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Phenotypic relationships between milk protein  
percentage, reproductive performance and body  
condition score in Irish dairy cattle

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## **Abstract**

A positive phenotypic correlation between milk protein percentage and reproductive performance in dairy cattle, especially during early lactation has been recently reported. The objective of this study was to quantify the relationship between milk protein percentage and different measures of fertility in Irish, seasonal calving, dairy cattle using data from experiments comparing strains of Holstein-Friesian cows under different feeding systems. The relationships between body condition score, milk production and fertility were also investigated.

The data used in this study consisted of 584 lactation records over a 5-yr period. Principal component analysis and logistic regression was used to study the relationship between milk protein percentage and fertility performance of the cow. Greater milk protein percentage during the first 60 days post-calving was associated with better reproductive performance. The probability of a cow being submitted in the first 21 days of the breeding season increased with increased milk protein percentage during early lactation. Similarly, the probability of a cow becoming pregnant to its first service or to the whole breeding season also increased. Cows were classified as either high or low milk protein percentage based on their protein percentage over the whole lactation. Cows in the high milk protein group had a 7% greater conception rate compared to cows in the low protein percentage group. In conclusion, cows with higher protein percentage, especially during early lactation are submitted earlier in the breeding season, and have a higher conception rate. Physiologically, the shortage of glucose caused by negative energy balance restricts the synthesis of milk protein in the udder. On the other side, negative energy balance also causes the reduction of IGF-I, LH and oestradiol, which consequently delay the ovarian follicular development and finally reduces fertility. Therefore, there is a biological explanation for the association between milk protein percentage and fertility performance.

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# Chapter 1

## INTRODUCTION

A seasonal calving pattern is recognised as an important feature of pasture-based milk production systems. This pattern is part of a system which involves the maximum utilisation of grazed pasture in the diet; with limited conservation of pasture as hay or silage, very little cropping, and almost no use of high energy or protein supplements. The timing of calving for the herd is a key factor in synchronising the herd's feed requirements with the seasonal pattern of pasture growth. Ideally, calving is planned to commence in late winter or early spring, with a large proportion of the herd calving during the first four weeks, and the remainder over the next 6 to 12 weeks. This means that calving should be completed by the time the herd has reached its peak demand for pasture dry matter, and the seasonal flush in pasture growth will have commenced to meet this demand.

A seasonal calving period requires seasonal mating seasons in the previous year. Cows in oestrus need to be identified promptly and accurately, typically using tail-painting plus regular observation. More than 90% of the cows in the herd should be mated in the first 3 to 4 weeks of the mating period (submission rate). A high conception rate (50 to 60%) is necessary, so that the high submission rate is translated into a compact calving pattern.

A common practice is to ensure that cows start the lactation with a body condition score of 5 (scale: 0 to 10) in New Zealand and 2.75 – 3.0 (scale: 0 to 5) in Ireland, because studies have demonstrated a positive relationship between body condition score at calving and fertility (Dechow et al., 2002; Pryce et al., 2001).

Previous research (Butler and Smith, 1989; Buckley et al., 2003; Roche et al., 2007a) has shown that changes in body condition score from calving to Day 60 of the lactation are also associated with cow fertility. More recently greater attention has been focused on the relationship between milk protein percentage and reproductive performance.

Analyses of fertility data collected from 168 commercial dairy herds in Australia (DRDC, 2000) showed that measures of fertility (6-week in-calf rate, 21-week in-calf rate, 3-week submission rate, first insemination conception rate) of dairy cows was strongly positively associated with milk protein percentage in the first 120 days of lactation. In New Zealand, Harris and Pryce (2004) reported that the positive genetic correlation between milk protein percentage and fertility.

An Irish study also showed that lower submission and conception rates were related with lower milk protein percentage around the time of artificial insemination (Buckley et al., 2003). The objective of the present research was to study the relationship between milk protein percentage and different measures of fertility in Irish dairy cattle, using data from experiments comparing strains of Holstein cows. The relationships between body condition score, milk production and fertility were also studied.

## **Chapter 2**

### **A review of the relationship between protein concentration in milk and fertility in dairy cattle**

The relationship between fertility and protein percentage in milk has been studied previously in Australia (Morton, 2000; Fahey et al., 2003) and New Zealand (Harris and Pryce, 2004) and some other European countries, including Ireland (Buckley et al., 2003). Protein percentage in milk was positively associated with submission rate, first service pregnancy rate and 21-day pregnancy rate (Fahey et al., 2003). The relationship between milk protein percentage and fertility was stronger during early lactation (Morton, 2001). Milk protein percentage was suggested as an indicator of reproductive efficiency.

Previous studies have shown that protein percentage in milk was closely related to the level of energy balance (Buckley et al., 2003; Patton et al., 2007). Energy balance, especially negative energy balance during early lactation, was associated with low fertility in lactating dairy cows (Butler, 2005). Therefore, in order to study the relationship between milk protein percentage and fertility, a good understanding of the relationships between energy balance, milk production and reproductive performance is very important.

The objective of this review is to give possible explanations of how milk protein percentage is related to fertility. The relationships between energy balance, body condition score, milk production and fertility is reviewed, together with some related physiological functions to establish a physiological background.

## **RESEARCH BACKGROUND**

### **Pastoral dairy system in Ireland**

The Irish dairy industry produces 5.3 million tonnes of milk annually. The number of dairy farmers has reduced from 68,000 in 1984 to the current figure of just over 22,000, which has resulted in larger farm sizes and increases in the average per farm quota to the current level of 231,000 litres (The National Dairy Council).

Before the middle 1980s, the dominant breed of dairy cow in Ireland was the British Friesian. With the importation of North American genetics, the North American Holstein-Friesian (NAHF) has become the dominant breed over the last 20 years, increasing from 9% in 1990 to 65% in 2001 (Horan et al., 2005). The importation of NAHF genetics was successful in achieving improved milk production, but resulted in a reduction in reproductive performance of the herd (Butler and Smith, 1989).

The production of milk in Ireland, facilitated by the country's mild climate, is mainly based on grazed grass system and is seasonal calving. Maintaining a calving interval of 365 days is therefore vital. Achieving a high pregnancy rate in a relatively short period after start of mating is required to ensure a concentrated calving pattern in next season. Therefore, the synchrony between changes of feed demand and pasture growth is important. Seasonal pastoral dairy production system is an economically-successful way of using cheap food to produce milk, and also to maximise the profitability of milk production.

## ENERGY BALANCE

Energy balance (EB) is used to describe the relationship between dietary energy intake and energy utilization. It is the difference between the net energy intake and the net energy required for maintenance, milk production, growth and activity (Lucy et al., 1991a).

After parturition, increase in milk yield is followed more slowly by an increase in dry matter intake (DMI). Hence, maximum milk production in early lactation is usually achieved prior to maximum feed intake (Coppock, 1985). This results in an increased mobilization of body adipose tissue to meet the energy deficit (Nebel and McGilliard, 1993). The severity and the duration of the energy deficit can be influenced by genetic merit for milk yield (Grainger et al., 1985; Veerkamp et al., 1994; Buckley et al., 2000a) and energy density of the feed. The deficit in energy is greatest in high producing cows. Therefore, a compensatory response, related to adipose tissue, liver, muscle and bone is triggered by this energy deficit, involving processes of increased lipolysis, gluconeogenesis, glycogenolysis and mobilization of protein and mineral reserves (Lucy et al., 1991a).

Energy deficit, or negative energy balance (NEB), is very common among dairy cattle during early lactation. NEB usually begins about one week before calving as the result of reduced feed intake. It becomes visible during early lactation as a loss in body condition, and reaches its nadir about two months postpartum (Butler, 2005). The degree of NEB differs between cows, and has been shown to be partly under genetic control (Berry et al., 2007).

NEB during early lactation has been linked phenotypically to low fertility in lactating cows (Butler, 2005). NEB affects follicular development and subsequent reproductive performance, particularly when it occurs during early postpartum folliculogenesis (Buckley et al., 2003). Many studies have shown that the reproductive performance of the cows, especially the probability of conception, is negatively associated with the degree and duration of NEB in early lactation (Butler and Smith, 1989; Nebel and McGilliard, 1993; Senatore et al., 1996; Domecq et al., 1997). Other studies have reported that NEB in dairy cattle is related to the interval to the first postpartum ovulation (Butler and Smith, 1989; Lucy, 2001) and the duration of anoestrous (Lucy,

2001). NEB during the first three to four weeks postpartum is highly correlated with the interval to first ovulation (Butler, 2000b). NEB delays the recovery process of postpartum reproductive function and also has other carryover effects such as on hormone secretion, which can reduce fertility during the breeding period (Butler, 2005). NEB and excessive body tissue mobilization are also related to an increase of metabolic disorders and poor fertility performance of the cow (Dechow et al., 2002; Buckley et al., 2003; Roche et al., 2006). Cows with greater DMI and a more positive energy balance are likely to have shorter calving to conception intervals (Patton et al., 2007).

## **BODY CONDITION SCORE (BCS)**

Body condition score (BCS) is a quick, noninvasive, inexpensive, visual and tactile way of assessing the degree of fatness of the cow (Waltner et al., 1993). BCS is used at the farm level as an indicator of EB during lactation (Wildman et al., 1982; Coffey et al., 2001).

### **BCS and milk production**

Cows that have high genetic merit for milk production usually lose more BCS in early lactation than do low genetic merit cows (Grainger et al., 1985; Veerkamp et al., 1994; Buckley et al., 2000a). According to Veerkamp et al. (2001), increasing the proportion of Holstein-Friesian genes from 50 to 100% results in a mean decrease of BCS by 1 unit (scale: 1 to 9). Also, Buckley et al. (2003) showed that every 100 kg increase in genetic merit for milk production was associated with 0.02 more BCS units loss between calving and first service.

According to Broster and Broster (1998), when milk fat percentage was at a relative low level, there was a positive response of milk fat percentage to increased BCS at calving. The average milk fat percentage during mid-lactation (days in milk 60 to 270) was positively correlated with greater BCS at both calving and nadir (Roche et al., 2007b). If fat percentage was very high, those responses could become negative, which meant that with an increase of BCS at calving the fat percentage may decrease rather than increase.

Compared with fat percentage in milk, the change of protein percentage in response to the change of BCS at calving is much narrower (Broster and Broster, 1998). In the review of Broster and Broster (1998), there was no response of milk protein percentage over the range of 27-30g/kg to different BCS at calving. However, two earlier studies (Moorby et al., 1996; Roche et al., 2007b) indicated that protein percentage over part or the whole lactation increased with higher average BCS. Roche et al. (2007b) concluded that milk protein percentage was not influenced by calving BCS, but was positively associated with nadir BCS and negatively associated with BCS loss between calving and nadir. Cows with greater BCS loss during early lactation had, on average, lower milk protein percentage (Roche et al., 2007b). However, Moorby et al. (1996) pointed

out that to understand the effect of calving BCS on protein percentage, the prepartum protein intake also needs to be considered.

### **BCS and reproduction**

Condition score of the cow is closely related with its reproductive, both phenotypically (Roche et al., 2006; Buckley et al., 2003) and genetically (Berry et al., 2003). BCS and BCS change are related to the reproductive performance of the cow since as they are indicators of NEB in early lactation (Butler and Smith, 1989). Not only the severity but also the duration of NEB influences fertility (Beam and Butler, 1999). Most recent studies indicate that increasing BCS or reducing BCS loss during early lactation is related to superior fertility (Markusteld et al., 1997; Butler and Smith, 1989; Gillund et al., 2001; Reksen et al., 2002; Buckley et al., 2003; Roche et al., 2007a).

Calving BCS is negative associated with the interval to first oestrous (Burke and Roche, 2007). Grainger et al. (1982) showed that each additional BCS at calving reduced the postpartum anoestrous interval by 5.7 days (scale: 1 to 8). However, heifers were reported to have longer interval to first oestrous when they calved in greater BCS, in the first week postpartum, every unit increase in BCS (scale: 0-5) was expected to increase the interval to first oestrous by 5.7 days (Pryce et al., 2001).

BCS during lactation have been reported to be associated with conception rate (Pryce et al., 2001; Buckley et al., 2003; Roche et al., 2007a). Most of those previous studies suggest that greater BCS loss during early lactation is related to longer calving to first service interval, lower conception rate, longer days open and longer calving intervals. Greater BCS loss during the first 30 days in milk is related to delayed first ovulation (Butler, 2005) and prolonged calving to conception interval. Cows in poor BCS at first service had a lower first service conception rate than cows with higher BCS during the same time (Patton et al., 2007). In the study of Butler and Smith (1989), cows that lost 0.5 to 1.0 point of BCS (scale: 1 to 6) between calving and first service achieved a first service pregnancy rate of 53%, which was only 17% for cows, who lost more than 1.0 point. According to Pryce et al. (2001), 1 unit increase in BCS at week 10 of lactation was associated with a reduction in the interval to first service by 6.2 days and an increase of first service conception rate by 9%. Lower nadir BCS was related to longer calving to conception interval (Patton et al., 2007). Generally, compared to calving BCS,

BCS during lactation especially the period around nadir BCS is suggested to be more strongly associated with fertility.

Genetically, greater BCS has been reported to be associated with a short interval to first service (Veerkamp et al., 2001; Berry et al., 2003b), higher submission rate (Pryce and Harris, 2006), higher conception rate (Veerkamp et al., 2001; Berry et al., 2003b; Pryce and Harris, 2006), higher non-return rate (Veerkamp et al., 2001; Wall et al., 2003; Banos et al., 2004), less number of service (Berry et al., 2003b; Wall et al., 2003), and shorter calving interval (Veerkamp et al., 2001; Wall et al., 2003; Banos et al., 2004).

## FERTILITY

In pasture-based seasonal calving dairy systems, management practices should focus on achieving the highest pregnancy rate in the shortest time after the start of the breeding season, resulting in a concentrated calving pattern in the next season. Therefore cows are required to conceive near to peak lactation.

Several reports have confirmed a decline in reproductive efficiency of dairy cattle, mainly in intensive feeding systems. This decline could be caused by a combined effect of physiological and management factors, together with a high selection for milk yield although neither is likely to be mutually exclusive. Butler (1998) showed that first service conception rate of NAHF cows has declined from 65% in 1951 to 40% in 1996. According to Lucy (2001), for cows that had been inseminated at observed oestrus, the conception rate was about 55% in 1950s and about 45% when inseminated at spontaneous oestrus and only approximately 35% when using timed AI in 1998. This trend not only exists in the United States, but many countries that rapidly adopted North American genetics have also faced declines in reproductive efficiency. Ireland, Australia and New Zealand, where the main dairy system is based on pasture grazing, the unsuitability of North American genetics with local feeding systems and management seems to have been the main cause of the declined fertility. Nowadays, poor reproductive efficiency appears to be a worldwide problem affecting the dairy industry (Lucy, 2001).

Lucy (2001) reported that the interval between calving and first ovulation was between 14 and 21 days, with a 5% anoestrous rate, in dairy herds during the period before 1970. However, the interval was approximately 10 days longer with greater anoestrous rate at the start of breeding season in 2000. The delay in the interval to first ovulation was thought to be partly caused by the greater NEB (Lucy, 2001). NEB during early postpartum period reduces postpartum LH pulsatility, thus, postpones the restart of ovarian activity (Butler, 2000b). A low nadir EB was correlated with a delay in luteal activity during postpartum period. However, only 3 to 4% of the delay in the interval could be explained by nadir EB or energy deficit (de Vries and Veerkamp, 2000). Therefore, there must be other factors that contributed to the prolonged interval to first ovulation in cows (Lucy, 2001).

## EFFECTS OF GENETIC SELECTION

Environmental factors such as nutrition, health and management, as well as genetic improvement, can all affect the milk production of the cow (Buckley et al., 2003). Genetic improvement has played an extremely important role in the increase of milk yield. According to statistics from the UK, nearly half of the total progress in milk yield can be attributed to genetic improvement (Pryce and Veerkamp, 2001). The same study also showed fertility of cows declined with increasing genetic merit for milk yield. The genetic correlation between fertility and milk production is unfavourable (Pryce and Veerkamp, 2001).

Previous experiments that compared animals of high and low genetic merit for milk production have slowly come to a consensus that increased genetic merit for milk production is associated with reduced fertility (Kelm et al., 1997; Pryce et al., 1999). At the genetic level, two explanations have been commonly put forward for this relationship. First, the pleiotropic gene effect, suggests that a single gene could control more than one trait; such that genes that affect milk yield also affect fertility. The second explanation is the linkage between genes, which suggests that genes which affect different traits are closely linked to each other at the chromosome level (Falconer and Mackay, 1996). The pleiotropy theory is thought to be more important than the gene linkage theory.

Differences in genetic merit are usually associated with different levels of feed intake, EB, and concentration of hormones such as growth hormone (GH), insulin-like growth factor I (IGF-I), insulin, prolactin, and progesterone; and metabolites such as glucose, NEFA and ketones (Veerkamp et al., 2003). Generally, selection for high yield increases metabolic load, and might reduce fertility through EB, the growth hormone axis or other pathways. Veerkamp et al. (2003) considered that regardless of pathways that might contribute to the reduction of fertility, the decline in available energy is more important than the change of hormone concentrations that cause poor fertility performance under the increase of genetic merit of the cow.

Buckley et al. (2003) showed that cows with a higher proportions of Holstein-Friesian genes or higher breeding values for milk production were associated with lower 21-day submission rates and 42-day pregnancy rates. However, no relationship with pregnancy rate to first service was found. Other studies, however, have shown that increased proportion of Holstein-Friesian genes is associated with a reduction in 56-day nonreturn rates (Hoekstra et al., 1994).

### **Effects on feed intake and EB**

Selecting for high milk yield increases the feed intake of the cow but also increases the difference between energy intake and energy output (Veerkamp, 1998; Veerkamp and Koenen, 1999). According to Veerkamp et al. (2003), the estimated genetic correlation between yield and dry matter intake ranges from 0.44 to 0.65 when animals were fed without regard to production, which meant only half of the energy requirements of increased milk production were covered by normal feed intake. The other half of the energy requirement, which is the energy gap of EB, is met by changing energy partition and fat mobilisation. High genetic merit is consequently associated with a greater degree of NEB (Gordon et al., 1995; Veerkamp et al., 1995; Buckley et al., 2000b). For a high genetic merit cow, increasing dietary energy density could not compensate for the extra body condition loss, since this extra energy would be directed into milk production, rather than filling the energy gap (Koenen and Veerkamp, 1997). Therefore, it is the genetically-controlled energy partitioning, rather than feed intake which is not keeping pace with increasing milk production and this is the main factor that causes a decline in EB in early lactation cows.

On the other hand, high milk production should not be equated with NEB. NEB occurs when the nutrition requirement for maintenance and milk production exceeds the ability of the cow to consume feed energy. Usually, the highest producing cows are not those with the lowest BCS or the greatest NEB (Lucy, 2001). According to Lucy et al. (1992), high producing cows have a greater DMI to try to meet metabolic demands for milk production. Therefore, their EB was similar to that of low producing cows. On the other hand, low producing cows, which also had a poor DMI had a greater risk for anoestrous and infertility than high producing cows.

## **Effects on NEFA and glucose**

*Nonesterified free fatty acids (NEFA).* During times of energy deficit, animals break down triglyceride (fat) stores in adipose tissue. This breakdown causes the release of NEFA, which enter the blood to be transported to organs and tissues throughout the body. The release of NEFA into blood only occurs when glucose concentrations fall. Plasma NEFA concentration reflects body fat mobilization: such that increased plasma NEFA indicates that dietary energy intake is insufficient for the cow's needs for milk production or foetal growth and that body fat is being broken down to supply the energy deficit.

NEFA is removed by the liver when sufficient oxalacetate is available. NEFA is converted into acetyl CoA, which is then oxidised via the citric acid cycle. However, when the oxalacetate availability is low, NEFA is not completely oxidized in the liver. This results in the production of ketone bodies, which cause ketosis, metabolic acidosis and the increased accumulation of triglycerides in the liver, resulting in fatty liver (Veerkamp et al., 2003).

NEFA is a useful measure of energy metabolism, especially in the immediate prepartum transition period. Before calving, the plasma NEFA concentration might be used to detect cows at risk for problems with severe NEB. NEFA concentrations seem to be higher in high genetic merit cows. However, according to Veerkamp et al. (2003), there were several studies that did not show any effect of selection for milk yield on NEFA concentrations.

*Glucose.* Glucose is a monosaccharide (or simple sugar) that is used by living cells as a source of energy and a metabolic intermediate. Some studies have shown that cows selected for high milk production had relatively low blood glucose concentration during lactation (Snijders et al., 2001), while others have shown that low glucose concentrations only appeared around the time of parturition (Lukes et al., 1989).

## **Effects on fertility**

Over the last few decades, the increase in milk production in dairy cows has been shown to be related to the decline of fertility. According to Butler (2005), the conception rate of mature cows in large commercial herds was 35-40%, compared to 51% for first lactation cows or over 65% for virgin heifers. With the increase in milk production through successive lactations, the fertility of the cow appears to be reduced.

Madsen (1975) pointed out that at the beginning of lactation, very high milk yield would put high physiological stresses on cows and this leads to reproductive disorders. Cows with the greatest milk production also have the highest incidence of infertility (Lucy, 2001).

Calving interval, days open, calving to first service interval and first service conception rate are the common traits used to estimate genetic correlations between yield and fertility. According to the review by Pryce and Veerkamp (2001), the estimated genetic correlations between production and fertility for calving interval range from 0.22 to 0.59, days open from 0.16 to 0.64, calving to first service interval from 0.22 to 0.44 and first service conception rate from -0.62 to 0.05. These clearly suggested an unfavorable correlation between production and fertility. If only those cows with an increased genetic merit of about 100 kg milk per year are kept for breeding, the calving interval could be expected to increase by 5 to 10 days over the next 10 years (Pryce et al., 1998).

High milk production will cause a greater incidence of multiple ovulations, which will in turn increase the percentage of twinning. Twinning is a problem in dairy farming because, after the birth of twins, research has found that both milk production and fertility of the cow are impaired (Beerepoot et al., 1992).

## **Nutrition and fertility**

An increase in milk production means that nutritional requirements also increase. Nutrition therefore also impacts on infertility (Lucy, 2003) (Figure 2.1). The rapid increase of dietary intake appears to be deleterious to oocyte development and subsequent establishment of pregnancy (O'Callaghan et al., 1999). The nutritional effects of high production cows on fertility are realized through the antecedent effects of NEB (Butler, 2000b).

*The effects mediated through NEB.* NEB is directly related to oestrous cyclicity after calving through the inhibition of gonadotropin releasing hormone (GnRH) secretion, which disrupts the LH pulse patterns (Butler and Smith, 1989). Re-establishment of the normal LH pulse pattern plays an important role in ovarian follicular development and the initiation of postpartum ovarian cyclicity (Butler and Smith, 1989). Consequently, an extreme energy deficit might impinge on the pulsatile secretion of LH (Butler and Smith, 1989; Lucy et al., 1991b), thereby delaying ovulation. Normally, during the first two or three weeks postpartum, the secretion of LH from the pituitary will increase in most cows (Lucy et al., 1991a). The function of LH in the cow is to act on ovarian follicles to induce ovulation of waves of follicular growth, leading to the ovulation of a dominant follicle between 15 and 25 days after calving (Stevenson and Britt, 1979). The length of the interval from calving to first ovulation represents an interaction of energy status with reproductive performance (Butler, 2000b). Low energy availability during NEB not only suppresses pulsatile LH secretion, but also causes a reduced ovarian responsiveness to LH stimulation (Butler, 2000a). Moreover, both plasma glucose and insulin are decreased in NEB cows (Beam and Butler, 1999; Butler, 2000a), whilst NEB also reduces serum progesterone concentrations, which may further affect fertility (Butler, 2000b).

IGF-I, which is directly related to energy status, is critical to ovarian follicular development (Beam and Butler, 1999). IGF-I serves as a hormonal mediator of nutritional regulation of ovarian function (Chase et al., 1998). Energy status partly controls the synthesis and secretion of IGF-I (Zulu et al., 2002), whilst dynamic changes in liver metabolism reduce its secretion of IGF-I during the postpartum period. Previous studies have found that IGF-I concentrations are significantly reduced when the animal suffers severe nutritional deficiency during early lactation (McGuire et al.,

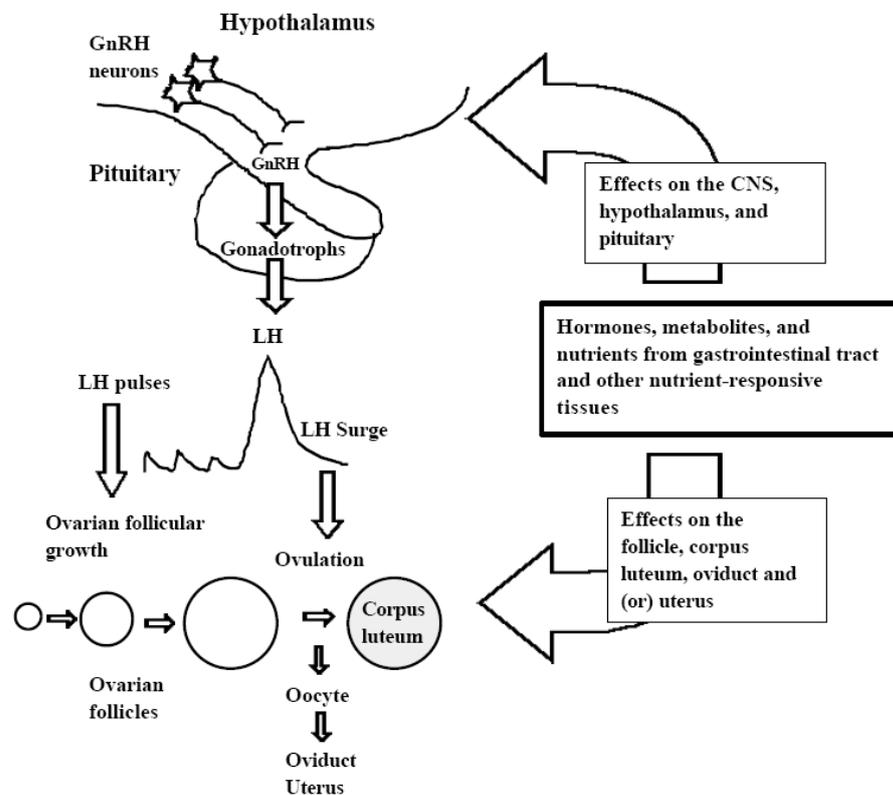
1992). However, this does not occur during mid lactation (McGuire et al., 1991). Serum IGF-I concentrations are also regulated by nutrition via IGF binding proteins (IGFBPs). Nutritional restriction decreases serum IGFBP-3 concentrations but increases those of IGFBP-1 and -2 (McCusker et al., 1991). IGFBPs are involved in the transport of IGF-I in the circulation and, hence the increase of serum IGFBP-1 and -2 may restrict IGF-I activity, whilst the reduction of IGFBP-3 may increase the availability of remaining IGFs to the body tissues (Zulu et al., 2002). Furthermore, in calves, restriction of food intake reduces binding of IGF-I in calves (Elasser et al., 1988).

The concentrations of plasma oestradiol were strongly correlated with plasma IGF-I concentrations (Beam and Butler, 1999). Oestradiol concentrations in follicular fluid and follicular development in heifers have been shown to be dependent on changes in concentrations of IGF-I and IGFBPs (Perks et al., 1999). Thus, the availability of insulin and serum IGF-I and the changing EB profile during the NEB period determines the ability of follicles to produce sufficient oestradiol for ovulation (Butler, 2000b).

***The effects through high protein intake.*** Regardless of energy intake, high milk production also depends on high protein intake. Various studies have found that high levels of dietary protein were associated with decreased reproductive performance (Butler, 1998; Westwood et al., 1998). In lactating cows, high dietary energy reduces plasma progesterone concentration (Nolan et al., 1998); and high dietary protein increases metabolic clearance rate of progesterone (Westwood et al., 1998). Both will result in reduced plasma progesterone concentrations. Low progesterone concentrations post breeding could reduce fertility (Larson et al., 1997).

High protein intake during the luteal phase could alter the pH and concentrations of other ions in uterine secretions (Butler, 2000b). High protein intake could also elevate blood ammonia and urea concentrations. An increase of plasma or milk urea nitrogen concentrations have been reported to be highly correlated with decreased fertility in cows (Butler, 1998; Westwood et al., 1998). Blood urea concentration could also be altered by diet and high urea concentration in cows was reported to reduce pregnancy rates as well (O'Callaghan et al., 1999). The elevated plasma urea concentrations caused by high protein intake are believed to raise uterine luminal pH via increased intra-uterine urea concentrations (Elrod and Butler, 1993) and, consequently, reduce pregnancy rate (Butler et al., 1996).

Plasma urea concentrations are highly correlated (0.88 to 0.98) with milk urea (Butler et al., 1996; Broderick and Clayton, 1997). The measurements of plasma and milk urea concentrations have been used as diagnostic tools to study reproductive performance of cows.



**Figure 2.1.** A model of the mechanisms through which nutrition affect the fertility of postpartum in dairy cattle. The gastrointestinal tract hormones, metabolites and nutrients, and nutrient-responsive tissues affect gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion through actions on the central nervous system (CNS) and hypothalamus. These hormones and metabolites may also directly affect ovarian function and the oocyte, oviduct and uterus. Postpartum fertility of the cow is determined by the combined effects of each axis (Lucy, 2003).

## MILK PROTEIN

Milk protein originates from ruminal ammonia nitrogen (N) through microbial protein synthesis in the rumen. The plasma-free amino acid pool is the major precursor of milk protein (Mepham, 1982). The efficiency of dietary N utilization for milk protein synthesis is about 19-20% in dairy cows (Tamminga, 1992). Milk protein percentage has been recognized as an important indicator of EB (Buckley et al., 2003; Patton et al., 2007). Fulkerson et al. (2001) showed that cows with low milk protein percentage suffered more severe and prolonged NEB compared to cows with higher milk protein percentage. Postpartum NEB is associated with increased milk fat percentage due to the mobilisation of adipose tissue and decreased milk protein percentage. This in turn is due to the shortage of glucose, which is used in the synthesis of milk protein in the udder (de Vries and Veerkamp, 2000). Milk protein percentage was positively associated with submission rate, first service pregnant rate and 21 days pregnant rate in the large field study carried by Morton (2000). Morton (2001) found that cows with a higher milk protein percentage in early lactation had substantially better reproductive performance. Positive relationships were identified between milk protein percentage, conception rate and the onset of luteal function (Patton et al., 2007). In the study carried by Fahey et al. (2003), the not-in-calf rate after a 21-week mating period was significantly higher for cows with the lowest quartiles for milk protein percentage compared to those with the highest.

Genetic selection for milk production has been reported to reduce the concentration of protein in milk (Kelm et al., 2000). A significant relationship between milk protein percentage and fertility performance has been reported in several previous studies. Phenotypically, milk protein percentage has been shown to be a good indicator of fertility performance in both Australia (Morton, 2000; Fahey et al., 2003) and New Zealand (Harris and Pryce, 2004). In year-round calving herds of New South Wales, the risk of subfertility in multiparous cows increased with lower milk protein percentage during the first 4 months of lactation or at the time of first service (Moss et al., 2002). Buckley et al. (2003) reported that, in Ireland, lower submission and conception rates were related to lower milk protein percentage around the time of artificial insemination. A Belgian study (Opsomer et al., 2000) showed that cows had an increased risk of

anoestrous if their average milk protein percentage during the first 3 months of lactation was low.

Conversely, genetic selection for milk protein percentage alone does not appear to have major effects on improving reproductive performance, and only 58% of the selection response in fertility performance was achieved (Harris and Pryce, 2004). Therefore, although the genetic correlation between milk protein percentage and fertility was reported to be positive in some studies (Haile-Mariam et al., 2003; Harris and Pryce, 2004), the use of protein percentage as a part of a breeding index has not proved to be efficient means for increasing fertility. However, phenotypically, milk protein percentage can be used as a good indicator of reproductive performance.

## HORMONES AND PHYSIOLOGY

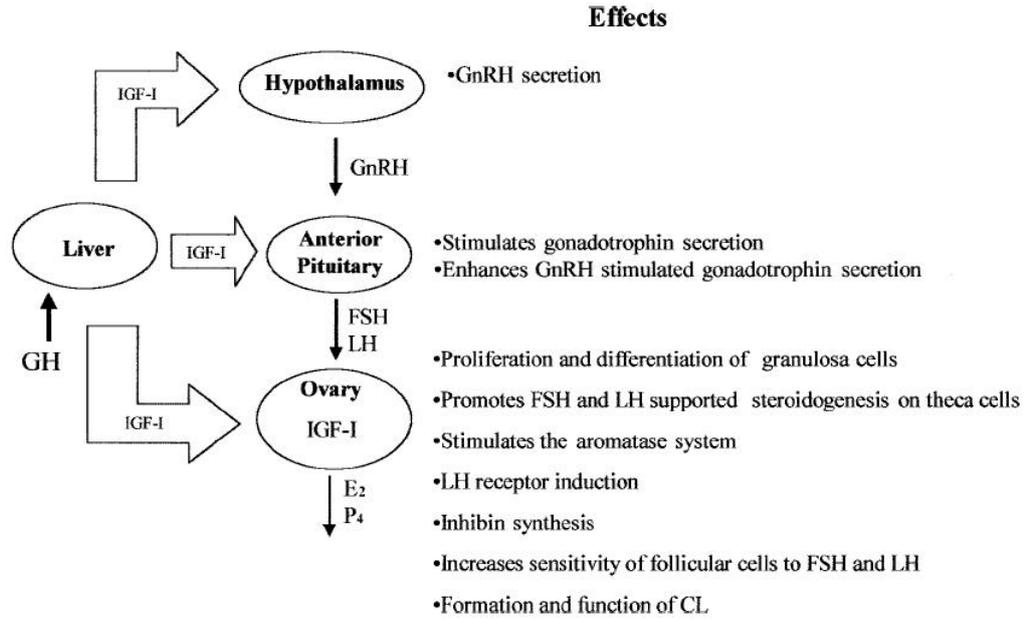
### Growth hormone and IGF-I

**Growth hormone.** Growth hormone (GH), or somatotrophin, is a pituitary hormone that directly or indirectly affects many aspects of growth, nutrient metabolism and reproduction (Lucy et al., 2001). Blood GH concentrations increase before calving. The increase of GH is thought to be related to some of the metabolic transition during early lactation. During lactation, higher GH concentrations results in more nutrients being partitioned towards the mammary gland rather than to other somatic processes (Taylor et al., 2004), through effects on adipose tissue and the liver. In adipose tissue, GH increases lipolysis, raising blood NEFA concentration (Lucy et al., 2001). In the liver, increased GH stimulate gluconeogenesis to meet the requirements of mammary lactose synthesis (Lucy et al., 2001). Increased milk production is thought to have been achieved, at least in past, by genetic selection for GH. Therefore, high overall plasma GH concentrations are present in high-yielding cows during early lactation. A study carried by Bauman (1999) showed that an increase of 10-15% in milk yield occurred in cows treated with recombinant bovine GH, because it stimulated various physiological processes that provided nutrients needed for milk synthesis (Etherton and Bauman, 1998). Nutritional changes can also influence somatotrophic axis, and affect ovarian and uterine systems (Taylor et al., 2004).

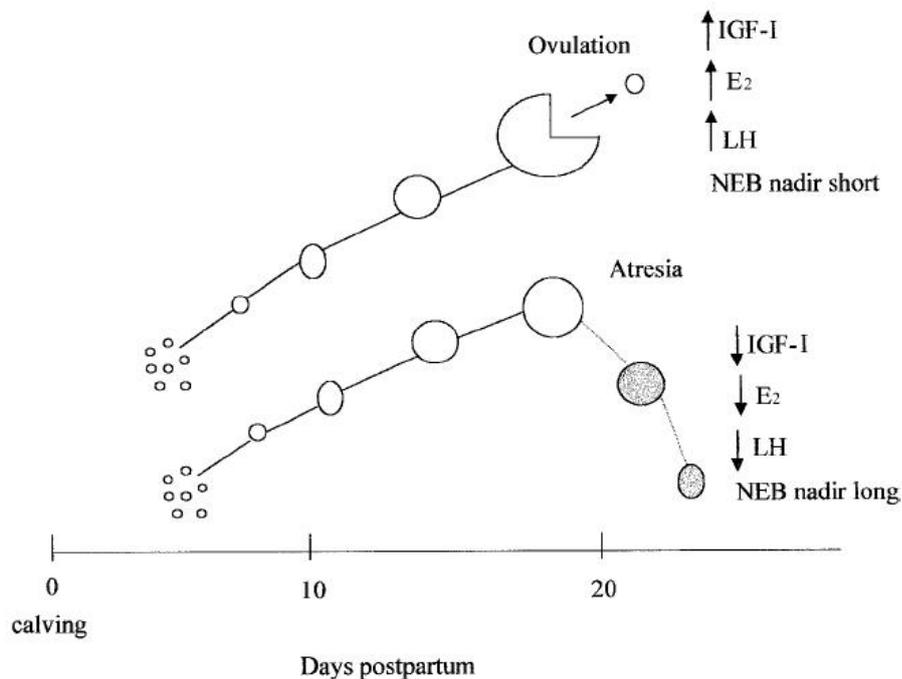
**IGF-I.** IGF-I plays a major role in reproductive function (Figure 2.2). Corpus luteum (CL) IGF-I concentrations are positively related to follicular development, ovulation, function and production of oestrogen and progesterone. Generally, low serum IGF-I leads to poor reproductive function (Zulu et al., 2002). IGF-I is produced mainly by the liver (Thissen et al., 1994). The release of IGF-I from the liver mediates many of the effects of GH on growth and reproduction (Jones and Clemmons, 1995), and controls the growth and function of cells and tissues throughout the body as well (Thissen et al., 1994). The ovary is another site of IGF-I gene expression and production. It also has receptors for IGF-I (Zulu et al., 2002). Postpartum ovarian function depends on LH pulsatility and blood IGF-I concentrations (Figure 2.3). Blood IGF-I affects ovarian function through follicular fluid IGF-I, which is derived from blood (Lucy, 2000). Intra-follicular concentrations of IGF-I increase in the final stages of follicular development

(Schams et al., 1999). IGF-I enhances GnRH, which stimulates LH release from the pituitary (Kanematsu et al., 1991). However, IGF- I is positively correlated to LH pulse frequency during postpartum NEB (Zurek et al., 1995). This suggests that LH release might be reduced by low IGF-I concentration during NEB. Moreover, IGF-I is required for normal CL formation and function (Chase et al., 1998). Studies of growth hormone receptors (GHR) deficient cattle, shows that low IGF-I concentrations reduced follicular growth and poor CL development (Chase et al., 1998). A sharp increase in IGF- I occurs around the time of the first ovulation postpartum. This increase of IGF- I may be associated with ovulation (Zulu et al., 2002). A greater plasma IGF-I concentration during the first 2 weeks of lactation was associated with a greater first service conception rate and shorter interval between calving and the onset of luteal activity (Patton et al., 2007). Anoestrous cows have lower plasma IGF-I concentrations than do cycling cows (Thatcher et al., 1996).

Plasma IGF-I concentrations are positively associated with circulating concentrations of glucose and insulin, BW and BCS, but are negatively associated with plasma concentrations of NEFAs and ketone bodies (Nishimura et al., 2000). Many previous studies agree that blood IGF-I concentrations are directly related to energy: basically, NEB is related to low blood IGF-I concentrations (Spicer et al., 1990; Ginger et al., 1997; Beam and Butler, 1998). In the period between calving and the start of breeding the IGF system undergoes dynamic changes (Zulu et al., 2002), since energy requirements exceed nutrient intake, so that blood IGF-I of the cow are decreased (Spicer et al., 1990). Conversely improving EB is associated with increased IGF-I concentrations in the blood (Breukink and Wensing, 1998). Serum IGF-I concentrations can also be influenced by variations in protein or energy intake (Breukink and Wensing, 1998; Zulu et al., 2002).



**Figure 2.2.** A summary of the effects of insulin-like growth factor I (IGF-I) on the reproductive axis in dairy cattle (Zulu et al., 2002).



**Figure 2.3.** A modelling description of the metabolic and reproductive changes postpartum in dairy cows (Zulu et al., 2002). ↓Decreased; ↑Increased.

***Somatotrophin and IGF-I in reproduction.*** GH is the dominant hormone that influences circulating concentrations of IGFs, especially IGF-I, which is completely depended on GH (Zulu et al., 2002). Cows have low blood GH concentration and high blood IGF-I concentration before parturition; at the time of calving and the onset of lactation, blood IGF-I concentrations decline while blood GH concentrations increase (Lucy, 2000). Blood concentrations of insulin and glucose also decline with the decrease of blood IGF-I. Several weeks into lactation, blood GH concentration gradually decrease while blood IGF-I and insulin concentrations gradually increase. Reduction in hepatic GH binding capacity and GHR number may be related to the fasting-induced decline in IGF-I (Zulu et al., 2002). Since nutritional deficits will lead to a decrease of GH concentrations, NEB may play an important role in these rapid metabolic changes during early lactation. On the other hand, IGF-I inhibits GH secretion through a negative feedback loop (Lucy et al., 2001), so the increase of GH concentrations after calving is also caused by the drop of IGF-I. Changes in blood IGF-I concentrations are directly link to changes of GHR expression or GHR second messenger systems in the liver (Lucy, 2000).

***Hypothalamic-pituitary-ovarian axis.*** During the period of energy deficit during early lactation, the hypothalamic-pituitary-ovarian axis recovers from the influence of pregnancy, and starts to undergo active changes which will lead to the reinitiation of oestrous cycles (Lucy et al., 1991a). During the first two or three weeks after parturition, the secretion of LH from the pituitary increases in most cows (Lucy et al., 1991a). The function of LH in the cow is to act on ovarian follicles to induce the waves of follicular growth, finally leads to the ovulation of a dominant follicle between 15 and 25 days after calving (Stevenson and Britt, 1979). The status of postpartum energy balance of the animal can affect this scenario, as an extreme energy deficit will impair the secretion of LH (Lucy et al., 1991b), therefore delaying the resumption of ovulation.

## **Reproductive physiology**

*The oestrous cycle.* High milk yielding cows have a high proportion of abnormal luteal phases. Longer luteal phases could delay breeding and make the prediction of oestrus harder. In a study comparing postpartum luteal function between moderate and high milk yielding cows, it was shown that high yielding cows have a three-fold higher incidence of anoestrous and also a higher chance of a prolonged luteal phase than moderate yielding cows (Opsomer et al., 1998). NEB, periparturient disorders and postpartum diseases can all result in prolonged luteal phases and delay the reestablishment of normal oestrous cycles.

A high blood oestradiol concentration is essential for triggering ovulation in cows. The secretion of oestradiol can be reduced by long luteal phases. In cows in NEB, the dominant follicle needs more time and a larger size to establish threshold blood oestradiol concentrations to trigger ovulation. This might be because the follicles in NEB cows were less estrogenic or might need a larger size to achieve greater estrogenic capacity. Furthermore, oestradiol metabolism might be greater during NEB. The consequence of all these effects is that a higher level of estrogen synthesis is necessary to achieve equivalent blood oestradiol concentrations. A comparison between lactating cows and heifers showed that larger preovulatory follicles with lower preovulatory blood oestradiol concentration were present in lactating cows (Sartori et al., 2000).

The fertility of oocytes is related to follicular size. Additional time and follicle size required for ovulation might create persistent follicles, in which premature activation of the oocytes occurs (Revah and Butler, 1996). Therefore, those oocytes were less fertile than oocytes from smaller growing follicles (Austin et al., 1999).

During early lactation, both natural nutrient partition and consequent NEB and loss of body weight have a negative effect on ovarian follicular growth and development. The mechanisms of how NEB inhibits follicular development are complex. In the review of Lucy (2000), two potential mediators were suggested, namely, LH and IGF-I. In cows in NEB, pulses of LH are decreased in frequency and plasma concentrations of IGF-I are also decreased. These two hormones act synergistically to promote follicular development. Therefore, the reduction of both of them is thought to delay the resumption of follicular growth during early lactation.

Based on all these foregoing studies, a possible relationship between milk protein percentage and fertility can be postulated. It is difficult to link milk protein percentage directly to the reproductive performance, but both of them are closely related to NEB. NEB causes a shortage of glucose, which is used in the synthesis of milk protein in the udder, therefore, NEB indirectly causes the reduction of protein percentage. Moreover, NEB causes a reduction of IGF-I, LH and oestradiol, all of which are critical for ovarian follicular development. Therefore, NEB delays the first postpartum ovulation and indirectly causes reduced fertility. NEB is the key part to connect the different physiological process together, not only the relationship between protein percentage and fertility, but also the relationship between milk production, BCS and fertility.

# Chapter 3

## MATERIALS AND METHODS

The data used in this study was obtained from a experiment carried out at Moorepark Research Centre in the Republic of Ireland over a 5-yr period (2001-2005) comparing three strains of Holstein-Friesian cows in three feeding systems (Horan et al. 2005; McCarthy et al., 2007)

### **Animals**

The three strains of Holstein-Friesian (HF) cows were compared: high production North American (HP), high durability North American (HD) and New Zealand (NZ). The HP strain represents the outcome of continuous aggressive selection for milk production; the HD strain represents a more balanced breeding policy, which also considers other traits like fertility when selecting milk production; the NZ strain represents the high NZ genetic cows (Horan et al. 2005; McCarthy et al., 2007).

To create the HP strain, the top 50% of HF cows in the Moorepark herds (based on pedigree index for milk production) were inseminated with semen from five North American Holstein-Friesian sires. These five sires were selected on predicted transmitting ability for milk, fat, and protein yields (Horan et al. 2005; McCarthy et al., 2007).

To create the HD strain, the bottom 50% of HF cows in the Moorepark herds (based on pedigree index for milk production) were inseminated with semen from five North American Holstein-Friesian sires. These five sires were selected base on combined pedigree indices for milk production, fertility and linear traits (Horan et al. 2005; McCarthy et al., 2007).

The NZ cows originated from embryos imported from NZ. The embryos were created by mating high genetic merit NZHF cows to five high genetic merit NZHF sires (Horan et al. 2005; McCarthy et al., 2007).

## Feeding systems

The three feeding systems were: a high grass allowance feeding system which is typical of spring calving herds in Ireland (MP, control); a higher concentrate system (HC) and a higher stocking rate system (HS) (Horan et al. 2005; McCarthy et al., 2007).

In the control group (MP), the overall stocking rate (SR) was 2.47 cows/ha with a nitrogen (N) fertilizer input of 290 kg N/ha (from early January to late September) and received 368 kg concentrate per cow during early lactation, the remainder of diet still came from grazed pasture. The HC group had similar SR and N input to the control group but with a higher concentrate input of 1,452 kg/cow. The HS group had similar concentrate (327 kg/cow) and N input to the control group but at a higher SR of 2.74 cows/ha (Horan et al. 2005; McCarthy et al., 2007).

The MP and HC feeding systems were designed to allow each strain to express its potential within each feed system largely unrestricted by limitations in feed supply. The HS feeding system was aim to reduce feed allowance by increasing SR (Horan et al. 2005; McCarthy et al., 2007).

A total of 99, 117, 117, 124 and 126 animals were used in Year 1, 2, 3, 4 and 5, respectively, divided between strains and feeding systems (Table 3.1).

**Table 3.1.** The number of dairy cow records included in the strain/feeding system trial.

Group	Strain of Holstein-Friesian <sup>1</sup>			Feed system <sup>2</sup>		
	HP	HD	NZ	MP	HS	HC
No. of animals						
Year 1	33	33	33	33	33	33
Year 2	39	39	39	39	39	39
Year 3	39	39	39	39	39	39
Year 4	40	42	42	40	42	42
Year 5	36	48	42	42	42	42

<sup>1</sup>HP = High production; HD = high durability; NZ = New Zealand.

<sup>2</sup>MP = High grass feed system; HS = high stocking rate feed system; HC = high concentration feed system.

## Animal measurements

Cows were milked twice daily in a 14-unit highline milking parlour. Milk yield was measured at each milking for each cow using electronic milk meters (McCarthy et al., 2007). The concentrations of fat, protein, and lactose in milk were determined using a Milkoscan 203 (Foss Electric DK-3400, Hillerød, Denmark) from successive morning and evening samples collected once weekly (Horan et al., 2005). The BCS was recorded every 3 weeks during lactation on a scale of 0 to 5 (0 = emaciated, 5 = extremely fat) with increments of 0.25. Fertility parameters included the interval from calving to first service, the interval from calving to conception, number of services per conception, pregnant to first service, pregnant to second service, pregnant to first and second services, submission within 3 weeks of the start of the breeding season and overall pregnancy rate.

## Statistical Analysis

Lactation curves for daily yields of milk, fat, protein and lactose and BCS were modelled using an orthogonal polynomial of order 2:

$$y_t = \alpha_0 + \alpha_1 p_t + \alpha_2 p_t^2 + e$$

where  $y$  is the daily yield of milk, fat, protein or lactose at day  $t$ . Legendre polynomials are conventionally defined in the range  $-1 \leq t \leq +1$  and are orthogonal within this range; days in milk were standardized to lie between  $-1$  and  $+1$  before evaluating the polynomials. Functions of  $p_t$  of Legendre polynomials were calculated as  $P_0(t) = 1$ ,  $P_1(t) = x_t$ ,  $P_2(t) = 1/2(3x_t^2 - 1)$ , with  $x_t = (2*t - (305 + 1)) / (305 - 1)$ , and  $\alpha_0$ ,  $\alpha_1$  and  $\alpha_2$  are regression coefficients.

Estimates of  $\alpha_0$ ,  $\alpha_1$  and  $\alpha_2$  for each lactation of each trait were obtained using PROC GLM of SAS based on the herd test records (SAS Institute, 2002). With these  $\alpha_0$ ,  $\alpha_1$  and  $\alpha_2$  values, the lactation curves for milk, fat, protein and lactose and BCS were modelled. Total lactation yields of milk, fat, protein and lactose were obtained as the sum of the yield of each lactating day. Accumulated yields were calculated at different periods postpartum: first 60, first 120, first 180 and actual lactation length. Based on these yields, protein percentages and fat to protein ratio during different periods postpartum were calculated. Different measures of BCS (BCS at calving, BCS on Day 60 and BCS

change from calving to Day 60) for each cow on each day of lactation were obtained using the lactation curve for BCS.

Pearson correlations among production, fertility and BCS traits were estimated. Logistic regression analysis (Hosmer and Lemeshow, 1989) was carried out in PROC GENOMD (SAS Institute, 2002) to investigate the associations between milk protein percentage and the binary fertility traits. The logistic regression model considered the fixed effect of genotype, feeding system, the interaction between genotype and feeding system, lactation number and year and the random effect of year.

The prediction of probability of reproductive performance was using the following model:

$$P = \frac{1}{1 + e^{-(\beta_0 + \beta_1 x_1 + \beta_2 x_2 \dots \beta_k x_k)}}$$

where  $P$  is the probability of reproductive performance, in the range  $0 \leq P \leq +1$ .  $\beta_0$  is a constant and  $\beta_k$  are coefficients of the predictor variables.

Another logistic regression analysis (Hosmer and Lemeshow, 1989) was carried out in PROC GENOMD (SAS Institute, 2002) to investigate the associations between BCS and production traits considered as independent variables and the binary fertility traits considered as dependent variables following a binomial distribution. The prediction of probability of reproductive performance was using the same model as shown above. Because of a large correlation between BCS traits and production traits, principal components analysis was applied to represent the BCS and production traits.

Principal component analysis is a method of transforming the original independent variables into new, uncorrelated variables (Lafi and Kaneene, 1992). The new, uncorrelated variables are called principal components. Each principal component is a linear combination of all the original independent variables.

The purpose of using principal component analysis is to reduce the dimensionality without losing much of the information. Since principal components labelled according to the size of their variance, the first principal component explains the largest amount of variation among the variables while the last principal component explains the least. Normally, the first few principal components are the most informative ones. Therefore,

the reduction of dimensionality can be achieved by choosing only the first few principal components to be used in the regression analysis (Lafi and Kaneene, 1992).

Let  $X$  be the original data set, and  $Y$  is a re-representation of that data set, which is related by a linear transformation  $P$ .

$$PX = Y$$

If  $p_i$  are the rows of  $P$ ,  $x_i$  are the columns of  $X$  (or individual  $\vec{X}$ ), and  $y_i$  are the columns of  $Y$ , then

$$PX = \begin{bmatrix} p_1 \\ \vdots \\ p_m \end{bmatrix} [x_1 \quad \dots \quad x_n]$$

$$Y = \begin{bmatrix} p_1 \cdot x_1 & \dots & p_1 \cdot x_n \\ \vdots & \ddots & \vdots \\ p_m \cdot x_1 & \dots & p_m \cdot x_n \end{bmatrix}$$

$$y_i = \begin{bmatrix} p_1 \cdot x_i \\ \vdots \\ p_m \cdot x_i \end{bmatrix}$$

In present study, principal components  $y_1, y_2, \dots, y_n$  which are uncorrelated with each other and account for decreasing proportions of the total variance of the original variables were defined as:

$$y_1 = p_{11}x_1 + p_{12}x_2 + \dots + p_{1n}x_n$$

$$y_2 = p_{21}x_1 + p_{22}x_2 + \dots + p_{2n}x_n$$

.....

$$y_n = p_{n1}x_1 + p_{n2}x_2 + \dots + p_{nn}x_n$$

with coefficients  $p$  was the respective eigenvalues, so that  $y_1, y_2, \dots, y_n$  account for decreasing proportions of the total variance of the original variables,  $x_1, x_2, \dots, x_n$ .

Principal component for BCS traits was the re-representation of BCS at calving and BCS change; principal component for milk production was the re-representation of accumulated milk, fat, protein and lactose yield; principal component for protein percentage was the re-representation of milk protein percentage. For each principal component, the  $p$  values were calculated using PROC PRINCOMP SAS software (SAS

Institute, 2002). Fertility performance was then analysed with a logistic regression model that considered the fixed effect of year and lactation number as class effects and the principal component for milk production, principal component for BCS and principal component for protein percentage as covariables and the random effect of cow to account for repeated measures on the same cow.

Lactations were divided in the two groups according to protein percentage over the whole lactation, low or high. The classification of lactation into each of these groups was based on the average protein percentage for each combination of strain and feeding system. Overall rate was then analysed with a logistic regression model using the GENMOD procedure of SAS (2002). The model included the fixed effect of P% group, strain, feeding system, the interaction of strain and feeding system, lactation number and year.

## **Chapter 4**

### **RESULTS**

#### **DESCRIPTIVE STATISTICS**

Descriptive statistics of milk production and fertility traits for each of the strains and feeding systems are given in Table 4.1. Amongst the different strains of HF cows, high production (HP) cows had the highest milk yield with New Zealand (NZ) cows had the lowest. However, the milk fat and protein percentage was greatest in NZ cows. High durability (HD) cows had the highest 21-day submission rate, while NZ cows had the highest first service and overall pregnant rates, as well as fewer services to achieve a success pregnancy.

Cows on the high concentrate (HC) feeding system produced more milk than cows on the other two feeding systems. As for reproductive performance, cows in the HC feeding system showed relatively high 21-day submission and first service conception rates, but similar overall pregnancy rates were found in both HC and Moorepark (MP) feeding system. Furthermore, cows in the MP feeding system needed the least number of services per pregnancy. Cows on the high stocking rate (HS) feeding system seemed to showed the worst reproductive performance.

**Table 4.1.** Mean ( $\pm$  SD) productive and fertility traits of three strains of Holstein-Friesians cows under three feeding systems.

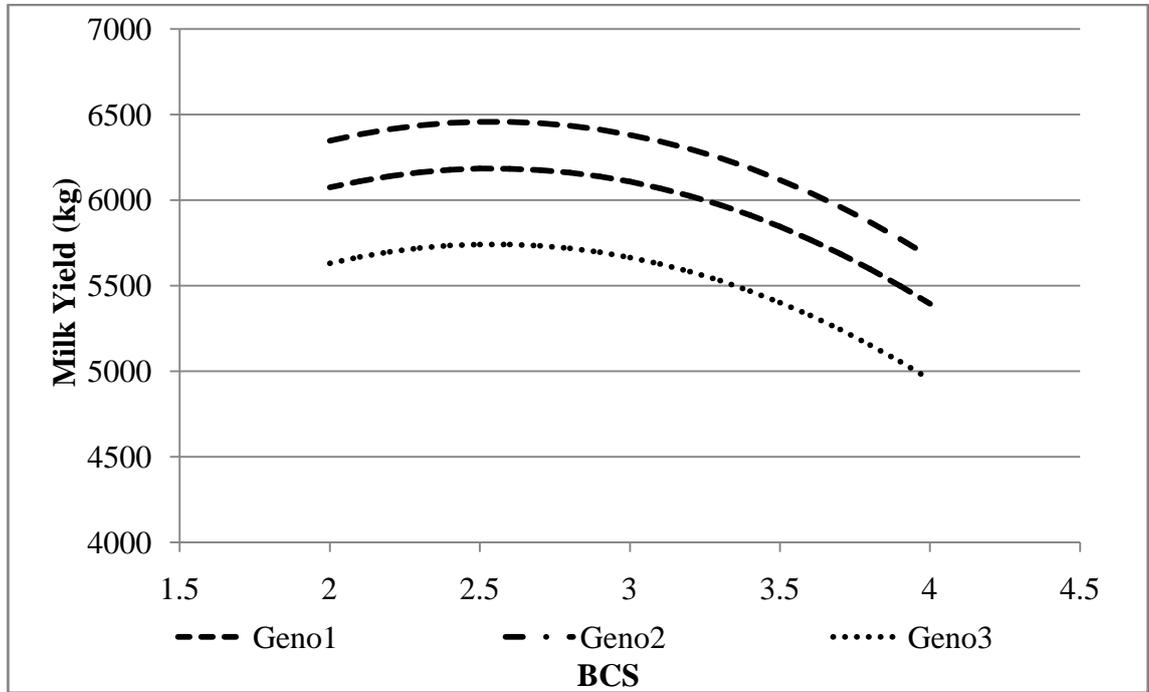
Effect	HP <sup>1</sup>			HD <sup>2</sup>			NZ <sup>3</sup>		
	MP <sup>4</sup>	HC <sup>5</sup>	HS <sup>6</sup>	MP	HC	HS	MP	HC	HS
Number of lactations	61	63	63	67	67	67	65	65	65
Milk production									
Milk, kg/cow	6346(1135)	7487(1135)	6420(1006)	6307(1024)	7202(1114)	6188(1019)	5881(1034)	6306(1165)	5601(906)
Fat, g/kg	41.1(4.3)	39.0(3.4)	39.6(4.3)	39.5(2.7)	39.1(3.8)	40.8(3.9)	44.0(3.0)	44.2(3.7)	44.2(3.3)
Protein, g/kg	34.6(1.7)	34.4(1.8)	34.0(1.8)	34.4(1.4)	35.2(2.0)	34.7(1.9)	35.9(1.6)	36.1(1.7)	35.5(2.2)
Lactose, g/kg	46.7(1.0)	47.6(0.9)	46.8(1.2)	46.9(1.0)	47.2(1.2)	46.9(1.3)	47.2(1.1)	47.8(1.0)	47.0(1.2)
Reproduction									
21-day submission rate, %	77(42)	78(42)	74(44)	94(24)	91(29)	77(41)	86(35)	91(29)	84(36)
1 <sup>st</sup> service conception rate, %	46(50)	49(50)	46(50)	58(49)	51(50)	48(50)	57(50)	70(46)	53(50)
Overall pregnancy rate, %	70(46)	76(43)	78(42)	95(21)	86(34)	74(44)	91(29)	94(24)	89(31)
Total services per cow	2.0(1.2)	2.0(1.1)	1.8(1.0)	1.6(0.9)	1.8(1.0)	1.9(1.1)	1.7(0.9)	1.5(1.0)	1.7(0.9)

<sup>1</sup>HP = high production; <sup>2</sup>HD = high durability; <sup>3</sup> NZ = New Zealand;

<sup>4</sup>MP = Moorepark feeding system; <sup>5</sup>HC = high concentrate feeding system; <sup>6</sup>HS = high stocking rate feeding system.

## ASSOCIATIONS AMONG TRAITS

### The relationship between BCS and milk production



**Figure 4.1.** Relationship between milk yield and body condition score (BCS) at calving for three different genotype cows under the same feeding system and same lactation. Geno1 represented the high durability North American Holstein-Friesian cows; Geno2 represented the high production North American Holstein-Friesian cows; Geno3 represented the New Zealand Holstein-Friesian cows.

The association between BCS at calving and milk production was non-linear. Milk production was positively associated with BCS at calving up to a BCS of about 2.5 to 2.75 units after which the association was negative. Similar trends were observed for BCS on Day 60.

## **The relationship between BCS and reproductive performance**

Table 4.2 shows estimates of Pearson correlations coefficients between measures of BCS and fertility traits. There were some significant correlations between measures of BCS and fertility traits.

Increased BCS at calving was associated with a reduced number of services ( $P < 0.05$ ), increased pregnancy rate to second service ( $P < 0.05$ ), increased pregnancy rate to first and second services ( $P < 0.01$ ), increased submission rate within 3 weeks after the start of breeding season ( $P < 0.001$ ), and increased overall pregnancy rate ( $P < 0.001$ ).

An increase of BCS on Day 60 was associated with an increased interval between calving and first service ( $P < 0.01$ ), increased pregnancy rate to first service ( $P < 0.05$ ), pregnancy rate to second service ( $P < 0.05$ ), pregnancy rate to first and second services ( $P < 0.001$ ), submission rate within 3 weeks after the start of breeding season ( $P < 0.001$ ), and overall pregnancy rate ( $P < 0.001$ ). Less number of service ( $P < 0.05$ ) were required for achieving a successful pregnancy as BCS<sub>60</sub> increased.

The greater the loss in BCS in early lactation, the longer the interval between calving and first service ( $P < 0.001$ ) and between calving and conception ( $P < 0.001$ ). There were no other significant correlations.

**Table 4.2.** Correlation coefficients between measures of body condition score and measures of fertility traits.

Trait	BCS_1	BCS_60	BCS_CH	CSI	CCI	NO_SERV	PREG1	PREG2	PREG1_2	SERV_3wk	PREG
BCS_1 <sup>1</sup>	1										
BCS_60 <sup>2</sup>	0.82***	1									
BCS_CH <sup>3</sup>	-0.56***	0.01	1								
CSI <sup>4</sup>	0.00	0.12**	0.19***	1							
CCI <sup>5</sup>	-0.03	0.08	0.17***	0.62***	1						
NO_SERV <sup>6</sup>	-0.10*	-0.09*	0.04	-0.20***	0.58***	1					
PREG1 <sup>7</sup>	0.08	0.09*	-0.00	0.19***	-0.48***	-0.81***	1				
PREG2 <sup>8</sup>	0.12*	0.14*	-0.01	0.10	-0.37***	-0.70***	.	1			
PREG1_2 <sup>9</sup>	0.13**	0.14***	-0.02	0.18***	-0.48***	-0.82***	0.63***	0.98***	1		
SERV_3wk <sup>10</sup>	0.15***	0.22***	0.07	0.06	0.10*	0.06	0.04	0.00	0.03	1	
PREG <sup>11</sup>	0.14***	0.19***	0.03	0.06	-0.19***	-0.46***	0.44***	0.41***	0.52***	0.18***	1

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

<sup>1</sup>BCS\_1 = body condition score at calving; <sup>2</sup>BCS\_60 = body condition score on Day 60;

<sup>3</sup>BCS\_CH = body condition score change (BCS\_60 – BCS\_1); <sup>4</sup>CSI = calving to submission interval; <sup>5</sup>CCI = calving to conception interval;

<sup>6</sup>NO\_SERV = number of services per conception; <sup>7</sup>PREG1 = pregnant to first service; <sup>8</sup>PREG2 = pregnant to second service;

<sup>9</sup>PREG1\_2 = pregnant to first and second services; <sup>10</sup>SERV\_3wk = submission within 3 weeks; <sup>11</sup>PREG = overall pregnancy rate.

### **Relationship between milk production and reproductive performance**

Estimates of correlation coefficients between production and fertility traits are shown in Table 4.3. Total milk, fat, protein and lactose yields were not correlated with calving to submission interval. However, milk, protein and lactose yields were positively correlated with calving to conception interval ( $P < 0.05$ ). Significant correlations were observed between yields in early lactation and calving to submission interval or calving to conception interval. The greater the yield of milk, fat, protein and lactose in the first 60 days of lactation, the shorter the calving to submission interval or calving to conception interval ( $P < 0.001$ ). Number of services per conception was positively correlated with milk ( $P < 0.01$ ) and lactose yields ( $P < 0.05$ ). Total milk, fat, protein and lactose yields were not correlated with first service pregnancy rate, but were negatively correlated with second service pregnancy rate ( $P < 0.05$ ). Negative correlations existed between both first and second service pregnancy rate and total milk ( $P < 0.01$ ), protein ( $P < 0.05$ ), fat ( $P < 0.01$ ) and lactose ( $P < 0.05$ ) yields, first 60 days milk ( $P < 0.05$ ), protein ( $P < 0.05$ ) and fat ( $P < 0.05$ ) yields, Overall pregnancy rate was negatively correlated with first 60 days milk ( $P < 0.01$ ), protein ( $P < 0.01$ ), fat ( $P < 0.01$ ) and lactose ( $P < 0.01$ ) yields, total milk ( $P < 0.01$ ), protein ( $P < 0.05$ ), fat ( $P < 0.05$ ) and lactose ( $P < 0.01$ ) yields. Reduced first three weeks submission rate was associated with increased first 60 days milk, protein, fat and lactose yields ( $P < 0.001$ ).

**Table 4.3.** Correlation coefficients between production traits and measures of fertility.

Trait	MY	MY_60	PY	PY_60	FY	FY_60	LY	LY_60	P%	P%_60	RATIO_FP	CSI	CCI	NO_SERV	PREG1	PREG2	PREG1_2	SERV_3wk	PREG	
MY <sup>1</sup>	1																			
MY_60 <sup>2</sup>	0.82***	1																		
PY <sup>3</sup>	0.95***	0.78***	1																	
PY_60 <sup>4</sup>	0.77***	0.97***	0.79***	1																
FY <sup>5</sup>	0.84***	0.76***	0.89***	0.76***	1															
FY_60 <sup>6</sup>	0.60***	0.86***	0.62***	0.86***	0.78***	1														
LY <sup>7</sup>	0.99***	0.78***	0.95***	0.73***	0.84***	0.57***	1													
LY_60 <sup>8</sup>	0.82***	0.99***	0.79***	0.96***	0.76***	0.85***	0.79***	1												
P% <sup>9</sup>	-0.24***	-0.18***	0.05	0.01	0.08*	-0.00	-0.23***	-0.17***	1											
P%_60 <sup>10</sup>	-0.10*	0.01	0.13**	0.26***	0.11**	0.14***	-0.09*	0.02	0.75***	1										
RATIO_FP <sup>11</sup>	-0.29***	-0.09*	-0.28***	-0.10*	0.19***	0.33***	-0.28***	-0.08	-0.04	0.06	1									
CSI <sup>12</sup>	0.04	-0.21***	0.08	-0.23***	0.06	-0.22***	0.05	-0.21***	0.11**	-0.13**	-0.06	1								
CCI <sup>13</sup>	0.09*	-0.15***	0.09*	-0.18***	0.07	-0.16***	0.09*	-0.16***	-0.01	-0.17***	-0.06	0.62***	1							
NO_SERV <sup>14</sup>	0.11**	0.07	0.08	0.05	0.08	0.05	0.10*	0.06	-0.12**	-0.07	0.00	-0.20***	0.58***	1						
PREG1 <sup>15</sup>	-0.06	-0.04	-0.02	-0.02	-0.03	-0.05	-0.04	-0.03	0.15***	0.09*	-0.03	0.19***	-0.48***	-0.81***	1					
PREG2 <sup>16</sup>	-0.13*	-0.10	-0.14*	-0.10	-0.13*	-0.07	-0.13*	-0.09	-0.04	-0.03	0.05	0.10	-0.37***	-0.70***	.	1				
PREG1_2 <sup>17</sup>	-0.12**	-0.09*	-0.10*	-0.08*	-0.11**	-0.09*	-0.10*	-0.08	0.06	0.03	-0.00	0.18***	-0.48***	-0.82***	0.63***	0.98***	1			
SERV_3wk <sup>18</sup>	-0.03	-0.18***	-0.00	-0.17***	-0.03	-0.19***	-0.01	-0.18***	0.11**	0.02	-0.08*	0.06	0.10*	0.06	0.04	0.00	0.03	1		
PREG <sup>19</sup>	-0.13**	-0.12**	-0.10*	-0.11**	-0.10*	-0.12**	-0.12**	-0.12**	0.11**	0.03	-0.01	0.06	-0.19***	-0.46***	0.44***	0.41***	0.52***	0.18***	1	

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

<sup>1</sup>MY = total lactation milk yield; <sup>2</sup>MY\_60 = 60 days postpartum milk yield; <sup>3</sup>PY = total lactation protein yield; <sup>4</sup>PY\_60 = 60 days postpartum protein yield; <sup>5</sup>FY = total lactation fat yield; <sup>6</sup>FY\_60 = 60 days postpartum fat yield;

<sup>7</sup>LY = total lactation lactose yield; <sup>8</sup>LY\_60 = 60 days postpartum lactose yield; <sup>9</sup>P% = whole lactation protein percentage; <sup>10</sup>P%\_60 = 60 days postpartum protein percentage; <sup>11</sup>RATIO\_FP = total lactation fat to protein ratio;

<sup>12</sup>CSI = calving to submission interval; <sup>13</sup>CCI = calving to conception interval; <sup>14</sup>NO\_SERV = number of services per conception; <sup>15</sup>PREG1 = pregnant to first service; <sup>16</sup>PREG2 = pregnant to second service;

<sup>17</sup>PREG1\_1 = pregnant to first and second services; <sup>18</sup>SERV\_3wk = submission within 3 weeks; <sup>19</sup>PREG = overall pregnancy rate.

### **Relationship between milk protein percentage and reproductive performance**

Some significant correlations existed between protein percentage and fertility (Table 4.4). First 60 days protein percentage was negatively correlated with calving to submission interval ( $P < 0.01$ ) and calving to conception interval ( $P < 0.001$ ). A slightly positive correlation was evident between first 60 days protein percentage and first service pregnancy rate ( $P < 0.05$ ). Significant negative correlations were found between first 120 days protein percentage and calving to conception interval ( $P < 0.05$ ), and between number of services per conception and first 180 days protein percentage ( $P < 0.05$ ). Both first 120 days protein percentage and first 180 days protein percentage were positively correlated with first service pregnancy rate ( $P < 0.05$ ). No other correlations between first 120 days protein percentage or first 180 days protein percentage and fertility traits were significant. High overall protein percentage was associated with longer calving to submission interval ( $P < 0.01$ ), high first service pregnancy rate ( $P < 0.001$ ), high first three weeks submission rate ( $P < 0.01$ ) and high overall pregnancy rate ( $P < 0.01$ ), but was negatively correlated with number of services per conception ( $P < 0.01$ ). Overall, higher protein percentage was associated improved fertility performance of the cow, as shown by the higher submission rate within the first 3 weeks after the start of breeding season, fewer services per conception, and higher pregnancy rate.

**Table 4.4.** Correlation coefficients between milk protein percentage and measures of fertility.

Trait	P%_60	P%_120	P%_180	P%	CSI	CCI	NO_SERV	PREG1	PREG2	PREG1_2	SERV_3wk	PREG
P%_60 <sup>1</sup>	1											
P%_120 <sup>2</sup>	0.96	1										
P%_180 <sup>3</sup>	0.87	0.98	1									
P% <sup>4</sup>	0.75	0.90	0.97	1								
CSI <sup>5</sup>	-0.13**	-0.04	0.02	0.11**	1							
CCI <sup>6</sup>	-0.17***	-0.10*	-0.05	-0.01	0.62***	1						
NO_SERV <sup>7</sup>	-0.07	-0.08	-0.09*	-0.12**	-0.20***	0.58***	1					
PREG1 <sup>8</sup>	0.09*	0.09*	0.10*	0.15***	0.19***	-0.48***	-0.81***	1				
PREG2 <sup>9</sup>	-0.03	-0.04	-0.04	-0.04	0.10	-0.37***	-0.70***	.	1			
PREG1_2 <sup>10</sup>	0.03	0.03	0.03	0.06	0.18***	-0.48***	-0.82***	0.63***	0.98***	1		
SERV_3wk <sup>11</sup>	0.02	0.04	0.06	0.11**	0.06	0.10*	0.06	0.04	0.00	0.03	1	
PREG <sup>12</sup>	0.03	0.06	0.08*	0.11**	0.06	-0.19***	-0.46***	0.44***	0.41***	0.52***	0.18***	1

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

<sup>1</sup>P%\_60 = 60 days postpartum protein percentage; <sup>2</sup>P%\_120 = 120 days postpartum protein percentage;

<sup>3</sup>P%\_180 = 180 days postpartum protein percentage; <sup>4</sup>P% = whole lactation protein percentage; <sup>5</sup>CSI = calving to submission interval;

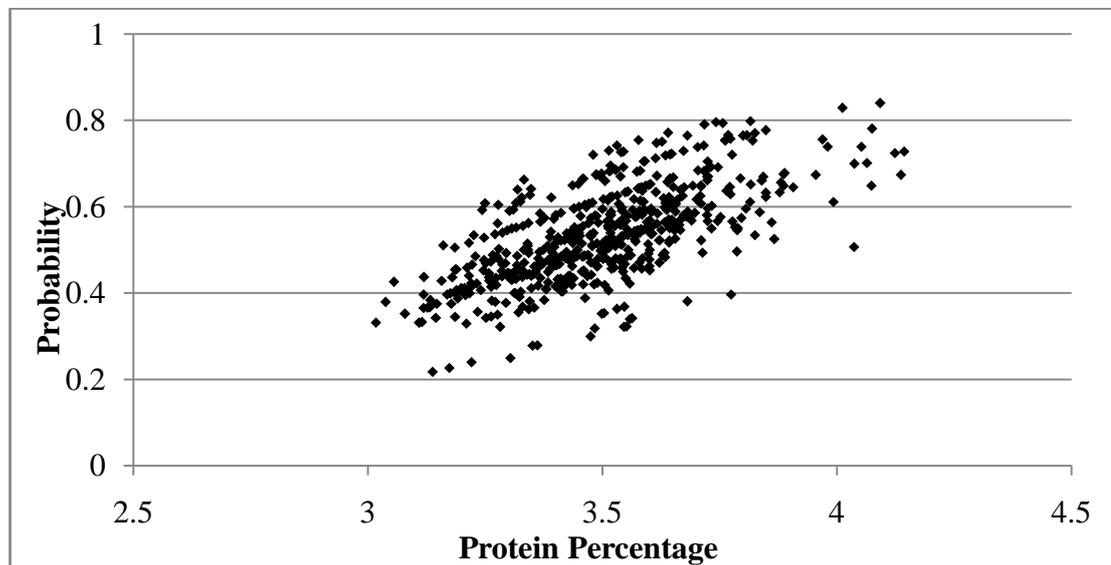
<sup>6</sup>CCI = calving to conception interval; <sup>7</sup>NO\_SERV = number of services per conception; <sup>8</sup>PREG1 = pregnant to first service;

<sup>9</sup>PREG2 = pregnant to second service; <sup>10</sup>PREG1\_2 = pregnant to first and second services; <sup>11</sup>SERV\_3wk = submission within 3 weeks;

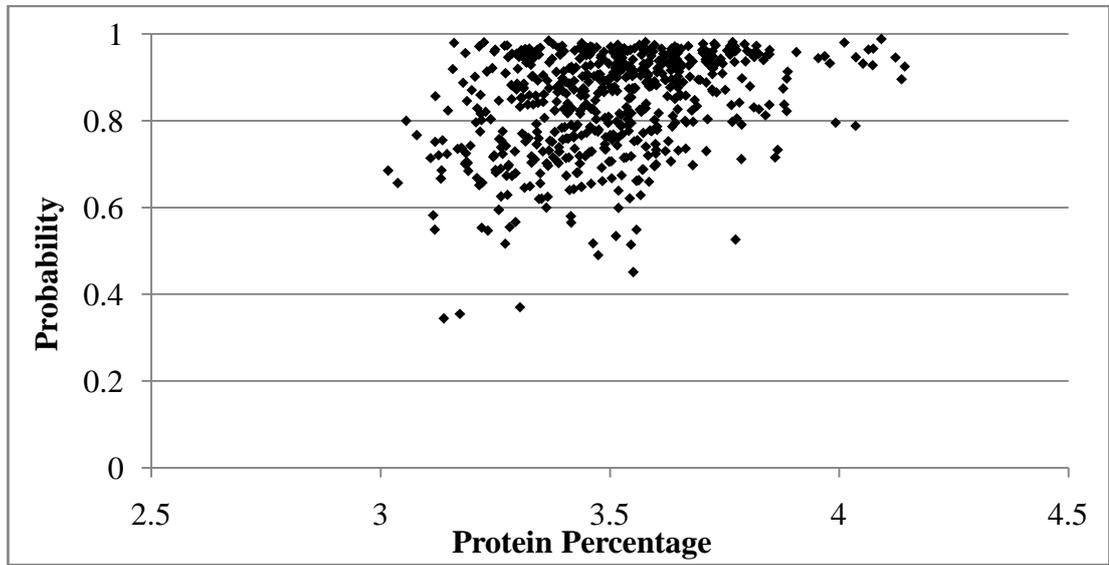
<sup>12</sup>PREG = overall pregnancy rate.

## LOGISTIC REGRESSION ANALYSIS

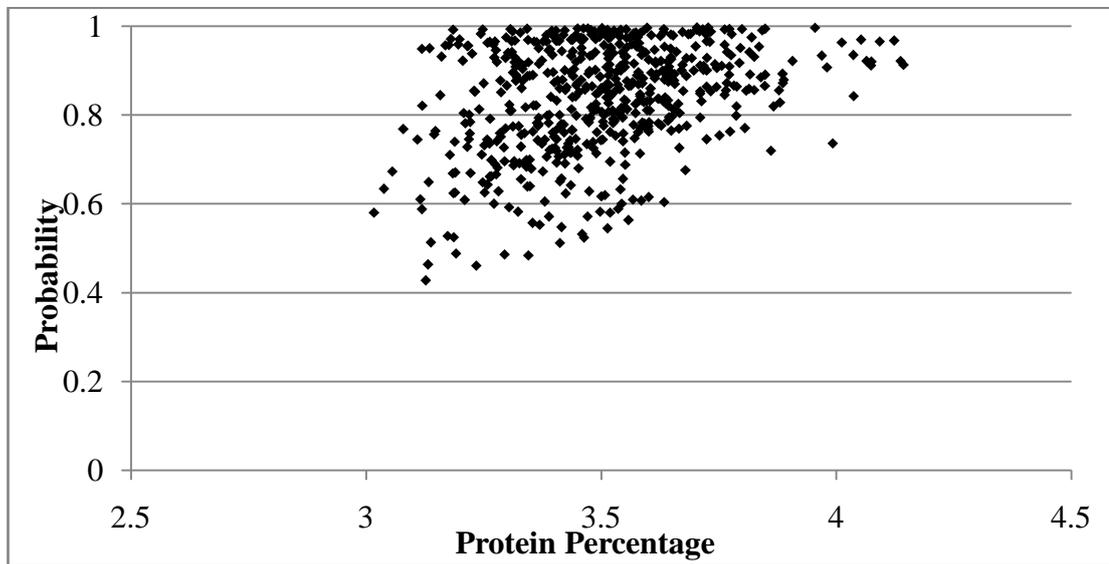
There was a positive relationship between milk protein percentage and fertility performance of the cow. Cows with high milk protein percentage during the whole lactation period had a higher probability of becoming pregnant to the first service after the start of the breeding season ( $P < 0.05$ ) (Figure 4.2) and a higher probability of becoming pregnant during the whole breeding season ( $P = 0.07$ ) (Figure 4.3). The probability of 3-week submission rate also increased ( $P = 0.09$ ) with milk protein percentage over the whole lactation (Figure 4.4).



**Figure 4.2.** Logistic regression of the probability of a cow becoming pregnant during the first service after the start of the breeding season on milk protein percentage of the whole lactation.



**Figure 4.3.** Logistic regression of the probability of a cow becoming pregnant during the whole breeding season on milk protein percentage of the whole lactation.



**Figure 4.4.** Logistic regression of the probability of a cow receiving first service within the first three weeks after the start of the breeding season on milk protein percentage of the whole lactation.

## LOGISTIC REGRESSION WITH PRINCIPAL COMPONENT ANALYSIS

The regression of the logit of the probability of the fertility performance on the principal components was weighted by the eigenvalues and eigenvectors. The primary eigenvalues and eigenvectors obtained from principal components analysis that were used in logistic regression are showed in Table 4.5 and Table 4.6 below. Table 4.5 was obtained by considering production and BCS traits and protein percentage only during early lactation (day 1 to day 60), while Table 4.6 considering the same traits but for the whole lactation period.

**Table 4.5.** Eigenvalues and eigenvectors of the three most important principal components obtained considering production and BCS traits and protein percentage in early lactation.

	<b>Principal Component</b>		
	1	2	3
Eigenvalue	3.89	1.53	1.00
Proportion of variation explained	0.55	0.22	0.14

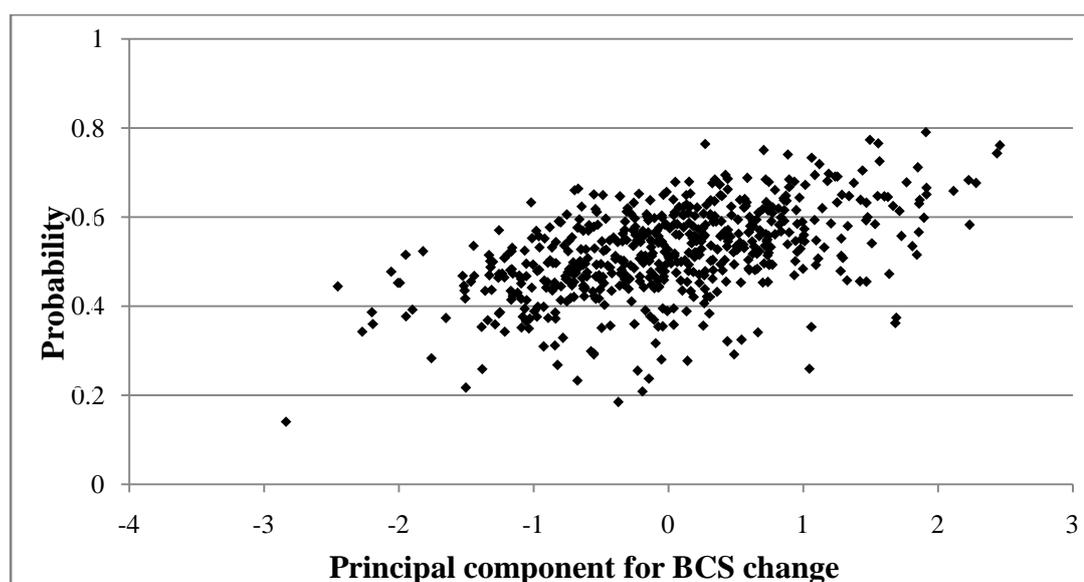
<b>Variable</b>	<b>Eigenvectors</b>		
Milk yield	0.4953	0.1112	-0.1091
Fat yield	0.4551	0.1744	0.1005
Protein yield	0.4989	0.0353	0.1193
Lactose yield	0.4938	0.1111	-0.0945
BCS at calving	-0.1987	0.5879	0.3671
BCS change	0.0909	-0.7118	-0.0811
Protein %	0.0840	-0.3023	0.9019

**Table 4.6.** Eigenvalues and eigenvectors of the three most important principal components obtained considering production and BCS traits and protein percentage during the whole lactation.

	Principal Component		
	1	2	3
Eigenvalue	3.82	1.27	0.74
Proportion of variation explained	0.64	0.21	0.12

Variable	Eigenvectors		
Milk yield	0.5013	-0.1485	-0.0348
Fat yield	0.4673	0.0956	0.2548
Protein yield	0.5018	0.0812	0.1304
Lactose yield	0.5005	-0.1364	-0.0293
Protein %	-0.0469	0.7764	0.5382
BCS change	0.1606	0.5837	-0.7914

#### BCS and reproductive performance



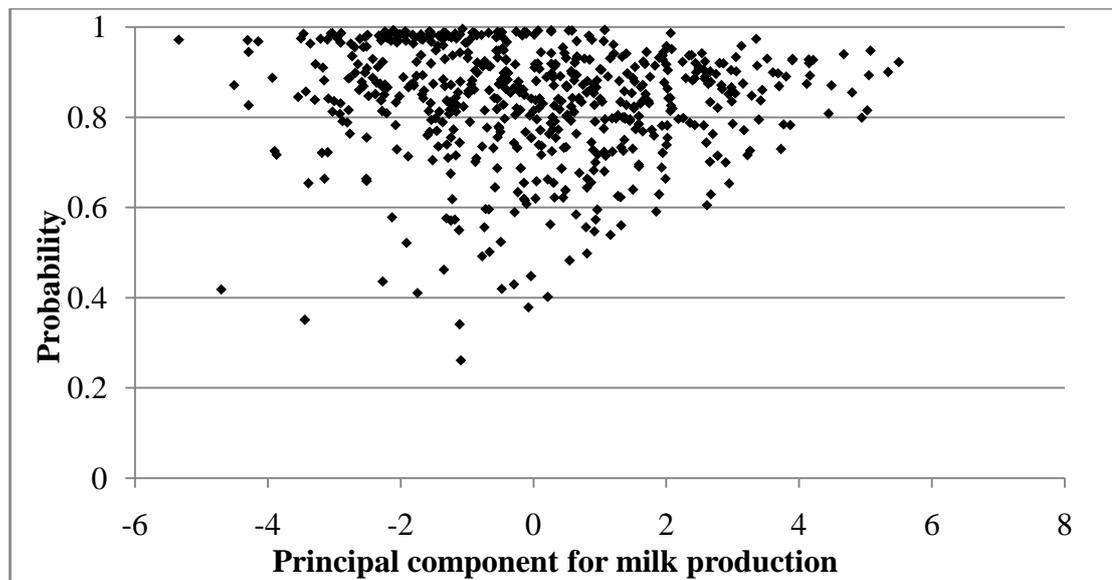
**Figure 4.5.** Logistic regression of the probability of a cow receiving first service within the first three weeks after the start of the breeding season on the principal component for body condition score change.

Figure 4.5 shows that an reduction in cow BCS change was associated with an increased probability of a cow to be submitted for insemination during the first three weeks after the start of the breeding season ( $P < 0.05$ ). Cow that lost less BCS during

early lactation presented themselves for service earlier in the breeding season. No other relationships between BCS and fertility were found from the logistic regression.

### **Milk production and reproductive performance**

Figure 4.6 shows that total milk production (milk, fat, protein, and lactose yields) was significantly negatively associated with the first three weeks submission rate of the herd ( $P<0.01$ ). Submission rate was lower in cows that produced more milk during the whole lactation period compared to their lower yielding contemporaries.



**Figure 4.6.** Logistic regression of the probability of a cow receiving first service within the first three weeks after the start of the breeding season on the principal component for whole lactation milk production.

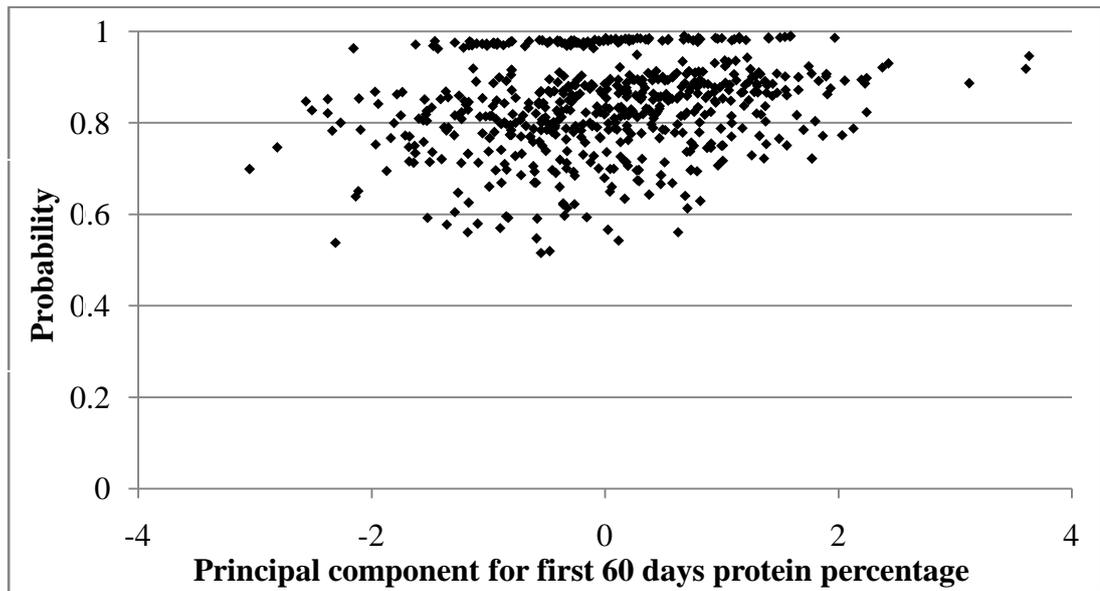
### **Protein percentage and reproductive performance**

Two different definitions of protein percentages in milk were used to investigate the relationship between protein percentages and fertility: average protein percentage for the first 60 days of lactation and whole lactation protein percentage.

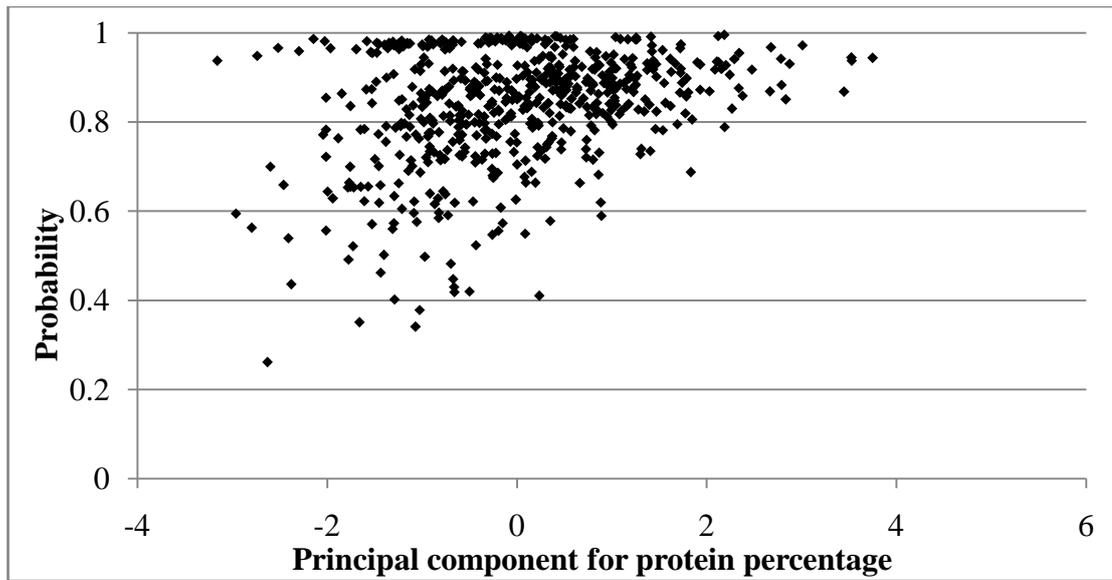
The probability of 3-week submission rate increased with protein percentage over the first 60 days ( $P<0.05$ ) (Figure 4.7) and the whole lactation ( $P<0.001$ ) (Figure 4.8). For the first service pregnancy rate, a similar positive relationship was shown. Both the first 60 days protein percentage (Figure 4.9) and the whole lactation protein percentage in milk (Figure 4.10) were positively related ( $P<0.05$ ,  $P=0.0502$ , respectively) to

pregnancy rate to first service. Protein percentage therefore showed a positive relationship with the fertility performance of the cow in early lactation.

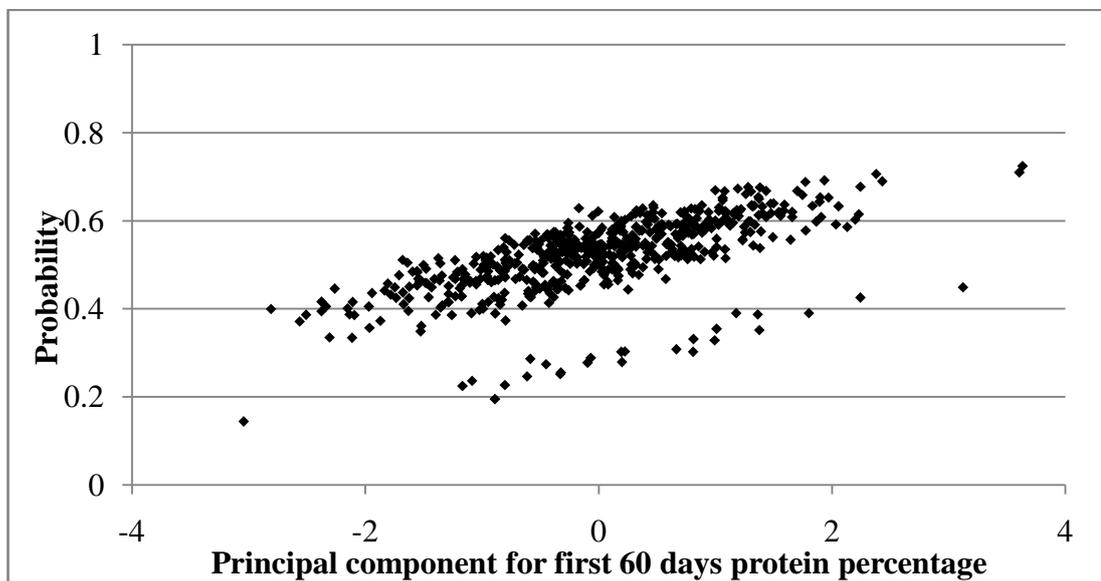
A significant relationship was found between protein percentage in milk and the pregnancy rate at the end of the milking season according to Figure 4.11 ( $P < 0.01$ ). An increase of protein percentage in milk was associated with a higher probability of becoming pregnant.



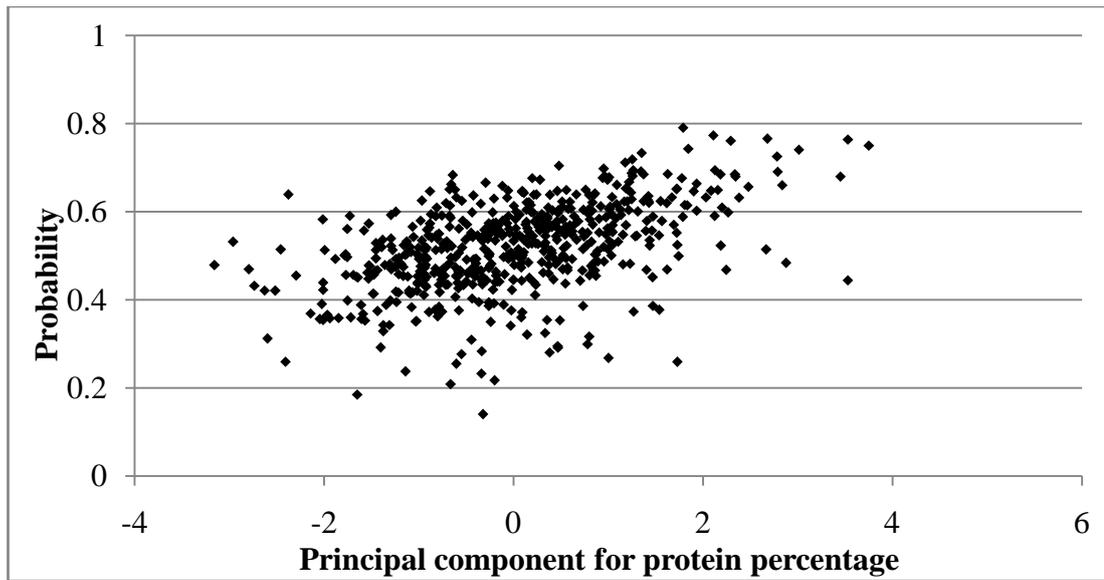
**Figure 4.7.** Logistic regression of the probability of a cow receiving first service within the first three weeks after the start of the breeding season on the principal component for milk protein percentage of the first 60 days in lactation.



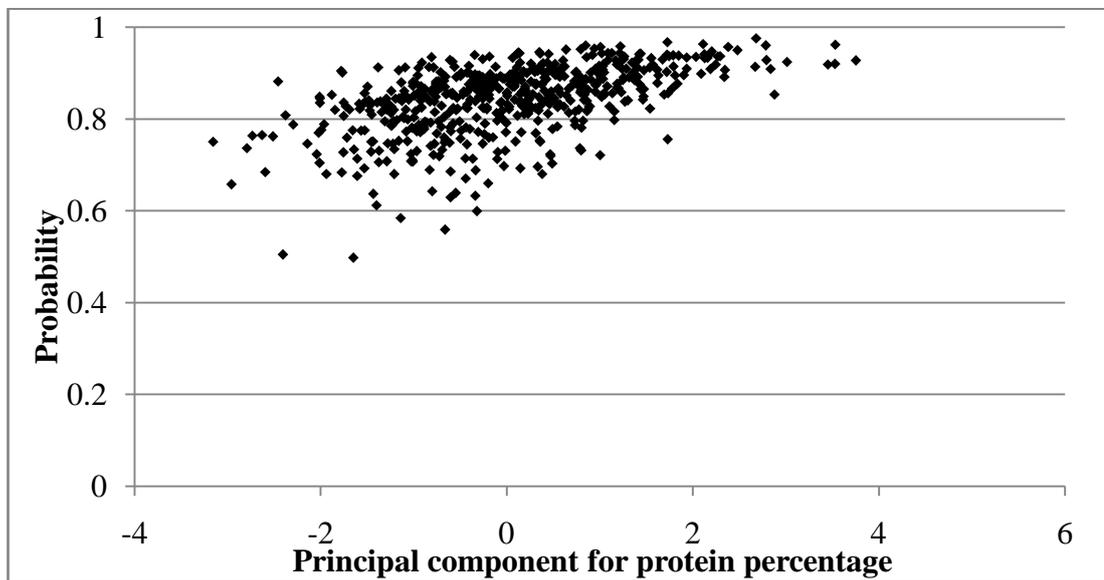
**Figure 4.8.** Logistic regression of the probability of a cow receiving first service within the first three weeks after the start of the breeding season on the principal component for milk protein percentage of the whole lactation.



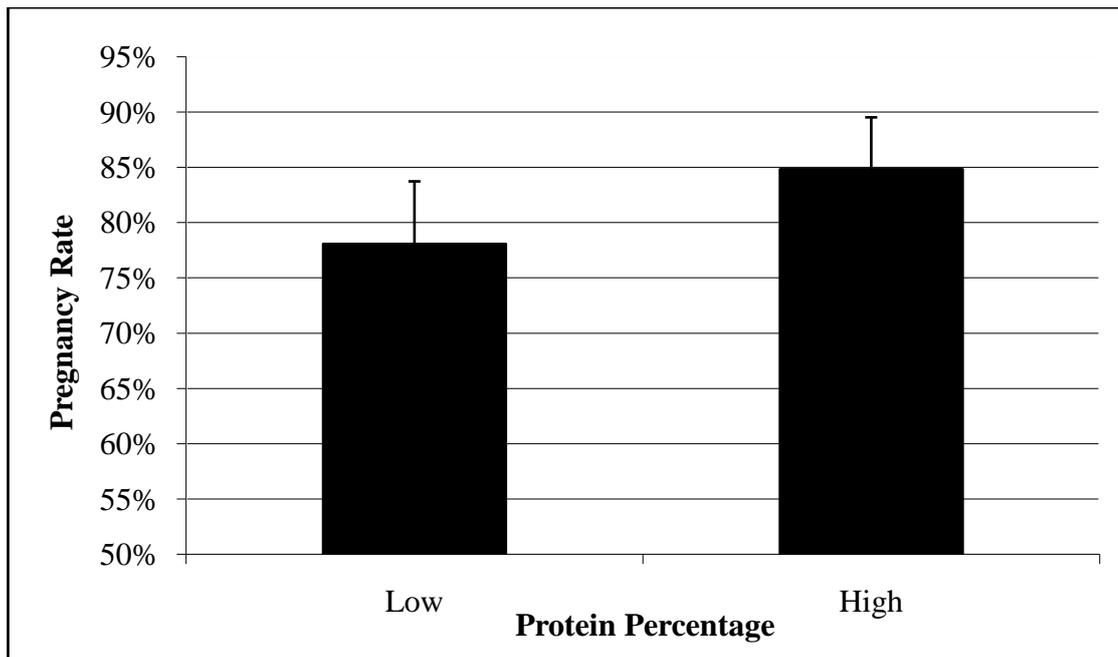
**Figure 4.9.** Logistic regression of the probability of a cow becoming pregnant during the first service after the start of the breeding season on the principal component for milk protein percentage of the first 60 days of lactation.



**Figure 4.10.** Logistic regression of the probability of a cow becoming pregnant during the first service after the start of the breeding season on the principal component for milk protein percentage of the whole lactation.



**Figure 4.11.** Logistic regression of the probability of a cow becoming pregnant during the whole breeding season on the principal component for milk protein percentage of the whole lactation.



**Figure 4.12.** Mean pregnancy rates of cows with high and low milk protein percentage. The error bars represent the 95% confidence interval.

Figure 4.12 shows the raw pregnancy rates of cows classified as high low protein percentage in milk within contemporary group of genotype, feeding system, parity and lactation. Cows with high milk protein percentage over the lactation had an average pregnancy rate of 85%, while the average pregnancy rate of cows with low protein percentage in milk was 78%. However, the difference was not significant.

# Chapter 5

## DISCUSSION

### **Relationships between BCS and milk production**

The decline in BCS that occurred during early lactation in the present study was in agreement with the results of previous studies (Veerkamp et al., 1994; Buckley et al., 2000a; Friggens et al., 2004). During early lactation, a rapid increase in milk production simultaneously with a slow rise in DMI normally causes the body fat reserves that were deposited during pregnancy to be rapidly mobilized in order to meet the increased energy demand for milk synthesis (Friggens et al., 2004).

In dairy cows in New Zealand, a positive and significant relationship between milk production and calving BCS has been reported (Roche et al., 2005). Later research (Roche et al., 2007a) suggested that this association between calving BCS and milk production is non-linear. The different results reported in previous studies (Roche et al., 2005; Roche et al., 2007a) might be caused by the range of BCS used for analysis. Only within a certain BCS range may the positive relationship reported in Roche et al. (2005) exist. In the present study, BCS at calving was also non-linearly related to the milk production. Obese cows with high BCS at calving produce less milk just like thin cows.

Earlier studies have concluded that every 100 kg increase in genetic merit for milk production was associated with an increase in BCS loss from calving to first service by 0.02 unit in a 5-point scale (Buckley, et al., 2003). The present study showed that milk yield increased rapidly during early lactation and was associated with a decrease in BCS. The greater the BCS loss of the cow during early lactation, the higher the milk production ( $P < 0.001$ ). Similar results have been reported in other previous studies (e.g. Waltner et al., 1993; Veerkamp et al., 1994; Buckley et al., 2000a; Pryce et al., 2001; Dechow et al., 2002). High producing cows normally partition a higher proportion of dietary energy into milk production than into body fat deposits. This may explain in part why increased milk production is associated with lower BCS.

## **Relationships between BCS and reproductive performance**

NEB during early lactation has been closely linked to low fertility performance of cows (Butler, 2005). A previous study showed that NEB had adverse effects on follicular development and subsequent reproductive performance of the cow (Buckley et al., 2003). The degree and duration of NEB during early lactation is consequently negatively associated with the probability of conception (Butler and Smith, 1989; Nebel and McGilliard, 1993; Senatore et al., 1996; Domezq et al., 1997).

BCS has been reported to be correlated with reproductive performance, both phenotypically (Buckley et al., 2003) and genetically (Berry et al., 2003a). In previous research undertaken in the seasonal grazing systems of Ireland, there were reduced 21-day submission rate, first service pregnancy rate and 42-day pregnancy rates for Holstein-Friesian cows in low BCS (Buckley et al., 2003). In the present study, higher BCS at calving was associated with a higher first 3-week submission rate ( $P < 0.001$ ), fewer services per conception ( $P < 0.05$ ) and a higher pregnancy rate ( $P < 0.001$ ). Greater loss of BCS was related to prolonged calving to submission ( $P < 0.001$ ) and calving to conception ( $P < 0.001$ ) interval. Similar results have been reported, in which the severity of BCS loss during the first 4 weeks postpartum was associated with a delay to first ovulation (Butler, 2005) and a prolonged calving to conception interval (Buckley et al., 2003).

During early lactation, the increased energy demand for milk production normally exceeds the energy intake of the cow. Body fat deposits start to be mobilized in order to fill the energy deficit. For cows with high genetic merit for milk production, their metabolic load is higher than low genetic merit cows. Simply increasing dietary energy density will not help fill the energy deficit, since this extra energy will be partitioned into production rather than body condition. Therefore, the decline of EB during early lactation is very common in high genetic merit cows. Interestingly, Lucy (2001) concluded that the highest producing cows were usually not those with the greatest NEB or lowest BCS.

Generally, high BCS loss during early lactation is associated with higher milk production but poorer reproductive performance (Pryce et al., 2001; Dechow et al., 2002). The amount of BCS loss during early lactation indicates the severity of NEB.

Physiologically, NEB reduces postpartum LH pulsatility (Butler, 2000b) and the ovarian responsiveness to LH stimulation (Butler, 2000a). Consequently, the restart of ovarian activity is delayed. Besides this, NEB has also been found to be related to decreased plasma glucose and insulin concentrations (Beam and Butler, 1999; Butler, 2000a), as well as reduced serum progesterone concentrations (Butler, 2000b). A positive association has been reported between plasma IGF-I concentration and BCS (Nishimura et al., 2000). Serum IGF-I concentration is positively associated with reproductive function, especially with the LH pulse frequency during postpartum NEB (Zurek et al., 1995). During postpartum NEB, IGF- I concentrations are low (McGuire et al., 1992), and such low concentrations would result in reduced follicular growth and poor corpus luteum development (Chase et al., 1998).

In year-round calving systems, Dechow et al. (2002) concluded that the greater the loss of BCS in early lactation, the longer the interval from calving to first service. In pasture-based seasonal-calving dairy systems, Grainger et al. (1982) showed that the postpartum anoestrous interval was reduced by 5.7 days with each additional increase in BCS at calving (scale: 1 to 8). In present study, the probability of cows being submitted within the first 3 weeks after the start of breeding season increased with the BCS at calving ( $P < 0.01$ ).

### **Relationships between milk production and reproductive performance**

Milk production in dairy cows has increased significantly over the past few decades. Whilst this has resulted in increased revenues from milk production, the costs of reduced fertility performance has also increased. In many breeding programs, emphasis has been put on milk production without cognisance of fertility traits. Previous research has shown that aggressive genetic selection for milk yield results in an increased risk of NEB, greater disease susceptibility (Pryce and Veerkamp, 2001) and decreased fertility (Veerkamp et al., 2003).

Genetically, there is a close relationship between milk yield and fertility, such that increase milk yield is associated with lower fertility of the cow (Pryce and Veerkamp, 2001), decreased conception rate to first service, prolonged calving to conception interval and increased number of service per conception (Gillund et al., 2001). Cows with high milk production generally conceive later during lactation (Roche et al., 2007b). Similar results were found in the present study. Higher milk production during

early lactation (first 60 days in milk) was associated with decreases in both the probability of cows becoming pregnant ( $P < 0.01$ ) and the probability of cows being submitted within the first three weeks of the breeding season ( $P < 0.001$ ). The probability of cows becoming pregnant also decreased as total milk production increased ( $P < 0.01$ ).

The present study showed that high milk production during early lactation was negatively related to calving to submission interval and calving to conception interval ( $P < 0.001$ ). In several previous studies, the postpartum anoestrous interval has been reported to be reduced with an increase of milk production (Pryce et al., 1998; Pryce and Veerkamp, 2001). However, such shorter postpartum anovulatory intervals did not result in a higher conception rate.

Evidence shows that a large increase in milk production in dairy cows is associated with a decline in fertility (Kelm et al., 1997; Pryce et al., 1999; Pryce and Veerkamp, 2001). These previous studies have not shown that the high yield of milk production is the cause of poor reproductive performance, and genetic relationships do not necessarily imply cause and effect (Veerkamp et al., 2003). Madsen (1975) pointed out that, at the beginning of the lactation, very high milk yield would put high physiological stresses on cows that finally lead to reproductive disorders.

Physiologically, the NEB associated with high levels of milk production (Butler, 2000b), disrupts the LH pulse patterns through an inhibition of GnRH secretion (Butler and Smith, 1989). Both ovarian follicular development and the initiation of postpartum ovarian cyclicity rely on the reestablishment of the normal LH pulse pattern (Butler and Smith, 1989). Besides this, the rapid increase of dietary intake is also deleterious to oocyte development and subsequent establishment of pregnancy (O'Callaghan et al., 1999). The length of the interval from calving to first ovulation represents an important interaction between energy status and reproductive performance (Butler, 2000b). Low energy availability during NEB not only suppresses pulsatile LH secretion, but also reduces ovarian responsiveness to LH stimulation (Butler, 2000a). The availability of insulin and serum IGF-I and the changing EB profile during NEB period determines the ability of follicles to produce sufficient oestradiol for ovulation (Butler, 2000b). Therefore, cows with the greatest milk production also have the highest incidence of infertility (Lucy, 2001).

## **Relationships between milk protein percentage and reproductive performance**

In seasonal-calving grazing systems, a positive relationship between milk protein percentage and reproductive performance has been reported in several previous studies worldwide (Buckley et al., 2003; Fahey et al., 2003; Harris and Pryce, 2004). Morton (2000) concluded that cows with higher milk protein percentage had higher submission and conception rates. Another study (Morton, 2001) emphasized that cows with greater milk protein percentage in early lactation had substantially better reproductive performance.

The present study, through the use of principal component analysis, showed that cows with high milk protein percentage in early lactation (up to 60 days in milk) had a higher probability of submission within 3 weeks of the start of breeding season ( $P < 0.05$ ) and a higher probability of becoming pregnant to first service ( $P < 0.05$ ). Increased milk protein percentage across the whole lactation was associated with the probability of a cow being submitted in the first 3 weeks of the breeding season ( $P < 0.01$ ), an increased probability of a cow become pregnant to its first service ( $P < 0.05$ ) and an increased probability of a cow become pregnant to the whole breeding season ( $P < 0.05$ ). When logistic regression was applied without using principal component analysis, cows with high milk protein percentage across the whole lactation had a higher probability of becoming pregnant to the first service after the start of the breeding season ( $P < 0.05$ ), a higher probability of becoming pregnant during the whole breeding season ( $P = 0.07$ ) and the probability of 3-week submission rate also increased ( $P = 0.09$ ). Hence, a positive relationship between milk protein percentage and reproductive performance existed. The difference between the average conception rate of cows with high milk protein percentage and low milk protein percentage was only 7% and this difference was not significant. A similar result was reported by Fahey (2003) in that there was only a 4% difference in first service conception rates between the highest and the lowest milk protein quartiles.

The relationship between milk protein percentage and reproductive performance is related to energy balance. Milk protein percentage is recognized as an important indicator of EB (Buckley et al., 2003; Patton et al., 2007). Cows with low milk protein percentage suffer more severe and prolonged NEB compared to cows with higher milk protein percentage (Fulkerson et al., 2001). Postpartum NEB is associated with

increased milk fat percentage due to the mobilisation of adipose tissue and decreased milk protein percentage due to the shortage of glucose (de Vries and Veerkamp, 2000). Other studies have reported that cows selected for high milk production had relatively low glucose levels during lactation (Jonsson *et al.*, 1999; Snijders *et al.*, 2001). NEB gives the explanation of how selection for high milk production can result in low fertility. Similarly, glucose may be the key linkage between milk protein percentage and reproductive performance. High milk production is related to low glucose levels, whose shortage would reduce the synthesis of milk protein; thus, low milk protein percentage would be found in cows with high milk production and low fertility. Morton (2000) suggested that the relationship between milk protein percentage and reproductive performance was affected by nutritional strategies which could increase milk protein percentage and cause better reproductive performance as well. The best way to avoid sub-optimal fertility was to achieve optimal BCS at calving and maximise postpartum energy intake.

Hormones also play an important role in the physiology of reproduction. Serum IGF-I concentrations are influenced by variations in protein synthesis or energy intake (Breukink and Wensing, 1998; Zulu *et al.*, 2002), and IGF-1 is also an indicator of energy balance (Spicer *et al.*, 1990). As mentioned above, severe NEB inhibits reproductive function through reduced LH pulse frequency, dominant follicle growth rate and diameter, concentrations of IGF-1, glucose and insulin and increased GH concentrations. All these changes would result in greater BCS loss and increased percentage of anoestrous cows (Roche *et al.*, 2000). As is well known, cows with prolonged anovulatory anoestrous periods have lower submission and conception rates, and higher culling rate for failure to conceive (Macmillan, 1997). An early study showed that cows that displayed oestrus more than once before the first insemination had higher fertility compared to those inseminated at the first oestrus (Macmillan and Clayton, 1980). Therefore, the reduced milk protein synthesis which is caused by NEB could influence the anovulatory anoestrous periods through the mediating effect of IGF-I concentrations.

## **Chapter 6**

### **CONCLUSION**

High milk protein percentage, especially in early lactation, was related to better reproductive performance. Cows with higher milk protein percentage during early lactation had a higher 21-day submission rate and higher pregnancy rate to first service. A potentially 7% higher conception rate was showed in cows classified as having high milk protein percentage compared to cows classified as low milk protein percentage. The study also focused on the relationship between milk production, BCS and fertility. Increased milk production was associated with reduced fertility: long anoestrous interval, long calving to conception interval and low conception rate. Relatively high BCS at calving as well as reduced BCS loss during early lactation was associated with better reproductive performance: a short anoestrous period, a reduced calving to conception interval and a high conception rate.

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