

Effect of beta-lactoglobulin polymorphism and seasonality on bovine milk composition

Bruno G Botaro¹, Ygor V R Lima², Adriana A Aquino³, Raquel H R Fernandes¹, José F Garcia⁴ and Marcos V Santos^{1*}

¹ Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Duque de Caxias Norte, 225, Pirassununga, 13635-900, Brazil

² Centro Universitário Anhangüera, Faculdade de Medicina Veterinária, Waldemar Silenci, 340, Leme, 13614-370, Brazil

³ Universidade Federal de Lavras, Faculdade de Zootecnia, PO Box 3037, Minas Gerais, 37200-000, Brazil

⁴ Universidade Estadual Paulista Júlio de Mesquita Filho, Laboratório de Bioquímica e Biologia Molecular Animal, Clóvis Pestana, 793, Araçatuba, 16050-680, Brazil

Received 10 September 2007; accepted for publication 16 January 2008

The objective was to evaluate the effect of β -lactoglobulin (β -lg) polymorphism and seasonality on milk composition (fat, lactose, total solids, milk urea nitrogen, total protein, true protein, casein and somatic cell counts) of Holstein and Girolando cows. Milk and blood samples from 278 Holsteins cows and 156 Girolando cows were taken during two dry seasons and two rainy seasons, for milk composition analysis and to determine β -lg genotypes, respectively. BB genotype was the most frequent for both breeds, followed by AA genotype for Holstein (BB>AA>AB) and by AB for Girolando cows (BB>AB>AA). No differences were found in milk compositional characteristics among genetic variants of β -lg (AA, AB and BB) either between Holstein or Girolando cows. No association between milk composition and β -lg genetic polymorphism was observed. During the dry season, independently of the breed considered, higher contents of lactose, true protein, casein and casein: true protein ratio were found.

Keywords: Milk protein, β -lactoglobulin, genetic variants, seasonality.

The discovery of electrophoretically distinct types of β -lactoglobulin (β -lg) by Aschaffenburg & Drewry (1955) initiated the beginning of extensive investigations of the different genotypes of this whey protein (Hambling et al. 1992). The two main genetic forms of β -lg differ in the substitution of a Gly and an Ala in the B variant, for an Asp and a Val in the A variant (Ghashghaei, 2003) and this slight molecular difference has been widely associated with significant differences in milk composition in dairy cows (Dove, 2000).

Several studies have reported the association of significantly higher fat content (Ng Kwai-Hang et al. 1986; Aleandri et al. 1990; Tsiaras et al. 2005), protein (Litwinczuk et al. 2006), casein (Robitaille et al. 2002), true protein (Bober et al. 1999) and total solids (Celik, 2003) with BB variant, while others have found higher milk component concentrations due to the AA variant (Ng Kwai-Hang et al. 2002b; Robitaille et al. 2002; Molina et al. 2006). Nonetheless, some authors have

found no relationship between β -lg polymorphism and milk composition, and attributed the differences observed in fat and protein contents to other factors (Ng Kwai-Hang et al. 2002a).

Factors other than genetic polymorphism of milk proteins, e.g., seasonal variations (Paquin & Lacroix, 1994; Carlsson et al. 1995; Lacroix et al. 1996; Allore et al. 1997; Auldist et al. 1998; Sharma et al. 2002; Lindmark-Månsson et al. 2003; Teixeira et al. 2003; Amenu et al. 2006) and breed (Auldist et al. 1998; Arunvipas et al. 2003) all affect milk composition but their interaction with β -lg genetic variants has not been studied. Moreover, data on the association between β -lg genetic variants of Girolando cows and milk composition of this breed are still rare. The Girolando breed is basically the result of crossing between the Holstein and Gir (indigenous Zebu breed) in standard proportions of 3/8 Zebu and 5/8 Taurine. This crossbred breed is very common in Brazil because it is claimed to have both the high resistance to heat and humidity characteristic of Indian cattle and the high productivity characteristic of European cattle (Bicalho et al. 2006). The objective of this study was to evaluate the

*For correspondence; e-mail: mveiga@usp.br

effect of genetic polymorphism of β -lg on milk composition (fat, lactose, total solids, milk urea nitrogen, total protein, true protein, casein and somatic cell counts) in Holstein and Girolando cows, during dry and rainy seasons.

Material and Methods

Selection of herds and cows for sampling

The study was carried out in eleven commercial dairy herds in the state of São Paulo, Brazil; among them six were composed of Girolando (crossbred Holstein-Zebu) and five of Holstein cows. Milk and blood samples were taken from 278 lactating Holstein cows and 156 lactating Girolando cows during two dry seasons and two rainy seasons (Sampling period 1, September and October, 2003; Sampling period 2, June and July, 2004; Sampling period 3, November and December, 2004; and Sampling period 4, January and February, 2005). In each dairy herd, the sampled cows had to fulfil the following criteria: first–third lactation and 30–250 d of lactation. Cows with clinical mastitis, or submitted to mastitis treatment 2 weeks before milk sampling, were excluded from this study. In addition, at the time of milk sampling, information such as age, days in lactation and type of feeding system (pasture, supplemented pasture or total mixed ration) for each selected dairy cow was registered.

Milk samples were collected during morning milking in order to represent the whole milking of each animal. The individual samples were collected directly from the collection bucket immediately after milking, or during milking, using flux measurement devices connected to the milk line. Collected samples were split into two flasks: one treated with Bronopol (Boots Microcheck, Nottingham, UK) was used for fat, lactose, total solids, milk urea nitrogen and somatic cell determinations; and the other was used for analysis of milk protein fractions.

Methods of analysis

Milk composition: Milk samples were analysed for fat, lactose and total solids by infrared absorption methodology (Bentley 2000, Bentley, Chasca MN, USA). Milk urea nitrogen (MUN) was determined by the colorimetric-enzymic method (Chemspec 150, Bentley Instruments Inc., Chasca MN, USA). Somatic cells were electronically counted (Somacount 300, Bentley Instruments Inc.) and converted to log scale (LogSCC). Milk protein fractions were determined for total nitrogen (AOAC, 1990; method 33.2.11; 991.20), non-casein nitrogen (NCN) (Lynch et al. 1998) and non-protein nitrogen (NPN) (AOAC, 1995; method 33.2.12; 991.21). In order to express the results as total protein (TP), total nitrogen values found were multiplied by 6.38 (Barbano et al. 1990). Milk true protein (MTP) and casein concentrations were obtained by

difference according to: $TP - (NPN \times 6.38) = \text{milk true protein}$, and $MTP - (NPN \times 6.38) = \text{casein}$, respectively.

***β*-lg genetic polymorphism analysis:** After DNA extraction from blood samples according to Ciulla et al. (1988), extracted DNA samples were submitted to PCR amplification as described by Schlee & Rottmann (1992). The oligonucleotides primers used for DNA amplification were synthesized by Invitrogen Custom Primers (Invitrogen Corp., Carlsbad CA 92008, USA), according to the sequence described by Faria et al. (2000):

Forward: 5'ACCTGGAGATCCTGCTGCAGAAATG3'

Reverse: 5'CATCGATCTTGAACACCGCAGGGAT3'

Each amplification reaction consisted of PCR buffer 1X (500 mM-KCl, 100 mM-Tris-Cl pH 8.3), 0.1 μ l of the described primers, 2.0 μ l dNTP (0.125 mM), 0.1 μ l Taq polymerase (Cenbiot/RS, PHN/MG), 0.75 μ l MgCl₂ (Cenbiot/RS, PHN/MG), 5.0 μ l of DNA and mili-Q water qsp 25 μ l. In all amplified reactions, a control (blank/no-DNA) was used to confirm the absence of contamination during the analysis. The amplification process was carried on a PTC 100-MJ Research thermocycler (MJ Research Inc., Watertown MA 02472, USA).

After DNA amplification of the targetted fragment, a 961-bp region of the β -lg gene, the PCR product (20 μ l) was submitted to RFLP in a 2% agarose gel electrophoresis, and digested by the restriction enzyme Hph-I (Invitrogen Corp.) according to Wilkins & Kuys (1992) to show the presence of either AA, AB or BB genotypes. Hph-I cleaved amplified fragments in 741 and 220 bp (AA genotype), 741, 166 and 54 bp (BB genotype), or a combination of both (AB genotype), with 741, 220, 166 and 54 bp (Wilkins & Kuys, 1992). After β -lg gene identification, the genotype and allele frequencies for the studied herd were obtained.

Statistical analysis

Effects of β -lg, breed and seasonality were analysed in a randomized block design with number of lactation (1, 2 and ≥ 3) and feeding system (pasture, supplemented pasture or total mixed ration) as blocks. For statistical analysis, selected cows were distributed according to the β -lg genetic variant (AA, AB or BB), breed (Holstein or Girolando) and seasonality (dry or rainy season). Descriptive statistics of the given results were stored (arithmetic mean and SEM) and analysis of variance was performed, evaluating the effects of breed, seasonality and genetic variation of β -lg on the response variables, by the SAS PROC GLM procedure (Statistical Analysis System, Version 8.02; SAS Institute Inc., Cary NC 2001, USA).

In the adopted model, dependent variables used were milk composition (MC) characteristics and fixed factors were β -lg polymorphism, breed and seasonality. The

Table 1. β -lactoglobulin gene and allele frequencies for Holstein and Girolando cows

Breed	Geno- types	Genotype frequencies		Allele frequencies \pm SEM [†]
		n_{observed}	%	
Holstein	AA	91	32.73	A=0.4694 \pm 0.0211 B=0.5305 \pm 0.0211
	AB	79	28.42	
	BB	108	38.85	
	Total	278		
Girolando	AA	33	21.15	A=0.3815 \pm 0.0278 B=0.6185 \pm 0.0278
	AB	53	33.97	
	BB	70	44.87	
	Total	156		

[†] SEM according to Oner & Elmaci (2006)

model is described as follows:

$$MC_{ilm} = \mu + L_i + F_j + B_l + P_m + S_n + B_l * P_m + B_l * S_n + P_m * S_n + B_l * P_m * S_n + e_{ijlmn}$$

MC is the variable considered for milk composition; μ = mean value for the characteristic; L_i = lactation number ($i=1, 2$ and ≥ 3), F_j = feeding system (j = pasture, supplemented pasture or total mixed ration), B_l = Breed (l = Holstein and Girolando); P_m = genotypes (m = AA, AB and BB); S_n = seasonality (n = rainy and dry season); e_{ijlmn} = error.

Results

Genetic polymorphism of β -lg in Holstein and Girolando cows

A total of 278 Holstein and 156 Girolando cows were tested for genetic polymorphism of β -lg. The distribution of genotype and allele frequencies for the β -lg gene of Holstein and Girolando cows, obtained by PCR-RFLP, is summarized in Table 1.

All three β -lg genotypes (AA, AB and BB) were present in both breeds. The allele B was present in a higher frequency both for Holstein and Girolando breed (0.5305 \pm 0.021 and 0.6185 \pm 0.027, respectively). The frequency of appearance of genotypes differed between Holstein and Girolando cows. BB genotype was the most frequent (38.85% and 44.87%) among studied breeds. AA and AB genotypes had frequencies of 32.73% and 28.42% for Holstein, but 21.15% and 33.97% for Girolando cows.

Effect of β -lg genetic polymorphism and breed on milk composition

Milk composition mean values and SEM for β -lg genotypes in Holstein and Girolando cows are shown in Table 2.

No statistical differences in milk composition of both breeds were observed among β -lg genotypes for any of the

studied variables except for milk urea nitrogen concentration, which was higher (16.64 mg/dl) for Holstein than for Girolando cows (14.38 mg/dl). No interaction between β -lg and breed was found for milk composition.

The average of milk fat and total solids content for the AA, AB and BB genotypes did not differ in any of the breeds. For Holstein cows, milk fat averages were 3.305, 3.267 and 3.359% for the AA, AB and BB genotypes, respectively, while for Girolando, the averages were 3.143, 3.274 and 3.166% for the same genotypes. Total solids averages 11.824, 11.823 and 11.876% among Holstein cows of AA, AB and BB genotypes, respectively, and 11.595, 11.808 and 11.755%, respectively to the cited genotypes for Girolando animals.

Concentrations of total protein, true protein, casein, MUN as well the casein: true protein ratio were not influenced by the β -lg genotypes in this study. Milk from Holstein and Girolando BB cows, respectively, had total protein of 3.125 and 3.091%, true protein of 2.892 and 2.859%, and casein of 2.095 and 2.070%. Averages for the casein: true protein ratio among BB genotype cows were 0.722 for the Holstein breed and 0.721 for Girolando.

Effect of β -lg genetic polymorphism and seasonality on milk composition

Effects of seasonality and β -lg polymorphism on milk composition are shown on Table 3.

Concentrations of lactose, MTP, casein and casein: MTP ratio were significantly higher during the dry season than in the rainy season. Average concentrations for the dry season were 4.501, 2.918 and 2.132 g/100 g of milk, respectively for lactose, TP, MTP and casein, while rainy season corresponding means were 4.349, 2.846 and 1.994 g/100 g of milk. No interaction between seasonality and β -lg polymorphism was observed.

When non-significant interactions were removed from the statistical model, casein concentrations observed during the rainy season were different among β -lg genetic variants (AA > BB) with higher averages for AA (2.067 g/100 g of milk) compared with BB cows (1.949 g/100 g of milk). But no differences were observed for milk composition variables among β -lg variants within the dry season.

Discussion

Ng Kwai-Hang et al. (1986), Van Eenennaam & Medrano, (1991), Celik (2003), Oner & Elmaci (2006) reported higher frequencies for the B allele for Holstein cows. In the present study, the frequency of genotype appearance differed between Holstein and Girolando cows. Although the BB genotype was the most frequent for both breeds (38.85% and 44.87%), AA genotype was the second higher in frequency level in Holstein breed (BB > AA > AB), while the AB was more frequent than AA genotype in

Table 2. Effect of *β*-lactoglobulin polymorphism in Holstein and Girolando cows on milk composition

Breed <i>β</i> -lg polymorphism	Girolando			Holstein			<i>P</i>		
	AA	AB	BB	AA	AB	BB	Breed	<i>β</i> -lg	B* <i>β</i> -lg
Fat, g/100 g	3.143	3.274	3.165	3.305	3.267	3.359	0.877	0.850	0.083
SEM	0.108	0.088	0.060	0.054	0.054	0.047			
Lactose, g/100 g	4.447	4.460	4.458	4.444	4.429	4.443	0.465	0.857	0.697
SEM	0.031	0.024	0.022	0.017	0.018	0.016			
Total Solids, g/100 g	11.595	11.808	11.755	11.824	11.823	11.876	0.497	0.782	0.534
SEM	0.126	0.103	0.081	0.064	0.067	0.057			
Total protein, g/100 g	3.150	3.066	3.091	3.146	3.144	3.125	0.914	0.213	0.716
SEM	0.039	0.031	0.025	0.020	0.020	0.018			
True protein, g/100 g	2.912	2.853	2.859	2.927	2.915	2.892	0.795	0.204	0.774
SEM	0.044	0.031	0.027	0.020	0.020	0.018			
Casein, g/100 g	2.123	2.065	2.070	2.129	2.111	2.095	0.449	0.123	0.804
SEM	0.048	0.032	0.030	0.023	0.023	0.020			
Casein:true protein	0.731	0.720	0.721	0.725	0.722	0.722	0.165	0.114	0.894
SEM	0.007	0.004	0.005	0.003	0.004	0.003			
Log (SCC × 10 ⁻³ cells/ml)	2.501	2.275	2.445	2.300	2.404	2.413	0.278	0.271	0.107
SEM	0.088	0.065	0.056	0.049	0.046	0.038			
Urea nitrogen, mg/100 ml	14.008	14.628	14.517	16.571	16.823	16.527	0.001	0.439	0.720
SEM	0.580	0.472	0.430	0.298	0.314	0.225			

Table 3. Effect of seasonality and *β*-lactoglobulin polymorphism on milk composition

Seasonality <i>β</i> -lg polymorphism	Rainy season			Dry season			<i>P</i>		
	AA	AB	BB	AA	AB	BB	Season	<i>β</i> -lg	S* <i>β</i> -lg
Fat, g/100 g	3.208	3.252	3.292	3.308	3.279	3.296	0.995	0.8501	0.988
SEM	0.080	0.082	0.063	0.060	0.057	0.046			
Lactose, g/100 g	4.354	4.353	4.339	4.500	4.488	4.514	0.001	0.857	0.272
SEM	0.023	0.023	0.019	0.019	0.017	0.016			
Total Solids, g/100 g	11.591	11.714	11.663	11.883	11.876	11.937	0.124	0.782	0.599
SEM	0.093	0.099	0.082	0.072	0.068	0.055			
Total protein, g/100 g	3.131	3.093	3.048	3.155	3.129	3.149	0.052	0.213	0.348
SEM	0.027	0.035	0.024	0.023	0.019	0.018			
True protein, g/100 g	2.920	2.842	2.775	2.934	2.901	2.922	0.019	0.204	0.218
SEM	0.032	0.037	0.027	0.023	0.019	0.018			
Casein, g/100 g	2.062	2.008	1.912	2.148	2.111	2.139	0.001	0.123	0.168
SEM	0.039	0.043	0.031	0.024	0.020	0.019			
Casein:Milk true protein	0.716	0.709	0.699	0.729	0.725	0.730	0.001	0.114	0.188
SEM	0.007	0.007	0.005	0.003	0.003	0.003			
Log (SCC × 10 ⁻³ cells/ml)	2.449	2.405	2.468	2.287	2.333	2.398	0.073	0.271	0.773
SEM	0.064	0.063	0.047	0.056	0.047	0.041			
Urea nitrogen, mg/100 ml	16.877	16.159	15.727	15.438	16.014	15.937	0.747	0.439	0.703
SEM	0.556	0.524	0.403	0.282	0.301	0.244			

Girolando cows (BB>AB>AA). Higher levels of BB genotype frequencies among Holstein cows were also observed by Celik (2003) but not by Hill (1993) and Oner & Elmaci (2006), who found a higher number of the heterozygous AB variants compared with homozygous cows.

Nevertheless, no report on *β*-lg genetic variants in Girolando animals was found in the literature, and data on *β*-lg allele frequency among *Bos indicus* breeds show a higher prevalence for the B gene (Silva & Del Lama, 1997; Neves et al. 1998; Faria et al. 2000) and a higher frequency for the BB genotype.

The present results differ from those of Ng Kwai-Hang et al. (1986), Aleandri et al. (1990), Bovenhuis et al. (1992) and Hill (1993). These authors all reported higher milk fat concentration in milk of Holstein cows comprising B variants of *β*-lg, and Hill (1993) concurred for significantly lower concentrations of fat and total solids in AA Holstein milk.

Milk nitrogen fractions were not influenced by *β*-lg genetic polymorphism in any of the breeds, although TP, MTP, casein means and casein:MTP ratio for AA cows were numerically higher for both breeds. Similarly, Molina

et al. (2006) and Bobe et al. (1999) found an increase in TP concentrations of milk when B allele was substituted for A. According to Bobe et al. (1999) such increment is due to the higher percentage of casein in β -lg A milk, while Molina et al. (2006) attributed this increase to the higher content of whey protein in milk with A variants. Mean values for casein and MTP concentrations in the present study, however, seem to be in disagreement with Robitaille et al. (2002), who found a tendency for higher casein concentrations in BB β -lg milk in Holsteins, and with a report of increased MTP when B allele was more frequent in the Holstein breed (Ng Kwai-Hang, Monardes & Hayes, 1990).

Although not statistically significant, the casein: MTP ratios for AA milk of both breeds were slightly higher than those observed for either AB or BB genotypes, and this is particularly relevant as MTP, especially β -lg concentrations in milk true protein, plays an important role in milk stability during heat treatment, since this protein strongly interacts with other milk molecules, particularly κ -casein (Karatzas & Turner, 1997; Fox & McSweeney, 1998; Singh, 2004).

MUN concentrations the present study ranged from 12 to 18 mg/dl within the normal minimum and maximum limits for lactating Holstein cows (Meyer et al. 2004). Higher MUN was observed for Holstein cows, but no effect of season and β -lg polymorphism was found. According to Arunvipas et al. (2003), effects of non-nutritional factors on MUN, such as breed, stage of lactation and cow pregnancy, production and milk composition explain 13.3% of the variability of MUN concentrations.

Based on the results of this study, there was no association between milk composition (fat, lactose, total solids, TP, MTP, casein, casein:MTP ratio, somatic cell count and MUN) and β -lg genetic variant itself. During the dry season, independently of breed considered, higher contents of lactose, TP, MTP, casein and casein:MTP ratio were found.

The authors thank the Fundação de Amparo a Pesquisa do Estado de São Paulo for the financial support to conduct this study (Grant n° 02/12058-9), and José Franchini Garcia Moreno and Lucinéia Mestieri, for their technical assistance.

References

- Aleandri R, Buttazzoni LG, Schneider JC, Caroli A & Davoli R 1990 The effects of milk protein polymorphisms on milk components and cheese producing ability. *Journal of Dairy Science* **73** 241–255
- Allore HG, Oltenacu PA & Erb HN 1997 Effects of season, herd size, and geographic region on the composition and quality of milk in the northeast. *Journal of Dairy Science* **80** 3040–3049
- Amenu B, Cowan T, Deeth H & Moss R 2006 Impacts of feeding system and season on milk composition and cheddar cheese yield in a subtropical environment. *Australian Journal of Experimental Agriculture* **46** 299–306
- AOAC 1995 *Official Methods of Analysis 16th Edn* Arlington VA, USA: Association of Official Analytical Chemists
- AOAC 1990 *Official Methods of Analysis 15th Edn* Arlington VA, USA: Association of Official Analytical Chemists
- Arunvipas P, Dohoo IR, Van Leeuwen JA & Keefe GP 2003 The effect of non-nutritional factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. *Preventive Veterinary Medicine* **59** 83–93
- Aschaffenburg R & Drewry J 1955 Occurrence of different β -lactoglobulin in cow's milk. *Nature* **176** 218–219
- Auldlist MJ, Walsh BJ & Thomson NA 1998 Seasonal and lactational influences on bovine milk composition in New Zealand. *Journal of Dairy Research* **65** 401–411
- Barbano DM, Clark JL, Dunham CE & Fleming JR 1990 Kjeldahl method for determination of total nitrogen content of milk: Collaborative study. *Journal of the Association of Official Analytical Chemists* **73** 849–859
- Bicalho HMS, Pimenta CG, Mendes IKP, Pena HB, Queiroz EM & Pena SDJ 2006 Determination of ancestral proportions in synthetic bovine breeds using commonly employed microsatellite markers. *Genetics and Molecular Research* **5** 432–437
- Bobe G, Beitz DC, Freeman AE & Lindberg GL 1999 Effect of milk protein genotypes on milk protein composition and its genetic parameter estimates. *Journal of Dairy Science* **82** 2797–2804
- Bovenhuis H, Arendonk JAM & Korver S 1992 Associations between milk protein polymorphisms and milk production traits. *Journal of Dairy Science* **75** 2549–2559
- Carlsson J, Bergstrom J & Pehrson B 1995 Variations with breed, age, season, yield, stage of lactation and herd in the concentration of urea in bulk milk and in individual cows milk. *Acta Veterinaria Scandinavica* **36** 245–254
- Celik S 2003 β -lactoglobulin genetic variants in Brown Swiss breed and its association with compositional properties and rennet clotting time of milk. *International Dairy Journal* **13** 727–731
- Ciulla TA, Sklar RM & Hauser SL 1988 A simple method for DNA purification from peripheral blood. *Analytical Biochemistry* **174** 485–488
- Dove P 2000 Genetic polymorphisms in milk protein genes and their impact on milk composition. In: *Biology of the Mammary Gland: Advances in Experimental Medicine and Biology* 225–230 (Eds JA Mol & RA Clegg). New York NY, USA: Kluwer Academic/Plenum Publishers
- Faria FJC, Guimarães SEF, Mourão GB, Lima RMG & Pinheiro LEL 2000 [Polymorphism analysis of β -lactoglobulin gene in Nellore cows and effects on weaning weight of the calves]. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **52** 261–265
- Fox PF & McSweeney PLH 1998 *Dairy Chemistry and Biochemistry*. London: Blackie Academic & Professional.
- Ghashghaei K 2003 Effect of cow phenotype and milk protein structure on biofouling rates in heat exchangers. MS thesis presented to the Faculty of California Polytechnic State University, San Luis Obispo, 98 p.
- Hambling SG, McAlpine AS & Sawyer L 1992 β -lactoglobulin. In *Advanced Dairy Chemistry, Vol. 1: Proteins, 2nd Edn* (Ed. PF Fox) pp. 141–189. London, Elsevier Applied Science.
- Hill JP 1993 The relationship between β -lactoglobulin phenotypes and milk composition in New Zealand dairy cattle. *Journal of Dairy Science* **76** 281–286
- Karatzas CN & Turner JD 1997 Toward altering milk composition by genetic manipulation: Current status and challenges. *Journal of Dairy Science* **80** 2225–2232
- Lacroix C, Verret P & Paquin P 1996 Regional and seasonal variations of nitrogen fractions in commingled milk. *International Dairy Journal* **6** 947–961
- Lindmark-Månsson H, Fonden R & Pettersson H 2003 Composition of Swedish dairy milk. *International Dairy Journal* **13** 409–425
- Litwinczuk A, Barłowska J, Krol J & Litwinczuk Z 2006 Milk protein polymorphism as markers of production traits in dairy and meat cattle. *Molymcyna Weterynaryjna* **62** 6–10
- Lynch JM, Barbano DM & Fleming JR 1998 Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen

- analysis: Collaborative study. *Journal of the Association of Official Analytical Chemists* **81** 763–774
- Meyer PM, Machado PF, Coldebella A, Corassin CH, Cassoli LD, Coelho KO & Rodrigues PHM** 2004 Development of models to estimate milk urea nitrogen concentrations. *Journal of Animal and Feed Sciences* **13** 527–530
- Molina LH, Kramm J, Brito C, Carrillo B, Pinto M & Ferrando A** 2006 Protein composition of milk from Holstein-Friesian dairy cows and its relationship with the genetic variants A and B of kappa-casein and β -lactoglobulin (part I). *International Journal of Dairy Technology* **59** 183–187
- Neves ALG, Guimarães SEF, Lima RMG, Penna VM & Pinheiro LEL** 1998 [Identification of A and B variants of the β -lactoglobulin gene in Brazilian Gir populations] *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **50** 409–414
- Ng Kwai-Hang KF, Dodds C, Boland MJ & Auldish MJ** 2002a The influence of genetic variants of β -lactoglobulin on gelation speed and firmness of rennet curd. *Milchwissenschaft* **57** 267–269
- Ng Kwai-Hang KF, Hayes JF, Moxley JE & Monardes HG** 1986 Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows. *Journal of Dairy Science* **69** 22–26
- Ng Kwai-Hang KF, Monardes HG & Hayes JF** 1990 Association between genetic polymorphism of milk proteins and production traits during three lactations. *Journal of Dairy Science* **73** 3414–3420
- Ng Kwai-Hang KF, Otter DE, Lowe E, Boland MJ & Auldish MJ** 2002b Influence of genetic variants of β -lactoglobulin on milk composition and size of casein micelles. *Milchwissenschaft* **57** 303–306
- Oner Y & Elmaci C** 2006 Milk protein polymorphisms in Holstein cattle. *International Journal of Dairy Technology* **59** 180–182
- Paquin P & Lacroix C** 1994 Seasonal and regional variations of different milk protein fractions: A survey of Quebec milk. In *Protein Definition* pp. 3–39. Brussels, Belgium: International Dairy Federation
- Robitaille G, Britten M, Morisset J & Petitclerc D** 2002 Quantitative analysis of β -lactoglobulin A and B genetic variants in milk of cows β -lactoglobulin AB throughout lactation. *Journal of Dairy Research* **69** 651–654
- Schlee P & Rottmann O** 1992 Genotyping of bovine beta-casein, β -lactoglobulin and alpha-lactalbumin using the polymerase chain-reaction. *Journal of Animal Breeding and Genetics* **109** 456–464
- Sharma RB, Kumar M & Pathak V** 2002 Effect of different seasons on crossbred cow milk composition and paneer yield in sub-Himalayan region. *Asian-Australasian Journal of Animal Sciences* **15** 528–530
- Silva IT & Del Lama MA** 1997 Milk protein polymorphisms in Brazilian zebu cattle. *Brazilian Journal of Genetics* **20** 625–630
- Singh H** 2004 Heat stability of milk. *International Journal of Dairy Technology* **57** 111–119
- Teixeira NM, Freitas AF & Barra RB** 2003 [Environmental factors influencing monthly variation of milk composition and somatic cell counts in herds of the State of Minas] *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **55** 491–499
- Tsiaras AM, Bargouli GG, Banos G & Boscos CM** 2005 Effect of kappa-casein and β -lactoglobulin loci on milk production traits and reproductive performance of Holstein cows. *Journal of Dairy Science* **88** 327–334
- Van Eenennaam A & Medrano JF** 1991 Milk protein polymorphisms in California dairy cattle. *Journal of Dairy Science* **74** 1730–1742
- Wilkins RJ & Kuys YM** 1992 Rapid β -lactoglobulin genotyping of cattle using the polymerase chain reaction. *Animal Genetics* **23** 175–178