Determination of Polymorphisms in Pituitary Genes of the Native Afghani Naked Neck Chicken

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We investigated means to improve the production of the indigenous Naked Neck chicken in Afghanistan. Specifically, we analyzed single nucleotide polymorphisms (SNPs) in the prolactin (PRL) (24 bp indel), growth hormone (GH) (T185G), and pituitary specific transcript factor 1 (PIT-1) (intron 5) genes. Blood samples were collected from 52 birds and genomic DNA was extracted. Polymorphisms in the mentioned loci were analyzed by PCR, allele-specific PCR, and PCR-restriction fragment length polymorphism (RFLP) using TaqI and MspI endonucleases. Cloning followed by DNA sequencing was performed to ascertain the accuracy of the PCR-RFLP analysis for PIT-1. Two alleles were found for the PRL 24 bp indel, GH (T185G), and PIT-1/TaqI, with the following respective allelic frequencies: PRL-Ins 0.64 and PRL-Dels 0.36, GH-T 0.91 and GH-G 0.09, and PIT-1-A 0.64 and PIT-1-B 0.36. Regarding the PIT-1/MspI polymorphism, three novel MspI recognition sites, as well as two reported MspI recognition sites, were detected in intron 5. Moreover, during sequence screening, two novel SNPs were found that generated restriction sites for MseI. Therefore, our results suggest that the PRL indel, GH T185G, and PIT-1/TaqI polymorphisms may be used as selection markers for Afghanistan Naked Neck chickens. Intron 5 of PIT-1 in the Afghani Naked Neck chicken was highly polymorphic compared to the reported Gallus gallus PIT-1 gene (GenBank accession no. NC_006088.4).

Key words: genetic polymorphism, Naked Neck, PIT-1, pituitary hormone

Introduction

The Naked Neck chicken breed is widespread and indigenous to numerous African and Asian countries (DAD-IS and FAO, 2012). This breed is native to Afghanistan and is well known for its egg production and resilience to disease and hot climates. An autosomal dominant gene (Na) controls the bared neck trait, which results in heat reduction and improvement in thermoregulation. The Na gene is known to be associated with heat tolerance and Na/na birds are superior in carcass yield, laying rate, mean egg weight, eggshell strength, and egg mass (Merat, 1990; Njenga, 2005). Furthermore, in high ambient temperatures and unfavorable environments, the dominant Na allele positively affects breast weight and growth rate, resulting in minimal loss of body weight during heat stress, high levels of heat shock protein 70 (HSP70), good feed conversion ratio, desirable carcass traits, and reduced effects of high ambient temperatures on fertility (Njenga, 2005; Islam and Nishibori, 2009). Thus, the Naked Neck chicken is a valuable breed for hot climates, including Afghanistan, and its characteristics can be further genetically investigated to enhance traits. Molecular markers are useful tools for improving production traits in farm animals and, to increase selection efficiency, marker-assisted selection linked to quantitative trait loci allows for the direct selection of genotypes in a population (Lamont et al., 1996).

The body weight and reproductive performance of vertebrates are regulated by the coordinated action of multiple factors (Sharma et al., 2008), including pituitary specific transcription factor 1 (PIT-1) that regulates vertebrate pituitary development, pituitary cell proliferation, and hormone expression. Moreover, PIT-1 also regulates the mRNA expression of growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone beta subunit (TSHB) in the

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pituitary gland (Bodner et al., 1988; Castrillo et al., 1991; Cohen et al., 1996). In the pituitary gland of post-hatched chicks, mRNA expression of PIT-1 was shown to correlate with GH and PRL expression (Tanaka et al., 1999). Additionally, PIT-1 reportedly transactivates the chicken GH and PRL promoters in vitro (Ohkubo et al., 2000; Ip et al., 2004), thus influencing growth and development. Consequently, mutations in PIT-1 may result in altered expression levels of PRL, GH, TSHB, and PIT-1 itself (Li et al., 1990; Cohen et al., 1996).

Polymorphisms in PRL, GH, and PIT-1 have been widely studied in domestic chickens with regard to reproduction, growth, and metabolism. For example, a 24 bp insertion and deletion in the promoter region of chicken PRL significantly affected egg production and occurrence of broodiness (Cui et al., 2006). Single nucleotide polymorphisms (SNPs) in this gene have also been associated with egg production in chickens (Kulibaba and Podstreshnyi, 2012; Li et al., 2012). Researchers have confirmed that chicken GH (cGH) has essential roles in growth, reproduction, body composition, aging, and egg production, and SNPs in cGH are associated with egg production and disease resistance (Kuhnelein et al., 1997), as well as growth and carcass traits (Yan et al., 2003; Wei et al., 2009). It was recently reported that the T185G substitution in cGH is significantly linked with growth and egg production (Su et al., 2014). Seventeen synonymous SNPs have been identified in chicken PIT-1, two of which were significantly associated with partial carcass traits (Xu et al., 2012). Restriction fragment length polymorphism (RFLP) analysis for TaqI and MspI in intron 5 of chicken PIT-1 in a cross of White Recessive Rock with Chinese Xinghua chickens (Nie et al., 2005, 2008) revealed an association between SNPs and egg production. Since the phenotypic and genotypic characteristics of any domestic breed, including the Naked Neck chicken, have not yet been characterized in Afghanistan, the present study aimed to determine whether economically valuable polymorphisms found in the PRL, GH, and PIT-1 genes of other chicken breeds are retained in Naked Neck chickens to address the possibility of using these polymorphisms to improve the traits of this breed.

## Materials and Methods

### Animals and DNA Extraction

Blood samples were collected from 52 Afghani Naked Neck chickens, comprising 25 females and 27 males reared in Kandahar, Afghanistan, and placed on FTATM ELUTE Micro Cards (GE Healthcare, Buckinghamshire, UK). Genomic DNA was extracted from the FTATM ELUTE Micro Cards according to the manufacturer’s protocol, and DNA concentrations were measured using a BioSpec-Nano (Shimadzu, Kyoto, Japan).

### PCR Detection of a PRL Polymorphism

A set of primers was designed for the PRL 24 bp indel (insertion/deletion) as previously reported by Cui et al. (2006) (Table 1) and used to amplify either a 130 bp or a 154 bp fragment containing the 24 bp indel in the chicken PRL promoter. Each PCR reaction (2 μL) contained 10 μL of 2× Green Master Mix (Promega, WI, USA), 1 μL of each forward and reverse primer (10 pmol/μL), 40–70 ng of genomic DNA as a template. PCR amplification was carried out for 35 cycles with an initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, with a final extension for 5 min at 72°C. Finally, the PCR products were electrophoresed on 3% agarose gels and stained with ethidium bromide to visualize the DNA under UV light.

### Allele-specific PCR Determination of a GH Polymorphism

Allele-specific PCR was used to detect the T185G polymorphism in the 5’ non-coding region of the chicken GH gene using a primer set (cGH_Common, cGH_T185, and cGH_G185) listed in Table 1. The PCR was performed in 20 μL reactions consisting of genomic DNA (40–70 ng), 10 pmol cGH_Common, 10 pmol cGH_T185 or cGH_G185, and 2× Green Master Mix (Promega). Amplification was performed for 35 cycles with an initial denaturation at 94°C for 5 min, then denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, with a final extension of 5 min at 72°C. Finally, the PCR products were electrophoresed on 2% agarose gels that were stained with ethidium bromide to visualize the DNA.

### PCR-RFLP Analysis of PIT-1

The polymorphic intron 5 of the chicken PIT-1 gene was

| Table 1. Primers used in the analysis for PRL, GH and Pit-1 |
|-----------------|------------------|------------------|
| Primer Name     | Primer sequence (5’ to 3’ )                          | Reference        |
| PRL 24bp Indel F| TTTAATATTTGTTGGAAGAGAAGACA                           | Cui et al., 2006 |
| PRL 24bp Indel R| ATGCCACTGTCCCTCGAAAAC                              |                  |
| cPIT-1F         | GGGACCTCTTCTACAGCTTCT                                 |                  |
| cPIT-1R         | GGGAGAATACAGGGAAAGGG                                  | Nie et al., 2008 |
| cGH_T185        | GGTGGAGTTTCTACCTGCGT                                 |                  |
| cGH_G185        | GGTGGAGTTTCTACCTGCGG                                 |                  |
| cGH_Common      | AATGCAGATGTTCGCCG                                    |                  |
| cPIT-1/SEQ1     | TCTCTAAACAGCTCTTGTC                                  |                  |
| cPIT-1/SEQ2     | ATACAGGGAAAGGCCG                                   |                  |
amplified by PCR in 20 μL reactions consisting of genomic DNA (40–70 ng); reverse and forward primers (10 pmol each of Pit-1R and cPit-1F; Table 1), dNTPs (5 mmol each), and TaKaRa Ex Taq DNA polymerase (1 unit; TaKaRa Bio, Shiga, Japan). Amplification was carried out for 30 cycles with an initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 45 s, and extension at 72°C for 1 min, with a final extension of 5 min at 72°C. Thereafter, 3 μL of each PCR product was separately digested with TaqI (New England Biolabs, Ipswich, MA, USA) and MspI (TaKaRa Bio) incubated at 65°C or 37°C, respectively for 4 to 8 h, and then electrophoresed on a 2% agarose gel stained with ethidium bromide to visualize the DNA.

**Sequencing of PIT-1 Intron 5**

After PCR-RFLP, amplified DNA that showed diverse digestion patterns for PIT-1/MspI was selected for direct sequencing. The PCR products separated on 2% agarose gels were purified using the MonoFas DNA purification kit 1 (GL Sciences Inc., Tokyo, Japan). Purified DNA (200 ng) served as sequencing template using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with each strand primer (cPIT-1/SEQ1 and cPIT-1/SEQ2; Table 1), according to the manufacturer’s instructions. The products were sequenced using the ABI 3130 Sequencer (Applied Biosystems).

To confirm the accuracy of the PCR-RFLP analysis and the results of direct sequencing, amplified DNA was subcloned into the pGEM-T Easy vector (Promega) followed by RFLP analysis for MspI. Cloned DNA was also sequenced in both directions, using the ABI 3130 Sequencer (Applied Biosystems).

**Genotyping and Statistical Analysis**

Based on the genotypes visualized on agarose gels, allelic frequencies were calculated via the maximum likelihood formula (Kulibaba and Podstreshnyi, 2012):

\[
P_A = \frac{2n_{AA} + n_{AB}}{2N}
\]

\[
P_B = \frac{2n_{BB} + n_{AB}}{2N}
\]

where, \(P_A\) and \(P_B\) are the frequencies of the existing alleles, \(n_{AA}\) and \(n_{BB}\) are the numbers of homozygous birds, \(n_{AB}\) is the number of heterozygous birds, and \(2N\) is the number of alleles (twice the number of individuals in the study group). Moreover, the Chi-square test (\(\chi^2 = \sum(O - E)^2 / E\), where \(O\) is the observed value and \(E\) is the expected value) was used to confirm whether this population was in Hardy–Weinberg equilibrium.

**Results**

**Frequency of the PRL 24 bp Indel Polymorphism in the Afghani Naked Neck Chicken**

For the PRL 24 bp indel, two alleles, \(In\) and \(Del\), were distinguishable with three genotypes, \(In/In\), \(In/Del\), and \(Del/Del\) (Fig. 1). The gene frequencies for \(In\) and \(Del\) were 0.64 and 0.36, respectively, and the genotypic frequencies for \(In/In\), \(In/Del\), and \(Del/Del\) were 0.40, 0.48, and 0.12, respectively (Table 2). The \(\chi^2\) test indicated that the genotypic distribution for the indel polymorphism was not in Hardy–Weinberg equilibrium.

**Frequency of the GH T185G Polymorphism in the Afghani Naked Neck Chicken**

For the GH T185G polymorphism, two alleles, \(T\) and \(G\), were distinguishable with three genotypes, \(TT\), \(TG\), and \(GG\), respectively (Table 2). The \(\chi^2\) test indicated that the genotypic distribution for the T185G polymorphism was not in Hardy–Weinberg equilibrium.

![Fig. 1. Determination of the PRL 24 bp indel mutation by agarose electrophoresis.](image)

**Table 2. The genotype and genetic frequency of PRL 24 bp indel, GH T185G and Pit-1/Taq1 in Afghani Naked Neck chicken population**

| Population          | Polymorphism | Genotypic frequency | Genetic frequency | Locus equilibrium |
|---------------------|--------------|---------------------|-------------------|------------------|
|                     | PRL 24 bp indel | \(In/In\) (\#\) | \(In/Del\) (\#\) | \(Del/Del\) (\#\) | \(In\) | \(Del\) | \(\chi^2\) test |
| Naked Neck (n=52)  | \(In/In\) (21) | 0.40 (21)           | 0.48 (25)         | 0.12 (6)         | 0.64   | 0.36   | \(P<0.05\)     |
|                     | \(In/Del\) (25) | 0.48 (25)           |                    |                  |        |        |               |
|                     | \(Del/Del\) (6) |                    | 0.12 (6)          |                  | 0.64   | 0.36   |               |
|                     | GH T185G      | \(TT\) (\#)        | \(TG\) (\#)       | \(GG\) (\#)      | \(T\)  | \(G\)  |               |
|                     | 0.83 (43)     | 0.17 (9)            | 0.00 (0)          |                  | 0.91   | 0.09   |               |
| PIT-1/Taq1 I       | \(AA\) (20)  | 0.38 (20)           | \(AB\) (27)       | \(BB\) (5)       | 0.64   | 0.36   |               |
|                     | \(AB\) (27)  | 0.52 (27)           |                    |                  |        |        |               |
|                     | \(BB\) (5)   | 0.10 (5)            |                    |                  |        |        |               |
were recognized via allele-specific PCR, in which two genotypes, TT and TG, were detected (Fig. 2). The genotypic frequencies of T and G alleles were 0.91 and 0.09, respectively (Table 2). The GG genotype was absent in this population (Table 2). The $\chi^2$ test indicated that the genotypic distribution of $GH\ T185G$ was not in Hardy–Weinberg equilibrium.

**PCR-RFLP Analysis of PIT-1**

For the $PIT-1$ /$TaqI$ polymorphism, two alleles (A and B) with three genotypes (AA, AB, and BB) were clearly distinguished (Fig. 3). The fragment size generated for allele A was 599 bp without the $TaqI$ restriction site, while for allele B, containing the $PIT-1$ /$TaqI$ restriction site, digestion yielded fragment sizes of 456 and 143 bp. The frequencies of the A and B alleles were 0.64 and 0.36, respectively, and the genotypic frequencies of AA, AB, and BB were 0.38, 0.52, and 0.10, respectively (Table 2). Based on the $\chi^2$ test, this population was not in Hardy–Weinberg equilibrium. Notably, the allelic frequency for allele A (0.64) was significantly higher than for allele B (0.36) (Fig. 3; Table 2).

Figure 4a shows the PCR-RFLP analysis for $PIT-1$/Mspl digestion. Although Mspl did not digest some samples completely (i.e., lanes 3, 4, and 6), multiple digestion patterns were observed in the PCR-RFLP analysis for Mspl. Two Mspl sites were found in intron 5 of the $PIT-1$ gene in the Iranian commercial broiler line (Rodbari et al., 2011), generating 599 bp (A allele), 500 bp, and 99 bp (B allele); and 321 bp and 278 bp (C allele) DNA fragments. Naked Neck chickens carrying the B allele (lane 2; BB homozygote) and C allele (lane 1; CC homozygote) were identified, but AA homozygotes were absent in the population. However, unexpected PCR-RFLP patterns from a previous study were observed (lanes 3 to 9); one pattern was speculated to represent a homozygote of unknown genotype (lane 8) and heterozygotes of an unknown allele and the B allele (lanes 5 and 7) or and the C allele (lane 9). Therefore, direct sequencing was performed to validate the accuracy of our observed PCR-RFLP results.

**Sequencing of the PIT-1 Polymorphic Region**

The DD homozygote was confirmed by direct sequencing (lane 8 in Fig. 4a). The sequencing results revealed an A-to-G transition at position 280 of the amplified DNA fragment, creating a new Mspl recognition site (Fig. 4b). Therefore, cloning and sequencing were performed for the unexpected digestion patterns observed by PCR-RFLP (Fig. 4a; lanes 3, 4, and 6). As a result, the observed individuals possessed two new point mutations, A-to-G and T-to-C transitions, which generated two new Mspl restriction sites at nucleotide positions 500 and 519, respectively. We named this allele E (Fig. 5a, lane 1 and Fig. 5b, panel 2). Three point mutations were detected for one individual (Fig. 4a, lane 6), in which T-to-C, A-to-G, and T-to-C transitions generated three Mspl recognition sites at nucleotide positions 280, 500, and 519, respectively. This allele was called F (Fig. 5a, lane 4 and Fig. 5b, panel 3).

Moreover, while screening the $PIT-1$ intron 5 sequence, we also found a double point mutation resulting in a CG-to-TA transition and a single point mutation resulting in an A-to-T transversion compared to the reported *Gallus gallus* $PIT-1$ sequence (GenBank accession no. NC_006088.4). These mutations generated new MseI restriction sites (Fig. 6).

**Discussion**

Even in environments that are poor and unfavorable for animal production, the Naked Neck chicken breed in Afghanistan still provides high-quality protein in the form of meat and eggs to the rural population, which comprises 75% of the total population of Afghanistan. The resilience of the Naked Neck chicken breed in high ambient temperatures promotes productivity (Islam and Nishibori, 2009). Genetic
selection, in addition to livestock management such as feeding and veterinary care, plays an important role in further improving the productivity of local breeds (Padhi, 2016). In the present study, PCR, allele-specific PCR and PCR-RFLP were used to scrutinize known mutations in somatotropic axis-related genes (PRL, GH, and PIT-1) that are significantly associated with growth and reproduction in diverse chicken breeds (Cui et al., 2006; Nie et al., 2008; Su et al., 2014), to determine the utility of adapting these polymorphisms to enhance the productive performance of the Naked Neck chicken in Afghanistan. Among these genes, PIT-1 regulates not only its own transcription but also that of PRL, GH and TSHB. In the pituitary gland, PIT-1 mRNA has been detected in somatotropic, thyrotrrophic, and lactotrophic cells (Simmons et al., 1990).

The PRL gene is known to be responsible for egg production and broodiness in chickens (Shimada et al., 1991). Polymorphisms in PRL were significantly correlated with egg production in the Muscovy duck (Zhang et al., 2015) and egg quality and number in chickens (Cui et al., 2006; Bhattacharya et al., 2012). Moreover, the indel polymorphism of PRL is also associated with egg laying and broodiness (Cui et al., 2006; Bagheri et al., 2013). The In allele has significant effects on egg production and the frequency of the In allele was higher (0.98) than that of the Del allele (0.02) in the White Leghorn chicken that produces more than 300 eggs in a year. The In and Del frequencies in Taihe chickens were 0.14 and 0.86, respectively, and this breed shows strong incubation behavior and reduced egg production (Cui et al., 2006). This group further concluded that the Del allele might also be associated with broodiness, which results in the loss of egg production (Cui et al., 2006). In this study, we found that the allelic frequency of In was higher than that of Del in Naked Neck chickens. This result
Fig. 5. RFLP analysis and sequencing result for PIT-1 intron 5 after cloning. (a) Lanes 1, 2 (cloned sample of lane 3 from Fig. 1a), 3, and 4 (cloned sample of lane 6 from Fig. 1a) represent alleles E, B, B, and F, respectively, in PIT-1 intron 5. The 19 bp fragment could not be visualized in this gel. M shows the molecular weight marker (100 bp ladder). (b) Panels (2) (named allele E) and (3) (named allele F) are the Naked Neck cloned sequences (of lane 3 or 4 and lane 6, respectively, from Fig. 1a) and panel (1) is the original sequence released by GenBank.

Fig. 6. Novel restriction sites for the MseI endonuclease in intron 5 of the PIT-1 gene in the Afghani Naked Neck chicken. Arrowhead indicates the SNPs in intron 5 of PIT-1 of the Naked Neck chicken. The upper sequence shows the original sequence reported by GenBank that contains only one MseI restriction site (GenBank accession no. NC_006088.4). The middle and lower sequences exhibit more than one MseI recognition site.
may indicate that the PRL genetic background is a limiting factor for productivity in Naked Neck chickens in Afghanistan.

Regarding the T185G polymorphism in GH, the genotypic frequency was significantly biased; TT and GT genotypes were found, but the GG genotype was not detected in the studied population (Table 2). The T185G polymorphism is known to be associated with increased total egg number in 300-day-old birds (EN 300), as well as egg and body weight in Recessive White chickens (RW) and Qingyuan partridges (QY). Birds possessing the TT and GT genotypes in QY and RW strains showed good performance in EN 300 (Su et al., 2014). Our results suggest that the T185G polymorphism in the GH gene has been almost fixed to T and further consideration of this SNP may not be relevant.

Polymorphism in intron 5 of the PIT-1 gene is also associated with productivity in highly selected exotic chicken breeds (Xu et al., 2012). In this study, PCR-RFLP and sequence analyses were carried out on the 599 bp fragment of intron 5 in the Naked Neck chicken. The results for the PIT-1/TaqI mutation were in accordance with both Nie et al. (2008) and Rodbari et al. (2011). However, the frequency of the B allele was significantly lower (36%) than that of the A allele (64%) in the Naked Neck chicken, which is an economically important locus for early growth rates in chickens. In the studied population, five novel polymorphisms were identified that generated three novel restriction sites for MspI and two for MseI. Only one restriction site for MspI was found in the originally reported sequence of the chicken PIT-1 gene and one recognition site for MseI (Nie et al., 2008). In the same region, Rodbari et al. (2011) found an extra allele, named C, in an Iranian line of broiler chicken by PCR-RFLP for MspI. It was previously shown that the PIT-1/MspI locus (BB genotype) had a positive correlation with average daily gain at 4-8 weeks of age in chickens (P<0.05). According to the different band patterns in the PCR-RFLP analysis, two alleles (A and B) with three genotypes (AA, AB, and BB) were identified for the PIT-1/MspI locus in Chinese broiler chickens (Nie et al., 2008). Remarkably, in Naked Neck chickens, the A allele for PIT-1/MspI was absent; this allele had previously been shown to have negative impacts on the early growth rate in Chinese native chickens and Iranian broiler chickens (Nie et al., 2008; Rodbari et al., 2011). In addition, the C allele found in Iranian broiler chickens was significantly associated with early growth rate (Rodbari et al., 2011). The absence of the

![Fig. 7. Schematic representation of MspI recognition sites in PIT-1 intron 5 that are generated by SNPs in the Naked Neck chicken of Afghanistan. Each box shows a different allele (A, B, C, D*, E*, and F*); the solid horizontal line in parentheses represents the 599 bp sequence of PIT-1 intron 5 in which / is the recognition site for MspI created by a point mutation. The double line between the solid circles represents the agarose gel fragment sizes visualized after digestion with MspI in agarose gel. Asterisk (*) indicates a new allele.](image-url)
A allele and presence of the C and D alleles in the Naked Neck chicken population were valuable observations noted in this study (Fig. 1a, lane 2). Importantly, the DD genotype found in the Naked Neck chicken comprised two different growth-associated alleles (B and C) that might influence the early growth rate of this breed (Fig. 4d). Additional research is needed to confirm this result. None of these SNPs have been associated with fat deposition and carcass traits in chickens (Nie et al., 2008).

The sequencing results after cloning and PCR-RFLP analysis revealed three novel SNPs that generated MspI recognition sites in intron 5 of Naked Neck chicken PIT-1 (Figs. 1b and 2), named alleles D, E, and F (Fig. 4). The mutations in the E and F alleles resulted in different fragment sizes after digestion with MspI (500 bp + 19 bp + 80 bp and 280 bp + 220 bp + 19 bp + 80 bp, respectively (Fig. 2a). It was difficult to determine the allelic frequency for the PIT-1/MspI locus of intron 5 in our studied population, as also demonstrated in other breeds (Nie et al., 2008; Rodbari et al., 2011). Two novel MseI sites were found in intron 5 of Naked Neck chicken PIT-1 that have yet to be reported in other chicken breeds and their association with productive traits have yet to be identified. Our results support the idea that nucleotide diversity in native chickens is greater than in commercial breeds (Nie et al., 2005).

In conclusion, the PRL 24 bp indel, GH T185G, and PIT-1/TaqI polymorphisms are fixed in Afghani Naked Neck chickens and may be viable selection markers to improve the breed. In addition, the 599 bp fragment from intron 5 of the PIT-1 gene in the Naked Neck chicken was highly polymorphic, and five novel SNPs were found in the population, generating three novel restriction sites for MspI and two for MseI. Further investigation is required to characterize these SNPs that may improve the productivity of this breed and thus enhance the economic value of the Naked Neck chicken in Afghanistan.

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