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Analysis of Polymorphism in Kappa-Casein and β-Lactoglobulin Genes in Egyptian Buffalo Bulls

Manal M. El-Bramony and M. A. Abo-Farw

Buffalo Breeding Research Department, Animal Production Research Institute, Agricultural Research Center, 12618, Dokki, Giza, Egypt

Abstract: Knowledge of polymorphic milk protein genes has a great economic importance in artificial insemination (AI) protocols for improving milk composition traits in native dairy livestock. The identification of polymorphism for both kappa-casein (κ-CN) and lactoglobulin (β-LG) genes and find for its association with genetic values of the important economic traits in Egyptian buffalo bulls was the objective of this study. Blood and fresh semen samples were collected from buffalo bulls experimental herds. Only one restriction pattern for both κ-CN and β-LG genes was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique with all the studied bulls. The positive findings of a share for both κ-CN and β-LG allele A and genotypes AA were detected among the bulls tested for milk yield, milk protein and milk fat yield. Consequently, accuracy of using milk protein genes to improve AI schemes differs according to the available phonotypical records, if the allelic association with interest economic traits is determined.

Key words: Artificial insemination · Kappa-casein · β-lactoglobulin · PCR-RFLP · Phonotypical records

INTRODUCTION

The selection of bulls for artificial insemination contributes largely to decisions affecting genetic progress of dairy cattle schemes [1]. In progeny testing protocols, the use of molecular markers as additional information to early predict the genetic value of young dairy bull has received considerable attention. In particular, molecular data provides information on inheritance of major qualitative loci, such as genetic defects or exact genotypes for desired qualitative traits [2]. The use of marker data as additional tool to improve breeding schemes depends on the phenotypic records available for dairy domestic populations. An association between a candidate gene polymorphism and the trait of interest is taken as evidence that the gene is involved in the genetic control of the trait [3]. Genetic polymorphism of milk proteins has been of great interest in animal breeding schemes and the dairy products for milk production traits [4-6].

In particular, kappa casein (κ-CN) and β-lactoglobulin (β-LG) genes have been associated genetically with milk production and composition of the milk protein fractions in dairy species [4-13]. The κ-CN and β-LG genes presence with common alleles are A and B genes being the most frequent. Thus, polymorphisms of these genes can be used for improvement accuracy estimation of breeding values of the production traits by selection programs [14]. In Egyptian buffalo population, design of the breeding schemes has focused on phenotypic data of predicted genetic values for quantitative traits such as milk yield. The bulls are evaluated, based on the phenotypic data available of their daughters and ancestors. This study aimed to find a polymorphism for both κ-CN and β-LG genes and search for its association with genetic values expressed by the breeding values of their daughters and ancestors (parents, grandfather and grandmother) with regard to milk yield, fat and protein yield and its content.

MATERIALS AND METHODS

The study was included sixteen buffalo bulls born between 2004 and 2012 progeny of 11 sires and 15 dams originated from experimental herds belonging to the Animal Production Research Institute (APRI), Agricultural Research Center (Mehalet Mousa, Kafr El-Sheikh Governorate and Egypt).

Corresponding Author: Manal M. El-Bramony, Buffalo Breeding Research Department, Animal Production Research Institute, Agricultural Research Center, 12618, Dokki, Giza, Egypt. E-mail: m.elbramony@gmail.com.

Management of Experimental Bulls Population: Bulls were housed in semi-open sheds and were kept under the regular system of feeding and management adopted by APRI. Bulls were fed formulated diets on the basis of adult buffalo bull requirements. They were fed Egyptian clover (*Trifoliumalx andrinum*); rice straw and concentrate feed mixture (CFM) during winter-spring period. During summer-autumn period; they were fed rice straw, the same CFM, maize silage and Egyptian clover hay. Mineral salt and vitamins was offered regularly. Feeds were given individually twice daily and clean water was available *ad libitum*. Drugs against diseases and parasites were applied twice a year. The body weights of bulls were recorded at sampling ranged between 380 and 750 kg.

Sampling and DNA Isolation: Blood samples (5-mL) were obtained from jugular vein into K_2EDTA sterile vacuum tubes (Vacutainer). About (3mL) semen was collected by sterile tubes. The isolation of DNA from whole blood and semen samples was done to the procedure (QIAampDNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's protocol. Briefly, 200 µl of the sample suspension was incubated with10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's protocol. Nucleic acid was eluted with 100 µl of elution buffer provided with the kit.

PCR Reaction and DNA Amplification: DNA fragment of κ-CN gene was amplified as described [15] with the Following nucleotide sequence: F: 5'-ATCATTTATGGCCATTCCACCAAAG-3' and R: 5'-GGCCATTTCGCCTTCTCTGTAACAGA-3'. Similarly, DNA fragment of buffalo β-LG gene was amplified as described [16] with the following nucleotide sequence: F: 5'-TGTGCTGGACACCGACTACAAAAA-3' and R: 5'-GCT CCCGGTATATGACCACCCTCT-3'.Primerswereutilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmolconcentration, 5.5 µl of water and 5 µl of DNA template. The reactions were performed in an applied biosystem 2720 thermal cycler (initial denaturation 94°C, 5 min; denaturation 94°C, 30 s; annealing 58°C, 45 s; elongation 72°C, 45 s; 35 cycles, final extending 72°C, 10 min). All the amplified DNA fragments were quantified by visualizing it on 0.8% agarose gel under UV lighting.

RFLP Analysis: The amplified κ-CN fragment gene was 350 bp and it was digested with restriction endonuclease *Hinf*I. The amplified β-LG fragment gene was 247 bp and it was digested with restriction endonuclease *Hae*III. The reaction done in a total volume of 10 µL amplified DNA fragment was digested separately with 1 µ each of *Hinf*I and *Hae*III restriction enzymes, 2 µL enzyme buffer (10X FastDigest Green buffer) and 17µl water, nuclease-free separate reaction tubes. Then the reaction was done at 37°C for 5 min in a thermoshaker (Biometra). After incubation, the fragments were separated to electrophoresis in an (1.5%) agarose gel (Applichem, Germany, GmbH), 1x TBE buffer at room temperature using gradients of 5V/cm. Gelpilot 100 bp DNA ladder (Qiagen, Germany, GmbH) and Generuler 50 bp ladder (Fermentas, Thermo Scientific, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Statistical Analysis: Breeding values for milk yield and its contents of the bulls genotyped over the period 1999-2010 were obtained from El-Bramony [17]. The statistical analysis was performed using the mixed model as follows:

$$y_{ijk} = \mu + g_i + b\,(X_{ijk} - \overline{X}) + a_{ij} + e_{ijk}$$

where, y_{ijk} is the estimated breeding value for milk yield or fat, protein yield or content of the bull; µ is the overall mean; g_i is the effect of their daughters and ancestors i; b is the linear regression coefficient of estimated breeding values on the year of bull birth; X_{ijk} is the year of bull birth; $\overline{X}$ is the average of the year of bull birth; a_{ij} is the random effect of the bull j nested within their daughters and ancestor i and e_{ijk} is the effect of random residual normally and independently distributed as $(0, I\sigma^2_e)$.

RESULTS AND DISCUSSION

PCR-RFLP Analysis of κ-CN and β-LG Genes: Genomic DNA was subjected to amplification with specific primers for both κ-CN and β-LG genes using the PCR technique. Amplified DNA fragment of κ-CN gene using a primer by PCR analysis was product 350 bp from blood (Figure 1) and semen samples (Figure 2). Similarly, Amplified DNA fragment of β-LG gene using a primer by PCR analysis was product 247 bp from blood (Figure 3) and semen samples (Figure 4). Digestion of amplified DNA fragment κ-CN gene with restriction endonuclease *Hinf*I generated two fragments of 132 and 84 bp (Figure 5 and Figure 6). For amplified DNA fragment β-LG gene also, generated two fragments of 148 and 99 bp with restriction endonuclease *Hae*III (Figure 7 and Figure 8).

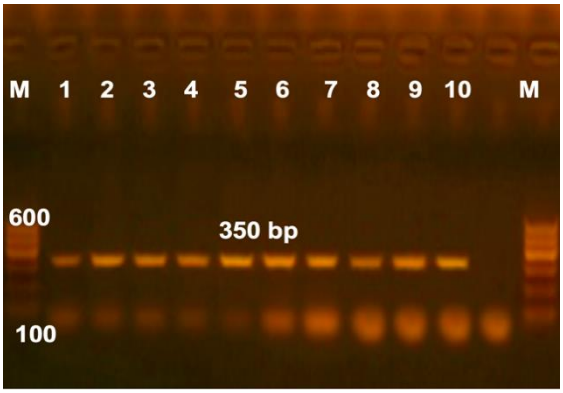

Fig. 1: Electrophoresis of the amplification a DNA fragment of κ-CN gene using a specific primer, M: ladder marker, lanes: 1, 2…, 10 of buffalo bulls

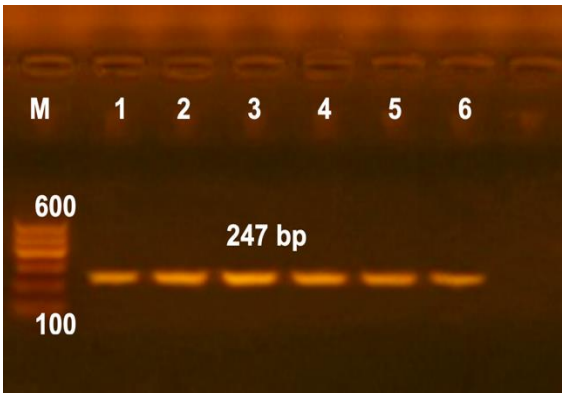

Fig. 4: Electrophoresis of the amplification a DNA fragment of β-LG gene using a specific primer, M: ladder marker, lanes: 1, 2…, 6 of buffalo bulls

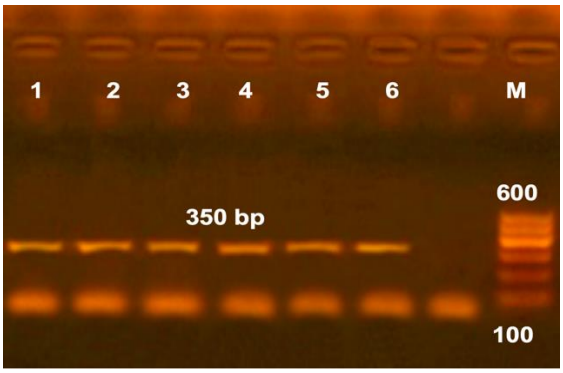

Fig. 2: Electrophoresis of the amplification a DNA fragment of κ-CN gene using a specific primer, M: ladder marker, lanes: 1, 2…, 6 of buffalo bulls

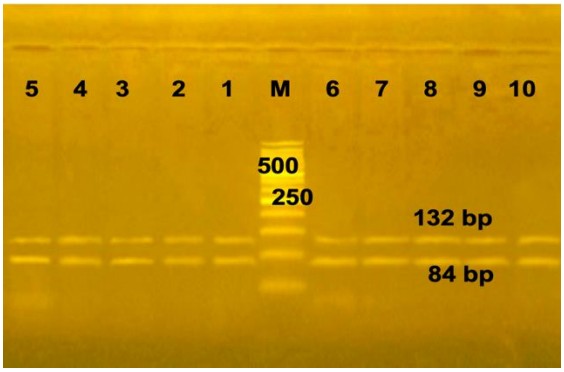

Fig. 5: The electrophoretic gel pattern of the digestion amplified DNA fragment κ-CN by restriction endonuclease *Hinf*I, M: ladder marker, lanes: 1, 2…, 10 are homozygous genotype AA from blood samples of buffalo bulls

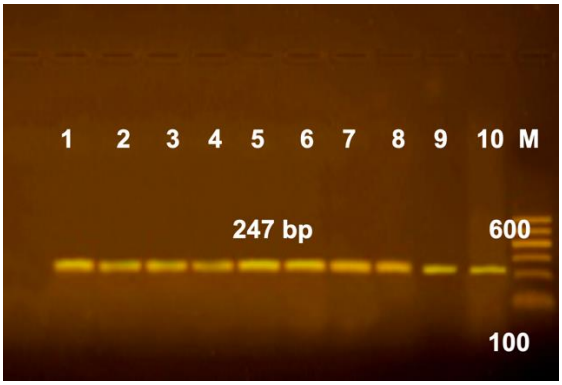

Fig. 3: Electrophoresis of the amplification a DNA fragment of β-LG gene using a specific primer, M: ladder marker, lanes: 1, 2…, 10 of buffalo bulls

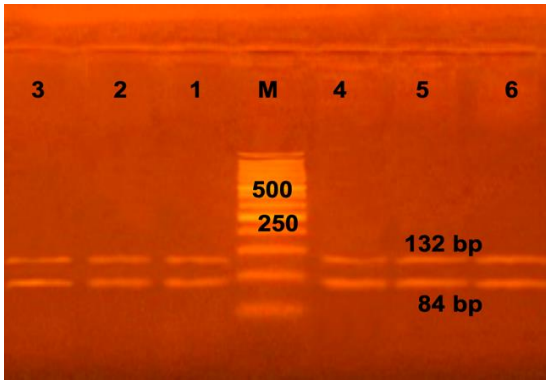

Fig. 6: The electrophoretic gel pattern of the digestion amplified DNA fragment κ-CN by restriction endonuclease *Hinf*I, M: ladder marker, lanes: 1, 2…, 6 are homozygous genotype AA from semen samples of buffalo bulls

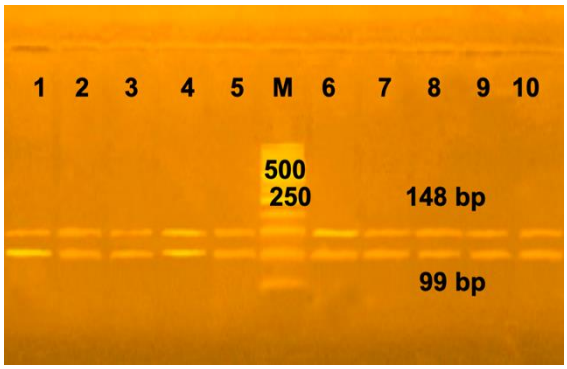

Fig. 7: The electrophoretic gel pattern of the digestion amplified DNA fragment β-LG by restriction endonuclease *Hae*III, M: ladder marker, lanes: 1, 2…, 10 are homozygous genotype AA from blood samples of buffalo bulls

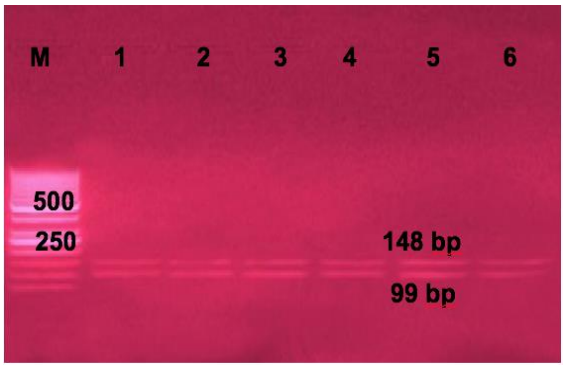

Fig. 8: The electrophoretic gel pattern of the digestion amplified DNA fragment β-LG by restriction endonuclease *Hae*III, M: ladder marker, lanes: 1, 2…, 6 are homozygous genotype AA from semen samples of buffalo bulls

Table 1: Least squares mean LSM (± standard errors) of estimated breeding values for milk composition traits of genotyped buffalo bulls

	Breeding value		
Trait	LSM	SE	No. of bull
Milk yield, kg	+30.4	51.7	16
Protein content, %	-0.03	0.06	16
Protein yield, kg	+1.3	1.9	16
Fat content, %	-0.01	0.03	14
Fat yield, kg	+2.1	1.7	14

These findings showed the existence of one allele A: for both κ-CN and β-LG genes, with all the tested bulls being homozygous for these genes. In general, only one restriction pattern could be detected among the bulls studied with κ-CN and β-LG genotypes AA. However, allele B: for both κ-CN and β-LG genes was not detected in the observed buffalo bulls. A different finding was found in Egyptian buffalo bulls by Abdel Dayem *et al.* [18] for κ -CN. In another study, Patel *et al*. [12] found the presence of κ-CN BB and AB genotypes in River buffalo bulls. Moreover, they detected β-LG AA, BB and AB genotypes among their bulls.

The least-squares mean (±standard errors) of the estimated breeding values for milk yield, fat and protein yield or content of all the tested bulls having both κ-CN and β-LG genotypes AA are shown in Table 1. The average breeding value was +30.4 kg and values ranged between -135 and +122 kg for milk yield among the bulls genotyped.

The positive estimate is higher than that obtained by Catillo *et al.* [19]; +10 kg of Italian bulls. Also, the mean breeding values for both protein and fat yield were +1.3 and +2.1 kg, respectively (Table 1) and values ranged between -2.3 and +5.9 and between -9.0 and +8.2 kg, respectively. Within the bulls tested, negative average breeding values were observed for both protein and fat content. Estimates were -0.03 and -0.01%, respectively (Table 1) and values ranged from -0.20 and +0.15% for protein % and from -0.66 to +0.27% for fat %.

In dairy bulls, Sabour *et al*. [8] and Kaminski *et al*. [20] reported using an association of bull genetic genotypes and their breeding values rather than comparison with the phenotypic values of milk yield traits. Association study showed that highest breeding values for milk yield related to κ-CN genotype AA in comparison with other genotypes BB and AB [20] in cattle bulls. While, lowest breeding values associated with κ-CN genotype AA for protein content. In contrast, Kučerova *et al*. [10] reported that AA κ-CN genotype was associated with the low average breeding value. The highest breeding values for both milk and protein yield were associated with genotype combination AB κ-CN and AA β-LG [11].

This finding is consistent with the finding of Kučerova et al. [10]. Who reported that the highest breeding values for milk and protein yield related to β-LG genotype AA. Kučerova *et al*. [10] and Matějíček *et al.* [11] found that the highest breeding values related to genotype AB β-LG for protein and fat content. In this context, Sabour *et al.* [8] suggest that increasing the

frequency of the variant A of β-LG in young bulls through progeny testing schemes improves genetic values of the bulls, but the expected genetic response is limited. In another study by Patel *et al*. [12] indicated predominance of B allele for κ-CN and β-LG with their allelic frequency 0.984 and 0.595 respectively, in River buffalo bulls.

Generally, according to literature, the common alleles for the κ-CN and β-LG genes are A and B with the most frequency of one allele B: in other buffalo populations.

CONCLUSION

Only one restriction pattern was observed among the bulls tested for both κ-CN and β-LG genes. These findings showed the existence of one allele A: for both κ-CN and β-LG genes, with all the studied bulls being homozygous for these genes. For young bulls, these findings provide early information in animal breeding schemes for improving milk composition traits. However, allele B: was not detected among the bulls studied.

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