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Genetic Polymorphism in Prolactin Gene and its Effect on Test Day Milk Production Traits in Sahiwal Cattle

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ABSTRACT

Background: The selection of genetically superior animals at an early stage of life, the molecular markers are used along with traditional selection. The study was carried out to identify the genetic polymorphism in the exon3 region of the Prolactin and enumerate its effect on milk production performance in Sahiwal cattle. Prolactin plays an imperative regulatory role in mammary gland development, milk emission and lactogenesis. Analysed the sequence of this gene to explore whether mutations in this sequence and it could be accountable for quantitative variations in milk production and its composition traits.

Methods: Total DNA was isolated from the blood samples of 98 pedigreed Sahiwal population. Using PCR-RFLP method and direct sequencing, noticed a single-nucleotide polymorphism in exon3 region of the Prolactin gene in 156 bp and also the effect of non-genetic factors on each trait was assessed by least-squares analysis for non-orthogonal data by a fixed model.

Result: PCR-RFLP was done with *RsaI* restriction endonuclease for the identification of different genotypes. The frequency of G and A alleles of the Prolactin gene was evaluated as 0.575 and 0.425, whereas the frequencies of GG, GA and AA genotypes for the Prolactin gene were 0.45, 0.25 and 0.30, respectively. SNP (G55A) conferred an increase in test-day milk yield around 321.5 g, in test day fat yield around 13.9 g and in test day SNF yield increase was 19.4 g, respectively. High correlation was perceived from test day (TD2) onwards between test day traits and lactation milk yield indicating that selection based on identified SNP in TD2 increased test day milk yield, fat yield and SNF yield by 1.1472 kg, 29.6 gm and 45.4 gm, respectively.

Key words: Genetic polymorphism, Production traits, Sahiwal cattle, Seasonal influence, Test day milk yield.

INTRODUCTION

Milk production is a multifaceted phenomenon regulation with diverse factors but among more conspicuous is genetic and non-genetic factors and there is always a coordinated between these factors. It is influenced by the interaction of a large number of genes of which, prolactin plays a pivotal role in the commencement of lactation in all animals. (Chrenek *et al.,* 1998; Dybus *et al.,* 2005) noted silent mutation in prolactin (exon3) in 156 bp and they also revealed this gene could be used as a popular genetic marker in dairy animals. Therefore, Prolactin (PRL) is a potential candidate gene for linkage analysis of Quantitative Trait Loci (QTL) and genetic marker for production traits in dairy cattle (Brym *et al.,* 2005). Many researchers (Alipanah *et al.,* 2008; Maksymiec *et al.,* 2008; Ghasemi *et al.,* 2009) reported that the PRL gene is highly polymorphic and had an association with milk production traits. Particularly, PRL-Rsa1 locus had a significant effect on milk production and fat percentage in dairy cattle (Chung *et al.,* 1996; Mitra *et al.,* 1995; Chrenek *et al*., 1998; Udina *et al.,* 2001; Dybus, 2001; Sacravarty *et al*., 2008; Wojdak *et al.,* 2008). The genetic evaluations incorporating genomic information of late have been adopted in many countries in dairy animals. A Genomic evaluation system has been implemented already in various developed countries for the selection of young elite bulls (Wiggan *et al.,* 2011).

Marker assisted selection (MAS) is a strategy to improve the accuracy of selection and genetic evaluation by combining the genetic information at markers with phenotypic information. The use of molecular markers will help to select genetically superior animals at an early age. This study aimed to point out the genetic polymorphism in (exon 3) prolactin gene and to assess the effect of SNP on milk production and composition traits so that the information could be useful for nascent the marker- assisted selection strategy in Sahiwal population.

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MATERIALS AND METHODS

Animal selection and sample collection

Under this study, the blood samples were collected from98 pedigree Sahiwal population comprising of 27 male calves, 22 female calves, 49 dams and 7 sires. The animals were

maintained at Livestock Research Centre, ICAR-NDRI and Karnal. Data on the production performance of Sahiwal animals' *viz.,* first and second lactation 305 days milk yield (kg), total milk yield (kg) and ten monthly test-day milk yield data of first and second lactation were collected from the Livestock Record Cell of Animal Genetics and Breeding Division and also milk compositions traits were obtained from Division of Livestock Production Management.

DNA isolation and quality checking

Isolation of DNA was done using a conventional method (Phenol-chloroform method) as described by Sambrook and Russel (2001) with negligible modifications. Quality of DNA was scrutinized by Agarose Gel Electrophoresis and concentration was appraised by Nanodrop Spectrophotometer. Samples showing sharp and intense bands and OD260/ OD280 ranging between 1.7- 1.9 were best quality and only those DNA samples were contemplated for this study. The concentration of DNA was estimated by the following formula:

$$\text{Concentration of DNA } (\mu g/ml) = \frac{(OD_{260} \times 50 \times \text{Dilution Factor})}{1000}$$

Primer designing

Targeted region of prolactin gene and primers; Both Forward (P1) and Reverse (P2) covering the complete targeted region are contrived by primer3 software. Primers designed were checked for specificity by BLAST program. Targeted region of Prolactin gene was amplified with a standard PCR program with genomic DNA and the corresponding set of forward and reverse primers with the help of thermal cycler. The master mixture for PCR comprised of 2.5 µl of 10× buffer, 0.5 µl each of dNTPs, forward and reverse primer, 0.25 µl of Taq polymerase, 18.75 µl of distilled water and 2 µl of template DNA. The final volume was made 25 µl. The size of the amplified product was 156 bp after PCR amplification and it was checked on 1.5% agarose gel to verify the amplification of the target region. The amplified PCR product was subjected to restriction fragment length polymorphism with a selected restriction endonuclease enzyme to generate a unique restriction polymorphic profile. To search the restriction enzyme site for typing SNPs and develop a PCR-RFLP test, two bioinformatics software was utilized *viz.* NEB cutter and cleaver. The restricted PCR products were checked by 2% agarose gel. The agarose gel documentation system under the UV light was then used to score for the respective genotypes.

Estimation gene and genotype frequency

The frequency of gene and genotype was estimated using gene counting method as suggested by (Falconer Mackay, 1996).

Estimating the effect of non-genetic factors

The mean, standard deviation, standard error and coefficients of variation (CV) of first and lactation milk yield and milk composition traits were evaluated using standard statistical procedures elucidated by (Snedecor and Cochran,1994). The effect of non- genetic factors on each trait was assessed by least-squares analysis for non-orthogonal data using a fixed model as narrated by Harvey (1990). The model used to study the effect of non-genetic factors on production traits was:

$$Y_{ijk} = \mu + S_i + P_j + e_{ijk}$$

Where,

Y_{ijk}= Observation of kth population under i^{th} season of calving and j^{th} parity.

µ= Overall mean.

S_i= i^{th} season of calving (fixed effect).

P_j= j^{th} parity (fixed effect).

e_{ijk}= Random error -NID (0, σ^2_e).

The effect of SNPs on each trait was judged by the partial regression coefficient (b) and the coefficient of Determination (R^2).

Effect of SNPs on the test day traits

Effect of SNP on prolactin gene (exon 3) on test day milk yield and composition traits was assessed with the help of model:

$$Y_{ij} = a + b_1 SNP_1 + e_{ij}$$

Where;

Y_{ij}= Test day milk yield and composition traits.

a= Intercept.

b_1= Regression coefficients.

SNP_1= Effect of SNP_1.

e_{ij}= Random residual, NID (0, σ^2_e) as proposed by (Wang *et al*., 2011).

RESULTS AND DISCUSSION

The location of the prolactin gene on chromosome number 23 (Barendse *et al.,* 1997) of *Bos indicus* (Gene ID 280901,

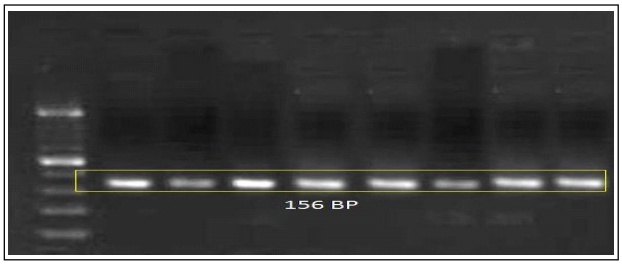

Fig 1: Prolactin (exon3): 156 bp PCR product.

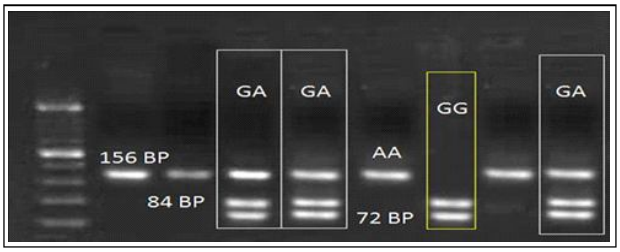

Fig 2: Prolactin (exon 3)- 156 bp, 84 bp and 72 bp PCR-RFLP product.

AC_00176.1) comprises 5 exons split by introns (Camper *et al.,* 1984; Dybus *et al.*, 2005) with a total length of 893bp. The targeted region (exon 3) of the prolactin gene was amplified by standard protocol (Fig 1). After amplification, Restriction fragment length polymorphism was performed using theRsa1 restriction enzyme. Result exhibited three bands at different base pairs 156 bp, 84 bp and 72 bp (Fig 2). Based on the band patterns, three genotypes *viz.,* AA (156 bp), GA (156 bp, 84 bp, 72 bp) and GG (84 bp, 72 bp) were classified. Genotype GA having 156 bp, 84 bp and 72 bp fragments, genotype GG with 84 bp and 72 bp fragments whereas the genotype AA had 156 bp fragments. The gene and genotype frequencies were estimated by gene counting method. The frequencies of A and G alleles were 0.425 and 0.575 and their genotypic frequencies of AA, GG and GA were estimated as 0.30, 0.45 and 0.25, respectively. A similar study was proposed by (Das *et al.,* 2012) the frequencies of A and B alleles 0.388 and 0.611 in Deoni cows.

Means and standard errors of test day milk yield and milk composition traits

The mean test-day milk, fat and SNF (Solid Not Fat) yield along with standard errors were presented in Table 1. The mean test day milk yield was estimated at 7.23±0.39 kg with a coefficient of variation (CV) 34.5 per cent. The mean test day fat yield was noticed 0.36±0.02 kg with CV 34.5 per cent. The mean test day SNF yield was estimated as 0.64±0.03 kg.

Least squares means of test day milk yield and milk composition traits

Least squares mean (LSM) test day milk yield were estimated in between 5.74±0.33 kg in test day 9 to 9.79±0.50 kg in test day 5. The different test day milk yields of LSM have revealed in Table 4 (a) and 4 (b). LSM of test day fat yield was found to be in between 0.18±0.031 kg in test day 10 to 0.45±0.028 kg in test day 4. The different test

Table 1: Means and standard errors of test day milk yield and milk composition traits in Sahiwal cattle.

Test day	Number of observations (N)	TDMY Mean±S.E (Kg)	TDFY Mean±S.E (Kg)	TDSNFY Mean±S.E (Kg)
T.Day1	45	6.05±0.29	0.33±0.02	0.63±0.04
T.Day2	44	8.27±0.47	0.40±0.03	0.71±0.04
T.Day3	45	9.32±0.42	0.42±0.02	0.78±0.04
T.Day4	45	9.68±0.39	0.43±0.02	0.80 ±0.03
T.Day5	45	9.35±0.41	0.42±0.02	0.79±0.03
T.Day6	43	8.40±0.38	0.36±0.02	0.68 ±0.03
T.Day7	38	7.70±0.40	0.31±0.01	0.60±0.03
T.Day8	36	6.90±0.44	0.26±0.02	0.48±0.03
T.Day9	35	5.79±0.33	0.21±0.01	0.38±0.02
T.Day10	31	4.89±0.37	0.30±0.02	0.27±0.02
Overall	49	7.23±0.39	0.36±0.02	0.64±0.04

TDMY= Test day milk yield, TDFY= Test day fat yield, TDSNFY= Test day solid-not-fat yield.

Table 2: Regression coefficients (b) of SNP of prolactin gene (exon 3) on test day milk yield and milk composition traits in Sahiwal cattle.

Test day	Number of observations (N)	TDMY Regression coefficients (b)	TDFY Regression coefficients (b)	TDSNFY Regression coefficients (b)
T.Day1	45	0.2574	0.0474	0.0247
T.Day2	40	1.1472	0.0296	0.0454
T.Day3	45	0.5187	0.0275	0.0335
T.Day4	44	-0.0142	0.0245	0.0885
T.Day5	45	0.6917	-0.0162	0.0624
T.Day6	44	-0.0892	-0.0403	-0.1122
T.Day7	40	-0.2981	-0.0335	-0.0416
T.Day8	39	0.4812	0.0014	0.0310
T.Day9	36	-0.0976	-0.0190	-0.0219
T.Day10	32	-0.7609	0.0630	-0.0148
Overall	49	0.3215	0.0139	0.0194

TDMY= Test day milk yield, TDFY = Test day fat yield, TDSNFY= Test day solid-not-fat yield.

been day fat yields of LSM have been shown in Table 5 (a) and 5 (b). Least squares mean of SNF yield were found to be in between 0.27±0.025 kg in test day10 to 0.56±0.4 kg in test day 7. The different test day SNF yields of LSM have presented in Table 6 (a) and 6 (b).

Effect of season of calving and parity on test day milk yield and milk composition traits

In case of test day milk yield, season of calving had a significant effect on test day 1 and test day 9 and also, observed age at first calving (AFC) was significantly affecting test day 4. Among all the test days, the maximum test day milk yield was noticed in test day 5 in winter season whereas the lowest milk yield was observed in test day 9 in winter season. In test day fat yield, the highest fat yield was found in test day 4 whereas the lowest test day fat yield was found in test day10 in summer season. In case of test day SNF yield, highest SNF yield was estimated in test day 5 in rainy season while the lowest SNF yield was revealed in test day10 in winter season. Age at first calving was found significantly affecting sixth test day milk yield and was subsequently, adjusted.

Effect of SNP of prolactin gene on test day milk and milk composition traits

The effect of SNP of prolactin (exon3) on milk production and composition traits was evaluated by regression analysis for different test days and their role in the form of increase or decrease in these traits was represented in Table 2. It was established that SNP of the prolactin gene increased overall test day milk yield by 321.5 gm, test day fat yield by 13.9 gm and test day SNF yield by 19.4 gm, respectively.

The maximum effect of SNP was perceived in second test day milk yield (b= 1.1472) which stipulated an increase in milk yield by 1.1472 kg in test day2 considering 13.8 per cent of the average second test day milk yield. The minimum effect of SNP was detected in tenth test day milk yield (b= -0.7609) which indicated that the decrease in milk yield was about 0.7609 kg in test day 10. The maximum effect of SNP was estimated in tenth test day fat yield (b= 0.0630) which designated an increase of 63.0 gm in test day10 fat yield which was about 21.00 per cent of the average tenth test day fat yield. The minimum effect of SNP was observed in sixth test day fat yield (b= -0.0403) which specifies that the decrease in fat yield was about 40.3 gm in test day 6.

Table 3: Correlation coefficients (r) of test day milk yield and milk composition traits with average lactation milk yield in Sahiwal cattle.

Test day	Number of observations (N)	TDMY and ALMY (r)	TDFY and ALMY (r)	TDSNFY and ALMY (r)
T.Day1	46	0.3216	0.2352	0.3742
T.Day2	44	0.4634	0.4741	0.4847
T.Day3	44	0.4273	0.4437	0.6329
T.Day4	44	0.7512	0.6213	0.7323
T.Day5	45	0.7364	0.5746	0.6146
T.Day6	44	0.6243	0.7347	0.7923
T.Day7	40	0.8277	0.6169	0.6537
T.Day8	40	0.6314	0.7120	0.8442
T.Day9	36	0.7325	-0.2492	0.6340
T.Day10	33	0.7617	0.7326	0.6326
Average	49	0.6012	0.6263	0.6746

TDMY= Test day milk yield, TDFY= Test day fat yield, TDSNFY= Test day solid-not-fat yield.
ALMY= Average lactation milk yield of pooled first and second lactation.

Table 4(a): Least squares means of test day milk yield in Sahiwal cattle.

Effect	Test day milk yield				
	TD1	TD2	TD3	TD4	TD5
Overall	6.21±0.35 (45)	8.69±0.57 (44)	9.60±0.53 (45)	9.71±0.46 (45)	9.79±0.50 (45)
			Season of calving		
Winter	5.31±0.72	0.08±1.30	9.15±1.10	9.72±0.46	10.68±1.17
Summer	5.40±0.46	6.79±0.77	8.40±0.68	9.83±0.66	8.68±0.68
Rainy	7.27±0.52	8.96±0.85	10.00±0.81	9.65±0.70	9.34±0.77
Autumn	6.88±0.93	10.94±1.42	10.84±1.42	9.88±1.12	10.45±1.22
			Parity		
First lactation	6.27±0.50	8.50±0.84	9.71±0.75	9.8±0.68	9.57±0.73
Second lactation	6.16±0.46	8.89±0.74	9.49±0.69	9.56±0.62	10.4±0.66

Table 4(b): Least squares means of test day milk yield in Sahiwal cattle.

Effect	Test day milk yield				
	TD6	TD7	TD8	TD9	TD10
Overall	8.65±0.46	7.45±0.48	6.94±0.45	5.74±0.33	8.69±0.57
	(44)	(40)	(39)	(36)	(44)
			Season of calving		
Winter	9.04±0.98	7.10±1.03	6.18±0.96	4.51±0.67	8.08±1.30
Summer	8.11±0.65	8.60±0.61	6.86±0.64	6.65±0.53	6.79±0.77
Rainy	8.12±0.73	6.42±0.73	5.84±0.79	4.94±0.56	8.96±0.85

Table 5(a): Least squares means of test day fat yield of Sahiwal cattle.

Effect	Test day fat yield				
	TD1	TD2	TD3	TD4	TD5
Overall	0.36±0.03	0.39±0.03	0.43±0.03	0.45±0.02	0.43±0.02
	(46)	(44)	(45)	(44)	(44)
			Season of calving		
Winter	0.31±0.07	0.28±0.09	0.42±0.06	0.42±0.06	0.44±0.06
Summer	0.28±0.04	0.34±0.05	0.39±0.04	0.41±0.03	0.43±0.03
Rainy	0.37±0.05	0.52±0.05	0.45±0.05	0.40±0.04	0.46±0.04
Autumn	0.47±0.09	0.41±0.01	0.46±0.09	0.55±0.06	0.38±0.06
			Parity		
First lactation	0.38±0.05	0.42±0.05	0.44±0.04	0.45±0.04	0.45±0.04
Second lactation	0.33±0.02	0.35±0.05	0.42±0.02	0.45±0.01	0.41±0.05

Table 5(b): Least squares means of test day fat yield of Sahiwal cattle.

Effect	Test day fat yield				
	TD6	TD7	TD8	TD9	TD10
Overall	0.45±0.02	0.37±0.02	0.28±0.02	0.20±0.01	0.18±0.03
	(44)	(44)	(39)	(36)	(33)
			Season of calving		
Winter	0.42±0.06	0.35±0.06	0.26±0.05	0.16±0.04	0.17±0.07
Summer	0.41±0.03	0.37±0.04	0.27±0.03	0.20±0.02	0.16±0.04
Rainy	0.40±0.04	0.35±0.04	0.26±0.04	0.18±0.02	0.21±0.04
Autumn	0.55±0.06	0.41±0.07	0.32±0.06	0.26±0.04	0.19±0.07
			Parity		
First lactation	0.45±0.04	0.36±0.04	0.28±0.03	0.17±0.02	0.16±0.04
Second lactation	0.45±0.03	0.38±0.03	0.27±0.07	0.23±0.01	0.20±0.06

Table 6(a): Least squares means of test day SNF yield of Sahiwal cattle.

Effect	Test day SNF yield				
	TD1	TD2	TD3	TD4	TD5
Overall	0.47±0.04	0.43±0.04	0.41±0.05	0.44±0.07	0.47±0.03
	(46)	(44)	(44)	(44)	(45)
			Season of calving		
Winter	0.43±0.09	0.35±0.09	0.19±0.11	0.35±0.13	0.42±0.13
Summer	0.50±0.05	0.57±0.05	0.52±0.06	0.57±0.07	0.59±0.07
Rainy	0.46±0.06	0.51±0.05	0.50±0.08	0.50±0.09	0.45±0.09
Autumn	0.48±0.12	0.30±0.11	0.42±0.14	0.35 ±0.13	0.44±0.14
			Parity		
First Lactation	0.42±0.06	0.40±0.06	0.43±0.07	0.44±0.08	0.49±0.84
Second Lactation	0.51±0.05	0.47±0.05	0.38±0.06	0.44±0.07	0.46±0.07

Table 6(b): Least Squares Means of Test day SNF yields of Sahiwal cattle.

Effect	Test day SNF yield				
	TD6	TD7	TD8	TD9	TD10
Overall	0.53±0.04 (44)	0.56±0.04 (40)	0.47±0.04 (40)	0.39±0.03 (36)	0.27±0.02 (32)
			Season of calving		
Winter	0.59±0.10	0.53±0.08	0.43±0.08	0.33±0.01	0.22±0.05
Summer	0.46±0.06	0.51±0.05	0.47±0.05	0.38±0.04	0.32±0.03
Rainy	0.68±0.07	0.56±0.07	0.45±0.07	0.38±0.05	0.25±0.04
Autumn	0.38±0.11	0.65±0.12	0.53±0.10	0.46±0.07	0.29±0.06
			Parity		
First lactation	0.56±0.07	0.52±0.06	0.42±0.05	0.36±0.04	0.27±0.03
Second lactation	0.50±0.09	0.61±0.05	0.51±0.05	0.41±0.03	0.32±0.04

The highest effect of SNP was found in fourth test day SNF yield (b=0.0885) which indicated an increase of 88.5 gm in test day 4 which was about 11.0 per cent of the fourth test day SNF yield. The minimum effect of SNP was revealed in tenth test day SNF yield (b= -0.0148) which specifies that the decrease in SNF yield was about 14.8 gm in test day 10.

Correlation between test day milk and milk composition traits and average lactation milk yield

The correlation analysis was used to find the early test days for the selection of animals between test day traits and average lactation milk yield of pooled first and second lactations in Sahiwal cattle. Correlation between average test day milks yield and average lactation milk yield of pooled first and second lactations was found to be very high (0.6012) and the maximum correlation was estimated between test day 7 milk yield (r=0.8277) and average lactation milk yield. Correlation between average test day fat yield and average lactation milk yield of pooled first and second lactations was found to be very high (0.6263) and the maximum correlation was found between test day 6 fat yield (r= 0.8277) and combined first and second lactation milk yield. Correlation between average test day SNF yield and average lactation milk yield of first and second lactations was also revealed very high (r = 0.6746). The maximum correlation was established between T. Day 8 (r= 0.8442) and average lactation milk yield. It was observed that high correlations were obtained from T. Day 2 onwards between test day traits and lactation milk yield indicating that selection based on identified SNP in T. Day 2 increased test day milk yield, fat yield and SNF yield by 1.1472 kg, 29.6 gm and 45.4 gm, respectively.

The study identified the genetic marker of prolactin in relation to monthly test day production performance in pedigreed Sahiwal cattle. PCR-RFLP method was performed with *Rsal* restriction endonuclease for the identification of genotypes. The frequency of G and A allele of prolactin was revealed as 0.575 and 0.425, whereas the frequencies of AA, GG and GA genotypes for prolactin gene were estimated as 0.30, 0.45 and 0.25, respectively. The SNP (G55A) contributed an increase in test day milk yield around 321.5 g, test day fat yield was around 13.9 g and test day SNF yield was increased by 19.4 g. High correlations were obtained from T. Day 2 onwards between test day traits and lactation milk yield indicating that selection based on identified SNP in T. Day 2 increased test day milk yield, fat yield and SNF yield by 1.1472 kg, 29.6 gm and 45.4 gm, respectively.

CONCLUSION

Sequencing analysis of Sahiwal animals for milk yield, fat yield and SNF yield, revealed that in SNP G55A was associated with test day milk production traits. Also, SNP (G55A) could be considered as a potential genetic marker for the selection of young Sahiwal animals. The SNP (G55A) contributed an increase in TMYD around 321.5 g, TFYD was around 13.9 g and TSNFYD was increased by 19.4 g, respectively. Marker-Assisted Selection strategy was developed for the selection of young Sahiwal animals (male and female) using the identified genetic marker of prolactin gene for increasing milk, fat yield and SNF yield.

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