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Research Article

PROP-1 Gene a Selective Marker of Wool Production in Sheep Breeds of Balochistan Pakistan

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Abstract | *PROP1* gene plays a vital role in growth and development of wool production in mammals. The genetic variations in *PROP1* gene in the sheep breeds of Pakistan are unknown. This study was conducted to compare wool color and fleece weight and to analyze the *PROP1* gene of Balochi, Bibrik, Harnai and Rakhshani sheep breeds of Balochistan. The wool was sheared, its color and fleece weight were observed. The result reveled that wool color and fleece weight were black and white with 1.91 kg, full white, white with black muzzles and limb extremities with 1.83 kg, white gray mix with 1.85 kg, white with black muzzles and limbs extremities with 1.77 kg, for Balochi, Bibrik, Harnai and Rakhshani sheep breeds respectively. For *PROP1* gene analysis, the blood sample were collected from the selected sheep breeds and processed for DNA extraction. Primers were designed using Primer 3 software and PCR was performed. PCR products were sequenced and analyzed for observing genetic variation in *PROP1* gene of these four sheep breeds of Balochistan. The result for *PROP1* gene analysis showed 7 variations including 3 frame shift and 4-point mutations with c.63G>A, c.83G>A, c.94G>T, c.104C>G, c.237G>A, c.432T>C and c.450C>T were observed in all three exons of *PROP1* gene of Balochi, Bibrik, Harnai and Rakhshani sheep breed of Balochistan. The phenotypic variation related to wool production traits including the fleece weight and wool color with diverse pigmentation and genetic variations observed in the coding region of the *PROP1* gene. This study indicates that the *PROP1* gene may be used as a selective marker for the future selection of the sheep breeds for different wool productive traits.

Keywords | Genotypic variation, Phenotypic variation, Sheep, *PROP1* gene, Wool trait.

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INTRODUCTION

Pakistan is the 11th sheep producing country in the world and has well known 28 breeds of sheep. (Anonymous et al., 2013). In Balochistan (Province of Pakistan), some sheep breeds and strains are found with diverse phenotypic characteristics including the production traits i.e., meat, milk and wool. Balochi, Bibrik, Harnai and Rakhshani are well-known sheep breeds of Balochistan; these breeds usually produce coarse wool and this type of wool is usually used for the manufacturing of carpet (Raziq et al., 2009). Wool production of these sheep breeds is low as compared to other sheep breeds of Pakistan. However, wool production can be improved by selecting the animals with high

genetic potency. Therefore, it is very important to identify the major genes which have effects on the growth and production of wool.

Homeobox protein prophet of Pit-1 (*PROP1*) gene is also called pituitary-specific homeodomain factor, *PROP1* Human and prophet of Pit1, paired-like homeodomain transcription factor. It controls and offer directions for protein development and other genes respectively. *PROP1* is also well known transcription factor due to their mood of action, and this protein is originated in the pituitary gland of the brain. In the pituitary gland this protein helps in the differentiation of various types of the cells (Sornson et al., 1996; Mehta et al., 2008). *PROP1* codes a transcription factor (paired-like homeodomain) in the mechanism of pituitary gland. It has dual activations skills for DNA binding, transcription and in sheep it is on chromosomes 5 with 3 exons and 2kb in size having a structure similar to other mammalian's genes such as the human and pig. It is a positive regulator for growth hormone (GH), prolactin (PRL) and thyroid stimulating hormone (TSH), in mammals (Cohen et al., 1997; Ribeca et al., 2014). GH promotes protein synthesis and the growth of nearly all body tissues as well as having key role in the regulation of carbohydrate metabolism (Carter-Su et al., 2000). Any alteration on this gene may results in absence of hormones GH, PRL, TSH, and *PROP-1* (Lan et al., 2009 b). It plays a vital role relating to the growth, reproductive and fiber traits in domestic animals. Growth is a highly orchestrated biological process involving a delicate interplay of different factors including genes (Ekegbu et al., 2019).

Consequently, *PROP1* gene can be considered as a marker gene to effect wool production in ovine. This marker substantially contributes to variance of the trait expression in small ruminants and have been increasingly focus in the area of livestock genetics. Such markers should be investigated in Pakistani sheep breeds that would be helpful in the selection of sheep breeds for high wool production. Presently no information about *PROP1* gene has been reported in the Pakistani sheep breeds. Therefore, the present study was designed to investigate the genotypic variation in *PROP1* gene in different sheep breeds of Balochistan with diverse phenotypic variations including wool, color, yield and fineness.

MATERIALS AND METHODS

The research study was carried out in the Laboratory of Molecular Genetics, Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS), Uthal, Balochistan. The trial conducted according to the guideline of the declaration of Lasbela University of Agriculture, Water and Marine Sciences and all of procedures were approved by the Ministry of Livestock, Government of Balochistan, Islamic Republic of Pakistan.

Animal Selection And Dna Extractions

A total 120 animals of four different sheep breeds (Balochi, Bibrik, Harnai and Rakhshani) of Balochistan (n = 30/breed) were randomly selected for the current research. All selected individuals were healthy and unrelated. Animals were selected from their respective breeds and home tracts by identifying them on the basis of phenotypic characteristics. A total blood samples (n=120) of four sheep breeds of Balochistan were aseptically collected. The 5ml blood sample was collected from jugular vein from each sheep breed by using vacuum tubes with EDTA (ethyline diamine tetra acetic acid) as an anticoagulant. The samples were stored in ice container at -20°C and were brought to laboratory of molecular genetics, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, for further analysis. Phenotypic data including weight of fleece and color of wool were also recorded. Animal's record was maintained, includes information about the animal age, phenotypic characters, and area where the samples were collected. The age of the animals was detected by physical appearance and observing their teeth using method as describe by (Kumar et al.,1990).

Dna Extractions

DNA was extracted by inorganic method (Sambrook & Russel, 2001). Quantity and quality of the DNA samples were measured by using spectrophotometer (SPUV1100). The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA.

Primers Designing And Optimizations

For the amplification of three exonic region of *PROP1* gene the specific primers were designed by using the software (primer 3 software (Rozen & Skaletsky, 2000). Complete *PROP1* gene of *Ovisaries* (KP229296) available on NCBI (http://www.ncbi. nlm.nih.gov. Sequence and product size of the specific primers are given in (Table 1). Primers were optimized for their annealing temperature by gradient PCR in which a range of annealing temperature (64°C to 54°C) was used in thermo cycler. The temperature at which primer showed best results was selected. The subsequent PCR were carried out at optimized annealing temperatures. Polymerase chain reactions were carried out in a 25µL reaction mixture containing, PCR Buffer(5x) 2.5µL, Taq Polymerase 0.5µL, dNTPs 2.5µL, MgCl2 2µL, Primer Forward 1µL, Primer Reverse 1µL, DNA 3µL, Distilled Water 12.5µL. The amplification conditions for primers of the *PROP1* gene were as follows: denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 30 sec; annealing at 57°C for 30 sec; and extension at 72°C for 45 sec; with a final extension at 72°C for

Table 1: List of the primers used in this study

Primers	Oligo Name	Sequence	Length	Annealing Temp:	Product size (bp)
Exon-1	Forward	CACCGAGGAGGTCACAGTCT	20	62.5	
	Reverse	GTGACCATGGACACAGAAG	20	60.5	112
Exon-2	Forward	AAGACTTTCTCGTGCCCAAA	20	56.4	238
	Reverse	ACTCAGCCCTACCGGAATCT	20	60.5	
Exon-3	Forward	AGCATGGTTGTAGGGGTGAG	20	60.5	340
	Reverse	AACCGCAGAGCTAAGCAGAG	20	60.5	

Table 2: Codon modification of *PROP 1* gene in Balochi, Bibrik and Harnai sheep breeds

Serial No.	Breed	Variation Position	Codon #	Reference Codon	Changed Codon	References Amino acids	Changed amino acids
1	Bibrik	63bp	21	CTG	CTA	Lysine	Lysine
2	Balochi	85bp	29	GCT	ACT	Alanine	Aspartic Acid
3	Bibrik, Balochi and Harnai	94bp	32	GTG	GGT	Valine	Glycine
4	Harnai	104bp	35	TCG	TCA	Serine	Serine
5	Balochi, Bibrik	237bp	79	GCG	GCA	Alanine	Alanine
6	Bibrik, Harnai	432bp	144	TCT	TCC	Serine	Serine
7	Harnai	450bp	148	CCC	CCT	Proline	Proline

Table 3: Per anum fleece weight, wool color and finesse of different sheep breeds of Balochistan

Breed Name	Fleece weight	Wool Color	Finesse
Balochi	1.91	Black and white, brown and white, gray and white, off white and white and sometimes white with black or brown muzzles and limbs extremities	Course wool
Bibrak	1.83	Full white, white with black muzzle and limbs extremities	Course wool
Harnai	1.85	White gray mix, white with black and sometimes brown muzzles and limbs extremities	Course Wool
Rakhshani	1.77	White with black muzzles and limbs extremities	Course Wool

10 min, on a Thermocycler. The PCR product was subjected to electrophoresis for detection of amplified DNA.

DNA Sequencing And Analysis

After preparation of PCR products, the samples were sent to Center of Applied and Molecular Biology, University of Punjab Lahore for sequencing of the samples.

Analysis Of Sequencing Data

DNA sequencing data analysis was performed by using the software Bioedit (Hall et al., 1999). The blast2 software was used for the subject sequences to align against the normal sequences. Single nucleotide polymorphism (SNPs) sequences with EU743938. Any change in DNA sequence was observed and confirmed by comparing each sense and antisense.

RESULTS

To investigate the genetic variation in *PROP1* gene and their effect on wool production and quality in four different sheep breeds of Balochistan including Balochi, Bibrik, Harnai and Rakhshani. Phenotypic data including weight of fleece and color of wool were also recorded. DNA samples were extracted from the collected blood samples and checked for quantification and quality analysis by gel electrophoresis (1%) (Figure 1).

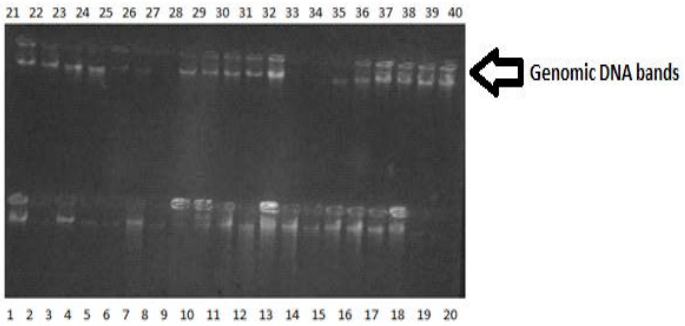

Figure 1: Gel Electrophoresis image showing genomic DNA bands of the samples

The exons 1-3 of the *PROP1* gene were amplified by using specific primers (Figure 2). Amplified PCR products were sequenced commercially.

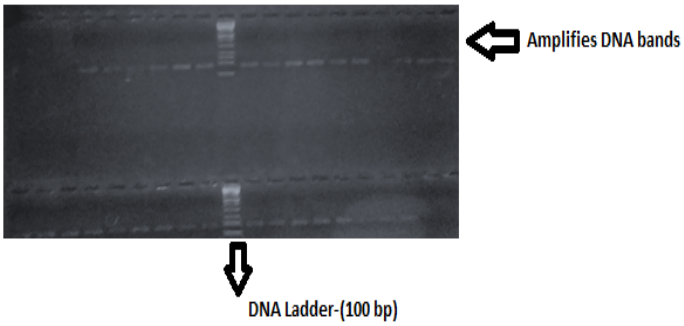

Figure 2: Amplified PCR products of exons 1-3.

Sequencing Results

The sequencing result revealed that 7 variations including 3 frame shift mutations and 4-point mutations were observed in all four sheep breeds of Balochistan. In exon-1 three variations were reported including c.63G>A (insertion of A, A at 63 and 64 nucleotide positions) a frame shift mutation (Figure 3), c.83G>A (pA29D) a missense heterozygous variation (Figure 4), and c.94G>T (insertion of G, G at nucleotide position 94,95) a frame shift mutation (Figure 5).

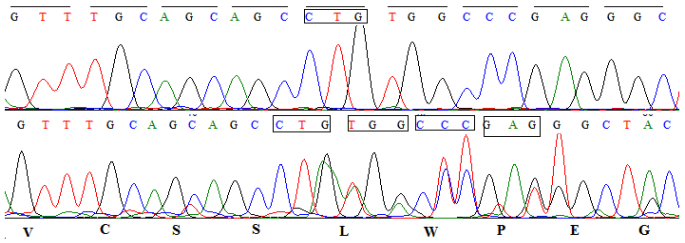

Figure 3: Insertion of A at the position, c.63 G>A (p.L21L) at exon 1 resultingframe shift mutation in the preceding codons

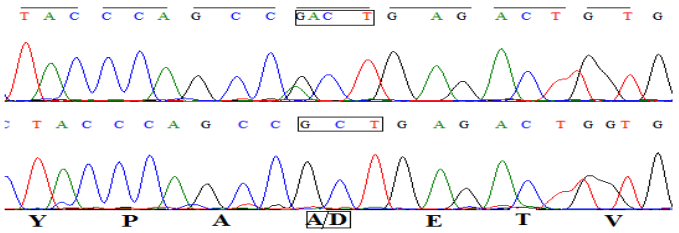

Figure 4: A Missense heterozygous variation at exon 1, c.85 G>A (p.A29D)

In exon 2, two variations were observed including c.104C>G and an insertion of A at 104 nucleotide position resulting a frame shift mutation (Figure 6) and c.237G>A (p.A79A) heterozygous silent variation (Figure 7).

In exon 3, two variations including c.432T>C (p.S144S) homozygous silent variation (Figure 8) and c.450 C>T (p.P148P) heterozygous silent variation (Figure 9) were reported.

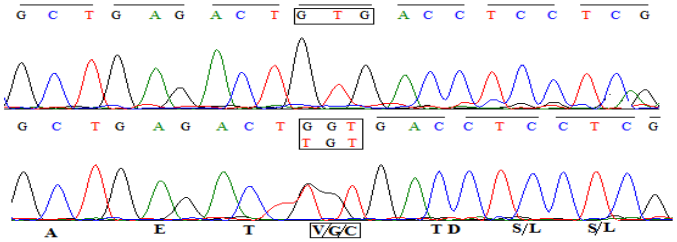

Figure 5: A frame shift mutation at exon 1 c.94 G>T and an insertion of G, changing codon into GGT and TGT resulting frame shift mutation causing change in preceding codons

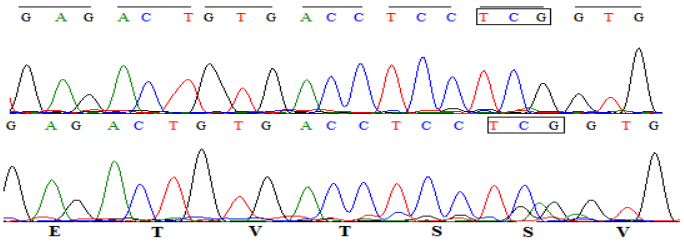

Figure 6: A frame shift nonsense mutation at exon 2 c.104C>G and an insertion of A resulting codon change from TCG into TCA and TGA (p.S35S and a stop codon)

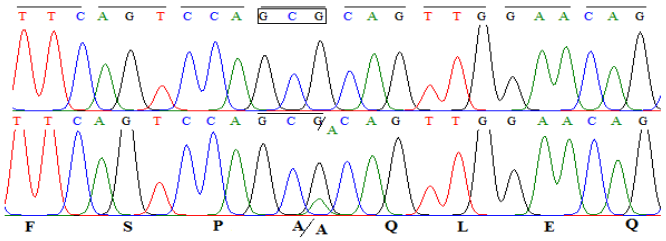

Figure 7: A silent heterozygous variation at exon 2, c.237 G>A (p.A79A)

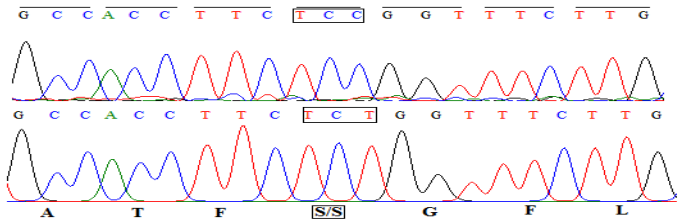

Figure 8: A silent homozygous variation at exon 3 c.432T>C (p.S144S)

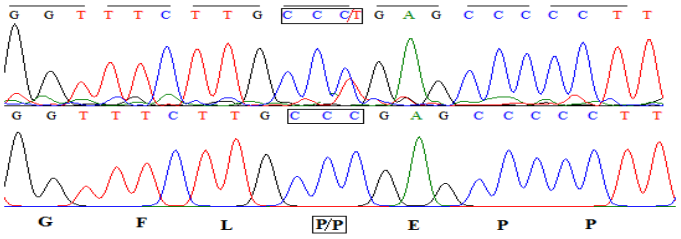

Figure 9: A silent heterozygous variation at exon 3, c.450C>T (p. P148P)

When subject sequences were compared with the control sequence (Accession No. KP228775.1*Ovisaries*). Variations reported in Balochi breed 85bp G>A, whereas GCT/ACT which coded Alanine into Aspartic acid on 29th codon, in 94bpG>T where GTG/GGT which coded Valine into Glycine on 32nd codon and 237bpG>A where GCG/GCA which coded same amino acid Alanine on 79th codon showed in Table 2.

In Bibrik breed 63bp G>A where CTG/CTA which coded same amino acid Lysine on 21st codon, on 94bp G>T where GTG/GGT which coded Valine into Glycine on 32nd codon, on 237bp G>A where GCG/GCA which coded same amino acid Alanine on 79th codon, on 432bp T>C where TCT>TCC which coded same amino acid Serine on 144th codon.

In Harnai breed 94bp G>T where GTG/GGT which coded Valine into Glycine on 32nd codon, on 104bp C>G where TCG/TCA which coded same amino acid Serine on 35th codon, on 432bp T>C where TCT>TCC which coded same amino acid Serine on 144th codon, on 540bp C>T where CCC/CCT which coded same amino acid Proline on 148th codon. In Rakhshani sheep breed on 540bp C>T where CCC/CCT which coded same amino acid Proline on 148th codon.

Phenotypic Variations

In this study phenotypic parameters including fleece weight, wool color, wool yield and finesse were also investigated.

Weight And Color Of Fleece

The average weight of fleece in animals per anum was recorded 1.91 kg, 1.83 kg, 1.85kg, 1.77 kg in Balochi, Bibrik, Harnai and Rakhshani sheep breeds of Balochistan respectively (Table 3).

The color of wool in Balochi sheep breed was observed in diverse variations including black and white, brown and white, gray and white, off white and white and sometimes white with black or brown muzzles and limbs extremities. In Bibrik the color of wool was observed as full white, white with black muzzle and limbs extremities. In Harnai breed the color of wool was observed as white gray mix, white with black and sometimes brown muzzles and limbs extremities whereas in Rakhshani sheep breed the color of wool was observed white with black muzzles and limbs extremities.

DISCUSSION

PROP1 gene consisting of 3 exons expressed in pituitary gland, play a vital role in growth and development in mammals (Nasonkin et al., 2004). It has been reported that this gene is associated with wool production trait and considered to be a candidate gene for selection of breeds of wool production (Zeng et al., 2011). In present study *PROP1* gene was investigated to analyze the variations in it and their association to the wool production trait in the sheep breeds. The sequencing results of *PROP1* gene revealed 7 variations including 3 frame shift mutations and 4-point mutations. In exon-1 three variations were reported including c63G>A (insertion of A, A at 63 and 64 nucleotide positions) a frame shit mutation, c.83G>A (p.A29D) a missense heterozygous variation and c.94G>T (insertion of G, G at nucleotide position 94,95) a frame shift mutation. In exon 2, 2 variations were observed including c.104C>G and an insertion of A at 104 nucleotide position resulting a frame shift mutation and c.237G>A (p.A79A) heterozygous silent variation. In exon 3, 2 variations including c.432T>C (p.S144S) homozygous silent variation and c.450C>T (p.P148P) heterozygous silent variation were reported. Studies suggested that *PROP1* gene play a vital role in determination of animal reproduction, growth and development (Andersen et al., 1995; Sornson et al., 1996; Gong et al., 2005). Polypeptide hormone prolactin (PRL) is a lactotroph due to its ubiquitous distribution beside other functions, also influence the growth of hair in domestic animals (Foitzik et al., 2009; Jung et al., 2021). Association of *PROP1* gene variation with wool diameter and suggest that *PROP1* gene could be used as molecular marker for sheep breeding and genetics through marker-assisted selection (Zeng et al., 2011). Association of *PROP1* gene polymorphisms with growth traits in sheep also reported by (Ekegbu et al., 2019). A study was performed on Loci association with wool production traits of Chinese Merino sheep (JunKen type) genotyped with 50 K single nucleotide. Twenty-Eight genome-wide significant SNPs detected for fiber diameter, fiber diameter coefficient of variation, fineness dispersion, and crimp trait and concluded that these novel SNP markers can be used for exploring the genetic control of wool traits in sheep (Zhipeng et al., 2014). In another study PROP1 gene mutation identified in the exon 1–3 and its association with wool traits in 345 Chinese Merino sheep. They reported ten novel SNPs within the sheep *PROP1* gene, namely, AY533708: g.45A [G resulting in Glu15Glu, g.1198A [G, .1341G [C resulting in Arg63Ser, g.1389G [A resulting in Ala79Ala, g.1402C [T resulting in Leu84Leu, g.1424A [G resulting in Asn91Ser, g.1522C[T, g.1556A [T, g.1574T [C, g.2430C [G. In addition, association analysis showed that three genotypes of P4 fragment were significantly associated with fiber diameter in the analyzed population (P = 0.044). They concluded that polymorphisms of the *PROP1* gene could be a useful molecular marker for sheep breeding and genetics through marker-assisted selection (MAS) (Zeng et al., 2011; Indraswari et al., 2021).

The results in present study revealed the phenotypic data regarding the wool production, the average fleece weight in breeds per anum was1.91 kg in Balochi, 1.83 kg in Bibrik, 1.85 in Harnai and 1.77 in Rakhshani respectively. The color of wool in Balochi sheep breeds was observed with diverse variations including black and white, brown and white, gray and white, off white and white and sometimes white with black or brown muzzles and limbs extremities. In Bibrik the color of wool was observed as full white, white with black muzzle and limbs extremities. In Harnai breed the color of wool was observed as white gray mix, white with black and sometimes brown muzzles and limbs extremities, whereas in Rakhshani sheep breed the color of wool was observed white with black muzzles and limbs extremities.

Whereas a study was conducted and reported white black and mixed color in Balochi and Rakhshani Breeds, white in Bibrik and white and mixed in Harnai breeds. The average wool production in Balochi, Bibrik, Harnai and Rakhshani 2.4, 1.8,1.8 and 1.3 kg respectively (Munir et al., 2010). Wool production performance of Harnai sheep breed of Balochistan was investigated and reported that overall mean yield of white and mixed wool from an adult sheep was 1.75 and 0.10 kg from young sheep was 1.58 and 0.10 kg respectively (Bukhari et al., 2016). Breed, management and environmental factors also influencing on wool production (Khan et al., 2012).

Environmental factors' influence on the wool production as well as the wool quality parameters of Mengali sheep. Season of shearing and location of flocks had significant effects ($P<0.05$) on fleece weight in different years. Sex and type of birth of animal were not statistically different ($P>0.05$) for fleece traits. Mengali wool characteristics are best suited for carpet manufacturing but color would be a limitation. The findings suggested that Mengali sheep wool production and quality can be improved through selection, management, and favorable environment (Tariq et al., 2013). Some other studies carried out in other parts of the world also reported spot pigmentations with white wool color (Sponenberg et al.,1988; Koseniuk et al., 2018).

CONCLUSION

The results of the current study indicate that sheep breeds of Balochistan having diverse genetic and phenotypic characteristic related to the different productive traits. The sequencing results of *PROP1* gene revealed 7 variations including 3 frame shift mutations and 4-point mutations. Genetic variations in *PROP1* gene suggest that this gene can be used as a suitable genetic marker for the future selection of the farm animals for different productive traits.

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AUTHORS CONTRIBUTION

Chandni Wajid and Abdul Hameed Baloch conceived and designed the project. Ahmed Nawaz Khosa wrote the paper, Hubdar Ali Kaleri and Nasrullah Bangulzai performed the experiments, Sarfaraz Ali Fazlani, Haleema Sadia and Saeeda kalsoom analyzed the data, Muhammad Bilawal Arain and Waseem Ali Vistro revised the paper. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal conflicts.

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