

Effects of *Dgat1*, *Leptin* and *Kappa Casein* Gene Variants on Milk Production in Sudanese Cattle

Siham Rahmatalla¹, Monika Reißmann², Balgees Atta Elmnan³, Ibrahim A. Ishag⁴, Gudrun A. Brockmann²

Department of Dairy Production¹, Nutrition³, and Animal Breeding⁴, University of Khartoum, Sudan

Department for Crop and Animal Sciences², Humboldt University of Berlin, Invalidenstrasse 42, D-10115 Berlin, Germany



Introduction

- Diacylglycerol acyltransferase1 gene (*DGAT1*), encodes a microsomal enzyme that catalyzes the terminal and committed step of triacylglycerol biosynthesis.
- became a functional candidate gene for lactation traits after studies indicated that female mice lacking both copies of *DGAT1* (gene knockout) did not lactate
- *DGAT1* has been mapped to a region on bovine chromosome 14 close to the centromere.
- known as strong positional and functional candidate gene affecting milk production traits.
- polymorphism in the promoter variable number of tandem repeat (VNTR) of *DGAT1* locus were associated with milk fat content in animals homozygous for the allele 232A.
- In cattle, the *Leptin* (*Lep/ob*) gene has been mapped to chromosome 4 and performs important roles in the regulation of food intake, fertility, immune functions and energy balance.
- a polymorphism located in the second intron (C/T transition) in *lep* gene is associated with milk production in dairy cattle and might affect feed intake and live weight.
- *Kappa casein* gene is located on chromosome 6 and involved in a number of important physiological processes.
- The mutation characterizing the A and B variants are located relatively closely to several glycosylation sites and probably affect the structure of the protein and glycosylation patterns.

Results

- At the *DGAT1* promoter VNTR, three different alleles were found in the Sudanese dairy cattle and denoted as 3, 4, and 5 according to their fragment length (**Figure 2**).
- Three different alleles were segregating in Butana dairy cattle, while only two alleles were found on Kenana dairy cattle.
- 33 genotype is the most frequent genotype of *DGAT1* VNTR mutation (**Table 1**).
- *DGAT1* promoter VNTR genotypes show significant effects on fat content ($P < 0.05$) in Butana and Kenana cows.
- Fat content is highest in homozygous 33 cows in both breeds, and significantly differing from other genotype [**Figure 3**].
- The VNTR allele 3 contributed to increase the fat and protein content (**Table 2**)
- For the *Leptin* *MboI* RFLP, the *MboI* digestion of the 400 bp PCR product produced two alleles, namely A and B.

Objectives

1. estimate the genotypes and alleles frequencies of the *DGAT1*, *Lep/ob*, and *CSN3* polymorphisms in two native Sudanese local cattle breeds.
2. investigate the relationships between the polymorphisms within the target genes in this study and milk yield and composition.
3. estimate the genetic and allelic effects of the gene variants on milk traits.

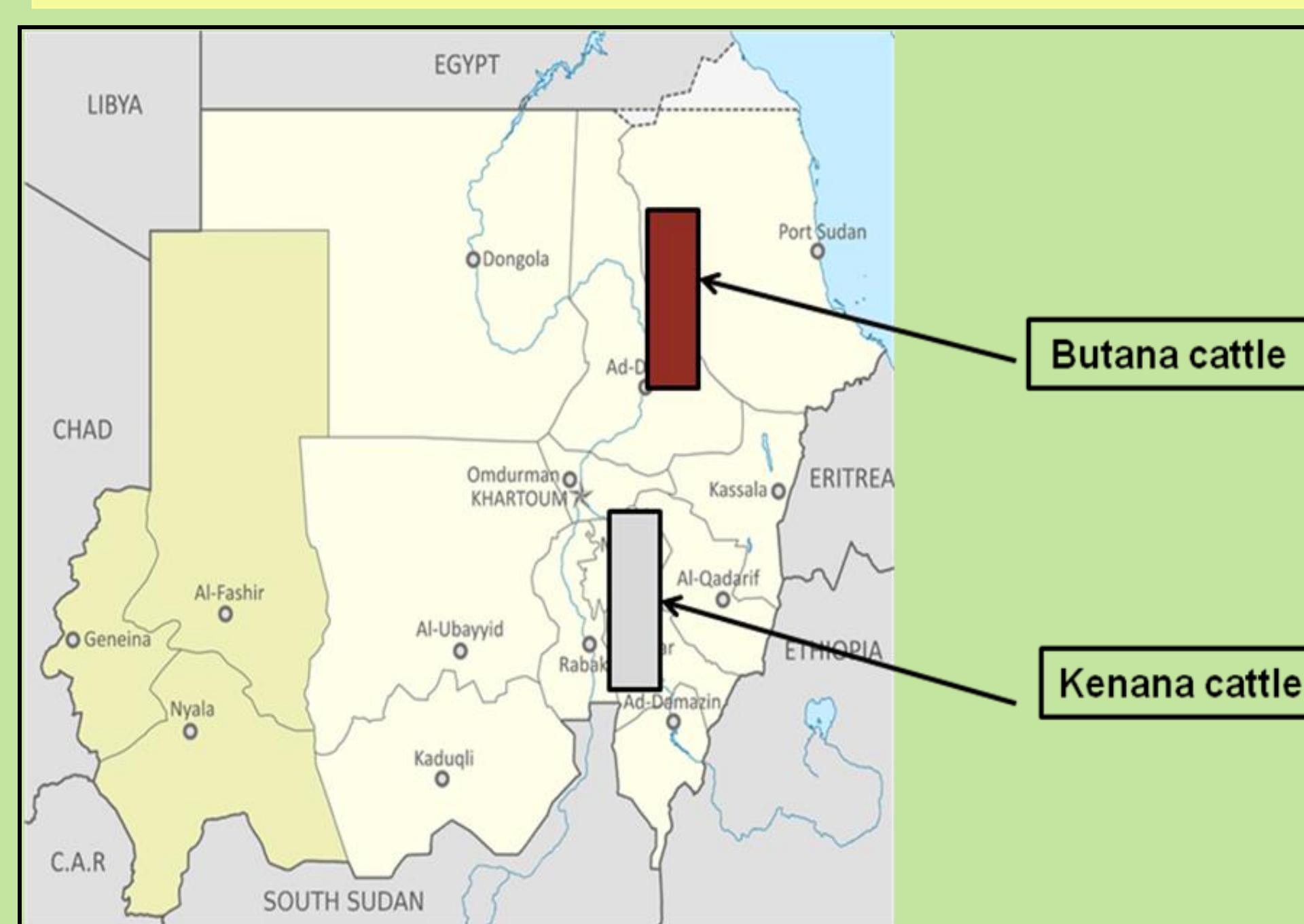


Figure 1: Location of Butana and Kenana dairy cattle in Sudan

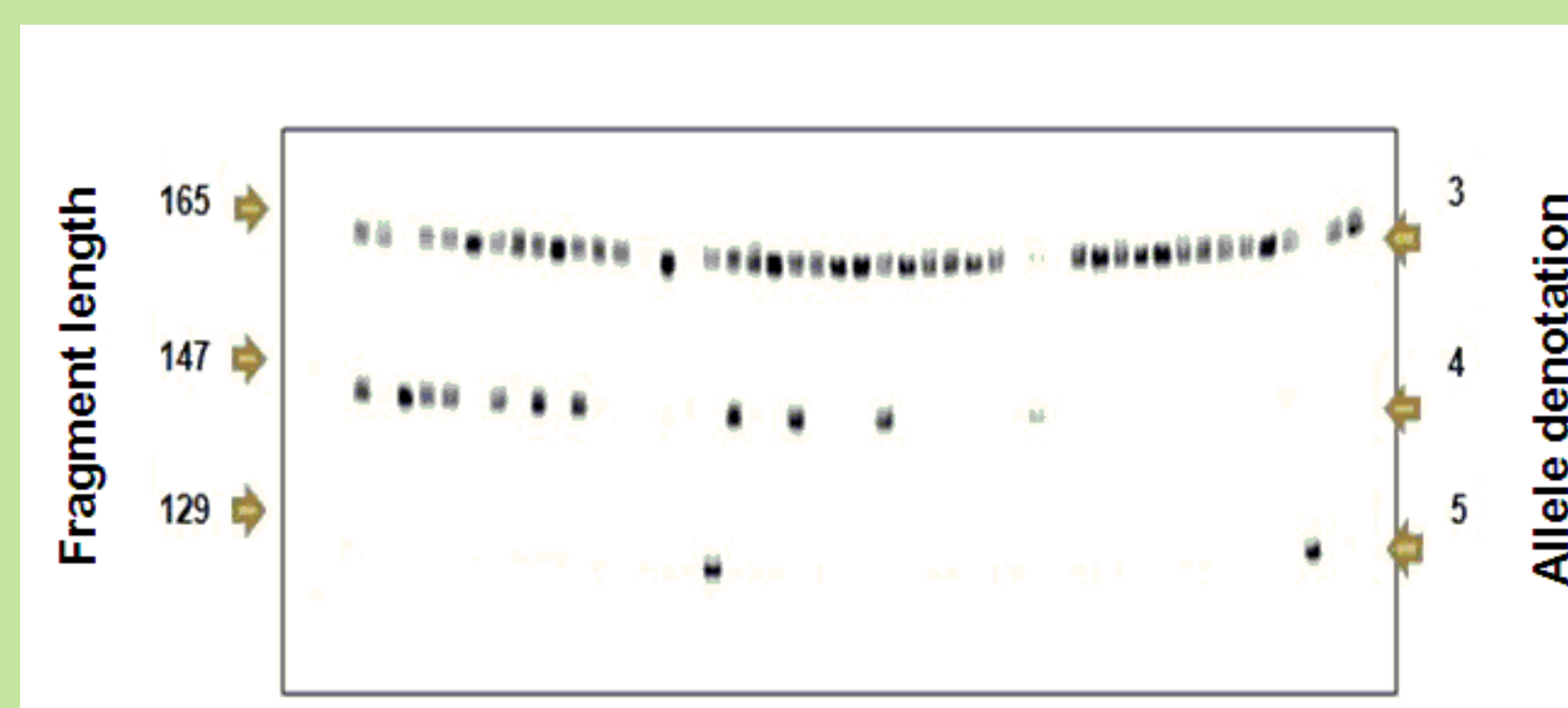


Figure 2: Polyacrylamide gel separating the 3 alleles detected of *DGAT1* promoter VNTR site

Breed	<i>DGAT1</i> VNTR			<i>Lep-MboI</i>		<i>CSN3</i>	
	3	4	5	A	B	A	B
Butana	70.5	27.2	2.5	97.5	2.5	86.3	13.8
Kenana	81.2	18.8	-	97.1	2.9	89.3	10.7

Table 1: Allele frequencies of the *DGAT1* VNTR, *Lep MboI* RFLP, *CSN3* polymorphism in Butana and Kenana cows

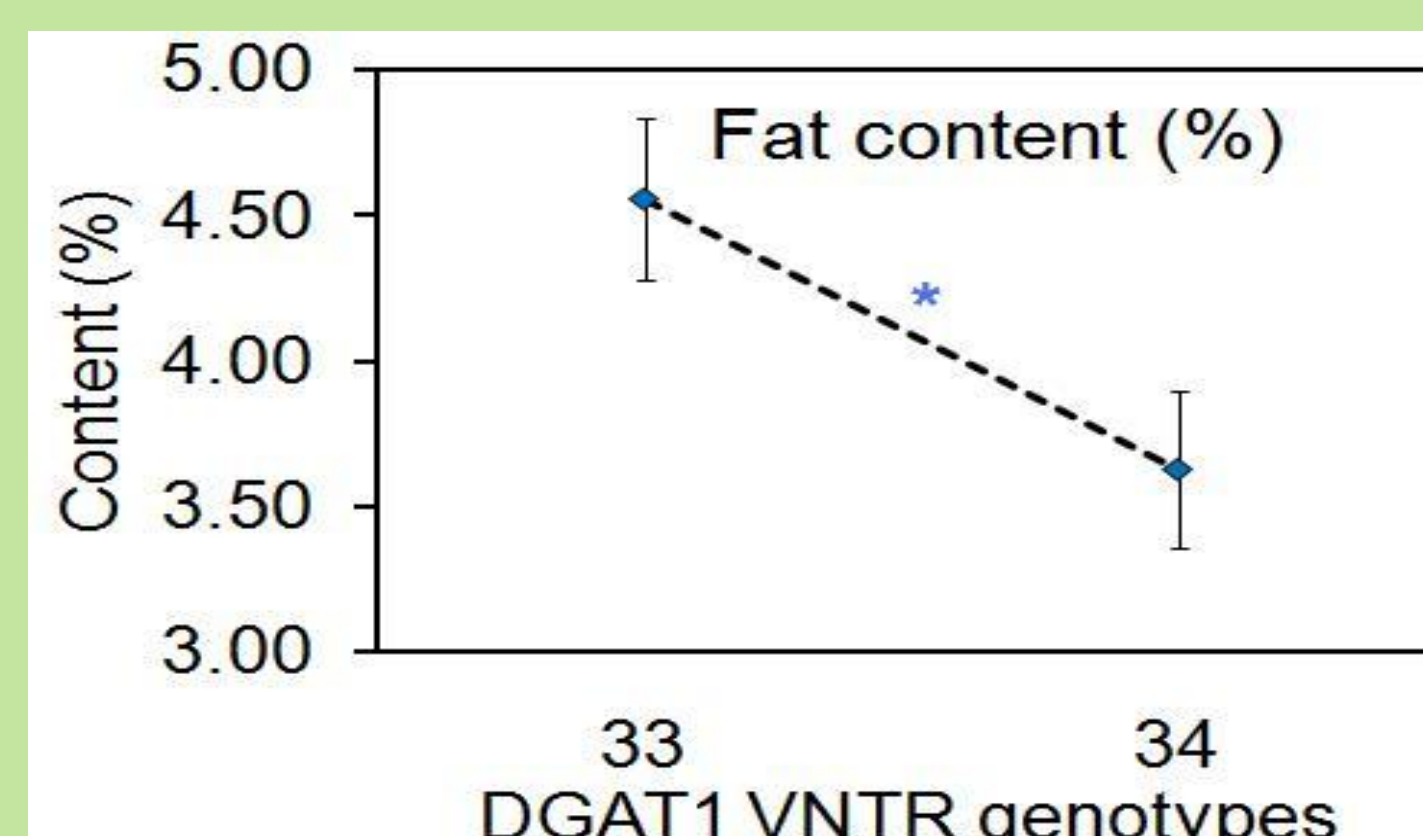


Figure 3: Influence of *DGAT1* promoter genotypes

Traits	VNTR – allele 3		
	α	SE	P-value
Milk yield (kg)	-0.24	0.49	0.6329
Fat yield (kg)	0.04	0.02	0.0923
Protein yield (kg)	0.002	0.02	0.9331
Fat content (%)	0.84	0.22	0.0013
Protein content (%)	0.19	0.08	0.0333

Table 2: Allele substitution effects (α) of the VNTR allele 3 at *DGAT1* locus, standard errors (SE), And p-value for milk traits

Materials and Methods

- 60 native Sudanese dairy cows from Butana and Kenana dairy cattle (**Fig 1**).
- genomic DNA extracted from whole blood
- The amplification of *DGAT1* promoter VNTR was conducted in a similar way as described by Kuehn et al. (2004).
- For the *Leptin MboI-RFLP*, the cows were genotyped according to Liefers et al. (2002).
- The *Kappa casein (CSN3)* locus was amplified according to the procedure proposed by Fernando Medrano and Aguilar-Cordova (1990).
- analysed traits: milk, fat and protein yield, and fat and protein content
- Allele and genotype frequencies calculated based on genotype counts observed in the population
- association analysis with “PROC MIXED” procedure of the SAS software package (release 8.02)

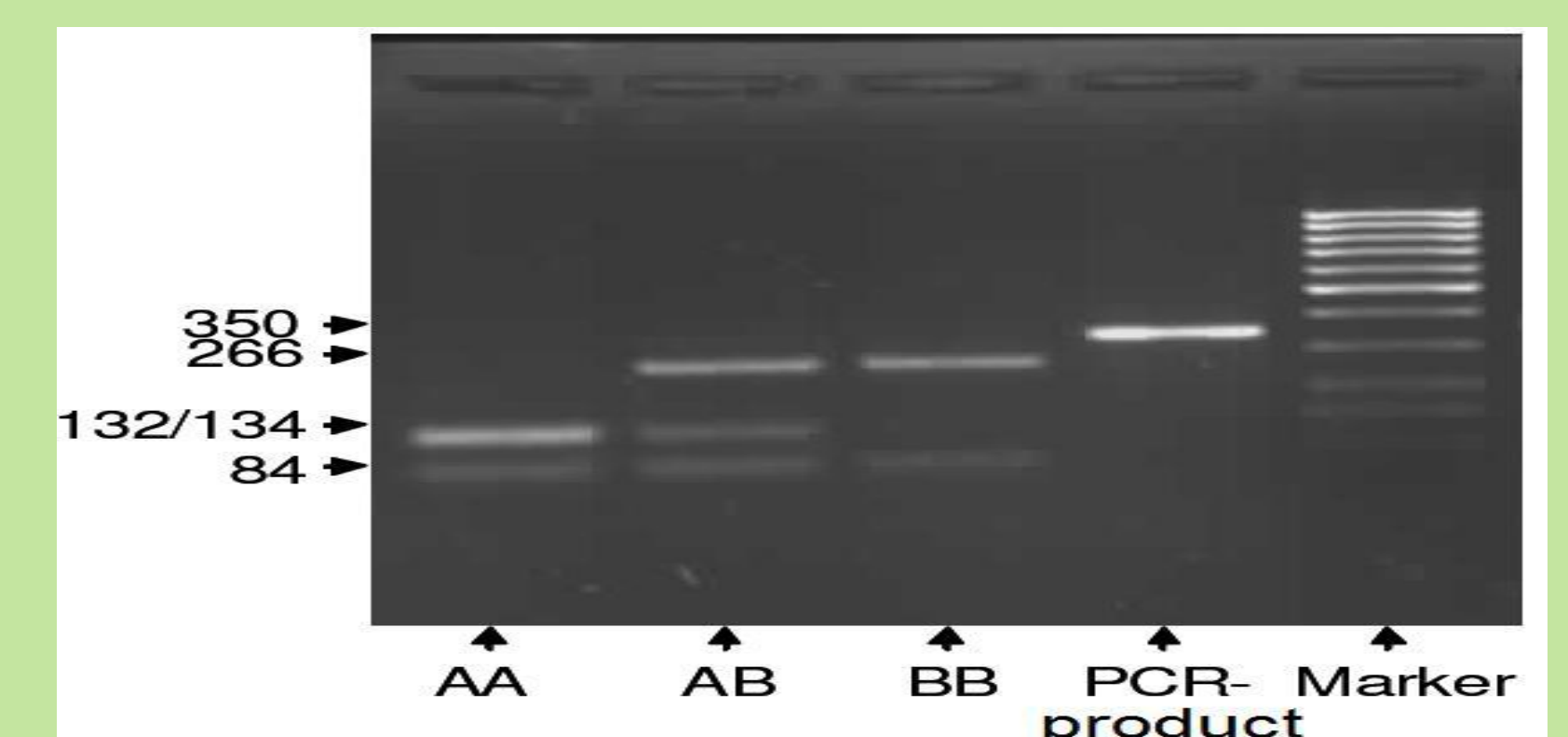


Figure 4: Separation of PCR products of *CSN3* gene in a 2% agarose gel by *HinfI* enzyme

- The frequencies of allele A at *lep* locus were 97.50% and 97.06% in Butana and Kenana breeds, respectively (**Table 1**).
- In general, the means of AB heterozygous cows were higher for milk, protein and fat yields when compared to those of AA homozygous cows in both breeds.
- The digestion of the 350 bp PCR product of *CSN3* (A/B variant) with *HinfI* restriction enzyme revealed three genotypes: AA, AB and BB (**Figure 4**).
- Frequencies of allele A for the two breeds were 86.25% and 89.29% for Butana and Kenana cows.
- Results from the statistical association analysis between *CSN3* genotypes and milk production parameters were not significant.

Conclusion

Allele 3 of the VNTR in the promoter of the *DGAT1* gene appeared favourable for fat and protein contents. For the *Leptin* locus, AB heterozygous cows were high milk, protein, and fat yield