

Kappa Casein Genetic Variants in Holstein Dairy Cattle and their Association with Yield and Quality of Milk

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Abstract: Polymorphism of kappa casein protein (κ -casein) in total 54 Holstein cattle was investigated using Horizontal Starch-urea Gel Electrophoresis Method to verify its effect on milk production traits and influence of other factors such as parity and season of calving; aiming at utilizing it as a genetic aid in selection to improve the milk production traits of this breed. The allelic frequencies of A and B were found to be 0.352 and 0.648, respectively. Genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. General Linear Model (GLM) was used to analyze differences between genotypes. The results indicated that κ -casein genotypes significantly ($p < 0.05$) affected 305 days milk yield, 305 days fat yield and fat percentage. But it had no effect on actual milk yield, actual fat yield and lactation length. Parity number had significant effect on milk production traits. Season of calving had significant effect 305 days milk yield, actual fat yield, 305 days fat yield and fat percentage but it had no effect on actual milk yield and lactation length. This study indicated that the κ -casein genetic variants may be used as a genetic aid through increasing the frequency of desired genotypes to improve the milk production traits of this breed.

Key words: κ -casein protein polymorphism, milk production traits, holstein, frequency, GLM

INTRODUCTION

Milk production traits in cattle are quantitative traits being influenced by environmental and allelic variations at many loci. Improvement of milk yield and its composition is the primary goal for animal selection in dairy industry. Milk protein polymorphism has attracted intensive research interest because of its potential use as an aid to genetic selection of bovine breeds (Kemenes *et al.*, 1999; Wu *et al.*, 2005). Many researches have been made on milk protein polymorphisms to determine frequencies of genetic variants and their associations with milk yield, composition and quality (Erhardt, 1996; Di Stasio and Mariani, 2000; Martin *et al.*, 2002; Vohra *et al.*, 2006). This genetic polymorphism of milk proteins can be helpful in selection in breeding research. In dairy cows kappa-casein (κ -casein) main variants A and B have been reported as the most important milk protein allelic (Mercier *et al.*, 1972; Grosclaude *et al.*, 1987; Mayer *et al.*, 1997) and rare variants of κ -casein C (Mariani, 1990) and κ -casein E (Erhardt, 1989).

Relationships between milk protein polymorphism and milk production yield, composition and manufacturing properties have been investigated and described in different studies. Earlier researches showed that κ -casein alleles could be used as a useful marker in improving milk

yield and composition selection programs. Lin *et al.* (1986), Ng-Kwai-Hang and Monardes (1990), Bech and Kristiansen (1990), Khatkar *et al.* (2004), Tsiaras *et al.* (2005), Rachagani and Gupta (2008), Riaz *et al.* (2008) and Ju *et al.* (2008). However, many studies described that this relationship was not important for milk production traits (Ng-Kwai-Hang *et al.*, 1984; McLean *et al.*, 1984; Van Eenennaam and Medrano, 1991; Lunden *et al.*, 1997; Cervantes *et al.*, 2007; Abad-Zavaleta *et al.*, 2012). Therefore, the aim of the present study was to investigate the effect of κ -casein protein polymorphism on milk production traits in Holstein cattle.

MATERIALS AND METHODS

Milk samples obtained from 54 multiparous Holstein cows reared in Research and Application Farm of College of Agriculture, Ataturk University were used to determine κ -casein protein. All animals were maintained the Research and Application Farm of College of Agriculture, Ataturk University, Erzurum, Turkey under similar welfare and nutritional conditions. Totally 244 production records were obtained from Holstein cows. Four calving season were included such that every 3 months of the year starting from the last month of the previous year were considered as one group of seasons as winter, spring,

summer and autumn. Five groups for parity number were included in the model. Lactation milk yields records were adjusted according to Anonymous.

About 10 mL of milk was collected from each animal and 20 mg potassium dichromate was added to each sample as a preservative. Fat-free milk samples were stored in a refrigerator at 4°C until they were analysed. Two or three drops of 2-mercaptoethanol were added to samples before electrophoresis. Milk protein genotyping was carried out by using horizontal starch-urea gel electrophoresis (Aschaffenburg and Michalak, 1968; Dogru, 1994). Direct counting was used to estimate gene and genotypic frequencies of the κ-casein proteins. The χ²-test was used to check whether the population was in Hardy-Weinberg equilibrium (Soysal, 1998). The data on the milk production traits of the different κ-casein genotypes were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) from the Statistical Analysis Software (SPSS Statistics 17.0). The following statistical model used was:

$$Y_{ijkl} = \mu + G_i + A_j + S_k + e_{ijkl}$$

Where:

- Y_{ijkl} = The observation on each trait of the ijklth animal
- μ = The general mean of each trait
- G_i = The fixed effect of ith κ-casein genotype (i = 1, 2, 3)
- A_j = The fixed effect of jth parity number (j = 1, 2, ..., 5; parity number > 5 were pooled with parity of 5)
- S_k = The fixed effect of the kth season of calving (k = 1, 2, ..., 4)
- e_{ijkl} = The random error effect associated to the ijklth observation

RESULTS AND DISCUSSION

The aim of this study was to identify κ-casein A and κ-casein B alleles and κ-casein AA, κ-casein AB and

κ-casein BB genotypes of κ-casein in a population of Brown-Swiss cows. Out of 54 studied cows, genotypic frequencies of κ-casein genotypes were: 12 cows of the κ-casein AA genotype, 14 of genotype AB and 28 of genotype BB. χ²-test for deviations from the Hardy-Weinberg equilibrium were carried out to determine statistical significance. Deviations from the Hardy-Weinberg equilibrium was significant (χ² = 10.05).

Table 1 shows the effect of κ-casein genotypes, parity and season of calving on milk production traits in Holstein cattle. The results indicated that κ-casein genotypes had significant (p<0.05) effect on 305 days milk yield, 305 days fat yield and fat percentage but it did not affect actual milk yield, actual fat yield and lactation length. It was observed that cows with genotype BB had significantly higher 305 days milk yield (3491.6 kg) than those of genotypes AA and AB (3301.2 and 3274.6 kg, respectively) and also had insignificantly higher actual milk yield (3797.6 kg) than those of other two genotypes (3658.9 and 3559.2 kg, respectively). The result indicate similarities with those of different researchers (Ng-Kwai-Hang and Monardes, 1990; Curic *et al.*, 1993; Lu *et al.*, 1995; Chung *et al.*, 1996; Ng-Kwai-Hang, 1998; Messina *et al.*, 1999; Khatkar *et al.*, 2004; Rachagani and Gupta, 2008) while they disagree with the result of some others who claimed that κ-casein (McLean *et al.*, 1984; Van Eenenaam and Medrano, 1991; Zitny *et al.*, 1996; Ng-Kwai-Hang, 1998; Lunden *et al.*, 1997; Ju *et al.*, 2008).

The results in Table 1 show that parity number had significant effect on all examined milk production traits. Season of calving had significant effect on 305 days milk yield, actual fat yield, 305 days fat yield and fat percentage. However, it had no effect on actual milk yield and lactation length. Milk production traits in cattle are quantitative traits being influenced by genetic and environmental factors.

Table 1: Effect of κ-casein genotypes, parity number and season of calving on milk production traits (least square mean±standard error)

Parameters	N	Actual milk yield (kg)	305 days milk yield (kg)	Actual fat yield (kg)	305 days fat yield (kg)	Fat in milk (%)	Lactation length (day)
κ-casein genotype							
AA	48	3658.9±173.7 ^{NS}	3301.2±126.5 ^{ab}	135.4±12.1 ^{NS}	125.9±5.6 ^b	3.65±6.6 ^b	330.1±12.8 ^{NS}
AB	52	3559.2±166.4	3274.6±121.2 ^b	137.2±11.6	115.6±5.4 ^b	3.50±6.3 ^b	316.5±12.2
BB	144	3797.6±98.60	3491.6±71.80 ^a	149.1±6.80	135.0±3.2 ^a	3.78±3.7 ^a	325.7±7.30
Parity							
1	54	3439.1±160.9 ^b	3046.8±117.2 ^c	127.8±11.2 ^{ab}	121.7±5.2 ^b	3.76±6.1 ^a	349.5±11.8 ^a
2	56	3681.7±157.8 ^{ab}	3272.4±114.9 ^b	137.6±10.9 ^{ab}	125.7±5.1 ^{ab}	3.71±6.0 ^a	341.9±11.6 ^a
3	44	3809.6±180.0 ^{ab}	3540.5±131.1 ^{ab}	157.0±12.5 ^a	126.8±5.8 ^{ab}	3.54±6.8 ^{ab}	314.3±13.2 ^{ab}
4	27	4007.8±230.4 ^a	3750.9±167.8 ^a	144.2±16.0 ^{ab}	139.6±7.5 ^a	3.61±8.7 ^{ab}	321.0±16.9 ^{ab}
5	63	3421.3±152.6 ^b	3168.3±111.1 ^c	119.7±10.6 ^b	114.0±4.9 ^b	3.50±5.8 ^b	293.7±11.2 ^b
Season of calving							
Spring	73	3460.4±137.1 ^{NS}	3025.1±99.90 ^b	122.4±9.50 ^b	115.1±4.4 ^b	3.49±5.2 ^b	321.0±10.1 ^{NS}
Summer	56	3766.0±167.3	3352.5±121.9 ^{ab}	155.3±11.6 ^a	129.9±5.4 ^a	3.71±6.4 ^a	327.9±12.3
Autumn	48	3676.5±175.5	3370.2±127.8 ^{ab}	136.4±12.2 ^{ab}	125.2±5.7 ^{ab}	3.64±6.7 ^a	320.4±12.9
Winter	67	3784.7±153.8	3495.3±112.0 ^a	134.9±10.7 ^{ab}	132.0±4.9 ^a	3.66±5.8 ^a	317.2±11.3
Overall	244	3671.9±88.80	3355.8±64.80	137.3±6.20	125.5±2.9	3.64±3.4	324.1±6.50

NS: Non-Significant; figures bearing different superscripts in a column for each parameter differ significantly (p<0.05)

CONCLUSION

This study demonstrated that κ -casein genotypes had significant effect on 305 days milk yield, 305 days fat yield and fat percentage but it did not affect actual milk yield, actual fat yield and lactation length. Further, researches with large numbers of animals and different breeds are required to investigate these relationship between κ -casein genotypes and milk yield, composition and manufacturing properties. This study also indicated that the κ -casein protein genetic variants may be used as a genetic aid through increasing the frequency of desired genotypes to improve the yield and quality of production in Holstein.

REFERENCES

- Abad-Zavaleta, J., S. del Moral, Cortez-Lopez, N.G., J.A. Rueda, C. Luna-Palomera and C.A. Meza-Herrera, 2012. Allelic and genotypic frequency of kappa casein gene in double purpose cattle. *Trop. Subtrop. Agroecosyst.*, 15: 47-55.
- Aschaffenburg, R. and W. Michalak, 1968. Simultaneous phenotyping procedure for milk proteins: Improved resolution of the β -Lactoglobulins. *J. Dairy Sci.*, 51: 1319-1320.
- Bech, A.M. and K.R. Kristiansen, 1990. Milk protein polymorphism in Danish dairy cattle and the influence of genetic variants on milk yield. *J. Dairy Sci.*, 57: 53-62.
- Cervantes, P., M. Luna, A. Hernandez, F. Perez-Gil, P. Ponce and O. Uffo, 2007. Genetic polymorphism in the kappa-casein locus in cows of different breeds and crossbreeds in the Mexican tropic. *Revista Salud Animal*, 29: 78-84.
- Chung, H.Y., H.K. Lee, K.J. Chen, C.H. You, K.D. Park, K.N. Kim and E.R. Chung, 1996. Studies on the relationships between biochemical polymorphism and production traits in dairy cattle. *Korean J. Dairy Sci.*, 18: 7-16.
- Curic, I., M. Kaps, J. Lukac-Havranek and N. Antunac, 1993. Associations of milk protein genotypes with first lactation traits in Croatian Simmentals. I effects of genotype. *Stocarstvo*, 471: 15-22.
- Di Stasio, L. and P. Mariani, 2000. The role of protein polymorphism in the genetic improvement of milk production. *Zootecnica e Nutrizione Animale*, 26: 69-90.
- Dogru, U., 1994. Genetic structure of several polymorphic blood and milk proteins of Brown-Swiss, Holstein, Simmental and Eastern Anatolian Red cattle breeds and their differences according to several production traits. Ph.D. Thesis, Ataturk University, University in Erzurum, Turkey.
- Erhardt, G., 1989. Kappa-casein in bovine milk-evidence of a further allele (κ -Cn E) in different breeds. *J. Anim. Breed. Genet.*, 106: 225-231.
- Erhardt, G., 1996. Detection of a new kappa-casein variant in milk of Pinzgauer cattle. *Anim. Genet.*, 27: 105-107.
- Grosclaude, F., M.F. Mahe and J.P. Ascolas, 1987. Note on genetic polymorphism of milk protein in Mongolian cattle and yaks. *Anim. Breed. Abst.*, 55: 4318-4318.
- Ju, Z., Q. Li, H. Wang, J. Li and O. An *et al.*, 2008. Genetic polymorphism of K-casein gene exon4 and its correlation with milk production traits in Chinese Holsteins. *Hereditas*, 10: 1312-1318.
- Kemenes, P.A., L.C.A. Regitano, A.J.M. Rosa, I.U. Paker and A.G. Razook *et al.*, 1999. κ -casein, β -lactoglobulin and growth hormone allele frequencies and genetic distances in Nelore, Gyr, Guzera, Caracu, Charolais, Canchim and Santa Gertrudis cattle. *Genet. Mol. Biol.*, 22: 539-541.
- Khatkar, M.S., P.C. Thomson, I. Tammen and H.W. Raadsma, 2004. Quantitative trait loci mapping in dairy cattle: Review and meta-analysis. *Genet. Sel. Evol.*, 36: 163-190.
- Lin, C.Y., A.J. McAllister, K.F. Ng-Kwai-Hang and J.F. Hayes, 1986. Effects of milk protein loci on first lactation production in dairy cattle. *J. Dairy Sci.*, 69: 704-712.
- Lu, J., H. Qiu and S. Wang, 1995. Genetic effects of milk protein loci on first lactation traits. *Acta Veterinaria et Zootechnica Sinica*, 27: 308-314.
- Lunden, A., M. Nilsson and L. Janson, 1997. Marked effect of β -lactoglobulin polymorphism on the ratio of casein to total protein in milk. *J. Dairy Sci.*, 80: 2996-3005.
- Mariani, P., 1990. Genetic polymorphism of casein in Italian Brown cows, Frequency of C variant at the κ -Cn lokus. *Anim. Breed. Abst.*, 58: 5040-5040.
- Martin, P., M. Szymanowski, L. Zwierzchowski and C. Leroux, 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reprod. Nutr. Dev.*, 42: 433-459.
- Mayer, H.K., A. Marchler, C. Prohaska and R. Norz, 1997. Milk protein polymorphism in Austrian dairy cattle breeds. *Milchwissenschaft*, 52: 366-369.
- McLean, D.M., E.R. Graham, R.W. Ponzoni and H.A. McKenzie, 1984. Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res.*, 51: 531-546.
- Mercier, J.C., F. Grosclaude and B.D. Ribadeau, 1972. Primary structure of Bovine casein: A review. *Milchwissenschaft*, 27: 402-408.

- Messina, M., E. Vrech, P. Pezzi and A. Prandi, 1999. Genetic markers associated with the somatotropin axis and milk protein polymorphism. *Scienza Tecnica Lattiero Casearia*, 50: 231-240.
- Ng-Kwai-Hang, K.F. and M. Monardes, 1990. Association between genetic polymorphism and milk production and composition. Proceedings of the Brief Communications of the 23rd International Dairy Congress, October 8-12, 1990, Montreal, Canada, pp: 65.
- Ng-Kwai-Hang, K.F., 1998. Genetic polymorphism of milk proteins: Relationships with production traits, milk composition and technological properties. *Can. J. Anim. Sci.*, 78: 131-147.
- Ng-Kwai-Hang, K.F., J.F. Hayes, J.E. Moxley and H.G. Monardes, 1984. Association of genetic variants of casein and milk serum proteins with milk, fat and protein production by dairy cattle. *J. Dairy Sci.*, 67: 835-840.
- Rachagani, S. and I.D. Gupta, 2008. Bovine kappa-casein gene polymorphism and its association with milk production traits. *Gen. Mol. Biol.*, 31: 893-897.
- Riaz, M.N., N.A. Malik, F. Nasreen and J.A. Qureshi, 2008. Molecular marker assisted study of kappa-casein gene in Nili-Ravi (buffalo) breed of Pakistan. *Pak. Vet. J.*, 28: 103-106.
- Soysal, M.I., 1998. Statistical methods. Agricultural Faculty Public No. 95, Notice No. 64, Namik Kemal University, Tekirdag, Turkey, (In Turkish).
- Tsiaras, A.M., G.G. Bargouli, G. Banos and C.M. Boscos, 2005. Effect of kappa-casein and beta-lactoglobulin loci on milk production traits and reproductive performance of Holstein cows. *J. Dairy Sci.*, 88: 327-334.
- Van Eenennaam, A. and J.F. Medrano, 1991. Milk protein polymorphisms in California dairy cattle. *J. Dairy Sci.*, 74: 1730-1742.
- Vohra, V., T.K. Bhattacharya, S. Dayal, P. Kumar and A. Sharma 2006. Genetic variants of β -lactoglobulin gene and its association with milk composition traits in riverine buffalo. *J. Dairy Res.*, 73: 499-503.
- Wu, X.L., M.D. MacNeil, S. De and Q.J. Xiao, 2005. Evaluation of candidate gene effects for beef backfat via Bayesian model selection. *Genetica*, 125: 103-113.
- Zitny, J., A. Trakovicka, A. Kubek, E. Michalickova and I. Ostertag, 1996. Differences in milk production efficiency of Slovakian Pied dairy cows with different kappa-casein genotypes. *Zivocisna Vyroba*, 41: 533-538.