

The use of frozen semen of Holstein-Friesian bulls with the bb genotype of the kappa casein gene in Indonesia¹

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ABSTRACT: The use of the kappa casein (CSN3) gene as MAS could be an effective way to identify a dairy bull with highly transmitted milk protein yield, as the CSN3 gene is one of dominant casein genes. The aims of this study was to identify the BB CSN3 genotype in Holstein-Friesian (HF) males as active and candidate AI services from the two national AI stations. Distribution of frozen semen of BB active AI bulls was studied continued by investigation of the use of the BB bulls in servicing HF females focused in dairy locations of BPPT SP Cikole and KPSBU Lembang. Two BB active AI bulls were identified at BBIB Singosari, namely Pipenary (No. 30 569) and Goldsy B (No. 30 667) of both from East Java. Pipenary produced large quantities of frozen semen distributed in Java, while B Goldsy produced frozen semen still at a low dose. At BIB Lembang was also identified two BB males, but the only bull of Kemang (No. 39 766) produced a lot of frozen semen, while another (P013) died before semen collection. Kemang's frozen semen was distributed in Java, Lampung and South Sulawesi. From 11 bulls actively used to serve HF lactating cows in KPSBU in 2007-2009 showed only Pipenary with the BB genotype. The two AI stations are suggested to increase the BB CSN3 active AI bulls.

Key words: HF bull, kappa casein gene, BB genotype

INTRODUCTION

At this time it seems that many domestic dairy consumens have been moving their altitude of drinking milk for a better nutritious from a lesser volume of milk. On the other condition, as the population under five years and school ages have increased, it could be a possible fact of inceasing demand of milk with a better quality specifically in its protein contents. By considering this possible demand, therefore, it is important to give attention of improving milk proteins through the available HF breeding stocks with highly transmitting milk protein yield.

Artificial insemination (AI) has been widely used to service Holstein-Friesian (HF) females in Indonesia. No less than 90% of HF females were serviced by AI mating using frozen semen of superior HF bulls. Most of active AI HF bulls as frozen semen producers were imported from several countries. In an effort of producing domestic superior dairy bulls, BBIB Singosari progeny tested HF bulls under the collaborative project with the Japan International Cooperation Agency for several years ago. Progeny testing have been continued up to now at surrounding local area in some dairy regions in East Java. Currently it has just been initiated a national progeny testing with the main target to produce domestic HF bulls for its highly transmitting milk ability under a wider area in Java.

One of major constraints to conduct progeny test for milk yield specifically at small dairy farmers that milk yield based selection facing complex process and more time consuming. Conversely, selection of superior bulls for milk protein content by kappa casein (CSN3) gene as MAS followed a simpler procedure and more reliable livestock can be identified earlier. Inconventional selection by using CSN3 gene as MAS, hence could be an effective way to select superior dairy bulls and cows for high milk protein ability. This is because caseins as the most milk protein fraction (80%) are controlled by only four genes, succesively CSN1S1, CSN1S2, CSN2 and CSN3. These caseins genes in the cattle genome are located on the length of 250 bp at the chromosome 6th or 6/BTA 6q31

¹ It was recommended that at both the two national AI sations of BBIB Singosari and BIB Lembang should increase the use of more the BB active AI HF bulls as producers of frozen semen.

(Threadgill and Womack, 1990 and Rijnkels *et al.*, 1997).

Verification of the CSN3 genes as MAS for the selection on high milk protein contents previously conducted in HF lactating cows in two different locations in west Java province by Anggraeni *et al.* (2009). The verification for a quite large numbers of lactating HF cows kept under an intensive dairy breeding station of BPPT SP Cikole and semi intensive of a number small dairy farmers of KPSBU Lembang proved that the BB cows compared to the AA cows had higher protein contents around 3.37-3, 84%. Nevertheless, the BB cows were identified very few around 0-9%. The low frequency of the BB females might be due to a limited number of BB actively AI HF bulls from the two national AI station.

Selection for superior milk protein HF bulls will have a synergistic position in optimizing the utilization of both national and local progeny tested bulls in their milk production ability. By considering the importances of both milk and protein traits, it was necessary to know more exactly the contribution of active AI HF bulls at the two national AI stations in affecting the BB frequency of domestic HF females. Therefore, it was necessary to do this research with specific purpose to identify distribution and utilization of actively AI HF bulls with the BB CSN3 genotype from the two national AI stations.

MATERIALS AND METHODS

Blood samplings were collected via vena cocsigalis of HF bulls for both active and candidate AI males from the two national AI Stations, in BIB Lembang for 25 heads and BBIB for 32 heads. Identification of the BB genotype of the CSN3 gene of those active and candidate AI bulls was based on polymerase chain reaction and restricted fragment length polymorphism (PCR-RFLP) as done by Sumantri *et al.* (2005) and Anggraeni *et al.* (2009). Data of distribution of frozen semen of each active AI HF bulls were collected from these two AI Stations.

Genetic frequencies of genotypes and alleles of the CSN3 gene from active and candidate HF males at BIB Lembang and BBIB Singosari were analyzed (Nei, 1987). Contribution of BB active AI HF bulls in affecting the BB frequency in domestic HF females was investigated through the amount of straw distributed by the two national AI stations.

As reported by Anggraeni *et al.* (2009) investigated variant genetic CSN3 gene in HF lactating cows at BBPT-SP Cikole and KPSBU Lembang in West Java. The study identified three types of variant genotype, succesively AA, AB and BB. The study also pointed very few of HF lactating heifers and cows with the BB genotype, 0-9%. Low frequencies of the BB HF females was predicted due to low uses of the BB active AI HF bulls by the two national AI stations.

It was therefore necessary to identify HF females serviced by frozen semen of the actively AI HF bulls both at national scale and at the prior two locations studied, BPPT-SP Cikole and KPSBU Lembang in West Java.

RESULTS AND DISCUSSION

Variant Genotypes of the CSN3 Gene

Development in PCR technology allows amplification of bovine genome, so polymorphic genotype and allele can be studied by RFLP method (Pinders *et al.*, 1991). PCR-RFLP technique was used to identify variant genotypes of the CSN3 gene at BPPT SP Cikole (82 hds) and small dairy farmers of two villages of Cilumber (98 hds) and Pasar Kemis (92 hds) of KPSBU Lembang in West Java (Anggraeni *et al.*, 2009). It was previously informed that the AA, AB and BB genotypes were identified. The DNA fragments of both AA and BB genotypes were characterized by the existence of only one fragment length, 152 bp and 183 bp respectively. Further, AB genotype had two DNA fragments, 152 bp and 183 bp. The pattern of those three BB CSN3 genotypes was expressed in Figure 1.

Association of the BB CSN3 Genotype and Milk Protein Contents

There was a strong tendency of beneficiary of the BB CSN3 genotype on milk quality especially for milk protein contents in dairy cattle as reported by a number of studies (Van Eenennaam dan

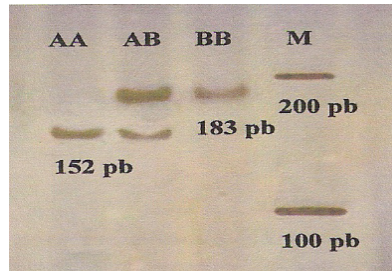


Figure 1. RFLP pattern of the CSN3 gene after restricted by Hinf I enzyme on achrilamide gel 1%. Banding patterns from the left were succesively AA, AB, BB genotypes and marker ladder 100 pb (M) Source: Anggraeni *et al.* (2009)

Table 1. Variant genotype of the Kappa Casein Gene in Active and Candidate AI HF bulls at BBIB Singosari

Number	Animal identity	Animal name	Origin	Birth date	Genotype
1	12753	-	-	-	AA
2	12765	-	-	-	AB
3	12771	-	-	-	AB
4	12905	-	-	-	AB
5	12927	-	-	-	BB
6	12947	-	-	-	AA
7	12955	-	-	-	AB
8	12962	-	-	-	AB
9	12992	-	-	-	AB
10	12995	-	-	-	AB
11	13019	-	-	-	AB
12	13032	-	-	-	AB
13	30047	Bootsy (47)	BBPTU Baturaden	28 February 2000	AB
14	30152	Surya (52)	Australia	1 April 2001	AB
15	30156	Roadgaru (56)	Australia	30 August 2001	AA
16	30157	Soulisa (57)	Australia	23 November 2001	AB
17	30158	Okky (58)	Australia	28 May 2001	AB
18	30160	Starko (60)	Australia	29 August 2001	AB
19	30193	-	-	-	AB
20	30566	Jaysy	Jawa Timur	24 October 2005	AB
21	30569	Pipenary	Jawa Timur	9 November 2005	BB
22	30571	Sanry	Jawa Timur	18 November 2005	AB
23	30572	Danilsy T	Jawa Timur	18 November 2005	AB
24	30662	Formerly (P. 017)	Jawa Timur	20 May 2006	AA
25	30664	-	-	-	AB
26	30665	Bullionary (P. 039)	Jawa Timur	9 October 2006	AA
27	30667	Goldsy B (P. 012)	Jawa Timur	15 February 2006	BB
28	30668	Ternisy H (P. 015)	Jawa Timur	13 March 2006	AB
29	39634	Starry SS (34)	Jawa Tengah	12 October 1996	AB
30	39941	Boisy (41)	BPTU Baturaden	17 February 2000	AA
31	39943	Cherry (43)	BPTU Baturaden	22 December 1999	AB
32	-	-	-	-	AB

Medrano, 1991; I konen *et al.*, 1999; dan Ojala *et al.*, 1997). Prior investigation of the benefit BB genotype on milk protein contents in domestic HF females was reported by Sumantri *et al.* (2005) at dairy breeding station of BBPTU Baturraden in Central Java. By classifying 249 cows into three different protein breeding values (BVs) showed the high BV group had the BB and AB genotypes at high frequencies, 0.36 and 0.59 respectively. By contrast, for the lowest group, the AA genotype had a high frequency (0.80) compared to the BB (0.20) and the AB (0.00). This result clearly showed a direct effect of the BB genotype on milk protein contents in HF females studied.

As prior study (Anggraeni *et al.*, 2009) verified in BPPT SP Cikole and KPSBU Lembang the BB CSN3 HF cows consistently produced higher milk protein contents compared to the AA cows, whereas the AB cows produced protein contents in between. The BB and AB cows also tended to produce milk dry matter contents higher than the AA cows. Therefore it was concluded that the BB CSN3 genotype so consistently in affecting higher milk protein contents.

Distribution of the BB CSN3 Genotype of HF Bulls

Table 1. listed the identity of individual active and non active AI HF males that were genotyped their CSN3 gene. From a total number 32 heads of HF bulls genotyped at BBIB Singosari showed 12 HF bulls were not found the information on their name, origin and birth year

Table 2. Variant genotype of the Kappa Casein Gene in active and candidate AI HF bulls at BIB Lembang

Num.	Animal identity	Animal name	Origin	Birth	Genotype
1	P009/30694	Flaunt	-	-	AB
2	P003	-	-	-	AA
3	39969/04991	FULLBERT	-	-	AB
4	P006/30693	Fervent	-	-	AA
5	39563	AZALEA	-	-	AA
6	-	Mindi	-	-	AA
7	-	Felicia	-	-	AB
8	-	Marin	-	-	AA
9	-	Casella	-	-	AA
10	-	Calender	-	-	AA
11	P008/30686	Farrel	-	-	AB
12	P007	-	-	-	AA
13	P010	-	-	-	AA
14	P011	-	-	-	AA
15	P019	-	-	-	AA
16	P013	-	-	-	BB
17	P21	-	-	-	AA
18	-	Tanjung Sari	-	-	AA
19	-	Cikole	-	-	AA
20	P020	-	-	-	AB
21	P024	-	-	-	AB
22	P026/30696	Furry	-	-	AA
23	P025/30695	Forturner	-	-	AB
24	-	Asep Pangalengan	-	-	-
25	-	Top Wine	-	-	AB
26	-	Bayu	-	-	AB
27	-	Iwan	-	-	AA
28	-	Baunday	-	-	AB
29	-	Ambic	-	-	AA
30	39766	Kemang	-	-	BB

All these bulls were presumably already finish in producing frozen semen, due to aging process or death. Majority HF bulls used for producing frozen semen in 1999 were came from East Java and Central Java, while others were imported from Australia. Majority of the HF bulls had the AA and AB genotypes.

Nevertheless, there were two BB HF bulls still be actively used to produce frozen semen, namely for the identity numbers of 30 569 named by Pipenary and 30 667 named by Goldsy. Pipenary was born at 9th November 2005 coming from East Java and Goldsy B was born 15th February 2006 also from East Java.

Information on distribution and utilization of frozen semen from the two BB active AI HF bulls at BBIB Singosari showed that Pipenary already produced large amounts of frozen semen that were distributed in three provinces of West Java, Central Java and East Java. In year 2009, the demand of frozen semen of this bull came from Indonesian Milk Cooperation Venture (GKSI Pusat) in West Java for 401 174 doses, Animal Husbandry Service of Boyolali District in Central Java for 426 doses, Milk Cooperation Unit (GKSI) in West Java for 1289, and Animal Husbandry Services in East Java for 903 doses. On the other side, Goldsy B produced frozen semen still at a low number so the spread of his frozen semen remained low, with the demand coming from Animal Husbandry Services of West Java and East Java for 150 doses respectively.

At BIB Lembang, it was identified two BB HF bulls, namely P013 and Kemang (Table 2). However, only Kemang was used as source of frozen semen and distributed toward a number of areas, while P013 dead before its semen was collected. The BB genotype of Kemang had the identity number of 39 766 and born 9th May 1997 came from BET Cipelang, Bogor. Kemang's frozen semen were produced and distributed to wider areas in and outside Java. Distribution in Java across Jakarta, West Java, and Central Java; while outside Java included for Lampung and South Sulawesi. In the period of 2001-2003, The amount of frozen semen were already produced around 52 826 doses. However, this bull could no longer produce frozen semen due to his dead.

Utilization of Frozen Semen of the BB HF Bulls

Table 3 presented the use of frozen semen of the BB actively AI HF bulls to serve HF lactating heifers and cows in a number of small dairy farmers under supervision of Milk Cooperation Unit of KPSBU Lembang in period 2002-2009. It was proved there were 30 HF bulls of whose their frozen used for AI services. The origins of these HF bulls were respectively from BBIB Singosari (11 hds), BIB Lembang (5 hds), Canada (6 hds), Australia (7 hds) and BET Cipelang (1 hd).

Table 3. The use of frozen semen of HF bulls in two villages of Cilumber and Pasar Kemis of KPSBU Lembang in 2007-2009¹

Number	Bull identity	Bull name	2007		2008		2009	
			CBR	KMS	CBR	KMS	CBR	KMS
1	30084	MINDI	743	374	23	-	-	-
2	30687	FILMORE	7	12	13	-	-	-
3	30686	FAREL	3	1	-	-	-	-
4	30156	RODGARD	725	406	-	-	-	-
5	30153	ARYANA	27	14	-	-	1	53
6	30045	YACOBI	1	1	-	-	-	-
7	30662	FERMENY	3	2	-	-	-	-
8	30157	SOULISA	-	-	3	5	68	-
9	30160	STRAKO	-	-	73	51	1433	839
10	20569	PIPENARY (BB)	-	-	-	-	109	47
11	30694	FLAUNT	-	-	8	1	-	-
TOTAL			1509	810	120	57	1611	971

¹CBR was Cilumber village, KMS was Pasar Kemis village

During the observation period 2007-2009 at the targetted villages of Pasar Kemis and Cilumber of KPSBU Lembang, it was known that the total number of the HF bulls functioned as the producers of AI frozen semen were approximately for 11 hds. However, only one HF bull with the BB genotype,

namely Pipenary with frozen semen used in 2009 in Cilumber for 109 doses and Pasar Kemis for 47 doses, resulting the total frozen semen were used for 156 doses. This proved a small number of frozen semen of the Pipenary used in these two villages. Based on this finding, it would give a little chance of the BB HF bulls in transmitting the BB CSN3 gene toward their offspring.

CONCLUSIONS

Two BB CSN3 Holstein-Friesian bulls were identified at BBIB Singosari and still actively used to produce frozen semen, namely Pipenary (No. 30 569) and Goldsy B (No. 30 667) of both from East Java. Pipenary produced in large quantities of frozen semen distributed in across Java, while B Goldsy produced frozen semen still low doses spread in West and East Java. Two BB CSN3 HF males were also identified at BIB Lembang, namely P013 and Kemang. Only Kemang produced frozen semen and distributed to Java, Lampung and South Sulawesi, while P013 was dead before semen collection. Observations in two villages of Cilumber and Pasar Kemis in the KPSBU' dairy field during 2007-2009 showed that from 11 HF bulls used to serve HF heifers and cows, the only Pipenary had the BB CSN3 genotype.

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