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T Karuthadurai
Department of Animal Genetics
and Breeding, ICAR-NDRI,
Karnal, Haryana, India

AK Chakravarty
Department of Animal Genetics
and Breeding, ICAR-NDRI,
Karnal, Haryana, India

Shrikant Dodamani
Department of Animal Genetics
and Breeding, KVAFSU,
Veterinary College, Bidar,
Karnataka, India

Pradeep Nag BS
Theriogenology Laboratory,
SRS of ICAR-NDRI, Bengaluru,
Karnataka, India

Corresponding Author:
T Karuthadurai
Department of Animal Genetics
and Breeding, ICAR-NDRI,
Karnal, Haryana, India

Effect of exon 3 region of polymorphism in prolactin gene on milk production and its composition traits in Sahiwal cattle

T Karuthadurai, AK Chakravarty, Shrikant Dodamani and Pradeep Nag BS

Abstract

The present study was undertaken to study the effect of SNP in exon 3 region of prolactin gene on milk production and its composition traits in Sahiwal cattle. The DNA was isolated from the blood samples of 98 pedigreed Sahiwal animals (including 27 male calf, 22 female calf and 49 Sahiwal adult female) PCR amplification was done, followed by PCR-RFLP. The phenotypic data was adjusted for non- genetic factors and regression analysis was performed. Multiple regression of SNP in exon 3 region of prolactin gene revealed that on an average, test day milk yield increased by 321.5g, test day fat yield by 13.9g and test day SNF yield increased by 19.4g. The SNP could be considered as potential genetic marker for early selection of animals based on test day milk production and its composition traits and could be incorporated in the Marker Assisted Selection strategy in future.

Keywords: prolactin gene, milk production, fat yield, SNF yield, polymorphism, Sahiwal

Introduction

In India agriculture sector contributes about 19.9 per cent in 2020-21 from 17.8 per cent in 2019-20 of the total GDP and livestock sector contributes about 25.6% to the total agriculture. This is due to 58% of the population were involved in the agriculture work. According to all India Livestock Census (Anonymous, 2019) ^[1] the cattle population is about 192.49 million in our country. Out of total cattle population, Indigenous/Non-descript cattle population in the country is 142.11 million with an average milk production of 3.01kg per day (BAHS, 2019) ^[3]. Our country has 50 recognized breeds of indigenous cattle of which Sahiwal is one of the best dairy breeds, was originated from Montgomery District of Pakistan. Their colour ranges from reddish brown through to the more predominant. The Sahiwal cattle has remarkable power of endurance to hot climate, ticks resistant, and resistance to various tropical diseases and has low cost of maintenance. Sahiwal breed has been taken to many tropical countries from India to use either for first crossing or later on for incorporating zebu genes, in the performance of cross breeds.

Over the years, Sahiwal cattle have been improved through selective breeding. However, the traditional breeding methodology increases the generation interval. (Karuthadurai *et al.*, 2019) ^[7]. Moreover, achieving genetic progress per year under traditional breeding is very low and therefore requires longer duration to improve the production performance. However, traditional breeding techniques in dairy cattle take more years and do not efficiently take into account all sources of genetic variability. The integration of genetic information along with the phenotypic data may help to increase accuracy of selection (Biochard *et al.*, 2016) ^[4] and hence, reduce generation interval. Marker assisted selection (MAS) is better than conventional selection for low heritability and sex-limited traits (Wakchaure *et al.*, 2015) ^[11]. MAS can be used to select the elite animals at a young age based on molecular markers associated with particular production traits like milk, fat, and SNF yield. Le provost *et al.* (1994) ^[8] reported that prolactin hormone imperative role in initiation and maintenance of lactation at the level of mammary alveoli, and help to promote the synthesis and secretion the components like protein, lactose, and lipids. Barendse *et al.* (1997) ^[2] stated that prolactin gene has many functions mainly related to milk production and milk composition traits. Therefore, the Prolactin gene could be considered as an excellent candidate gene for milk production and milk composition traits in Sahiwal cattle.

However, scanty literatures are available concerning the quantification of SNP effect of prolactin gene on milk production and its composition traits in Sahiwal cattle. This is required to infer the contribution of individual SNPs in the total trait breeding value which will help in Marker Assisted Selection of animals. So, this study was taken up to estimate the effect of SNPs found in the exon 3 region of prolactin gene in Sahiwal cattle.

Materials and Methods

Sample Collection

A total number of 98 pedigrees, Sahiwal population (49 dams, 7 sires, 27 male calves and 22 female calves) were utilized for the used. The blood samples were collected from animals maintained at the Livestock Research Centre (LRC) of ICAR-NDRI, Karnal. Blood samples were collected from the animals and DNA was isolated by Phenol-chloroform method (Sambrook and Russel, 2001) [9]. The quality and concentration of DNA was checked using Agarose Gel and Nanodrop Spectrophotometer, respectively.

PCR Amplification and RFLP technique

PCR amplification was carried out for exon 3 region of prolactin gene using primers designed by Primer 3 software. The primers for exon 3 region of prolactin gene were: F- CGA GTC CTT ATG AGC TTG ATT CTT (23) and R - GCC TTC CAG AAG TCG TTT GTT TTC (23). The amplified PCR product was checked on 1.7% agarose gel to verify the amplification of the targeted region. Restriction endonucleases designed using NEB cutter and cleaver were used to cleave the targeted regions in PCR-RFLP.

Statistical Analysis

The gene and genotype frequency were estimated by Gene Counting method as suggested by Falconer and Mackay (1996) [5] as: Genotype frequency = Total number of Sahiwal cattle of a particular genotype / Total number of Sahiwal cattle of all genotypes. Gene frequency = (2D + H) / 2N, where, D = Number of homozygotes, H = Number of heterozygote and N = Total number of Sahiwal cattle. The phenotypic data of Sahiwal animals' viz. first and second lactation 305-day milk yield (kg), ten monthly test day milk yield (TDMY, in kg), test day fat percentage and SNF percentage of first and second lactation were collected. The data was normalised (Snedecor and Cochran, 1994) [10] and the effect of significant non-genetic factors were adjusted using least-squares analysis (Harvey, 1990) [6], using the following model: $Y_{ijk} = \mu + S_i + P_j + e_{ijk}$, where Y_{ijk} = observation of k^{th} cattle under i^{th} season of calving and j^{th} parity, μ = overall mean, S_i = fixed effect of i^{th} season of calving, P_j = fixed effect of j^{th} parity and e_{ijk} = random error - NID (0, σ_e^2). The effect of SNP in the targeted region was estimated using the following multiple regression model: $Y_{ij} = a + b \text{ SNP} + e_{ij}$, where Y_{ij} = test day milk yield or milk composition traits, a = intercept, b = partial regression coefficient for SNP in exon 3 region, SNP = effect of SNP exon 3 region, and e_{ij} = random residual, NID (0, σ_e^2) (Wang *et al.*, 2011) [12].

Result and Discussion

PCR amplification of exon 3 region of prolactin gene revealed a 156 bp PCR product at an annealing temperature of 57.4 °C for 30 sec. After amplification, Restriction fragment length polymorphism was performed. The digestion of 156bp

fragment of prolactin gene (exon3) with RsaI restriction enzyme, 3 genotypes – AA, GG, and GA were observed. The genotype GA having 156bp, 84bp and 72bp fragments whereas the genotype AA had 156bp fragment (Fig.1). The frequencies of A and G allele were 0.425 and 0.575, and the frequencies of AA, GG, and GA genotypes were estimated as 0.30, 0.45, and 0.25, which has presented in Table 1, respectively.

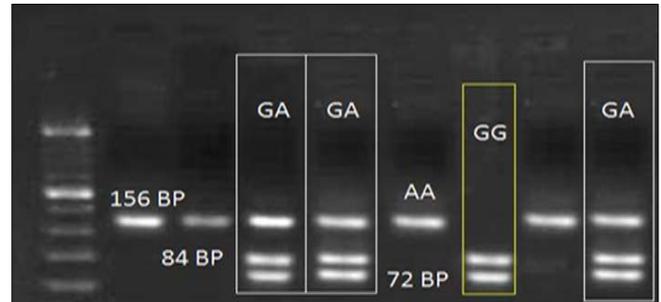


Fig 1: Prolactin (exon3) - 156bp, 84bp and 72bp PCR- RFLP product

Table 1: Estimation of Gene and genotype frequencies of exon 3 region of prolactin gene

SNP	Genotypes	Frequency	Allele	Frequency
G55A	GG	0.45	G	0.575
	GA	0.25	A	0.425
	AA	0.30		

Estimation of average different test day milk, fat, SNF yield in Sahiwal Cattle

The average test day milk yield was estimated as 7.23 ± 0.39 kg with coefficient of variation 34.54%. The highest test day milk yield was observed on test day 4 which was about 9.68 ± 0.39 kg with coefficient of variation 27.36%. The lowest test day milk yield was observed as 4.89 ± 0.37 kg with coefficient of variation 27.36%. The average milk yield in different test days of combined lactation of 1st and 2nd has been depicted in Table 2. Age at first calving (AFC) was found significantly affected Test Day 4. Season of calving had significant effect on Test Day 1 and Test Day 9, respectively. The average test day fat yield was estimated as 0.36 ± 0.02 kg with coefficient of variation 46.24%. The highest test day fat yield was revealed (0.43 ± 0.02 kg) on fourth test day with coefficient of variation 37.13%. The lowest test day fat yield was reported (0.21 ± 0.02 kg) on 9th test day with coefficient of variation 41.45 percent. The average fat yield in different test days of combined lactation of 1st and 2nd has been presented in Table 3. The average test day SNF yield was estimated 0.64 ± 0.03 Kg with coefficient of variation 32.21%. The highest test day SNF yield was estimated (0.80 ± 0.03 Kg) on fourth test day with coefficient of variation 31.91%. The lowest test day SNF yield was observed on tenth test day (0.27 ± 0.02 kg) with coefficient of variation 38.32 percent. The average SNF yield in different test days of combined lactation of 1st and 2nd has been depicted in Table 4, respectively. The effect of SNP in exon 3 region of prolactin gene was analysed for test day milk yield, test day fat yield and test day SNF yield and partial regression coefficients were estimated for the different test days were depicted in Table 5. The SNP in exon 3 region of prolactin gene was contributed an increased test day milk yield by 321.5g, test day fat yield by 13.9g and test day SNF yield increased by 19.4g, respectively. The highest correlation was observed in between Test Day 7 milk yield ($r=0.8277$)

and the lowest association was observed in between Test Day 1 milk yield and combined first and second lactation ($r = 0.3216$) in Sahiwal cattle. So, this SNP could be incorporated

as potential markers for selection of young animals at an early age for milk production and its composition traits.

Table 2: The mean milk yield in different test days of combined lactation of 1st and 2nd lactations in Sahiwal cattle

Test day	Number of animals	Mean \pm Std error (kg)	Coefficient of Variation (%)
Test day 1	45	6.05 \pm 0.29	32.65
Test day 2	44	8.27 \pm 0.47	38.11
Test day 3	45	9.32 \pm 0.42	30.22
Test day 4	45	9.68 \pm 0.39	27.36
Test day 5	45	9.35 \pm 0.41	29.52
Test day 6	43	8.40 \pm 0.38	30.24
Test day 7	38	7.70 \pm 0.40	32.25
Test day 8	36	6.90 \pm 0.44	38.97
Test day 9	35	5.79 \pm 0.33	34.28
Test day 10	31	4.89 \pm 0.37	42.92
Average	49	7.23 \pm 0.39	34.54

Table 3: The mean milk yield in different test days of combined lactation of 1st and 2nd lactations in Sahiwal cattle

Test day	Number of animals	Mean \pm Std error (kg)	Coefficient of Variation (%)
Test day 1	46	0.33 \pm 0.02	57.72
Test day 2	44	0.40 \pm 0.03	52.67
Test day 3	45	0.42 \pm 0.02	38.54
Test day 4	44	0.43 \pm 0.02	37.13
Test day 5	44	0.42 \pm 0.02	38.46
Test day 6	44	0.36 \pm 0.02	43.53
Test day 7	40	0.31 \pm 0.01	39.01
Test day 8	39	0.26 \pm 0.02	48.28
Test day 9	36	0.21 \pm 0.01	41.45
Test day 10	33	0.30 \pm 0.02	48.24
Average	39	0.36 \pm 0.02	46.24

Table 4: The mean SNF yield in different test days of combined lactation of 1st and 2nd in Sahiwal cattle

Test day	Number of animals	Mean \pm Std error (kg)	Coefficient of Variation (%)
Test day 1	46	0.63 \pm 0.04	52.93
Test day 2	44	0.71 \pm 0.04	43.28
Test day 3	44	0.78 \pm 0.04	36.73
Test day 4	44	0.80 \pm 0.03	31.91
Test day 5	45	0.79 \pm 0.03	31.97
Test day 6	44	0.68 \pm 0.03	37.16
Test day 7	40	0.60 \pm 0.03	36.10
Test day 8	40	0.48 \pm 0.03	50.60
Test day 9	36	0.38 \pm 0.02	39.93
Test day 10	33	0.27 \pm 0.02	38.32
Average	49	0.64 \pm 0.03	32.21

Table 5: Effect of SNP in exon 3 (b) region of prolactin gene on Test Day milk yield, Test Day fat yield and Test Day SNF yield

Test day	Test day milk yield (b)	Test day fat yield (b)	Test day SNF yield (b)
Test day 1	0.2574	0.0474	0.0247
Test day 2	1.1472	0.0296	0.0454
Test day 3	0.5187	0.0275	0.0335
Test day 4	-0.0142	0.0245	0.0885
Test day 5	0.6917	-0.0162	0.0624
Test day 6	-0.0892	-0.0403	-0.1122
Test day 7	-0.2981	-0.0335	-0.0416
Test day 8	0.4812	0.0014	0.0310
Test day 9	-0.0976	-0.0190	-0.0219
Test day 10	-0.7609	0.0630	-0.0148
Average	0.3215	0.0139	0.0194

Conclusion

The effect of SNP in exon 3 region of prolactin gene was

contributed an increased test day milk yield by 321.5g, test day fat yield by 13.9g and test day SNF yield increased by 19.4g, respectively. So, these SNP could be considered as potential markers for these traits and could be incorporated in any future Marker Assisted Selection strategy for selection of young Sahiwal animals.

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