Indian Journal of Biotechnology
Vol. 19, July 2020, pp 211-218

Genotyping of cattle based on kappa-casein and alpha-lactalbumin genes

Rahman Sumaiya[1,2], Hossain Md. Imam[1], Hossain Mridha Md. Kamal[2], Alim Md. Abdul[2], Jahan Nusrat[2], Islam Md. Ariful[3], Rubaya[2], Salimullah Md[2], Bhuyan Anjuman Ara[2], Alam Jahangir[2*]

[1]Mawlana Bhashani Science and Technology University, Tangail, Bangladesh

[2]National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka. Bangladesh

[3]Upazila Livestock Officer, Monohordi, Narsingdi, Department of Livestock Services, Bangladesh

Received 15 May 2020; revised & accepted 29 June 2020

Milk yield, protein and fat content are regulated by milk protein genes. However, genetic makeup of different milk protein genes of cattle available in Bangladesh is not known. This study determined the genetic variants of kappa-casein (κ-CN) and α-lactalbumin (α-LA) gene in cattle. DNA was extracted from 50 blood samples collected from indigenous and crossbred cattle. Polymerase chain reaction (PCR) was performed with gene specific primers. For genotyping PCR products of κ-casein gene was digested with *Hind*III and *Hae*III restriction enzymes while the PCR products of α-LA gene was digested with *Bsp1286*I. Overall from all the tested samples we could amplify specific DNA viz. 935 and 429 bp for κ-CN and α-LA genes, respectively. In case of κ-CN gene 30 samples were analyzed for genotyping and of these 66.67, 30.00 and 3.33% samples were genotyped as AA, AB and BB, respectively. AA genotype was found dominant for κ-CN gene. Allele frequency for A was 82%. AA genotype was found higher in indigenous (0.366) than crossbred (0.30) animals. Homozygous BB genotype was not found in crossbred animals. For α-LA gene 50 samples were genotyped and all the samples (100%) were belonging to AA genotype. Three genetic variants of κ-CN gene viz. AA, AB & BB and one genotype (AA) of α-LA gene were detected in tested samples. Alleles of both the genes are reported to be associated with higher milk, protein, and fat yields.

Keywords: Cattle, genotypes, kappa-casein, alpha - lactalbumin, allele frequency

Introduction

In an agriculture based country like Bangladesh, livestock plays a major part in economy. About eighty percent of the total population of Bangladesh lives in the rural area and their livelihood is mostly based on cultivation and farming. The densities of livestock in Bangladesh are quite higher in the world, 145 large ruminants/km compared with India, Ethiopia and Brazil which have 90, 30 and 20 ruminants/km, respectively[1]. However, the productivity of indigenous cattle is far below the world average. Low milk yield of the local cattle may be the resultant effect of inferior breed, low genetic potentialities, poor nutrition, lack of proper management practices and late age at puberty. Improvement of milk production through selective breeding is one of the very useful tools for livestock industry. Emphasis is given to improve the milk production traits for more than 100 years.

Conventional methods for selective breeding are time-consuming and expensive. However, identification of molecular markers associated with milk production traits of an animal can be even before phenotypes are expressed and independent of age and gender[2]. Milk protein genes regulate different milk production traits of cattle including milk yield, milk protein content, fat content, fat percentage etc[3-4]. There are several variants for each of the six milk protein genes have been identified[5-6]. Kappa casein (κ-CN) is one of the major casein proteins and alpha-lactalbumin (α-LA) is a major whey protein of milk. The κ-casein gene has been mapped on chromosome 6 (6q31) in cattle[7] and to date eleven (A, B, C, E, F1, F2, G1, G2, H, I and J) alleles are known. Two κ-CN variants have been described in detail as κ-CN A and B. 'A' variant shows Asp (GAT) and Thr (ACC) at positions 148 and 136, respectively, whereas the variant 'B' shows Ala (GCT) and Ile (ATC) at the same positions[8]. κ-CN plays an important role in milk chemistry by providing colloidal stability to the casein micelle. The association between κ-CN alleles and the total protein content, fat percentage and milk

*Author for correspondence:
alamjahan2003@yahoo.com

production has been reported in several studies[9-12]. κ-CN 'B' allele was reported to have a positive and significant effect on both milk yield and milk protein content in dairy local cattle, buffalos in different countries[13-15]. Also, its favorable effect on milk technological properties and cheese yield in taurine and zebu cattle breeds[16] and Brazilian cattle[16], desirable coagulation properties reported in Finnish Ayrshire cows[18].

Alpha-lactalbumin (α-La), the major whey protein, essential for the biosynthesis of lactose at the level of mammary glands. It directly influences the quality and the volume of the milk since it is directly involved in the lactose synthesis[19]. It is coded by the *LAA* gene, mapped on chromosomes 5[20]. Three variants (A, B and C) of (α-LA) have been reported so far. Variant A differs from variant B because glutamine at 10^{th} position in variant B replaces with arginine. The most common B variant consists of 123 amino acids with a molecular weight of 14.175 kDa. α-LA has most important function related to milk productivity as well as quality in cows[21]. Besides, it has several important biological functions, such as lactose synthesis and Ca^{2+} binding[22]. In addition, it has been linked to antimicrobial activity[23], reduction of stress[24], immunomodulation[25], regulation of cell growth[26], anti-ulcer activity[27] and anti-hypertensive activity[28]. There is no report about κ-CN and α-LA gene variants of cattle in Bangladesh. The objective was to determine the allelic and genotypic frequencies, genetic diversity and polymorphic information for κ-CN and α-LA in cattle.

Materials and Methods

Blood Sample and DNA Extraction

Fifty blood samples were retrieved from the sample repository of Animal Biotechnology Division of National Institute of Biotechnology along with their relevant information. These samples were collected from Shibpur Upazila, Narsingdi during 2013 - 2014. The samples were derived from both indigenous (n=25) and crossbred (n=25) animals. Detail information about crossbred animals is not available. However, it is to be mentioned that semen from Shahiwal, Holstein, Friesian etc, are being using for artificial insemination in Bangladesh including Shibpur Upazila. DNA was extracted from the samples using phenol chloroform method according to the method described earlier[29]. Extracted DNA was analyzed in NanoDrop spectrophotometer to measure the DNA concentration as well as purity. Among the 50 samples, 30 samples were subjected to κ-CN genotype analysis and all 50 were subjected to α-LA genotype analysis.

Primer

The primers used in this study are reported previously. About 935 bp product of the kappa casein gene was amplified with κ-CN F 5'-AGCGCTGTGAGAAAGATG-3' and κ-CN R 5'-GTGCAACAACACTGGTAT-3' primers reported earlier[30]. On the other hand about 429 bp fragment of the alpha-lactalbumin gene was amplified with α-LA F 5'-GATCAGTCCTGGGTGGTCATT-3' and α-LA R 5'-CAGTGGGTACCCATCCTAAGT-3' primers reported by Voelker and Bleck[31]. The primers were synthesized from BioBasic, Canada and supplied Biotech Concern Ltd., Bangladesh.

Amplification of κ-CN and α-LA Genes by Polymerase Chain Reaction

The PCR reaction consisted of 12.5 µL of 2X master mix (Gene Amp® Fast PCR Master Mix, Applied Biosystems), 0.2 µL *Taq* DNA polymerase, 0.5 µL forward primer (10 pmole), 0.5 µL reverse primer (10 pmole), approximately 50 ng of DNA and molecular grade water to a final volume of 25 µL. Gene specific primers reported earlier were used for amplification of DNA[30-31]. The cycling profile for κ-CN comprised an initial denaturing step for 5 min at 94°C, followed by 32 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min. The cycling condition for α-LA was also same except annealing temperature which was 54°C instead of 60°C. Amplicons were analyzed by gel electrophoresis in a 1.5% agarose gel using TAE buffer and stained with ethidium bromide.

Genotyping of κ-CN and α-LA

Genotyping of κ-CN and α-LA gene was performed by restriction fragment length polymorphism (RFLP) analysis. For this purpose, PCR product of κ-CN gene was digested with *Hind*III and *Hae*III endonucleases and PCR product of α-LA gene was genotyped by using *Bsp1286*I endonuclease. All the restriction enzymes were derived from New England Bio-Labs, USA. Each digestion reaction (10 µl) consisted of 1.5 µl nuclease free water, 1 µl of compatible 10X buffer, specific restriction enzyme 0.5 µl and PCR product 7 µl. The reaction mixture was incubated at 37°C in water bath for 1 hour. Upon digestion the products were run in 4% agarose gel. Genotyping was done based on the digestion pattern (Table 1).

Data Analysis

Polymorphic amplicons were considered to estimate the allelic diversity and effective number of alleles. Allelic and genotypic frequencies were estimated using the software Popgen32[32].

Analysis of Restriction Sites for *Hind*III and *Hae*III in κ-CN Gene of Different Species of Animals

Sequences of κ-CN gene of cattle (*Bos indicus, Bos taurus)* buffalo (*Bubalus bubalis*), goat (*Capra hircus*) and sheep (*Ovis aries*) were retrieved from GenBank and analysed for restriction sites of *Hind*III and *Hae*III. Sequence ID X14908 was considered as standard and analysed against this sequence.

Results and Discussions

Genotyping of Cattle Based on Two Milk Protein Genes

Genotyping of cattle based on two milk protein genes are performed in this study. Samples were collected from both local and crossbred animals. PCR and restriction analysis were performed to genotype the genes.

κ-CN Genotype Analysis

Tested samples were successfully amplified and genotyped with *Hind*III and *Hae*III restriction enzymes (Fig. 1). Distributions of gene and genotype frequencies in local and crossbred animals are presented in Table 2. Three genotypes, viz. AA, AB and BB were found in all the tested samples. Genotypes AA and AB were found in both indigenous and crossbred cattle. On the other hand BB genotype was found only in indigenous cattle. Two alleles, A and B of κ-CN gene were detected in tested samples. The frequency of A and B allele was 0.82 and 0.18,

Table 1 — Expected fragment pattern of PCR products of κ-CN and α-LA genes digested with different restriction enzymes.

Genotype	Fragment length		
	κ-CN (935 bp)		α-LA (429 bp)
	HindIII	*HaeIII*	*Bsp1286I*
AA	935	641 + 294	429
AB	935 + 520 + 415	641 + 294	429 + 260 + 169
BB	520 + 415	641 + 294	260 + 169

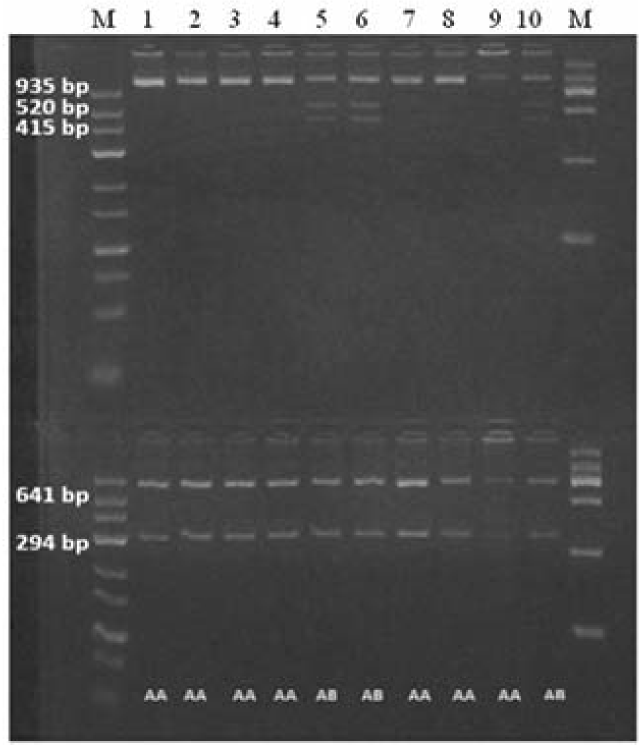

Fig. 1 — Fragment length analysis of κ-casein gene. PCR products of 935 bp were digested with *Hind*III (upper panel) and *Hae*III (lower panel) and DNA fragments were visualized in 4% agarose gel. Lane M: marker; Lane 1-10: Tes samples. Genotype of each sample is indicated as AA, AB and BB based on digestion pattern

Table 2 — Genotype and allele frequencies of κ-casein and α-lactalbumin gene found in the tested samples

Gene	No. of sample	Genotype	Frequency in indigenous cattle	Frequency in crossbred cattle	Overall frequency	Allele frequency	
						A	B
κ-CN	30	AA	0.366	0.30	0.666	0.82	0.18
			n = 11	n = 9	n = 20		
		AB	0.13	0.17	0.30		
			n = 04	n = 05	N = 09		
		BB	0.03	-	0.03		
			n = 01	n = 00	n = 01		
α-LA	50	AA	0.50	0.50	1	1.00	0.00
			n = 25	n = 25	n = 50		
		AB	-	-	-		
			n = 0	n = 0	n = 0		
		BB	-	-	-		
			n = 0	n = 0	n = 0		

respectively. This result is in agreement with the several studies reported previously. For example, our results were found similar to the findings[33] from Friesian Holstein cattle where frequency of A allele is 0.78 - 0.85. Higher frequencies of A allele (0.62 - 0.63) were reported from Egyptian Baladi and Holstein cattle[34], Turkey native cattle, Anatolian black and East Anatolian red cattle (0.75 - 0.78)[35], Holstein populations (0.68-0.89) etc[36]. In a study Khaizaran and Al-Razem[37] found A and B allele were favorable alleles in κ-CN with higher frequencies of A allele (0.81) than B allele (0.19) in Palestinian Holstein-Friesian cattle. Keating *et al* (2007)[38] found that A allele was dominant over B allele among dairy, beef and dual purpose cattle population. Beata *et al*, (2008)[39] reported that Holstein-Friesian cattle in Poland carried 0.83 and 0.17 frequencies of A and B allele, respectively. Crossbred cattle of meat and dairy type reported to carry dominant A allele. It is reported that frequency of A allele is more frequent than B allele in crossbred of Simmental and Holstein cattle[40]. Frieswal cattle (Friesian x Sahiwal) carried dominant A allele (0.58) over B allele (0.42)[41]. On the contrary, Zepeda-Batista *et al* (2015)[42] found higher B allele frequency (0.69) than the A (0.26) and E (0.05) in the Mexican Jersey cattle population. Higher B allele frequencies (0.71-0.89) were also reported from Colombian, German, and Chinese Jersey populations[43-45].

Kappa-casein A allele was predominantly observed in the studied cattle populations and reported to be associated with high milk yield[46-47]. It has been reported that, BB form of κ-CN is responsible for higher yield in cheese making as well as milk and milk protein yield[47-49]. It is reported that the cheese production can be increased by 10% by using milk from cows of genotype BB of κ-CN[50]. Vidović *et al* (2013)[51] reported that Holstein cows with BB had higher percentage of milk fat than AA and AB genotypes. Besides, Alipanah *et al* (2008)[52] mentioned that polymorphism of κ-CN gene affect fat percentage in a Russian indigenous cattle namely Black Pied breed. Especially, BB genotype showed a higher fat percentage (4.79 ± 0.23) than AA and AB alleles. Higher solid non fat (SNF) percentage and monthly milk yield was reported from BB genotype of Sahiwal and Tharparker cattle than AA and AB genotypes[53]. In a study, Deb *et al* (2014)[41] reported that Frieswal cattle of AB genotype had a significant higher total milk yield, peak yield and SNF for 300 days than AA genotypes41. Similar results were also found by Gurses and Yuce (2012) from Turkey local cattle[35]. They reported that AB genotype of East Anatolian Red cattle had a higher percentage of milk solid non fat and protein than AA genotype. Bittante *et al* (2012)[54] reported that κ-CN BB variant of milk has superior renneting properties with shorter rennet coagulation time (RCT), faster rates of curd firming and producing a firmer curd compared to other κ-casein variants. All these studies showed an individual effect of the κ-casein gene on milk yield and milk fat yield. Increasing the frequency of B allele and BB genotype in cattle population could help to improve milk production traits (milk yield, milk protein content) through selection based on allele and genotype. Selected animals having BB and AB genotypes could be crossed to generate more animals with BB genotypes.

Alpha Lactalbuimin (α-LA) Genotype Analysis

All 50 samples were successfully amplified and genotyped with *Bsp1286I* restriction enzyme. Only A allele of the α-LA gene was detected in 100% samples obtained from indigenous and crossbred animals. alleles B and C were not detected in the tested samples. Hence, all samples were genotyped as AA (Table 2 & Fig. 2). The A allele of α-LA is reported to be associated with greater milk, protein and fat yields, and the B allele is associated with greater protein and fat percentage. Mao and Bremel[55] examined the α-LA variants in Holstein cattle from four different farms in Taiwan and found both A and B allele with AA, AB and BB genotype. They found that cows with α-LA AA or AB genotype produced 300 kg more milk (305 d) than BB type cows. alleles A and B were detected in Taiwan Holstein, Italian Friesian, Italian Red Pied, Swedish Red and White, and Italian Brown[56]. Aschaffenburg reported that the frequency of α-LA A allele was higher Indian zebu cattle than in the most African zebu cattle[57]. Genotyping of Sahiwal cattle in India based on the α-LA gene was performed by Mir *et al* (2014) and found AB and BB genotypes with frequencies of 0.14 and 0.86, respectively. They could not find the genotype AA. They also reported that animals having AB genotypes had a higher average milk yield than the animals with BB genotypes. Reports also indicated that BB genotype in zebu cattle (Sahiwal, Hariana, Tharparkar) showed higher milk yield[58]. Zhang *et al* (2007) screened Chinese Holstein cattle for α-LA locus and found no significant association between the two genotypes found and milk production traits in these cattle[59]. Recently, Zhou and Dong (2013) identified single nucleotide substitution C→T (α-LA2516) at position 2516 of the α-LA gene in Chinese Holstein cattle with frequencies of T and C as 0.67 and 0.32, respectively. There was no significant association between genotypes resulting from this SNP and production traits in cattle[60].

Heterozygosity and Hardy–Weinberg Equilibrium

This study showed that there were no differences between observed (Ho) and expected (He) heterozygosity (Table 3) in the population analyzed. Expected (He) heterozygosity was the same as observed (Ho) in κ-CN locus. Based on a Chi-square (χ^2), test heterozygosity was out of Hardy-Weinberg disequilibrium ($P > 0.05$) were within in κ-CN locus. This result implied that, there was no artificial selection applied for reproductive management and genetic improvement programs in the studied population against κ-CN locus. χ^2 test P value ($P > 0.05$, not consistent with Hardy-Weinberg Equilibrium).

Analysis of Restriction Sites for *Hind*III and *Hae*III in κ-CN Gene of Different Species of Animals

A total of 387 sequences of the κ-CN gene of different species of animals such as cattle (*Bos indicus, Bos taurus)* buffalo (*Bubalus bubalis*) goat (*Capra hircus*) and sheep (*Ovis aries*) were analysed for restriction sites of *Hind*III *and Hae*III (Table 4). For this purpose, 935 bp sequences of κ-CN gene of

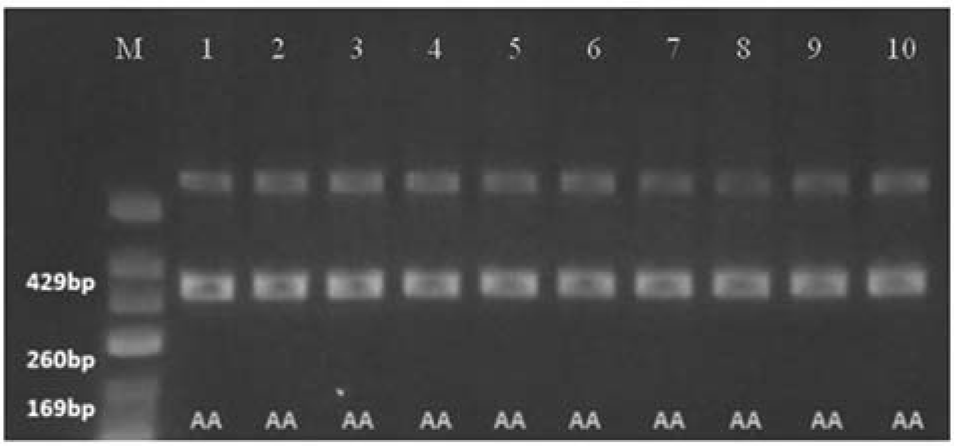

Fig. 2 — Fragment length analysis of α-lactalbumin gene. PCR products of 429 bp were digested with Bsp1286I and DNA fragments were visualized in 4% agarose gel. Lane M: marker; Lane 1-10: Test samples. Genotype of each sample is indicated as AA based on digestion pattern.

Table 3 — Observed heterozygosity (Ho) and expected heterozygosity (He) for the loci κ-CN, and α-LA in the indigenous and crossbred cattle

Locus	Genotypic frequency			Allele frequency		Ho (observed)	He (expected)	Chi-squire (χ^2)	Average effective number of alleles	Polymorphic loci (%)
	AA	AB	BB	A	B					
κ-CN	0.66 n = 20	0.30 n = 9	0.03 n = 1	0.82	0.18	0.30	0.30	0.007	1.4	100
α-LA	1 n = 50	0 n = 0	0 n = 0	1	0	0	0	0	1	0

Table 4 — Analysis of restriction sites for *Hind*III and *Hae*III in κ-CN gene of different species of animals

Species	Number of seq analyzed	Quarry coverage (%)	Maximum homology with seq ID X14908 (%)	*Hind*III site (A*AGCTT) in number			*Hae*III site (GG*CC) in number			Number of seq have both sites
				Present	Absent	No seq	Present	Absent	No seq	
Bos indicus	29	29 - 93	99.54	8	20	1	27	0	2	8
Bos taurus	87	2 - 100	100	41	43	3	77	0	10	36
Bubalus bubalis	75	2 - 99	100	30	30	15	30	0	45	30
Capra hircus	114	2 - 80	100	91	0	23	98	1	15	91
Ovis aries	82	2 - 65	100	9	29	44	9	62	11	7
Total	387			179	122	86	241	63	83	172

GenBank seq ID X14908 was considered standard because the primers used in this study for κ-CN genotyping was reported to be based on this sequence. Analysed data are present in Table 4. Over all, the quarry coverage and homology was found varied from 2 - 100 and 99.54 - 100%, respectively. Irrespective of animal species sites for *Hind*III and *Hae*III were found in 179 (46.25%) and 241 (62.27%) sequences, respectively, while absent in 122 (31.52%) and 63 (16.28%) sequences, respectively. On the other hand, both sites were found in 44.44% (172/387) sequences. Out of 387 sequences, 86 (22.22%) and 83 (21.45%) sequences have no sequences spanning the restriction sites of *Hind*III and *Hae*III, respectively. Restriction site of *Hae*III was found higher than *Hind*III site in all animal species. Hundred percent (100%) quarry coverage and homology was not found in sequences of in any species. Hundred percent (100%) homology was found while coverage was lower than 10%. Hundred percent (100%) quarry coverage was found only in two sequences (Accession nos. AY380228.1 and AY380229.1) of *Bos taurus*, but homology was 99.89%. In *Bos taurus* two sites for *Hind*III were found in six sequences (Accession nos. AF030326, XM_014478623, XM_005897042, MH378281, AY095311, JX862174) and two sites for *Hae*III were found in one sequence (Accession no. HQ589917.1). From this analysis it is revealed that two sites for *Hind*III are located at 415^{th} position and 427^{th} position in relation 935 bases X14908. So, three fragments of 11, 415 and 509 bp would be generated. Out of 179 sequences having *Hind*III site, only six sequences (3.35%) have two sites and should be genotyped as BB. In our study, we have found one (3.33%) out thirty samples as BB genotype complies with the findings of studied sequences.

In conclusion, present study focuses on the genotyping of animals based on κ-casein and α-lactalbumin gene variants. Three variants (AA, AB and BB) of κ-casein and one variant of α-LA (AA) were detected. For both genes A allele was found dominant. Heterozygosity in κ-CN locus was found out of Hardy-Weinberg disequilibrium.

Acknowledgement

This study was conducted with partial support from the project (ID No. BS-180) entitled "Polymorphism of beta casein gene in animals in Bangladesh." The project was funded by Ministry of Science and Technology (MoST), Government of the People's Republic of Bangladesh under Special Allocation Program during the year 2016-2017.

References

1 Uddin M M, Huylenbroeck G V, Hagedorn K, Sultana N & Peters K J, Institutional and organizational issues in livestock services delivery in Bangladesh, *J Intl Agril,* 49 (2010) 111-125.

2 Ontaviano R, Tonhati H, Desiderio J A & Munoz F C, Kappa-casein gene study with molecular marker in female buffalos, *Gene & Mol Biol,* 28 (2005) 237-241.

3 McLean D M, Graham E R, Ponzoni R W & McKenzie H A, Effects of milk protein genetic variants on milk yield and composition, *J Dairy Res,* 51 (1984) 531-546.

4 Mir S N, Ullah O & Sheikh R, Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle, *African J Biotechnol,* 13 (2014) 555-565.

5 Farrell H M Jr, Jimenez-Flores R, Bleck G T, Brown E M, Butler J E *et al*, Nomenclature of the proteins of cows' milk-sixth revision, *J Dairy Sci,* 87 (2004) 1641-1674.

6 Caroli A, Chessa S, Chiatti F, Rignanese D, Melendez B *et al*, Carora cattle show high variability in alpha (s1)-casein, *J Dairy Sci,* 91 (2008) 354-359.

7 Threadgill D W & Womack J E, Genomic analysis of the major bovine milk protein genes, *Nucleic Acids Res,* 18 (1990) 6935-6942.

8 Eigel W N, Butler J E, Ernstrom C A, Farrell H M Jr, Harwalkar V R *et al*, Nomenclature of proteins of cow's milk: Fifth revision1, *J Dairy Sci,* 67 (1984) 1599-1631.

9 Verdier-Metz I, Coulon J B & Pradel P, Relationship between milk fat and protein contents and cheese yield, *Anim Res*, 50 (2001) 365-371.

10 Boettcher P J, Caroli A, Stella A, Chessa S, Budelli E *et al*, Effects of casein haplotypes on milk production traits in Italian Holstein and Brown Swiss cattle, *J Dairy Sci,* 87 (2004) 4311-4317.

11 Kubarsepp I M, Henno M, Viinalass H & Sabre D, Effect of κ-casein and β-lactoglobulin genotypes on the milk rennet coagulation properties, *Agr Res,*1 (2005) 55-64.

12 Tsiaras A M, Bargouli G G, Banos G & Boscos C M, Effect of kappa-casein and beta-lactoglobulin loci on milk production traits and reproductive performance of Holstein cows, *J Dairy Sci,* 88 (2005) 327-34.

13 Caroli A, Chessa S, Bolla P, Budelli E & Gandini G C, Genetic structure of milk protein polymorphism and effects on milk production traits in local dairy cattle, *J Anim Breed Genet,* 121 (2004) 119-127.

14 Raj G D, Shetty S, Govindaiah M G, Nagaraja C S, Byregowda S M *et al*, Molecular characterization of kappa-casein in buffaloes, *Sci Asia*, 34 (2008) 435-549.

15 Riaz M N, Malik N A, Nasreen F & Qureshi J A, Molecular marker assisted study of kappa-casein gene in nili-ravi (buffalo) breed of Pakistan, *Pakistan Vet J,* 28 (2008) 103-106.

16 Ceriotti G, Marletta D, Caroli A & Erhardt G, Milk protein loci polymorphism in taurine (*Bos taurus*) and zebu (*Bos indicus*) populations bred in hot climate, *J Anim Breed Genet,* 121 (2004) 404-415.

17 Azevedo A L S, Nascimento C S, Steinberg R S, Carvalho M R S, Peixoto M G C D *et al*, Genetic polymorphism of

the kappa casein gene in Brazilian cattle, *Genet Mol Res,* 7 (2008) 623-630.

18 Ikonen T, Ojala M & Ruottinen O, Association between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows, *J Dairy Sci,* 82 (1997) 205-214.

19 Ashwell M S, Rexroad Jr C E, Miller R H, Van Raden P M & Da Y, Detection of loci affecting milk production and health traits in an elite US Holstein population using microsatellite markers, *Anim Genet,* 28 (1997) 216-222.

20 Hayes H, Petit E, Bouniol C & Popescu P, Localisation of the alpha-S2-casein gene (CASAS2) to the homologous cattle, sheep and goat chromosome 4 by *in situ* hybridization, *Cytogenet Cell Genet,* 64 (1993) 282-285.

21 Bleck G T & Bremel R D, Correlation of the α-lactalbumin (+15) polymorphism to milk production and milk composition of Holsteins, *J Dairy Sci,* 76 (1993) 2292-2298.

22 Bleck G T, White B R, Miller D J & Wheeler M B, Production of bovine α-lactalbumin in the milk of transgenic pigs, *J Anim Sci,* 76 (1998) 3072-3078.

23 Kilara A & Panyam D, Peptides from milk proteins and their properties, *Crit Rev Food Sci Nutr,* 43 (2003) 607-633.

24 Markus C R, Olivier B, Panhuysen G E, Gugten J V D, Alles M S *et al*, The bovine protein α-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress, *Am J Clin Nutr,* 71 (2000) 1536-1544.

25 Cross M L & Gill H S, Immunomodulatory properties of milk, *Br J Nutr,* 84 (2000) S81-S89.

26 Sternhagen L G & Allen J C, Growth rates of a human colon adenocarcinoma cell line are regulated by the milk protein α-lactalbumin, *Adv Exp Med Biol,* 501 (2001) 115-120.

27 Matsumoto H, Shimokawa Y, Ushida Y, Toida T & Hayasawa H, New biological function of bovine α-lactalbumin: Protective effect against ethanol- and stress-induced gastric mucosal injury in rats, *Biosci Biotechnol Biochem,* 65 (2001) 1104-1111.

28 Fitzgerald R J, Murray B A & Walsh D J, Hypotensive peptides from milk proteins, *J Nutr,* 134 (2004) 980-988.

29 Nag M, Rahman M M, Bhuyan A A, Hossain M M K, Alim M A *et al*, Avian influenza resistant gene (*Mx)* and its diversity in chicken and duck, *Intl J Anim Biol* 1 (2015) 78-85.

30 Soria L A, Iglesias G M, Huguet M J & Mirande S L, A PCR-RFLP test to detect allelic variants of the bovine kappa-casein gene, *Anim Biotechnol,* 14 (2003) 1-5.

31 Voelker G R, Bleck G T & Wheeler M B, Single-base polymorphisms within the 5' flanking region of the bovine alpha-lactalbumin gene, *J Dairy Sci*, 80 (1997) 194-197.

32 Yeh C F, Yang R C and Boyle T, PopGen32 program. Canada: University of Alberta, 1999.

33 Volkandari S D, Indriawati I & Margawati E T, Genetic polymorphism of kappa-casein gene in Friesian Holstein: A basic selection of dairy cattle superiority, *J Indonesian Trop Anim Agric,* 42 (2017) 213-219.

34 Gouda E M, Galal Kh M & Abdelaziz S A, Genetic variants and allele frequencies of kappa casein in Egyptian cattle and buffalo using PCR-RFLP, *J Agril Sci*, 5 (2013) 197-203.

35 Gurses M & Yuce H, Determination of kappa casein gene polymorphisms and their effects on milk composition in some native cattle breeds of Turkey, *J Anim Vet Advan,* 11 (2012) 1023-1027.

36 Gurcan E K, Association between milk protein polymorphism and milk production traits in black and white dairy cattle in Turkey, *African J Biotechnol,* 10 (2011) 1044-1048.

37 Khaizaran Z A & Al-Razem F, Analysis of selected milk traits in Palestinian Holstein-Friesian cattle in relation to genetic polymorphism, *J Cell Anim Biol,* 8 (2014) 74-85.

38 Keating A F, Davoren P, Smith T J, Ross R P & Cairns M T, Bovine kappa-casein promoter haplotypes with potential implications for milk protein expression, *J Dairy Sci,* 90 (2007) 4092-4099.

39 Beata S, Wojciech N E J A & Ewa W, Relations between kappa-casein polymorphism (csn3) and milk performance traits in heifer cows, *J Centl European Agric,* 9 (2008) 641-644.

40 Trakovicka A, Moravčikova N & Navratilova A, Kappa-casein gene polymorphism (CSN3) and its effect on milk production traits, *Acta fytotechnica et zootechnica*, *Nitra, Slovaca Universitas Agriculturae Nitriae Suppl*, 3 (2012) 61-64.

41 Deb R, Singh U, Kumar S, Singh R, Sengar G *et al*, Genetic polymorphism and association of kappa-casein gene with milk production traits among Frieswal (HF X Sahiwal) cross breed of Indian origin, *Iran J Vet Res,* 15 (2014) 406-408.

42 Zepeda-Batista J L, Alarcon-Zuniga B, Ruiz-Flores A, Nunez-Dominguez R & Ramirez-Valverde R, Polymorphism of three milk protein genes in Mexican Jersey cattle, *Electron J Biotechnol*, 18 (2015) 1-4.

43 Trujillo E, Noriega D & Camargo M, Genotipificación de kappa-caseína bovina yevaluación de las frecuencias genotípicas y alélicas de sus polimorfismos en cuatrorazas, *Actual Biol,* 22 (2000) 145-52.

44 Jann O C, Ibeagha-Awemu E M, Özbeyaz C, Zaragoza P, Williams J L *et al*, Geographic distribution of haplotype diversity at the bovine casein locus, *Genet Sel Evol,* 36 (2004) 243-57.

45 Ren D X, Miao S Y, Chen Y L, Zou C X, Liang X W *et al*, Genotyping of the k-casein and β-lactoglobulin genes in Chinese Holstein, Jersey and water Buffalo by PCR-RFLP, *J Genet,* 90 (2011) 1-5.

46 Alim M A, Dong T, Xie Y, Wu X P, Yi Zhang *et al*, Effect of polymorphisms in the CSN3 (j-casein) gene on milk production traits in Chinese Holstein Cattle, *Mol Biol Rep,* 41 (2014) 7585–7593.

47 Morkūnienė K, Baltrėnaitė L, Puišytė A, Bižienė R, Pečiulaitienė N *et al*, Association of kappa casein polymorphism with milk yield and milk protein genomic values in cows reared in Lithuania, *Veterinarija IR Zootechnika*, 74 (2016) 27-32.

48 Caroli A M, Chessa S & Erhardt G J, Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition, *J Dairy Sci,* 92 (2009) 5335-5352.

49 Burbano G L Z, Cabrera Y M E, Portilla C E S & Galindo C Y R, Kappa casein genotypes and curd yield in Holstein cows, *Rev Colomb Ciene Pecu,* 23 (2010) 422-428.

50 Marziali A & Ng-Kwai-Hang K, Relationships between milk

protein polymorphisms and cheese yielding capacity, *J Dairy Sci,* 69 (1986) 1193-1201.

51 Vidović V, Nemes Z, Popović-Vranjes A, Lucač D, Cvetanovic D *et al*, Heritability and correlations of milk traits in the view of kappa-casein genotypes in Vojvodina Holstein-Friesian dairy cattle, *Mljekarstvo,* 63 (2013) 91-97.

52 Alipanah M, Kalashnikova L A & Rodionov G V, Kappa-casein and PRL-RsaI genotypic frequencies in two Russian cattle breeds, *Arch Zootech* 57 (2008) 131-138.

53 Rachagani S & Gupta I D, Bovine kappa-casein gene polymorphism and its association with milk production traits, *Genet Mol Biol*, 31 (2008) 893-897.

54 Bittante G, Penasa M & Cecchinato A, Genetics and modeling of milk coagulation properties, *J Dairy Sci* 95 (2012) 6843-6870.

55 Mao F C & Bremel R D, Distribution of bovine alpha-lactalbumin and k-casein genotypes in Taiwan, Pp 331-332. http://www.wcgalp.org/system/files/proceedings/1994/distribution-bovine-alpha-lactalbumin-and-kappa-casein-genotypes-taiwan.pdf. Accessed on 06/07/2018

56 Formaggioni P, Summer A, Malacarne M & Mariani P, Milk protein polymorphism: Detection and diffusion of the genetic variants in *Bos* genus, *Ann Fac Med Vet Un Parma,* 19 (1999) 127-165.

57 Aschaffenburg R, Genetic variants of milk proteins: Their breed distribution, *J Dairy Res*, 35 (1968) 447-460.

58 Sashikanth M A & Yadev B R, Alpha lactalbumin polymorphism in three breeds of Indian zebu cattle, *J Anim Breed Genet*, 115 (2011) 403-405.

59 Zhang J, Sun D, Womack J E, Zhang Y, Wang Y *et al*, Polymorphism Identification, RH mapping and association of α-lactalbumin gene with milk performance traits in Chinese Holstein, *Asian-Aust J Anim Sci,* 20 (2007) 1327-1333.

60 Zhou J P & Dong C H, Association between a polymorphism of the α-lactalbumin gene and milk production traits in Chinese Holstein cows, *Genet Mol Res,* 12 (2013) 3375-3382.