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Polymorphisms of LEP, LGB and PRLR in water buffalo

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Abstract : The polymorphisms of several genes including Leptin (LEP), beta-lactoglobulin (LGB) and Prolactin receptor (PRLR) have been shown to affect milk composition traits in dairy cattle. But, the effects of these polymorphisms on the milk traits of Philippine water buffalo are still unclear. In the Philippines, buffalo are the major milk producers most of which are the Philippine carabao (PC), the American Murrah Buffalo (AMB) and Bulgarian Murrah Buffalo (BMB). The LEP, LGB and PRLR genes are considered to be associated with milk production traits. The objective of the present study was to identify the single nucleotide polymorphisms (SNPs) in the LEP, LGB and PRLR genes of PC, AMB and BMB and to investigate the effect of the SNPs on milk production traits in these buffalo. Genetic polymorphisms were screened by DNA sequencing and 12 SNPs were detected in BMB; 5 SNPs were in LEP exon3 region (G14227A, G14343A, T14502C, C14526T, G14603A); 5 SNPs were in LGB exon 2 region (G1861C, A1900G, G1901T, T1948C, G1949A); 2 SNPs were in PRLR exon 6 (T59047C, T59109C). Also, 12 polymorphism sites between cattle and buffalo were identified. Our analysis of the association between SNPs and milk production traits should be useful in future studies of buffalo breeding to improve lactation performance.

Key words **:** Buffalo, Philippine carabao, LEP, LGB, PRLR, Milk production traits

I. Introduction

The water buffalo (*Bubalus bubalis*) are important domestic animals distributed in the tropical and sub-tropical regions (Shi et al., 2011). They are of particular importance for meat and milk production in tropical and subtropical countries because of their ability to adapt to harsh environmental conditions (Das and Khan, 2010; Yindee et al., 2011). There are two distinct types, the riverine and swamp types. Native water buffalo or Carabao that are found in the Philippines and in the South and Southeast Asian regions belong to the swamp type. American Murrah Buffalo (AMB) and the Bulgarian Murrah Buffalo (BMB) are found in India, Europe and the Americas and belong to the riverine type. Swamp buffalo have 48 chromosomes, and riverine buffalo have 50 chromosomes (Nanda et al., 2003; Nanda et al., 2006; Lei et al., 2007; Groeneveld et al., 2010; Mingala et al., 2011).

The Korean cattle, *Bos taurus*, is a breed native to the Korean peninsula and the Japanese islands, and is considered to descend from a breed of European cattle (Seong et al., 2012)

In a previous study, Leptin (LEP), beta-lactoglobulin (LGB) and Prolactin receptor (PRLR) were shown to be genes that are associated with milk production traits in cattle (Ruheena, et al., 2011; Shi, et al., 2011; Heidari, et al., 2012; Tanpure, et al., 2012). LEP, the fat derived protein hormone, has a variety of physiological functions such as regulation of feed intake, energy balance, and fertility and immune functions (Tanpure et al., 2012). Genetic polymorphisms within the bovine LEP gene were found to be associated with growth, fertility and milk production in Holstein cows (Clempson et al., 2011; Tanpure et al., 2012). LGB is one of the major components of bovine milk. The SNPs in the exon2 region of the LGB gene were

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shown to affect milk performance traits (i.e., milk yield, protein and fat contents) in Chinese Holstein cows (Yang et al., 2011). PRLR is one of the main proteins of bovine milk (Ganai et al., 2009). The PRLR-S18N polymorphism (Ser to Asn) is the major QTL associated with milk production traits. Holstein, Chinese Holstein and Montebeliard cows with the AG nucleotide genotype showed the highest milk yield, while GG genotype cows showed the highest fat content (Shi et al., 2011).

Genetic analysis of these genes in the Philippine buffalo is limited. In this study, we detected SNPs in the LEP, LGB and PRLR genes using DNA sequencing technology in BMB, AMB, PC and KC. This study not only provides insight into milk production of dairy cattle but it also provides helpful information that may be used to improve the milk production, in both quantity and quality, through genetic manipulation in water buffalo.

II. Materials and Methods

1. DNA samples and Genomic DNA Extraction

A total of 390 buffaloes from the Philippine Carabao Center (PCC) were used with the following distribution: 20 American Murrah Buffalo, 20 Philippine Carabao, 350 Bulgarian Murrah Buffalo and 20 Korean cattle (KC). DNA was extracted from blood using the Promega Blood and Tissue Kit and DNA concentration was quantified using a ND-2000 UV-Vis spectrophotometer (Nano Drop Technologies, USA).

2. PCR Amplification and Sequencing

The LEP, LGB and PRLR gene regions were amplified using five pairs of primers (Table 1). The Polymerase Chain Reaction (PCR) amplification was performed in 20 μl volume and each reaction contained 100 ng of genomic DNA, 10 pmole of each primer, 2.5 μl of 10x PCR buffer (10 mM Tris-HCl,50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 2.5 μl dNTP (2.5 mM), and 0.1 μl Taq polymerase(10 unit/μl)(GENET Bio, Korea). The PCR condition was as follows: first denaturation step of 5 min at 94℃ followed by 35 cycles, each step consisting of 1 min at 94℃, 30 sec at each temperature, 30sec at 72℃ and then, a final step of 5 min at 72℃. The reactions were performed using a PTC200 Pettier thermal cycler (MJ Research, USA). DNA sequencing was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems, USA) to determine each of the nucleotide sequences. Sequences were determined using the Seq Man II program (DNA Star, USA) for each SNP.

3. Statistical analysis

Allele and genotype frequencies were calculated by simple counting. Hardy-Weinberg equilibrium was tested by comparing the expected and observed genotypes of frequencies using the Chi-square test.

III. Results

1. Polymorphisms of the LEP, LGB and PRLR genes using BMB

We identified 12 single nucleotide polymorphisms

Table 1. Primers and length of each amplified fragment and annealing temperature.

Primer Name		Primer Sequence	Size (bp)	Annealing Temp (℃)
LEP exon 3	F	GAGGAAGCACCTCTACGCTCTA	815	61
	R	GATTCTCAGAGGGAATCTTGCTT		
LGB exon 2	F	CTGGCCCTCAGTTCATCCT	243	57
	R	ATCACCACCAGCCCCTTAG		
PRLR exon 6	F	GACCTACATACTGGCTTCTCTGC	280	58
	R	GCAGATTTCAGGCAGAATCC		

(SNPs) in the LEP, LGB, and PRLR genes. Of these SNPs, 5 were located in LEP exon 3 region (G14227A, G14343A, T14502C, C14526T, G14603A), 5 SNPs were in LGB exon 2 region and intron (G1861C, A1900G, G1901T, T1948C, G1949A), and 2 SNPs were in PRLR exon 6 and intron (T59047C, T59109C). The estimated LEP, LGB and PRLR allele and genotype frequencies are shown in Table 2.

In the LEP gene, G14343A is a synonymous mutation where amino acid 159 changes from arginine to glutamine. The G14343A frequency for allele G was higher than that for allele A and 0.68, 0.29 and 0.03 displayed the GG, GA and AA genotypes, respectively. Also, the C14526T mutation for the LEP gene had a frequency for allele C that was higher than allele T and 0.35, 0.45 and 0.20 displayed the CC, CT and TT genotypes, respectively.

In the LGB gene, the A1900G frequency for allele A was higher than that for allele G and 0.78, 0.21 and 0.01 displayed the GG, GA, and AA genotypes, respectively. For the G1901T SNP, the frequency for allele G was higher than that for allele T and 0.78, 0.21 and 0.01 displayed the GG, GT, and TT genotypes, respectively. Also, two haplotypes A-G and G-T, were found at the 1900th-1901st site.

In the PRLR gene, the T59109C SNP had a frequency for allele C that was higher than that for allele T and 0.90 and 0.10 displayed the CC and CT genotypes, respectively.

2. Comparison of polymorphism sites between cattle and buffalo

We identified 12 SNPs in the three sequenced gene fragments between buffalo and KC, including nine intra specific SNPs among the three buffalo groups (Table 3).

For the LEP gene, KC, AMB and PC species fixed the G allele genotype of the G14343A SNP and BMB had the G and A allele fixed. Thus, KC, AMB and PC species fixed the R allele genotype of R159Q and BMB have both R and Q at this same amino acid.

For the LGB gene, KC has the T allele genotype of the G1901T SNP, BMB and AMB have both the G and T alleles and PC has both the A and T alleles.

For the PRLR gene, all buffalo species fixed the A allele genotype of the G59222A SNP, and KC fixed both the A and G allele genotypes.

Table 2. Genotype and allele frequencies of specific gene regions in BMB.

Gene	SNPs	AA change	Genotype freq			Allele freq	
			11	12	22		
LEP	G14227A		GG (0.80)	GA (0.19)	AA (0.01)	G (0.90)	A (0.10)
	G14343A	R159Q	GG (0.68)	GA (0.29)	AA (0.03)	G (0.82)	A (0.18)
	T14502C		CC (0.52)	CT (0.41)	TT (0.07)	C (0.73)	T (0.27)
	C14526T		CC (0.35)	CT (0.45)	TT (0.20)	C (0.58)	T (0.43)
	G14603A		AA (0.19)	GA (0.46)	GG (0.35)	A (0.42)	G (0.57)
LGB	G1861C		CC (0.79)	CG (0.19)	GG (0.02)	C (0.89)	G (0.11)
	A1900G		AA (0.78)	AG (0.21)	GG (0.01)	A (0.88)	G (0.12)
	G1901T		GG (0.78)	GT (0.21)	TT (0.01)	G (0.88)	T (0.12)
	T1948C		TT (0.28)	TC (0.53)	CC (0.18)	T (0.55)	C (0.45)
	G1949A		GG (0.29)	GA (0.53)	AA (0.19)	G (0.55)	A (0.45)
PRLR	T59047C		CC (0.34)	CT (0.49)	TT (0.17)	C (0.59)	T (0.41)
	T59109C		CC (0.90)	CT (0.10)	TT (0.00)	C (0.95)	T (0.05)

Table 3. The polymorphism sites of three gene fragments between water buffalo and cattle.

Gene Fragment	Position	*Bos Taurus*	*Bubalus bubalis*		
		Hanwoo	BMB	AMB	PC
LEP	14263	C/T	C	C	-[1)]
	14266	C/T	T	C	-
	14278	C/T	C	C	-
	14362	C/T	T	T	-
	14474	G/A	G	G	-
	14227	G	G/A	G/A	A
	14343	G	G/A	G	G
LGB	1900	C	A/G	A/G	A/G
	1901	T	G/T	G/T	A/T
	1950	G/A	G/A	G/A	G
PRLR	59047	T	T/C	T/C	T
	59222	A/G	A	A	A

[1)] No data

IV. Discussion

Asia has an appreciably rich water buffalo population that has the ability to adapt to harsh environmental conditions, and although these buffalo have poor milk yields their milk has a higher percentage of fat and protein compared to dairy cattle (Shi et al., 2011). In the Philippines, water buffalo are grown mainly for milk and meat. Given the increasing demands for milk, the water buffalo genetic improvement program is currently underway. To develop efficient milk producers, government efforts have focused on transforming the swamp buffalo population from draft animals to milk producers by cross-breeding the riverine species with local breeds. This program focuses on improving milk traits and institutionalizing an organized evaluation and selection system. To achieve this, the program continually back- crosses the water buffalo with the Murrah breed with the aim of producing close to purebred dairy cattle by the third or fourth generation.

In recent years, a number of candidate genes related to milk production traits have been identified, and many studies have been undertaken to investigate the relationship between these candidate genes and the lactation performance in dairy cattle. However, the polymorphisms of these genes and their effect on milk traits in Philippine water buffalo are still unclear.

LEP is a candidate gene related to milk production traits. In previous studies, genetic polymorphisms within the bovine leptin gene were found to be associated with growth, fertility and milk production in Holstein cows (Clempson et al., 2011; Tanpure et al., 2012). A novel synonymous SNP (R159Q) was found in BMB.

In the LGB gene, SNPs in the exon 2 region affected milk performance traits (i.e., milk yield, protein and fat contents) in Chinese Holstein cows (Yang et al., 2011). Two interesting haplotypes A-G, G-T, were found at the 1900th-1901st site in BMB.

In the PRLR gene, a previous study showed that there was a synonymous SNP (c/t) in exon 3 in the Chinese swamp buffalo group, and all buffalo groups shared the same N allele of the S18N amino acid mutation (Shi et al., 2011). In this study, a novel SNP was found in all buffalo where the A allele genotype of G59222A were fixed, and KC have the A and G allele genotypes fixed.

The present study identified SNPs of the LEP, LGB and PRLR genes of Philippine buffalo and KC. These findings should provide insight for further study in water buffalo breeding programs that focus on increasing

lactation performance.

V. Conclusions

The objective of the present study was to identify the single nucleotide polymorphisms (SNPs) in the LEP, LGB and PRLR genes of PC, AMB and BMB and to investigate the effect of the SNPs on milk production traits in these buffalo. We identified 12 single nucleotide polymorphisms (SNPs) in the LEP, LGB, and PRLR genes. Of these SNPs, 5 were located in LEP exon 3 region (G14227A, G14343A, T14502C, C14526T, G14603A), 5 SNPs were in LGB exon 2 region and intron (G1861C, A1900G, G1901T, T1948C, G1949A), and 2 SNPs were in PRLR exon 6 and intron (T59047C, T59109C). Information provided in this study should provide insights for further studies in improving Philippine buffalo breeding for better lactation performances by analysis of association between SNPs and milk production traits.

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