

Milk composition and productive and reproductive performance of cows from A1 and A2 β -casein variants, milked once or twice a day

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Abstract

Protein is an important component of milk and it plays an essential role in all living organisms. β -casomorphins-7 (BCM-7) is derived from A1 β -casein and has been implicated in some human health issues. This A1 β -casein is produced by cows with the A1A1 or A1A2 genotype, whilst cows with the A2A2 genotype produce A2 β -casein, which has not been implicated in the same human health issues. Given the potential importance of A2 milk for public health and its apparent commercial potential, selection based on the A2 genotype and its impact on production and reproduction traits should be investigated. The objective of the current study was to compare the productive and reproductive performance of dairy cows based on A2 genotype in two different dairy farms. From July 2017 to May 2018, 206 cows (including 122 A2A2 genotype; “A2 cows”) were milked once a day at Dairy 1 and 451 cows (including 217 A2 cows) were milked twice a day at Dairy 4. Records of lactation yields of milk, fat and protein, fat percentage, protein percentage, days from start of mating to conception, pregnancy rate to first service, the submission rate at 21 days and the pregnancy rate at 21 and 42 days (PR42) after the start of mating from 642 cows in two herds were analysed. The effects of A2 genotype on production and reproduction traits were not significant. The interaction between farm and β -casein genotype was significant for PR42 ($P < 0.05$) but not for any other traits. The interactions between parity number and genotype were not significant for any of the traits. The results indicated that cows of different β -casein genotypes have similar production and reproductive performance.

Keywords: β -casein genotype; A2 milk; protein percentage; pregnancy rate at 42 days

Introduction

Proteins play an essential role in the formation, maintenance and repair of the body tissue in all living organisms. In addition to providing a source of energy, proteins are important because they also provide essential amino acids for the human body. Milk is an important food and source of protein for both infants and adults. Caseins and whey proteins are two major groups of milk protein. The four major caseins in cow's milk are α_{s1} -, α_{s2} -, β - and κ -casein (Eigel et al. 1984) with β -casein comprising about 30% of total protein (Walstra et al. 1984). A1 and A2 types are two major genetic variants of β -casein proteins in bovine milk (Ng-Kwai-Hang et al. 1990; Caroli et al. 2009; Massella et al. 2017). Cows with homozygous genes (A1A1 or A2A2) produce milk exclusively with A1 or A2 β -casein, whereas heterozygous cows (A1A2) produce milk with both types of β -casein.

Food-derived peptides are cut away and released from protein molecules under the influence of enzymatic hydrolysis in the process of digestion (Kamiński et al. 2007). One of the bioactive peptides derived from β -casein digestion is known as β -casomorphins-7 (BCM-7). There is a histidine at position 67 of the milk protein sequence in A1 β -casein and a BCM-7 can be cut off from it, whereas the proline residue in A2 β -casein protects the bond between Ile⁶⁶ and Pro⁶⁷ from hydrolysis by digestive enzymes (Jinsmaa et al. 1999). A1 β -casein and BCM-7 have been implicated in some human health issues, as it has been suggested that bioactive BCM-7 may have detrimental impacts throughout the body, such as on the gastrointestinal tract, and the central nervous, cardiovascular and immune

systems, by acting as an μ -opioid receptor agonist (Korhonen et al. 2006; Kamiński et al. 2007).

The A2 Milk Company was founded in New Zealand in 2000 and markets milk and dairy products only with the A2 β -casein variant to New Zealand, Australian and US markets. With the commercial success of the A2 Milk Company, a large number of dairy farms within New Zealand contain herds with a higher percentage of the A2 allele (Woodford 2007). According to the Livestock Improvement Corporation (LIC 2020), in the year 2019 about 30% of dairy cows in New Zealand produced milk containing only A2 β -casein.

Given the potential importance of A2 milk for public health and its apparent commercial potential, the influences of the A2 β -casein variant on production and composition of milk should be investigated before using A2 genotype as an additional criterion in bull selection. In addition, reproduction traits are also economically important. Poor fertility is the biggest cause of culling of dairy cows in New Zealand (Xu & Burton 2000; Martinez Rocha 2017), resulting in substantial economic losses to dairy farmers. Therefore, the effects of selection for certain milk casein genotypes on cow fertility warrants investigation. The association of β -casein polymorphism with milk production (Ng-Kwai-Hang et al. 1986; Çardak 2005; Heck et al. 2009), milk composition (Aleandri et al. 1990; Winkelman et al. 1997; Ikonen et al. 1999) and fertility (Lin et al. 1987; Ruottinen et al. 2004; Demeter et al. 2010) in dairy cows has been investigated. However, the literature in relation to the productive performance and fertility of the cows with A2 β -casein genotype in New Zealand is scarce. The objective

of the current study was to compare the productive and reproductive performance of dairy cows with A1 and A2 β -casein genotypes in two different dairy farms.

Materials and methods

The data were collected from Dairy 1 and Dairy 4 farms at Massey University, Palmerston North. Dairy 1 farm is managed as a low-input farm and has a spring-calving, once-a-day (OAD) milking system. It is pasture based with paddocks containing ryegrass with white and red clover mix (100 ha), plantain and chicory with white and red clover mix (10 ha) and a lucerne crop (10 ha). Dairy 4 farm is managed as a high-input farm with a spring-calving and twice-a-day (TAD) milking system. Dairy 4 farm is pasture based and the pastures are predominantly perennial ryegrass and white clover.

Cows in Dairy 1 farm were milked once daily at 6:30 am, whereas those in Dairy 4 were milked twice a day at 5:30 am and 2:30 pm throughout lactation. Calving began in mid-July on both farms in 2017 and cows were milked until May the following year. The breeding season began on October 18th and ended on December 23rd.

Animal information consisted of breed composition, lactation length and genotype. A radio frequency electronic identification system (Allflex New Zealand Ltd., Palmerston North, New Zealand) was used to identify each cow. Lactation length was calculated as the number of days in milk between calving and drying off. The genetic test for the β -casein genotype of a cow has been developed by LIC, and can be either a test of the milk sample via herd testing, or a test of some hairs along with the skin follicle cells collected from the animal (tissue test). In the current study, the A1/A2 status of cows was determined based on a tissue test.

The production traits comprised milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP) and protein percentage (PP). The yield of milk solids (MSY) was calculated as the sum of FY and PY. The records of MY, FY, PY and somatic cell count (SCC) were obtained from monthly herd testing conducted by LIC. The SCC from herd-test records was log-transformed to somatic cell score (SCS). The average SCS was calculated as the mean of SCS obtained in all herd tests over the lactation year. The reproduction traits measured on each cow included days from start of mating to conception (SMCO), pregnancy rate at first service (PRFS), the submission rate at 21 days (SR21) and the pregnancy rate at 21 (PR21) and 42 days (PR42) after the start of mating.

Genotypic information of all the first lactation cows in Dairy 4 was unavailable in the original data set. In order to keep an identical data structure for the comparison between two farms, the first lactation cows of Dairy 1 were also excluded from this study. Complete records were available for 642 cows of parity ≥ 2 and these were used in this analysis.

The cows were Holstein Friesian (F), Jersey (J) and crossbred (F $\times$ J). In general, a cow was considered

purebred when she had $\geq 87.5\%$ of F or J, otherwise she was considered F $\times$ J. There were 206 cows from Dairy 1 farm in the analysis. The breed structure was 49 F, 50 J and 107 F $\times$ J. By comparison, there were 451 dairy cows from Dairy 4 including 139 F, 4J and 308 F $\times$ J. It is worth noting that the proportion of F (pF) was used as a variable rather than cattle breeds in the current study when analysing the fixed effect of breed on milk production, milk composition and fertility.

The lactation number of the cows included in the current study ranged from two to six. For statistical analysis, the cows were divided into two groups: (1) second parity and (2) third or greater than third parity. Due to the fact that both A1A1 and A1A2 cows produce A1 protein, cows were grouped by A1 cows (cows with either A1A1 or A1A2 genotypes) and A2 cows (cows with only A2A2 genotypes) to minimise the inaccuracy of statistical analysis caused by small sample size, as there were only six cows with homozygous A1A1 genotypes in Dairy 1. There were 84 A1 and 122 A2 cows in Dairy 1, and 234 A1 and 217 A2 cows in Dairy 4.

The dataset was analysed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were generated with the MEANS procedure. Analysis of variance for MY, FY, PY, FP, PP, SMCO, PRFS, SR21, PR21 and PR42 were performed using the MIXED procedure with the following mixed linear model:

$$y_{ijkm} = \mu + F_i + L_j + G_k + FG_{ik} + LG_{jk} + \beta_1 p^F + \beta_2 h_{F \times J} + \beta_3 d + e_{ijkm}$$

where y_{ijkm} is the dependent trait measured in a cow m^{th} ; μ is a general mean; F_i is the fixed effect of farm; L_j is the fixed effect of the parity; G_k is the fixed effect of the genotype; FG_{ik} is the interaction between farm i and genotype k ; LG_{jk} is the interaction between lactation number j and genotype k ; β_1 is the regression coefficient of the dependent variable on pF; β_2 is the regression coefficient of the dependent variable on F $\times$ J heterosis ($h_{F \times J}$); β_3 is the regression coefficient of the dependent variable on deviation from median calving date (d); e_{ijkm} is the random residual error associated with the observation of y_{ijkm} . Binominal variables (PRFS, SR21, PR21 and PR42) were analysed using the GLIMMIX procedure with the same mixed linear model described above after a logit transformation. Least-squares means and standard errors were obtained and used for multiple mean comparisons using Fisher's least-significant difference as implemented in the LSMEAN option. Significant differences among means were declared at $P < 0.05$.

Results

The milk yield ranged from 1,491 kg to 7,371 kg per lactation with a mean value of 4,696 kg (Table 1). The range for FP was from 2.91 to 7.22% and was less than PP which ranged from 3.13 to 4.94%. The mean value of SMCO was 16 days with a range from 0 to 69 days. Pregnancy rate at

first service averaged 50%, and SR21 was high (>90%). The pregnancy rates at 21 and 42 days were 54% and 77%, respectively.

The MY and PY were both significantly greater ($P<0.05$) in Dairy 4 than in Dairy 1 (Table 2), whereas FY was not significantly different between two farms. The FP and PP were both significantly greater ($P<0.05$)

Table 1 Mean, standard deviation (SD), minimum and maximum values of lactation yields of milk, fat and protein, fat percentage, protein percentage, days from start of mating to conception (SMCO), pregnancy rate at first service (PRFS), submission rate at 21 days (SR21) and pregnancy rate at 21 (PR21) and 42 days (PR42) after the start of mating in Massey University Dairy 1 and 4 farms in 2017.

Traits	Mean	SD	Minimum	Maximum
Milk yield, kg	4,696.3	1,063.60	1,491	7,371
Fat yield, kg	222.5	46.7	59	353
Protein yield, kg	179.4	38.2	56	278
Fat percentage	4.81	0.69	2.91	7.22
Protein percentage	3.85	0.32	3.13	4.94
SMCO, days	16.8	14.1	0	67
PRFS, %	50	50		
SR21, %	90	31		
PR21, %	54	50		
PR42, %	77	42		

in Dairy 1 than in Dairy 4. The milk composition was not affected by parity number, whereas the MY, FY and PY were all significantly greater ($P<0.05$) in parity ≥ 3 . The effect of interaction between farm and β -casein genotype was not significant on any of the production traits. The interaction between parity and β -casein genotype was not significant for any of the production traits. The effect of pF was significant ($P<0.05$) for all the traits except for FY. Heterosis had no significant effect on any of the production traits, whereas deviation from median calving date had a significant effect ($P<0.05$) on all production traits.

The PRFS, PR21 and PR42 were all significantly higher ($P<0.05$) in Dairy 1 than in Dairy 4, whereas SR21 was significantly lower ($P<0.05$) in Dairy 1 than in Dairy 4 (Table 3). The SMCO was significantly greater ($P<0.05$) in second parity than in ≥ 3 parity cows, whereas the other reproduction traits were not significantly different between parity. The β -casein genotype had no significant effect on any reproduction trait. The effect of interaction between farm and β -casein genotype was significant ($P<0.05$) on PR42 but not on any other reproduction traits. The interaction between parity and β -casein genotype was not significant for any of the reproduction traits. The SR21 was significantly affected ($P<0.05$) by pF, but the effect of pF was not significant for any other reproduction traits. Heterosis was not significant for any of the reproduction

Table 2 Least squares means, standard errors (SE) and P values of factors affecting productive performance of cows in Massey University Dairy 1 and Dairy 4 farms in 2017.

Effect	Trait ¹									
	MY		FY		PY		FP		PP	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Farm										
Dairy 1	4,188 ^b	71	210.3	3.4	165.4 ^b	2.7	5.10 ^a	0.04	3.97 ^a	0.02
Dairy 4	4,639 ^a	59	213.1	2.8	173.9 ^a	2.2	4.67 ^b	0.03	3.78 ^b	0.02
Parity										
2	4,110 ^b	91	196.3 ^b	4.4	157.3 ^b	3.5	4.9	0.05	3.87	0.02
≥ 3	4,717 ^a	43	227.1 ^a	2.1	182.0 ^a	1.6	4.88	0.02	3.88	0.01
β -casein genotype ²										
A1	4,444	76	212.4	3.6	169.7	2.9	4.87	0.04	3.85	0.02
A2	4,383	65	211.0	3.1	169.6	2.5	4.91	0.04	3.90	0.02
Interaction Farm $\times$ β -casein genotype										
P-value	0.127		0.564		0.321		0.146		0.106	
Interaction Parity $\times$ β -casein genotype										
P-value	0.438		0.709		0.386		0.421		0.688	
pF ³										
Effect	1,107.20	125.8	3.62	6.04	22.22	4.78	-1.21	0.07	-0.5	0.03
P-value	<0.0001		0.549		<0.0001		<0.0001		<0.0001	
Heterosis										
Effect	-82.7	121.1	6.21	5.82	2.51	4.6	0.09	0.07	0.05	0.03
P-value	0.495		0.286		0.585		0.178		0.124	
dmcd ⁴										
Effect	-15.5	2.23	-0.97	0.11	-0.76	0.08	-0.005	0.001	-0.004	0.001
P-value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

¹MY = milk yield, FY = fat yield, PY = protein yield, FP = fat percentage and PP = protein percentage. ² β -casein genotype A1 = cows with either A1A1 or A1A2 genotypes, and A2 = cows with only A2A2 genotype. ³pF = proportion of Holstein-Friesian. ⁴dmcd = deviation from median calving date. ^{a, b} Least-squares means with different superscripts within column are significantly different ($P<0.05$).

Table 3 Least-squares means, standard errors and P values of factors affecting fertility traits of cows in Massey University Dairy 1 and Dairy 4 farms in 2017.

Effect	Trait ¹									
	SMCO		PRFS		SR21		PR21		PR42	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Farm										
Dairy 1	16.8	1.2	58.2 ^a	4.0	79.8 ^b	3.4	67.7 ^a	3.8	82.7 ^a	3.2
Dairy 4	18.3	1.0	41.7 ^b	3.3	93.4 ^a	1.5	44.4 ^b	3.4	73.8 ^b	3.1
Parity										
2	19.9 ^a	1.5	45.7	5.1	86.4	33.6	52.9	5.3	76.8	4.5
≥3	15.3 ^b	0.7	54.3	2.5	89.8	1.5	59.9	2.6	80.3	2.1
β -casein genotype ²										
A1	17.4	1.3	50.6	4.3	87.9	2.6	60.0	4.4	81.3	3.6
A2	17.9	1.1	49.3	3.7	88.6	2.4	52.8	3.7	75.6	3.2
Interaction Farm× β -casein genotype										
P value	0.884		0.925		0.470		0.232		0.036	
Interaction Parity× β -casein genotype										
P value	0.1322		0.2424		0.1259		0.2336		0.9286	
pF ³										
Effect	0.57	2.17	0.13	0.07	-0.09	0.04	0.05	0.07	-0.07	0.06
P value	0.793		0.053		0.045		0.435		0.281	
Heterosis										
Effect	-0.40	2.12	0.04	0.07	-0.02	0.04	-0.01	0.07	-0.02	0.06
P value	0.851		0.568		0.780		0.876		0.699	
dmcd4										
Effect	0.17	0.04	-0.003	0.001	-0.002	0.001	-0.004	0.001	-0.003	0.001
P value	<0.0001		0.023		0.034		0.002		0.004	

¹SMCO = start of mating to conception, PRFS = pregnancy rate at first service, SR21 = submission rate at 21 days after the start of mating, PR21 = pregnancy rate at 21 days after the start of mating and PR42 = pregnancy rate at 42 days after the start of mating. ² β -casein genotype A1 = cows with either A1A1 or A1A2 genotypes, and A2 = cows with only A2A2 genotype. ³pF = proportion of Holstein-Friesian. ⁴dmcd = deviation from median calving date. ^{a, b} Least-squares means with different superscripts within column are significantly different ($P < 0.05$).

traits, whereas the effect of deviation from median calving date was significant ($P < 0.05$) for all binomial reproduction traits.

Discussion

Analysis of variance in the current study showed that the β -casein genotype had no significant effect on total MY, FY or PY. The effects of β -casein polymorphism on milk production traits have been investigated, but the results from previous research conflict in relation to the significance and the size of genetic effects. For example, some studies reported that β -casein variants A1 and A2 did not significantly affect MY, FY or PY in F cows (Ng-Kwai-Hang et al. 1986; Çardak 2005), which was in agreement with the current study. In herds with a mixed population of J and F cattle, McLean et al. (1984) also reported that β -casein A1 and A2 genotypes had no significant effect on total MY and FY over a complete lactation. The first study to examine the influence of protein phenotypes on productive performance in New Zealand dairy cows was carried out by Winkelman et al. (1997), and similar to the present study, no relationship between β -casein variants and production traits was reported.

Conversely, Bech et al. (1990) and Ng-Kwai-Hang et al. (1990) investigated the relationship between A1 and A2

β -casein genotypes and MY during the first three lactations in Holstein herds, and A2A2 cows produced more milk than did A1A1 cows. The increase in PY resulting from greater milk production by cows with the A1 allele has also been reported (Heck et al. 2009). Although Lin et al. (1986) suggested that the loci of A1 and A2 β -caseins had no significant effect on the 308-day MY of first-lactation cows, they found that the effect was significant on PY ($P < 0.05$). Their results suggested that the A2 genotype was superior to the A1 genotype, and increasing A2 allele frequency over A1 would improve first-lactation MSY. In New Zealand dairy cows, Morris et al. (2005) found that carriers of the A2A2 variant had significant higher FY ($P < 0.05$) and PY ($P < 0.10$) than did those of the A1A1 variant. The productive advantage of the A2 allele over the A1 allele has been also reported by others (Ng-Kwai-Hang et al. 1984; Bech et al. 1990; Ikonen et al. 1999). However, the results from those studies in relation to the influence of genetic variants of β -casein on MY and MSY were inconsistent. The reason for contradictory results might be gene linkage. Casein genes on bovine chromosome 6 are closely linked in the sequence of α_{s1} -, β -, α_{s2} -, and κ -casein (Threadgill et al. 1990; Rijnkels et al. 1997). Therefore, sometimes it is difficult to distinguish whether the influence of the β -casein genotype is due to the effect of its linked gene or the loci of

β -casein themselves. Some of the aforementioned studies were conducted in F cows and others were in herds with a mixed-breed population. The difference between MY, FY and PY were significant for A1 and A2 β -casein genotypes in Simmentaler cows, but not in F cows (Çardak 2005), which likely indicated the differential effect of a linked gene in different breeds.

The current study found no significant difference in FP between β -casein variants, which was supported by early studies (Ng-Kwai-Hang et al. 1986; Gonyon et al. 1987). A significant relationship between the β -casein A2A2 genotype and milk fat content has been previously reported, but the results were less consistent. Several studies highlighted the association of β -casein A2A2 genotype with reduced FP in Holstein cows (Aleandri et al. 1990; Ng-Kwai-Hang et al. 1990) and Finnish Ayrshire cows (Ikonen et al. 1999), whereas one study reported the opposite results for a mixed population of J and F cows (McLean et al. 1984).

The difference in PP for β -casein A1 and A2 genotypes was not significant, which was supported by early studies with Holstein cows (Ng-Kwai-Hang et al. 1986; Ng-Kwai-Hang et al. 1990), Guernsey (Haenlein et al. 1987) and a mixed herd (McLean et al. 1984). Çardak (2005) examined the effects of the β -casein genotype and reported a significant increase in the protein concentration for A2A2 over the A1A2 genotype in Simmentaler cows. However, the differences in PP between the A1A1 and A2A2 genotypes were not significant in the Holstein cattle in the same study. In addition, some studies reported that the A2 genotype had a detrimental effect on whey protein concentration (McLean et al. 1984) and casein (Ng-Kwai-Hang et al. 1986), but not on the concentration of PP. The conflicting observations in milk composition may be partially explained by gene linkage in different breeds of the herds as discussed previously.

The effects of interactions between farm and β -casein genotype and between parity and β -casein genotype on production traits were no significant. To the best of our knowledge, few previous studies have investigated the interaction of the β -casein genotype A1 and A2 with other effects. Only one study reported the presence of a significant interaction between β -casein genotype and breed (Winkelman et al. 1997). Their results showed that about 2% more milk, fat and protein was produced by A2A2 F than A1A1 F cows, whereas A2A2 J cows produced 3-4% less milk than did A1A1 J cows.

The influence of the β -casein genotype was not significant for all fertility traits in the current study. The effect of milk protein polymorphism on reproductive performance of the cows has been reported in limited literature (Hargrove et al. 1980; Lin et al. 1987; Demeter et al. 2010), but supported the current study with respect to β -casein genotype A1 and A2.

Pregnancy rate at 42 days after the start of mating of A1 and A2 cows in Dairy 1 were 87.1 ± 5.1 % and 75.3 ± 4.1 % and corresponding values of the genotypes in Dairy

4 were 72.0 ± 4.0 % and 75.2 ± 3.9 %, which explains the significant interaction between farm and β -casein genotype. The A1 cows had higher PR42 in Dairy 1 whereas the A2 cows had higher PR42 in Dairy 4. However, the association between β -casein genotype and fertility was examined by analysing a relatively small dataset, which could impair the consistency of the results from the present study. The major issue in determining the effect of the β -casein variant on fertility traits is that the majority of the variation in fertility traits is due to environmental factors (Hodel et al. 1995). Consequently, most of the fertility traits have very low heritability (Weigel et al. 2000). Therefore, for future studies on the effect of the A2 β -casein genotype on fertility traits, analyses of large and accurate data sets are necessary.

Conclusions

The results indicated that cows of different β -casein genotypes have similar production and reproductive performance. Selection of animals based on the A2 genotype should have no negative impact on their production and fertility.

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