GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Effect of casein genes - *beta-LGB*, *DGAT1*, *GH*, and *LHR* - on milk production and milk composition traits in crossbred Holsteins

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Genet. Mol. Res. 14 (1): 2561-2571 (2015)
Received March 5, 2014
Accepted August 21, 2014
Published March 30, 2015
DOI http://dx.doi.org/10.4238/2015.March.30.15

ABSTRACT. The objectives of this study were to determine the effects of a single gene and composite genotype of the casein gene family, including the beta-lactoglobulin gene (*beta-LGB*), acyl-CoA: diacylglycerol acyltransferase 1 gene (DGAT1), growth hormone gene (*GH*), and luteinizing hormone receptor gene (*LHR*) on milk yield, milk composition, the percentage of fat, protein, solids-not-fat, and total solid in crossbred Holsteins. A total of 231 crossbred Holstein cows were examined for the study. The genotype of the beta-casein gene was analyzed by allele-specific polymerase chain reaction, while the alpha-S1, alpha-S2, kappa-casein, *DGAT1*, *beta-LGB*, and *GH* genes were analyzed using a polymerase chain reaction-restriction fragment length polymorphism assay. The association between genes and milk yield and milk composition was analyzed. Three pairs of genes, for which significant associations were detected, were beta + kappa-casein, *DGAT1* + beta-casein, and *GH* + *beta-LGB*. In the single-gene model, most loci are significantly associated with traits. A significant

association between the composite genotype and the traits was detected in all composite genotypes. *GH* + *beta-LGB* appears to be the most suitable variants for improving milk production and percentage of milk protein. Overall, the effects of the composite genotype and single gene were different. A physical or functional relationship between genes is necessary for investigating gene markers.

Key words: Acyl-CoA:diacylglycerol acyltransferase 1 gene; Beta-lactoglobulin gene; Casein gene family; Growth hormone gene; Luteinizing hormone receptor gene; Milk production

INTRODUCTION

More than 20 years ago, several studies investigated the relationship between genes and milk production and milk composition traits in dairy cattle. Some candidate genes were found to have significant effects on the traits, including the casein gene family and the beta-lactoglobulin (*LGB*) (Boettcher et al., 2004; Comin et al., 2008), acyl-CoA:diacylglycerol acyltransferase 1 (*DGAT1*) (Kuehn et al., 2007; Nowacka-Woszuk et al., 2008; Signorelli et al., 2009), growth hormone (*GH*) (Pawar et al., 2007; Mullen et al., 2011; Heidari et al., 2012; Krasnopiorova et al., 2012), and luteinizing hormone receptor (*LHR*) genes (Hastings et al., 2006). There is strong evidence that a group of genes may be used as markers for selection programs. The use of gene markers in a selection program, however, is not well-understood, particularly with regard to the number of gene markers and the genes that should be used in a selection program.

The variable effect of genes on milk production and composition traits has been observed previously (Hastings et al., 2006; Comin et al., 2008; Signorelli et al., 2009; Krasnopiorova et al., 2012). In theory, genes do not function alone, but have a close relationship with a number of genes in a genome; some of these genes are involved in regulating expression, particularly for quantitative traits. Thus, the effects of genes depend on the genetic structure. The additive effect, dominance effect, and epistasis effect are the main factors causing fluctuations in the effects of genes. Because animals have a different genetic structure, they have different alleles and genotypes, which may influence gene function. Moreover, the physical relationship between genes on the same chromosome and different chromosomes result in varied expression patterns. Linkage disequilibrium (LD) occurs when non-random association of an allele at 1 locus is found together with a specific allele at a second locus (Falconer and Mackay, 1996). LD typically refers to the association between 2 alleles on the same chromosome; however, alleles at 2 loci on different chromosome can also be associated. Therefore, LD between all genes should be analyzed regardless of their locations.

When the effects of gene are considered for gene markers, single genes that do not provide information for other genes may not be suitable.

In the current study, we investigated the effect of candidate genes on milk production and milk composition traits, LD between these genes, and whether the effect of the gene was greater independently or in combination with other candidate genes. The genes selected for this study included the *DGAT1* gene, which is located on the bovine chromosome 14 and encodes acyl-CoA:diacylglycerol acyltransferase 1 enzyme (Grisart et al., 2002); the casein gene

family, which is composed of the alpha-S1, alpha-S2, beta-, and kappa-casein genes (Mercier and Viloite, 1993; Jann et al., 2004) and is located on the bovine chromosome 6 at q31-q33; the *beta-LGB* gene, which is located on chromosome 11 and encodes the main protein of whey protein (Eigel et al., 1984); the *GH* gene, which is located on chromosome 19 in the region of bands q26-qter (Hediger et al., 1990); and the *LHR* gene, which is located on chromosome 11 (Marson et al., 2008). All of these genes affect milk production and milk composition. We investigated the physical relationships between these genes, which may be useful for studies aimed at increasing desirable traits. We hypothesize that some of the genes would show significant LD. Therefore, a composite genotype was used to estimate effects. We also hypothesized that the effects of a single gene and the composite genotype would differ. The results will be useful for selection programs for crossbred Holsteins. Therefore, the objectives of this study were to determine the effects of single genes and the interaction effects of composite genotypes of the casein gene family and the *beta-LGB*, *DGAT1*, *GH*, and *LHR* genes on milk production and milk composition in crossbred Holsteins.

MATERIAL AND METHODS

Animals and data

We used 231 crossbred Holstein cows that had the following data: number of tested days; approximately 2300 records of milk yield; and percentage of fat, protein, total solid, and solids-not-fat. The percentage level of Holstein cattle varied from 75-90%. Grading up to increase the level of Holsteins with imported semen was carried out for the breeding program. North American Holstein was the main source of semen used.

Genotyping

Genomic DNA was extracted from whole blood using the DNA Mini Kit Protocol-Blood (Geneaid, New Taipei City, Taiwan). The allele and genotypes of the beta- and kappa-casein genes were analyzed by allele-specific polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP), respectively; these methods followed those used by Aroondechachai et al. (2004). The allele and genotype of alpha-S1 casein gene and alpha-S2 casein gene were analyzed based on the methods described by Koczan et al. (1991) and Ibeagha-Awemu et al. (2007), respectively. The method described by Kuehn et al. (2007) was used to study polymorphisms of the *DGAT1* gene. PCR-RFLP and the methods described by Marson et al. (2008) and Rachagani et al. (2006) were used to genotype the *LHR*, *beta-LGB*, and *GH* genes, respectively.

Statistical analysis

LD between each gene was analyzed using GENEPOP version 3.4 (Raymond and Rousset, 2003). An association was considered to be significant when $P < 0.05$.

A general linear model was used to estimate the effects of the genes. The first model was used to estimate the single gene effect for every gene on the traits. The pair of genes with significant associations were re-arranged as the composite genotype and the second model

was used to estimate the effects of the composite genotype on the traits. The lactation level of Holstein cattle was used as the fixed effect in both models, while herd-year-season and days in milk were used as covariates. The relationship between genotypes and traits was considered to be significant when $P < 0.05$. The least significant difference was used to compare the effects of each genotype. SPSS for windows (Release 10) (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

Alleles, genotypes, and composite genotype frequency

The allele, genotype, and composite genotype frequencies of all genes are shown in Tables 1 and 2. Most of the loci (beta-casein, kappa-casein, *beta-LGB*, *DGAT1*, *LHR*, and *GH* genes) showed potential for use as gene markers in a selection program. At each locus, there was more than 1 genotype, and each genotype showed a suitable frequency, although there were some genotypes of some loci with very low frequencies (<3%). These genotypes were eliminated from the analysis.

Table 1. Frequency of allele and genotype (number) of beta- and kappa-casein, acyl-CoA:diacylglycerol acyltransferase 1 (*DGAT1*), beta-lactoglobulin (*beta-LGB*), luteinizing-releasing hormone receptor (*LHR*), and growth hormone (*GH*) genes.

	Allele			Genotype				
Beta-casein	A1	A2	B	A1A1	A1A2	A1B	A2A2	A2B
	34.8	61.0	4.2	5.3 (12)	57.1 (129)	1.8 (4)	29.2 (66)	6.6 (15)
Kappa-casein	A	B	E	AA	AB	AE	BB	BE
	77.2	19.0	3.8	57.0 (129)	34.5 (78)	5.8 (13)	0.9 (2)	1.8 (4)
	Allele			Genotype				
Alpha-S1 casein	B		C	BB		BC	CC	
	0.5		0.5	0		1	0	
Alpha-S2 casein	A		B	AA		AB	BB	
	0.96		0.04	0.92		0.08	0	
DGAT1	K		A	KK		KA	AA	
	35.5		64.5	11.4 (26)		48.2 (110)	40.4 (92)	
beta-LGB	A		B	AA		AB	BB	
	44.3		55.7	17.5 (40)		53.5 (122)	28.9 (66)	
LHR	C		T	CC		CT	TT	
	95.0		5.0	91.2 (166)		7.7 (14)	1.1 (2)	
GH	V		L	VV		VL	LL	
	57.2		42.8	14.3 (26)		85.7 (156)	0	

The alpha-S1 casein and alpha-S2 casein genes were eliminated from the analysis because only 1 genotype of the alpha-S1 casein gene (BC) was observed; for the alpha-S2 casein gene, the frequency of genotype AA exceeded to 0.92, while the other 2 genotypes, AB and BB, were very rare (0.08) and absent, respectively.

Table 2. Frequency of composite genotype (number) of beta + kappa-casein, acyl-CoA:diacylglycerol acyl transferase 1 (*DGAT1*) + beta-casein, and growth hormone (*GH*) + beta-lactoglobulin (*beta-LGB*).

Gene	Frequency of composite genotype					
beta + kappa-casein	A1A2AA	A1A2AB	A1A2AE	A2A2AA	A2A2AB	A2BAB
	36.4 (83)	14.9 (34)	4.8 (11)	17.5 (40)	11.4 (26)	6.6 (15)
DGAT1 + beta-casein	AAA1A2	AAA2A2	KAA1A2	KAA2A2	KKA1A2	
	20.6 (47)	12.7 (29)	28.5 (65)	13.6 (31)	7.5 (17)	
GH + *beta-LGB*	VLAB	VVAA	VVAB	VVBB		
	11.0 (20)	18.7 (34)	42.9 (78)	24.2 (44)		

The composite genotype shown is that with a frequency higher than 3%.

Only 2 alleles, B and C, of the alpha-S1 casein gene were observed in the current study, which is consistent with the results of previous studies such as Heck et al. (2009). However, the results did not agree with those of Kučerová et al. (2006), who studied Czech Fleckvieh dairy cattle with a very high frequency of the variant AB. However, the observed genotype for the alpha-S2 casein gene in the current study was similar to that found by Ibeagha-Awemu et al. (2007), who studied British Friesian and Jersey cattle and found a very high frequency of allele A. Different results were found in populations of Brahman and Nelore cattle, for which the frequency of alleles A and B were similar (Ibeagha-Awemu et al., 2007). Both loci were observable for most dairy cattle breeds investigated, which rarely show allele A for the alpha-S1 casein gene and allele B and D for the alpha-S2 casein gene. This may be because of the specific character of dairy cattle or the result of the selection in the population.

For the beta-casein gene, the gene frequency was consistent with that observed by Aroondechachai et al. (2004) (*Bos taurus* x *Bos indicus*), Jann et al. (2004) (*B. indicus*), and Comin et al. (2008) (*B. taurus*).

For the kappa-casein gene, for which the most common allele was allele A, the results were in agreement with those of Aroondechachai et al. (2004), Azevedo et al. (2008) (*B. taurus* x *B. indicus*), Tsiaras et al. (2005), and Comin et al. (2008) (*B. taurus*).

For the *beta-LGB* gene, a similar allele and genotype frequency was found in the current study as was observed in some previous studies (Rachagani et al., 2006), showing that the frequency of allele A was similar to that of allele B.

For the *DGAT1* gene, allele A and genotype KA were the most frequent in crossbred Holstein cattle. This result is consistent with those of previous studies of different breeds, including UK Holsteins (Banos et al., 2008), German Holsteins (Kaupe et al., 2004), Polish Holsteins (Nowacka-Woszuk et al., 2008), and Chinese Holsteins (Sun et al., 2009).

For the *LHR* gene, allele C and genotype CC were the most frequently observed in the current study. This result is consistent with that of a previous study (Shirasuna et al., 2011), which examined a Holstein population, but the frequency was different from the study of Marson et al. (2008), who examined crossbred dairy cattle in their study.

For the *GH* gene, alleles V and L showed similar frequencies; however, genotype LL was absent from the population. These results differ from those of Heidari et al. (2012), who found that the frequency of allele L was very high in a study of Holstein dairy cattle.

As described above, the group of genes examined may have a specific pattern of allele and genotype frequencies in each dairy cattle breed or line, which may result in a different performance of various breeds or lines of dairy cattle. Such patterns suggest that all genes examined in this study affect milk production and milk composition, as the patterns of allele and genotype frequencies depend on the selection program used for each population.

LD

As shown in Table 3, there were 3 pairs of loci for which a significant LD was found, including the beta + kappa-casein genes, *DGAT1* + beta-casein genes, and *GH* + *beta-LGB* genes. There were 3 interesting observations based on these results. First, a composite genotype of these genes may be an appropriate form for use as a genetic marker if it has a significant effect on traits. Second, the 3 pairs of genes with significant LDs indicate that the whole genome may have numerous LDs between genes, and therefore LD should be considered when choosing genetic markers. Finally, many factors affect LD, including size of population, whether the line was purebred and crossbred, and selection. Therefore, when the population is changed (with a different genetic structure), LD should be re-analyzed. In particular, if the population is crossbred, it is more important to follow the changes in LD as a study conducted by Toosi et al. (2010) found that LD decays faster in crossbreds than in purebreds.

Table 3. P value of linkage disequilibrium analysis.

	Kappa-casein	*DGAT1*	*LHR*	*GH*	*beta-LGB*
Beta-casein	0.0001	0.03	0.67	0.40	0.63
Kappa-casein		0.47	0.48	0.09	0.12
DGAT1			0.15	0.06	0.61
LHR				0.19	0.22
GH					0.02

Effects of a single gene on milk production and milk composition

The effects and the least square means of a single gene on milk production and milk composition traits are shown in Table 4. These results indicate that most genes selected for investigation have a significant effect on milk production and milk composition traits, with the single exception of the *GH* gene, for which no significant effect was found. The negative and positive effects of the genes depend on the traits and genotype. The variant AA of the *beta-LGB* gene had a very large positive effect on MY. This result is consistent with those of many previous studies (e.g., Heidari et al., 2012). The variant AB of the kappa-casein gene showed a very large effect on the percentages of fat, protein, solids-not-fat, and total solid. The studies of Ikonen et al. (2001), Boettcher et al. (2004), and Comin et al. (2008) showed similar results. The largest negative effect on the traits was detected for the variant AA of the *DGAT1* gene. Banos et al. (2008), Bennewitz et al. (2004), Grisart et al. (2002), and Kuehn et al. (2007) showed that there is a significant effect of this locus on milk composition traits, but with a different trend.

The milk protein genes (beta-casein, kappa-casein, and *beta-LGB*), fat protein gene (*DGAT1*), and fertility fitness gene (*LHR*) not only affected their own traits but also affected other traits. This may have occurred through pleiotropic effects of all genes on milk production and milk composition traits (Bovenhuis et al., 1992). Therefore, when choosing gene markers for selection programs, the impact of decreasing genotype frequency that positively affects other traits that are not current breeding goals must be considered.

The genotypes of milk protein genes (except for the kappa-casein gene) *DGAT1* and *LHR* genes, were found to have negative effects on milk protein and other milk composition

traits, while other genes had a positive effect. However, the *GH* gene showed no significant effect on any and was not included in the analysis. According to previous studies (Ikonen et al., 2001; Boettcher et al., 2004; Comin et al., 2008; Sun et al., 2009), inconsistent effects, both positive and negative, have been detected depending on the breed or line of dairy cattle. If the studies were conducted using different breeds or lines of dairy cattle, different genetic structures and epistasis effects may have been observed. This may explain the negative effects of some genotypes in the current study.

However, because the kappa-casein gene showed LD with the beta-casein gene, the *DGAT1* gene has LD with the beta-casein gene, and the *beta-LGB* gene with the *GH* gene, the effects of the composite genotype must also be considered.

Table 4. Effect of a single gene [standard error (SE)] and the least square mean (SE) of milk yield (MY) and milk composition (fat percentage, FAT%; protein percentage, PROT%; solids-not-fat percentage, SNF%; and total solid percentage, TS%).

Traits/gene	Effect of genotype (SE)			Least square mean (SE)		
Beta-casein gene	A1A2	A2A2	A2B	A1A2	A2A2	A2B
MY ($g \cdot cow^{-1} \cdot day^{-1}$)	0.00 (0.30)	-0.54 (0.32)	0	10.12 (0.18)^{b}	9.58 (0.21)^{a}	10.12 (0.29)^{b}
FAT%	-0.03 (0.06)	0.01 (0.07)	0	3.93 (0.04)	3.96 (0.04)	3.96 (0.06)
PROT%	-0.07 (0.03)	-0.01 (0.03)	0	2.96 (0.02)^{a}	3.02 (0.02)^{b}	3.03 (0.03)^{b}
SNF%	-0.10 (0.06)	-0.02 (0.05)	0	8.34 (0.03)^{a}	8.42 (0.03)^{b}	8.44 (0.04)^{b}
TS%	-0.03 (0.06)	0.01 (0.07)	0	12.50 (0.06)^{a}	12.44 (0.07)^{b}	12.47 (0.09)^{b}
Kappa-casein gene	AA	AB	AE	AA	AB	AE
MY ($g \cdot cow^{-1} \cdot day^{-1}$)	0.11 (0.07)	-0.61 (0.08)	0	10.31 (0.18)^{b}	9.59 (0.12)^{a}	10.20 (0.36)^{ab}
FAT%	0.11 (0.07)	0.17 (0.08)	0	3.91 (0.04)^{ab}	3.98 (0.04)^{b}	3.81 (0.08)^{a}
PROT%	0.06 (0.03)	0.09 (0.03)	0	2.98 (0.02)^{a}	3.01 (0.02)^{b}	2.92 (0.04)^{a}
SNF%	0.05 (0.05)	0.09 (0.05)	0	8.36 (0.03)	8.40 (0.03)	8.31 (0.05)
TS%	0.17 (0.11)	0.29 (0.11)	0	12.32 (0.06)^{a}	12.44 (0.06)^{b}	12.15 (0.12)^{a}
LGB gene	AA	AB	BB	AA	AB	BB
MY ($g \cdot cow^{-1} \cdot day^{-1}$)	1.25 (0.22)	1.06 (0.17)	0	10.39 (0.23)^{b}	10.20 (0.17)^{b}	9.14 (0.21)^{a}
FAT%	-0.13 (0.05)	-0.11 (0.04)	0	3.90 (0.05)^{a}	3.92 (0.04)^{a}	4.03 (0.05)^{b}
PROT%	0.00 (0.02)	-0.29 (0.02)	0	3.01 (0.02)	2.98 (0.02)	3.00 (0.02)
SNF%	-0.04 (0.03)	-0.09 (0.03)	0	8.40 (0.04)^{ab}	8.35 (0.03)^{a}	8.44 (0.03)^{b}
TS%	-0.20 (0.07)	-0.22 (0.06)	0	12.34 (0.07)^{a}	12.31 (0.05)^{a}	12.53 (0.07)^{b}
DGAT1 gene	AA	KA	KK	AA	KA	KK
MY ($g \cdot cow^{-1} \cdot day^{-1}$)	0.92 (0.26)	0.64 (0.25)	0	10.36 (0.20)^{b}	10.09 (0.18)^{b}	9.44 (0.25)^{a}
FAT%	-0.45 (0.05)	-0.26 (0.05)	0	3.73 (0.04)^{a}	3.93 (0.04)^{b}	4.18 (0.05)^{c}
PROT%	-0.10 (0.02)	-0.05 (0.02)	0	2.93 (0.02)^{a}	2.99 (0.02)^{b}	3.03 (0.02)^{c}
SNF%	-0.18 (0.04)	-0.13 (0.04)	0	8.31 (0.03)^{a}	8.36 (0.03)^{a}	8.49 (0.04)^{b}
TS%	-0.69 (0.08)	-0.44 (0.08)	0	12.07 (0.07)^{a}	12.32 (0.06)^{b}	12.76 (0.08)^{c}
LHR gene	CC	CT	TT	CC	CT	TT
MY ($g \cdot cow^{-1} \cdot day^{-1}$)	-0.02 (0.72)	-0.34 (0.76)	0	10.26 (0.16)	9.93 (0.32)	10.29 (0.73)
FAT%	-0.03 (0.16)	0.14 (0.17)	0	3.95 (0.04)^{a}	4.13 (0.07)^{b}	3.99 (0.16)^{a}
PROT%	-0.08 (0.07)	-0.05 (0.07)	0	2.96 (0.02)	2.98 (0.03)	3.03 (0.07)
SNF%	-0.14 (0.11)	-0.03 (0.12)	0	8.33 (0.03)	8.44 (0.05)	8.45 (0.12)
TS%	-0.17 (0.24)	0.19 (0.26)	0	12.32 (0.06)^{a}	12.68 (0.11)^{b}	12.49 (0.25)^{ab}
GH gene	VL	VV	-	VL	VV	-
MY ($g \cdot cow^{-1} \cdot day^{-1}$)	-0.00 (0.27)	0	-	10.23 (0.17)	10.23 (0.17)	-
FAT%	0.10 (0.03)	0	-	4.05 (0.06)	3.95 (0.37)	-
PROT%	-0.04 (0.03)	0	-	2.93 (0.03)	2.96 (0.02)	-
SNF%	-0.01 (0.04)	0	-	8.34 (0.04)	8.34 (0.03)	-
TS%	0.10 (0.09)	0	-	12.43 (0.09)	12.34 (0.06)	-

^{a,b,c}Indicate significant difference at $P < 0.05$.

Effects of composite genotype on milk production and milk composition

Table 5 shows that the variant VVAA of the composite genotype *GH* + *beta-LGB* had the highest effect on milk production and a moderate effect on milk protein. Therefore, the variant VVAA of the composite genotype *GH* + *beta-LGB* may be the most suitable variant for a breeding program if the goal is to improve milk production and milk protein; however, milk fat levels will be reduced.

Table 5. Effect of the composite genotype [standard error (SE)] and the least square mean (SE) of milk yield (MY) and milk composition (fat percentage, FAT%; protein percentage, PROT%; solids-not-fat percentage, SNF%; and total solid percentage, TS%).

Traits/gene	Effect of genotype (SE) (upper)			Least square mean (SE) (lower)		
Beta + kappa-casein	A1A2AA	A1A2AB	A1A2AE	A2A2AA	A2A2AB	A2BAB
MY (g·cow^{-1}·day^{-1})	0.19 (0.31)	-0.42 (0.44)	0.00 (0.44)	-0.52 (0.34)	-1.23 (0.57)	0
	10.28 (0.19)^{c}	9.67 (0.23)^{b}	10.89 (0.36)^{b}	10.04 (0.24)^{bc}	8.85 (0.28)^{a}	10.09 (0.29)^{bc}
FAT%	-0.03 (0.07)	-0.01 (0.07)	-0.14 (0.09)	-0.05 (0.07)	0.10 (0.08)	0
	3.93 (0.04)	3.96 (0.05)	3.82 (0.08)	3.91 (0.05)	4.06 (0.06)	3.96 (0.06)
PROT%	-0.06 (0.03)	-0.07 (0.03)	-0.11 (0.04)	-0.04 (0.03)	0.03 (0.03)	0
	2.97 (0.02)^{ab}	2.96 (0.02)^{ab}	2.92 (0.03)^{a}	2.99 (0.02)^{b}	3.06 (0.03)^{c}	3.03 (0.03)^{c}
SNF%	-0.09 (0.05)	-0.12 (0.05)	-0.13 (0.07)	-0.07 (0.05)	-0.08 (0.06)	0
	8.35 (0.03)^{b}	8.32 (0.04)^{a}	8.31 (0.06)^{ab}	8.37 (0.04)^{ab}	8.52 (0.04)^{c}	8.44 (0.04)^{bc}
TS%	-0.16 (0.1)	-0.16 (0.11)	-0.32 (0.14)	-0.15 (0.11)	0.15 (0.12)	0
	12.32 (0.06)^{ab}	12.31 (0.08)^{ab}	12.15 (0.12)^{a}	12.33 (0.12)^{ab}	12.62 (0.09)^{c}	12.48 (0.09)^{b}
DGAT1 + beta-casein	AAA1A2	AAA2A2	KAA1A2	KAA2A2	KKA1A2	
MY (g·cow^{-1}·day^{-1})	1.23 (0.33)	0.33 (0.36)	0.91 (0.31)	0.57 (0.35)	0	
	10.72 (0.23)^{c}	9.81 (0.27)^{a}	10.39 (0.19)^{bc}	10.05 (0.25)^{ab}	9.48 (0.29)^{a}	
FAT%	-0.47 (0.07)	-0.50 (0.07)	-0.34 (0.06)	-0.16 (0.07)	0	
	3.74 (0.05)^{a}	3.70 (0.06)^{a}	3.86 (0.04)^{b}	4.04 (0.05)^{c}	4.20 (0.06)^{d}	
PROT%	-0.02 (0.03)	-0.01 (0.03)	0.02 (0.03)	0.07 (0.03)	0	
	2.91 (0.02)^{a}	2.93 (0.02)^{ab}	2.96 (0.02)^{b}	3.00 (0.02)^{c}	2.94 (0.03)^{ab}	
SNF%	-0.14 (0.05)	-0.09 (0.05)	-0.11 (0.04)	-0.02 (0.05)	0	
	8.26 (0.03)^{a}	8.32 (0.04)^{ab}	8.30 (0.03)^{a}	8.39 (0.04)^{b}	8.41 (0.04)^{b}	
TS%	-0.65 (0.1)	-0.60 (0.11)	-0.48 (0.09)	-0.22 (0.10)	0	
	12.03 (0.07)^{a}	12.07 (0.08)^{ab}	12.20 (0.06)^{b}	12.45 (0.07)^{c}	12.67 (0.09)^{d}	
GH + *beta-LGB*	VLAB	VVAA	VVAB	VVBB		
MY (g·cow^{-1}·day^{-1})	0.53 (0.35)	1.61 (0.25)	1.10 (0.21)	0		
	9.89 (0.32)^{ab}	10.52 (0.24)^{b}	10.46 (0.18)^{b}	9.36 (0.23)^{a}		
FAT%	0.02 (0.08)	-0.14 (0.06)	-0.14 (0.05)	0		
	4.05 (0.07)^{b}	3.89 (0.05)^{a}	3.88 (0.04)^{a}	4.03 (0.05)^{b}		
PROT%	-0.02 (0.03)	0.05 (0.02)	-0.01 (0.02)	0		
	2.94 (0.03)^{a}	3.00 (0.02)^{b}	2.95 (0.02)^{ab}	2.96 (0.02)^{ab}		
SNF%	-0.04 (0.06)	0.01 (0.04)	-0.05 (0.03)	0		
	8.33 (0.05)	8.38 (0.04)	8.32 (0.03)	8.37 (0.04)		
TS%	-0.04 (0.12)	-0.16 (0.09)	-0.22 (0.07)	0		
	12.42 (0.11)^{ab}	12.31 (0.08)^{ab}	12.25 (0.06)^{a}	12.46 (0.08)^{b}		

^{a,b,c}Indicate significant difference at $P < 0.05$.

An interaction effect between genes (*GH* and *beta-LGB*, beta-casein and kappa-casein, and *DGAT1* and beta-casein genes) was observed. No significant effect was detected when only the *GH* gene was analyzed, while the effect of only the *beta-LGB* gene was less than that of the composite genotype or the effect of beta + kappa - casein gene on milk production and milk composition. Most variants of the kappa-casein gene showed a positive effect on most traits, but the composite genotype of this gene with the beta-casein gene had a negative effect on most traits; when the *DGAT1* + beta-casein gene was considered, the effect of the

beta-casein gene was dominated by the *DGAT1* gene.

The association between genes should be determined. With regard to the beta + kappa-casein genes, both were located very closely within 250 kilobases on chromosome 6 (Bonfatti et al., 2010), so it is possible that both genes have a co-regulation mechanism (Bonfatti et al., 2010), which would explain the association between these genes. A dominant effect of the beta-casein gene was observed, as the composition of milk protein and the proportion of the beta-casein protein (30%) was higher than for the kappa-casein protein (10%) (Choi et al., 1988). Therefore, the effect of the beta-casein gene may be stronger than for the other genes. When the composite genotype of *GH* + *beta-LGB* and the composite genotype of *DGAT1* + beta-casein were investigated, they were found to be located on different chromosomes, but these genes may function together.

Regarding the composite genotype of *GH* + *beta-LGB*, the interaction effect may have been caused by the product of the *GH* gene, a growth hormone. The protein is thought to increase hormone secretion and increase the diversion of nutrients from the body stores to the mammary gland (Tucker, 1985). This mechanism may stimulate milk production and increase the levels of amino acid used in protein synthesis, resulting in the production of the beta-LGB, which may increase the beta-LGB protein. However, the role of the *beta-LGB* gene in milk production remains unclear, but some previous studies (Doosti et al., 2011; Heidari et al., 2012) found significant effects on this trait. Bovenhuis et al. (1992) suggested that this is due to pleiotropic effects. When both of the genes have effects on similar traits, an interaction may occur.

For *DGAT1* + beta-casein, the traits observed include the interaction effects on milk production and milk fat. For milk production, both genes were found to have a significant effect on the traits in many previous studies as described above. However, the mechanism through which the genes exert their effects on the milk production remains unclear. It is possible that the *DGAT1* gene and the beta-casein gene have important effects on the composition of milk, fat, and protein, respectively; therefore, they may have an indirect effect on milk synthesis, explaining why an interaction effect on milk production was observed. In the case of milk fat, a dominant effect of the *DGAT1* gene on the beta-casein gene was observed, as the *DGAT1* gene has a primary role in milk fat synthesis. Similarly, for protein, it has been observed that the effect of the beta-casein gene dominates the effect of the *DGAT1* gene.

CONCLUSION

In this study, we found that the variant VVAA of the composite genotype *GH* + *beta-LGB* had the highest effect on milk production and a moderate effect on milk protein; therefore, this genotype is the most suitable for use as a gene marker in selection programs for the crossbred Holstein population used in the current study. Furthermore, because all genes showed significant LD with each other, the effects of the single gene and composite genotype were different; therefore, a study of the gene marker with a single gene or several independent genes is not a suitable design for such a study. A study of a group of genes with similar or related roles and their LDs should be conducted. Moreover, a deeper understanding of the interaction between genes with LD is necessary to elucidate the role of the effects of genes on traits and on each other.

ACKNOWLEDGMENTS

The authors are grateful to the Farm of Suranaree University of Technology for providing the data and blood collection and also to the Suranaree University of Technology, Thailand Research Fund, the National Research Council of Thailand and the Office of the Higher Education Commission for their financial support.

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