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Associations of exon 2 of leptin gene polymorphism with carcass traits in cross bred pigs

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Abstract
The association between various polymorphic variants of leptin gene and carcass traits were investigated by PCR-RFLP method in crossbred pigs. The 604 bp amplified PCR product was digested with *Bam* HI revealed restriction fragments 604 bp, 495 bp and 109 bp sizes for exon 2. The population in respect to various genotypes at exon 2 in crossbred pigs was not in equilibrium. The results of one-way ANOVA for dressing percentage, carcass length, loin eye area, Backfat thickness, belly girth and thigh girth showed non significance (p<0.05) in exon 2 locus of leptin gene except for heart girth which showed significance (p<0.05) Higher heart girth was observed for AB genotype which clearly indicates the superiority of AB genotypes than AA and BB genotypes.

Keywords: Carcass traits, crossbred, gene polymorphism, leptin gene, PCR-RFLP

1. Introduction
Livestock play an important role in rural economic development. Among the various livestock species, piggery is the most potential source of meat production and more efficient feed converters after the broiler (Vikaspedia, 2022) [1]. Pig farming will provide employment opportunities to seasonally employed rural farmers and additional income to improve their living standards. Leptin gene is one of the potential gene that are involved intricately in the metabolism and growth of animals. The leptin gene has been mapped to Porcine chromosome 18 (Neuenschwander *et al.*, 1996) [2, 15]. In pigs, it consists of 167 amino acids with a predicted molecular weight of 18.661 kDa. The product of this gene is a 16 kDa protein that is primarily produced by white adipocytes (Houseknecht *et al.*, 1998) [3]. Leptin has been implicated in the regulation of feed intake, energy expenditure, and whole-body energy (Houseknecht *et al.*, 1998) [3]. Leptin gene are considered as candidate genes related with meat quality and fatness traits in farm animals (Bauer *et al*., 2006) [4]. New research studies suggest that the Leptin gene should be considered as a candidate gene which can influence carcass traits in pigs (Barb *et al*., 2001) [5].

2. Materials and Methods
2.1 Blood samples
The experimental material for the present study comprised blood samples of 50 crossbred pigs from All India Co-ordianted Research Project on Pig, College of Veterinary Science & A.H, an organized piggery farm in Jablapur, Madhya Pradesh. 5 ml of blood was collected from 50 healthy animals at the time of slaughter. Genomic DNA was extracted from blood as per the method described by John *et al*. (1991) [6] with minor modifications. Quality of DNA was assessed through 0.8% horizontal submarine agarose gel electrophoresis. The DNA having good quality intact bands with no smearing was used for further analysis. The study was carried with approval of Institutional Animal Ethics Committee, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh 482001, India.

2.2 Point of care – PCR
The primers for exon 2 (forward 5'-GTGGGGTCCAGATATCCGTT–3', reverse 5'-CCAGGCTAGGGGTCTAATCG–3') of leptin gene were used for amplification of PCR product as described by de Oliveira Peixoto *et al*. (2006) [7]. PCR was carried out for exon 2 of leptin gene in a final reaction volume of 25 µl.

Reaction mixture consists of 2X PCR mastermix (Fermentas), primers (forward and reverse), DNA sample and DNAase free water (Table 1). The amplified PCR product of each sample was digested using BamHI restriction endonucleases (RE). 10 µl PCR product of each sample was digested with 1 µl of BamHI in manufacturer's recommended assay buffers in a final reaction volume of 30 µl. The reaction mixture was incubated at 37°C for overnight respectively in water bath. The PCR products were analyzed on 2.0% agarose gel and it was prepared in 0.5 X TBE in a similar way as used for checking the quality of DNA and subsequently 10 µl of RE digested PCR product mixed with 2 µl of 6x gel loading dye (Bromophenol blue) was loaded along with 100 bp DNA ladder as a molecular size marker in a separate lane. The electrophoresis at constant voltage of 80 volt for 45 mins at 370 C using 0.5 X TBE buffer was performed. After completion of gel electrophoresis, the digested PCR products were visualized by UV transilluminator and photographed (Gel documentation system, Bio-Rad, USA) to detect the banding pattern/ genotype of cGHI gene of each sample.

2.3 Statistical Analysis

Genotype frequencies, gene frequencies and genetic equilibrium at different loci were estimated using software POPGENE 32 version 1.32, the user-friendly software for Population Genetic Analysis. (Yeh *et al*., 1999) [8]. The following statistical mode was used to find out the association of various polymorphic variants of leptin gene with the carcass traits by using one way analysis of variance with MSTAT software.

$$Y_{ij} = \mu + a_i + e_{ij}$$

Where,

Y_{ij} = the carcass traits of j^{th} crossbred pig of the i^{th} genotype.

μ = is the overall mean

a_I = is the set of random cross classified effects due to genotpyes.

e_{ij} = is the random error

3. Results

The present investigation revealed amplified PCR product of 604 bp for exon 2. Peixoto *et al.* (2006) [7] and Peixoto *et al.* (2009) [9] reported PCR product of 604 bp for exon 2 in the population created by crossing two boars of the native Brazilian Piau with 18 commercial sows composed of Landrace, Large White and Pietrain breeds [12, 13]. The PCR product of various bp sizes in different breeds and strains of pigs may be due to insertion/duplication and deletion in leptin gene, which can only be confirmed by sequencing. The study revealed restriction fragments 604 bp, 495 bp and 109 bp sizes for exon 2 using BamHI restriction endonuclease among crossbred pigs (Figures-1 and 2). The RFLP fragments and patterns of genotypes obtained in the present study are in agreement with the RFLP fragments and patterns of genotypes as reported by Peixoto *et al.* (2006) [7] and Peixoto *et al.* (2009) [9].

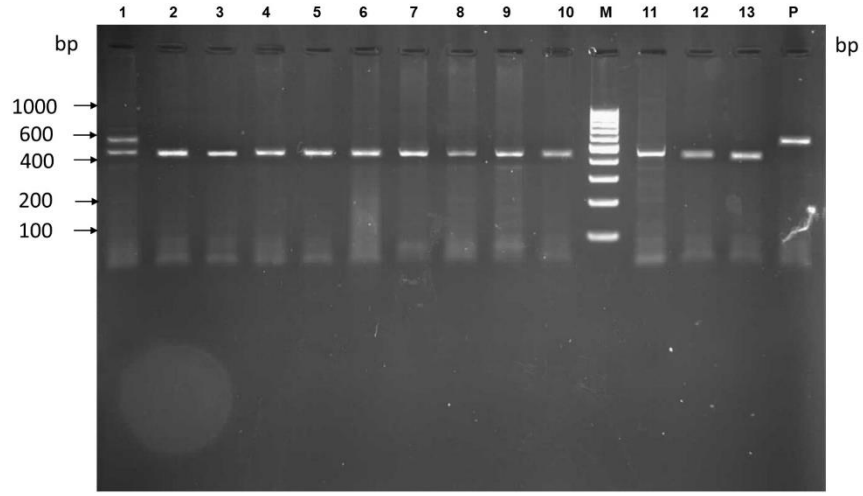

Figure 1: PCR-RFLP pattern in exon 2 of leptin gene digested with BamHI Restriction enzyme on 2% agarose.

M = 100bp DNA ladder; P = PCR product; Lane 1 = AB genotype (604/495/109bp); Lane 2-10, 11-13 = BB genotype (495/109bp)

Genotypic frequencies for exon 2 were 0.54 for AA genotype, 0.10 for AB genotype and 0.36 for BB genotype. The allele frequencies were 0.59 for A allele and 0.41 for B allele in crossbred pigs. The highly significant Chi-square value indicates that the population in respect to various genotypes in crossbred pigs was not in equilibrium. The probable reason might be that two different populations used for generating the crossbreds were significantly different from each other in respect to gene frequency.

Table 1: Reaction mixture for PCR of exon 2 of leptin gene

PCR Components	Quantity
2X PCR mastermix (Fermentas)	12.5 µl
Primers: Forward : Reverse	1.0 µl 1.0 µl
Genomic DNA	3.0 µl
DNAase free water	7.5 µl
Total	25.0µl

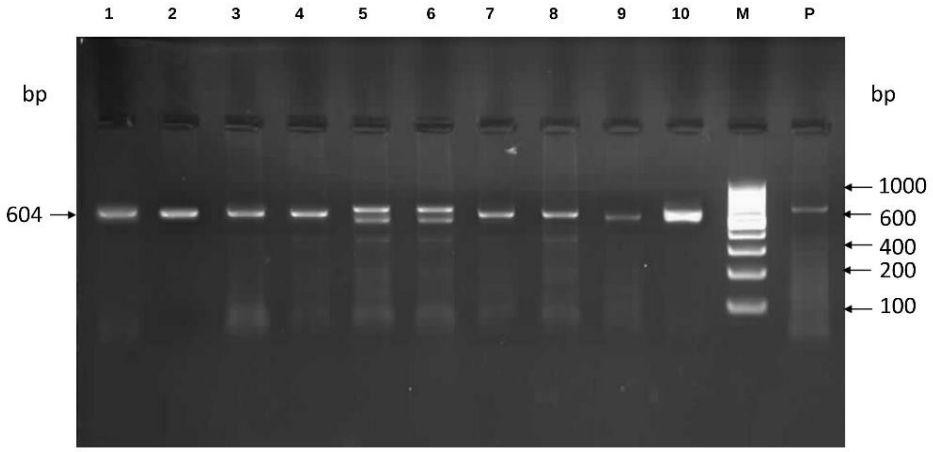

Figure 2: PCR-RFLP pattern in exon 2 of leptin gene digested with BamHI Restriction enzyme on 2% agarose.

M = 100bp DNA ladder; P = PCR product; Lane 1-4, 7-10 = AA genotype (604bp); Lane 5-6= AB genotype (604/495/109bp)

Table 2: Various carcass traits for breed and genotypes for exon 2 locus (Mean±SE)

Factors / Breed	Dressing percentage	Carcass length	Loin eye area	Back fat thickness	Heart girth	Belly girth	Thigh girth
Crossbred	73.77±0.29	82.39±0.60	25.47±0.41	2.67±0.03	110.93±0.83	111.28±0.70	41.22±0.36
Genotype							
AA	73.97±0.40	82.17±0.83	25.58±0.55	2.70±0.04	111.66±1.08^{ab}	111.92±0.91	41.69±0.48
AB	74.03±0.93	84.33±1.93	27.02±1.28	2.74±0.10	115.21±2.51^{a}	114.44±2.13	42.23±1.11
BB	73.41±0.49	82.39±1.02	24.89±0.67	2.59±0.05	108.67±1.32^{b}	109.44±1.12	40.22±0.59

Different superscripts (a,b) in a row show significant difference (p<0.05)

4. Discussion

The means along with standard error for different carcass traits of leptin gene at exon 2 are depicted in Table 2. The results of one-way ANOVA for dressing percentage, carcass length, loin eye area, backfat thickness, heart girth, belly girth and thigh are as below.

4.1 Dressing percentage

The results of analysis of variance showed that the dressing percentage showed non significance (p<0.05). Non significance (p<0.05) differences were observed in this crossbred pigs. In the present study crossbred pig was only considered and no other breed was considered. All the genotype did not show any relationship with carcass traits. No comparable results are available in available literature.

4.2 Carcass length

The results of one-way ANOVA showed that the Carcass length showed non significance (p<0.05). Non significance (p<0.05) differences were observed in this crossbred pigs. In the present study crossbred pig was only considered and no other breed was considered. All the genotype did not show any relationship with carcass traits. No comparable results are available in available literature.

4.3 Loin eye area

The results of one-way ANOVA showed that the Loin eye area showed non significance (p<0.05). Non significance (p<0.05) differences were observed in this crossbred pigs. In the present study crossbred pig was only considered and no other breed was considered. All the genotype did not show any relationship with carcass traits. No comparable results are available in available literature.

4.4 Back fat thickness

The results of one-way ANOVA showed that the back fat thickness showed non significance (p<0.05). Non significance (p<0.05) differences were observed in this crossbred pigs. In the present study crossbred pig was only considered and no other breed was considered. All the genotype did not show any relationship with carcass traits. But significance was found in the back fat thickness as reported by Bauer *et al.* (2006) [4], Hoa *et al.* (2021) [10] and Peixoto *et al.* (2009) [9] as compared to non – significance obtained by Szydlowski *et al.* (2004) [11].

4.5 Heart girth

The results of one-way ANOVA showed that the heart girth showed significance (p<0.05). Significance (p<0.05) differences were observed in the crossbred pigs for leptin gene exon 2 locus. In the present study crossbred pig was only considered and no other breed was considered. All the genotype did not show any relationship with carcass traits except heart girth with exon 2 of leptin gene [14]. Higher heart girth was observed for AB genotype which clearly indicates the superiority of AB genotypes than AA and BB genotypes in case of exon 2 of leptin gene. No comparable results are available in available literature.

4.6 Belly girth

The results of one-way ANOVA showed that the belly girth showed non significance (p<0.05). Non significance (p<0.05) differences were observed in this crossbred pigs. In the present study crossbred pig was only considered and no other breed was considered. All the genotype did not show any relationship with carcass traits. No comparable results are available in available literature.

4.7 Thigh girth
The results of one-way ANOVA showed that the thigh showed non significance ($p<0.05$). Non significance ($p<0.05$) differences were observed in this crossbred pigs. In the present study crossbred pig was only considered and no other breed was considered. All the genotype did not show any relationship with carcass traits. No comparable results are available in available literature.

5. Conclusions
In crossbred pigs of AB genotypes at exon 2 was superior to AA and BB genotypes. Hence it is recommended that crossbred pigs having AB genotype may be selected as parents for future breeding.

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