±37.32	300-320	65 g	00.70	00.30	0.15	0.85	0.50	0.40	0.10	0.15	0.00	0.85
<b>Moulard</b>	4021.00±20.49	200-230	60 g	00.60	00.40	00.74	00.26	0.35	0.50	0.15	0.61	0.26	0.13
<b>Pekin</b>	2846.00±30.02	200-300	60 g	00.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
<b>Muscovy</b>	3740.00±47.37	100-180	77 g	1.00	00.00	00.85	00.15	1.00	0.00	0.00	0.75	0.20	0.05

In similar manner, *Dra*I/RFLP for *PRL* (exon 5) yielded two homozygous genotypes were GG (400 bp), CC (235 and 165 bp) and heterozygotes are known as genotypes GC (400, 235 and 165 bp, Figure 1c). As illustrated in Table 1, all races are polymorphic, except Pekin which was monomorphic (C). Besides, the allele G was more dominant than allele C in the four races. Additionally, *PRL* gene exon 5 of the four manipulated duck breeds, at 10-weeks-body-old weight, had two alleles (G and C) and three genotypes GG, GC, CC, ducks that had high frequency of allele C were superior at egg production compared with the other ducks (Table 1).

Genetic polymorphisms of *PRL* gene covers the coding region of *PRL* gene (promoter, exon 1 and part of intron 1 and exon 5) between the four Egyptian duck breeds also can be detected by DNA-sequencing methods, Clustal Omega was used to aligned each breed of the four Egyptian duck breeds with each other and with *Anas platyrhynchos*, *PRL* gene, cds, AB158611 (*A. platyrhynchos*, *PRL*) presented in the database. The Moulard breed samples revealed that Moulard-1 sequences shared similarity (97.36%) with Moulard-2. Also, the DNA sequence from Campbell breed samples revealed that the Campbell-1 sequence shared similarity (97.36%) with Campbell-2. However, Moulard-1 sequences shared high similarity (100%) with Campbell-2, and Moulard-2 sequences shared high similarity (100%) with Campbell-1. The alignment between Moulard-1 and Campbell-2 with both Moulard-2 and Campbell-1 showed that Four nucleotide substitutions at nucleotide 132 (A/G), 166 (T/C), 213 (C/T) and 378 (T/C), this mutation are considered from the transition type, the other seven nucleotide substitutions at nucleotide 319, 325, 332 (T/G), 318, 322, 326, 330 (G/T) the SNPs between the sequences are considered from the transversion type.

Furthermore, *A. platyrhynchos*, *PRL* shared similarity (99.04%) with Moulard-1 and Campbell-2 sample, two nucleotide substitutions at nucleotide 267 (G/A) and at nucleotide 412 (A/G), these mutations are considered from the transition type, the other two nucleotide substitutions at nucleotides 312 (T/G) and 314 (G/T), are considered from the transversion type. Both Moulard-2 and Campbell-1 samples shared similarity (96.40%) with *A. platyrhynchos*, *PRL*, six nucleotide substitutions at nucleotide 132 (A/G), 166 (T/C), 213 (C/T), 267 (G/A), 378 (T/C) and 412 (A/G), the mutations are considered from the transition type, the other nine nucleotide substitutions at nucleotides 312, 319, 325, 332 (T/G), 314, 318, 322, 326, 330 (G/T), are considered from

the transversion type (Figure 2) and percent identity matrix of the multiple sequence alignment (Table 2).

The genomic sequences of *PRL* gene from Pekin and Muscovy breeds revealed that the sequences (Pekin-1 and Pekin-2) also, (Muscovy-1 and Muscovy-2) shared high similarity (100%) between each other as there is no SNPs between the sequences. However, all Pekin breed samples shared similarity (98.32%) with *A. platyrhynchos*, *PRL* (Table 2). Eight transitions at nucleotides; 267 (G/A) and 412 (A/G). In addition, five transversions at nucleotides; 135 (T/A), 137(C/G), 213(C/T), 312 (T/G) and 314 (G/T). All Muscovy ducks shared similarity (96.40%) with *A. platyrhynchos*. Six transitions at nucleotides; 132 (A/G), 166 (T/C), 213 (C/T), 267 (G/A), 378 (T/C), 412 (A/G), and nine transversions at 312, 319, 325, 332 (T/G), 314, 318, 322, 326, 330 (G/T), were found (Figure 2).

Sequence comparisons among the four studied breeds *PRL* gene exon1 sequence revealed that Moulard-1 revealed high similarity (100%) with Campbell-2, (99.28%) with Pekin and (97.36%) with Moulard-2, Campbell-1 and Muscovy. As Muscovy resulted high similarity (100%) with Campbell-1 and Moulard-2, (97.12%) with Pekin (Table 2).

The four examined duck lines, *PRL* gene exon 5 sequence, aligned with *A. platyrhynchos*, *PRL* disclosed upraised similarity (100%) with all Pekin (Pekin-1 and Pekin-2), Moulard-2, Campbell-2 and also Muscovy-1. However, *A. platyrhynchos*, *PRL* illustrated similarity (97.25%) with Moulard-1, Campbell-1 and Muscovy-2 (Table 3). There were five transitions at nucleotides; 5780 (G/A), 5808(T/C), 5822(C/T), 5871(A/G), 5926(G/A), and six transversions at nucleotides; 5766 (G/T), 5792 (A/T), 5834 (G/T), 6004 (A/C), 6007(G/C) and 6029(G/T) (Figure 3). For the sequence comparisons among the four checked duck breeds exon 5 revealed that Moulard-1 exhibited high similarity (100%) with Campbell-1, Muscovy-2, but 97.25% with Moulard-2, Campbell-2, Muscovy-1, Pekin-1 and Pekin-2 (Table 3).

The obtained sequences were submitted to Gen bank and have been assigned with accession number; Campbell *PRL* exon1 allele A (LC565022) and allele B (LC565023), Moulard *PRL* exon 1 allele A (LC576823) and allele B (LC565024), Pekin *PRL* exon 1 (LC565026), Muscovy *PRL* exon 1 (LC565025). Also, the obtained sequences for exon 5 were submitted, Campbell *PRL* exon 5 allele A (LC565015) and allele B (LC565016), Moulard *PRL* exon 5 allele A (LC565017) and allele B (LC565018), Pekin *PRL* exon 5 (LC565021), Muscovy *PRL* exon 5 allele A (LC565019) and allele B (LC565020).

**Table 2.** Percent Identity Matrix of DNA multiple sequence alignment for the four Egyptian duck breeds (Campbell, Moulard, Pekin and Muscovy) with each other and other avian species sequences of *PRL* gene exon 1 published in GenBank database

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1: <i>M. gallopavo</i>	100.00	77.27	78.64	76.50	76.50	76.50	76.92	76.50	76.50	76.50	85.88	84.71	97.37	94.12
2: <i>C. moschata</i>		100.00	91.86	98.72	98.72	98.72	97.28	97.45	97.45	97.02	90.10	86.96	87.55	86.25
3: <i>A. anser</i>			100.00	94.19	94.19	94.19	93.84	95.64	95.64	95.40	90.50	87.90	85.89	82.89
4: Campbell-1, allele A				100.00	100.00	100.00	96.40	97.36	97.36	97.12	89.30	86.75	88.44	84.73
5: Moulard-2 allele A					100.00	100.00	96.40	97.36	97.36	97.12	89.30	86.75	88.44	84.73
6: Muscovy-1,2						100.00	96.40	97.36	97.36	97.12	89.30	86.75	88.44	84.73
7: <i>A. platyrhynchos</i>							100.00	99.04	99.04	98.32	88.48	85.94	87.85	84.18
8: Campbell-2 allele B								100.00	100.00	99.28	88.89	86.35	88.00	84.36
9: Moulard-1 allele B									100.00	99.28	88.89	86.35	88.00	84.36
10: Pekin-1,2										100.00	88.48	85.94	87.56	84.00
11: <i>C. japonica</i>											100.00	97.11	96.41	91.67
12: <i>P. cristatus</i>												100.00	95.57	92.34
13: <i>G. gallus</i> B allele													100.00	94.20
14: <i>P. colchicus</i>														100.00

1: *Meleagris gallopavo* PRL AB605394, 2: *Cairina moschata* PRL, KM390982, 3: *Anser anser* PRL, GU984377, 4: Campbell-1 PRL Ex-1, LC565022, 5: Moulard-2 allele A, LC576823, 6: Muscovy PRL Ex1, LC56502, 7: *A. platyrhynchos* PRL, AB158611, 8: Campbell-2 allele B, LC565023, 9: Moulard-1 allele B, LC565024, 10: Pekin, PRL Ex-1, LC565026, 11: *Coturnix japonica* PRL, AB452962, 12: *Pavo cristatus* PRL, AB452960, 13: *Gallus gallus* PRL B allele, JN661569, 14: *Phasianus colchicus* PRL, AB452961

**Table 3.** Percent Identity Matrix of DNA multiple sequence alignment for the four Egyptian duck breeds (Campbell, Moulard, Pekin and Muscovy) with each other and other avian species sequences of *PRL* gene exon 5 published in GenBank database

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1: <i>Struthio camelus</i>	100.0	87.17	87.17	87.17	87.96	87.96	87.96	87.96	87.96	88.74	81.98	81.34	81.68	83.76
2: Campbell-1 allele A Ex5		100.00	100.00	100.00	97.75	97.75	97.75	97.75	97.75	97.50	83.89	84.20	84.48	83.54
3: Moulard-1 allele A Ex5			100.00	100.00	97.75	97.75	97.75	97.75	97.75	97.50	83.89	84.20	84.48	83.54
4: Muscovy-2 allele B Ex5				100.00	97.75	97.75	97.75	97.75	97.75	97.50	83.89	84.20	84.48	83.54
5: <i>A. platyrhynchos</i>					100.00	100.00	100.00	100.00	100.00	98.75	85.68	86.65	85.75	85.09
6: Campbell-2 allele B Ex5						100.00	100.00	100.00	100.00	98.75	85.68	86.65	85.75	85.09
7: Moulard-2 allele B Ex5							100.00	100.00	100.00	98.75	85.68	86.65	85.75	85.09
8: Muscovy-1 allele A Ex5								100.00	100.00	98.75	85.68	86.65	85.75	85.09
9: Pekin-1,2									100.00	98.75	85.68	86.65	85.75	85.09
10: <i>A. anser</i>										100.00	85.93	86.38	86.51	86.34
11: <i>G. gallus</i>											100.00	94.57	93.61	93.50
12: <i>P. cristatus</i>												100.00	94.57	94.43
13: <i>M. gallopavo</i>													100.00	98.45
14: <i>Ph. Colchicus</i>														100.00

1: *Struthio camelus* PRL AB36288, 2: Campbell-1 PRL gene Ex5, LC56501, 3: Moulard-1 PRL gene Ex5, LC565017, 4: Muscovy-2 PRL gene Ex5, LC565020, 5: *A. platyrhynchos* PRL, AB158611, 6: Campbell-2 PRL gene Ex 5, LC565016, 7: Moulard-2 PRL gene Ex5, LC565018, 8: Muscovy-1 PRL gene Ex5, LC565019, 9: Pekin PRL gene Ex5, LC565021, 10: *Anser anser* PRL, GU984377, 11: *Gallus gallus* PRL, AB011438, 12: *Pavo cristatus* PRL, AB605393, 13: *Meleagris gallopavo* PRL, AH005340, 14: *Phasianus colchicus* PRL, AB605395

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Anas platyrhynchos, PRL gene Ex 1, AB158611          TCCCACGACTGAAATTCCTAATGAAATCCCTCTCACAGTTACAAATAATAAAAAAATAC      60
Pekin-1 duck breed PRL gene Ex 1, alleleB           -----AAATTCCTCTCACAGTTACAAATAATAAAAAAATAC      38
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----A-----T-----                        38
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----A-----T-----                        38
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----A-----T-----                        38
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----A-----T-----                        38
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----A-----T-----                        38
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----A-----T-----                        38
Muscovy-2 duck breed PRL gene Ex 1, alleleA         -----A-----T-----                        38
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Anas platyrhynchos, PRL gene Ex 1, AB158611          TGAATATGAATGTGGAAGAAAGGCAGTTTGATGTTGTAATTATCGAGGTAACCTCCAGC    120
Pekin-1 duck breed PRL gene Ex 1, alleleB           TGAATATGAATGTGGAAGAAAGGCAGTTTGATGTTGTAATTATCGAGGTAACCTCCAGC    98
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----T-----T-----                        98
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----T-----T-----                        98
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----T-----T-----                        98
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                        98
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----T-----T-----                        98
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                        98
Muscovy-2 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                        98
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Anas platyrhynchos, PRL gene Ex 1, AB158611          ACCTGTTGAATATATGCAAAATGGACCCGGATGGTGTATATAAATCTGGTATGTGCAGA    180
Pekin-1 duck breed PRL gene Ex 1, alleleB           ACCTGTTGAATATATAAGAAAATGGACCCGGATGGTGTATATAAATCTGGTATGTGCAGA    158
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----A-A-G-----T-----                    158
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----A-T-C-----T-----                    158
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----A-T-C-----T-----                    158
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----G-T-C-----C-----                    158
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----G-T-C-----C-----                    158
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----G-T-C-----C-----                    158
Muscovy-2 duck breed PRL gene Ex 1, alleleA         -----G-T-C-----C-----                    158
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Anas platyrhynchos, PRL gene Ex 1, AB158611          AAATAAAAGCAAGTATTGAGACTTCTTTCTGGCAGAGCAAGTCATCCTACAGGGTCTCTA    240
Pekin-1 duck breed PRL gene Ex 1, alleleB           AAATAAAAGCAAGTATTGAGACTTCTTTCTGGCAGAGCAAGTCATCCTACAGGGTCTCTA    218
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----T-----T-----                    218
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----C-----C-----                    218
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----C-----C-----                    218
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                    218
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----T-----T-----                    218
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                    218
Muscovy-2 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                    218
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Anas platyrhynchos, PRL gene Ex 1, AB158611          CCATGAGCACCAAGGGGGATTCGTTGGAAGGTAAGACTTTAGCCATTCACCTGTGCGATAA    300
Pekin-1 duck breed PRL gene Ex 1, alleleB           CCATGAGCACCAAGGGGGATTCGTTGGAAGGTAAGACTTTAGCCATTCACCTGTGCGATAA    278
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----A-----A-----                    278
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----A-----A-----                    278
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----A-----A-----                    278
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----A-----A-----                    278
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----A-----A-----                    278
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----A-----A-----                    278
Muscovy-2 duck breed gene Ex 1, alleleA             -----A-----A-----                    278
*****

Anas platyrhynchos, PRL gene Ex 1, AB158611          TTTTATGTTTTTGTGTTGTTGTTGTTGTTTTTACTTTAGATTGAATTTGGAAGTAA      360
Pekin-1 duck breed PRL gene Ex 1, alleleB           TTTTATGTTTTTGTGTTGTTGTTGTTGTTTTTACTTTAGATTGAATTTGGAAGTAA      338
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----G-T---GT---G---TG---G-T-----T-----  338
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----G-T---GT---G---TG---G-T-----T-----  338
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----G-T---GT---G---TG---G-T-----T-----  338
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----G-T---TG---T---GT---T-G-----T-----  338
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----G-T---TG---T---GT---T-G-----T-----  338
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----G-T---TG---T---GT---T-G-----T-----  338
Muscovy-2 duck breed PRL gene Ex 1, alleleA         -----G-T---TG---T---GT---T-G-----T-----  338
*****

TCTAGA
Anas platyrhynchos, PRL gene Ex 1, AB158611          CTGACAGGTAACAACCTCTAGA AACTGAGGCTCTCCAGACATCCAGTTTTCAAGTATAGG    420
Pekin-1 duck breed PRL gene Ex 1, alleleB           CTGACAGGTAACAACCTCTAGA AACTGAGGCTCTCCAGACATCCAGTTTTCCAGTATAGG    398
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----T-A-----G-----                    398
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----T-A-----G-----                    398
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----T-A-----G-----                    398
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----C-A-----G-----                    398
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----C-A-----G-----                    398
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----C-A-----G-----                    398
Muscovy-2 duck breed PRL gene Ex 1, alleleA         -----C-A-----G-----                    398
*****

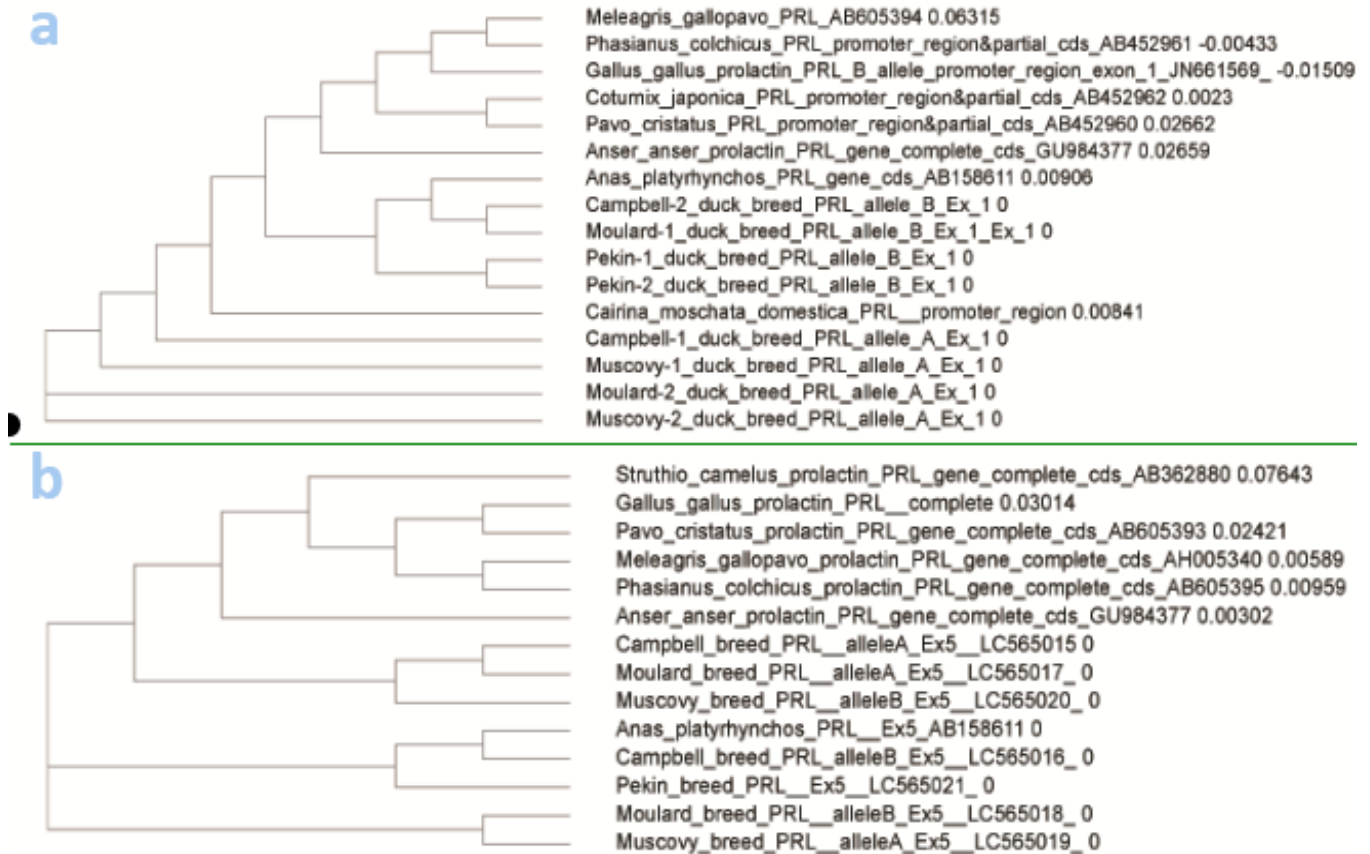
Anas platyrhynchos, PRL gene Ex 1, AB158611          TGAAACTTGTCTCTGCATC      439
Pekin-1 duck breed PRL gene Ex 1, alleleB           TGAAACTTGTCTCTGCATC      417
Pekin-2 duck breed PRL gene Ex 1, alleleB           -----T-----T-----                    417
Moulard-1 duck breed PRL gene Ex 1, alleleB         -----T-----T-----                    417
Campbell-2 duck breed PRL gene Ex 1, alleleB        -----T-----T-----                    417
Moulard-2 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                    417
Campbell-1 duck breed PRL gene Ex 1, alleleA        -----T-----T-----                    417
Muscovy-1 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                    417
Muscovy-2 duck breed PRL gene Ex 1, alleleA         -----T-----T-----                    417
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**Figure 2.** Multiple DNA sequence alignment of *PRL* Ex1 of the four Egyptian duck breeds (Campbell, Moulard, Pekin and Muscovy) with the *Anas platyrhynchos PRL* gene Ex 1, AB158611, and nucleotide sequences of alleles A and B showing SNPs at nt 356 and the presence of *Xba*I restriction site (T↓CTAGA) in alleles B.



**Figure 3.** Multiple DNA sequence alignment of PRL Ex5 of the four Egyptian duck breeds (Campbell, Moulard, Pekin and Muscovy) with the *Anas platyrhynchos* PRL gene Ex 5, AB158611, and nucleotide sequences of alleles G and C showing SNPs position at nt 165 and the presence of *Dra*I restriction site (3'AAA↓TTT 5') (underlined) in alleles C.



**Figure 4a.** Phylogenetic tree of the Multiple DNA sequence alignment of the four Egyptian duck breeds (Moulard, Pekin, Campbell and Muscovy) *PRL* gene exon 1 with the other sequences of avian prolactin gene species published in GenBank database. **Figure 4b.** Phylogenetic tree of the Multiple DNA sequence alignment of the Egyptian duck breed *PRL* gene exon 5 with the other sequences of avian prolactin gene species published in GenBank database.

Sequence comparison of *PRL* gene exon 1, between the four Egyptian duck breeds and other avian Species, appeared in percent identity matrix of DNA multiple sequence alignment of exon 1 (Table 2) and phylogenetic tree (Figure 4a). Likewise, DNA sequence comparison of exon 5, between the four breeds, and other birds, were illustrated in percent identity matrix of DNA multiple sequence alignment of exon 1 (Table 3) and phylogenetic tree (Figure 4b).

Regards to the phylogenetic tree, of Egyptian duck breeds, *PRL* gene exon1, the locus of Moulard-1 was related to Campbell-2 and Pekin, more than Moulard-2, Campbell-1 and Muscovy. However, the locus of Muscovy was related to Moulard-2, Campbell-1, more than Pekin (Figure 4a). For exon 5 tree, the locus of Moulard-1 was related to Campbell-2, Muscovy-2, more

than Moulard-2, Campbell-2, Muscovy-1 and Pekin (Figure 4b).

Lastly, prolactin gene sequence, in the four studied strains, was aligned with Duck genome, by using the BLAST program. The results of these alignments represent Duck prolactin (*PRL*) gene sequence on chromosome 2 (APL 2), *Anas platyrhynchos* isolate PK-2015 chromosome 2, IASCAAS Peking Duck PBH1.5 ranging from 49241262 to 49241678 for prolactin promoter exon 1 gene, and ranging from 49246949 to 49247348 for exon 5.

## DISCUSSION

Research on the *PRL* gene have been widely carried out, the avian *PRL* gene is highly conserved and most



sequence polymorphisms in the PRL gene occur in 5' flanking region, 3' flanking region (Kansaku et al., 2008). The literatures focused on polymorphisms of PRL gene in 5' flanking region (promoter region) which has been considered as an excellent experimental model for studying both tissue-specific and hormonally regulated activation of gene transcription. The literature lightened up PRL gene polymorphism, in 5' flanking region (promoter region) which has been respected as a perfect experiential example, for exploring both tissue-special and hormonally managed activation of gene transcription (Elsholtz et al., 1991; Li et al., 2009; Wang et al., 2011; Chang et al., 2012).

In this study, the results showed that Genetic polymorphisms of PRL gene by RFLP analysis, so the restriction enzymes *XbaI* and *DraI* have been used. The results of RFLP characterization analysis using the *XbaI* enzyme showed that the four Egyptian duck breeds PRL gene exon 1 and part of intron 1 at 10-weeks-old body weight had two alleles (A and B) and three genotypes AA, AB, BB, ducks with high frequency of allele A superior at egg weight compared to others. Into the bargain, the association of intron 1 polymorphism of PRL gene with egg weight was studied (Li et al., 2009) and agreed with present study. PCR-RFLP produced three genotypes AA, BB, and AB, and ducks of BB genotype were higher egg production and superior egg weight at 30 weeks than AB genotypes, which in agreement with this study. Wang et al. (2011) found variations in exons 2, 4 and 5 in local Chinese ducks, but the relationship with annual egg production was shown by exon 5. The prolactin gene SNP at intron 1, C→A mutation at position 386 at introns which can be detected by *XbaI* enzyme (Mazurowski et al., 2016), identifying two alleles G and T, and three genotypes GG, TG and TT. PRL/*XbaI* locus was found polymorphic in Pekin and Moulard duck populations, while monomorphic in Muscovy duck breed. Thus, the result would be useful as a control for genetic equilibrium in Muscovy ducks. In addition, Bai et al. (2019) studied the association between PRL gene with egg production. They found three genotypes AA, AG, GG and ducks that have GG genotype were greater in egg weight and egg production when compared to the other genotypes. Prolactin hormone gene can use as a genetic marker for reproductive traits (Bai et al., 2019; Basumatary et al., 2019). The C359A polymorphism was reported in Khaki Campbell duck as being associated with egg production at 300 days, with ducks with the GT genotype producing a

greater number of eggs than those with TT and GG genotypes (Chuekwon and Boonlum, 2017).

RFLP characterization analysis results by using the *DraI* enzyme on the exon 5 PRL gene fragment resulted that four Egyptian duck breed PRL gene exon 5 at 10-weeks-body-old weight had two alleles (G and C) and three genotypes GG, GC, CC, ducks that had high frequency of allele C were superior at egg production compared with others. Also, the results of PRL/*DraI* in Bayang ducks resulted three genotypes consisting of homozygotes (CC), heterozygotes (CT) and homozygotes (TT) with frequencies of 0.684, 0.293 and 0.21, respectively. The proportion of alleles obtained in the Prolactin (PRL/*XbaI*) gene fragments in the Bayang duck studied has a frequency of genotype and allele not much different from the genotype and allele frequency values in the study of Shaoxing local Chinese ducks (Yurnalis et al., 2019). These are in accordance with present results. The different distribution of genotypes in different duck populations may be described to the different genetic background of these populations (Wang et al., 2011). Since the chicken PRL gene had been cloned and sequenced (Watahikiet al., 1989), most research focused on polymorphism detection in this gene.

For genetic polymorphism of PRL gene, comprising the promoter, exon 1, part of intron 1 and exon 5, the genomic sequence comparisons between four Egyptian duck breeds PRL gene exon 1 sequence revealed that Moulard-1 breed sample shared high similarity (100%) with Campbell-2, (99.28%) with Pekin and (97.36%) with Moulard-2, Campbell-1 and Muscovy breed samples. As Muscovy resulted high similarity (100%) with Campbell-1 and Moulard-2, and (97.12%) with Pekin. For the PRL gene exon 5 the sequence comparisons revealed that Moulard-1 shared high similarity (100%) with Campbell-1, Muscovy-2, but shared similarity (97.25%) with Moulard-2, Campbell-2, Muscovy-1 and two samples of Pekin-1 and Pekin-2. Several SNPs were revealed from the both type (transition and transversion) for the two exons 1 and 5. However, the difference in genotypes between four Egyptian duck breed resulted from the presence of SNPs at T/C mutation position 378 bp in intron 1 region and A/G mutation at position 5871 bp in exon 5 region (Accession no. AB158611).

A great number of SNPs were reported in PRL gene for chicken. From direct sequencing and association analysis, Cui et al. (2006) got six SNPs (C-2402T, C-2161G, T-2101G, C-2062G, T-2054A and G-2040A) and a 24-bp indel (insertion-deletion) showed that the 24-bp indel was correlated to egg production and chicken

broodiness. Also, three mutations screened by Liu *et al.* (2007) (C-1607T, C-5749T and T-5821C) showed a link between different haplotypes and production of eggs. In geese, three SNPs (A-401G, G-268A and T-266A) were screened in the 5'-proximal region of *PRL* gene and statistical analysis indicated that these polymorphisms have the potential to be used for egg production in molecular breeding (Jiang *et al.*, 2009). All the above studies showed that *PRL* is an effective candidate gene for production eggs.

Assorted gene SNPs, concerning egg-laying traits in both chicken and geese, have been exposed, with few reviews in ducks (Kang *et al.*, 2012; Kulibaba, 2015; Alsiddig *et al.*, 2017; Mohamed *et al.*, 2017; Feng *et al.*, 2018). There was a number of new SNPs, not the 12 SNPs hit in the six native Chinese duck strains (Wang *et al.*, 2011). These results showed that the duck *PRL* gene in these duck populations is rich in polymorphisms in these duck populations.

Sequence comparisons between genomic sequence of *PRL* gene exon 1 from the four Egyptian duck lines and from the other avian species showed that sequence alignments of Moulard allele B and Campbell allele B shared similarity (99.04%, 97.45%, 95.64%, 88.00%, 76.50%, 88.89%, 86.35% and 84.36%), Muscovy exon 1, Moulard allele A and Campbell allele A shared similarity (96.40%, 98.72%, 94.19%, 88.44%, 76.50%, 89.30%, 86.75% and 84.73%) and also, Pekin exon 1 shared similarity (98.08%, 97.02%, 95.16%, 87.56%, 76.00%, 88.48%, 85.94% and 84.00%) with *PRL* gene of *A. platyrhynchos* (Moulard duck), Muscovy duck, geese, chicken, turkey, Japanese quail, Indian peafowl, and ring-necked pheasant, respectively. Also, the sequence comparisons results of Egyptian duck breeds *PRL* gene exon 5 and the other avian species revealed that Moulard allele A, Campbell allele A, and Muscovy allele B shared similarity (97.75%, 97.50%, 83.89%, 84.48%, 83.54%, 87.17% and 84.20%) and (Moulard allele B, Campbell allele B, Muscovy allele A and Pekin) shared similarity (100.00%, 98.75%, 85.68%, 85.75%, 85.09%, 87.96% and 86.65) with *A. platyrhynchos* (Moulard duck), goose, chicken, turkey, ring-necked pheasant, Ostrich and Indian peafowl, respectively.

The *PRL* gene has been cloned formerly in divergent avian species like pigeon, duck, chicken, quail, turkey and pigeon (Liu *et al.* 2008). Duck *PRL* was found to have sequence identity (92.0%, 91.7% and 91.4%) at the cDNA level compared to *PRL* of chicken, turkey and quail, respectively. The mature duck *PRL* has an overall similarity with a comparable region of chicken (95.5%),

turkey (92.5%) and quail (95.5%) *PRL* (Kansaku *et al.*, 2005). Also, the sequence analysis of the proximal region, of duck *PRL* promoter, displayed a high plane of similarity to turkey and chicken *PRL* promoter. These results viewed that the mechanisms, modulating the gene expression, may be vastly conserved in avian species (Kansaku *et al.*, 2005). Over and above, since the avian *PRL* gene was cloned and sequenced, most researches illuminate new polymorphic sites in this gene (Rashidi *et al.*, 2012).

The phylogenetic tree of exons 1 and 5 for DNA *PRL* gene in the different avian species showed that the locus of four Egyptian duck was related to *PRL* gene of *A. platyrhynchos*, *A. anser* more than of *G. gallus*, *M. gallopavo*, *P. colchicus*, *S. camelus* and *P. cristatus*. This clustering based on both of nucleotide of *PRL* gene clearly showed that the phylogenetic inter-relationship among these species and is generally in agreement with the known species relationships. This tree was constructed using Mr. Bayes, employing a previously calculated gene family with multiple sequence alignment (Huelsenbeck and Ronquist, 2001; Siltberg and Liberles, 2002).

## CONCLUSION

The results of this study manipulating the four Egyptian duck breeds; Campbell, Moulard, Muscovy and Pekin, supported previous findings. That there are many SNPs found in the sequences in the four Egyptian duck breeds. The utmost vital ones, are those found at the restriction sites of *Xba*I, for the amplified fragment contained the promotor, exon 1 and intron 1 (T378C), and exon 5/*Dra*I (A5871G). These data could serve as a basis for further insight into this avian gene. Owing to the fact that the genotypes distribution in this studied between the four Egyptian duck breeds was not in Hardy–Weinberg equilibrium, to confirm the observed associations, further research should be applied to a broad and more homogeneous population of ducks. In addition, a survival evaluation and post slaughter examination of ducks must be carried out to ascertain any potential connections with polymorphic variants in the *PRL* gene present in duck.

## DECLARATIONS

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### Authors' contributions

All authors contributed evenly throughout the manuscript. All authors participated equally in the study plan and design. EME, NMS, DMM and MAM collected the samples from different locations and isolated the nucleic acids. NMS, DMM and MAM carried out PCR and sequencing analyses. NMS, DMM, MAM and KFM carried out the statistical analysis of data and reported the results of the molecular analysis. KFM, NMS and MAM collaborated on writing, revising, and improvement of the article for publication. All authors read and approved the final.

### Competing interests

The authors declare that they don't have any conflict of interest.

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