

GENETIC POLYMORPHISM OF THE KAPPA CASEIN GENE IN HOLSTEIN- FRIESIAN DAIRY CATTLE IN WEST JAVA PROVINCE

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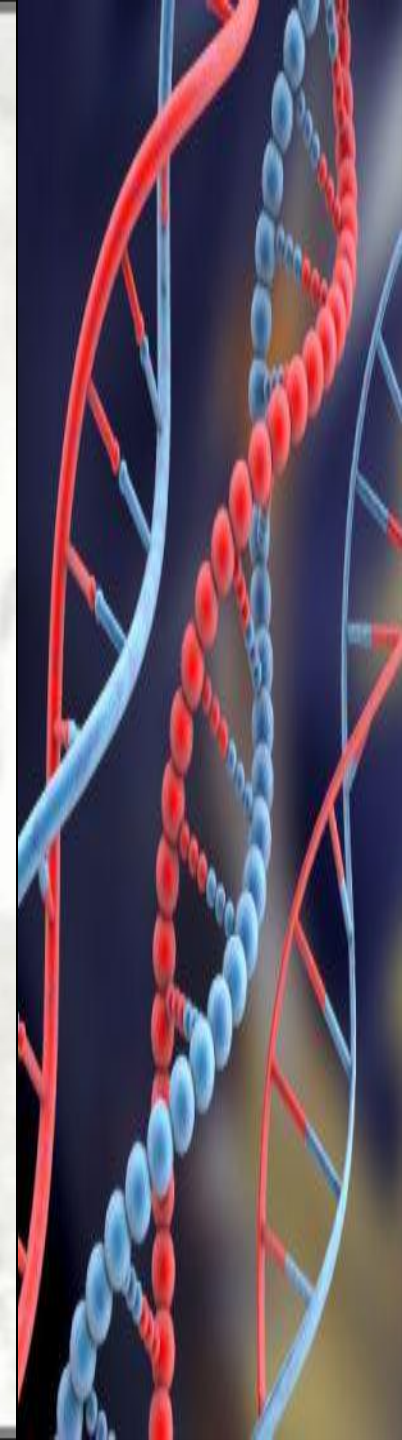
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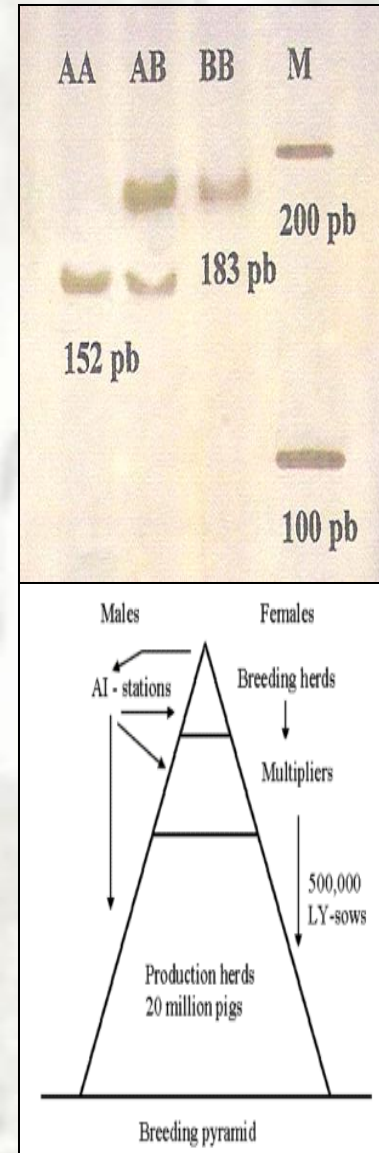
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Introduction

- DNA polymorphic markers → candidate genes in selection
- Marker-assisted selection (MAS) + traditional method of selection → an effective way to improving quantitative traits.
 - ❖ increase the accuracy of selection
 - ❖ reduce interval generation
 - ❖ accelerating selection responses

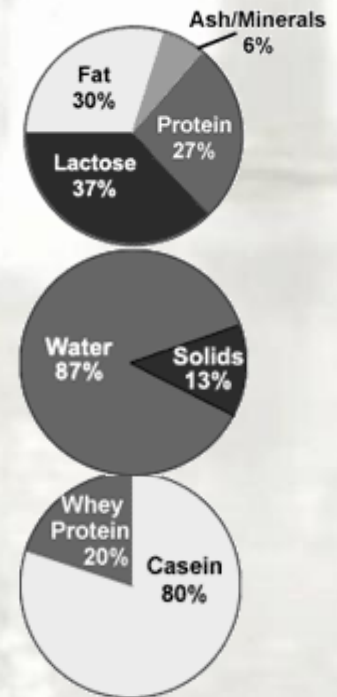


➤ Milk proteins are mainly divided into two fractions

1. soluble fraction → whey protein (α -lactalbumin and β -lactoglobulin)

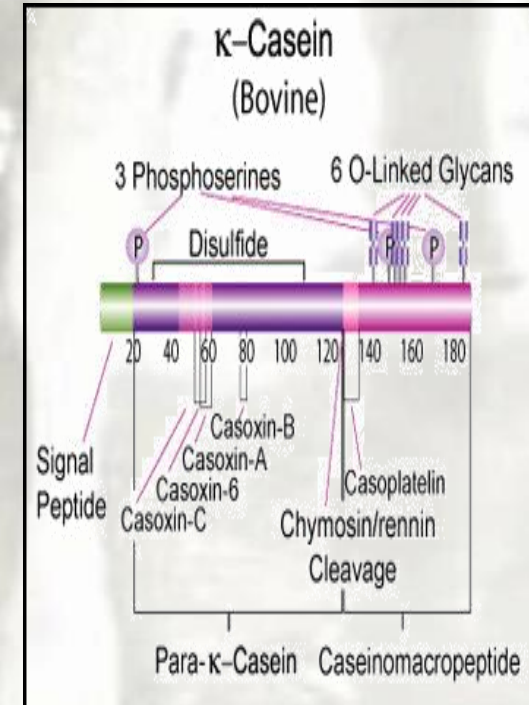
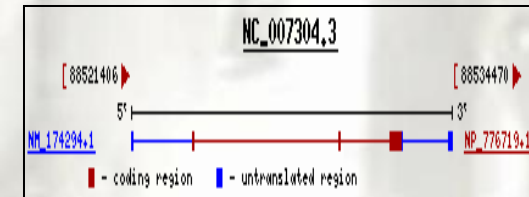
2. insoluble fraction → casein (78-82% milk proteins)

➤ κ -Cn approximately 12% of the whole milk casein



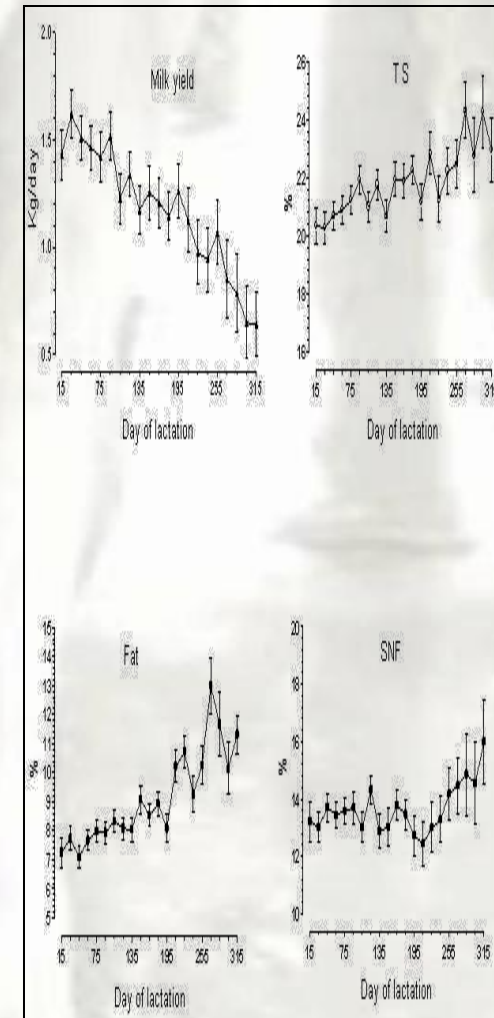
Introduction

- κ-Cn gene there is in the chromosome 6q31, consisting 5 exon and 4 intron
- two alleles are the most commonly identified in *Bos taurus* dairy cattles
 1. A allele with threonine (136) and aspartic acid (148)
 2. B allele with isoleucine (136) and alanine (148)



Introduction

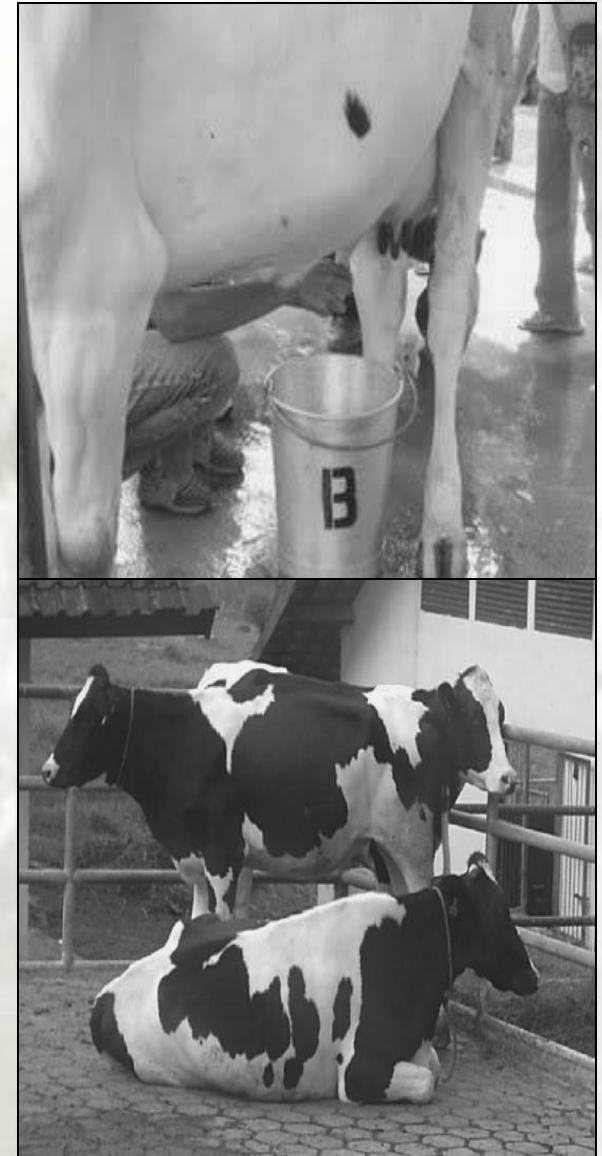
- Milk protein variants influence of milk composition, proteins and cheese production → Milk protein genes could be useful as genetic markers for selection in dairy cattle breeding
- Several studies showed association of κ -Cn variants with lactation performance and effect on milk composition and its processing properties



- Relationships between variants κ -Cn genotypes for different milk proteins and yield traits have been reported by several authors
 - ❖ κ -Cn BB cows produced milk with a 0.08 percent higher protein content than that of AA cows
 - ❖ κ -Cn variants had a significant effect on milk production ($p < 0.001$), with cows of the BB genotype producing 173 kg less milk than AA cows

Introduction

The aim of this research was to know the frequencies of both genotype and allele of the κ -CN gene in HF under the two different research locations, both from government dairy breeding stations and a number of small dairy farmers in central dairy region in West Java Province, Indonesia



Material and Method

Animal and blood samplings

- Total of 342 Holstein-Friesian (HF) females, reared under two different managements → intensive and semi intensive management
- Total of 57 males both actively used (bulls) and candidates (young sires) artificial insemination (AI) → AI Stations in Lembang and in Singosari
- Blood samplings were collected via vena jugularis/vena cocsigalis

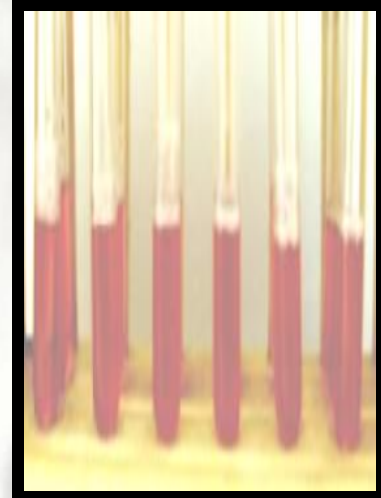
Material and Method

Tabel Total Sample were Used in This Resaerch

Location	Number of Blood Sample	Sex
Eco Farm	20	Female
BET Cipelang	50	Female
BPPT-SP Cikole	82	Female
Sub Total	152	
KPSBU-Cilumber	98	Female
KPSBU-Pasar Kemis	92	Female
Sub Total	190	
Total	342	
BIB Lembang	25	Male
BBIB Singosari	32	Male
Total	57	

DNA Extraction

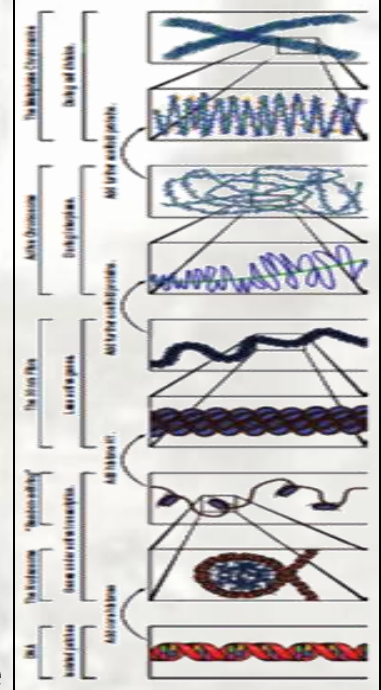
DNA from blood sample was extracted by method as described by Sambrook *et al.* (1989) with minor modification



Polymerase chain reaction (PCR)

PCR with volume 17.5 μ l was followed:

- 2 μ l DNA sample
- 1 pmol kappa-casein (κ -casein) primer
- 200 μ M dNTPs
- 0.5 unit AmpliTaq gold DNA polymerase



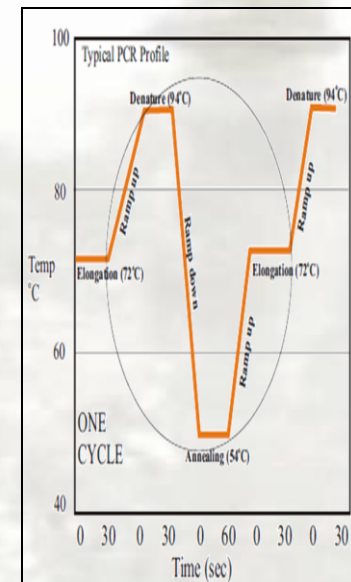
Material and Method

Table Sequence Primer were Used in This Resaerch

Primer	Sequent	PCR Product
Forward	5'-AAA TCC CTA CCA TCA ATA CC -3'	183 bp
Reverse	5' CTT CTT TGA TGT CTC CTT AG-3'	

Table PCR Condition were Used in This Resaerch

Step	Temperature (°C)	Time	Cycle
First Denaturation	95	10.00	1
Denaturation	95	00.30	
Annealing	55	00.30	40
Extention	72	01.00	
Final Extention	72	05.00	1



PCR-RFLP

Analysis of PCR-RFLP with volume 10 μ l was followed:

- 4 μ l PCR Product
- 2.5 unit PstI

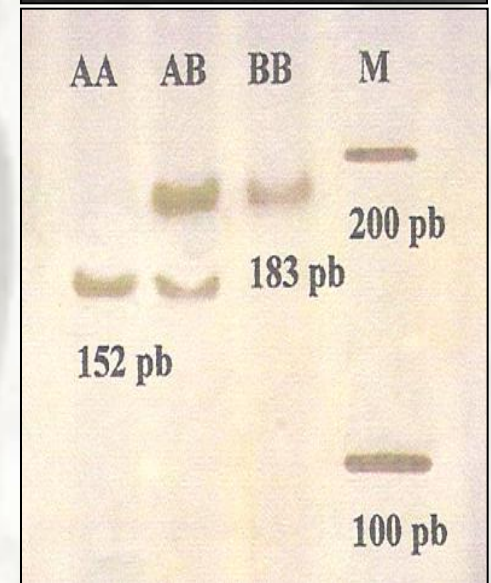
Data Analysis

The genotype frequencies were

calculated by
$$\chi_{ii} = \frac{n_{ii}}{n}$$

The allelic frequencies were calculated by Nei (1987)

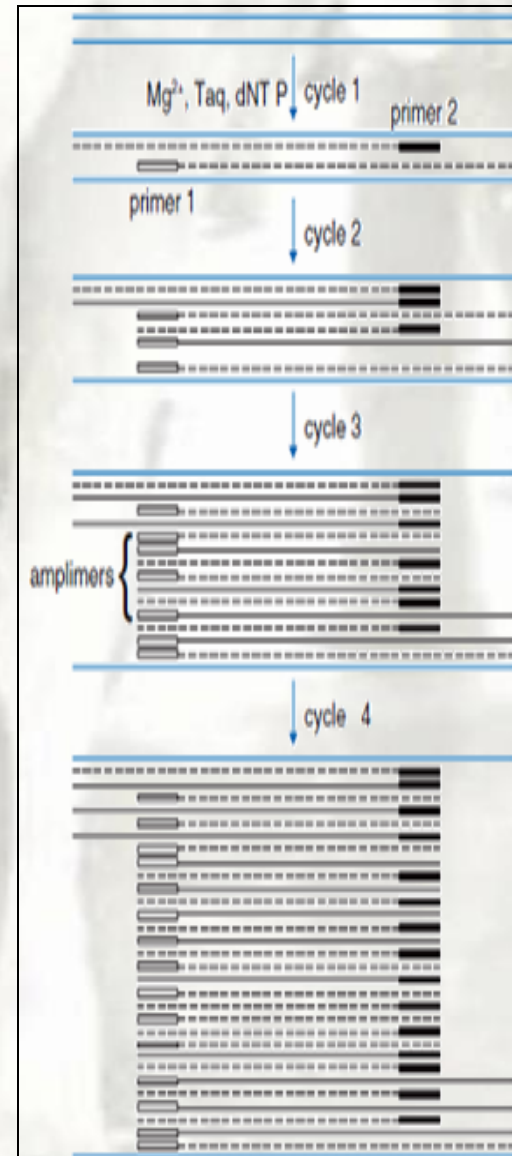
$$\chi_i = \left(2n_{ii} + \sum_{j \neq i} n_{ij} \right) / (2n)$$



Result and Discussion

Amplification

- The existing technology of PCR makes possible to amplify segments of the genome containing genotypic (allelic) variations
- The DNA fragment amplified from the A and B allele were characterized by the presence the fragment lengths of 152 bp and 183 bp respectively



Result and Discussion

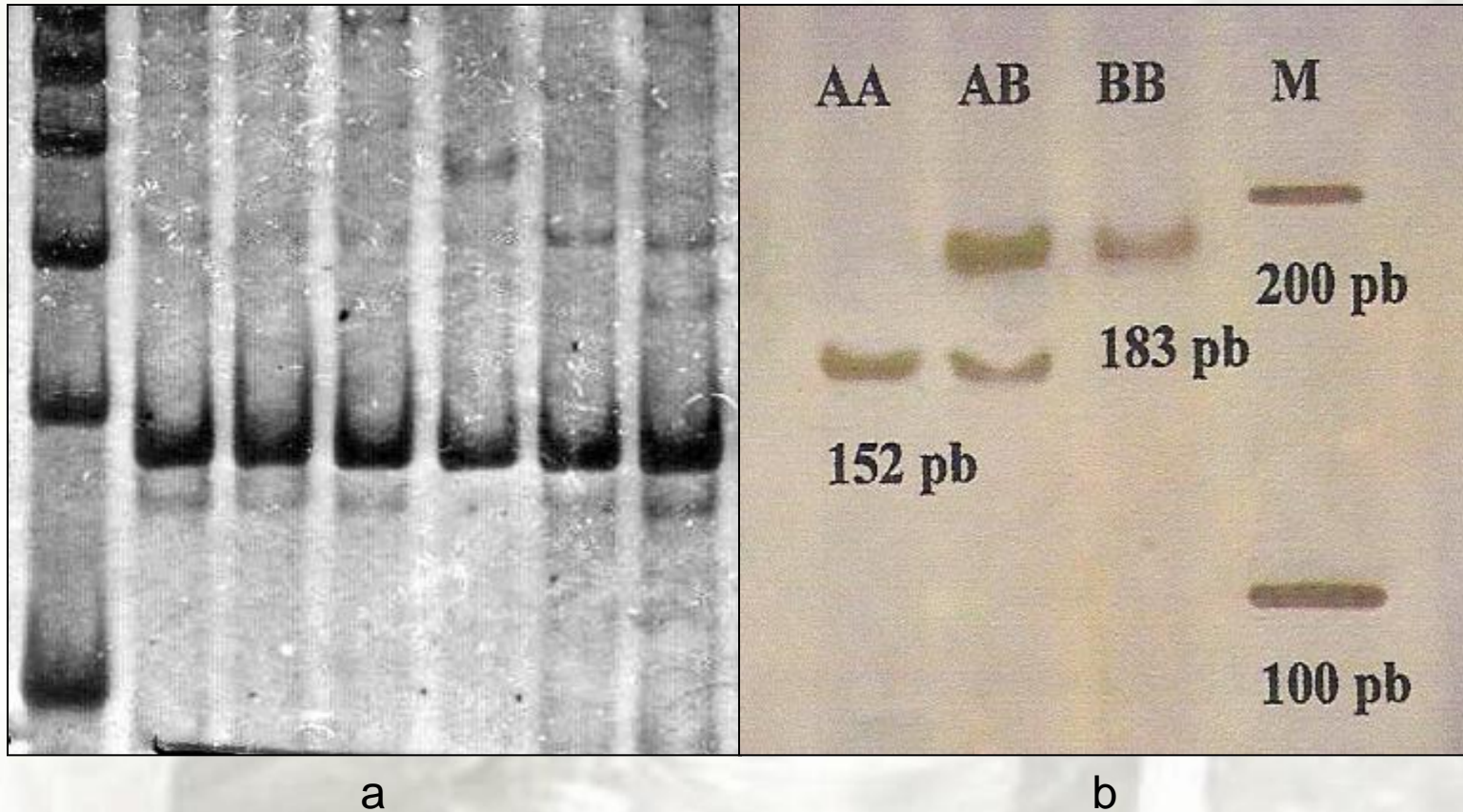


Figure PCR Product 183 bp (a). The RFLP patterns of the k-casein after digestion with PstI on the 6% PAGE AA= 152 bp, AB=183 and 152 bp, and BB= 183 bp

Result and Discussion

Table Frequencies of Genotypes and Alleles of the κ -Casein Gene in Holstein-Friesian by Location

Location and Blood Sample (Number)	Genotype frequency (Sample)			Allele frequency	
	AA	AB	BB	A	B
Eco Farm Fapet IPB (20)	0,45 (9)	0,55 (11)	0,00 (0)	0,73	0,27
BET-Cipelang (50)	0,54 (27)	0,46 (23)	0,00 (0)	0,77	0,23
BPPT-SP Cikole (82)	0,10 (8)	0,85 (70)	0,05 (4)	0,52	0,48
Sub total (152)	0,29 (44)	0,68 (104)	0,03 (4)	0,63	0,37
KPSBU - Cilumber (98)	0,32 (31)	0,62 (61)	0,06 (6)	0,63	0,37
KPSBU - Pasar Kemis (92)	0,34 (31)	0,64 (59)	0,02 (2)	0,66	0,34
Sub total (190)	0,33 (62)	0,63 (120)	0,04 (8)	0,64	0,36
Total (342)	0,31 (106)	0,65 (224)	0,04 (12)	0,64	0,36

Result and Discussion

- The results showed that those animals with the BB genotype were very low, at a range of 0 - 6 % with the average of 4 %
- Samples at the Eco Farm Anim Sci. IPB and BET Cipelang showed that none of these animals had the BB genotype
- Frequency BB genotype in BPPT-SP Cikole is 5 %
- Frequency BB genotype in Pasar Kemis and Cilumber villages are 6% and 2% respectively.

Result and Discussion

Table Frequencies of Genotypes and Alleles of the κ -Casein Gene at BIB Lembang and BBIB Singosari

Location / Animal (hd)	Genotype			Allele		
	frequency (%)			frequency (%)		
	AA	AB	BB	A	B	
BIB Lembang	Active AI bulls (10)	0,60 (6)	0,40 (4)	0,00 (0)	0,80	0,20
	<i>Progeny test</i> candidates (15)	0,56 (8)	0,33 (5)	0,13 (2)	0,70	0,30
	Sub total (25)	0,56 (14)	0,36 (9)	0,08 (2)	0,74	0,26
BBIB Singosari	Active AI bulls (23)	0,17 (4)	0,78 (18)	0,04 (1)	0,57	0,43
	<i>Progeny test</i> candidates (9)	0,22 (2)	0,56 (5)	0,22 (2)	0,50	0,50
	Sub total (32)	0,19 (6)	0,72 (23)	0,09 (3)	0,55	0,45
Total	0,35 (20)	0,56 (32)	0,09 (5)	0,63	0,37	

Result and Discussion

- BBIB Singosari have the only one bull with the BB genotype
- BIB Lembang have none active bull with the BB genotype
- Total BB frequency of the κ -Cn gene both at BIB Lembang (0,26) and Singosari (0,45) was sufficient
- non active AI (young) males not gave any contribution to increase the BB genotype females

Result and Discussion

- at KPSBU Lembang, it was generally found that the A allele was higher compared to the B allele, both in Cilumber (0,63 vs 0,37) and Pasar Kemis (0,66 vs 0,34) villages
- at BPPT-SP Cikole had the frequencies of the A and B alleles almost similar (0,52 vs 0,48).
- However, all of observed HF females had a higher frequency for the A allele over the B (0,64 vs 0,36)

- The occurrence of more higher the B allele than the A allele was also found for the active and non active AI males at the two AI stations of BIB Lembang (0,74 vs 0,26) and BBIB Singosari (0,63 vs 0,37)
- With regarding to the lower BB genotype females at Ecofarm Fapet IPB, the reason for the lower frequency of the B allele over the A allele (0.27 vs 0.73) was that only one bull continuously used for mating females and this bull was identified for the AA genotype

Result and Discussion

- The A allele frequency in this study was still lower than USA, Europe and Japan (0,8-0,9)
- This was due to the main purpose to develop dairy cattle for high milk yield, the A allele is more related to higher milk yield, while the B allele closely associated with high protein yield
- This can be associated with decision for the imported HF bull policy mainly based on high values of *Estimated Transmitting Ability* (ETA) of their milk yield

Conclusion

- Genotyping κ -casein gene in HF dairy cattle resulted two alleles were identified (A and B) → three genotypes (AA, AB, and BB)
- The BB genotype HF females in this study were identified at a very low frequency in the range of 0-6 %
- All of this finding seemingly be a possible indication for a small number of the BB genotype HF females in some central dairy region in West Java



Thanks For You

Attention

