B-4705
[1-6]

RESEARCH ARTICLE Indian Journal of Animal Research

Genetic Profiling of *PHKA2* Gene and its Association with Udder Type Traits in Indian Dairy Cattle

Oshin Togla, S.M. Deb, Sagar Kadyan, P.B. Nandhini, Sushil Kumar, Linda George 10.18805/IJAR.B-4705

ABSTRACT

Background: The experiment aimed to investigate polymorphisms in exon 2 of the *PHKA2* gene and investigate the relationship between identified single nucleotide polymorphism and udder type traits in Sahiwal cows. Udder morphometry is being used as a forecaster of production performance in cows since older times. Finding the variants associated with these traits in largely variable region of *PHKA2 gene* can prove to be highly beneficial.

Methods: DNA isolated from Sahiwal cows was analysed by DNA sequencing. Nine udder type and five teat types were measured for each animal according to procedure followed by International committee of animal recording (2012).

Result: Three SNPs *g.124497381C>T, X:124497248 G>A* and *X:124497189 C>T* were identified. Recessive homozygotes were negligible as mutant allele exhibited very low ranging frequency (from 0.02 to 0.08) for the targeted loci. Identified point mutation *g.124497381C>T* was found to be significantly ($p<0.05$) associated with distance between teats and central ligament, SNP *X: 124497248G>A* with rear udder width, fore udder attachment, udder depth, udder length and teat length ($p<0.05$) and SNP *X: 124497189 C>T* with udder length and rear udder height ($p<0.05$). Interaction of identified SNPs and udder traits highlighted the gene's potential as a candidate gene for selecting for conformation traits in Indian Sahiwal cattle.

Key words: Association, Exon:II, PHKA2 gene, Polymorphism, Sahiwal cattle, Udder type traits.

INTRODUCTION

PHK, the prototype of protein kinases, acts as in charge in cascade of enzymatic reactions regulating glycogen breakdown (Hendrickx *et al.*, 1999). Cytogenetic and molecular Location of *PHKA2* gene is Xp22.13; from base pairs 18,892,298 to 18,984,362 (*Homo sapiens* Annotation Release 109, GRCh38.p12) (NCBI). Mutations in gene cause X-linked recessive liver-specific *PHK* deficiency leading to glycogen accumulation in cell resulting in hepatomegaly and cirrhosis (Choi, 2016). If liver is overwhelmed, it is unable to "clear" or metabolize fat coming in and becomes infiltrated with fat. There are chances of fatty liver condition turning into subclinical/clinical ketosis. Liver reaching this state is not able to perform and one defence mechanism is a reduction in milk production in an attempt to reduce the metabolic energy demand. Milk production is directly and indirectly related to the udder morphometry and health. Phosphorylase b kinase actuates glycogen phosphorylase b by transforming it to glycogen phosphorylase a. When active, this enzyme breaks down glycogen (Newgard *et al.*, 1989). Since the significance of gene is in regulation of glycogen, thus, gene expression is directly associated with milk production and it is justifiable to consider it as a good candidate gene for MAS.

Udder configuration is of prime importance when it comes to predict production performance of animal. Indeed, *PHKA2* gene polymorphisms have been significantly associated with udder attachment, stature, strength and body depth, rump width, fore udder attachment and udder height (Cole *et al.*, 2011). Udder traits are crucial while selecting animals, directly or indirectly influence milk production, culling and longevity decisions and thus, there are great benefits to considering most of the udder traits in the study. The information on genetic polymorphisms of *PHKA2* and their association with glycogen disorder has been reported in Homo sapiens (Tsilianidis *et al.*, 2013) but so far, no research has been carried out to check the genetic effect of *PHKA2* gene variants with udder configuration in Bos indicus.

Sahiwal (Bos indicus) breed of cattle is the dominant milch breed of Indian origin having native tract in North-Western region but a much broader breeding tract in the country (Chopra *et al.*, 2020). Of all the zebu milch breeds, it is the heaviest displaying well-developed udder and is popular for higher milk production, remarkable power of endurance for hot climate of subtropics, comparatively resistant to diseases and low maintenance cost (Ratwan *et al.*, 2019a). The study aims to identify the polymorphisms

Division of Animal Genetics and Breeding, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.

Corresponding Author: Oshin Togla, Division of Animal Genetics and Breeding, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India. Email: Toglaoshin2121@gmail.com

How to cite this article: Togla, O., Deb, S.M., Kadyan, S., Nandhini, P.B., Kumar, S., George, L. (2021). Genetic Profiling of *PHKA2* Gene and its Association with Udder Type Traits in Indian Dairy Cattle. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4705.

Submitted: 06-07-2021 **Accepted:** 02-09-2021 **Online:** 11-10-2021

in the *PHKA2* gene exon aII and flanking region and perform association analysis of identified SNPs with udder type traits in Indian Dairy cattle.

MATERIALS AND METHODS

Ethics statement

All the animal experiments had prior approval of Institutional Animal Ethics Committee (IAEC) of ICAR-NDRI and the experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experimentation in Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India.

Location and subject of study

Blood samples from random 100 lactating Sahiwal cows maintained at Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, India, were collected. Location of NDRI cattle farm is between 29° 420N latitude and 72° 020E longitude at an altitude of 250 m above the mean sea level (MSL). Subtropical climate prevails with range of temperature varying from 45°C to 2°C. Annual rainfall is 760 to 960 mm and relative humidity of the region ranges from 40 to 85%. It can be said that cows are exposed to a vivid range of climatic conditions typically subtropical in nature.

DNA isolation

Genomic DNA isolated from blood samples using a blood DNA Kit (Wizard Genomic DNA Purification Kit (Promega, cat no # 1620A, USA). Integrity of isolated DNA was checked by 0.8% agarose gel electrophoresis and purity of DNA was assessed by UV-vis spectrophotometer (Biophotometer Plus, Eppendorf) immediately after extraction. Samples having 1.7-2.0 ratio of absorbance at 260 and 280 nm were considered as pure DNA and were aliquoted to 50 ng/µL before PCR amplification.

Primer designing and SNP identification

According to the exon II of *PHKA2* gene bovine sequence (NCBI Ref Seq: NC_037357.1) and flanking regions of, the following PCR primer was designed: Forward: 5'-GTGGTAGCAGGGCAAGGATT -3' and Reverse: 5'-GCCTCCCCCAAATAGCCTAC -3', using Primer 3 software (Rozen and Skaletsky, 2000). PCR amplification was performed in a total volume of 25 µl with 100 ng DNA template, 1x PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTPs, 20 pmol of each primer and 1 unit of Taq DNA polymerase. Amplification process was executed in thermal cycler in following stages-initial denaturation at 92°C for 5 min., followed by 34 cycles of 94°C for 30 s, annealing at 54.6°C for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min. The PCR products were separated on 1.5% agarose gel including 0.5 µg/ml of ethidium bromide and documented under UV light. The amplified PCR products were sent to 1st base sequencing for purification and custom sequencing from both ends (5' and 3' ends). The sequences were analysed using Bioedit software for the confirmation of variants. The reference sequence (NC_037357.1 for *Bos taurus PHKA2* gene) was aligned with sequencing results for each animal and each target region using Clustal W multiple alignment programme.

Udder type traits

Udder type traits were measured for each animal, spanning over period from August, 2018 to July, 2019. The traits consist of nine udder type traits *viz.* Fore udder attachment (FUA), rear udder height (RUH), udder depth (UD), udder balance (UB), rear udder width (RUW), central ligament (CL), udder length (UL), udder width (UW), udder circumference (UC) and five teat type traits *viz.* Teat thickness (TT), teat length (TL), teat circumference (TC), average distance between teats (DBT), average shortest distance from teat ends to floor (DFF). Procedure of International committee of animal recording (2012) were followed for measurement and scoring of traits. All traits were measured on centimetre scale except FUA, measured in degrees.

Statistical analysis

Genotype frequency, gene frequency and Hardy-Weinberg equilibrium (HWE) were calculated through χ^2 test *via* POPGENE 1.32 software. General linear model (GLM) procedure of SAS (Statistical Analysis System 9.3, SAS Institute, Cary, NC) including genotype as fixed effect was used to find association among genotypes and traits under study. The model used was:

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

Where,

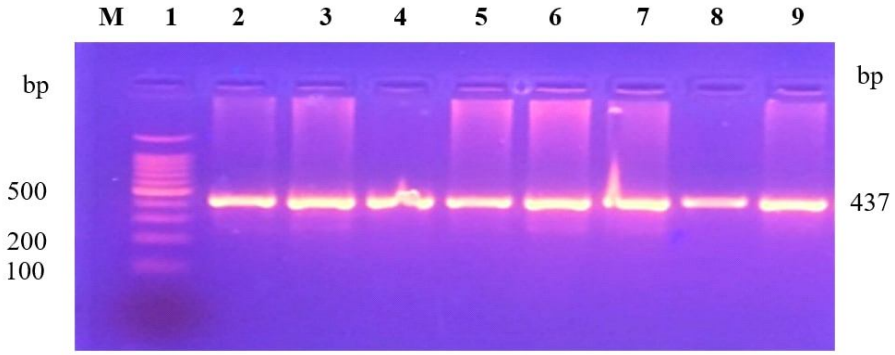
Y_{ij} is the observed value of targeted traits, µ is overall mean, G_i is the fixed effect of i^{th} genotype and e_{ijk} is the random residual error NID (0, σ^2_e). Data were presented as mean ± SE and significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

According to the presumption made for this study, an attempt was made to study the variants of exon II region of *PHKA2* gene present on X chromosome and to associate with udder traits in Bos Indicus for the first time. Primers for exon 2 and its flanking region of bovine *PHKA2* gene were used for amplification of genomic DNA of 100 Sahiwal cows. A 437 bp band was successfully amplified by PCR in all DNA samples providing a clear band with good specificity (Fig 1). This gene is present on bovine X chromosome. Various studies on X chromosome polymorphic regions associated with udder traits have been reported (Yan *et al.* (2020), Devani *et al.* (2020), Ogorevc *et al.* (2009). In Charolais cattle, *myostatin* gene (*GDF*8) was significantly associated with udder volume and teat size (Vallée *et al.*, 2016). According to Cole *et al.* (2011), the X chromosome was highly variable and the most significant X chromosome SNP effects were associated with udder attachment, rear teat placement, specifically in context of udder traits.

DNA sequence analysis revealed three-point mutations *i.e* g.124497381C>T in exon 2, X:124497248G>A and X: 124497189 C>T in our targeted population. Chromatogram changes in *PHKA2* gene in targeted regions are shown in Fig 2-4. Genetic architecture of targeted loci in resource population exhibited very low ranging frequency of mutant allele (from 0.02 to 0.08). Detailed genotypic and allelic frequencies are presented in Table 1. Chi-square test showed that SNP X: 124497248G>A; X: 124497189 C>T were not in Hardy-Weinberg equilibrium ($p<0.05$) due to the kind of genetic architecture. The recessive homozygotes were negligible and therefore the variant could be seen in the heterozygote genotypes only. This specific kind of selection for dominant homozygotes in the present population against recessive gene might be an example of directional selection. The unfavourable form (recessive allele) might probably have been present in the previous generations, which now is in process of removal by selection. Also, selection against recessive alleles must have been very efficient initially, which generally becomes slower as larger proportion of the latter is protected in heterozygotes. Also, these variants in non-coding region might have role in the gene activity, protein assembly *etc*. Noncoding DNA has wide implications for a range of phenotypic traits such as cell size, growth rate, metabolism and life history and is an important yet often neglected part of the genotype-phenotype link (Hessen, 2017).

However, an association analysis was performed and least square means of udder traits grouped based on genotype at different loci were analysed (Table 2). It was observed that SNP g.124497381C>T was significantly ($p<0.05$) associated with DBT and CL. It is inferred that selection of heterozygote genotype will help to select the animal having more distance between teats and shallower CL than homozygote animals. The homozygote animals were having closely placed teats and deeper udder cleft which is a very desirable udder conformation. Widely placed teats are undesirable as is evident by various reports. Türkyılmaz *et al.* (2018) found a negative relationship between distance between udder teat places and milk



PCR amplification of 2nd exonic region of PHKA2 gene
Lane 1- 9 : PCR Products, 437bp
Lane M : 100 bp ladder

Fig 1: PCR amplified product of target region of *PHKA2* gene of Sahiwal cattle.

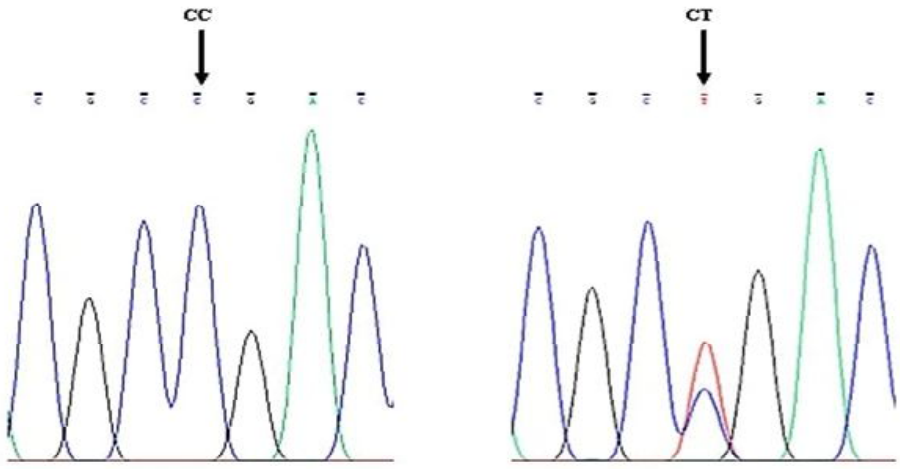


Fig 2: Sequence variants of PCR products, depicting SNP at g.124497381C>T in Sahiwal cattle.

components in sheep. Berry *et al.* (2004) stated that teats at either extremity of the scale in cows increases the likelihood of being culled while long CL stipulates a strong median suspensory ligament.

Additionally, SNP X: 124497248G>A was significantly ($p<0.05$) associated with RUW, FUA, UD, UL and TL. Homozygote genotype were having higher values for RUW, UL, FUA and TL than heterozygote animals except UD. Furthermore, SNP X: 124497189C>T was significantly ($p<0.05$) associated with UL and RUH.

Homozygote genotype were selected and favoured in the population. Higher the RUW, higher is the udder capacity. Medium range positive phenotypic correlation was reported between RUW and 305-day milk yield and between UL and Milk yield by Khan and Khan (2016). FUA indicates how strongly the fore udder is attached to the body wall *via* lateral

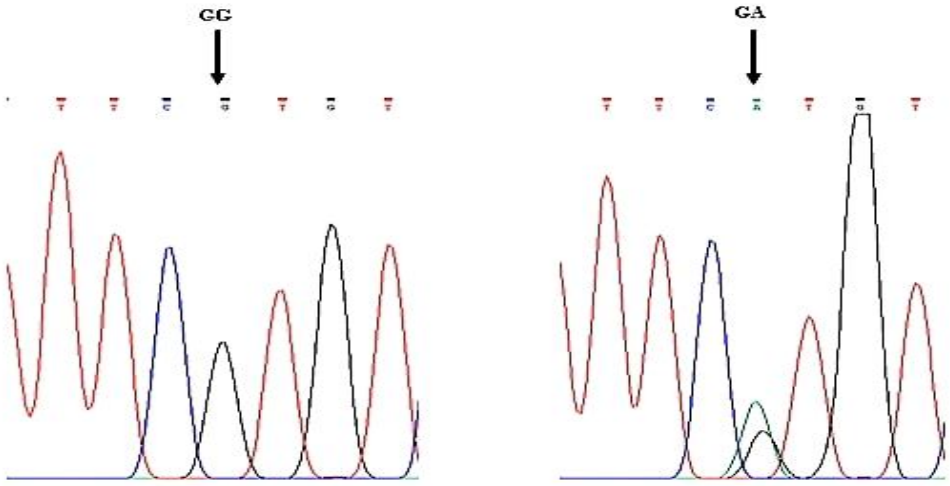


Fig 3: Sequence variants of PCR products, depicting SNP at X:124497248 in Sahiwal cattle.

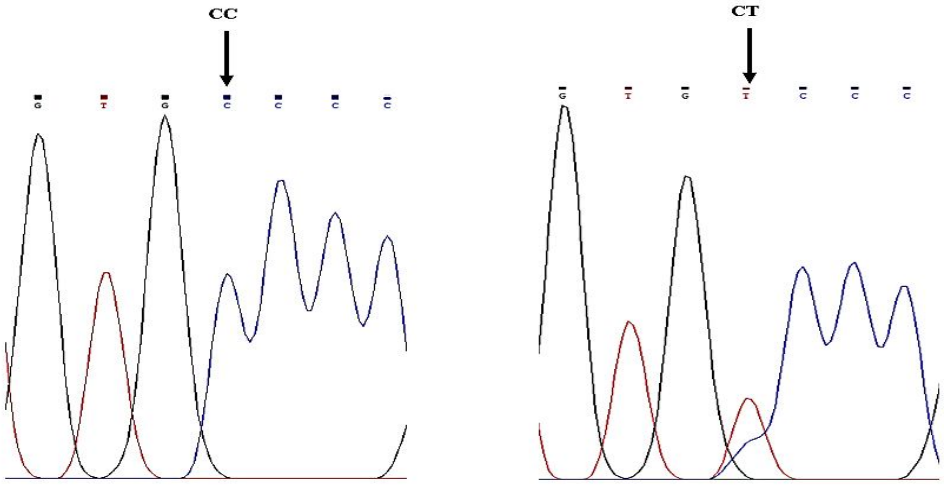


Fig 4: Sequence variants of PCR products, depicting SNP at X:124497189 in Sahiwal cattle.

Table 1: Gene and genotype frequency of the SNPs of exon II and flanking region of *PHKA2* gene in Sahiwal cattle.

Mutation	SNP Id	Gene region	Frequency					Chi-square	P-value
			Genotype			Allele			
C>T	X:124497381	Exon II	CC	CT	TT	C	T	0.718	0.39
			0.86	0.13	-	0.93	0.07		
G>A	X: 124497248	Intron II	GG	GA	AA	G	A	2.76	0.08
			0.90	0.09	-	0.94	0.05		
C>T	X: 124497189	Intron II	CC	CT	CC	C	T	18.49	0.0017*
			0.96	0.03	-	0.97	0.02		

(*$p<0.05$).

Table 2: Least square means of udder traits grouped based on genotype at different loci.

Trait	g.124497381 (C>T)			X: 124497248 (G>A)			X: 124497189 (C>T)		
	Genotype	Mean square± Std error	P value	Genotype	Mean square± Std error	P value	Genotype	Mean square ± Std error	P value
Rear udder height	CC	24.56±0.65	0.076	GG	23.67±0.85	0.826	CC	25.27±1.09	0.057*
	CT	23.99±0.78		GA	23.47±0.61		CT	21.78±0.99	
Rear udder width	CC	8.90±1.00	0.460	GG	9.00±0.53	0.026*	CC	7.15±1.37	0.348
	CT	6.28±0.86		GA	7.56±0.84		CT	7.89±1.01	
Udder width	CC	61.53±1.41	0.826	GG	67.43±1.22	0.332	CC	71.57±0.94	0.323
	CT	63.61±0.85		GA	58.78±2.98		CT	74.26±1.83	
Fore udder attachment	CC	118.96±2.91	0.681	GG	132.25±2.71	0.057*	CC	106.24±2.95	0.735
	CT	111.50±3.23		GA	119.22±2.92		CT	105.91±3.16	
Udder circumference	CC	119.57±1.70	0.936	GG	122.76±1.83	0.808	CC	128.07±2.35	0.684
	CT	128.27±2.35		GA	121.76±1.53		CT	119.57±1.70	
Udder balance	CC	-1.23±0.66	0.583	GG	-1.65±0.49	0.116	CC	-1.14±0.44	0.946
	CT	-1.98±0.40		GA	-1.37±0.45		CT	-1.35±0.48	
Central ligament	CC	5.62±0.16	0.025*	GG	5.58±0.19	0.655	CC	5.41±0.27	0.477
	CT	2.48±0.20		GA	5.60±0.18		CT	5.63±0.19	
Udder depth	CC	39.04±0.76	0.308	GG	37.05±0.83	0.057*	CC	38.89±0.88	0.334
	CT	38.22±0.90		GA	39.36±0.82		CT	37.65±1.22	
Udder length	CC	50.92±1.01	0.226	GG	50.80±0.85	0.050*	CC	51.94±1.00	0.038*
	CT	50.73±1.15		GA	44.73±1.01		CT	48.30±0.93	
DBT	CC	4.87±1.66	0.026*	GG	5.36±0.17	0.833	CC	7.18±0.01	0.958
	CT	6.29±1.89		GA	5.51±1.36		CT	5.59±1.04	
DFF	CC	45.20±0.58	0.142	GG	39.41±0.89	0.821	CC	42.60±1.57	0.496
	CT	41.17±1.36		GA	42.21±1.71		CT	41.29±1.76	
Teat circumference	CC	8.54±0.91	0.511	GG	7.33±1.08	0.579	CC	8.88±1.48	0.322
	CT	8.60±0.72		GA	7.80±1.31		CT	9.09±1.05	
Teat diameter	CC	2.34±0.56	0.120	GG	3.40±0.67	0.120	CC	3.84±0.65	0.125
	CT	3.27±0.89		GA	2.38±0.75		CT	4.23±0.99	
Teat length	CC	6.99±0.82	0.750	GG	5.87±0.82	0.049*	CC	5.54±0.32	0.742
	CT	7.18±1.21		GA	3.57±1.21		CT	6.00±1.11	

*p- value significant at p<0.05.

ligaments. The strong and tight fore udder attachment is the most desirable (Godara *et al.*, 2015). In context of TL, intermediate values of TL are preferred over smaller TL agreeing with the findings of study. Short teats cause problem for milking machines (ICAR, 2001) while more longer teats are genetically predisposed to a higher incidence of mastitis. Klein *et al.* (2005) and Paulrud and Rasmussen (2004) stated that teat length and thickness play an important role in preventing mastitis, since the longer the canal length, the more pronounced the keratin cap, acting as a natural barrier, prevents contamination with the pathogens causing mastitis. Therefore, it is crucial to establish a balance between functionality and health for this trait, given that extremes are undesirable (Panetto *et al.*, 2017). However, lower value of UD which is not advantageous. This condition might have happened in the resource population because of selection of high milk producing cows eventually resulting in deep udders. Shallow udder depth is most desirable. Cows with deeper udders have more chances of udder injury and mastitis (Rogers, 1993). Cows with deeper udders and less udder clearance are more susceptible to high SCS (Carlström *et al.* 2016; Dadpasand *et al.* 2012; Stefani *et al.* 2018). Similar to RUW, high rear udder attachment is also an index of more udder capacity, so higher values are beneficial.

CONCLUSION

The current study is the first to show a connection between the *PHKA2* gene and udder traits in Sahiwal cattle. The results show that the population is not in HW equilibrium with respect to the identified SNPs *i.e.*, X:124497248G>A and X: 124497189C>T. Association analysis uncovered that these SNPs in *PHKA2* gene can be used as a candidate marker in *Bos indicus* to select animals with claimed udder conformation for enhancing milk producing ability.

ACKNOWLEDGEMENT

Facilities provided by Director, Indian Council of Agricultural Research-National Dairy Research Institute, Karnal, India to conduct this research is duly acknowledged.

REFERENCES

Berry, D.P., Buckley, F., Dillon, P., Evans, R.D. and Veerkamp, R.F. (2004). Genetic relationships among linear type traits, milk yield, body weight, fertility and somatic cell count in primiparous dairy cows. Irish Journal of Agricultural and Food Research. 161-176.

Carlström, C., Strandberg, E., Johansson, K., Pettersson, G., Stålhammar, H. and Philipsson, J. (2016). Genetic associations of in-line recorded milkability traits and udder conformation with udder health. Acta Agriculturae Scandinavica, Section A-Animal Science. 66: 84-91.

Choi, R., Park, H.D., Kang, B., Choi, S.Y., Ki, C.S., Lee, S.Y., Kim, J.W., Song, J. and Choe, Y.H. (2016). PHKA2 mutation spectrum in Korean patients with glycogen storage disease type IX: Prevalence of deletion mutations. BMC Medical Genetics. 17(1): 1-9.

Chopra, A., Ali, S.A., Bathla, S., Rawat, P., Vohra, V., Kumar, S. and Mohanty, A.K. (2020). High-resolution mass spectrometer-based ultra-deep profile of milk whey proteome in Indian Zebu (Sahiwal) cattle. Frontiers in Nutrition. 7.

Clustal Omega Multiple Sequence Alignment EMBL-EBI [Internet]. [cited 2021 24 March]. Available from: https:/www.ebi.ac.uk/Tools/msa/clustalo

Cole, J.B., Wiggans, G.R., Ma, L., Sonstegard, T.S., Lawlor, T.J., Crooker, B.A., Van Tassell, C.P., Yang, J., Wang, S., Matukumalli, L.K. and Da, Y. (2011). Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary US Holstein cows. BMC Genomics. 12(1): 1-17.

Dadpasand, M., Zamiri, M.J., Atashi, H. and Akhlaghi, A. (2012). Genetic relationship of conformation traits with average somatic cell score at 150 and 305 days in milk in Holstein cows of Iran. Journal of Dairy Science. 95: 7340-7345.

Devani, K., Plastow, G., Orsel, K. and Valente, T.S. (2020). Genome-wide association study for mammary structure in Canadian Angus cows. Plos One. 15(8): e0237818.

Godara, A.S., Tomar, A.K.S., Patel, M., Godara, R.S., Bhat, S.A. and Bharati, P. (2015). Body Conformation in Tharparkar cattle as a tool of selection. Journal of Animal Research. 5(3): 423.

Hendrickx, J., Lee, P., Keating, J.P., Carton, D., Sardharwalla, I.B., Tuchman, M., Baussan, C. and Willems, P.J. (1999). Complete genomic structure and mutational spectrum of *PHKA2* in patients with x-linked liver glycogenosis type I and II. The American Journal of Human Genetics. 64(6): 1541-1549.

Hessen, D.O. (2017). Impact of Noncoding DNA on Phenotype Evolution. eLS. 1.6.

ICAR, (2012). International agreement of recording practices. guidelines approved by the general assembly Riga Latvia June 2010. International Committee for Animal Recording Rome Italy.

International Committee for Animal Recording (2001). Conformation Recording of Dairy Cattle. Guidelines approved by the General Assembly, Rome, Italy. www.icar.org/Documents/.../Guidelines/Guidelines_2001.pdf

Khan, M.A. and Khan, M.S. (2016). Genetic and phenotypic correlations between linear type traits and milk yield in Sahiwal cows. Pakistan Journal of Agricultural Sciences. 53(2).

Klein, D., Flock, M., Khol, J.L., Franz, S., Stuger, H.P. and Baumgartner, W. (2005). Ultrasonographic measurement of the bovine teat: Breed differences and the significance of the measurements for udder health. Journal of Dairy Research. 72: 296-302.

Newgard, C.B., Hwang, P.K. and Fletterick, R.J. (1989). The family of glycogen phosphorylases: Structure and functio. Critical Reviews in Biochemistry and Molecular Biology. 24(1): 69-99.

Ogorevc, J., Kunej, T., Razpet, A. and Dovc, P. (2009). Database of cattle candidate genes and genetic markers for milk production and mastitis. Animal Genetics. 40(6): 832-851.

Panetto, J.C.D.C., Machado, M.A., da Silva, M.V.G., Barbosa, R.S., dos Santos, G.G., de MH Leite, R. and Peixoto, M.G.C. (2017). Parentage assignment using SNP markers, inbreeding and population size for the Brazilian Red Sindhi cattle. Livestock Science. 204: 33-38.

Paulrud, C.O. and Rasmussen, M.D. (2004). How teat canal keratin depends on the length and diameter of the teat canal in dairy cows. Journal of Dairy Research. 71(2): 253.

Ratwan, P., Chakravarty, A.K. and Kumar, M. (2019a). Assessment of relation among production and reproduction traits in Sahiwal cattle at an organized herd of northern India. Biological Rhythm Research. 1-9.

Rodenhouse, N.L., Best, L.B., O'Connor, R.J. and Bollinger, E.K. (2004). SAS Institute Inc. In SAS, 9(1): 3.

Rogers, G.W. (1993). Index selection using milk yield, somatic cell score, udder depth, teat placement and foot angle. Journal of Dairy Science. 76: 664-670.

Rozen, S., Skaletsk, H. (2000). Primer3 on the WWW for General users and for Biologist Programmers. In: Bioinformatics Methods and Protocols: [Krawetz, S., Misener, S., editors]. Methods in Molecular Biology. 1st Edn. Totowa: Humana Press. 365-386.

Stefani, G., El Faro, L., Santana Júnior, M.L. and Tonhati, H. (2018). Association of longevity with type traits, milk yield and udder health in Holstein cows. Livestock Science. 218: 1-7.

Tsilianidis, L.A., Fiske, L.M., Siegel, S., Lumpkin, C., Hoyt, K., Wasserstein, M. and Weinstein, D.A. (2013). Aggressive therapy improves cirrhosis in glycogen storage disease type IX. Molecular Genetics and Metabolism. 109(2): 179-182.

Türkyılmaz, D., Özyürek, S., Esenbuğa, N. and Yaprak, M. (2018). Correlation between various udder measurements and milk components in Morkaraman, Tuj and Awassi sheep. Pakistan Journal of Zoology. 50(5).

Vallée, A., Daures, J., Van Arendonk, J.A.M. and Bovenhuis, H. (2016). Genome-wide association study for behavior, type traits and muscular development in Charolais beef cattle. Journal of Animal Science. 94(6): 2307-2316.

Yan, Z., Wang, Z., Zhang, Q., Yue, S., Yin, B., Jiang, Y. and Shi, K. (2020). Identification of whole genome significant single nucleotide polymorphisms in candidate genes associated with body conformation traits in Chinese Holstein cattle. Animal Genetics. 51(1): 141.

Yeh, F.C. (1999). POPGENE (version 1.3.1). Microsoft Window-Bases Freeware for Population Genetic Analysis; [cited 2021 March 24]. Available from: http://www. ualberta. ca/~ fyeh.