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CYP11B1 and *PPARGC1A* genes polymorphism controlling reproductive traits and estimation of breeding value of first lactation milk yield in *Bos indicus* (Deoni) cattle

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ABSTRACT

In the present study, in Deoni, the dual purpose cattle breed of Southern India, maintained in a National Dairy Research Institute herd, Southern Regional Station, Bengaluru, molecular characterization of the putative exon 1 of *CYP11B1* and putative intron 9 and 3'UTR of *PPARGC1A* genes was carried out in 146 animals using the PCR-RFLP technique. Three restriction enzymes, namely PstI, HaeIII and NheI, were used for digestion of the amplicons of the genes, respectively. In the putative exon 1 of the *CYP11B1* gene, two genotypes, VV and VA, were detected with frequencies of 0.23 and 0.77, respectively. The frequencies of allele V and A in the population were found to be 0.62 and 0.38, respectively. The allelic frequencies of C and T types were observed as 0.63 and 0.37, with frequencies of CC, TC and TT genotypes as 0.38, 0.51 and 0.11 in the putative intron 9 of the *PPARGC1A* gene, respectively. Three genotypes, namely AA, AC and CC were detected in 3'UTR of the *PPARGC1A* gene, with respective frequencies of 0.75, 0.21 and 0.04. The allelic frequencies of A and C types were 0.86 and 0.14, respectively. The locus (c.1892+19T>C) in the putative intron 9 of the *PPARGC1A* gene was found to be significant (P<0.05), with unadjusted data of age of first calving (AFC). Adjusted data of first lactation milk yield (FLMY) showed a significant (P<0.10) association with the loci of the *CYP11B1* (p.Val30Ala) and *PPARGC1A* (c.3359A>C) genes. No significant (P<0.10) association was observed between the loci of the genes and breeding value of FLMY in the studied cattle population.

Key words: Deoni; first lactation milk yield; C*YP11B1* gene*; PPARGC1A* gene

Introduction

The *CYP11B1* (Cytochrome P45011 beta hydroxylase 1) gene is a positional and functional candidate gene for milk production traits, present in BTA14q12 (KAUPE et al.,

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2004), near marker ILSTS039, which is associated with milk yield as well as with milk component yields. The gene has p.Val30Ala polymorphism in the first exon, with positive association with milk production traits in Czech Fleckvieh cattle (BOLECKOVA et al. 2012). The bovine *PPARGC1A* (Peroxisome Proliferator Activated Receptor Gamma Co-activator 1 Alpha) gene has been mapped to chromosome 6 (BTA6) (KHATIB et al., 2007), consists of 14 exons and is expressed at different levels in a great number of tissues. The gene has interaction with the activity of many nuclear hormone receptors and transcriptional factors involved in regulation of adaptive thermogenesis, fiber-type switching in skeletal muscle, adipogenesis and gluconeogenesis. Genetic variants of the *PPARGC1A* gene have been found to be associated with production and reproduction traits.

Deoni is a medium sized heavy cattle breed among 41 well-established breeds of India. It has a massive and upstanding body with considerable depth, with a prominent, broad and slightly bulging forehead. Its horns are medium and thick, emerging from the side of the poll with a characteristic outward and backward curve. They have bright and alert eyes. The cows have bowl-shaped udders with fairly well-developed quarters and squarely placed black teats. They have straight powerful legs, with broad and strong, systematically placed, black hooves *i.e.* they are well suited to the local conditions of cultivation and transportation in home tracts, and are admired very much by farmers for their draft capacity and endurance. This breed is hardy and well-adapted for tropical wind prone areas (DAS et al., 2011). The literature revealed that genetically, the Deoni breed evolved through the crossbreeding of the Gir cattle of the Kathiawar region of Gujarat with the Dangi breeds of Marathawada and local desi cattle of Nizam state from Bidar and Osmanabad, more than 300 years ago (JOSHI and PHILLIPS, 1953). The improvement of this dual purpose breed is necessary in order to maintain this breed, by obtaining suitable breeding objectives with proper selection strategies.

The animal's dual purpose productivity depends heavily on how well the animal responds to the management system, involving a production strategy and diverse natural environment including particular combinations of favorable and unfavorable foraging and watering conditions. Phenotypic performance profiles, for traits associated with productivity, adaptation and the molecular information of known genes with putative effects on traits of current and future interest, will be helpful to estimate quantitative genetic merit and genetic variability. Genomic knowledge increases the prospects for applying molecular technology and provides better selection for sustainable, healthy and productive animals. Thus it is necessary to ensure dual purpose cattle are profitable, by improving genetic performance, and sustainable in the environmental conditions so that the farmers are able to maintain these cattle in their homes and preserve domestic

animal biodiversity. Genomic knowledge increases the prospects for applying molecular technology and provides for better selection of sustainable healthy productive animals.

Materials and methods

Source of data. The data pertaining to the productive and reproductive performance of Deoni cattle were collected from history sheets and breeding cards maintained at the cattle yard and these records were used for estimation of production and reproduction performance (first lactation milk yield, age at first calving, and first service period), genetic parameters, the breeding value of first lactation milk yield, and the association of the genetic variants of the *CYP11B1* and *PPARGC1A* genes with FLMY, AFC and FSP. To ensure normal distribution the outliers were removed and only data within the range of mean ± 2 SD were considered. For genetic studies, only sires with three or more daughters were considered.

Blood sample collection. Blood samples of about 5-10 mLof were collected from 146 Deoni cattle maintained at the National Dairy Research Institute, Southern Campus, Bengaluru. After collection, the samples were stored at 4 °C and DNA isolation was performed as soon as possible, the delay not exceeding 24 hours. DNA was isolated from the blood samples using a modified high salt method, as described by MILLER et al. (1988).

Quality and quantity estimation of DNA. Quality of DNA was checked by loading 2-5 mL of DNA onto 0.8% agarose gel electrophoresis in a horizontal mini electrophoresis unit using 1x TBE as the running buffer. After electrophoresis, the gel was stained with ethidium bromide solution (0.5 mg/mL). The DNA was quantified using a UV spectrophotometer (Eppendorf Biophotometer, Germany). The samples showing Optical Density (O.D.) ratio (260/280 nm) of between 1.7 to 1.9 were stored at -20 °C, used for further analysis, and diluted to 100 ng/µL for utilization as a DNA template in polymerase chain reaction (PCR).

Primers and PCR Conditions. Published primers of the *CYP11B1* (KAUPE et al., 2007) and *PPARGC1A* (KHATIB et al., 2007) genes were used for amplification of the genomic DNA of Deoni cattle. Pst I (CTGCAG) restriction enzyme was used for detection of p.Val30Ala polymorphism at exon 1 of the *CYP11B1* gene and for the *PPARGC1A* gene Hae III (GGCC) and Nhe I (GCTAGC) restriction enzymes were used to find the polymorphisms of c.1892+19T>C and c.3359A>C loci, respectively. The sequence of forward and reverse primers, their product size and the enzyme used are presented in Table 1. The primers were ordered and procured from Amnion Biosciences Pvt. Ltd., Bengaluru.

PCR conditions were standardized for each primer set. The PCR reactions were carried out on 100 ng of genomic DNA in 25 µL per reaction volume, containing 200

μM each of dNTP, 10X Taq polymerase assay buffer, 1 U of Taq polymerase enzyme, and 20 pM of each primer and nuclease free water. The standardization was carried out with minor modifications to ensure proper amplification for both the genes. The PCR products were electrophoresed at 100 V in 1.5% agarose gel in 1X TBE buffer, containing 0.5 μg/mL ethidium bromide, along with a 100 bp molecular size marker. The gels were visualized and documented using a gel documentation system (Gel Doc 1000, Bio-Rad, USA).

Table 1. Details of primer sequences (5' to 3' sequences) used for amplification

Gene	Position	Primer type	Sequence	Product size (bp)	Enzyme used	Annealing Temp. (ºC)
CYP11B1	Putative exon 1	FP	ATACTGGAGG GGGAGGAGG	568	*Pst I*	63
		RP	GGACAGAACG TGAGGGTGTT			55
PPARGC1A	Putative intron 9	FP	CATAGCCGGCGCCCCAG GTAAGATGCACGTTGGC	195	*Hae III*	65
		RP	CTGGTACTCC TCGTAGCTGTC			57
	3'UTR	FP	GCGAGCACGGTGTTACATT ACTAAGGAGAGTTGGCTAG	358	*Nhe I*	50
		RP	GTTGTGTTGC ACTCAATGGAC			48

PCR-RFLP analysis. For PCR- RFLP analysis, the PCR amplified product was digested with PstI, HaeIII and NheI restriction enzymes (New England Biolabs), with the respective regions of putative exon 1 of the *CYP11B1* gene and putative intron 9 and 3'UTR of the *PPARGC1A* gene.

The reaction mixture of 20 μL contained 10 μL of the PCR sample, 0.5 μL of the restriction enzyme, 2 L 10 X buffer, and 7.5 μL of nuclease free water. The reaction mixture was prepared at 4 °C and incubated at 37 °C for digestion of the PCR product, followed by heat inactivation for the respective enzyme. The restriction fragments were resolved on 2.5% agarose gel. Different band patterns obtained were custom-sequenced by Xcelris Lab. Ltd., Ahmedabad.

Evaluation of breeding values in cows using a single record of individual animals for FLMY. Most Probable Producing Ability (MPPA) for estimation of individual animals for FLMY. The most probable producing ability (MPPA) of the cattle was computed using a single record of the first lactation milk yield. Deoni cows were evaluated on the basis of the deviation of the population mean of FLMY from its phenotypic value. This is not the true breeding value of the cattle. However, this method is useful to evaluate the cattle

when very little information is available about the cow other than heritability of the traits. Environmental factors led to variations in the predicted values from the population mean. The following method, described by LUSH (1943), was used to compute MPPA:

MPPA of the cow = $\mu + h^2 (Y_i - \mu)$

Where,

μ = Population mean

h_2 = Heritability of first lactation milk yield of Deoni cattle

Y_i = Phenotypic value of FLMY of ith cow

LSA for predicting breeding value excluding and including genotype information. Generalized linear models were used to predict the breeding value of the first lactation milk yield of the Deoni cows through the LSA method using SAS software. The mixed linear model was chosen, incorporating and without incorporating conventional and molecular information on the loci of the *CYP11B1* and *PPARGC1A* genes. The following models were used:

$$Y_{ijklmnox} = \mu + S_i + POB_j + LND_k + POC_l + SOC_m + CAFC_n + CFLL_o + e_{ijklmnox}$$

$$Y_{ijklmnogx} = \mu + S_i + POB_j + LND_k + POC_l + SOC_m + CAFC_n + CFLL_o + G_{1p} + G_{2q} + G_{3r} e_{ijklmnogx}$$

Where,

$Y_{ijklmnogx}$ = x^{th} cow of i^{th} sire born in j^{th} period at k^{th} lactation of its dam, calving at m^{th} season of l^{th} period under n^{th} and o^{th} group of AFC and FLL and g^{th} genotypes.

μ = Population mean

S_i = i_{th} sire (i = 1, 2, 3….7)

POB = j^{th} period of birth (j = 1, 2 and 3)

LND = k^{th} lactation number of dam (k = 1, 2, 3….5)

POC = l^{th} period of calving (l = 1, 2, 3 and 4)

SOC = m^{th} season of calving (m = 1, 2 and 3)

CAFC = n^{th} class of age of first calving (n = 1, 2, 3…..7)

CFLL = o^{th} class of first lactation length (o = 1, 2, 3….6)

G_{1p} = p^{th} genotype of Val.p.30.Ala locus of *CYP11B1* gene (p = VV and VA)

G_{2q} = q^{th} genotype of c.1892 + 19T>C locus of *PPARGC1A* gene (q = TT, TC and CC)

G_{3r} = r^{th} genotype of c.3359A> C locus of *PPARGC1A* gene (r = AA, AC and CC)

$e_{ijklmnox}$ = random error of x^{th} individual, NID (0, $\sigma^2 e$)

$e_{ijklmnogx}$ = random error of x^{th} individual, NID (0, $\sigma^2 e$)

Comparison between the LSA models. The effectiveness of the predictive mixed linear model was compared using coefficient of determination (R^2), Akaike information

criterion (AIC), Pearson and Spearman's correlation. The parameters for determination of a better model were estimated using SAS Software Ver. 9.2 (2003). The higher R^2 value of the model is taken as a better model for prediction. The model with the lowest AIC value was considered as the optimum model. A higher correlation between the true phenotypic and the predicted value reflects a better model for prediction.

Statistical analysis and association study of the studied loci with AFC, FSP and FLMY. Gene and genotype frequencies of the *CYP11B1* and *PPARGC1A* genes were calculated according to FALCONER (1998). Association studies of the respective polymorphic loci of the *CYP11B1* and *PPARGC1A* genes were performed with age at first calving, first service period and first lactation milk yield. However, associations of the loci of the *CYP11B1* and *PPARGC1A* genes were also seen with the MPPA and predicted breeding value of the cows. The GLM procedure of SAS 9.2 version was used for the study, with unadjusted and adjusted data on the respective traits. The model used under the association studies is given below:

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

Where,

Y_{ij} = Data (Unadjusted, adjusted and breeding value) of j^{th} animal of i^{th} genotypes of the loci of *CYP11B1* and *PPARGC1A* genes

μ = Overall population mean of respective traits of AFC, FSP and FLMY

e_{ij} = Random error NID (0, $\sigma^2 e$)

The effects of the loci of the respective genes on AFC, FSP and FLMY were observed through the analysis of variance procedure of the General Linear Model (GLM). Duncan's multiple range test (DMRT) as modified by KRAMER (1956), was applied for testing differences among the least squares means (using an inverse coefficient matrix) by SAS software.

Results and discussion

Polymorphism and gene and genotypic frequencies. Two genotypes, namely VV and VA, were observed for the digested product of the *CYP11B1* gene putative exon 1 with PstI, restriction enzyme. Three band patterns revealed VV, and four band patterns VA genotypes, respectively (Fig. 1). The frequencies of VV and VA genotypes were observed as 0.23 and 0.77, respectively, with the allelic frequencies of 0.62 and 0.38 for V and A types. The *PPARGC1A* gene revealed polymorphisms at intron 9 using the HaeIII restriction enzyme. Two band patterns were observed in CC, three in TC and a single band pattern in TT genotypes, respectively (Fig. 2).

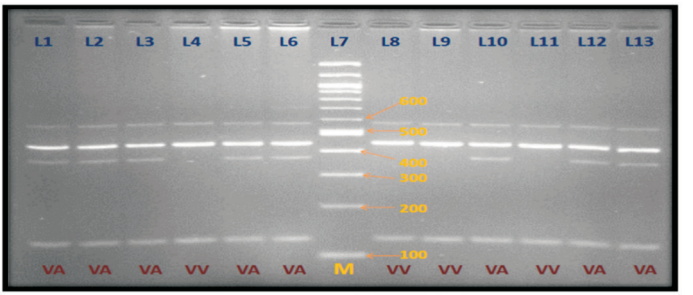

Fig. 1. Genotypic patterns of exon 1 of *CYP11B1* gene in Deoni cattle. M = 100 bases DNA marker (L7); L1, L2, L3, L5, L6, L10, L12 and L13 = VA genotypes L4, L8, L9 and L11 = VV genotypes.

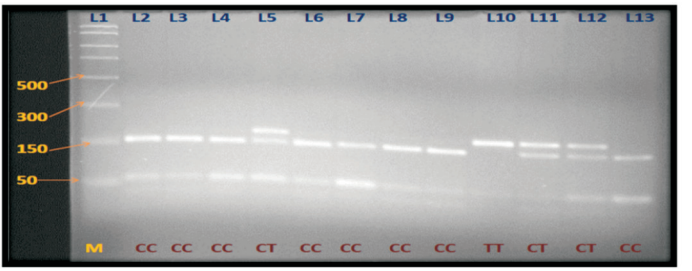

Fig. 2. Genotypic patterns of putative intron 9 of *PPARGC1A* gene in Deoni cattle. M = 100 bases DNA marker (L1); L2, L3, L4, L6, L7, L8, L9 and L13 = CC genotypes, L5, L11 and L12 = CT genotypes and L10 = TT genotype.

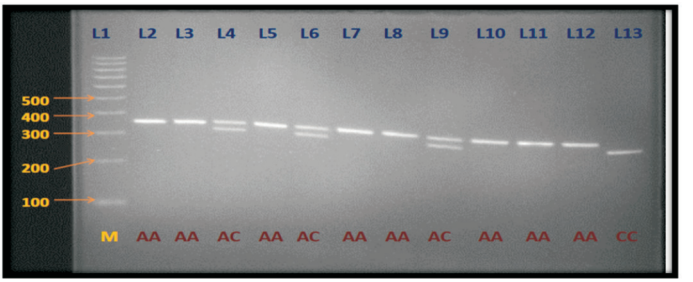

Fig. 3. Genotypic patterns of 3'UTR in *PPARGC1A* gene in Deoni cattle. M = 100 bases DNA marker (L1); L2, L3, L5, L7, L8, L10, L11 and L12 = AA genotypes, L4, L6 and L9 = AC genotype and L13 = CC genotype.

Three genotypes TT, TC and CC were observed with frequencies of 0.11, 0.51 and 0.38, respectively, at putative intron 9 of the *PPARGC1A* gene. The respective frequencies of T and C alleles were estimated as 0.37 and 0.63. Two band patterns were observed

for AC genotypes, whereas a single band pattern was seen in AA and CC genotypes, respectively. Three types of genotypes, AA, AC and CC (Fig. 3), were detected at 3'UTR of the *PPARGC1A* gene using NheI restriction enzymes, with genotypic frequencies of 0.75, 0.21 and 0.04, respectively. Allelic frequencies were observed as 0.86 and 0.14 for the A and C alleles. (Table 2).

Table 2. Genotype and allele frequencies of *CYP11B1* and *PPARGC1A* genes

Gene	Loci	Genotypes (n)	Genotype frequencies	Alleles	Allelic frequencies
CYP11B1	p.Val.30Ala	VV (34)	0.23	V	0.62
		VA (112)	0.77	A	0.38
		AA (0)	0		
PPARGC1A	c.1892+19T>C	CC (55)	0.38	C	0.63
		TC (75)	0.51	T	0.37
		TT (16)	0.11		
	c.3359A>C	AA (110)	0.75	A	0.86
		AC (31)	0.21	C	0.14
		CC (5)	0.04		

n = Number of cattle under genotypes

Sequence variability of the sample. Sequence analysis revealed sixteen single nucleotide polymorphisms in putative exon 1 of *CYP11B1* (14) and 3'UTR of the *PPARGC1A* (2) gene of Deoni cattle, respectively (Figs. 4 and 5). The sequences of the *CYP11B1* and *PPARGC1A* genes were submitted online to the NCBI gene bank and received the Accession Numbers KF471016 and KF691739, respectively.

Level of heterozygosity, Chi square (χ^2) test effective number of allele (n_e). The degree of heterozygosity, and the χ^2 test for the Hardy Weinberg equilibrium for the cattle population were performed using the population genetic analysis software POPGENE version 1.31 (YEH et al., 2006). The observed heterozygosity value was 0.767, which was higher than the expected heterozygosity value (0.475). This clearly indicated that there was a prevalence of heterozygotes to a higher degree in the studied cattle population in exon 1 of the *CYP11B1* gene. The POPGENE analysis revealed that the estimated χ^2 value (55.95) for the genotype at p.Val30Ala locus of the *CYP11B1* gene was highly significant (<0.01). Hence, the population was not consistent with the HW equilibrium for the locus. In the case of the *PPARGC1A* gene, the X2 values for the genotypes of c.1892+19T>C and c.3359A>C loci were 1.55 and 2.27, with the probability values of 0.21 (P>0.05) and 0.13 (P>0.05), respectively. This indicated that the studied cattle population had not deviated from the HW equilibrium in respect to the two loci of the *PPARGC1A* gene (Table 3).

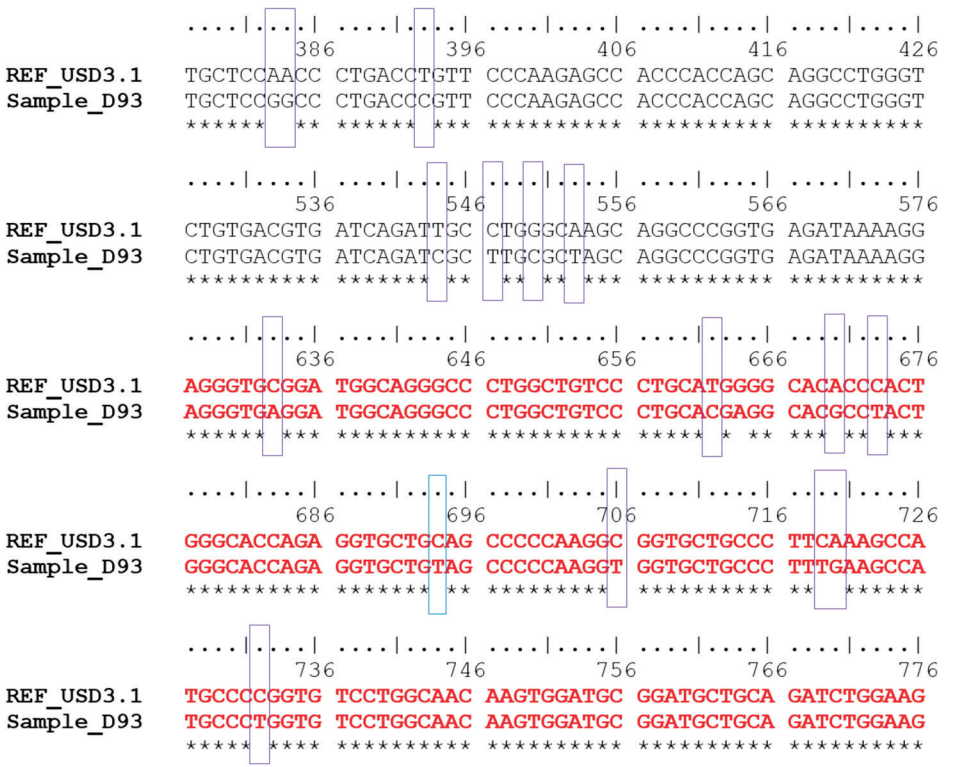

Fig. 4. Clustal W Multiple alignment sequence of putative exon 1 of *CYP11B1* gene. Alignment: Putative exon 1 of *CYP11B1* of *PPARGC1A* gene of Deoni cattle with USD3.1 sequence of *PPARGC1A* gene of *Bos tarus* cattle. Red marking indicated exon 1 of bovine *CYP11B1* gene nucleotide in rectangular box showing polymorphisms.

Table 3. Heterozygosity Statistics and H-W equilibrium for *CYP11B1* and *PPARGC1A* genes

Gene	Loci	Heterozygosity		Effective number of alleles (n_e)	Chi-square value	Probability
		Observed	Expected			
CYP11B1	p.Val30Ala	0.767	0.475	1.897	55.95	<0.01
PPARGC1A	c.1892+19T>C	0.514	0.466	1.867	1.55	0.21
	c.3359A>C	0.212	0.242	1.318	2.27	0.13

Fig. 5. Clustal W Multiple alignment sequences at 3'UTR of *PPARGC1A* gene. Alignment: 3'UTR of bovine *PPARGC1A* gene with USD3.1 sequence of *PPARGC1A* gene of *Bos tarus* cattle. Red marking indicated partial 3'UTR sequence of bovine *PPARGC1A* gene nucleotide in rectangular box showing polymorphisms.

Association study. The mean sum of squares due to genotype obtained from ANOVA for AFC, FSP and FLMY traits, with unadjusted, adjusted and computed breeding value data of the cattle, are presented in Table 4. It was found that the c.1892+19T>C locus of the *PPARGC1A* gene was significantly (P<0.05) associated with the unadjusted data of age at first calving. However, the effect of this locus was found to be non-significant in relation to the first service period and first lactation milk yield, with both adjusted and unadjusted data. The effect of the p.Val30Ala locus of *CYP11B1* and the c.3359A>C locus of the *PPARGC1A* genes was found to be non-significant with both unadjusted and adjusted data of AFC, FSP and FLMY. However, the P values obtained from ANOVA for the adjusted first lactation milk yield were low for the p.Val30Ala (0.0592) and c.3359A>C (0.0919) loci of the *CYP11B1* and *PPARGC1A* genes, respectively. The effects of the three loci were found to be non-significant (P<0.05) in relation to the breeding values for the first lactation milk yield, estimated by least squares analysis and MPPA methods (Table 4).

Table 4. Analysis of variances (F value and P value) of AFC, FSP and FLMY for the loci of *CYP11B1* and *PPARGC1A* genes

Traits	Data type	*CYP11B1* gene	*PPARGC1A* gene	
		p.Val30Ala	c.1892+19T>C	c.3359A>C
AFC	Unadjusted (137)	0.64 0.4245	3.85 0.0238	0.76 0.4675
	Adjusted (98)	0.26 0.6127	0.56 0.5766	0.79 0.4567
FSP	Unadjusted (116)	0.01 0.9946	0.84 0.4336	0.33 0.7181
	Adjusted (84)	0.46 0.4991	1.65 0.20	0.70 0.50
FLMY	Unadjusted (128)	3.72 0.0562	1.19 0.3073	1.21 0.3004
	Adjusted (90)	3.69 0.0592	0.50 0.6060	2.48 0.0919
	MPPA (90)	1.71 0.1944	0.41 0.6617	2.16 0.1213
	LSA_without genotype (90)	1.44 0.2338	0.65 0.5229	1.90 0.158

Parentheses indicated number of observation under each locus

No previous reports are available on the association of the p.Val30Ala locus of the *CYP11B1* gene with AFC and FSP. However, KAUPE et al. (2007) reported in German Holstein cattle that the V (*CYP11B1*V) allele was poorer than the A allele in respect to milk yield, with De-regressed breeding values (DRBV). The effect of half the allele substitution effect of the *CYP11B1*V allele was -182 ± 23 kg, which was highly significant (P<0.001). BOLECKOVA, et al. (2012) reported that the least squares means of AA genotypes (7599.88 ± 220.98 kg) were better than VV (Val/Val) genotypes (7533.90 ± 98.87 kg) in respect to the milk yield of Czech Fleckvieh cattle. It was also found that the effect of the genotypes was non-significant at P<0.05, which is similar to the present study. Regarding the association of EBVMY, the same study found that the Ala/Ala genotype was better than the Val/Ala and Val/Val genotypes.

No previous reports are available on the association of c.1892+19T>C genotypes with AFC. However, KOMISAREK and WALENDOWSKA (2012) reported the association of the genotypes with age at first insemination (AFI) and calving to conception (CCI) in Polish Holstein Friesian cattle. In the study, the least squares means for AFI traits were found to be in higher TT genotypes (805.36 + 64.10 days) than CC genotypes (801.69 + 71 days), but it was non-significant (P = 0.92). Also, the least squares mean for CCI

was higher in TT genotypes (163.95 + 79.16 days) than CC genotypes (129.32 + 64.14 days), and this was found to be significant (P<0.05). Similar results were found with the LS means of AFC and FSP traits of the genotype in the present study on Deoni cattle, although the effect of the genotypes was non-significant (P = 0.43 with unadjusted data and P = 0.20 with unadjusted data) with FSP. It may be that environmental factors play a major role in the AFC of this indigenous breed of cattle. WEIKARD et al. (2005) and KHATIB et al. (2007) in German Holstein, KOWALEWSKA-LUCZAK et al. (2010) in Jersey and BOLECKOVA et al. (2012) in Czech Fleckvieh cattle reported higher milk yield with the TT genotype than the CC genotype which is similar to the present study in Deoni cattle.

No previous reports are available on the association of c.3359A>C genotypes with AFC, FSP in indigenous cattle. However, reports on the association of the genotypes are available with milk yield traits. WEIKARD et al. (2005) and KHATIB et al. (2007) reported higher milk yield in AA genotypes than CC genotypes in German Holstein. KOWALEWSKA-LUCZAK et al. (2010) reported heterozygote (CA) animals having higher milk yield in comparison to AA homozygotes, whereas, no homozygote for the CC genotype was found in 181 Jersey cows.

Computation of breeding value for first lactation milk yield. The breeding values for the FLMY of Deoni cows were computed on the basis of the records up to the first lactation using three different models (Tables 6 and 7).

Estimation of MPPA of Deoni cows. The most probable producing ability of first lactation milk of Deoni cattle was estimated on the basis of a single record of milk yield in first lactation, and estimated heritability. In the present study, the MPPA of Deoni cattle for FLMY was estimated and ranged from 375.77 to 897.25 kg, with an accuracy of 62.01 per cent. The estimated MPPA is the genetic superiority or inferiority of the individual in comparison to the population mean of 109 cattle. Here, the non-additive gene effect and the environmental factors bring the MPPA nearer to the estimated population mean of 516.11 ± 40.30 kg rather than the phenotypic value of the individuals.

Estimation of breeding value through least squares analysis method. Least squares analysis (LSA) was performed to compute the breeding value of Deoni cattle for the first lactation milk yield, based on single record, including and excluding genotype information of the p.Val.30Ala locus in *CYP11B1* and the c.1892+19T>C and c.3359A>C loci in the *PPARGC1A* gene. The predicted breeding values (PBV) of the cattle obtained by the LSA method without taking genotype information were in the range of 139.32 to 1520.15 kg, with a CV value of 79.59 per cent. When genotypic information was incorporated in the LSA model the range of PBV were estimated from 724.2 to 1156.31 kg with 79.73 per cent CV (Table 7).

Table 5. LS Mean ± SE of AFC, FSP and FLMY based of different genotypes of the loci of *CYP11B1* and *PPARGC1A* genes

Traits	Data types	*CYP11B1* gene		*PPARGC1A* gene					
		p.Val30Ala		c.1892+19T>C (105)			c.3359A>C		
		VV	VA	CC	CT	TT	CC	CA	AA
AFC	Unadjusted	42.82 ± 1.3[a]	44.04 ± 0.7[a]	41.81 ± 1.9[a]	45.57 ± 0.9[a]	42.11 ± 1.1[a]	43.64 ± 0.8[a]	44.64 ± 1.3[a]	40.20 ± 2.3[a]
	Adjusted	44.88 ± 1.35[a]	45.12 ± 1.76[a]	44.06 ± 2.18[a]	46.45 ± 1.49[a]	44.48 ± 1.57[a]	46.48 ± 1.11[b]	45.84 ± 1.45[b]	42.67 ± 3.20[a]
FSP	Unadjusted	191.7 ± 15.5[a]	191.6 ± 9.3[a]	181.13 ± 11.4[a]	203.64 ± 12.5[a]	186.13 ± 23.7[a]	192.9 ± 9.3[a]	181.3 ± 18.2[a]	213.4 ± 24.4[a]
	Adjusted	181.96 ± 24.21[a]	198.76 ± 18.97[a]	184.41 ± 29.05[a]	198.38 ± 21.13[a]	188.29 ± 21.78[a]	183.34 ± 14.39[a]	181.24 ± 23.31[a]	206.51 ± 44.99[a]
FLMY	Unadjusted	529.0 ± 65.7[a]	698.2 ± 45.7[a]	564.6 ± 105.0[b]	619.0 ± 57.1[a]	723.5 ± 59.3[a]	622.4 ± 41.4[a]	767.2 ± 97.4[a]	681.5 ± 253.5[a]
	Adjusted	592.28 ± 116.49[a]	804.46 ± 98.24[b]	564.6 ± 143.27[a]	750.34 ± 105.35[b]	764.53 ± 110.75[b]	552.05 ± 68.03[a]	819.49 ± 93.45[b]	723.56 ± 251.36[b]
	MPPA	488.38 ± 29.85a	540.94 ± 21.52[a]	483.45 ± 41.88[a]	534.64 ± 23.39[a]	530.0 ± 35.10[a]	504.08 ± 18.84[a]	588.15 ± 41.92[a]	551.72 ± 130.66[a]
	LSA without genotype	458.79 ± 74.20[a]	580.9 ± 55.1[a]	413.64 ± 92.96[c]	576.76 ± 60.12[a]	548.47 ± 88.94[b]	495.99 ± 49.15[a]	696.75 ± 403.24[a]	527.46 ± 263.23[a]

Different superscript among the genotypes in the loci indicated statistically distinct group

Table 6. Descriptive statistics of FLMY through various methods

	LS Mean ± SE	SD	CV (%)	Minimum (kg)	Maximum (kg)
True FLMY	516.11 ± 40.30 (110)	415.74	81.88	231.5	1725.00
MPPA	516.11 ± 15.19 (109)	160.59	30.59	407.96	975.49
LSA excluding genotypes	518.33 ± 39.51 (109)	409.38	79.59	139.32	1520.20
LSA including genotypes	547.98 ± 46.05 (98)	436.91	79.73	131.37	1156.31

SE = Standard error; SD = Standard deviation; CV = Coefficient of variation; MPPA = Most probable producing ability and LSA = Least squares analysis.

Table 7. Ranking of Deoni cows based on predicted breeding value incorporating genotypes information for FLMY with other methods

Animal No.	LSA with incorporating Genotypes	Rank	LSA without Genotypes	Rank	MPPA	Rank	FLMY
555	1156.31	1	1481.17	3	832.99	10	1350
533	1078.24	2	738.83	26	650.59	21	870
311	1074.2	3	1520.15	1	936.73	2	1623
316	933.05	4	688.03	27	605.94	25	752.5
399	897.52	5	1421.1	7	929.51	3	1604
341	888.06	6	399.29	29	423.92	37	273.5
308	859.97	7	1025.13	18	707.78	17	1020.5
307	849.59	8	1029.49	17	583.52	26	693.5
347	830.03	9	1386	10	856.74	9	1412.5
349	808.82	10	1414.59	9	897.02	5	1518.5
350	793.44	11	986.48	21	720.51	16	1054
343	783.87	12	305.08	46	414.42	84	248.5
394	780.99	13	322.66	39	421.07	61	266
348	760.8	14	962.9	22	675.48	20	935.5
313	749.58	15	1447.27	5	861.87	8	1426
445	742.52	16	139.32	90	422.59	47	270
465	739.59	17	257.63	77	421.64	57	267.5
435	726.35	18	257.78	76	418.79	77	260
377	724.2	19	1456.51	4	886.95	6	1492
358	724.2	20	270.05	67	410.81	86	239

Comparison of the models. In the present study two models for prediction of first lactation milk was developed incorporating and without incorporating genotypes information of the *CYP11B1* and *PPARGC1A* genes. Comparison between the two models was performed using Coefficient of determination (R2), Akaike information criterion (AIC), Pearson (r^2_p) and Spearman's rank (r^2_s) correlation. The lower AIC value (830.16) and higher R^2 value (95.11%) of the second model indicated that genotype information provides more information than the first model for prediction of first lactation milk yield in the cattle. Besides, the higher Pearson correlation (0.975) and Spearman's rank correlation (0.726) of the second model, with the phenotypic value, than the first model shows that the second model provides a better prediction than first one, which lacks genotype information (Table 8).

Table 8. Comparison between the prediction models

Model	R^2	AIC	r^2_s	r^2_p
LSA without genotype	94.69	1001.70	0.97312	0.680
LSA with genotype	95.11	830.16	0.97526	0.726

R^2 = Coefficient of determinants; AIC = Akaike information criterion; r^2_p = Pearson correlation coefficient; r^2_s = Spearman's correlation coefficient

Conclusions

In the present investigation frequencies of V, C and A alleles were higher than A, T and C alleles at the putative exon 1 of the *CYP11B1* gene, and the putative intron 9 and 3'UTR of the *PPARGC1A* genes in the studied population. The Chi-square test revealed that the population was in H-W equilibrium with respect to genotypes of the *PPARGC1A* gene and the opposite was observed in the genotypes of the *CYP11B1* gene. A significant ($P<0.05$) association of the genotypes at putative intron 9 was found with unadjusted data of AFC. A significant ($P<0.10$) association was found with the genotypes at the putative exon 1 of the *CYP11B1* gene and 3'UTR of the *PPARGC1A* gene with adjusted FLMY. This study suggests that these SNPs could be used as molecular markers based on next generation validates for selection of superior animals in terms of better milk production and improvement ofreproductive traits. However further study could be carried out for identification of genetic markers and their association with economically important traits in a larger number of animals for marker assisted selection.

References

BOLECKOVA, J., J. MATEJICKOVA, M. STIPKOVA, J. KYSELOVA, L. BARTON (2012): The association of five polymorphisms with milk production traits in Czech Fleckvieh cattle. Czech J. Anim. Sci. 57, 45-53.

DOI: 10.17221/5131-cjas

DAS, D. N., M. A. KATAKTALWARE, K. P. RAMESHA, A. O. REDDY (2011): Productive and reproductive performances of Deoni cattle under intensive management system. Indian J. Anim. Sci. 81, 1186-1188.

FALCONER, D. S. (1998): Introduction to Quantitative genetics. 4 ed., Addison Wesley Longman Ltd. Essex, England.

JOSHI, N. R., R. W. PHILLIPS (1953): Zebu cattle of India and Pakistan. FAO Agricultural studies No. 19. 146-151. FAO Publication. Rome.

KAUPE, B. S., S. KOLLERS, R. FRIES, G. ERHARDT (2004): Mapping of *CYP11B1* and putative CYHR1 paralogous gene to bovine chromosome 14 by FISH. Anim. Genet. 35, 6.

DOI: 10.1111/j.1365-2052.2004.01200.x

KAUPE, B., H. BRANDT, E. M. PRINZENBERG, G. ERHARDT (2007): Joint analysis of the influence of *CYP11B1* and *DGAT1* genetic variation on milk production, Somatic cell score, conformation, reproduction and productive lifespan in German Holstein cattle. J. Anim. Sci. 85, 11-21.

DOI: 10.2527/jas.2005-753

KHATIB, H., I. ZAITOUN, J. WIEBELHAUS-FINGER, Y. M., CHANG, G. J. M. ROSA (2007): The association of bovine *PPARGC1A* and *OPN* genes with milk composition in two independent Holstein cattle populations. J. Dairy Sci. 90, 2966-2970.

DOI: 10.3168/jds.2006-812

KOMISAREK, J., A. WALENDOWSKA (2012): Analysis of the *PPARGC1A* gene as a potential marker for productive and reproductive traits in cattle. Folia Biol-Krakow 60, 171-174.

DOI: 10.3409/fb60_3-4.171-174

KOWALEWSKA-LUCZAK, I., H. KULIG, M. KMIEC (2010): Association between the bovine *PPARGC1A* gene and milk production traits. Czech J. Anim. Sci. 55, 195-199.

DOI: 10.17221/101/2009-cjas

KRAMER, C. Y. (1956): Extension of multiple range tests to group means with unequal numbers of replication. Biometrics 12, 307-310.

DOI: 10.2307/3001469

LUSH, J. L. (1943): Animal breeding plans, Iowa State College Press, p. 168.

MILLER, S. A., D. D. DYKES, H. F. POLESKY (1988): A simple salting out procedure for extraction of DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

DOI: 10.1093/nar/16.3.1215

SAS INSTITUTE INC., (2003): Release 9.2 (TS2MO) of the SAS system for Microsoft Windows. SAS Institute. Inc., Cary. NC. USA.

WEIKARD, R. C. KUHN, T. GOLDAMMER, G. FREYER, M. SCHWERIN (2005): The bovine *PPARGC1A* gene: molecular characterization and association of an SNP with variation of milk fat synthesis. Physiol. Genomics 21, 1-13.

DOI: 10.1152/physiolgenomics.00103.2004

YEH, F., R. YOUNG, T. BOYLE (2006): POPGENE version 1.31, Microsoft Windows based freeware for population genetics analysis. University of Alberta and Centre for International Forest Research, Canada.

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BASAK, S., D. N. DAS, U. T. MUNDHE: Polimorfizam CYP11B1 i PPARGC1A gena i njegov utjecaj na kontrolu reproduktivnih obilježja te procjenu uzgojne vrijednosti za proizvodnju mlijeka u prvoj laktaciji *Bos indicus* (deoni) goveda. Vet. arhiv 89, 463-479, 2019.

SAŽETAK

U ovom istraživanju je za molekularnu karakterizaciju pretpostavljenog egzona 1 CYP11B1 gena i pretpostavljenog introna 9 i 3'UTR gena PPARGC1A korištena PCR-RFLP tehnika na 146 goveda kombinirane deoni pasmine iz južne Indije. Za odvajanje genskih aplikona upotrijebljena su tri restrikcijska enzima PstI, HaeIII i NheI. U pretpostavljenom egzonu 1 gena CYP11B1 otkrivena su dva genotipa, VV i VA, s učestalošću 0,23, odnosno 0,77. Učestalost u populaciji alela V iznosila je 0,62 a alela A 0,38. Frekvencija alela C i T iznosile su 0,63 i 0,37, a frekvencije CC, TC i TT genotipova 0,38, 0,51 i 0,11. U pretpostavljenom intronu 9 te u 3´UTR gena PPARGC1A otkriveni su AA, AC i CC genotipovi s učestalostima 0,75, 0,21 i 0,04. Učestalost alela A iznosila je 0,86 a alela C 0,14. Za lokus (c.1892+19T>C) u pretpostavljenom intronu 9 PPARGC1A gena utvrđen je statistički znakovit ($P<0,05$) utjecaj na neprilagođene podatke o dobi pri prvom teljenju. Prilagođeni podaci za proizvodnju mlijeka u prvoj laktaciji pokazali su znakovitu ($P<0,10$) povezanost s lokusima gena CYP11B1 (p.Val30Ala) i PPARGC1A (c.3359A>C). U istraženih goveda nije opažena znakovita ($P<0,10$) povezanost između lokusa gena i uzgojne vrijednosti za proizvodnju mlijeka u prvoj laktaciji.

Ključne riječi: deoni govedo; proizvodnja mlijeka u prvoj laktaciji; CYP11B1 gen; PPARGC1A gen