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ASSOCIATION OF PROLACTIN GENE POLYMORPHISM WITH SOME BIOCHEMICAL AND LACTATION TRAITS IN DAIRY COW IN KARBALA PROVINCE

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ABSTRACT
The purpose of this study was to evaluate the genotype and alleles frequency prolactin gene polymorphism associated with milk traits dairy cow in kerbala province . The study included 120 Cross breed and Horstein-Frisian cows that were genotyping using ARMS PCR assay, One hundred twenty apparently healthy dairy crossbred cows were used in this study , their aged ranged from four to six years ,and weight ranged from 278 – 305 KG in mid stage 40-120 days of lactation, according heart girth equation to the obtained from unorganized fields of rural area in different region of Karbala city during the period from December 2020 to the end May 2021, blood was put in a gel tube for somehormones estimation in cattle serum which including Prolactin (PRL), Oxytoxine (OXY) , Thyroid stimulating hormones (TSH) ,Growth hormone (GH) , Estrogen (ES), Progestron (PRO).Oligonucleotides primer was designed to obtain ARMS Primers ARMS- PCR primers for detection Prolactin gene polymorphism. The concentration of Growth hormone μIU/mL in the blood serum of cross-bred cows was non-significant ($p > 0.05$) differences in the Growth hormone μIU/mL with prolactin gene polymorphism, The protein concentration was significant ($P < 0.05$) differences and recorded as 3.49 ± 0.65, 3.56 ± 0.25, The concentration freezing point in the milk of cross-bred cows was nonsignificant ($p > 0.05$) differences in the freezing point with prolactin gene polymorphism, it was found a lactose concentration was significant ($P < 0.05$) recorded as 5.05 ± 0.99, 5.1 ± 0.4 and 6.27 ± 1.45 for AA wild, AG heterozygot, GG mutation, respectively. In conclusion: milk composition and some of hormones were significant within prolactin genotype polymorphism.

INTRODUCTION
Prolactin (PRL), also known as lactotropin, is a polypeptide hormone, secreted mainly by the anterior pituitary gland and It has a molecular weight of appxoximately 22-kDa. It is a single-chain polypeptide of 198 amino acids and is apparently the result of removal of some amino acids. involved in many endocrine activities (Skorupski & Kmieć, 2012).

Bovine PRL gene is located on chromosome 23 and comprises five exons spanning a 10 kb genomic segment and encodes a 199 amino acid mature protein (Uddin *et al*., 2013). Previously several polymorphic sites have been detected within PRL gene and statistically significant associations between PRL variants and milk production traits have been described in dairy cattle (Dong *et al*., 2013)

Based on its genetic, structural, binding and functional properties, prolactin belongs to the prolactin/growth hormone/placental lactogen family [group I of the helix bundle protein hormones (Wojdak-Maksymiec et al., 2008). genes encoding prolactin, growth hormone, and placental lactogen evolved from a common ancestral gene by gene duplication (Forsyth & Wallis, 2002).
Significant associations between PRL variants and milk production traits in dairy cattle have been statistically confirmed. But, the information of PRL gene effects on milk production traits was still limited. and Prolactin plays an essential role in metabolism, regulation of the immune system and pancreatic development. prolactin is a peptide hormone, encoded by the PRL gene (Weikard *et al*., 2005 ; Akyuz *et al*., 2012).

The divergence of the prolactin and growth hormone lineages occurred ;400 million years ago (Takahashi et al., 2013) (Cooke et al., 1981 and Cooke et al., 1980). for example, in the bovine genome, a single gene, found on chromosome 20, encodes prolactin. The prolactin gene is about 10 kb in size and is composed of 5 exons and 4 introns. The bovine prolactin (bPRL) cDNA is 917 nucleotides long and contains a 699-nucleotide open reading frame encoding the prolactin prohormone. The signal peptide contains 30 amino acids, thus the mature bovine prolactin is composed of 199 amino acids (Marc et al., 2000). The goal of study was to evaluate the genotype and alleles frequency prolactin gene polymorphism associated with milk traits and hormones levels in dairy cow in karbala province.

MATERIALS AND METHODS

Animal and blood sampling

One hundred twenty apparently healthy dairy crossbred cows were used in this study , their aged ranged from (4-6) years ,and weight ranged from (278 – 305 KG) in mid stage (40-120) days of lactation, according heart girth equation (Wangchuk et al., 2018) to the obtained from unorganized fields of rural area in different region of Karbala city during the period from December 2020 to the end May 2021. Ten milliliters of blood have beentaken from all animals from jugular vein by sterile disposable syringe andcollected in three tubes for following analysis. 3 ml of blood was put into EDTA tubes for prolactingene polymorphism analysis. 4 ml of blood was put in a gel tube for somehormones estimation in cattle serum which including Prolactin (PRL), Oxytoxine (OXY) , Thyroid stimulating hormones (TSH) ,Growth hormone (GH) , Estrogen (ES), Progestron (PRO). On the other hand, 30ml of milk have been taken from lactation cattle and collected in sterile disposable cup and stored in ice box at (4C) until the milk was analyzed by (EKOMILK Ultrasonic milk analyzers/ USA) according to manufacture company.

Genetic Analysis

3 ml of blood in EDTA tubes was used to extract DNA by using Mini DNA extraction Kit with whole Blood Protocol according to the manufacture company. Oligonucleotides primer was designed to obtain ARMS Primers by using website Search Frame (soton.ac.uk) ,ARMS- PCR primers for detection Prolactin gene polymorphism. These primers was provided from Macrogen company, Korea as following table 1

Table 1: Nucleotides primers for detection Prolactine and gene polymorphism in cross breed and holestin cattle.

Type of gene	Primer	Sequence 5'-3'	Amplicon	References
PRL. gene	IF	ACCCTCTGTATCACCTAGTCACCGAGTTG	164 bp	Present study
	IR	CATCTGGGGCTCCTTTCATACCCAGT	191 bp	
	OF	GGTCAATCACTCTGAGCAAAAATCACATG	300 bp	
	OR	AATAGCAAGGAAGCTTTCATGAAGCTGC		

IF= inner forward, IR= inner reverse, OF= Outer forward, OR= Outer reverse

The reactions of ARMS-PCR were optimized for 25 µl final volume using 50-100 ng genomic DNA, 200µM each dNTP, 15 mM MgCl2; 1µl from 7 Picomole of each primer (four different primers), 5x Green Go Taq Reaction buffer, 0.5 U of GoTaq DNA Polymerase (Promega) and completed with nuclease-free water**,** The amplification products were hold through 1.5% agarose gel stained with and ethidium bromide, the procedure involved all the four different primers in one reaction tube, the mutant-type of Prolactin gene polymorphism (denoted by the letters G) and the wild-type denoted as (A)**,** The PCR optimization for prolactin gens were clarify in the following95C 5min as initial denaturation . followed by35 cycles consist of denaturation 95C for 35 Sec, annealing temperature 58C for 5 sec, externsion 72 C for 55 sec and final extension 72 c for 7 min.

Statistical analysis

All the data were written in Excel sheet , the experience was designaccording to the Complete Randomly Design(CRD) ,the results wereanalyzed using Statistical Software (SAS Institute 2002 Ver. 9) andarithmetic averages were measured on Duncan Multidimensional scale(Duncun,1955).

RESULTS AND DISCUSSION

High quality genomic DNA was used as a template forthe amplification of milk PRL gene, The amplicons of the this gene (PRL) were presented (Figure 1) and sequence results clearly indicate that the correct target genes were investigated in this study.

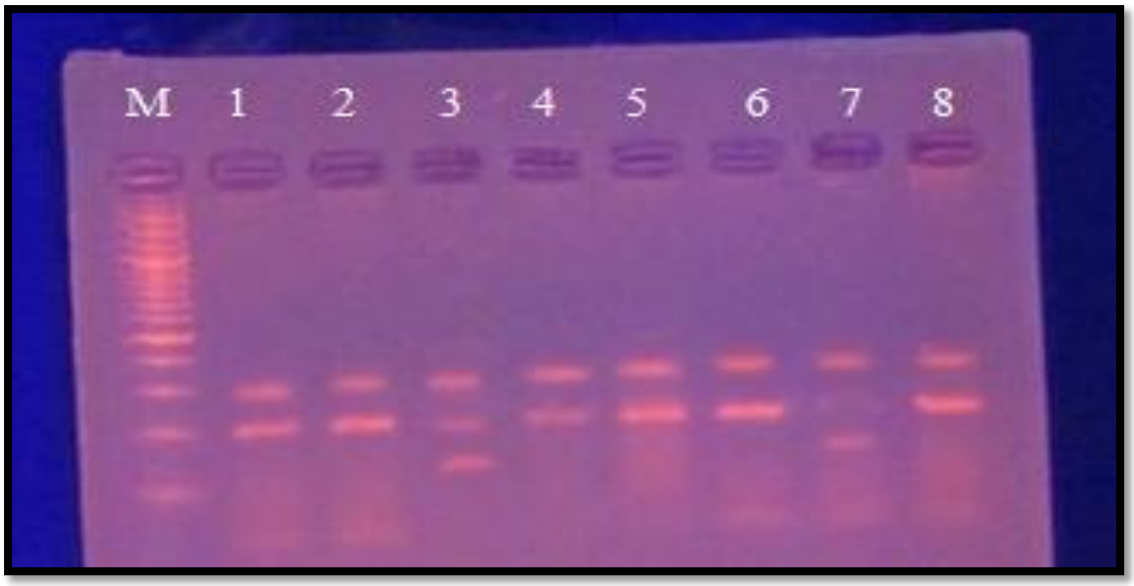

Figure (1)The genotypic analysis of PRL gene in cross bread Iraqi cattle. PCR products amplified from genomic DNA collected from blood samples extracted from Kerbala city cattle.The homozygous (GG) genotypes appeared a two bands (163 bp and 300 bp) which represented as 7 wells, The homozygous (AA) genotypes appeared a two bands (191 bp and 300 bp) which represented as 1,2,4,5,6 and 8 wells and heterozygous (AG) genotype appeared a three bands (191 bp , 163 bp and 300 bp), which represented 3 well

The protein concentration was significant (P< 0.05) differences and recorded as 3.49 ± 0.65, 3.56 ± 0.25 and 4.31 ± 0.93 forAA wild, AG heterozygot, GG mutation, respectively, as well as lactose concentration was significant (P< 0.05) recorded as 5.05 ± 0.99, 5.1 ± 0.4 and 6.27 ± 1.45 for AA wild, AG heterozygot, GG mutation,respectively, figure 2.

The concentration freezing point in the milk of cross-bred cows was nonsignificant (p> 0.05) differences in the freezing point with prolactin gene polymorphism, it was found 59.86± 11.45, 60.11± 4.73 and 59.21±4.43 for AA wild, GA heterozygot, GG mutation, respectively, as well as , the mean and standered deviation of density g/cm3 was non-significant (p> 0.05) difference, it was found 30.71±7.15, 30.47±4.22 and 59.4.43±4.43, for AA wild, GA heterozygot, GG mutation, respectively,

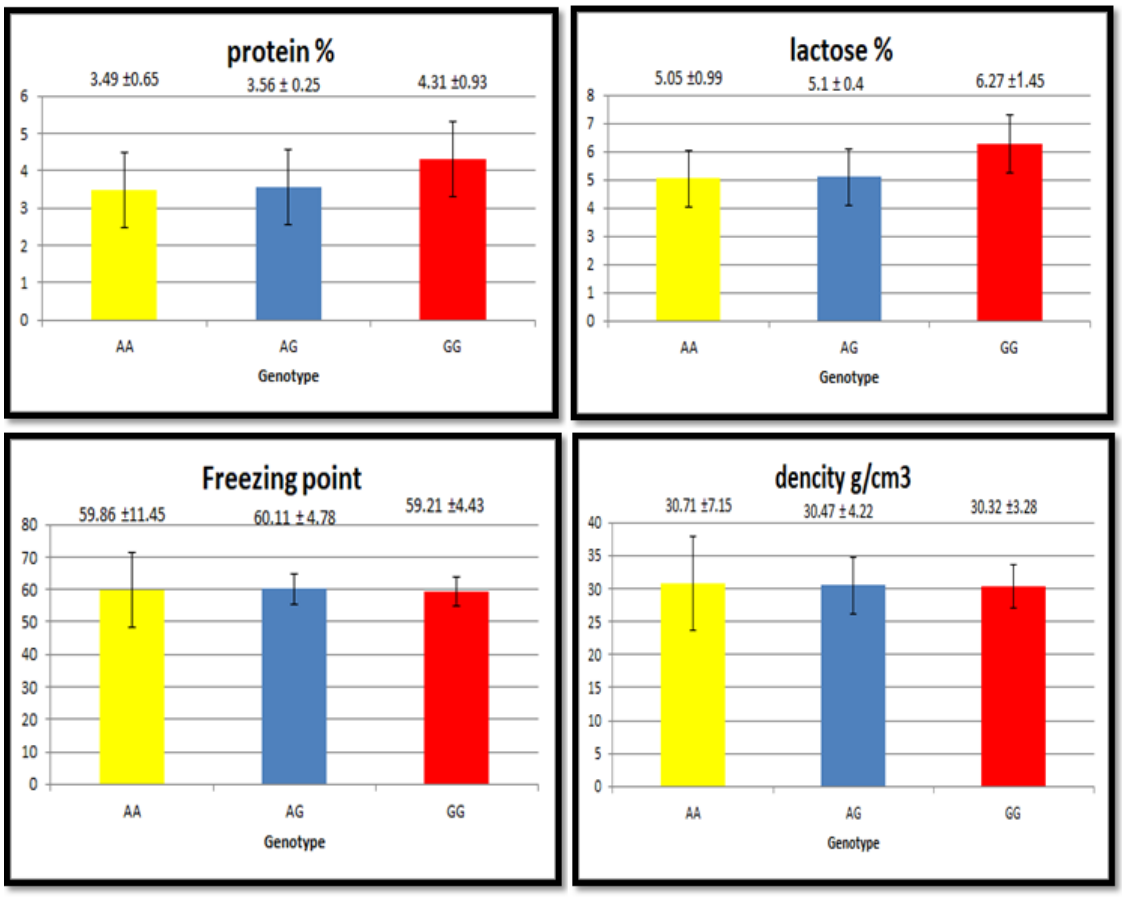

Figure 2: mean and standard deviation of protein , lactose , freezing point and dencity of milk cattle among genotype

The concentration of Growth hormone µIU/mL in the blood serum of cross-bred cows was non-significant (p> 0.05) differences in the Growth hormone µIU/mL with prolactin gene polymorphism, it was found 2.84±1.65, 2.61±2.11 and 2.38±1.94 for AA wild, GA heterozygot, GG mutation, respectively, Otherwise , the mean and standard deviation of Thyroid Stimulating Hormone (TSH) mIU/L was non-significant (p> 0.05) difference, it was found 22.48±3.62, 23.94±3.54 and 25.21±5.24, for AA wild, GA heterozygot, GG mutation, respectively , on the other hand , the mean and standard deviation of Prolactin mIU/L was significant (P< 0.05) difference, it was found 91.77±18.99, 119.69±32.99 and 127.7±37.42 , for AA wild, GA heterozygot, GG mutation, respectively figure 3.

Milk protein yield is of great significance for the dairy industry, the amount and composition of proteins in milk is largely determined by the genetics of the animal, and is difficult to change through nutrition (Heck *et al*., 2009).

However, due to the high requirement of protein synthesis for energy, the milk protein yield can be affected by the energy content in the diet, physiological status of animal and genetic factors (Dai *et al*., 2017).

our result found all milk composition were significant within prolactin genotype, otherwise, protein and lactose were given significant associated between milk composition and genotype. the protein concentration was recorded as 3.49 ± 0.65, 3.56 ± 0.25 and 4.31 ± 0.93 for AA wild, GA heterozygot, GG mutation.

The concentration of Progesterone ng/mL in the blood serum of cross-bred cows was significant (P< 0.05) differences in the Progesterone ng/mL with prolactin gene polymorphism, it was found 4.51±3.76, 7.93±4.39 and 7.52±6.21 for AA wild, GAheterozygot, GG mutation, respectively, Otherwise , the mean and standard

deviation of Estrogen (Estradiol) pg/mL was non-significant (p> 0.05) difference, it was found10.15±0.533, 10.76±9.94 and 10.01±0.523, for AA wild, GA heterozygot,GG mutation, respectively , on the other hand , the mean and standard deviation of Oxytocin was non-significant (p> 0.05) difference, it was found 17.3±3.69,17.68±3.58 and 18.15±3.31, for AA wild, GA heterozygot, GG mutation, respectively figure4.

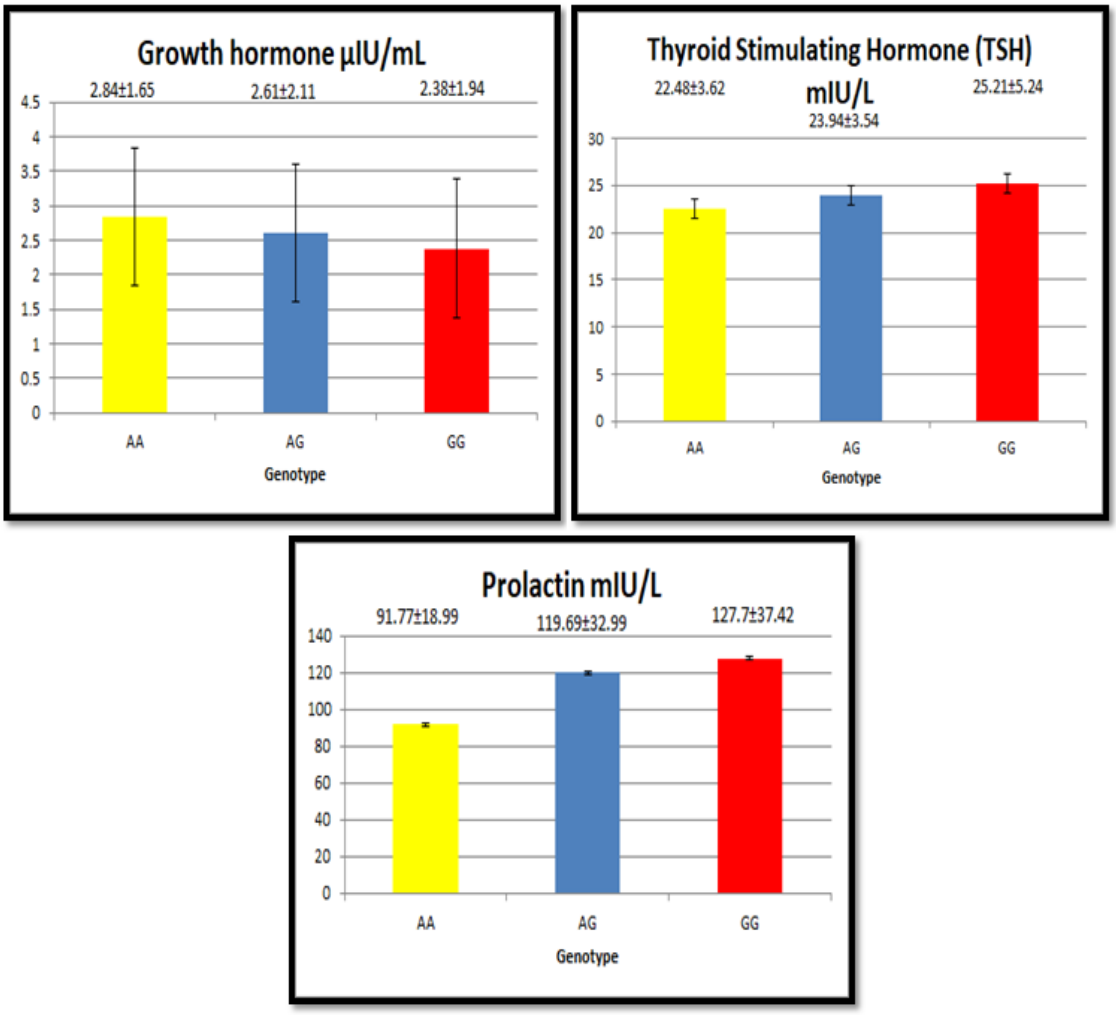

Figure 3: mean and standard deviation of Growth hormone, thyroid stimulating hormone, prolactin hormone with cow cross-bred among genotype.

This results can be investigated that a positive express of AG heterozygote, allele on the milk and protein yield , otherwise it was less extended on fat yield Fat percentage was lower because of the higher milk yield ,but nearly constant fat yield, associated with the AG heterozygote, allele (Boleckova *et al*., 2012) ,in addition the effect of prolactin hormone on milk components was clear ,because the prolactin regulates several secreted milk proteins ,including the caseins ,lactoglobulin ,lactalbumin and whey acidic protein (Pegolo *et al*. ,2018).

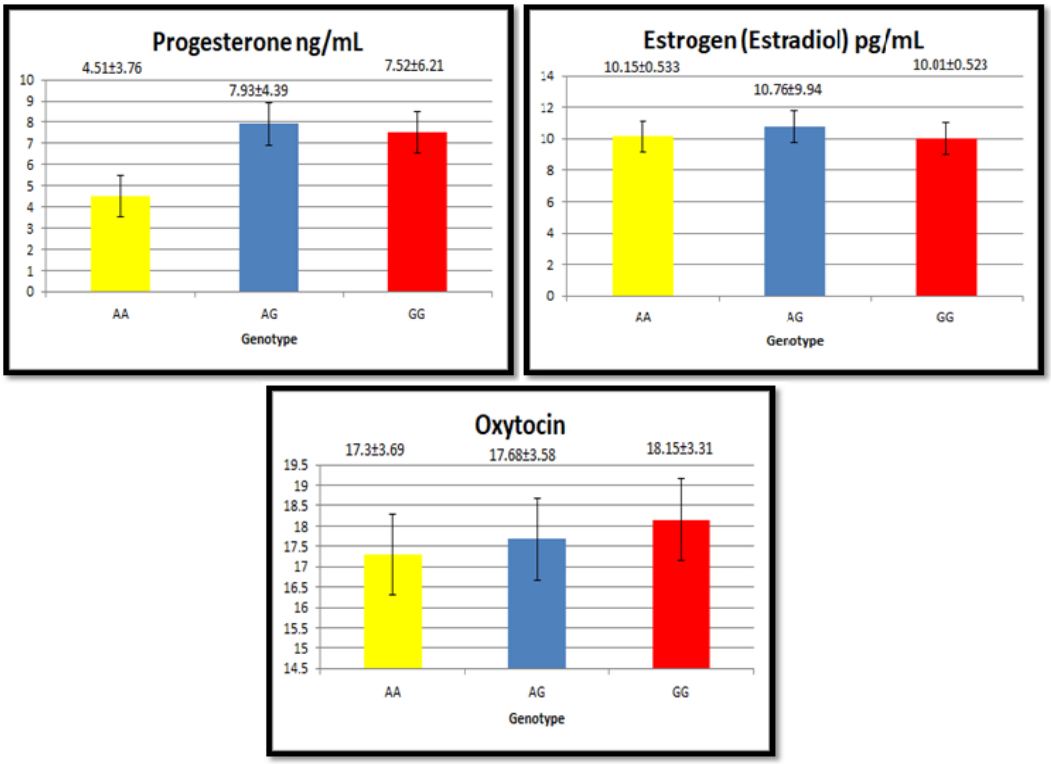

Figure 4: mean and standard deviation of Progesterone hormone, Estrogen hormone, Oxytocin Hormone with cow cross-bred among genotype,

REFERENCES

1. Skorupski, J., & Kmieć, M. (2012). Structural and functional characteristics of prolactin and its gene in the American mink (Neovison vison Schreb., 1777). A rewiev. Annales Universitatis Mariae Curie-Skłodowska. Sectio EE: Zootechnica, 30(4).
2. Uddin, R. M., Babar, M. E., Nadeem, A., Hussain, T., Ahmad, S., Munir, S., ... & Ahmad, F. J. (2013). Genetic analysis of prolactin gene in Pakistani cattle. Molecular biology reports, 40(10), 5685-5689.
3. Dong, C. H., Song, X. M., Zhang, L., Jiang, J. F., Zhou, J. P., & Jiang, Y. Q. (2013). New insights into the prolactin-RsaI (PRL-RsaI) locus in Chinese Holstein cows and its effect on milk performance traits. Genet Mol Res, 12(4), 5766-5773.
4. Wojdak-Maksymiec, K., Kmic, M., Strzalaka, J., & Judyma, U. D. (2008). Prolactin gene polymorphism and somatic cell count in dairy cattle. Journal of Animal and Veterinary Advances, 7(1), 35-40.
5. Forsyth, I. A., & Wallis, M. (2002). Growth hormone and prolactin—molecular and functional evolution. Journal of mammary gland biology and neoplasia, 7(3), 291-312.
6. Weikard, R., Kühn, C., Goldammer, T., Freyer, G., & Schwerin, M. (2005). The bovine PPARGC1A gene: molecular characterization and association of an SNP with variation of milk fat synthesis. Physiological Genomics, 21(1), 1-13.

7. Akyuz, B., Agaoglu, O. K., & Ertugrul, O. (2012). Genetic polymorphism of kappa-casein, growth hormone and prolactin genes in Turkish native cattle breeds. International Journal of Dairy Technology, 65(1), 38-44.
8. Takahashi, T., Hayashi, K. G., & Hosoe, M. (2013). Biology of the placental proteins in domestic ruminants: expression, proposed roles and practical applications. Japan Agricultural Research Quarterly: JARQ, 47(1), 43-51.
9. COOKE NE, COIT D, SHINE J, BAXTER JD, AND MARTIAL JA. Human prolactin cDNA structural analysis and evolutionary comparisons. J Biol Chem 256: 4007–4016, 1981.
10. COOKE NE, COIT D, WEINER RI, BAXTER JD, AND MARTIAL JA. Structure of cloned DNA complementary to rat prolactin messenger RNA. J Biol Chem 255: 6502–6510, 1980.
11. Marc, E. F., K. BeLa, L. Anna and N. GyoRgy. 2000. Prolactin: structure, function and regulation of secretion. Physiol. Reviews. 80(4):1523-1631.
12. Wangchuk, K., Wangdi, J., & Mindu, M. (2018). Comparison and reliability of techniques to estimate live cattle body weight. Journal of Applied Animal Research, 46(1), 349-352.
13. Duncun,D.B.(1955).Multiple Range and F.test.Biometric ,11,42.
14. Heck, J. M. L., Schennink, A., Van Valenberg, H. J. F., Bovenhuis, H., Visker, M. H. P. W., Van Arendonk, J. A. M., & Van Hooijdonk, A. C. M. (2009). Effects of milk protein variants on the protein composition of bovine milk. Journal of dairy science, 92(3), 1192-1202.
15. Dai, W., Chen, Q., Wang, Q., White, R. R., Liu, J., & Liu, H. (2017). Complementary transcriptomic and proteomic analyses reveal regulatory mechanisms of milk protein production in dairy cows consuming different forages. Scientific reports, 7(1), 1-14.
16. Boleckova, J., Matejickova, J., Stipkova, M., Kyselova, J., & Barton, L. (2012). The association of five polymorphisms with milk production traits in Czech Fleckvieh cattle. Czech J Anim Sci, 57(2), 45-53.
17. Pegolo, S., Mach, N., Ramayo-Caldas, Y., Schiavon, S., Bittante, G., & Cecchinato, A. (2018). Integration of GWAS, pathway and network analyses reveals novel mechanistic insights into the synthesis of milk proteins in dairy cows. Scientific reports, 8(1), 1-15.