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 type and its impact on production and reproductive traits should be investigated. The objective of the current study was to compare the productive and reproductive performance of dairy cows based on A2 type 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 type on productive and reproductive 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 types have similar production and reproduction performance.

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List of Abbreviations

A1 cows	Cows with either A1A1 or A1A2 genotypes
A2 cows	Cows with only A2A2 genotypes
BBB	Blood-brain barrier
BCM-7	β-casomorphins-7
CNS	Central nervous system
Dmcd	Deviation from median calving date
F	Holstein-Friesian
F×J	Holstein-Friesian × Jersey crossbreds
FP	Fat percentage
FY	Fat yield
IDDM	Type I (insulin-dependent) diabetes mellitus
IHD	Ischaemic heart disease
J	Jersey
MY	Milk yield
OAD	Once-a-day milking system
PP	Protein percentage
PR21	Pregnancy rate at 21 days
PR42	Pregnancy rate at 42 days
PRFS	Pregnancy rate at first service
PY	Protein yield
SCC	Somatic cell count
SCS	Somatic cell score
SMCO	Start of mating to conception
SR21	Submission rate at 21 days
TAD	Twice-a-day milking system

Chapter 1

General 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 alleles (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 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 Ile^{66} and Pro^{67} 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 mu-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, US and Chinese 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 type as an additional criterion in bull selection. In addition, reproductive 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 β-casein types 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 type in New Zealand is scarce. The objective of this thesis was to compare the productive and reproductive performance of dairy cows with A1 and A2 β-casein types in two different dairy farms.

Chapter 2

Literature Review

2.1 The grazing system in New Zealand

Around the world there are a number of different farming systems. In contrast to the majority of dairy systems in North America and Europe, New Zealand has a pasture-based system which highly relies upon the growth of the pasture that is consumed by grazing cows. The major component of the diet is grazed pasture, and the quantities of feeds other than pasture (concentrate and silage) fed to cows are low. The use of pasture as a cheap source of feed throughout the year for dairy animals in pastoral system results in lower production costs (White et al. 2002) and high milk output per hectare as feed is always the largest cost in a milk production system. The typical pasture in New Zealand are predominantly composed of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) (Kemp et al. 1999).

The synchrony between pasture growth and feed demand is of great importance for a pasture-based system. In order to maximise the pasture utilisation, New Zealand cows normally start calving in spring (July to August) before rapid pasture growth and are dried off in late autumn as the pasture growth gradually decreases. Peak pasture growth is coincided with peak feed demand in October (Roche et al. 2017). Surplus pasture is usually conserved as silage or hay and will be consumed during the periods of slow pasture growth to avoid production losses when the pasture fails to satisfied the energy requirements of the cows.

Cows are typically milked twice a day (TAD) in the New Zealand grazing system. Once a day (OAD) milking in pasture-based systems can be tactically used as a management tool to reduce the energy requirement of the herd when feed supply is insufficient. Long-term OAD milking can increase the productivity and flexibility of the labour, improve life quality of farmer and their families, and reduce expenses on farm facilities (Bewsell et al. 2008; Stachowicz et al. 2014). Although the practice of OAD milking can lead to lower milk production per cow, shorter lactations and higher somatic cell count (SCC) (Holmes et al. 1992), some New Zealand farmers have switched to a full-

lactation OAD system over the last decade as they believe that OAD herds can be as profitable as TAD herds (Edwards 2019).

The typical breeds in New Zealand herds are Jersey (J), Holstein Friesian (F) and their crossbred (F×J). According to recent New Zealand dairy statistics, the crossbred was the predominant breed group (47.8%), whereas F and J accounted for 33.4% and 9.0% of the population, respectively (LIC and DairyNZ 2018). The rest of the population were Ayrshire (0.5%) and other breeds (9.2%).

The study by Bryant et al. (1985) demonstrated that HF and Jersey cows had a similar efficiency of converting feed into profit. Some of the major difference between the two breeds in relation to productive traits are milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP) and protein percentage (PP). HF cows had highest lactation yields of milk and Jersey cows produced milk with highest percentage of fat and protein (Table 2.1).

Table 2.1 Breed composition (%), lactation yields of milk (MY), fat (FY) and protein (FY), and percentage of fat (FP) and protein (PP) from J, F and F×J cows in season 2017/18 (LIC and DairyNZ 2018).

Breed	%	MY (litres)	FY (kg)	PY (kg)	FP (%)	PP (%)
J	9.0	3208	180.1	132.6	5.65	4.14
F×J	47.8	4102	201.8	161.0	4.97	3.94
F	33.4	4470	198.0	166.2	4.48	3.73

Crossbreeding is a common practice in New Zealand dairy industry. Crossbred animals will have an additional genetic gain compared to the average genetic level of their parent breeds for a number of profit-related traits. Crossbreeding allows dairy farmers of New Zealand to take advantage of favorable heterosis effects to increase dairy cows' performance.

2.1.1 The effects of productive performance on farm profitability

In spite of the milking frequency and the types of breed used in a system, dairy farmers always aim for the maximum profitability. Farm profitability can be affected by many factors. In typical pasture-based dairy systems, the efficiency of milk production is often considered to have major impacts on profitability (Harris et al. 2007). It is associated with the production and utilisation of the grazed pasture, and the efficiency of milksolids production by each cow (Penno 1998).

A cow will be more profitable if she produces more milk and uses the feed more efficiently. High-genetic-merit cows which have higher gross feed conversion efficiency and produce more milk per unit of feed consumed are used in New Zealand dairy farms (Holmes et al. 1987). Farm profitability can also be improved by increasing the sales price of the milk. Although milk price highly depends on the market, dairy farmers try to achieve higher profitability by improving the quality of the milk. The proportion of milksolids determines the quality and nutritional value of raw milk and its products (Kefford et al. 1995). The concentration of some particular milk proteins can also change the price of the milk.

2.1.2 The effects of reproductive performance on farm profitability

Profitability will be improved by increasing the longevity of cows because cows with more lactations are expected to be more profitable (Pritchard et al. 2013). Fertility is one of the major factors affecting the longevity of the cow and it is of critical importance to New Zealand dairy production system. Poor fertility is the biggest cause of culling of dairy cows in New Zealand (Xu & Burton 2000; Martinez Rocha 2017). Due to the request for synchronisation between pasture supply and animal energy demand, the breeding and calving are restricted to a very compact period of time (Holmes et al.

1987). Early culling of a cow due to reproductive failure means not only a waste of productivity but also the cost of animal rearing is diluted by fewer lactations. A high calving percentage are essential to maximising the profitability of a dairy farm. However, late calving caused by poor reproductive performance is still undesirable even if the cows were pregnant. It increases the calving to calving interval and shortens the lactation. A relatively earlier date of calving with a more compact calving period will result in a higher level of milksolids yield per cow since the lactation is actually prolonged (Dillon et al. 1995; Macmillan et al. 1996).

Several fertility traits are measured on cows to assess their reproductive performance in New Zealand dairy herds. The submission rate at 21 days (SR21) is defined as the percentage of oestrous cows receiving at least one insemination in first three weeks of the mating period. The national goal for New Zealand herds for SR21 is 90% (LIC and DairyNZ 2018). Pregnancy rate at 21 (PR21) and 42 days (PR42) is the percentage of cows conceived at 21 and 42 days after the start of mating, whereas pregnancy rate at first service (PRFS) is the percentage of cows conceived after the first insemination. Start of mating to conception (SMCO) is defined as the days from the first day of breeding season to the date when the cow become pregnant.

2.2 Milk as the source of bioactive peptides

Proteins play an essential role in the formation, maintenance and repair of the body tissue in all living organisms. Proteins are polymer chains formed from different amino acids linked by peptide bonds. Proteins have the same energy density as carbohydrates and each gram of protein provides four calories. Despite providing a source of energy, proteins are of significant importance because they also provide essential amino acids which cannot be synthesised in the human body (Young 1994). Dietary proteins are broken down into polypeptide chains in the stomach, and then further broken down to amino acids and then absorbed by the small intestine. Proteins may also be cut into peptides by proteolytic enzymes during digestion or food processing (Neurath 1984).

Unlike proteins, peptides are a shorter chain of amino acid residues, ranging in size from 2 to 50 amino acids (McNaught et al. 1997).

Milk is an important food and source of proteins for both infants and adults. Caseins and whey proteins are two major groups of milk protein, and the ratio of casein/whey protein is around 80:20 (Groenen et al. 1994). There are four major caseins in cow's milk which are α_{s1}-, α_{s2}-, β- and κ-casein (Eigel et al. 1984).

Milk and dairy products are the main source of β-casein, and β-casein families comprise about 30% of total milk proteins in bovine milk (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). Genetic variation between herds and breeds determines the sequence of milk protein. For instance, a Proline residue is present at position 67 in A2 variant while it is replaced by a Histidine residue in the A1 variant in the same position (Ng-Kwai-Hang et al. 2003). This point mutation of one amino acid resulted in the original occurrence of A1 β-casein in European herds 5000-10000 years ago, and it eventually became widespread around herds in Europe and America (Ng-Kwai-Hang et al. 2003). Dairy cows from modern herds in a large number of western countries have a roughly similar gene frequency of A1 and A2 (1:1) due to the changes in herd composition over time (De Noni et al. 2009). By contrast, purebred cattle from other regions, such as Asia and Africa, produce milk only having A2 β-casein. Genetic polymorphism is also associated with cattle breeds, as Southern European breeds usually have a higher A2 allele frequency than Northern European breeds (Pal et al. 2015). A higher frequency of A1 appears in Holstein-Friesian, Ayrshire and Red cows (McLean et al. 1984; Bech et al. 1990), whereas A2 is most frequently observed in Jersey and Guernsey cows (Ehrmann et al. 1997). However, the distribution of allele frequency in various cattle breeds depends on the local breeding history of the dominant breeds (De Noni et al. 2009). It is unreliable to estimate the allele frequency of β-casein merely based on the information of cattle breed within any particular herds. Nowadays, it is commercially available to conduct herd testing for gene frequency with DNA analysis in many

countries. The different genetics of the individual cows dictate which type will be expressed in their milk. Cows with homozygous genes (A1A1 or A2A2) produce milk exclusively with A1 or A2 β-casein. In comparison, heterozygous cows (A1A2) produce milk with both types of β-casein. Milk containing solely A2 β-casein is generally called A2 milk, whereas milk with both A1 and A2 β-casein variants is commonly categorised into A1 milk.

2.3 The formation of BCM-7 during digestion and milk processing

Once food is ingested, some fragments will be cut away and released from the food protein molecules under the influence of enzymatic hydrolysis in the process of digestion (Kamiński et al. 2007). These protein fragments are known as food-derived peptides. Each peptide molecule contains 2-20 amino acid residues in most cases, but sometimes it could be longer than 20 amino acids. Some of these peptides are biologically active and have physiological effects such as regulation of peripheral blood pressure (Murray et al. 2007) and antibiotic activity (Clare et al. 2003). Some also involve the modulation of gastrointestinal (Chabance et al. 1998; Shimizu 2004) and immune system (Meisel 2004). Up to the present the biological property of milk protein-derived peptides has been widely studied while less work has focused on the pharmacology of bioactive peptides from other food sources.

One of the bioactive peptides derived from β-casein digestion is known as β-casomorphins-7 (BCM-7). The structure of BCM-7 is Tyr^{60}-Pro^{61}-Phe^{62}-Pro^{63}-Gly^{64}-Pro^{65}-Ile^{66}, with a molecular formula of $C_{41}H_{55}N_7O_9$ and molecular weight of 789.9 g/mol. The enzymatic release of BCM-7 via digestion of β-casein is determined by the sequences of amino acid of this casein. It has been identified that the amino acid at position 67 of the protein sequence is of significance to the generation of BCM-7 (Jinsmaa et al. 1999). As described above, there is a Histidine at this position in A1 β-casein and a BCM-7 can be cut off from it, whereas the Proline residue in A2 β-casein protect the bond between Ile^{66} and Pro^{67} from hydrolysis by digestive enzymes (Figure

2.1). A3, H1, H2, I, J, K and L β-casein are sub-variants belong to A2 β-casein family. Some sub-variants within A1 group, including B, C, F and G β-casein, also have a Histidine residue at position 67 (Kamiński et al. 2007), which allows them to be cleaved enzymatically and release BCM-7 just like A1 β-casein. In fact, the generation of BCM-7 from B variant could be even higher than that from A1 β-casein (De Noni 2008). The gastrointestinal digestion of yoghurt and cheese made from A1 milk will release BCM-7 into human body (De Noni et al. 2010). In addition, BCM-7 will also be released and retained during the production and processing of dairy product like cheese (Sienkiewicz-Szłapka et al. 2009) but not yoghurt (De Noni et al. 2010).

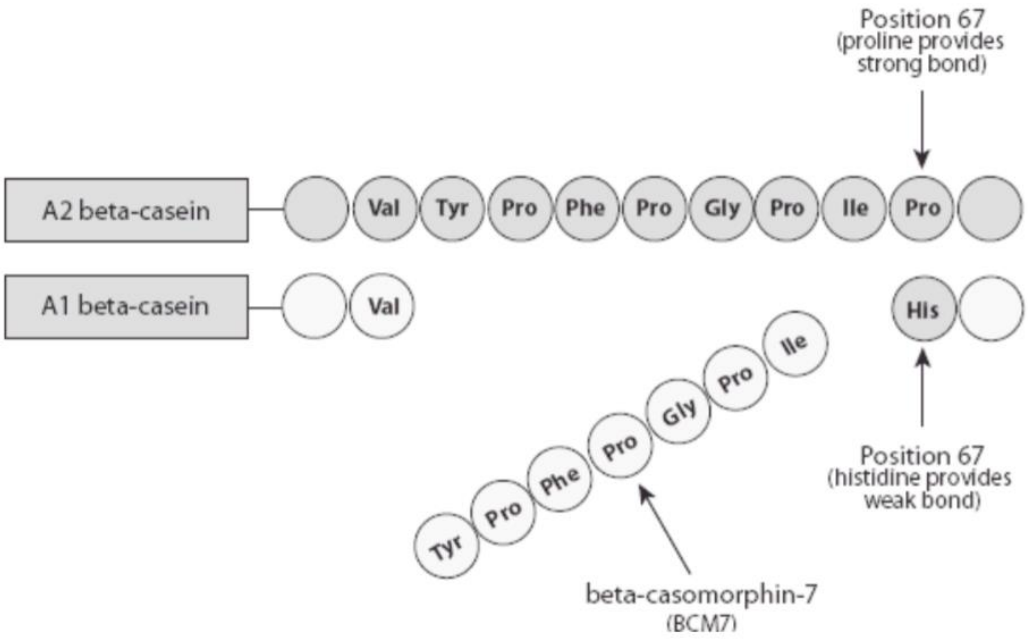

Figure 2.1 Release of β-casomorphins-7 with the breakdown of amino acid chain in A1 β-casein (Woodford 2008).

2.4 The significance of opioids systems and its interactions with BCM-7

Endogenous opioids peptides, including endorphins, enkephalins, and dynorphins, are naturally produced by the human body and function as neurotransmitters (Brownstein 1993). It is clear that some casein derived peptides have similar biological activities which involve the mediation of the opioid system (Korhonen et al. 2006). The term 'opioid' refers to a class of substances that can elicit morphine-like effects via opioid

receptors in the body. Opioids peptides bind to different types of receptors which are capable of modulating the body reactions to pain.

The opioid bioactive peptides derived from caseins during protein digestion or food processing (e.g., milk fermentation or cheese ripening) are exogenous ligands which have either agonistic or antagonistic action on opioid receptors. The interaction of exogenous opioid ligands (exorphins) or endogenous opioid ligands with their receptors can mediate various physiological effects (Clare et al. 2000; Teschemacher 2003). Opioid receptors are widely distributed throughout the central nervous system (CNS) and can be found in immune tissues (Wittert et al. 1996) and mammal's gastrointestinal tract (Fickel et al. 1997).

There are three different classes of opioid receptors, which are μ-, δ- and κ-opioid receptors. Opioid ligands can activate μ-opioid receptors, resulting in a variety of effects such as analgesia, depression of breathing, miosis, euphoria and reduction of gastrointestinal motility (Brownstein 1993; Waldhoer et al. 2004). There are some reports suggesting that the expression of μ-opioid receptors may be involved in autism (Wakefield et al. 2002; Sokolov et al. 2014) and schizophrenia (Volk et al. 2011). The opioid property of peptides isolated from casein peptone has been revealed for decades (Brantl et al. 1979; Henschen et al. 1979). It has been well established that bioactive BCM-7 can have various impacts throughout human physiology, such as on gastrointestinal tract, CNS, cardiovascular and immune system by acting as an μ-opioid receptor agonist (Korhonen et al. 2006; Kamiński et al. 2007).

2.5 The possible health effects of milk-derived peptides

Although some early researchers proposed that dairy proteins might have the possibility to increase the risk of some diseases (Popham et al. 1983; Dahl-Jørgensen et al. 1991), the moderate consumption of dairy products is not considered to be harmful to human health in most major studies. However, it has come to scientists' notice later on that it

was milk protein polymorphism that could result in some negative health effects. The hypothesis that the A1 β-casein derived BCM-7 is an important contributor to type I diabetes in children was developed in late 1990s by Elliott et al. (1999). Two years later, another study by McLachlan (2001) suggested that there was a positive correlation between the consumption of A1 β-casein and the mortality of ischaemic heart disease (IHD). The comparative data from these studies has drawn considerable attention to the distinct physiological properties of different milk components and stimulated the extensive research into the health effects of some specific milk proteins – namely, A1 β-casein. The majority of the research to date is associated with the potential effects of BCM-7 on the CNS, the cardiovascular and gastrointestinal function.

2.5.1 The transport of milk-derived peptides

Biologically active peptides must be delivered to the target sites in the first place. Following the breakdown of milk proteins, peptide molecules will be absorbed from the gastrointestinal tract into the blood circulation and transferred to their potential target tissues in the body. In order to maintain a bioactive form, the peptides have to first withstand the action of brush border peptidases and then be absorbed across the intestinal mucosa. The transepithelial transport of opioid peptides is usually difficult due to their hydrophobic character (Ganapathy et al. 2005). Enzymatic hydrolysis has been identified as the major limiting factor for the half-life of opioid peptides in human small intestine (Iwan et al. 2008). The poor intestinal absorption of oral administered peptides results not only from their instability against enzymatic degradation but also from low membrane permeability across the intestinal mucosa owing to their undesirable physicochemical property (Pauletti et al. 1996). Nevertheless, since there is no barrier or mechanism which can cormpletely prevent intestinal absorption of peptides (De Noni et al. 2009), it is still feasible for BCM-7 to penetrate human intestinal mucosa regardless of their inconsistent bioavailability. The possibility of transfer of food-derived opioid peptides though human intestinal epithelium has been

demonstrated in a number of studies (Iwan et al. 2008; Sienkiewicz-Szłapka et al. 2009; Vij et al. 2016).

Milk-derived biofunctional peptides must have the ability to cross the blood-brain barrier (BBB) and still stay active so as to exert influences on the CNS. BBB is a physiological barrier that separates the peripheral circulation from the CNS due to the tight junctions between the endothelial cells. As the gatekeeper of the CNS, this highly selective border allows the blood vessels to regulate the movement of substance between the brain and blood, thus providing the neural tissue with a defence against disease-causing pathogens and toxins in the blood (Daneman et al. 2015). Opioid peptides include BCM-7 can be transported across BBB via the peptide transport system-1 (PTS-1) (Ermisch et al. 1985; Banks et al. 1986). Therefore, the delivery of opioid peptides in the blood to the possible target tissue in the CNS seems achievable.

2.5.2 The possible effects of A1 β-casein on Type I diabetes mellitus

Type I (insulin-dependent) diabetes mellitus (IDDM) is also known as juvenile diabetes, as it occurs mostly in childhood and adolescence. IDDM is generally considered to be an autoimmune disease which involves an incorrect attack and destruction of insulin-secreting islet cells in the pancreas by T lymphocytes (Alberti et al. 1998). Consequently, very little or no insulin will be produced within the body to maintain normal blood glucose levels. The exact cause of IDDM is still unknown, but development of the disease results probably from both genetic and environmental factors (Association 2010). As the environment in which children are raised has been changed during the last several decades, the incidence of IDDM has been increasing remarkably worldwide (Wild et al. 2004). In order to explain the rising prevalence of IDDM, one hypothesis which involves the consumption of milk and dairy products has been suggested.

The relationship between dietary factors and the increased incidence of childhood diabetes has been widely investigated but the findings have been inconsistent. For instance, it was observed in many studies that the development of IDDM may be triggered by milk consumption in childhood (Dahl-Jørgensen et al. 1991; Fava et al. 1994; Saukkonen et al. 1998; Thorsdottir et al. 2003), shorter duration of breast feeding (Borch-Johnsen et al. 1984; Gimeno et al. 1997) and early exposure to dairy products (Virtanen et al. 1993). Nevertheless, the connection between IDDM and consumption of cow's milk is not demonstrated in some other studies (Thorsdottir et al. 2000; Strotmeyer et al. 2004). Furthermore, the study by Meloni et al. (1997) suggested that early introduction of cow's milk has no effect on the development of IDDM, which is supported by Esfarjani et al. (2001) and Savilahti et al. (2009). A short breast-feeding period showed no significant effect on reducing the risk of IDDM (Esfarjani et al. 2001; Ziegler et al. 2003).

Studies have looked into the role of gut-related immune function and A1 β-casein has been identified as a possible triggering factor of IDDM (Bell et al. 2006; Kamiński et al. 2007), which support the early study by Elliott et al. (1999). In fact, dairy proteins in conventional formula could induce an enhanced humoral immune response in infants, whereas those given hydrolysed formula expressed a reduced immunological response to proteins in cow's milk (Åkerblom et al. 2005). A hypothesis has been proposed that BCM-7 exclusively derived from A1 β-casein impairs the development of gastrointestinal immune tolerance or inhibits the body defences against enterovirus by acting as an immune suppressant, which may lead to the development of IDDM in genetically predisposed individuals (Kamiński et al. 2007).

Nevertheless, the finding reported by Elliott et al. (1999) that the positive correlation between the incidence of IDDM in infants and children (from 0 to 14-year-old) from 10 countries or areas and the per capita consumption of A1 β-casein based on ecological and epidemiological data is controversial. For example, the measurement of A1 β-casein consumption in this study is flawed. Most of the formulas used to feed infants

are based on the content of an increased whey protein and a reduced casein, there might be a difference between the adult per capita milk consumption and milk intake from formulas by infants in this case (Truswell 2005). Additionally, a more recent study showed that the gastrointestinal digestion of cheese also leads to the release of BCM-7 into the body (De Noni et al. 2010). The correlation will be weakened as a result of incorporation of A1 β-casein derived from cheeses as an additional source of BCM-7 (Lacroix et al. 2014). It is also worth considering that the robustness of the results in this study is limited by the dataset from a relatively small number of selected countries probably due to insufficient available data. One larger ecological study led to opposite conclusion as the increase in IDDM incidence (a 3% yearly increase) in 37 world areas from 1961 to 2000 was reviewed and its correlation with daily milk intake has not been confirmed (Muntoni et al. 2006).

The biological evidence from animal experiments and human studies which support the supposition that A1 β-casein plays a causative role in the development of IDDM is weak and insufficient. One large, multi-centre animal experiment investigated the diabetogenic effect of A1 β-casein on biobreeding (BB) rats and non-obese diabetic (NOD) mice which are both genetically prone to develop IDDM (Beales et al. 2002). However, although the authors suggested that milk caseins could facilitate the induction of diabetes in some cases, the study did not support the previous results that A1 β-casein is more diabetogenic than A2 β-casein. In the human study, the levels of A1 β-casein antibodies in the serum of IDDM patients were compared with those of their siblings, parents and controls (Padberg et al. 1999). However, the results from this study suggested that the differences in the concentration of A1 antibodies were probably related to age rather than IDDM.

In conclusion, the hypothesis that A1 β-casein in cow's milk is a diabetogenic factor is yet to be supported by convincing evidence. A1 β-casein itself, however, does not seem to work as an exclusive contributing factor of IDDM, although it may enhance the outcome of the disease to some extent.

2.5.3 The possible effects of A1 β-casein on cardiovascular health

The major cardiovascular disease is ischaemic heart disease (or coronary artery disease), which has underlying mechanisms involving atherosclerosis and thrombosis. High blood pressure, high blood cholesterol, obesity and smoking are some of the well-known risk factors for cardiovascular disease. The significant relationship between consumption of A1 β-casein and cardiovascular disease has been established in several ecological studies (McLachlan 2001; Birgisdottir et al. 2002; Laugesen et al. 2003). The discovery of this possible risk factor for cardiovascular disease arouses considerable interest.

In fact, some biofunctional peptides derived from milk proteins even have beneficial cardiovascular effects including the reduction of blood pressure (Murray et al. 2007). In regard to A1 milk protein, however, studies suggested that BCM-7 could lead to atherosclerosis (Tailford et al. 2003; Allison et al. 2006). The proatherogenic effect of BCM-7 involves the promotion of oxidation of human low-density lipoproteins (LDL) (Torreilles et al. 1995). The oxidation of LDL within artery walls can contribute to the development of atherosclerosis and cardiovascular disease.

The animal study carried out by Tailford et al. (2003) demonstrated the detrimental effects of A1 β-casein on the progression of atherosclerosis. However, the methodology of the experiment is somewhat concerning. The sample groups given specific diet were very small (only 6 per group). The duration of the trial was relatively short (for only 6 weeks), especially compared to natural development of atherosclerosis which usually takes years in man (De Noni et al. 2009). This single study in a rabbit model may provide only insubstantial evidence to support the causative role of A1 β-casein in human atherosclerosis. Extrapolating these results from animal experiments to human could be very problematic.

The study by Laugesen et al. (2003) failed to find the association between IHD and some well-recognised risk factors (e.g., the consumption of tobacco products and saturated fat), which reflects the fact that it is difficult to adjust for all the confounding factors and demonstrate a cause-effect relation in an ecological study. The proportion of A1 β-casein in milk was rising over the years, whereas the association between the A1 β-casein consumption and IHD was much weaker in the 1990s than in the 1970s (Hill et al. 2002). These findings obviously contradict the results from previous ecological studies suggesting the proatherogenic effect of A1 β-casein. Also, no evidence has been found to confirm the adverse effect of A1 β-casein on cardiovascular health in human studies (Chin-Dusting et al. 2006; Venn et al. 2006). Therefore, to support the role of A1 β-casein in the pathogenesis of cardiovascular disease in humans, more strong and convincing evidence is required.

2.5.4 The physiological effects of A1 β-casein on CNS

The μ-opioid receptors which bind to BCMs are highly expressed in the brain and spinal cord (Besse et al. 1990; Wittert et al. 1996). BCMs was reported to exert analgesic effects on experimental animals and the duration was much longer than that of endogenous peptides (Brantl et al. 1981). These analgesic effects of BCMs could be totally blocked by the opioid receptor antagonist naloxone. Other CNS-associated effects of BCMs were also reported in animal studies include stimulation of food intake (Lin et al. 1996) and dose-dependent modulation of memory and learning (Sakaguchi et al. 2006).

Many opioids receptors are located in the nuclei which are also involved in regulation of active sleep (Aghajanian 1978). In fact, opioid peptides may play a role in induction and maintenance of the sleep (Wilson et al. 1984) and have a tonic effect on breathing (Santiago et al. 1985). The positive correlation between chronic opioid use for pain

management and sleep-disordered breathing has been found (Farney et al. 2003; Webster et al. 2007). It is suggested that long-term use of opioid may promote the development of central sleep apnea syndrome (CSAS) and ataxic breathing (Walker et al. 2007).

It is clear that the binding of opioid with μ-opioid receptors at some particular neuronal sites in the CNS leads to opioids-induced respiration depression. Sudden infant death syndrome (SIDS) is the sudden and unexpected death of a seemingly heathy child less than one year old (Sun et al. 2003). The death usually happens during sleep and is unexplained even after a complete autopsy. Infants who later develop SIDS show an abnormality of respiratory center function (Valdes-Dapena et al. 1983). It has been hypothesised that BCMs are the possible etiological factors for SIDS (Ramabadran et al. 1988; Sun et al. 2003; Wasilewska et al. 2011). The accumulation of BCMs in immature CNS of the infants may depress brain-stem respiratory centers. The antibodies against BCMs have been found in the brain stem of the SIDS victims (Pasi et al. 1993), which validates that BCMs can pass through BBB and interact with their receptors in CNS.

Autism spectrum disorder (ASD) refers to a group of neurodevelopmental disorders characterised by difficulties with social interaction, impaired communication and repetitive, restricted patterns of behaviour (Baio 2014). Autism is a spectrum condition and generally considered incurable. The etiology of autism remains unclear. However, there has been a growing interest lately in the role of gastrointestinal pathology in psychiatric disorders including autism and schizophrenia. Gastrointestinal symptoms are common in children with developmental disorders (Horvath et al. 2002; Wakefield et al. 2002). Studies found the urine concentrations of BCM-7 were significantly higher in children with autism (Reichelt et al. 2012; Sokolov et al. 2014). They claimed that early child development could be impaired by increased BCM-7 levels, leading to predisposition to autism. However, contradictory results have also been presented in other studies which failed to confirm the presence of opioid peptides in urine of autistic

children (Hunter et al. 2003; Cass et al. 2008).

It has also been reported in some studies that BCM-7 may be implicated in schizophrenia (Sun et al. 1999; Severance et al. 2010). The possible link between elevated milk casein antibodies and the risk of schizophrenia has been suggested (Niebuhr et al. 2011). Casein-free diets seems to improve some neurological symptoms of schizophrenia (Okusaga et al. 2013). Nonetheless, the current evidence supporting the role of BCM-7 in schizophrenia is limited and weak.

It is important to note that abnormal BCM-7 levels have never been found in the CNS of patients with autism or schizophrenia according to current literature. In summary, the association of BCM-7 with autism or schizophrenia is yet to be supported by stronger evidence, despite the fact that a casein-free diet may benefit a subgroup of individuals with these mental illnesses.

2.5.5 The physiological effects of A1 β-casein on GI tract and milk intolerance

Dietary proteins affect the release of gut hormones by stomach and small intestine, which in turn modulate gastrointestinal digestion and metabolic process (Lichtenberger 1982). Research has shown that casein-derived peptides, including BCM-7 can stimulate the secretion of mucin and enhance gene expression in human intestinal goblet cells by direct interacting with endogenous opioid systems (Zoghbi et al. 2006; Martínez-Maqueda et al. 2013). Current evidence clearly indicates the regulatory effects of food-derived peptides on gastrointestinal function and digestive process. It has been demonstrated in different animal models that a casein meal decelerated gut motility and increased gastrointestinal transit time compared with a soy protein meal or a whey protein meal (Daniel et al. 1990; Defilippi et al. 1995; Crowley et al. 2013). Young rats fed hydrolysed casein have shown a faster gastrointestinal transit than those given normal casein due to no release of BCMs from hydrolysed casein during digestion

(Mihatsch et al. 2005). Likewise, Wistar rats fed A1 β-casein had significantly longer gastrointestinal transit time than A2 group (Barnett et al. 2014).

Milk intolerance, which is a commonly reported gastrointestinal disorder, is generally considered to be caused by in sufficient lactase enzyme activity. However, there is increasing body of evidence in human studies that milk-derived BCM-7, rather than lactose per se, may play a causative role in milk intolerance. Adults humans consuming A1 β-casein milk were reported to have significantly higher Bristol Stool Scale than those consuming A2 β-casein milk, suggesting that A1 group had softer stools (Ho et al. 2014). The consumption of conventional milk was associated with delayed gastrointestinal transit times and significant increased gastrointestinal inflammation (Jianqin et al. 2015). Substituting conventional milk with milk containing only A2 β-casein resulted in reduced gastrointestinal response caused by milk intolerance in Chinese preschool children (Sheng et al. 2019). However, these responses are direct gastrointestinal effects of BCM-7, or indirect effects caused by longer gastrointestinal transit times, is yet to be elucidated by further studies.

2.6 The effects of milk protein variants on productive and reproductive performance of cows

The A2 Milk Company was set up in New Zealand in 2000. It focuses on the selection of the cows that produce only A2 milk protein and markets milk and dairy products only with the A2 β-casein variant to New Zealand, Australian, US and Chinese markets. The A2 Milk Company also developed a method for identifying A1 and A2 types of the cows. After realising the commercial potential of the A2 milk in global market, a large number of dairy farms within New Zealand chose to develop their 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. The A2 Milk Company is experiencing strong sales growth in China.

There is growing interest in using the A2 casein type as an additional criterion in bull selection for artificial insemination due not to the potential importance of A2 milk for public health but also to its apparent commercial potential. The effects of A2 β-casein type on milk production, milk composition warrants investigation. Furthermore, fertility is a major factor affecting the profitability of New Zealand dairy production systems as poor fertility is the biggest cause of culling of dairy cows in New Zealand (Xu & Burton 2000; Martinez Rocha 2017). Early culling of a cow due to reproductive failure can result in substantial economic losses to dairy farmers because it is not only a waste of productivity but also the cost of animal rearing is diluted by fewer lactations. Therefore, reproductive traits are also economically important and the effects of selection for certain β-casein types on cow fertility should also be investigated.

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 in New Zealand is scarce.

Chapter 3

Material and Methods

3.1 Farms and animals

The data was collected from Dairy 1 and Dairy 4 farms at Massey University, Palmerston North. Dairy 1 farm is 142.7 hectares with 65 paddocks, and 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). The number of cows in Dairy 1 was 249 in the season 2017/18.

Dairy 4 farm is 224 hectares (effective grazable area) with 90 paddocks. It 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. The number of cows in Dairy 4 was 593 in the season 2017/18.

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 23th.

3.2 Description and handling of the data set

Animal information consisted of breed composition, liveweight (LW), lactation length and genotype. A radio frequency electronic identification system (Allflex New Zealand Ltd., Palmerston North, New Zealand) was used to identify each cow. The records for Daily LW were obtained with an automatic race walkover scale (WoW xR-3000, Tru-Test Ltd. Auckland, New Zealand) after each milking in both farms (Correa-Luna et al. 2018). The LW of each cow for the whole season was calculated as the average of all the LW measurements. Lactation length was calculated as the number of days in milk (DIM) 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 (milk test), 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 in both farms was determined based on a tissue test.

The productive 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 MY 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 reproductive traits measured on each cow include 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.

The objective of the current study was to investigate the productive and reproductive performance of cows with different β-casein types (A1 and A2) in two farms. However, genotypic information of all the first lactation cows in Dairy 4 were 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. As a result, only 733 cows of parity ≥2 were selected. Due to lack of complete individual information from some of these animals, complete records were available for 642 cows and these were used in this analysis. The final number of cows used for statistical analysis (642) accounts for approximately 88% of the total number of cows selected (733).

3.2.1 Breed

The cows used for data analysis were Holstein Friesian (F), Jersey (J) and crossbred (F×J). In general, a cow was considered purebred when she had ≥87.5% of F or J,

otherwise she was considered crossbred. There were 206 dairy cows in Dairy 1 farm in the season 2017/18. The breed structure of Dairy 1 was 49 F, 50J and 107 F×J. By comparison, there were 451 dairy cows in Dairy 4 including 139 F, 4J and 308 F×J. It is worth noting that proportion of Holstein-Friesian (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 summary of the number of cows and breed proportion in each farm is presented in Table 3.1.

Table 3.1 Number of cows (N) and breed composition (%) of each herd.

		Breed[1]			
Farm		F	F×J	J	Total
Dairy 1	N	49	107	50	206
	%	23.8	51.9	24.3	
Dairy 4	N	139	308	4	451
	%	30.8	68.3	0.9	

[1]F = Holstein Friesian, J = Jersey and F×J = crossbred.

3.2.2 Parity

The lactation number of the cows included in this study ranged from 2 to 6 as the first lactation cows were excluded in both farms. For statistical analysis, the cows were divided into two groups: (1) second parity and (2) third or greater than third parity. The summary of the parity of cows in each farm is presented in Table 3.2.

Table 3.2 Number of cows (N) in different parity group and its proportion (%) in each farm.

Farm		Parity		Total
		2	≥3	
Dairy 1	N	57	149	206
	%	27.7	72.3	
Dairy 4	N	48	403	451
	%	10.6	89.4	

3.2.3 Genotype

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. The summary of the β-casein types of cows in each farm is presented in Table 3.3.

Table 3.3 Number of cows (N) in different genotypes and its proportion (%) in each farm.

Farm		β-casein type		Total
		A1 cows[1]	A2 cows[2]	
Dairy 1	N	84	122	206
	%	40.8	59.2	
Dairy 4	N	234	217	451
	%	51.9	48.1	

[1]A1 cows = cows with either A1A1 or A1A2 genotypes.

[2]A2 cows = cows with only A2A2 genotypes.

3.3 Statistical methods

The data set 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 type; FG_{ik} is the interaction between farm *i* and type *k*; LG_{jk} is the interaction between lactation number *j* and type *k*; β_1 is the regression coefficient of the dependent variable on proportion of Holstein-Friesian (pF); β_2 is the regression coefficient of the dependent variable on F×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 observations 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 between means were declared at $P<0.05$.

Chapter 4

Results

4.1 Descriptive statistics

Descriptive statistics for milk production and reproductive traits of total 642 cows (186 F, 53 J, 403 F×J) from Dairy 1 and 4 in the season 2017/18 are shown in Table 4.1. The lactation milk yield in 2017 ranged from 1,491 kg to 7,371 kg with a mean value of 4,696 kg. The means for yields of fat and protein were 223 kg and 179 kg, respectively. The range for FP was from 2.91 to 7.22% and was more than PP which ranged from 3.13 to 4.94%. For fertility traits, the mean value of SMCO was 16 days with a range from 0 to 69 days. Pregnancy rate at first service was 50% on average. Submission rate at 21 days was high (>90%). The average pregnancy rates at 21 and 42 days were 54% and 77%, respectively.

Table 4.1 Mean, standard deviation (SD), minimum and maximum values of lactation yields of milk (kg), fat (kg) and protein (kg), 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
Total milk yield	4696.3	1,063.6	1,491	7,371
Total fat yield	222.5	46.7	59	353
Total protein yield	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	16.8	14.1	0	67
PRFS	50	50		
SR21	90	30		
PR21	54	50		
PR42	77	42		

4.2 Analysis of variance

Table 4.2 shows the least squares means, standard errors and P-values of the factors affecting the productive performance of the dairy cows in two farms. The MY and PY were both significantly greater ($P<0.05$) in Dairy 4 than in Dairy 1, whereas FY was not significantly different between two farms. The FP and PP were both significantly greater ($P<0.05$) 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 difference of PP between β-casein variants was 0.0567 and its effect on PP tended to be significant ($P<0.10$). A2 cows had a greater PP than A1 cows. The effect of interaction between farm and β-casein type was not significant on any of the productive traits, although its effect on PP approached significance at $P=0.106$. The interaction between parity and β-casein type was not significant for any of the productive 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 productive traits, whereas deviation from median calving date had a significant effect ($P<0.05$) on all productive traits.

Table 4.2 Least squares means (Mean), standard errors (SE) and P-values of factors affecting productive performance of cows in Massey University Dairy 1 and Dairy 4 farms in 2017.

	Trait[1]									
	MY		FY		PY		FP		PP	
Effect	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
P-value	<0.0001		0.4860		0.0082		<0.0001		<0.0001	
Parity										
2	4,110[b]	91	196.3[b]	4.4	157.3[b]	3.5	4.90	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
P-value	<0.0001		<0.0001		<0.0001		0.7871		0.7082	
β-casein type										
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
P-value	0.5407		0.7608		0.9861		0.4477		0.0567	
Interaction Farm×β-casein type										
P-value	0.1270		0.5641		0.3208		0.1464		0.1055	
Interaction Parity×β-casein type										
P-value	0.4381		0.7094		0.3856		0.4213		0.6877	
pF[3]										
Effect	1,107.2	125.8	3.62	6.04	22.22	4.78	-1.21	0.07	-0.50	0.03
P-value	<0.0001		0.5492		<0.0001		<0.0001		<0.0001	
Heterosis										
Effect	-82.7	121.1	6.21	5.82	2.51	4.60	0.09	0.07	0.05	0.03
P-value	0.4947		0.2860		0.5854		0.1783		0.1236	
dmcd[4]										
Effect	-15.50	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	

[1]MY = milk yield, FY = fat yield, PY = protein yield, FP = fat percentage and PP = protein percentage.

[2]β-casein type A1 = cows with either A1A1 or A1A2 genotypes, and A2 = cows with only A2A2 genotype.

[3]pF = proportion of Holstein-Friesian.

[5]dmcd = deviation from median calving date.

[a, b] Least squares means with different superscripts within column are significantly different (P<0.05).

Table 4.3 shows the least squares means, standard errors and P-values of the factors affecting fertility traits of the dairy cows in two farms. 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. The SMCO was significantly greater (P<0.05) in second parity than in ≥3 parity cows, whereas the other reproductive traits were not significantly different between parity. The β-casein type had no significant effect on any other reproductive traits. The effect of interaction between farm and β-casein type was significant (P<0.05) on PR42 but not on any other reproductive traits. The interaction between parity and β-casein type was not significant for any of the reproductive traits. The SR21 was significantly affected (P<0.05) by pF, but the effect of pF was not significant for any other reproductive traits. Heterosis was not significant for any of the reproductive traits, whereas the effect of deviation from median calving date was significant (P<0.05) for all binomial reproductive traits.

Table 4.3 Least squares means (Mean), standard errors (SE) and P-values of factors affecting fertility traits of cows in Massey University Dairy 1 and Dairy 4 farms in 2017.

	Trait[1]									
	SMCO		PRFS		SR21		PR21		PR42	
Effect	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
P-value	0.3582		0.0007		<0.0001		<0.0001		0.0375	
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
P-value	0.0084		0.1334		0.3491		0.2271		0.4577	
β-casein type[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
P-value	0.7476		0.8167		0.8367		0.2071		0.2460	
Interaction Farm×β-casein type										
P-value	0.8840		0.9247		0.4704		0.2321		0.0360	
Interaction Parity×β-casein type										
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	0.7934		0.0534		0.0446		0.4352		0.2809	
Heterosis										
Effect	-0.40	2.12	0.04	0.07	-0.02	0.04	-0.01	0.07	-0.02	0.06
P-value	0.8505		0.5682		0.7795		0.8762		0.6998	
dmcd[4]										
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		0.0227		0.0343		0.0017		0.0036	

[1]SMCO = days from start of mating to conception.

[2]β-casein type A1 = cows with either A1A1 or A1A2 genotypes, and A2 = cows with only A2A2 genotype.

[3]pF = proportion of Holstein-Friesian.

[5]dmcd = deviation from median calving date.

[a, b] Least squares means with different superscripts within column are significantly different (P<0.05).

Chapter 5

Discussion

5.1 Productive performance

Productive and reproductive performance of cows based on A2 type in Massey University Dairy 1 and 4 farms in the season 2017/18 was analysed in the current study. Cows in Dairy 1 produced 4,188 kg of milk, 210.3 kg of fat and 165.4 kg of protein over the lactation, whereas those in Dairy 4 produced 4,639 kg of milk, 213.1 kg of fat and 173.9 kg of protein. Cows milked OAD in Dairy 1 produced 9.7, 1.3 and 4.9% less milk, fat and protein per lactation than cows milked TAD in Dairy 4, but cows in Dairy 1 produced milk with higher percentages of fat and protein. The productive performance of these cows from two farms was higher than another study by Lembeye et al. (2015) who reported 2,950 kg of MY, 153.4 kg of FY and 118.0 kg of PY for cows milked OAD and 3,836 kg of MY, 188.0 kg of FY and 145.9 kg of PY for cows milked TAD from total lactation records for the period 2008 to 2012. Despite the difference in breed composition and feeding, selection within the herds over the years for cows with better genetic and ability to adapt well to their own OAD/TAD system contributed to the increase in milk and solids production. Lembeye et al. (2015) also reported 23.1, 18.4 and 19.1% less milk, fat and protein produced by cows milked OAD than cows milked TAD, which was larger than the differences of production between OAD and TAD cows in the current study. However, it is not possible to directly compare the milk and solids production by OAD and TAD cows in the current study due to the different feeding strategies. Cows in Dairy 1 farm were milked OAD with a low stocking rate and more pasture, whereas cows in Dairy 4 were milked TAD with a higher stocking rate and higher use of supplementary feed. Fat percentage and PP of the milk from cows milked OAD were significantly greater than the milk from cows milked TAD.

5.2 Effect of β-casein type on milk and milk solid production

Analysis of variance in the current study showed that β-casein type had no significant effect on total MY, FY or PY. The effects of β-casein polymorphism on milk productive

traits have been investigated in a number of studies, 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 Holstein Friesian cows (Ng-Kwai-Hang et al. 1986; Çardak 2005), which was in agreement with the current study. In herds with a mixed population of Jersey and Friesian cattle, McLean et al. (1984) also reported that β-casein A1 and A2 types 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). In line with the present study, no relationship between β-casein variants and productive traits was reported.

Conversely, the association between A1 and A2 β-casein types and MY during first three lactations in Holstein herds was demonstrated, and A2A2 cows produced more milk than A1A1 cows (Bech et al. 1990; Ng-Kwai-Hang et al. 1990). The increase in PY resulting from more milk production by cows with A1 allele has also been suggested (Heck et al. 2009). Although Lin et al. (1986) suggested that the loci of A1 and A2 β-caseins had no significant effect on 308-day MY of first lactation cows, they found that the effect was significant on PY ($P<0.05$) and approached significance ($P=0.09$) for FY. The results suggested that the A2 type was superior to A1 type, 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 those of the A1A1 variant. The productive advantage of the A2 allele over the A1 allele has been also reported by other published work (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 type is due to the effect of its linked gene or the loci of β-casein themselves. Some of the aforementioned studies were conducted in Friesian 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 types in Simmentaler cows but not in Friesian cows (Çardak 2005), which likely indicated the different effect of a linked gene in different breeds.

5.3 Effect of β-casein type on milk composition

The current study found no significant difference in fat concentration between β-casein variants, which was support by early studies (Ng-Kwai-Hang et al. 1986; Gonyon et al. 1987). However, 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 Friesian 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 Jersey and Friesian cows (McLean et al. 1984).

In the present study, the difference in PP was reported for β-casein A1 and A2 types at $P=0.0567$, which was significant at $P<0.10$. Milk produced by A2 cows contained 3.90% protein, which was greater than the PP (3.85%) in the milk produced by A1 cows. In comparison, no association was found between β-casein A1 and A2 types and milk protein content in the previous studies with Friesian cows (Ng-Kwai-Hang et al. 1986; Ng-Kwai-Hang et al. 1990), Guernsey (Haenlein et al. 1987) and a mixed population (McLean et al. 1984). Çardak (2005) examined the effects of the β-casein genotype and reported a significant increase in protein concentration for A2A2 over the A1A2 genotype in Simmentaler cows. However, the A1A1 genotype was not significantly different from A2A2 genotype (A2A2=A1A1>A1A2), and no significant effect was observed in Friesian cows in the same study. Only in one case that a negative association

between β-casein A2 type and PP was indicated (Gonyon et al. 1987). In addition, some studies reported that the A2 type had a detrimental effect on concentration of whey proteins (McLean et al. 1984) and milk casein (Ng-Kwai-Hang et al. 1986), but not on concentration of crude total protein. The conflicting observations in milk composition may partially explained by gene linkage in different breeds of the herds as discussed previously.

5.4 Effect of interaction between farm and β-casein type and between parity and β-casein type

The present study found no significant interaction between farm and β-casein type and between parity and β-casein type. However, the interaction between farm and β-casein type approached significance at P=0.1055. A2 cows in Dairy 1 farm produced 2% more milk than A1 cows, while A2 cows in Dairy 4 farm produced only 0.4% more milk than A1 cows. To the best of author's knowledge, few studies published previously investigated the interaction of the β-casein genotype A1 and A2 with other effects. Among them there are only one paper 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 Friesians than A1A1 Friesians, whereas A2A2 Jerseys produced 3-4% less milk than A1A1 Jerseys.

5.5 Effect of β-casein type on fertility

The influence of β-casein type 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 supporting 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 types in Dairy 4 were

72.0±4.0 % and 75.2±3.9 %, which explains the significant interaction between farm and β-casein type. 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 type 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.

5.6 Conclusion

The results indicated that cows of different β-casein types have similar production and reproduction performance. A2 cows tended to produce milk with higher PP ($P<0.10$), and selection of animals based on the A2 type should have no negative impact on their fertility.

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