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POLYMORPHISMS IN GROWTH HORMONE (GH) AND INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) GENE AND THEIR ASSOCIATION WITH GROWTH TRAITS IN BEETAL GOAT

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Beetal is one of the most famous goat breed of Pakistan which is also present in parts of Indian Punjab. It is popular for its larger body size, higher body weight, and good ability to produce milk. In current study, polymorphism in Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) genes and their association with growth-related traits were studied in Beetal goat. On the basis of previously reported mutations, a set of 7 primer pairs were designed to screen PCR-RFLP based polymorphisms in 60 random selected Beetal goats varying in body weight. The IGF-1 gene was found polymorphic and following three genotypes (AA, AB, and BB) were detected in tested animals with the frequency of mutant (B) and wild-type (A) alleles as 0.35 and 0.65 respectively. Homozygous carrier genotype (BB) was significantly associated ($P<0.05$) with higher body weight (47.13±0.78kg) followed by heterozygous carrier (BB) and wild-type (AA) genotype associated with 40.45±0.31kg and 38.18±0.37kg body weight respectively. Additionally, maximum wither height and body length were observed in BB (32.25 ± 0.33, 31.93±0.27 inches) genotype followed by AB (31.93±0.27, 31.16±0.35) and AA (31.62±0.68, 30.87±0.86) genotype. Moreover, maximum heart girth was also observed in BB (33.65±0.11) genotype. All known point mutations of GH gene were found monomorphic in the tested animals. Upon sequencing of GH gene, one non-synonymous SNP was identified in exon 2 at 825bp position that caused a change of amino acid Threonine into Alanine. And likewise one insertion variant in intron 4 was observed at position 1546bp in this gene. The results showed that homozygous carrier animals, having BB genotype in IGF-1 gene, were having the highest values for growth traits suggesting the additive effects of mutant (B) allele on growth traits in this breed. On the basis of our results it is suggested that the identified markers can be used in marker-assisted-selection (MAS) in Beetal goat.

Keywords: GH gene, IGF-1 gene, PCR-RFLP, growth traits, Beetal goat, DNA sequencing

INTRODUCTION

Beetal is the most well-known and famous goat breed of Pakistan which is also present in parts of Indian Punjab (Khan *et al.,* 2008). It is considered a noteworthy goat breed due to its high body weight, large body size, good milking strength, and reasonable rate of fecundity (Khan and Ashfaq, 2012). Its most significant characteristic is better adaptability in a range of environmental conditions which makes it a highly preferred breed for intensive goat farming in tropical and sub-tropical regions of the world (Khan and Ashfaq, 2012). Goat meat is a preferred type of mutton in a number of Asian and African countries compared with other types of meat. Moreover, during outbreaks of some avian diseases like bird flu most of the poultry meat consumers switch to mutton which further increases its demand.

In small ruminant production, growth traits are always of foremost concern of breeders due to ever growing demand for mutton and its high economic value (Zhang *et al.,* 2008c; Hua *et al.,* 2009). Growth hormone (GH) is of high biological significance because of its role in affecting different traits in livestock species such as growth rate, body structure, health, milk yield, reproduction (Scaramuzzi *et al.,* 1999; Malveiro *et al.,* 2001) and metabolism (Jiang and Lucy 2001; Supakorn *et al.,* 2007; An *et al.,* 2011). The growth hormone (GH) is synthesized as well as released by the somatotroph cells of anterior pituitary and its secretion is strongly controlled and activated by growth hormone releasing hormone (GHRH) (Dettori *et al.,* 2015).

GH gene have been the main focus of genetic studies on small ruminant and a number of variations like the identification of haplotypes in exon 4 and 5 of GH gene in Black Bengal goat (Gupta *et al.,* 2009), PCR-RFLP based polymorphism in Sirohi and Barbari breeds (Singh *et al.* 2015), 5 point mutations in in Osmanabadi and Sangamneri goat breeds (Wickramaratne *et al.,* 2011), and some variants associated with growth in Boer goat (Hua *et al.,* 2009; Ishida *et al.,* 2010) have been reported. Likewise, Ishida *et al.* (2010) reported genetic polymorphism of bovine growth hormone gene associated with calf weight in Japanese Black cattle. Additionally, some polymorphisms in GH gene had also been found associated with milk yield in goat (Malveiro *et al.,*

2001; Lan *et al.,* 2007) and milk composition in sheep (Dettori *et al.,* 2015).

GH secretion leads to switching on of IGF-1 gene and results in its enhanced expression in the liver. IGF-1 is single-chain polypeptide fundamentally homologous with insulin which plays a vital role in a many biological processes such as embryogenesis, growth and lactation, increase in glucose uptake, stimulation of myogenesis, inhibition of apoptosis and reproduction, (Bai *et al.,* 2013). Some QTLs for growth and body confirmation traits have been found in goat on chromosome 5 (Tu *et al.,* 2016) encompassing IGF-1 gene (De la Rosa Reyna *et al.,* 2010). Moreover, polymorphism in IGF-1 gene and its flanking regions had been found associated with birth weight, growth rate, live weight, and carcass weight in different breeds of sheep and goat like in Zel sheep (Kazemi *et al.,* 2011), in Kurdish goat (Rasouli *et al.,* 2017), in Nanjiang Huang goats (Zhang *et al.,* 2008c), in Egyptian sheep and goat breeds (Othman *et al.,* 2016), in Indian goat breeds (Sharma *et al.,* 2014) and in Russian Merino sheep (Trukhachev *et al.,* 2016).

However, the numbers of studies on the exploration of candidate genes for growth traits in Beetal goat are very few which is the major hurdle in the implementation of marker-assisted selection in this breed. In order to bridge the growing gap between demand and supply of goat meat there is need to find out the genetic variants affecting growth in this breed in order to enhance its genetic potential for growth-related traits.

MATERIALS AND METHODS

***Ethical statement*:** Permission (number DR/152) for the collection of blood samples was obtained from the Ethical Review Committee for animal research of University of Veterinary and Animal Sciences, Lahore, Pakistan.

***Experimental Animals/blood sampling and DNA extraction*:** In total 60 healthy adult Beetal goats were selected on the basis of variation in their body weight. Animals were divided into two body weight categories i.e. 30 heavy and 30 low weight animals. Phenotypic data for different traits including body weight, wither height, body length, and heart girth were recorded. Blood samples with a volume of 5ml from each goat were collected aseptically from jugular vein into 50ml falcon tubes containing 160µl (0.5M) EDTA. DNA extraction was performed by using inorganic method described by (Sambrook and Russell, 2001)**.**

***DNA amplification by PCR*:** Polymerase chain reaction (PCR) was carried out in a final reaction volume of 25µl in C1000 Touch™ Thermal Cycler (BIO-RAD, USA). A set of 7 primers pairs for the amplification of different regions of GH and IGF1 gene were taken from previously published literature (Table 1). The reaction mixture consisted of 200µM dNTPs, 1.5mM MgCl2, 2.5mM 10x buffer, 30ng each of forward and reverse primer and 1 Unit Taq DNA polymerase. The PCR reaction cycle was accomplished by denaturation for 1min at 94°C; 30 cycles of denaturation at 94°C for 45s, annealing 56-63°C, and extension step at 72°C for 45s with a final extension at 72°C for 5 min. The PCR products were visualized in Gel Doc™ EZ imager (BIO-RAD, USA) following electrophoresis through 2% agarose gel.

***PCR-RFLP analysis*:** Amplified PCR products of all the primer sets (Table 1) were digested with restriction enzyme HaeIII at 37°C for 12 hours. After digestion, the samples were stored at 4°C. The digested product was visualized in Gel Doc™ EZ imager (BIO-RAD, USA) following electrophoresis through 2% agarose gel. The gel images were recorded in a gel documentation system.

***Sequencing and Analysis*:** The amplified regions were sequenced using an ABI3130 automated DNA Sequencer (Applied Biosystems, USA). Multiple sequence alignments were performed with MEGA 7 (Sudhir *et al.* 2016) software using CLUSTALW algorithm (Thompson *et al.,* 1994) to identify polymorphisms. The coding DNA sequences of

Table 1. List of Primers along with their amplified region, amplicon size, and references

Gene	Primer Sequence	Amplified Region	Amplicon Size (bp)	Reference
GH1	F:CTCTGCCTGCCCTGGACT R:GGAGAAGCAGAAGGCAACC	Exon 2 and exon 3	422	(Hua *et al.* 2009)
GH2	F:TCAGCAGAGTCTTCACCAAC R:CAACAACGCCATCCT CAC	Exon 4	116	(Hua *et al.* 2009)
GH3	F: CGACGCCATAGACAGCAG R: CATTTATGCAAGGACCACTGG	Partial exon 1	419	(Supakorn *et al.* 2007)
GH4	F:TAGGGGAGGGTGGAAAATGGA R:GACACCTACTCAGACAATGCG	Exon 5	404	(Silveira *et al.* 2008)
GH5	F: GCCAGTGGTCCTTGCATAAA R: AGTCCAGGGCAGGCAGAG	Exon 1 and partial exon 2	500	(Supakorn *et al.* 2007)
GH6	F: CCATCCAGAACACCCAGGT R: CCAAGCTGTTGGTGAAGACTC	Partial exon 3 and partial exon4	417	(Supakorn *et al.* 2007)
IGF-1	F:CACAGCGTATTATCCCAC R:GACACTATGAGCCAGAAG	Exon 4 and partial intron 4	363	(Liu *et al.* 2010)

different exon regions were translated to amino acid sequences using Chromaspro software.

***Statistical Analysis*:** The General Linear Model procedure of Genstat, version 18 (Payne *et al.,* 2011) was used to test the effects of genotypes on the body weight, body length, body height, and heart girth. The linear model used to analyzed the data is as follow

$Y_{ijkl} = \mu + S_i + P_j + W_k + G_l + e_{ijkl}$

Where, μ = Population mean

S = Season of Birth, i=[1, and 2]

P = Parity, j=[1, 2, and 3]

W = Year of Birth, k=[1, 2, 3, and 4]

G =Genotype, l=[1, 2, and 3]

e = Random Residual Error

RESULTS

Present study was conducted in order to identify polymorphism in GH and IGF-1 candidate genes and to determine their association with growth traits in Beetal goat breed.

Polymerase Chain Reaction (PCR) of Growth Hormone Gene: The five exons of Growth hormone (GH1) gene were amplified by PCR using exon specific primer sets (Table 1). The amplified products were run on 1.2% (W/V) agarose gel electrophoresis along with DNA ladder that was used as size standard.

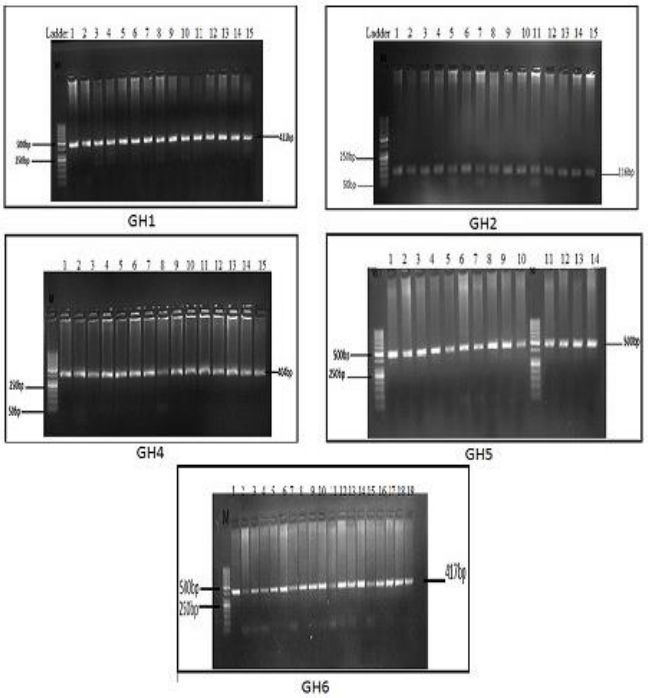

Figure 1. Amplified PCR products GH1 was 422bp, GH2 was 116bp, GH3 could not be amplified, GH4 was 404bp, GH5 was 500bp and GH6 was 417bp.

The sizes of target amplified products for GH1, GH2, GH4, GH5, and GH6 were 422 base pair (bp), 116bp, 404bp, 500bp, and 417bp, respectively, as shown in Figure 1. However, the PCR conditions for the GH3 primer set could not be optimized.

***PCR-Restriction Fragment Length Analysis of Growth hormone (GH) gene*:** Several techniques are available to identify the single nucleotide polymorphism (SNP) in target sequence. However, one of the most common method is the PCR-RFLP analysis to detect the genetic polymorphism in target gene. It is a powerful method for identifying nucleotide sequence variation in amplified DNA and can detect single base variations in enzymatic restriction sites.

In this study, we used the PCR-RFLP technique for the identification of SNP in five exons of growth hormone (GH) gene. All the selected exons of GH gene were amplified through specific primer sets. The HaeIII enzyme was used for the detection of polymorphism in all the amplified PCR products (GH1, GH2, GH4, GH5, and GH6) of growth hormone gene. The HaeIII-based RFLP results showed that all the growth hormone gene regions were monomorphic in both high and low body weight goats (Figures 2).

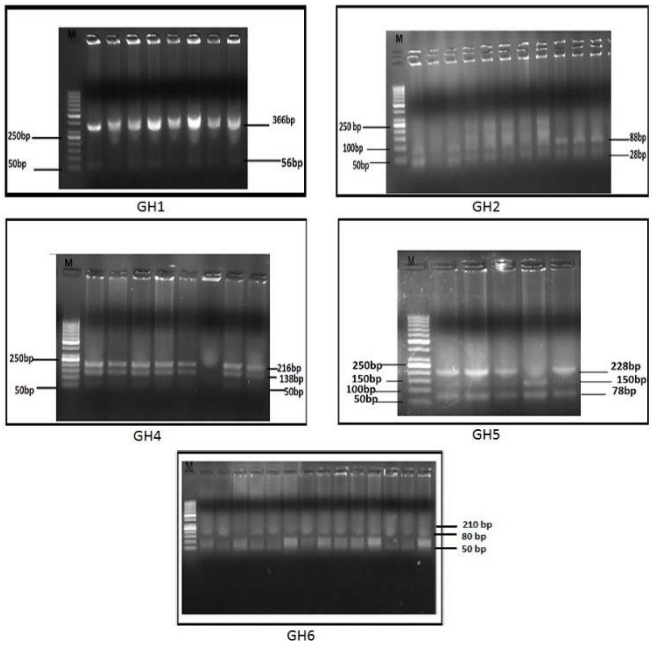

Figure 2. GH1, GH2, GH4, GH5 and GH6 are digested with HaeIII enzymes. M represent 50 bp DNA ladder. After digestion with restriction enzyme, GH1 showed two fragment of 366 bp and 56 bp. GH2 showed 88 bp and 28bp. GH4 showed three fragments 216 bp, 138 bp, 50-bp respectively.GH5 showed three fragments 228 bp, 150 bp, 78 bp respectively while GH6 showed three fragments of size 210 bp, 80 bp, and 50 bp respectively

***Sequencing Results of Growth Hormone Gene (GH)*:** Finally, five exons of growth hormone gene were sequenced. The sequences from low body weight animals were compared with sequences of high body weight, and one single nucleotide polymorphism was identified in GH1 (exon 2). This substitution mutation (A > G) at position 825bp was changing CGA codon to CAA resulting in an amino acid change from Threonine to Alanine (Figure4, and Figure 5).

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                        10         20         30         40         50
High_BW          --AGCTTTGC GTCTTCAGCC ATGTCCTTGT CAGGCCTGTT TGCCACGCTG
High_BW          --AGCTTTGC GTCTTCAGCC ATGTCCTTGT CAGGCCTGTT TGCCACGCTG
Low_BW           AGGCCTGTGA TGCTTCAGCC ATGTCCTTGT CAGGCCTGTT TGCCACGCTG
Low_BW           --GGCGGGGG TCCTTCAGCC ATGTCCTTGT CAGGCCTGTT TGCCACGCTG
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       60         70         80         90        100
High_BW          TGCTCCGGGC TCAGCACCTG CATCAGCTGG CTGCTGACAC CTTCAAGAGT
High_BW          TGCTCCGGGC TCAGCACCTG CATCAGCTGG CTGCTGACAC CTTCAAGAGT
Low_BW           TGCTCCGGGC TCAGCACCTG CATCAACTGG CTGCTGACAC CTTCAAGAGT
Low_BW           TGCTCCGGGC TCAGCACCTG CATCAACTGG CTGCTGACAC CTTCAAGAGT
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       110        120        130        140        150
High_BW          TTGTAAGCTC CCCAGAGATG TGTCCTAGAG GTGGGGAGGC AGGAAGGGGT
High_BW          TTGTAAGCTC CCCAGAGATG TGTCCTAGAG GTGGGGAGGC AGGAAGGGGT
Low_BW           TTGTAAGCTC CCCAGAGATG TGTCCTAGAG GTGGGGAGGC AGGAAGGGGT
Low_BW           TTGTAAGCTC CCCAGAGATG TGTCCTAGAG GTGGGGAGGC AGGAAGGGGT
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       160        170        180        190        200
High_BW          GAATCCGCAC CCCCTCCACA CAATGGGAGG GAACTGAGGA CCTCAGTGGT
High_BW          GAATCCGCAC CCCCTCCACA CAATGGGAGG GAACTGAGGA CCTCAGTGGT
Low_BW           GAATCCGCAC CCCCTCCACA CAATGGGAGG GAACTGAGGA CCTCAGTGGT
Low_BW           GAATCCGCAC CCCCTCCACA CAATGGGAGG GAACTGAGGA CCTCAGTGGT
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250
High_BW          ATTTTATCCA AGTAAGGATG TGGTCAGGGG AGTAGAAATG GGGGTGTGTG
High_BW          ATTTTATCCA AGTAAGGATG TGGTCAGGGG AGTAGAAATG GGGGTGTGTG
Low_BW           ATTTTATCCA AGTAAGGATG TGGTCAGGGG AGTAGAAATG GGGGTGTGTG
Low_BW           ATTTTATCCA AGTAAGGATG TGGTCAGGGG AGTAGAAATG GGGGTGTGTG
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       260        270        280        290        300
High_BW          GGGTGGGGAG GGTTCCGAAT AAGGCAGTGA GGGGAACCAC ACACCAGCTT
High_BW          GGGTGGGGAG GGTTCCGAAT AAGGCAGTGA GGGGAACCAC ACACCAGCTT
Low_BW           GGGTGGGGAG GGTTCCGAAT AAGGCAGTGA GGGGAACCAC ACACCAGCTT
Low_BW           GGGTGGGGAG GGTTCCGAAT AAGGCAGTGA GGGGAACCAC ACACCAGCTT
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       310        320        330        340        350
High_BW          AGACCCGGGT GGGTGTGTTC TCCCCCCAGG AGCGCACCTA CATCCCGGAG
High_BW          AGACCCGGGT GGGTGTGTTC TCCCCCCAGG AGCGCACCTA CATCCCGGAG
Low_BW           AGACCCGGGT GGGTGTGTTC TCCCCCCAGG AGCGCACCTA CATCCCGGAG
Low_BW           AGACCCGGGT GGGTGTGTTC TCCCCCCAGG AGCGCACCTA CATCCCGGAG
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       360        370        380        390        400
High_BW          GGACAGAGAT ACTCCATCCA AAACACCCAG GTTGCTTTCT GCTTCTCCAT
High_BW          GGACAGAGAT ACTCCATCCA AAACACCCAG GTTGCTTTCT GCTTCTCCAT
Low_BW           GGACAGAGAT ACTCCATCCA AAACACCCAG GTTGCTTTCT GCTTCTCCAT
Low_BW           GGACAGAGAT ACTCCATCCA AAACACCCAG GTTGCTTTCT GCTTCTCCAA
                 ....
High_BW          AC--
High_BW          AC--
Low_BW           TC--
Low_BW           ACGT
```

Figure 3. Sequence Alignment of GH1 of high body weight (High_BW) and low body weight (Low_BW) animals.

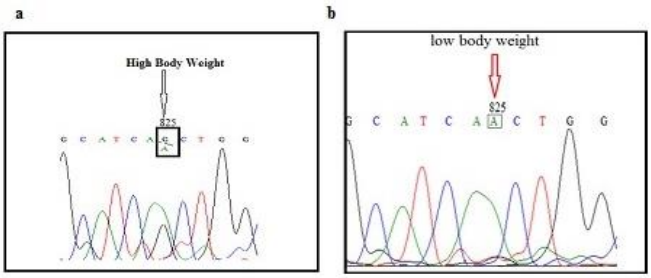

Figure 4. Mutation at position 825 A>G (a, b).

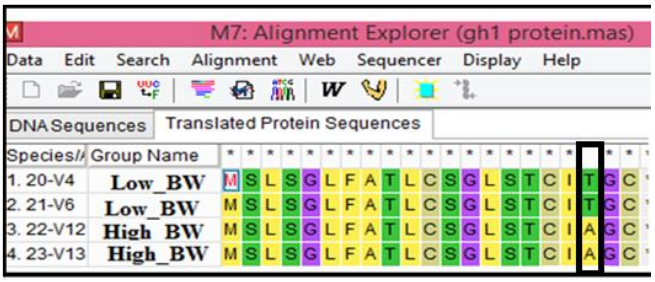

Figure 5. Pictorial view of Protein Translation. The substitution mutation (A > G) of CGA to CAA at position 825bp caused an amino acid change from Threonine to Alanine.

***Sequence alignment of GH6*:** One insertion mutation was identified at position 1546bp in GH6 (intron 4) by comparing the sequences of low and high body weight animals (Figure 6, and Figure 7).

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                        10         20         30         40         50
High_BW          ---CTTGGGA CTCTCTGACA TCCAGGCCCC ACGAGGCAGA TGAGGCCCAG
High_BW          -TGTTTGGTT CTCTGT-ACA TCCAGGCCCC ACGAGGCAGA TGAGGCCCAG
Low_BW           TCTTTTGGTT ACTCAGTTCA TCCAGGCCCC ACGGGCAGAC TGAGGCCCAG
Low_BW           TCTTTTGGTT ACTCAGTTCA TCCAGGCCCC ACGGGCAGAC TGAGGCCCAG
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       60         70         80         90        100
High_BW          CAGAATCAGT GAGTGGCCAC CTAGGACCGA GGAGCAGGGG ACCTCCTTCA
High_BW          CAGAATCAGT GAGTGGCCAC CTAGGACCGA GGAGCAGGGG ACCTCCTTCA
Low_BW           CAGAATCAGT GAGTGGCCAC CTAGGACCGA GGAGCAGGGG ACCTCCTTCA
Low_BW           CAGAATCAGT GAGTGGCCAC CTAGGACCGA GGAGCAGGGG ACCTCCTTCA
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       110        120        130        140        150
High_BW          TCTTA-GTAG GCTGCCCCAG CTCTCTGCAC CGGGCCTGGG GTGGCCTTCT
High_BW          TCTTA-GTAG GCTGCCCCAG CTCTCTGCAC CGGGCCTGGG GTGGCCTTCT
Low_BW           TCTTAAGTAG GCTGCCCCAG CTCTCTGCAC CGGGCCTGGG GTGGCCTTCT
Low_BW           TCTTAAGTAG GCTGCCCCAG CTCTCTGCAC CGGGCCTGGG GTGGCCTTCT
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       160        170        180        190        200
High_BW          CCCTGAGGTG GCAGAGGGTG TTGGATGGCA GTGGAGGATG ATGGTTGGTG
High_BW          CCCTGAGGTG GCAGAGGGTG TTGGATGGCA GTGGAGGATG ATGGTTGGTG
Low_BW           CCCTGAGGTG GCAGAGGGTG TTGGATGGCA GTGGAGGATG ATGGTTGGTG
Low_BW           CCCTGAGGTG GCAGAGGGTG TTGGATGGCA GTGGAGGATG ATGGTTGGTG
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250
High_BW          GTGGTGGCAG GAGGTCCTCG GGCAGAGGCC GACCTTGCAG GGCTGCCCCG
High_BW          GTGGTGGCAG GAGGTCCTCG GGCAGAGGCC GACCTTGCAG GGCTGCCCCG
Low_BW           GTGGTGGCAG GAGGTCCTCG GGCAGAGGCC GACCTTGCAG GGCTGCCCCG
Low_BW           GTGGTGGCAG GAGGTCCTCG GGCAGAGGCC GACCTTGCAG GGCTGCCCCG
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       260        270        280        290        300
High_BW          AGCCCGCGGC ACCCACCAAC CACCCATCTG CCAGCAGGAC TTGGAGCTGC
High_BW          AGCCCGCGGC ACCCACCAAC CACCCATCTG CCAGCAGGAC TTGGAGCTGC
Low_BW           AGCCCGCGGC ACCCACCAAC CACCCATCTG CCAGCAGGAC TTGGAGCTGC
Low_BW           AGCCCGCGGC ACCCACCAAC CACCCATCTG CCAGCAGGAC TTGGAGCTGC
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                       310        320        330        340        350
High_BW          TTCGCATCTC ACTGCTCCTT ATCCAGTCGT GGCTTGGGCC CCTGCAGTTC
High_BW          TTCGCATCTC ACTGCTCCTT ATCCAGTCGT GGCTTGGGCC CCTGCAGTTC
Low_BW           TTCGCATCTC ACTGCTCCTT ATCCAGTCGT GGCTTGGGCC CCTGCAGTTC
Low_BW           TTCGCATCTC ACTGCTCCTT ATCCAGTCGT GGCTTGGGCC CCTGCAGTTC
                 ....|....| ....|....| ....|....| .
                       360        370        380
High_BW          CTCAGCAGAG TCTTCACCAA CAGCTTGGAA
High_BW          CTCAGCAGAG TCTTCCCCAA CAGCTTGGAA
Low_BW           CTCAGCAGAG TCTTCCCCAA CAGCTTGGGA
Low_BW           CTCAGCAGAG TCTTCCCCAA CAGCTTGGGA
```

Figure 6. Sequence alignment of GH6 gene region

Pictorial view of Mutation and its position.

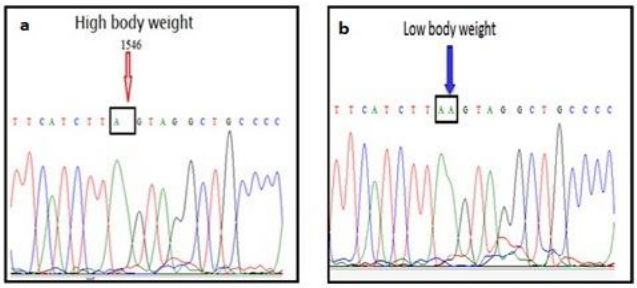

Figure 7. Deletion Mutation at 1546 position (a, b)

Insulin–like growth factor-1 (IGF-1) gene

The amplified product of exon 4 and partial intron 4 of IGF-1 gene was 363bp long. The IGF-1 gene fragments were amplified by PCR and run on 1.2% (W/V) agarose gel electrophoresis as shown in Figure 8.

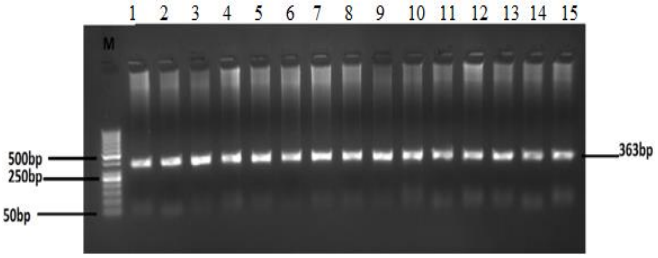

Figure 8. IGF-1 fragments amplified by PCR

***PCR-Restriction Fragment Length Analysis of Insulin-like growth factor -1 (IGF-1) gene*:** The genotyping of IGF-1 gene was done after digesting the PCR product (363bp) by the restriction enzyme HaeIII and the digested fragments were run on agarose gel electrophoresis. The fragments obtained were of different sizes and categorized into different genotypes as follows, 363bp for AA genotype, 273bp and 90bp for BB genotype and, 363 bp, 273bp, and 90bp for AB genotype after digestion with HaeIII, as shown in Figure 9.

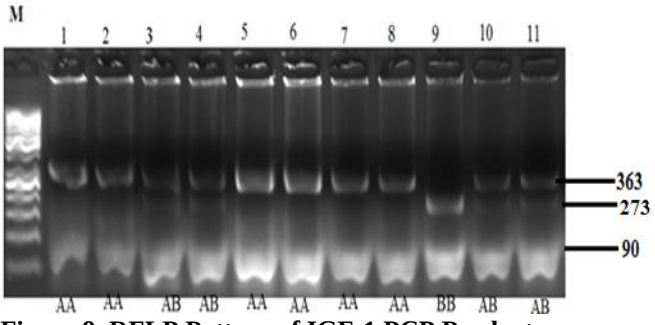

Figure 9. RFLP Pattern of IGF-1 PCP Product

***Statistical Analysis of Insulin-like Growth Factor-1 gene*:** The observed genotypes were (AA, AB, and BB) and genotypic frequencies of AA, AB, and BB were 0.39, 0.47, and 0.086 respectively (Table 2). The frequency for allele A and B was 0.65 and 0.35 respectively (Table 2). The association analysis between different IGF-1 genotypes and growth traits including adult body weight, and body measurements (body length, body height, and chest girth), taken at adult age, was performed. The results of association analysis of different genotypes with body weight and body measurements are shown in Table 3. Genotype AA was found significantly ($P<0.05$) associated with low body weight (38.18 + 0.37kg) while BB genotype was associated ($P<0.05$) with high body weight 47.13 + 0.78 kg. Moreover, genotype AB showed additive effect and significant association with body weight of up to 40.45 + 0.31 kg. Similarly maximum wither height and body length was found in BB (32.25 + 0.33, 31.93 + 0.27 inches) genotype following AB (31.93 + 0.27 , 31.16 + 0.35) and AA (31.62 + 0.68, 30.87 + 0.86) genotype. Likewise, maximum heart girth was observed in BB (33.65 + 0.11) genotype followed by AB (31.52 + 0.90) and AA (33.25 + 0.24) genotype.

DISCUSSION

In livestock production, animal growth traits are always of foremost concern for breeders due to their economic value

Table 2. Observed genotype, Allelic frequency and Genotype frequency of IGF-1 gene in Beetal goats

Breed	Observed genotype			Allelic Frequency		Genotype Frequency		
Beetal goat	AA	AB	BB	A	B	AA	AB	BB
	23	30	7	0.65	0.35	0.39	0.47	0.09

Table 3. Association of IGF-1 genotypes with Body weight, Wither height, Body length and Heart girth in Beetal goats

Genotype	Body weight	Withers height	Body length	Heart girth
	-----(kg)----	----------(inch)----------		
AA	38.18 ± 0.37^{b}	31.62 ± 0.68	30.87 ± 0.86	33.25 ± 0.24
AB	40.45 ± 0.31^{b}	31.93 ± 0.27	31.16 ± 0.35	31.52 ± 0.90
BB	47.13 ± 0.78^{a}	32.25 ± 0.33	31.97 ± 0.42	33.65 ± 0.11

Means with different superscripts within the same column are significant at $P<0.05$

(Zhang *et al.,* 2008a; Zhang *et al.,* 2008b). In the current study, we used the GH and IGF-1 as candidate genes to find out the polymorphism and its association with the growth traits in Beetal goats.

***Growth hormone gene (GH)*:** In the current study, five exons of GH were amplified to detect the PCR-RFLP based mutation by digesting with HaeIII restriction enzyme. However, all tested animals of low and high body weight were found monomorphic (Table 1) in the present study. Although the same RFLP pattern was also observed by Hua *et al.* (2009) who reported the polymorphic nature of mutation in first four exons of growth hormone gene in Boer goat. In contrast to our results, An *et al.* (2010) reported polymorphism in exon 1, 3, 4, and 5 of growth hormone gene in four different populations of goats in China. Moreover, sequence data of exonic regions of growth hormone gene revealed two point mutations in this study. One SNP was observed in exon 2 and this was a substitution mutation (A > G) changing CGA to CAA at position 825bp. This was non-synonymous mutation causing an amino acid change from Threonine to Alanine. Additionally, one deletion was identified at position 1546bp in intron 4 region of growth hormone gene. From our results, it is likely that the said mutation at 825bp position which causes change in the amino acid from hydrophobic to hydrophilic group may be one of reason of variation in the body weight in Beetal goat. Additionally, another insertion mutation was found in the intron 4 region of GH gene. There is possibility that these observed mutations might be responsible for variation in body weight by changing the biological function in Beetal goat. It also seems likely because growth hormone plays an important role in the growth of different livestock species as well as in other biological processes such as metabolism and lactation (Jiang and Lucy, 2001; Ge *et al.,* 2003; Supakorn *et al.,* 2007; An *et al.,* 2011) and reproduction (Scaramuzzi *et al.,* 1999). In consistent with our resutls, Hua *et al.* (2009) found two mutations, one in the exon 2 at position 781bp and another at position 1575bp. Although the mutation found by them in exon 2 was very close to the mutaion observed in our tested animal of Beetal goats. It is likely that breed difference might be one of the reason for the difference of nucleotide polymorphism between these two studies.

***Insulin-like growth factor-1 gene (IGF-1)*:** In our study, three genotypes (363 for AA genotype, 264 and 90 for BB genotype and, 363, 264 and 90 for AB) were observed in exon 4 and intron 4 of IGF-1 gene by using PCR-RFLP method. The results showed the polymorphic nature of mutation in Beetal goat. These results are in accordance with the findings of Wu-Jun *et al.,* (2010) and Negahdary *et al.,* (2013) who also found three genotypes for IGF-1 in their goat population.

In the current study, we also assessed the association between different IGF-1 genotypes and growth traits including the body weight, body length, body height, chest girth and body weight. Our results showed that genotype AA was associated with low body weight (38.18Kg) and while BB genotype was significantly associated ($P<0.05$) with higher body weight 47.13Kg. However, genotype AB was significantly associated with 40.45kg body weight showing the additive effects of allele B. In consistent with our results, Zhang *et al.* (2008c) observed mutation in intron 4 of IGF-1 gene and found significant association with birth weight, and body weight at 6 and 12 months in Nanjiang Huang goats. Similar results were found in the in Markhoz goat breed of Iran by Rasouli *et al.* (2017) who estimated a significant effect of different genotypes of IGF-1 and IGFBP-3 gene with the weaning weight, and average daily weight gain. In our study, the frequency of mutant allele (B) was low (0.35) as compared to 0.65 frequency of wild allele (A), therefore, the BB genotype was observed in a small proportion of samples. Likewise, same mutation was observed in Guanzhong and Xinong-Saanen breeds by (Deng *et al.,* 2010). Additionally, the frequency of mutant allele was low in both of goat breeds but in contrast to our findings, this polymorphism was not associated with any of growth traits. Similarly, contrasting results were also found by Zhang *et al.* (2008) who revealed no single nucleotide polymorphism (SNP) in the 5´ flanking region of the IGF-I gene. However, the reason for the low frequency of BB genotype in our population might be the limited number of tested animal in current study. The association studies related to IGF-I gene polymorphism and growth traits showed different pattern of results in different breeds. Significant association of the polymorphism in IGF-1 gene was observed with daily gain in Baluchi sheep (Tahmoorespur *et al.,* 2009). Likewise, same polymorphism was associated with growth traits in sheep and in Kurdish goat (Kazemi *et al.,* 2011; Kurdistani *et al.,* 2013). In contrast to our results, Wang *et al.,* (2011) studied the same polymorphism in three breeds of goats and found no significant association with birth weight. These inconsistent results might be due to linkage disequilibrium of the IGF-I gene with QTLs of these observed traits. However to validate the effect of IGF-1 gene with growth traits in Beetal goat, more studies are required with larger number of animals.

***Conclusion*:** On the basis of our data it is concluded that mutations already reported in the exonic regions of Growth hormone gene were monomorphic in Beetal goat. Moreover, one substitution mutation in exon 2 G>A at position 825bp along with one insertion mutation in intron 4 at position 1546bp was also observed in the tested animals of Beetal goat. The IGF-1 gene was proven of prime importance because IGF-1 gene was polymorphic and had significant association with body weight and body measurements. It is suggested that identified variants can be used as genetic markers in order to select the Beetal goat for the growth-related traits in future.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that they have no conflict of interest.

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