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**Effect of feeding high or low protein supplements with fodder beet  
in late gestation on cow and calf performance**

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A thesis  
submitted in partial fulfilment  
of the requirements for the Degree of  
Doctor of Philosophy

at  
Lincoln University  
by  
Yingluck Moonsan

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Abstract of a thesis submitted in partial fulfilment of the  
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Yingluck Moonsan

New Zealand dairy production is a predominantly pasture-based system, with crops often used as winter feed. Mitigation of N losses to the environment has led to dry cows wintering on low crude protein (CP) crops such as fodder beet (FB), in late gestation. However, there are emerging questions about the low protein offered to late gestation cows fed these crops, which is below recommended feeding standards, that it may have immediate or long term effects on the dam and in particular the *in-utero* foetus. To address these questions, three experiments were done to evaluate the impact of restricting protein supply to the maternal diet during late gestation on short and long term responses of both dam and offspring.

The objective of the first experiment was to examine the effect of restricting protein by up to 45% for 50 days in late gestation on the dam's performance and the subsequent growth and development of her *in-utero* foetus. The treatment feeding occurred between week 31 to 38 of gestation, where 190 crossbreed (Holstein x Jersey) non-lactating dairy cows were allocated into two isoenergetic diet treatments; 1) a moderate CP (MCP; 5 kg DM of FB + 5 kg DM of lucerne silage) as a control diet and 2) a low CP (LCP; 5 kg DM of FB + 5 kg DM of maize silage plus straw). Treatment diets offered approximately 120 MJ ME/cow/day at 11.5 MJME/kgDM which provided 7.8 and 13.6% CP to cows in LCP and MCP, respectively. Low CP during late gestation altered the energy/protein balance of the diet but cows apparently consumed 95% (LCP) and 100% (MCP) of ME requirements and were able to maintain BCS throughout the eight-week experiment irrespective of treatment. Serum urea concentration of the MCP cows were higher than the LCP (6.37 vs 1.67 and 5.70 vs 2.41 mmol/L, at week 4 and 8, respectively) ( $P < 0.01$ ) and urinary N excretion was lower in LCP compared with MCP. Low Ca in the LCP diet may result in more health issues during the transition phase and in the subsequent lactation (43 vs 32 interventions for LCP and MCP, respectively,  $P = NS$ ). There were

no evident carryover effects from winter feeding treatments on the consequent milk yield (average 5,147 litres/lactation) or milk composition of the dams. Among the calves, no difference between maternal treatments was observed for average birth LW ( $33.2\pm0.66$  kg), heart girth ( $74.1\pm0.4$ ), neck length ( $33.8\pm0.6$ ), spine length ( $54.4\pm0.4$ ), wither height ( $71.0\pm0.4$ ), hip height ( $73.1\pm0.4$ ) and waist circumference ( $73.2\pm0.6$ ) ( $P>0.05$ ). Colostrum composition and colostral IgG level from both treatments were similar. Calf serum protein, albumin, globulin, IgG and EAAs did not differ due to the maternal diet, but early colostrum intake had a significant impact on these parameters. These findings suggest that feeding a low protein supplement with fodder beet during late gestation can be used to reduce urinary N loss without compromising milk yield or calf development. However, any late gestation diet should be used with awareness of mineral requirements to avoid metabolic disorders during the transition phase.

The objective of the second experiment was to examine the effect of maternal diet and milk feeding regime on pre-weaning calf response between day 4 and 39 of age, post-weaning growth and first lactation performance. Sixty 4 day old heifer calves from experiment 1 were allocated to a factorial 2 x 2 design, with the first factor being the maternal treatment (LCP and MCP) and the second factor being the milk allowance regime. A commercial calf milk replacer (CMR) was fed twice daily depending on allowances; 1) a conventional regime (CON at the rate of 2 litres/calf/feed or 10% of BW) and 2) high allowance (HA, at the rate of 4 litres/calf/feed or 20% of BW). Calf starter and water were offered ad-lib. Intake of CMR and starter, general health and behaviour and faeces score were recorded daily. There was no significant impact of maternal treatment on pre-weaning growth, stature measurements, weaning weight or average daily growth rate (ADG). Faeces score was not different between maternal or allowance treatment. The HA calves had greater weaning weight ( $88.0$  vs  $79.0$  kgLW) and overall ADG ( $0.90$  vs  $0.75$  kg/day) than the CON calves, and these differences were also evident in their stature measurements. There was no carryover benefit from HA on heifer growth rate and ADG after weaning. Fifteen of the 60 heifers continued as replacements in the milking herd, the HA heifers had greater DIM and a tendency for increased milk yield in the 1<sup>st</sup> lactation compared with CON heifers. There was no effect of maternal treatment on milk yield. These outcomes highlight the importance of pre-weaning nutrition on calf development and long term productivity. There was no evidence of an effect of moderate maternal protein restriction on calf performance.

Due to limited numbers of animals in the first and second experiments, the objective of the third experiment was to use a larger database of cows to examine the long term effects of feeding different winter crops, kale or FB, to late gestation cows on the growth and milk production of their offspring. Heifer data from a commercial herd of 300 multiparous dairy cows wintered on kale or FB

over three consecutive winters (2012-2014) were collected from Livestock Improvement Corporation's MINDA™ Live herd-recording software (MINDA) and Livestock Improvement Corporation Ltd (LIC). Effect of maternal diet on heifer growth and production was compared. There was no significant difference between maternal diets on LW of calves which were well managed and, on average, exceeded industry recommended target weights. Overall LW gain of calves from the age of 3 -22 months averaged  $0.66\pm 0.006$  kg/day and were not affected by the maternal winter feeding regime ( $P>0.05$ ). The first and second lactation milk yield, milk composition and days in milk of those progeny were also similar across maternal treatments. Average milk yield for the first two lactations was  $4,625\pm 59.4$  litres with  $439.7\pm 4.3$  kg milksolids ( $P>0.05$ ). Percentage of milk protein and fat were similar between treatments at an average of  $4.19\pm 0.02$  and  $4.99\pm 0.04$  %, respectively ( $P>0.05$ ). These results suggest that under conditions of adequate energy, the late gestation feeding regime is unlikely to alter the long term survival, growth and their first two lactation performance of the progeny.

Overall, based on the conditions of the present study, feeding FB with low CP supplement to late gestation cows resulted in reduced nitrogen excretion with no apparent adverse effects on either the dam herself or her calf. The cows maintained BCS and achieved high milk yields. There was no significant adverse effect of maternal low protein on short and long term performance of the offspring and passive immunity in colostrum. Also, colostrum management was critical for neonatal survival and later health. A high allowance regime can accelerate pre-weaning ADG and increased weaning LW. These results may suggest that, under the conditions of adequate energy, cows in late gestation have an effective ability to manipulate nutrition to maintain and respond to *in-utero* foetal requirements. While the small sample size is a limitation of this study, research outcomes in the wider science community support our results that moderate protein restriction of short duration during gestation can be buffered by the dam to avoid lasting negative effects on the progeny.

**Keywords:** maternal nutrition, late gestation, crude protein, fodder beet, maize silage, lucerne silage, *in-utero* growth, stature measurements, Refractometer, immunoglobulin G, passive immunity, pre-weaning rearing, post-weaning growth, milk production.

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## List of Abbreviations

| <b>Abbreviation</b> | <b>Description</b>                           | <b>Units</b> |
|---------------------|--|--------------|
| AA                  | Amino acid                                   |              |
| ADF                 | Acid detergent fibre                         |              |
| ADG                 | Average daily gain                           | kg/d         |
| ADRDS               | Ashley Dene Research and Development Station |              |
| ANOVA               | Analysis of variance                         |              |
| BAT                 | Brown adipose tissue                         |              |
| BCS                 | Body condition score                         |              |
| BHBA                | Beta-hydroxybutyric acid                     | mmol/L       |
| BUN                 | Blood urea nitrogen                          |              |
| BW                  | Birth weight                                 | kg LW        |
| Ca                  | Calcium                                      |              |
| Cl                  | Chloride                                     |              |
| cm                  | Centimetre                                   |              |
| CMR                 | Calf milk replacer                           |              |
| CON                 | Conventional regime, 10% BW                  |              |
| CP                  | Crude protein                                |              |
| d                   | Day  |              |
| DCONC               | Days since conception                        | days         |
| DCP                 | Dicalcium phosphate                          |              |
| DE                  | Digestible energy                            |              |
| DM                  | Dry matter                                   |              |
| DMI                 | Dry matter intake                            |              |
| DOMD                | Digestible organic matter in DM              |              |
| EAAAs               | Essential amino acids                        |              |
| EE                  | Ether extract                                |              |
| EK                  | Early sown kale                              |              |
| FB                  | Fodder beet                                  |              |
| FCE                 | Feed conversion efficiency                   |              |
| FPT                 | Failure of passive transfer                  |              |
| g                   | Gram   |              |
| GE                  | Gross energy                                 | Mcal/kg      |
| HA                  | High allowance, 20% BW                       |              |
| HF                  | Holstein Friesian                            |              |
| ha                  | Hectare                                      |              |
| Ig                  | Immunoglobulin                               | g/L          |
| IgG                 | Immunoglobulin G                             | g/L          |
| K                   | Potassium                                    |              |
| kg                  | Kilogram                                     |              |
| L                   | Litre  |              |
| LCP                 | Low crude protein                            |              |
| LK                  | Late sown kale                               |              |
| LS                  | Lucerne silage                               |              |
| LW                  | Live weight                                  | kgLW         |
| LWG                 | Live weight gain                             | kgLW         |
| m                   | Metre  |              |
| m <sup>2</sup>      | Metre square                                 |              |
| MADF                | Modified acid detergent fibre                |              |
| MCP                 | Moderate crude protein                       |              |
| ME                  | Metabolisable energy                         | MJME         |

| <b>Abbreviation</b> | <b>Description</b>              | <b>Units</b>  |
|---------------------|---------------------------------|---------------|
| Mg                  | Magnesium                       |               |
| MJ                  | Mega joule                      |               |
| MP                  | Metabolisable protein           |               |
| MS                  | Maize silage                    |               |
| MUN                 | Milk urea nitrogen              |               |
| N                   | Nitrogen                        |               |
| n                   | Number                          |               |
| Na                  | Sodium                          |               |
| ND                  | Nutrient deficiency             |               |
| NDF                 | Neutral detergent fibre         |               |
| NEAAs               | Non-essential amino acids       |               |
| NE <sub>B</sub>     | Negative energy balance         |               |
| NEFA                | Non-esterified fatty acid       | mmol/L        |
| NE <sub>L</sub>     | Net energy for lactation        | MJ/kg of DM   |
| NH <sub>3</sub>     | Ammonia                         |               |
| NIRS                | Near-infrared spectrophotometer |               |
| NSC                 | Not suckled colostrum           |               |
| NZ                  | New Zealand                     |               |
| OM                  | Organic matter                  |               |
| P                   | Phosphorus                      |               |
| RID                 | Radial immunodiffusion          |               |
| S                   | Sulphur                         |               |
| SC                  | Suckled colostrum               |               |
| SCC                 | Somatic cell count              | 1,000 cell/ml |
| SEM                 | Standard error of mean          |               |
| TIA                 | Turbidimetric immunoassay       |               |
| WSC                 | Water-soluble carbohydrate      |               |

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# Chapter 1

## Introduction

### 1.1 Introduction

The New Zealand economy benefits significantly from the primary industries, of which the dairy sector is a significant contributor. New Zealand pasture-based dairy production has continuously increased in terms of number and size of herds, and milk produced. In 2020, the total number of 6.35 million dairy cows (Granwal, 2020) produced 21.8 million tonnes of milk with 1.90 million kilograms of milksolids (DairyNZ, 2021). Each year, approximately 95% of New Zealand's milk products are exported worldwide, accounting for one-third of national exporting (DCANZ, 2020). The export value of New Zealand dairy commodities was almost 20.0 billion dollars in year to June 2020 (DCANZ, 2020).

Each year, approximately 20% of the national herd is replaced by young stock with improved genetics for production and longevity. The long term productivity of the national herd relies on successful rearing of replacement stock. The factors influencing this success begin *in-utero*. In this regard, the nutritional management of pregnant cows is an important consideration in the rearing of replacement heifers (Caton et al., 2007, Dunlap et al., 2015). Early life nutrition is important because it can impact all stages of reproductive system development of the *in-utero* foetus (Rhind, 2004), and alter the postnatal growth pathways, metabolism, and body composition of the offspring (Redmer et al., 2004). Furthermore, the nutrition of a foetus during gestation is also shown to affect their subsequent milk production (Funston et al., 2010a). Proper management of this critical stage is necessary for sustainable and prosperous dairy production. In New Zealand, the adoption of high energy, low protein crops, such as fodder beet (FB), for feeding cows in late gestation has become commonplace, particularly in the South Island where cows leave the milking platform for much of the winter. The advantages of FB include its low cost, high yield and off-farm feeding enables pasture covers on-farm to be regained on the milking platform (Edwards et al., 2014b). More importantly perhaps, is that it provides mitigation options for the damaging environmental impacts of excessive nitrogen (N) loading from livestock manure and subsequent nitrate leaching (Edwards et al., 2014a).

However, the protein content in FB at approximately 7 - 8% crude protein (CP) is very low compared to dietary recommendations of 12% CP (NRC, 2001) or 13% CP (AFRC., 1993) for late gestation cows. Chew et al. (1984) reported that feeding low CP at 80% of NRC requirement to cows during late gestation may lead to reduced dry matter intake (DMI) (Chew et al., 1984) and reduce the subsequent milk yield. Lower DMI may also indicate poor nutrient supply which greatly influences the response in foetal growth during the final stages of gestation (Eley et al., 1978). This status can cause a negative energy and protein balance in dams and lead to intrauterine growth restriction (IUGR), low birth weight (BW) and subsequent poor growth of calves (Wu et al., 2004, LeMaster et al., 2017, Bell et al., 2000, Grummer, 1995). Preliminary studies comparing skeletal development of calves born from cows fed kale or FB in late gestation showed reduced body weight of calves from cows fed FB diets (Bryant and Pirat, 2014). One conclusion was that the higher protein in the kale and pasture diets better met foetal amino acid requirements through improved protein supply compared with FB. With the adoption of FB already widespread in order to meet environmental nutrient limits, further reductions in protein feeding during late gestation are a potential risk for the industry. There is no information on the immediate and long term impact of feeding a combination of low protein crop with low protein supplement. This research set out to determine the effect of protein restriction on late gestation dairy cows – and their calves - by adopting and modifying the existing commercial practice of fodder beet feeding in June and July.

## **1.2 Research objectives**

### **1.2.1 Significance of the study**

There is insufficient information on the impact of feeding late gestation dairy cows a high energy low protein diet, on foetal development and neonatal growth. The results of this research will provide valuable information on the effect of supplementing late gestation dairy cows on a low protein crop, with high or low, protein silage.

## **1.2.2 Objectives**

The objective of this research was to investigate the effect of combining feeds varying in protein to energy ratio, which are fed to late gestation dairy cows, on the subsequent development and growth of calves.

## **1.2.3 Specific objectives**

- i) To compare the immediate effects of late gestation nutrition (low protein ration using fodder beet) on the dam and calf concerning development *in-utero*, colostrum quality and transfer of passive immunity.
- ii) To compare the carryover effects of late gestation nutrition (low or high N supplement feeds) on heifer progeny growth and lactation performance.
- iii) To compare the long term effects of late gestation nutrition (such as kale or fodder beet) on productivity with regards to milk yield in the first two lactations.

## **1.3 Hypothesis**

### **1.3.1 General Hypothesis**

Feeding low protein feeds to cows in late gestation, to reduce environmental impact, has a negative effect on calf development, growth and long term performance. Adjusting the feeding regime of dams and offspring by providing appropriate protein nutrition will off-set potential negative effects on the offspring without compromising environmental impacts.

### **1.3.2 Specific hypothesis**

Null hypothesis 1: Feeding a low protein supplement with a low protein crop to late gestation dairy cows will not affect dam performance or calf development and colostrum quality.

Null hypothesis 2: Increasing milk allowance to offspring of dams fed a protein-restricted diet will not increase heifer growth rates or lactation performance.

Null hypothesis 3: Farm systems which provide a moderate protein diet to late gestation dairy cows will not produce more productive progeny than farm systems which feed restricted protein diets.

## 1.4 Thesis structure

This thesis consists of seven chapters, as showed in detail in Figure 1.1. Briefly, Chapter two reviews the literature for the breeding cycle in New Zealand seasonal farming systems, *in-utero* nutrient transfer and the nutrient requirements for gestation. The effect of protein nutrition on dams and progeny at various stages and pre-weaning nutrition for supporting progeny growth were also reviewed. The remaining chapters report on 3 experiments (two field studies and one data analysis) considering the effect of feeding differing protein diets to late gestation cows and subsequent impact on calf development and immunity transfer (Chapter 4), carryover effect of maternal treatment and pre-weaning allowance on heifer growth and first lactation performance (Chapter 5) and longterm impact of maternal diets on lactation (Chapter 6) of the heifer progeny.

Figure 1.2 depicts the allocation of the experimental animals throughout the two field experiments. Firstly, 190 late gestation non-production cows were stratified into two groups differing in CP level, after calving, the cows were allocated to a farmllet experiment (not related to the present study) for the remaining lactation period. The newborn calves were measured for *in-utero* development. Secondly, 60 heifer progeny from those dams were then allocated into the pre-weaning experiment to study the response of feeding level on pre and post-weaning growth and 1<sup>st</sup> lactation milk performance. A separate data set using cows from the Lincoln University demonstration farm (LUDF) evaluated the longterm impact of maternal diets on the heifer progeny lactation performance, which is not indicated in Figure 1.2 here.

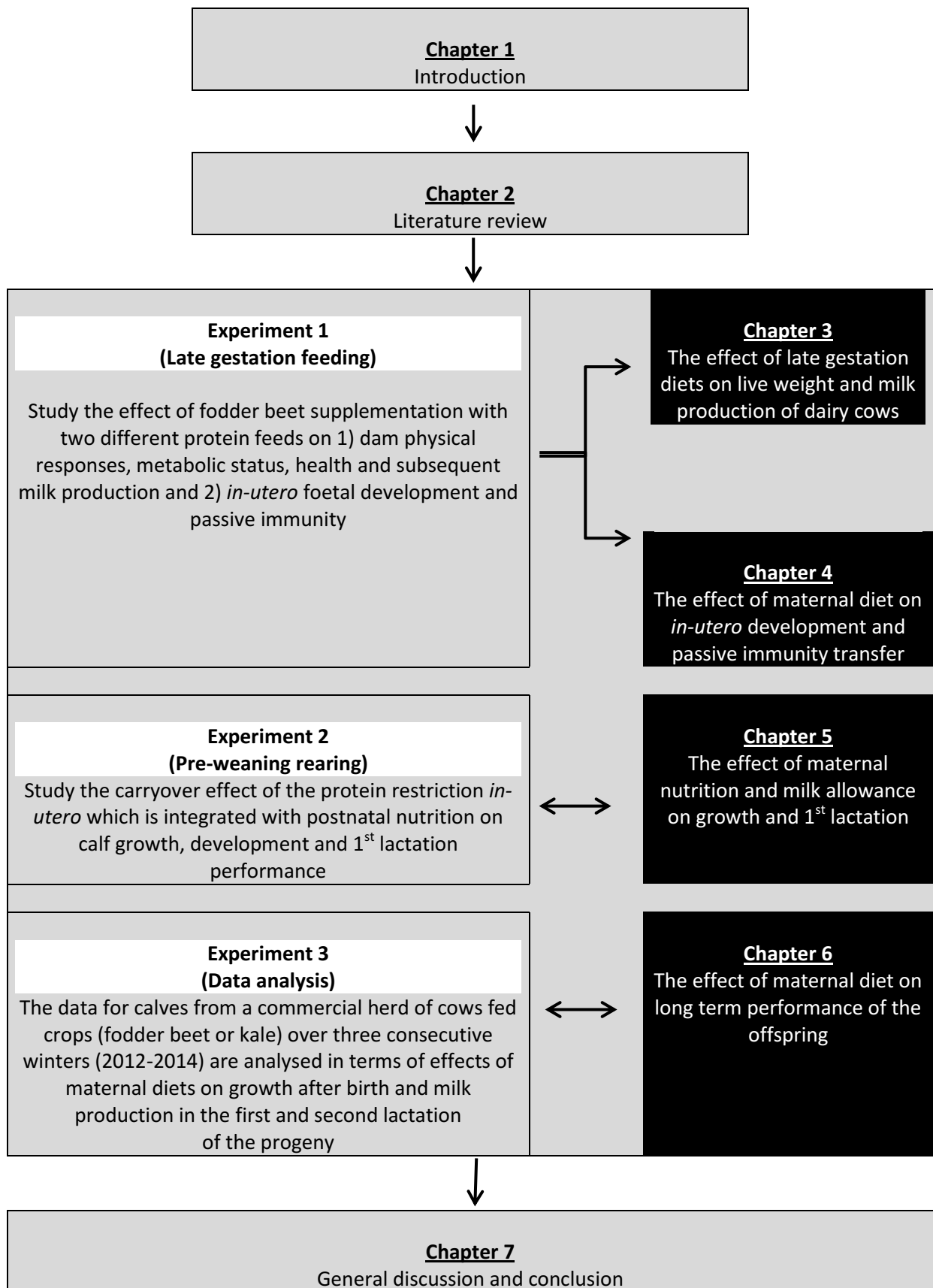
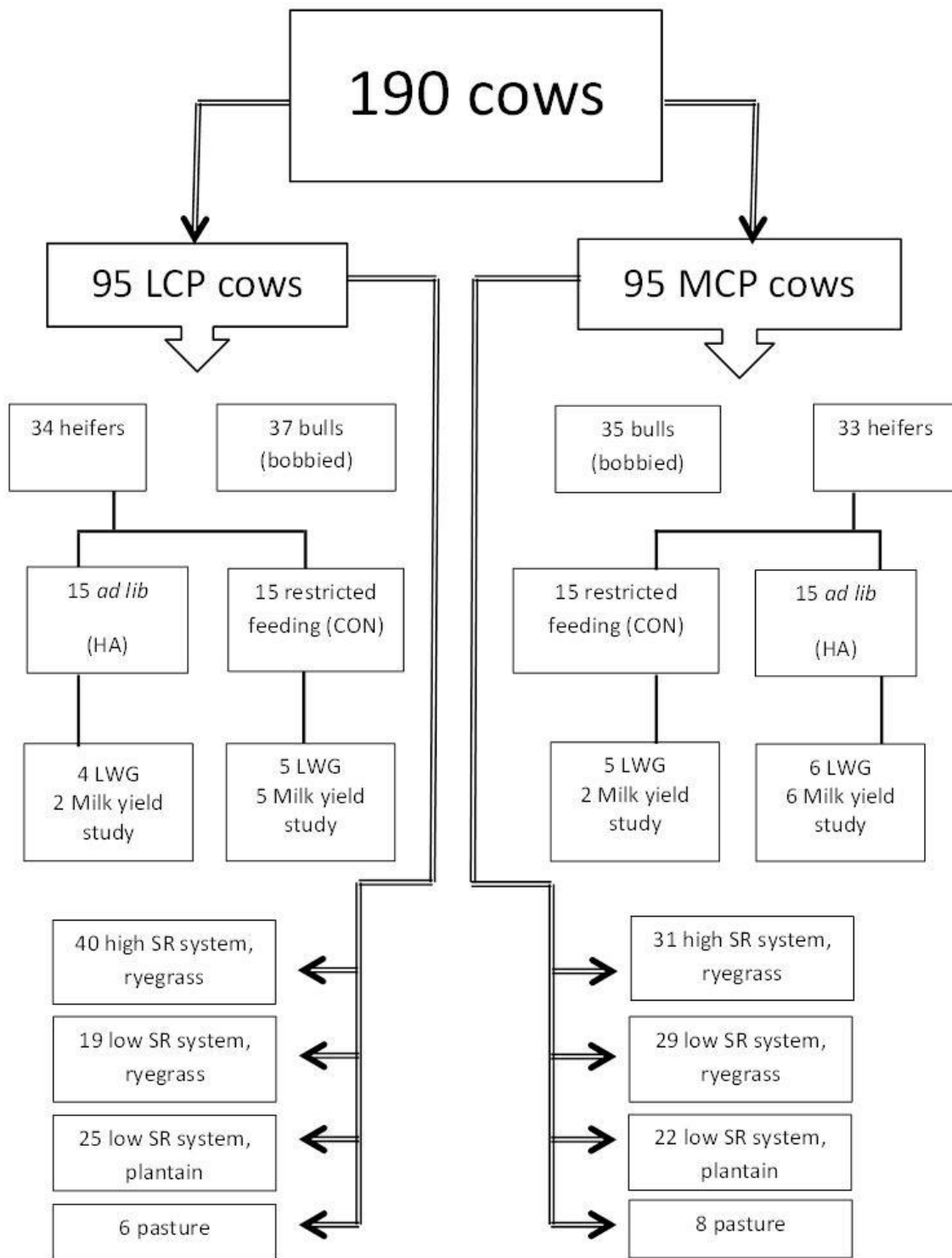


Figure 1.1 Diagram of the thesis structure



**Figure 1.2 Flow chart of animal assignment in the experiments**

== Cows in wintering experiment (Chapter 3) — heifers born to the experimental cows and were assigned to the pre-weaning experiment (Chapter 5)

## Chapter 2

### Literature Review

#### 2.1 Introduction

Dairy is one of the key players in New Zealand primary production. New Zealand dairy farming operates primarily under a seasonal supply regime. This seasonal production depends on low-cost pastures and crops which are used to feed livestock in such a way as to match peak animal demand with peak pasture growth and thus, take full advantage of pasture utilisation. Perennial ryegrass (*Lolium perenne*) is typically used as the primary forage across the production year. However, its growth becomes low during winter and it may not be sufficient to meet cow nutrient requirements (Judson and Edwards, 2008). Thus alternative forages such as fodder beet (FB) are used to fill these feed shortages by accumulating high yields of biomass during the growing season which maintains feed quality even at yields of over 25 t DM/ha (de Ruiter et al., 2007).

In New Zealand, the breeding cycle of dairy cows is aligned with the seasonal forage growth cycle. Calving occurs in late winter to early spring (July - September). Generally, after calving, milk yield will continually increase until a peak at about eight weeks post-partum, then decline at about 2% per week (Knight, 2001). The lactation lasts typically for about 8-9 months. During that time, oestrus is expected to occur within 6-8 weeks of calving with the herd expected to become pregnant again within two ovulation cycles (Brownlie et al., 2015) and recalved within 365 days (Knight, 2001). A non-lactating cow is referred to as a dry cow. Generally, dry cows are pregnant, as farmers will remove non-pregnant or non-lactating cows which are no longer economic to retain. Late gestation, dry cow feed management aims to allow the cow to retain or regain the body energy reserves (Edwards et al., 2014b), which were mobilised during the previous lactation and repair and rebuild mammary tissue for the next lactation (Keogh et al., 2009). Further, the *in-utero* foetus is in rapid growth during this period (Funston et al., 2010a, Eley et al., 1978).

As described above, FB has advantages for dairy production, namely high yield and high digestible energy but low protein which has shown to provide options for reduced urinary nitrogen (N) losses and subsequent nitrogen leaching to the environment when fed

to animals. However, feeding dry cows with low protein diet (i.e. FB) may have a negative impact on foetal development. Shortly before calving in late gestation, the dry matter intake (DMI) of cows declines, which compromises nutrient balance (Garnsworthy and Jones, 2010). Inadequate protein was reported to worsen suppressed DMI (Chew et al., 1984, Wu et al., 2006), thus leading to increased nutrient restriction (NRC, 2001). A negative impact of poor late gestation nutrition on the dam and foetal responses have been reported in previous studies (LeMaster et al., 2017, Van Saun et al., 1993, Funston et al., 2010b).

The long term animal response of nutrient restriction in the pasture-based dairy system is not well studied, so this literature search aims to review the impacts of maternal nutrition on the outcomes of dam and progeny including that of passive immunology of the progeny.

## **2.2 Nutritional regimes for cows in late gestation**

In New Zealand, calving commences in late winter (between mid-July to mid-August), so late gestation feeding occurs during the winter period. Other than meeting energy requirements for gestation, the objectives for the farm system take little account of the neonate as farmers tend to set goals around cow body condition score, cost of feeding, environmental effects and actions to minimise metabolic disorders around calving (Edwards et al., 2014a). In early to mid-gestation, cows are lactating and are fed pasture while on the milking platform. Feeding levels are typically high at 2.5-3.0 times maintenance. In late gestation, cows are fed at 1.2 – 2.0 times maintenance depending on weight gain targets and climate. On more intensive farms with high stocking rates, cows are grazed away from the milking platform, often on smaller parcels of land. Consequently, many feeding regimes include inexpensive, high yielding, low protein crops (such as FB), with a cheap source of fibre (silage or straw) to produce a low crude protein (CP) diet (<12% CP) to reduce nitrate leaching from urinary N (Edwards et al., 2014a).

### **2.2.1 Fodder beet**

Fodder beet (*Beta vulgaris*) is an annual forage crop which is widely used as an energy source during winter for dairy production in Canterbury (Judson and Edwards, 2008, Jenkinson, 2013, Edwards et al., 2014a). Fodder beet can produce high yields of dry matter

(DM, up to 30 T DM/ha), that can be carried through the winter with minimal nutritional degradation (Judson and Edwards, 2008).

The nutritional composition of the plant varies between leaf and bulb. The leaf of FB contains 14-18 % crude protein (DM basis) which is comparable with ryegrass silage (14.1%) and higher than oat straw (11.8%). The bulb of FB consists primarily of sucrose which is highly digestible but low in CP at 7.3-8.7% (Jenkinson, 2013, Edwards et al., 2014b). On average, FB plant has 10.4-10.8% CP (Jenkinson, 2013, Rehfeldt et al., 2011). Furthermore, FB maintains metabolisable energy contents of greater than 12 MJ ME/kg DM and typical yields of FB reach 18-25 t DM/ha (Judson and Edwards, 2008, Westwood and Mulcock, 2012).

Because FB alone cannot meet the nutritional needs of livestock in terms of fibre, minerals and protein, allocation of FB is typically recommended to make up no more than 60% of the ration. Also, its high content of readily fermentable carbohydrates increases the risk of ruminal acidosis (Waghorn et al. 2018), so moderation is cautioned to prevent health concerns. As such, cows need a long transition of 10-14 days or increase by 1 kg DM FB/2 days, from a pasture diet to one that consists of FB crop. A typical feed allocation after the transition is approximately 6-10 kg DM FB plus 3-6 kg of another supplement per cow per day, depending on the size and requirement of the cow (Gibbs, 2014). Edwards et al. (2014b) reported the average DM utilisation of FB to be around 90%. As a result, cows offered a sufficient quantity of FB have regained a body condition score (BCS) over the 8-week winter-feeding period (Edwards et al., 2014b).

Previous studies reported that feeding crops such as FB or low protein kale during late gestation in dairy cows might have adverse effects on BW and skeletal development of calves (Bryant and Pirat, 2014). Pacheco et al. (2016) reported a reduction in plasma amino acids (AAs); arginine, citrulline and ornithine in the total amino acid of lactating dairy cows fed FB. These changes may be related to an insufficiency of essential nutrients, especially protein, which might result in deficits of particular amino acids.

The choice of supplement is very important in balancing the deficiencies of FB rations, though little consideration has been given to the impact of different supplements. Common supplements fed to livestock in New Zealand include silages made from pasture, lucerne, maize or cereal crops, and to a lesser extent dried forages such as straw or pasture

hay are fed. Conserved feeds made from pasture often reflect the nutritional composition of those feeds with increased fibre and reduced protein compared to the fresh forage. Maize silage (*Zea mays* L.) is widely cultivated and used as a major forage feed for dairy cows in New Zealand pastoral dairy systems (de Ruiter et al., 2007). Good quality maize silage consists typically of 28-35% dry matter content, 7-8% of crude protein, 38-45% neutral detergent fibre (NDF), 23-28% acid detergent fibre (ADF) and energy content of 10.8 MJME/kgDM (Kolver et al., 2001). The NDF content of maize silage is highly variable, which is mainly caused by maturity when harvested (Khan et al., 2015). Lucerne silage, on the other hand, is high in terms of crude protein and rumen digestible protein (RDP) but low in rumen undigestible protein (Sinclair et al., 2015). Ultimately a greater understanding of the adequacy of crops and supplements as a late gestation feed is required.

## **2.3 Foetal development during gestation**

### **2.3.1 Early to mid-gestation**

Placental growth is highest in the early-stage of gestation between days 28 – 80 (Funston et al., 2010a). The placenta plays an essential role in substance exchange between maternal and foetal circulation (Redmer et al., 2004, Bell and Ehrhardt, 2002, Zhang et al., 2015). The placenta is developed to support the foetus using two functions. Firstly, the large surface area of the placenta is necessary for nutrient exchange by the epithelial barrier and foetus blood vessels. Secondly, the placental trophoblast cells deliver nutrients, growth factors and immune cell regulators from the uterus to the foetus (Bell and Ehrhardt, 2002).

During gestation in ruminants, the caruncular crypts (caruncle) of the maternal uterus and the foetal cotyledonary villi will interdigitate to form the placentome (Figure 2.1). The placentome is responsible for uterus-foetus nutrient and oxygen transfer (Hradecký et al., 1988). These structures are continually developing and are responsible for blood flow throughout gestation. Igwebuiké and Ezeasor (2013) reported that placentomes of sheep increased in size during late gestation in response to the more significant nutrient and metabolic demand of the foetus.

Foetal growth is minimal during early to mid-gestation. However, during this period, the survival organs of the foetus (i.e. brain, heart and nephrons) are entirely formed and

continue to develop (Funston et al., 2010a). Therefore any nutritional restriction which occurs during this phase can reduce the growth and function of those organs (Gopalakrishnan et al., 2005).



**Figure 2.1 Placentome formation in bovine (Anonymous, 2017)**

### **2.3.2 Late gestation**

In the cow, late gestation is the period from day 200 to parturition, signifies the period of rapid growth of the foetus, which requires careful nutritional management of the dam to ensure she is able to meet both her needs and that of the foetus (Knight, 2001, Zhang et al., 2002). Even though the growth of the placenta is minimal towards the end of gestation, maternal undernutrition at this time can reduce uterine blood flow, which results in a 14% reduction in placenta morphology and function (Reynolds et al., 2006). Thus, the failure of placentome development and function could be associated with underdevelopment or even loss of the foetus. Although the genesis of the organs of the foetus has already occurred by mid-gestation, approximately 80% of foetal growth occurs during late gestation (Moisá et al., 2015, Greenwood and Cafe, 2007, Caton et al., 2007, Redmer et al., 2004). The accelerated growth of the foetus is about 7.4%/day or more than 200 g/day - until it reaches the maximum at around 6-7 weeks prepartum or 230 -232 days of gestation (Eley et al.,

1978, Prior and Laster, 1979). Dry matter intake of the dam, on the other hand, reduces by approximately 10–35%, three weeks prepartum (Bell, 1995, Grummer, 1995). The growth rate of the foetus then declines at a rate of 0.125%/day or less than 100 g/day afterwards (Prior and Laster, 1979, Eley et al., 1978) (Figure 2.2).



**Figure 2.2 Rate of foetal weight gain at day 40 – 280 (Eley et al., 1978)**

(Note: the flaw at around 100 days of pregnancy represents dividing lines between two groups of foetuses, one measured from 40 to 100 days of gestation and the other measured from day 100 to day 280).

During late gestation, maternal nutrition plays a pivotal role in the offspring's gene expression which will influence the programming of nutrient partitioning and ultimately growth and production of the offspring (Moisá et al., 2015, Dunlap et al., 2015). Numerous studies have reported maternal feed quality affects the newborn calf either pre or post-weaning, two stages which are essential to heifer growth and development (Armstrong et al., 2001, Bell et al., 2000, Bell, 2005, Caton et al., 2007, Faichney and White, 1987, Gao et al., 2013b, Gao et al., 2012a, Duarte et al., 2013). Those studies concluded that the effects

of variation in nutrient intake of the dam would be more significant in late, compared with early gestation.

## **2.4 Nutrition and gestation**

### **2.4.1 Energy and protein requirements**

Late gestation is the most energy-demanding period of the dam and foetus, and the rapid increase of nutrient requirements risks leading to nutrient deficiencies for the foetus. During this critical period, the foetus and placental tissue take priority in the demands on maternal nutrient supply (Redmer et al., 2004). Ensuring the foetus a supply of crucial nutrients; glucose and amino acids, but it is also necessary to prevent metabolic disorders occurring in the dam (Ingvarsen et al., 2003). Nutrients, especially protein, during gestation may have numerous impacts on livestock production characteristics (Wu et al., 2006). Prepartum maternal nutrition during different stages of foetal development can cause significant changes in intrauterine foetal growth and development, altering sufficiency of structures, physiology and metabolism throughout postnatal life (Zhang et al., 2002, Gao et al., 2012a, Peñagaricano et al., 2014). Moreover, feeding management during gestation is proposed to support the mammary gland, avoid intake depression and reduce postpartum related metabolic disorders in dams (Moorby et al., 2000).

The estimation of the metabolisable energy and protein requirements for a typical kiwicross dairy cow in the last eight weeks of gestation increases from roughly 12 to 37 MJ ME/day, and from 67 to 156 g MP/day (AFRC., 1993). Glucose and AAs are important building blocks for growth and metabolism of the foetus (Bell, 1995). Glucose can be either used directly as glucose or converted to lactate (a placental intermediate form of glucose) (Regnault et al., 2005).

As with energy, the requirement for protein depends on the type of animal, growth stage, health status, activity level and production (Bell et al., 2000, Ørskov, 1992). Amino acids, which make up protein, are essential for the development and growth of the foetus and neonate. *In-utero*, AAs play an essential role in foetal and placental development, tissue protein synthesis, they are significant fuels for foetal growth, regulators of hormone secretion and are cell signalling molecules (Kwon et al., 2004, Dunlap et al., 2015). Amino

acids are necessary precursors for the synthesis of non-protein substances, for example, polyamines, nitric oxide, amino sugars, purine and pyrimidine, nucleotides and some hormones. These non-protein substances are necessary for DNA and protein synthesis, placenta angiogenesis (Huang et al., 2021) and foetal-placental blood flow regulation (Gao et al., 2012). Also, in the circumstance of low nutrient supply, AAs will be used as the predominant metabolic fuel for the foetus (Thomas et al., 1998). For example, L-arginine administration in twin bearing ewes fed pasture resulted in improvement of BW and brown fat stores (McCoard et al., 2013). Restricted protein during gestation (e.g. 80% of NRC recommendation) can lead to reduced DMI of the cows (Chew et al., 1984), low BW and system impairments (e.g. cardiovascular, reproductive system) of the offspring in cattle (Mossa et al., 2013), sheep (Wallace et al., 2011) or rats (Rinaldi et al., 2013). In contrast, maternal over-nutrition typically leads to excessive BW of calves, a cause of dystocia (Bell et al., 1995, Bryant and Pirat, 2014).

The requirements for dietary CP depend on the stage of gestation and the size of the calf (Holmes et al., 2007). In general, the dietary CP requirements of the dam during pre-parturition are not expected to be higher than 1.1 kg/d (Bell et al., 1995). NRC (2001) recommended that the total CP content in a diet for maintenance of late gestation cow should be not lower than 12% of DM. These guidelines were established to ensure that the dam's metabolisable protein (MP) requirements for maintenance and gestation are being met. However, AFRC (1993) recommended that a crossbred dairy cow at 250 days since conception (DCONC) requires 320-340 g MP/d for maintenance and gestation when fed a diet of reasonable quality, which equates to around 13% dietary crude protein. At these low levels of MP requirements, all the MP needs can be met by microbial source of protein. However, a higher level of protein in the maternal diet may also encourage the development of the calves *in-utero*. Some studies, such as Martin et al. (2007), indicated that protein supplementation during late gestation might reduce some adverse effects of nutrient restriction in dams and may improve the reproductive capability of the offspring. Further, nutrient adjustment in the ewe diet increased the availability of amino acids and polyamines during gestation, resulting in increased concentrations of total amino acids and prevented foetal growth retardation (Kwon et al., 2004). Thus the recommendation of 12% of DM as CP appears practical (Quigley and Drewry, 1998).

#### **2.4.2 Effect of undernutrition on the dam**

In general, inadequate nutrition during gestation leads to a decrease in energy reserve, commonly measured as BCS, resulting in adverse effects on reproduction and milk production after calving, (Stockdale, 2008, Roche et al., 2009). Body condition score is one of the critical indicators affecting post-calving reproductive performance in bovine (Roche et al., 2007, Selk et al., 1988, Funston, 2006). Stockdale (2008) reported that BCS at calving correlated with consequent milk production; BCS at 4.1 and 5.6 units (on an 8-point scale) resulted in daily milk yield differences of 2 L per cow per day (31.6 v. 33.7 kg/cow).

Insufficient protein intake during late gestation to supply maternal and conceptus tissue requirements resulted in increased protein mobilization from peripheral tissues, suppressed tissue protein synthesis and possible to increased proteolysis (Bell et al., 2000). The physiology and function of the gastrointestinal tract and small intestine of the cow with poor nutrition are altered to increase nutrient absorption to meet the gestational requirement (Meyer and Caton, 2016).

Improving the protein nutrition of the cow during gestation resulted in improved DMI and successful maintenance of animal live weight (LW) and BCS (Van Emon et al., 2017). The possible reason is related to a reduction in prepartum tissue mobilization (Park et al., 2002, Quigley and Drewry, 1998). This improvement resulted in energy and protein intake balance (Bell, 1995), efficient nutrient metabolism (Quigley and Drewry, 1998) and enhanced milk production (Park et al., 2002, Bell et al., 2000). Protein supplementation also increased ADG in pregnant heifers (Summers et al., 2015, LeMaster et al., 2017).

#### **2.4.3 Effect of undernutrition on the foetus**

Although the maternal body can mobilize nutrient reserves from body tissue to maintain gestation (Gao et al., 2007, Knight, 2001), both nutrient restriction of the adult dam and over nourishment of the adolescent dam during gestation, suppresses placental cell proliferation and vascularity.

Nutrition for the foetus is vital for skeletal muscle development, as muscle fibre proliferation only takes place *in-utero* (Funston et al., 2010a). Maternal under-feeding can cause intrauterine growth restriction and retardation (IUGR), or weak calf syndrome (Zhang

et al., 2002), which results in decreased BW (Bell, 2005). Furthermore, restricted protein or energy (40% lower than the control) during late gestation in goats limited development of the gastrointestinal tract of the foetus, especially the small intestine (He et al., 2013). In late gestation of sheep, the growth restriction of the foetus has shown to delay lung development from the *in-utero* period to 8 weeks after birth (Maritz et al., 2004). Other disorders as a result of malnutrition of the dam include alteration of gene expression in foetal muscle and adipose tissues (Peñagaricano et al., 2014), perturbations to renin-angiotensin homeostasis, kidney disorders and adult hypertension (Ryan et al., 2003, Woods et al., 2001). Gao et al. (2013b) reported severe maternal undernutrition in ewes during late gestation caused negative impacts on foetal body weight, body size and immune status.

Several studies have investigated the impact of energy restriction on the dam and offspring. Corah et al. (1975) reported that pregnant heifer cows fed 65% of their calculated energy requirements during the last 100 days of gestation lost 5.8 kg of their live weight compared to the control (+36.1 kg) with subsequent adverse effects on their reproductive capacity. Importantly, the heifers from the low plane of energy produced calves with 13 kg lower BW and had increased morbidity and mortality rates (10% compared with 3%). Gao et al. (2012a) reported that reducing the maternal energy density (in the form of net energy for lactation;  $NE_L$ ) from 6.48 MJ/kg of DM to 5.25 MJ/kg of DM, during the last 21 days of gestation, had little effect on cow live weight. However, concentrations of plasma glucose, non-esterified fatty acids (NEFA), and beta-hydroxybutyric acid (BHBA) were changed. Also, there was a reduction in BW (43.9 kg compared with 39.2 kg) and physiological structure; body height, body length, thoracic girth, and umbilical girth, of the calves from the low energy maternal group. A similar response to nutrient restriction was observed in goats. He et al. (2013) reported decreased BW, body length, weight of the abomasum and small intestine, thymus and heart of kids born to dams which were restricted by up to 40%. However, it was also reported that after six weeks, the compensatory growth of the kids from a restricted mother reduced the effects of treatment by 13% (He et al., 2013).

Dams fed a low protein diet produced calves with low accumulated protein in the carcass (Bell et al., 2000). Quigley and Drewry (1998) found that cows fed inadequate protein intake during late gestation resulted in calves with a high rate of morbidity and mortality. Maternal protein restriction (50% of NRC requirement) during mid and late gestation showed a significant decrease of total AA in maternal and foetal plasma by 8.8%

and 8.3%, respectively (Kwon et al., 2004). Bryant and Pirat (2014) compared maternal diets differing in protein (11% CP compared with 12-13%) but high energy (160 MJ ME/cow/day). They found that when feeding either kale or FB, BW tended to be lower in the low protein treatment, but post-weaning growth was not negatively affected. Those results raised concerns that feeding late gestation cows on crops with more severe protein restrictions, chosen by farmers for economic and environmental reasons, may have negative effects on calf development. More research is required to validate these concerns.

#### **2.4.4 Colostrum and immunoglobulins**

Colostrum is the first milk secreted after a cow has calved. It is an essential source of nutrients, growth factors and immunoglobulin (Ig) for the neonate (Gulliksen et al., 2008, Conneely et al., 2013, Roche et al., 2015). Other nutrients measured in colostrum include fat-soluble vitamins, water-soluble vitamins and minerals. The concentration of these essential nutrients is dependent on maternal reserve status, diet and season (Kehoe et al., 2007b).

Several factors, especially nutrition during gestation are correlated with bovine colostrum IgG, including breed (Muller and Ellinger, 1981, Funston et al., 2010a), parity (Pritchett et al., 1991, Gulliksen et al., 2008, Kehoe et al., 2007b), length of the dry period (Pritchett et al., 1991), BCS during the dry period (Shearer et al., 1992), weight of the calf (Conneely et al., 2013), volume or weight of colostrum produced (Conneely et al., 2013, Pritchett et al., 1991) and order of colostrum milking, i.e. first versus second milking (Jaster, 2005). However, storage conditions, i.e. temperature and time of storage did not affect the concentration of colostral IgG (Cummins et al., 2016). Cows with heavier calves produced more colostrum (Conneely et al., 2013).

Immunoglobulin is a plasma protein, comprising of 5 classifications: IgG, IgM, IgA, IgD and IgE. Immunoglobulins are produced from lymphocytes in the bloodstream of animals in response to foreign antigens and play an essential role in the immune response to pathogenic organisms, providing resistance to disease (Conneely et al., 2013, Lora et al., 2018). In dairy cattle, three types of Ig have been found in the colostrum: IgG, IgM and IgA, with IgG accounting for 85% to 90% of the total (Kehoe et al., 2007b, Pritchett et al., 1991, Nowak et al., 2012). IgG has two subtypes: IgG<sub>1</sub> and IgG<sub>2</sub> and typically, in dairy cattle, the

concentration of IgG<sub>1</sub> is around 85% of total IgG (Kehoe et al., 2007b, Le Cozler et al., 2016). The IgG concentration is considered to be a useful indicator of colostrum quality, which is internationally defined as being of high quality when the concentration is greater than 50 g/l of IgG (Conneely et al., 2013, Gulliksen et al., 2008, Godden, 2008).

The concentration of IgG in colostrum and serum can be evaluated by Radial immunodiffusion (RID) (Jaster, 2005) or Turbidimetric immunoassay (TIA) (Conneely et al., 2014, Helander et al., 2015). Both laboratory procedures are considered as the reference standard, with high correlations to each other (Figure 2.3), but do, however, require a skilled laboratory specialist (Quigley et al., 2013). The procedures are time-consuming (approximately 18 to 24 h to determine the results) and expensive (Hernandez et al., 2016, Morrill et al., 2013). As a result, this technique is impractical to use for the failure of passive transfer (FPT) of immune determination in neonates (Hernandez et al., 2016). Total serum protein, as a routine blood measurement parameter has also been used as a proxy for IgG in calves (Cuttance et al., 2018). Recently, refractometry, which uses an inexpensive Brix refractometer, is reported to be a reliable, practical procedure to estimate IgG concentration in colostrum and serum (Bielmann et al., 2010, Quigley et al., 2013).

In principle, a refractometer can estimate the concentration of a substance in a solution by its light refraction properties. Total protein in a solution can refract light and be evaluated. IgG is the most significant source of protein in colostrum and serum and therefore measuring total protein in these solutions can result in a value which is highly correlated with IgG concentration (Morrill et al., 2012, Morrill et al., 2013). The Brix percentage was highly correlated with IgG analyzed by RID and TIA ( $r$  between 0.71-0.93) (Quigley et al., 2013, Deelen et al., 2014), for either fresh or frozen samples (0.71 and 0.74) (Bielmann et al., 2010). Similarly, Morrill et al. (2012) reported that freeze-thaw cycles had no significant effect on the relationship between IgG concentration and refractometry.



**Figure 2.3 Regression of colostral IgG indicated a high correlation ( $P<0.01$ ) between the Radial immunodiffusion (RID) and Turbidimetric immunoassay (TIA) (Quigley et al., 2013)**



**Figure 2.4 Correlation between the Brix percentage reading of IgG for frozen colostrum samples from digital and optical refractometer and the Radial immunodiffusion (RID) (Bielmann et al., 2010)**

Either optical or digital refractometers can be used to evaluate FPT (Elsohaby et al., 2015, Biemann et al., 2010) with a similar correlation (Figure 2.4). However, the digital method is more convenient and reduces the problem of a blurred band from the high-fat content in colostrum (Ishler et al., 2016). Furthermore, due to an upper limit of the optical refractometer on the scale, the tail of the distribution might be reduced (Biemann et al., 2010). Consequently, reports of colostrum quality with respect to IgG can be variable depending on the method used to measure it, in addition to the timing of measurement. The impact of dam nutrition on colostrum quality is less clear however. Quigley and Drewry (1998) reported that a reduction of immunoglobulin G (IgG) absorption could be found as a result of low protein feeding during the dry period even though the concentration of colostrum Ig was not directly affected by dietary CP. This finding has important implications for dairy production systems which seek to restrict protein feeding in late gestation if the risk is associated with compromised passive immunity. There is limited information regarding the effect of crop feeding to late gestation cows on colostrum quality and transfer of passive immunity.

## **2.5 Passive immunity and health**

### **2.5.1 Health**

The Ministry of Primary Industries of New Zealand (MPI, 2017) reported that the three most common diseases in dairy calves are enteritis (inflamed gut), inflamed umbilical's and scours. The first two diseases are caused by pathogen infection and can subsequently lead to scours. Scours, or diarrhea, is a common health problem in newborn calves (especially during the first three weeks of life) (Lorenz et al., 2011), this problem is defined as increased water movement through the digestive tract. The scouring calves have an increased amount of soft or watery faeces and increased frequency of defecation, causing a loss of body fluids, minerals and therefore dehydration (Bazeley, 2003). The depressed calves experience weight loss, weakness and possibly death in severe cases. Scours can be classified into two types; infectious and non-infectious (Al Mawly et al., 2015). The infectious scours is caused by pathogens, i.e. bacteria, viruses, protozoa and parasites, and frequently complicated by multilevel pathogens at one time (Lorenz et al., 2011, Abuelo, 2016). While the non-

infectious or nutritional scours is usually the result of stress from a change of routine in feeding program and management, or environmental stress (Drackley, 2007, Muir et al., 2002). Nutritional scouring calves can usually recover in a few days, though under elevated pathogenic levels, the calves can develop infectious scours (Bazeley, 2003). Monitoring and rapid treatment during the first weeks are necessary to prevent severe symptoms and reduce the fatality rate in scouring calves (Muir et al., 2002, Cuttance et al., 2017).

### **2.5.2 Passive immunity**

Neonatal calves are born agammaglobulinemic (Conneely et al., 2014). Colostrum is the primary source of immunoglobulins to provide passive immunity (Gulliksen et al., 2008; Mann et al., 2016). As a result, immediate intake of good quality colostrum is necessary for reducing neonatal morbidity and mortality, supporting growth and improving long term productivity (Conneely et al., 2013; Jaster, 2005).

Newborn calves have hypogammaglobulinemia as the maternal antibodies are unable to cross the placental structure and the calf is born without an immune system (Borghesi et al., 2014). Although the calf starts to develop an active immune system within days of being born, the immediate protection of the calf from pathogens is through passive immunity and transfer of antibodies in colostrum produced by the dam. This primary immunodeficiency affects calf health and survival which is also associated with economical cost and welfare of dairy farms (Lora et al., 2018, Atkinson et al., 2017). In pastoral systems like New Zealand, calf management practices are to promote calf health include allowing newborn calves to remain with their dams for 12-24 hours to encourage suckling (Cuttance et al., 2018), and using good hygiene practices in rearing facilities to minimize exposure to harmful pathogens (Cuttance et al., 2017).

### **2.5.3 Passive immunity transfer**

To achieve passive immunity, calves should, within 6 hours of birth, receive at least 4 L of colostrum with IgG concentrations >50 mg/mL (Morrill et al., 2012). Immunoglobulin IgG is absorbed through the gut and passed to the bloodstream and is crucial for passive immunity transfer before the calf can synthesize endogenous antibodies (Morrill et al., 2012). The

failure of passive transfer of IgG in neonatal calves increases the pathogen susceptibility leading to diseases and increases in morbidity and mortality (Bielmann et al., 2010). Effective transfer of passive immunity is determined as calf serum IgG concentration >10 mg/mL at 24 to 48 h after birth (Godden, 2008, Morrill et al., 2013). Consequently, feeding colostrum with higher IgG concentration to calves has resulted in more significant serum protein and IgG concentration (Jaster, 2005). Villarroel et al. (2013) Investigated factors affecting serum total protein (TP) and IgG concentration, found differences due to age and breed. Between breeds, they found Jersey calves had a significantly higher concentration of TP and IgG than those in Holstein calves. Furthermore, concentrations of both TP and IgG have a similar maximum concentration at 2-3 days of age which decreased by around 0.07 g per day of TP and 74 mg/dL per day of IgG (Villarroel et al., 2013).

External and internal factors may affect the concentration of colostrum IgG. The external factors include the parity of the cows, BCS and bodyweight of dams related to nutrition during late gestation. Time post-calving has a negative correlation with the quality of colostrum, every hour post-calving, colostrum IgG has shown to be reduced by 1.1% (Conneely et al., 2013). The internal factor is the position of the teat, with reports of differences in IgG level between the front and hind teats (Le Cozler et al., 2015, Gomes et al., 2011b). However, the time during a milking event (each minute from start to the end of each milking) did not alter the level of IgG for the pool colostrum of each cow (Le Cozler et al., 2015).

## **2.6 Maternal nutrition and postnatal development**

Maternal undernutrition not only compromises foetal development but may also affect subsequent growth. Replacement heifers are a significant investment and because of this, a potential source of environmental wastage if target outcomes are not achieved. Expenses in raising dairy heifers are about 25% of production cost with substantial resources, i.e. feed, labour and facilities (Akins, 2016). As a pre-ruminant on a milk-based diet, the relative weight gain and body development during the pre-weaning period are the highest compared to other stages of life (Akins, 2016). Any prolonged severe growth retardation in early life can result in adverse effects on long term subsequent growth and productivity (Greenwood & Cafe, 2007).

Several studies reported that gestational nutrient status affects foetal tissue and organ development and the result can be exposed to the new-born through postnatal life (Duarte et al., 2013, Gao et al., 2012a). The gastrointestinal tract (GI tract) including the small intestine is known as a highly plastic tissue and can be changed by nutrient intake and physiological state throughout the animal's lifetime (Johnson et al., 1990, Meyer and Caton, 2016). Meyer and Caton (2016) stated that in mid to late gestation nutrition altered foetal and postnatal small intestine growth and development, digestive enzyme activity and gene expression in lamb and cattle offspring. The small intestine is a crucial system for immunoglobulin absorption in newborn calves (Duarte et al., 2013). Poor intrauterine nutrition or IUGR also found altered small intestine mass, villi morphology and gene expression of the offspring in ruminants (Meyer and Caton, 2016, Duarte et al., 2013).

### **2.6.1 Gastrointestinal tract development and growth**

The digestive tract of the pre-weaned calf is not fully developed and does not function as a developed rumen (Heinrichs and Lesmeister, 2007). The ability to digest and utilize solid feed is typically low in young calves (Khan et al., 2016). Consequently, nutrients from liquid feed, especially metabolizable protein and energy (Akins, 2016, Heinrichs and Lesmeister, 2007), and intake level (Van Amburgh and Drackley, 2007, Ganchev et al., 2015) are the major factors which impact on the growth response of the calves. Energy intake drives protein requirement and growth rate (Bartlett et al., 2006). Weight gain and growth rate are the outcome of muscle and skeletal growth with minimal fat deposition (Akins, 2016). To reach the optimum growth response, both protein and energy intake need to be equally adequate. High allowance with low protein content in calf milk replacer (CMR) can result in excess fat gain rather than muscle growth. In contrast, high protein CMR at lower allowance leads to inadequate energy intake to metabolise proteins for muscle growth (Akins, 2016, Van Amburgh and Drackley, 2007).

To optimize calf growth and economics, dairy farmers typically adopt feeding regimes which encourage early solid feed intake to promote rumen development. Calves are capable of consuming 20% of their weight in milk (>10 litres per day or 3% of live weight as dry weight). Also, it is believed that the capacity of calf abomasum is about 2 litres, and the excess milk will enter or backflow into the rumen (Van Den Borne et al., 2004). Some

previous studies reported that calves could drink milk up to 12 L/day *ad libitum* from a feeder (de Passillé et al., 2011) or in small 8-12 meals/day from their dam (Eckert et al., 2015, Reinhardt and Reinhardt, 1981). A radiography study revealed that the calf abomasum has high distention ability. Dairy calves (19-23 days old) fed more than 6 litres in one meal, show an increase of approximately 13% in body weight, without demonstrating any discomfort, or with no radiographed digestive injuries being observed (Ellingsen et al., 2016).

Holstein calves offered *ad libitum* milk naturally consume approximately 10-12 L of whole milk (equivalent to 20% of BW) (Jasper and Weary, 2002, Khan et al., 2011). Further, calves fed high allocation of milk replacer showed greater growth rate compared to low milk replacer allowance during the pre-weaning stage (Bach et al., 2013). In contrast, some studies reported that calves offered limited milk feeding typically showed hunger behaviours and also resulted in reduced growth rate, behaviour and welfare problem (Khan et al., 2011). However, more commonly in New Zealand, dairy calf rearing practices feed 4 to 5 litres per day or equal to 10% of the calves live weight. This practice is regarded as restricted feeding, with the purpose to encourage solid feed consumption, rumen development and reduce rearing costs (MacPherson et al., 2016, Khan et al., 2011). Calves fed restricted diets have reduced growth rates compared with calves on high allocation or *ad libitum* diets. For example, Rosenberger et al. (2017) reported that 5-17% increased pre-weaning ADG related to higher milk allowances from 6 to 10-12 L/calves/day. Similarly, Morrison et al. (2009) who reported 19% higher LW gain in pre-weaning calves fed CMR at the rate of 10 L compared to 5 L/day ( $P<0.001$ ).

### **2.6.2 Feed intervention to remedy *in-utero* growth retardation**

Several studies indicate that nutrient intervention to increase protein and energy intake during pre-weaning can promote growth (Chester-Jones et al., 2017), increase body size (Davis Rincker et al., 2011), improve immune function (Khan et al., 2011), development of mammary gland (Geiger et al., 2016) reducing the age at first calving and milk yield in the first lactation (Soberon et al., 2012, Khan et al., 2011). Higher live weight and growth rate pre-weaning correlated to high milk yield at first lactation. Every 1 kg of increased average daily growth (ADG) during the pre-weaning period correlated with an increase in milk yield

in the first lactation from more than 400 heifers (Chester-Jones et al., 2017) to 1,000 kg (Soberon et al., 2012).

Hill et al. (2008) also reported the potential of high feed volume on increasing stature measurements of calves. Furthermore, during eight weeks of the pre-weaning stage, the mammary glands were found to grow at a greater rate than other tissues (Esselburn et al., 2015) and more likely to have a greater response to external factors such as feeding (Kesser et al., 2017). Thus, higher pre-weaning growth from intensive feeding was noted as a positive effect on long term milk production and lactation related to the increased number of cells available for milk synthesis and mammary parenchyma mass (Soberon et al., 2012, Davis Rincker et al., 2011, Brown et al., 2005a, Brown et al., 2005b, Roche et al., 2015).

## **2.7 Maternal nutrition and lifetime productivity**

Maternal nutrition also impacts on lifetime productivity (i.e. reproductive and milk production) of the offspring (Funston et al., 2010a, Wallace et al., 2011, Wu et al., 2006). Poor intrauterine nutrition results in IUGR, causing a permanent stunting effect on long-term growth and impairs health and performance (Wu et al., 2006, McCoard et al., 2017). The intrauterine nutrition during the period of foetal development induces changes in gene expression (Zeisel, 2009) which consequently effects lifetime performance (Wu et al., 2006). Even though the mammary gland of the bovine foetus is generated in early gestation, the most rapid growth occurs from the 7<sup>th</sup> month by enlargement of the mammary fat pad and development of other mammary tissues and systems (Rowson et al., 2012).

Further, several studies reported the alteration of organs and metabolism from the *in-utero* nutritional effect, mainly related to insulin metabolism and glucose tolerance (Bell et al., 2000, Guilloteau et al., 2010), skeletal muscle growth and development (Du et al., 2010, Paradis et al., 2017), GI tract (Meyer and Caton, 2016, Guilloteau et al., 2010, Trahair et al., 1997), adipose tissue (Symonds et al., 2016, McCoard et al., 2013, Wallace et al., 2015). These organs and tissues are crucial for early life growth and health, and further influence growth, reproductive and milk production in later life (Meyer and Caton, 2016, Wu et al., 2006) and the alteration may lead to trans-generational epigenetic programming (Paradis et al., 2017). Wallace et al. (2011) and Vi et al. (2004) also reported that adverse effects of *in-utero* malnutrition on the metabolic phenotypes of the offspring, such as

abnormal glucose metabolism, might be improved by postnatal nutrition management in lambs.

Lower BW (28.6 compared to 38.8 kg on average), a typical sign of IUGR, can reduce postnatal growth, metabolism, and health throughout life in beef cattle (Greenwood and Cafe, 2007). Low BW calves also usually have low growth rates and grow to be smaller heifers with a higher risk of failing to reach puberty in an acceptable time and subsequent poor milk yield (Roche et al., 2015). Further, beef heifers fed 20% less dry matter between weaning breeding tended to reach puberty 5 d later than well-fed heifers (Freetly and Cundiff, 1998).

Martin et al. (2007) reported that 42% CP supplementation (a mix of sunflower meal, cottonseed and urea) at a rate of 0.45 kg/d/cow, three times/week, in beef cows during late gestation had positive effects on weight at puberty and reproductive capability of heifer progeny. However, nutrition of the dams did not influence heifers' average calving date, calving difficulty, and calf BW.

## **2.8 Conclusions**

Maternal nutrition during late gestation is important for the *in-utero* foetus because the majority of foetal growth and development happens in this stage. Restricted protein can lead to negative impacts on the cow herself and the offspring in many aspects including growth, immunity and health and lifelong production. Late gestation cows then require increased metabolisable protein and energy to ascertained adequate nutrient availability. However, in NZ pasture-based systems, late gestation cows are wintered on low protein forages (i.e. fodder beet), with supplements (i.e. maize or lucerne silage) used to raise the nutrient intake; DM, ME and CP. Feeding low CP fodder beet to late gestation dairy cows has shown to be a cost effective, adoptable option for farmers. However, accentuating low protein intake to reduce urinary N losses maintain knowledge gaps of this feeding practice:

- 1) It is important to understand how feeding low CP fodder beet with low or high CP silage supplement may alter response in late gestation cows, health status and subsequent reproduction and lactation.

2) There is limited information about the impact of restricted protein during late gestation on the fetal growth and development, colostrum quality and passive immune transfer to newborn calves.

3) What are the carryover effects of maternal restricted protein on life time productivity of heifer progeny, and to what extent does pre-weaning nutrition impact their life time performance.

## Chapter 3

### The effect of late gestation diet on the dam

#### 3.1 Introduction

Throughout gestation the nutritional requirement of the dam increases, reaching peak *in-utero* demands in the final 3-4 weeks before parturition (Dunlap et al., 2015, Moisé et al., 2015). In Chapter 2 (section 2.4), nutritional risk factors were identified where undernutrition of the dam during late gestation can influence her energy status and subsequent lactation performance. In New Zealand's temperate dairy farm systems, cows are grazed outdoors year-round, often with low protein feeding regimes during late gestation (Edwards et al., 2016, de Ruiter et al., 2019). Farmers target low protein feeding regimes for several reasons which include: avoiding metabolic problems at calving, reduce feed costs and to reduce nitrogen (N) surplus by offering low N feeds (Moisé et al., 2015, Edwards et al., 2014a). Because of its affordability, high yield, high digestibility and low protein, the feeding of fodder beet (FB) to late gestation cows, has become commonplace, particularly in South Island dairy systems (Edwards et al., 2016).

In New Zealand pastoral systems, late gestation, occurs during the winter period with the start of the calving period targeted for the end of July, early August. At the end of the previous late lactation and subsequent dry period, farmer decision making will balance priorities with regards to reducing environmental impacts of dairy systems and ensuring animal welfare with regards to the cows having adequate body condition to support energy demands of early lactation. Feeding high energy and low protein feeds such as fodder beet is one way of achieving this balance. During late gestation, a dairy cow requires a ration with a metabolisable energy (ME) concentration of 11.0 to 11.5 MJ/kgDM and a crude protein (CP) concentration between 12 and 14% of the dry matter (DM) (AFRC., 1993, NRC, 2001). This ration should provide total metabolisable protein (MP) of approximately 300-340 g MP/day, including 95-100 g MP/day for gestation (AFRC., 1993). Previous studies show that despite low dietary protein, cows are able to gain weight or body condition score (BCS) on generous allocations of winter feed (>120 MJ ME/cow/day) because the high dry

matter intake (DMI) enables the animals to meet their MP requirements (gMP/day) (Edwards et al., 2014b, Greenwood et al., 2011, Cheng et al., 2018).

For many farmers achieving a target BCS of 5.0 for calving can be attained during late lactation prior to dry off, so feeding requirements during the late gestation winter period can be reduced to save costs and reduce N losses. Edwards et al. (2014b) showed that feeding FB could reduce urinary N concentration, but there were no reports showing the effect of FB feeding on urinary N excretion. Furthermore, there is concern that in striving to reduce urinary N losses, farmers will further limit protein - and in some cases energy - supply to late gestation dairy cows and compromise the long term needs of the dam and her progeny. For example, Chew et al. (1984) found that offering 80% of the recommended protein requirements to late gestation cows resulted in approximately an 80% reduction in DMI and 15% reduction in subsequent milk yield and milksolids compared to the control group. More information is required to understand the longer term effects of feeding high energy low protein feeds such as FB.

While many farmers have chosen FB as the main feed source for non-lactating cows during winter (Edwards et al., 2016), there may be limited flexibility to alter this crop as part of their system. However, there is scope to alter supplementation as farmers do not use 100% beet diets. Choice of supplements provides the opportunity to offset nutrient limitations. The purpose of this research was to investigate the impact of supplementing a fodder beet ration with differing protein contents:

- 1) the impact on cow energy status and body condition score,
- 2) the impact on excretion of N in urine and faeces
- 3) the impact on cow milk production during subsequent lactation

## **3.2 Materials & methods**

### **3.2.1 Experimental site and design**

This study was a longitudinal observational experiment to investigate the impact of two late gestation feeding regimes on dam productivity and neonatal development. The study was

conducted at Ashley Dene Research and Development Station (ADRDS) Lincoln University (-43.65 ° North, 172.33 ° East), on Lismore/Balmoral shallow stony loam soil structure (Landcare Research, 2013) between 1 June 2017 and 9 May 2018. Each feeding regime included 95 cows which had previously experienced similar management during the lactation period.

The two feeding regimes were designed to be isoenergetic but not isonitrogenous (AFRC., 1993) with the following composition:

1) The control diet of FB (5 kg DM/cow/day) plus lucerne silage (5 kg DM/cow/day) (moderate crude protein, MCP) offering approximately 120 MJ ME/cow/day, and offering a diet of equivalent to 16 % CP (MCP).

2) A low protein diet of FB (5 kg DM/cow/day) plus maize silage (5 kg DM/cow/day) (low crude protein, LCP) offering approximately 120 MJ ME/cow/day, and offering a diet equivalent to 10 % CP (LCP).

Determination of the energy requirements was based on findings from Roche et al. (2005) and Mandok et al. (2013) who reported that in pastoral systems non-lactating late gestation cows in ambient conditions below thermoneutral conditions require 1.05 MJ ME/kg BW<sup>0.75</sup> for maintenance and pregnancy. This is equivalent to 107 MJ ME/day for a HF x J crossbred cow (live weight of 475 kg). Assuming a combined utilisation of 90% for crop and supplement (Edwards et al., 2014b) allocation of 110% of requirement was estimated to meet ME requirements. As the cows were expected to have reached target BCS of 5.0 at dry off, prior to starting the study, no additional allocation for live weight gain was included in ME requirement calculations.

Determination of protein requirements was based on AFRC calculations for metabolisable protein which in late gestation (220 to 260 days since conception, DCONC), pregnancy and maintenance is between 280-330 g MP/day (AFRC., 1993). This requirement could be met by microbial protein sources if the average dietary crude protein content was 12.5% of the dry matter. Consequently, through management of supplement we aimed to

supply diets 20% above or below requirement for CP% (i.e. diets differing by 40% in protein supply) to achieve a target CP intake of 1.4 or 0.9 kg CP/cow/d.

The crops and supplements were all grown on the experimental property. In October 2016 an irrigated pasture of 2.5 ha (approximately size of 80 x 300 m<sup>2</sup>), was sprayed, cultivated and sown with FB (cv. Rivage) at sowing rate 100,000 seeds/ha. Weed control and fertiliser management was conducted using the standard management guidelines (see Edwards et al. (2014b) for details). The maize for the maize silage was grown under irrigation near the FB area. The farm included a non-irrigated dryland block on which a lucerne crop had previously been established and which was harvested for silage throughout the growing season. The maize was harvested and compressed in a silage stack while the lucerne was harvested into baleage.

### **3.2.2 Animals and management**

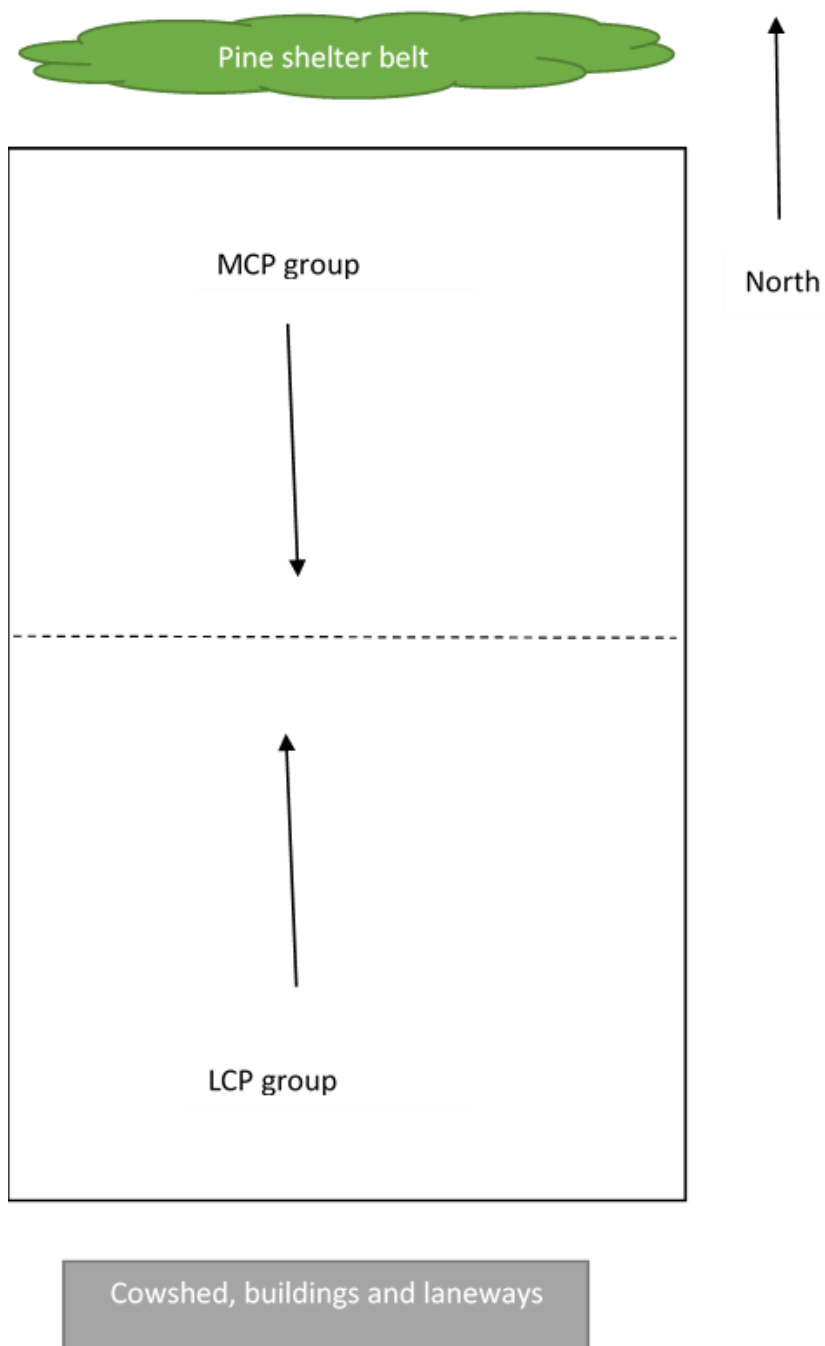
A total of 190 pregnant, non-lactating spring calving Friesian x Jersey dairy cows, with an average LW of 499±4.5 kg, average age of 4.3±0.1 years, BCS of 4.5±0.5, and an average calving date 12<sup>th</sup> August 2017 (±8 days) were used. The cows were allocated to one of two treatment groups using stratified randomisation. First order stratification was based on the estimated mature weight of progeny (stratified into >489.0, 489-521 and <521 kg), followed by the expected calving date (stratified into before 05/08/2017, from 05/08/2017 to 19/08/2017 and after 19/08/2017) and dam BCS (stratified into >5.0, 4.5-5.0 and <4.5).

The experiment continued through 3 periods (343 days from 1<sup>st</sup> of June 2017 to 9<sup>th</sup> of May 2018), from wintering, calving and lactating period. The first 60 day period was the fodder beet feeding period from 1<sup>st</sup> of June to 30<sup>th</sup> of July 2017, where the cows were wintered on experimental diet treatments. The second period was the calving period, from 26<sup>th</sup> July to 25<sup>th</sup> August 2017. The third was the lactation period from August to 9<sup>th</sup> May 2018. The overlap of the periods reflect the transition of a large number of cows from one state to another.

### 3.2.2.1 Non-lactating (dry) period

Although the impact of feeding regimes on cow health was a variable of interest, decision rules in the management of experimental animals aimed to minimize any adverse effects of treatments on cow health. One of the first health risks was related to transition feeding onto FB to avoid acidosis. The experimental cows were already receiving FB during late lactation as a supplement to their base pasture diet, so the allocation of FB was increased from 2.0 kg DM per cow per day by 0.5 kg increments every second day from the 17<sup>th</sup> to 30<sup>th</sup> May 2017, to a final full allocation of 5 kg DM of FB on 1<sup>st</sup> of June. At the same time, allocation of pasture (reduced) and silage (increased) was adjusted to meet daily nutrient requirements and desired treatment diets. Cows in each treatment group were allowed to strip graze from each end of the FB area (Figure 3.1, Picture 3.1). The daily allocation area was determined weekly based on the number of cows, target DMI and crop yield of FB. Although the MCP group grazed in front of a pine shelter belt on the North, the majority of any cold wet weather arrives from the south, so both groups had similar exposure to southerly weather conditions.

Supplement allocation for each treatment was based on the nutritional requirements for fibre, ME and minerals. Forage and supplement samples were collected before the study and analysed by Elementar (Variomax CN Analyser, Analysensysteme GmbH, Hanau, Germany) and by inductively coupled plasma mass spectrophotometry (ICP-MS) to determine the content of minerals: N, Ca, Mg, K, P, Cl, S and Na. The maize silage was mixed with straw at 50 % (as fed) to provide sufficient fibre at the same ME value as the control diet. The supplements for both groups were fed in the paddock each day between 0700 and 0800 h using a silage wagon. To improve utilization, silage was fed in long strips, close to the electric fence to prevent trampling (Picture 3.2). The new allocation of FB was offered at approximately 10.00 h. Dicalcium phosphate (DCP) was added to supplements at the rate of 50 g/cow/day. Water was available *ad lib* in troughs throughout the experiment. Essential minerals (Neville and Scott, 2012) provided in trough water included Copper, Zinc, Cobalt, Iodine, Selenium and Magnesium at a rate of 2.5 mg/cow/day, with additional Mg as magnesium oxide at 60-80 mg/cow/d (Jeff Curtis, farmer operations manager, pers. Comm. 2017).



**Figure 3.1 Fodder beet paddock layout showing the location of treatment groups in relation to buildings and trees and direction of break-feeding of crop**

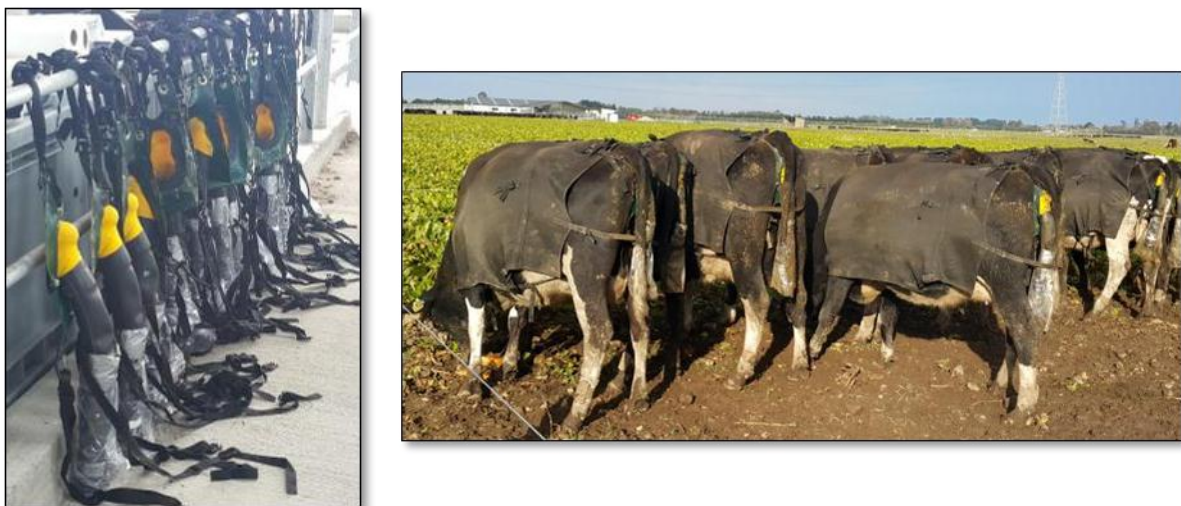


**Picture 3.1 The experimental cows in the fodder beet paddock**



**Picture 3.2 The experimental cows were eating maize silage supplement**

To determine the effect of diet treatment on urination behaviour 10 cows balanced for live weight, from each treatment, were selected for urine measurements from 19<sup>th</sup> – 25<sup>th</sup> June 2017. The urination behaviour study was a joint collaboration between this PhD and the Forages for Reduced Nitrate Leaching project (Urine sensor work carried out by AgResearch). The selected cows were separated into a small area of the same paddock as the larger group, and diet was offered with the same allowance. In brief, the urine sensor was glued around the vulva of the cow and retained on the cow by a harness (Picture 3.3). The urine volume of each urination event was measured and recorded using a calibrated pressure sensor. The concentration of urine nitrogen was measured using a refractive index (RI) sensor. Data was stored in a data logger and downloaded via a wireless network system (as described in Shepherd et al. (2017)). This data was used to evaluate the impact of feeding regime on N loss.



**Picture 3.3 AgResearch urine sensor assembly (left) and the experimental cows wearing AgResearch urine sensors at another end of the fodder beet paddock (right)**

### **3.2.2.2 Calving period**

From early July, cows were monitored daily for physical signs of parturition, based on visual assessment of tightness of the udder and swollen vulva (around 7-10 days before calving). At which point those cows – referred to as ‘springers’ -were transferred from the FB paddock onto pasture by farm staff. Although FB was no longer provided, cows continued to be fed the same supplement (including the DCP at the same rate until calving). Pasture allocation for springer cows was approximately 40-60 m<sup>2</sup>/cow/day with *ad lib* access to clean water. Once the cows calved the cow and calf were collected within 24 hours (see Chapter 4 part 4.2.2.1 for details on calf management) and taken to the cow shed and yards. Cows were milked and after their first post calving milking were transferred to a new paddock, referred to as the ‘colostrum mob’ – for a further 4-5 days. Colostrum cows were milked once per day and all cows received the same feeding management during this period. After five days all cows were milked twice per day until dry off the following autumn.

### **3.2.2.3 Lactation period**

Prior to calving cows were pre-allocated to participate in a farmlet study investigating the effects of using plantain pastures in farm systems (see Al-Marashdeh et al. (2021) for details). Of the 190 cows in the maternal nutrition study, 166 were included in the farmlet study, 14 cows were retained within a non-experimental herd and the remaining 10 cows were excluded from further data collection as they calved outside the experimental period. For clarity the allocation of cows from the current study into the farmlets are given in Figure 1.2 (page 6). In the farmlet study, the three feeding systems were: 1. high input, ryegrass system (HIRG); 2. low input ryegrass system (LIRG); 3. low input ryegrass and plantain system (LIRG+PL). The lactating cows were managed according to the decision rules of each farm system, and milk production was monitored by monthly herd testing.

### 3.2.3 Sampling and analysis

#### 3.2.3.1 Crop, supplement and pasture

##### *Fodder beet*

Pre-grazing FB DM yield was determined weekly (total 9 measurements conducted) by harvesting all bulbs along 3 x 6m transects per treatment (total 6 sites per measurement) as described by Gibbs (2011). Fodder beet sampling procedure is shown in Appendix 2. Briefly, fresh weight was recorded in the field after all soil around the bulb was removed. Firstly, the whole bulbs with leaves were weighed, and then bulb and leaf were separated, weighed and recorded separately. Additionally, two intact FB plants were randomly collected from each site to and were washed to removed soil and dead material (i.e. mushy particle). After that, the sample of FB bulb or leaves were chopped, mixed together and subsampled (100-200g) again. The first subsample was oven-dried at 90°C for 48 h for determination of DM% and calculation of DM yield. A second subsample was freeze-dried, ground through a 1 mm sieve (ZM200, Retsch) analysed in triplicate for dry matter, ash, digestible organic matter in DM (DOMD), crude protein (CP), water-soluble carbohydrate (WSC), neutral detergent fibre (NDF) and acid detergent fibre (ADF) by wet chemistry analysis (AOAC, 1990). Means across triplicate samples were collated, except where variance of more than 10% occurred within triplicate resulting in removal of outlier or exclusion of the sample replicate. Metabolisable energy (ME) was calculated as MJ ME/kg DM = 0.16 x DOMD (CSIRO, 2007).

Post-grazing DM yield was determined weekly by harvesting all residual FB in three randomly placed 1 m<sup>2</sup> quadrats in areas that had been grazed in the previous week. All FB residues within the quadrat were collected and washed to remove soil, faeces and dead material. The sample was weighed fresh, oven-dried at 60°C for 48 h and re-weighed. The percentage utilisation of FB was determined (pre DM yield – post DM yield/pre DM yield x 100) and used to estimate apparent intake (kg DM/cow/day, % utilisation x allowance).

##### *Supplement*

Supplement composition was measured weekly by weighing the mass of maize or lucerne silage in three random areas of 1 m<sup>2</sup> in each treatment group before it was eaten by cows (Picture 3.4). The sampling areas were marked for post-feeding sampling the day after

allocation. Approximately 100 g fresh weight of each sample was collected to determine the DM content by oven drying at 60°C for 48 h. A second subsample was kept frozen at -20°C before being freeze-dried then ground through a 1 mm sieve (ZM200, Retsch, Haan, Germany) and kept for chemical analysis by near-infrared spectrophotometer (NIRSystems 5000, Foss, Maryland, USA). Metabolisable energy (ME) was calculated as MJ ME/kg DM = 0.16 x DOMD (CSIRO, 2007). Supplement utilisation was determined by measuring silage refusals (Picture 3.5) the day after allocation. All refusals in the three pre-sampling areas of each group were collected and weighed fresh in the field at that time. The refusals were then brought to the lab, washed and left to drain for 15 minutes before weighing again (fresh weight). Dry matter content of the clean sample was determined by oven drying at 60°C for 48 h, to calculate utilisation percentage. On the day of post-feeding sampling, the new supplement was offered on the other side of the paddock to avoid mixing of pre and post-feeding sample. Supplement fed to springer cows on pasture was also weighed and sub-sampled for determination of nutrient intake.



**Picture 3.4 The supplement sampling**



**Picture 3.5 The supplement post feeding**

### *Pasture*

In the pasture areas where LCP and MCP groups were transferred prior to calving, a folding rising plate meter (Jenquip, New Zealand) was used to measure pre-grazing pasture yield for the purpose of allocation. Pasture mass estimation was based on the manufacturers equation for winter pasture:  $\text{kg DM/ha} = (\text{RPM clicks} \times 140) + 500$ .

To determine nutrient intake on pasture, apparent intake was first determined by collecting quadrat cuts pre (n=24) and post (n=24) grazing. The pre and post grazing samples were cut to grazing height (3.5 cm) using electric hand shears. The samples were oven-dried at 60°C for 48 h and dry weight recorded. Nutrient content was determined from snip samples which were collected weekly at 1200 h by harvesting pasture to grazing height (cm) before grazing (n = 12 samples/treatment). All pasture samples were homogenised and subsampled with the first subsample used for DM content analysis, and the second subsample was used for chemical composition by NIRS (see section 3.2.3.1). A third subsample was assessed botanical composition of ryegrass and white clover.

### 3.2.3.2 Animal measurements

The key variables of interest included measures which reflected the energy and protein status of the dams at a specific time point during gestation.

#### *Energy and protein status*

To determine the effect of maternal nutrition feeding regimes on energy and protein status of the animal; live weight, BCS and blood parameters were measured. To indicate body condition change, all of the cows were individually assessed for BCS on two occasions during the study period; 30<sup>th</sup> May 2017 and 26<sup>th</sup> July 2017 (at the beginning and after eight weeks). A certified scorer carried out the assessment using the 'hands-on' New Zealand scoring (1-10) (Roche et al., 2009). Live weight was measured concurrently using portable scales.

#### *Blood parameters*

Blood sampling took place before a new FB allocation between 0800 and 1000 h, on three occasions during the study. The sampling dates were at the beginning of the study at 31 weeks (208 days since conception, DCONC) of gestation (30<sup>th</sup> May 2017 or week 0), at 35 weeks (235 DCONC) of gestation (the end of June or week 4 of the study) and prior to calving, 38 weeks (265 DCONC) of gestation (26<sup>th</sup> July 2017 or week 8 in the study) for expected mean calving date of 12 August. A random selection of 20 cows from each treatment was moved to nearby yards, and vet race and 10 ml of blood via coccygeal vein were collected. The blood samples were collected into 1) a fluoride oxalate tube (coated with FE Sodium Fluoride/K3EDTA) for plasma glucose, and 2) a plain tube (without anticoagulant) for serum non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA) and urea. All samples were placed on ice immediately after collection and later centrifuged at 3000 x g for 10 min at 4°C. Plasma/serum was collected into clean tubes, then frozen to -20°C. Before analysis, plasma/serum samples were thawed at 4°C. The serum was analysed for BHBA and NEFA (Cat. No. FA115) by Randox Daytona analyser (Rx Daytona, Randox, Ireland) and plasma glucose using hexokinase method (Cat. No. GL 3816). Serum urea concentration was determined by Daytona using an enzymatic kinetic method (UR 3825). Dam serum amino acid (AA) profile was analysed by the high-performance liquid

chromatography (HPLC) analysis (an Agilent 1100 series, Agilent Technologies, Walbronn, Germany).

#### *Excretion of N in urine and Faeces*

Faeces and urine were sampled from 20 random cows in each group (total 40 cows, the same as those blood sampled) at week 0, 4 and 8 of the study, following voluntary excretion or manual stimulation by rubbing the vulva (Greenwood et al., 2012). Urine (40-70 ml) was acidified by adding sulphuric acid (1 ml of 6 m H<sub>2</sub>SO<sub>4</sub>) to a pH of approximately 4.0, to prevent volatilization. Then all samples were stored frozen to -20°C until subsampling at the end of the calving.

Individual faecal samples were thawed in the fridge and mixed thoroughly to gain two representative subsamples for analysis. The first subsample was weighed and oven-dried at 65°C for 48 h then reweighed to determine DM %. The second subsample (approximately 40 g/sample) was freeze-dried at 0.5 mbar (supplier; Cuddon Limited, New Zealand Model E. D. 5.3), then ground through 1 mm sieve (ZM200, Retsch, Haan, Germany). The ground faeces were analysed for N percentage by combustion of the sample under oxygen supply and high temperature using the Varimox CN analyser; Elementar (supplier; Elementar Analysensysteme GmbH; Germany).

Urine samples were thawed overnight and a subsample of approximately 2 ml analysed for urine ammonia, urea, creatinine concentration and total N%. The total N% obtained by combustion using Variomax CN Analyzer. Urine ammonia (NH<sub>3</sub>) concentration was determined by an enzymatic UV method using Radox ammonia kit (AM 1054), creatinine and urea concentrations were determined by Daytona (CR 3814 and UR 3825).

#### *Milk yield*

To determine the effect of late gestation nutrition on dam productivity (within the season), milk yield (kg) was automatically measured and recorded at each milking (Waikato milking systems, Hamilton, New Zealand). Milk samples from all cows were collected monthly between September 2017 and May 2018. Samples were then analysed by Livestock Improvement Corporation Ltd (LIC; Christchurch, New Zealand) for somatic cell count,

percentage of milk protein, fat, lactose and urea. Any sick cows recorded as being treated were excluded from the analysis.

### **3.3 Statistical Analysis**

To compare the effect of wintering treatments on cow blood metabolites and AA profile, composition of urine and faecal samples, ANOVA was performed on the individuals at each sampling date. For example: to compare the effect of maternal protein treatment on blood metabolites, maternal nutrition treatment was included as a fixed term and animal as a random term in the GenStat 18.1 (VSN International, Hemel, Hempstead, UK).

For full season milk yield and composition, a repeated-measures ANOVA was performed in GenStat using maternal nutrition treatment as the fixed term, herd test date as the time effect and animal as the random variable. Days in milk was included as a covariate and farmlet system used as a blocking error term. Results presented are means  $\pm$  standard error of mean.

## 3.4 Results

### 3.4.1 Climate conditions

#### *Climate*

The temperature and rainfall during the non-lactating wintering period through to the calving period varied from the overall average of the previous year, 2007-2016 with higher rainfall occurring (Table 3.1). The crop and supplement area (as described in material and method, part 3.2.2.1) became very muddy as a result of wet conditions as shown in Picture 3.6. The minimum mean air temperatures were lower than the long term average. More detail about the climate during the experiments is shown in Appendix 1-2.

**Table 3.1 Climate condition including accumulated rainfall (mm) during year 2017 and previous years of 2006-2017 (values are means  $\pm$  SEM)**

| Period                          | Rainfall (mm)  | Temperature ( $^{\circ}$ C) |                 |                 |
|---------------------------------|----------------|-----------------------------|-----------------|-----------------|
|                                 |                | Minimum                     | Maximum         | Average         |
| 1 June to 30 July 2017          | 162            | 0.2 $\pm$ 0.40              | 10.8 $\pm$ 0.27 | 5.5 $\pm$ 0.25  |
| 1 August to 31 August 2017      | 56             | 0.6 $\pm$ 0.71              | 12.6 $\pm$ 0.43 | 6.6 $\pm$ 0.42  |
| 1 September 2017 to 30 May 2018 | 507            | 7.4 $\pm$ 0.27              | 19.8 $\pm$ 0.33 | 13.6 $\pm$ 0.27 |
| Long term (2007-2016) average   |                |                             |                 |                 |
| 1 June to 30 July               | 137 $\pm$ 20.3 | 1.6 $\pm$ 0.16              | 10.5 $\pm$ 0.11 | 6.1 $\pm$ 0.12  |
| 1 August to 31 August           | 65 $\pm$ 11.1  | 2.9 $\pm$ 0.19              | 12.8 $\pm$ 0.16 | 7.8 $\pm$ 0.14  |
| 1 September to 30 May           | 435 $\pm$ 28.2 | 9.5 $\pm$ 0.08              | 20.3 $\pm$ 0.09 | 14.8 $\pm$ 0.08 |



Picture 3.6 The muddy paddock after the rain (photo taken on 1/07/2017)

### ***Diet composition***

By mixing the maize silage supplement with straw, the fibre content (NDF) of the supplement in the LCP diet was increased from 44% to 55% (DM basis), and ME decreased from 11.0 to 9.4 MJME/kgDM. However, the protein content of maize silage and straw did not differ at approximately 7% (Table 3.2).

**Table 3.2 Nutrient (% of dry matter, DM) and energy (MJ ME/kg DM) composition of straw and maize silage fed to cows in the low crude protein treatment (mean± SEM)**

| <b>Nutrient composition</b> | <b>Straw</b> | <b>Maize silage</b> |
|-----------------------------|--------------|---------------------|
| Organic matter              | 91.9±0.7     | 95.5±0.2            |
| Crude protein               | 7.0±0.3      | 6.7±0.2             |
| Neutral detergent fibre     | 66.8±1.3     | 44.1±1.1            |
| Acid detergent fibre        | 42.3±0.4     | 25.7±0.2            |
| Water soluble carbohydrate  | 5.0±0.9      | 3.4±0.1             |
| Metabolisable energy        | 7.2±0.4      | 11.0±0.06           |

Fodder beet yield, bulb and leaf proportion were similar for each group with leaf:bulb ratios at approximately 0.09:1 vs 0.1:1 in LCP and MCP, respectively. The crude protein and other nutrient composition, including fibre, ME and minerals, were not different for FB fed in the two treatments (Table 3.3). Fodder beet was high in ME, WSC, potassium and sodium content compared with supplements, but low in phosphorus.

Aside from protein content, the maize silage supplement was higher in organic matter ( $P < 0.01$ ), NDF ( $P < 0.001$ ) and WSC ( $P < 0.05$ ) than the lucerne silage, though both silages had similar ME concentration (Table 3.3). Starch content of maize silage was approximately 2.0 % of DM, while lucerne silage had no detectable starch content. Calcium, potassium and sulphur content were greater in lucerne silage than in mixed maize silage ( $P < 0.001$ ) (Table 3.3). The crude protein percentage of lucerne silage was approximately three times higher than the mixed maize silage (Table 3.3), resulting in a higher CP level for MCP of 13.6 % compared to 7.4% in LCP. The combined metabolisable energy of both treatment rations was similar at approximately 11.5 MJME/kg DM.

**Table 3.3 Feed qualities of fodder beet (FB) and supplements (maize silage, MS or lucerne silage, LS) offered to cows in low crude protein (LCP) and moderate crude protein (MCP). Data include means and standard errors of means.**

|                                       | LCP (7.4% CP) |                 | MCP (13.6% CP) |             |
|---------------------------------------|---------------|-----------------|----------------|-------------|
|                                       | FB            | MS <sup>1</sup> | FB             | LS          |
| <b>Crop composition</b>               |               |                 |                |             |
| Bulb (% DM)                           | 91.5 ± 0.4    | -               | 91.2 ± 0.5     | -           |
| Leaf (% DM)                           | 8.5 ± 0.4     | -               | 8.8 ± 0.5      | -           |
| Pre grazing (t DM/ha)                 | 23.1 ± 0.4    | -               | 23.0 ± 0.3     | -           |
| Post grazing (t DM/ha)                | 1.0 ± 0.1     | -               | 0.6 ± 0.2      | -           |
| Dry matter utilisation (%)            | 95.7 ± 0.5    | -               | 97.4 ± 0.6     | -           |
| <b>Nutrient composition (% of DM)</b> |               |                 |                |             |
| Dry matter % (as fed basis)           | 14.7 ± 0.4    | 42.2 ± 1.3      | 16.6 ± 0.2     | 42.2 ± 0.7  |
| Crude protein                         | 7.6 ± 0.7     | 7.3±0.7         | 6.7 ± 0.3      | 20.5 ± 0.3  |
| Organic matter                        | 94.4 ± 0.3    | 55.1±0.7        | 95.4 ± 0.1     | 89.6 ± 0.3  |
| Neutral detergent fibre               | 13.0 ± 0.3    | 53.1 ± 0.7      | 12.0 ± 0.2     | 44.2 ± 0.5  |
| Acid detergent fibre                  | 6.9 ± 0.2     | 31.2 ± 0.5      | 6.4 ± 0.1      | 34.1 ± 0.4  |
| Water soluble carbohydrate            | 59.4 ± 0.9    | 4.6 ± 0.4       | 62.6 ± 0.6     | 3.4 ± 0.3   |
| Starch                                | nd            | 1.8 ± 0.4       | nd             | nd          |
| Calcium (g/kg)                        | 3.6±1.02      | 2.0±0.09        | 3.6±1.04       | 23.3±0.15   |
| Phosphorus (g/kg)                     | 1.7±0.22      | 2.1±0.03        | 1.7±0.22       | 2.9±0.37    |
| Potassium (g/kg)                      | 23.2±3.39     | 5.1±0.20        | 23.4±3.40      | 17.89±0.23  |
| Magnesium (g/kg)                      | 2.3±0.64      | 1.3±0.10        | 2.3±0.65       | 2.6±0.11    |
| Sodium (g/kg)                         | 5.9±1.50      | 0.2±0.01        | 6.0±1.51       | 0.7±0.06    |
| Sulphur (g/kg)                        | 0.8±0.12      | 0.8±0.03        | 0.8±0.12       | 2.0±0.21    |
| Zinc (g/kg)                           | 0.02±0.001    | 0.03±0.002      | 0.02±0.001     | 0.03±0.0001 |
| Metabolisable energy<br>(MJ ME/kg DM) | 13.5 ± 0.03   | 9.4 ± 0.10      | 13.6 ± 0.01    | 9.4 ± 0.10  |

**Note 1)** MS<sup>1</sup> is maize silage mixed with straw

### Apparent intake

There was no difference in crop yield, but cows in the LCP treatment left slightly more residual resulting in a 2 % lower utilization of the FB crop compared with the MCP treatment ( $P < 0.05$ ). Maize and lucerne supplements were offered at 5.0 kg DM/cow/day, and feeding in the paddock resulted in an average wastage of 1.11 and 0.61 kg DM/cow/day equivalent to 78 and 88% utilization for MS and LS respectively (Table 3.4).

**Table 3.4 Nutrient intake (/cow/day) during wintering from low crude protein diet (LCP) and moderate crude protein diet (MCP)**

| Parameter                       | LCP        | MCP        | Difference (%) | P-value |
|---------------------------------|------------|------------|----------------|---------|
| Fodder beet intake (kg DM)      | 4.7±0.07   | 4.9±0.03   | 2.7            | 0.130   |
| Supplement intake (kg DM)       | 3.9±0.07   | 4.9±0.14   | 12.8           | 0.002   |
| Total intake (kg DM)            | 8.8±0.10   | 9.3±0.15   | 5.9            | 0.010   |
| <b>Nutrient intake</b>          |            |            |                |         |
| Organic matter (kg)             | 7.0±0.04   | 8.9±0.13   | 21.3           | 0.005   |
| Crude protein (kg)              | 0.6±0.01   | 1.3±0.02   | 117.0          | <0.001  |
| Nitrogen (g)                    | 102.5±0.11 | 208.2±0.23 | 103.1          | <0.001  |
| Neutral detergent fibre (kg)    | 2.8±0.06   | 2.6±0.04   | -7.7           | 0.786   |
| Acid detergent fibre (kg)       | 1.6±0.04   | 1.9±0.02   | 15.8           | 0.005   |
| Water soluble carbohydrate (kg) | 2.8±0.05   | 3.1±0.03   | 10.7           | 0.003   |
| Calcium (g)                     | 24.5±0.01  | 126.0±0.02 | 414.3          | <0.001  |
| Phosphorus (g)                  | 16.8±0.01  | 21.5±0.01  | 28.0           | 0.001   |
| Potassium (g)                   | 125.2±0.01 | 192.8±0.03 | 54.0           | <0.001  |
| Magnesium (g)                   | 15.9±0.01  | 22.9±0.01  | 44.0           | <0.001  |
| Sodium (g)                      | 27.1±0.01  | 31.3±0.01  | 15.4           | 0.001   |
| Sulphur (g)                     | 7.2±0.001  | 13.4±0.001 | 86.1           | <0.001  |
| Zinc (g)                        | 0.2±0.000  | 0.2±0.000  | 0.0            | 0.075   |
| Metabolisable energy (MJ ME)    | 100.8±1.22 | 107.0±1.59 | 6.2            | 0.065   |

The combined effect of lower utilisation of crop and supplement in LCP was lower total dry matter intake for the LCP cows. The ME intake for cows in MCP was at target requirements and tended to be higher than for cows in LCP which apparently consumed 95% of target ME. While apparent ME intake was at or close to target, apparent crude protein intake differed from planned.

As expected, due to greater CP in the lucerne silage, the protein intake of MCP was higher than in the LCP. However, the actual CP intake for both groups were 7% and 35% below the target of 1.4 and 0.9 kg CP/cow/d for MCP and LCP respectively. For the MCP group apparent CP was close to that expected but the poor utilisation of supplement in the LCP treatment lead to the reduced CP intake. Variation in composition of supplements resulted in organic matter and WSC intake being similar between treatments. Fibre intake was less than 3 kg/cow/day, similar in LCP and MCP. Mineral intake was higher in the MCP, particularly in calcium and sulphur was 415 and 86 % higher than those in LCP (Table 3.4).

On average, cows in LCP were removed from crop and onto pasture  $7.6 \pm 0.9$  days before calving, though the number of days on pasture ranged from 1 to 18 days. Cows in MCP were removed from crop on average  $9.8 \pm 1.1$  days before calving, with a range of 2 to 26 days ( $P = 0.125$ ). The herbage mass and nutritional composition of pre-grazing pasture offered to cows before calving is presented in Table 3.5. Botanical composition was dominated by perennial ryegrass, (*Lolium perenne* L., 78%) and dead material (16%). Clover (*Trifolium repens*) and weeds accounted for 5% of the remaining biomass. A single calibration equation was derived for both pastures to determine herbage mass, resulting in the following equation:  $\text{kg DM/ha} = 94.63 \text{ RPM} + 674.4$ ,  $R^2=88.9$ . Pre grazing pasture yield (kg DM/ha), as determined by the calibrated plate meter showed similar pre graze pasture mass for both groups ( $P>0.05$ ).

However, cows were provided large areas on which to calve, so DM allocation was not restricted. The quality of the pasture provided immediately before calving to the LCP group did not differ to the MCP group (Table 3.5). Further, silage utilisation on pasture was similar to that on crop with greater utilisation ( $P<0.01$ ) of lucerne silage (90.0%) than maize silage (77.6 %).

**Table 3.5 Nutrient composition of pre-grazing pasture for calving cows on a low crude protein diet (LCP) and moderate crude protein diet (MCP)**

| Parameter                            | LCP       | MCP       | P-value |
|--------------------------------------|-----------|-----------|---------|
| Pasture mass (kg DM/ha) <sup>1</sup> | 1,870±183 | 1,562±112 | 0.085   |
| Dry matter % (as fed basis)          | 23.8±2.10 | 23.0±1.83 | 0.760   |
| Organic matter (%)                   | 88.3±0.43 | 87.0±0.34 | 0.048   |
| Crude protein (%)                    | 17.6±1.20 | 16.5±1.22 | 0.101   |
| Neutral detergent fibre (%)          | 37.2±0.96 | 35.0±0.97 | 0.149   |
| Acid detergent fibre (%)             | 20.0±0.44 | 18.8±0.48 | 0.087   |
| Water soluble carbohydrate (%)       | 30.4±1.10 | 28.8±1.40 | 0.416   |
| Digestible organic matter (%)        | 80.1±0.66 | 78.6±0.56 | 0.145   |
| Metabolisable energy (MJ ME/kgDM)    | 12.1±0.18 | 11.9±0.28 | 0.096   |

**Note 1)** <sup>1</sup> Pasture mass as determined from calibrated plate meter compressed height

### 3.4.2 Energy and protein status

#### *Energy status*

The effect of diet in late gestation on dam responses is shown in Table 3.6. As expected, live weight increased with the increasing development of the conceptus, resulting in an average weight gain of approximately 17 kg in both groups. Cows in both groups maintained a BCS of 4.5 over the study period. The target was to dry cows off at a BCS at 5.0 or near 5.0, which was not achieved, nor was the management regime able to increase BCS to 5.0 over the winter. While the target was for cows to calve at an average BCS of 5.0 more than half of the cows were at 4.5 BCS or lower – giving an average BCS of 4.55 for both groups (Figure 3.2). There was no difference between treatments for final BCS though the MCP group tended ( $P < 0.10$ ) to gain more BCS over the treatment period (Table 3.6).

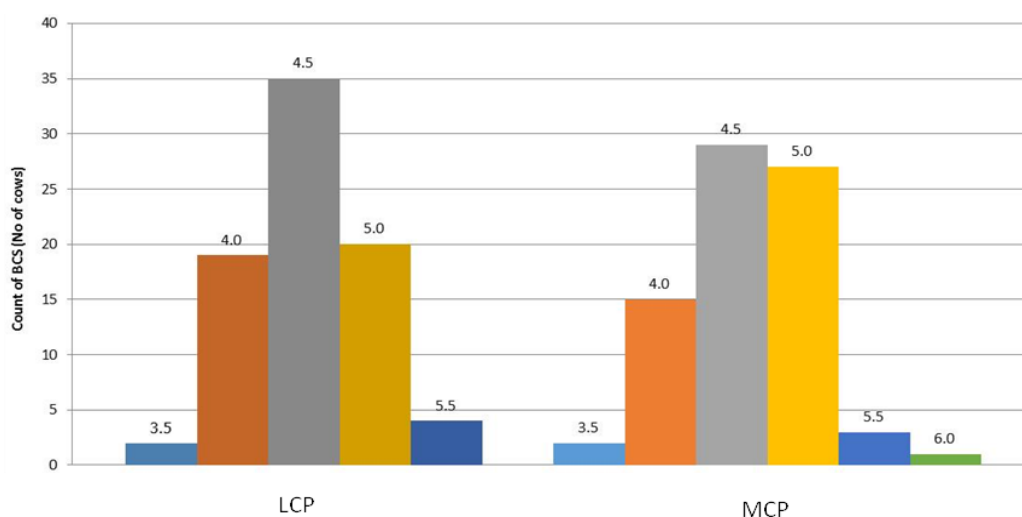
#### *Blood parameters*

Blood parameters at the starting point (week 0) were within the normal range and were similar for both groups (Figure 3.3). Overall, dams in both groups were within the normal range for plasma glucose and serum NEFA, BHBA and urea at respective averages of 3.7±0.04, 0.5±0.04, 0.4±0.03 and 3.6±0.18 mmol/L. There was no difference in plasma

glucose concentrations due to nutritional treatment (Figure 3.3 a) though glucose did vary according to sampling date. At week 4, plasma glucose was 20% lower than observed at week 0 (beginning) or 8 (end of the experiment before cows moved to springer mobs). Generally the blood parameters differed with sampling date, with little effect of nutritional regime evident after the first four weeks, but by week 8 on their respective treatments, cows in the MCP group had higher BHBA and lower NEFA ( $P<0.05$ ) compared with cows on the LCP feeding regime (Figures 3.3 b & c).

**Table 3.6 Body condition score (BCS) and Live weight (LW) of dairy cows in low crude protein (LCP) and moderate (MCP) measured at the beginning and the end (8 weeks) of the experiment**

| Parameter           | LCP         | MCP         | P- value |
|---------------------|-------------|-------------|----------|
| <b>Dam measures</b> |             |             |          |
| Initial BCS         | 4.48 ± 0.05 | 4.44 ± 0.05 | 0.613    |
| Final BCS           | 4.53 ± 0.05 | 4.61 ± 0.05 | 0.281    |
| BCS gain            | 0.05 ± 0.04 | 0.17 ± 0.05 | 0.061    |
| Initial LW (kg)     | 501.3 ± 6.6 | 497.2 ± 6.2 | 0.754    |
| Final LW (kg)       | 518.7 ± 7.5 | 514.2 ± 7.6 | 0.743    |
| LW gain (kg)        | 17.5 ± 5.6  | 17.5 ± 4.8  | 0.941    |



**Figure 3.2 The distribution of BCS of the cow in low crude protein (LCP) and moderate crude protein (MCP) group at the end of trial**

### *Protein status*

There was an interaction between treatment and sampling date for urea ( $P < 0.001$ ) showing that serum urea increased over time for the MCP treatment and decreased over time in the LCP treatment. At week 4 and 8, serum urea concentration of MCP was approximately twice the initial concentration at week 0. Differences between treatments showed higher serum urea for the MCP cows than the LCP at week 4 and 8 respectively,  $P < 0.01$  (Figure 3.3 d). This is in contrast to the LCP group, in which the final serum urea concentration is lower than the initial concentration ( $P < 0.01$ ).

Total serum amino acid from week 0 to week 4 was not different between treatments (Table 3.7). The total AA concentration increased by around 30 % between week 0 and week 8. In general, NEAAs represented the dominant AA supply, comprising approximately 60% of the total of serum AA, for cows in both LCP and MCP.

The EAA concentration in the MCP group was higher than in LCP group ( $P = 0.046$ ), at approximately 41% compared to 38% of total AA. Threonine and valine were the main EAAs, comprising 41% of serum EAA concentration. Cows in MCP group had a significantly higher serum threonine and valine concentration ( $P < 0.05$ ), arginine, lysine and tryptophan ( $P < 0.01$ ). For both groups histidine concentration increased throughout 8 weeks ( $P < 0.01$ ) while tryptophan concentration continually decreased during the study ( $P < 0.01$ ). Other essential amino acids varied throughout the trial with no consistent pattern observed.

The NEAA's glycine, glutamine and alanine accounted for over a third of total AA at around 42% and 39% of total AA in LCP and MCP cows, respectively. Most serum NEAA concentrations were affected by the time of sampling, excluding tyrosine which was constant at around 2% of total AA throughout the experiment. An interaction between treatment and time of sampling was found in some NEAAs, including glutamine, asparagine, cysteine and aspartic acid (Table 3.7).

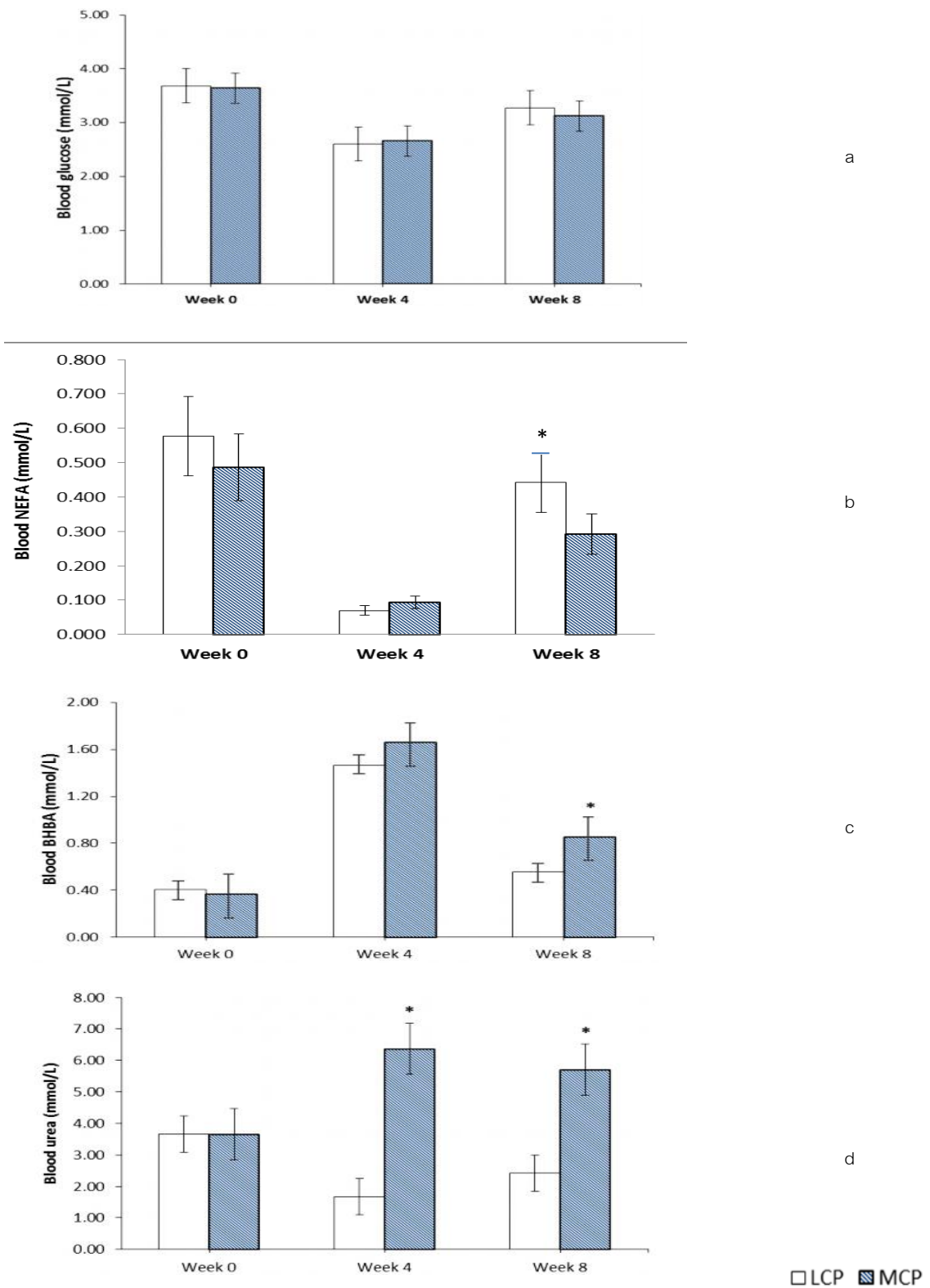


Figure 3.3 Effect of feeding different crude protein levels to dairy cows (LCP; □ and MCP; ■) during late gestation on blood (a) glucose (b) non-esterified fatty acid (NEFA), (c) beta-hydroxybutyric acid (BHBA) and (d) urea concentration. Error bars represent standard error, \* indicates significant differences ( $P < 0.05$ ) between means at a particular sampling point.

**Table 3.7 Effect of feeding low crude protein (LCP) and moderate crude protein (MCP) in dairy cows during late gestation on total amino acid (uM/L) and percentage of serum amino acids (AAs) separated by the time of sampling (at the beginning, four weeks and eight weeks of the study)**

| Amino acid<br>(%) | Treatment   |             |             | time        |             |             |             | P-value      |              |              |
|-------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|--------------|--------------|
|                   | LCP         | MCP         | SEM         | start       | 4 weeks     | 8 weeks     | SEM         | Trt          | time         | Trt x time   |
| Total AA (uM/L)   | 2,310.8     | 2,286.7     | 71.8        | 2,109.7     | 2,143.9     | 2,675.1     | 59.1        | 0.712        | <0.001       | 0.796        |
| Arginine          | 4.6         | 5.1         | 0.3         | 3.0         | 6.0         | 5.5         | 0.1         | <0.001       | <0.001       | 0.132        |
| Histidine         | 2.3         | 2.4         | 0.1         | 2.1         | 2.4         | 2.4         | 0.1         | 0.073        | 0.002        | 0.979        |
| Isoleucine        | 4.0         | 3.8         | 0.1         | 4.1         | 3.6         | 4.0         | 0.1         | 0.288        | 0.073        | 0.218        |
| Leucine           | 4.4         | 4.4         | 0.7         | 5.1         | 3.7         | 4.5         | 0.2         | 0.721        | <0.001       | 0.748        |
| Lysine            | 2.6         | 3.4         | 0.1         | 3.0         | 3.0         | 3.3         | 0.2         | <0.001       | 0.611        | 0.655        |
| Methionine        | 1.0         | 1.1         | 0.02        | 1.0         | 1.0         | 1.1         | 0.03        | 0.139        | 0.114        | 0.796        |
| Phenylalanine     | 2.2         | 2.2         | 0.1         | 2.3         | 2.2         | 2.1         | 0.1         | 0.480        | 0.300        | 0.577        |
| Threonine         | 8.2         | 9.2         | 0.3         | 9.0         | 8.1         | 9.5         | 0.3         | 0.037        | 0.013        | 0.262        |
| Tryptophan        | 1.4         | 1.6         | 0.1         | 1.8         | 1.6         | 1.2         | 0.1         | 0.005        | <0.001       | 0.078        |
| Valine            | 7.2         | 7.8         | 0.2         | 8.5         | 6.9         | 7.4         | 0.2         | 0.043        | <0.001       | 0.727        |
| <b>Total EAA</b>  | <b>38.4</b> | <b>41.1</b> | <b>0.61</b> | <b>40.1</b> | <b>38.6</b> | <b>41.0</b> | <b>0.61</b> | <b>0.046</b> | <b>0.167</b> | <b>0.908</b> |
| Alanine           | 10.6        | 9.8         | 0.3         | 10.8        | 10.3        | 9.1         | 0.3         | 0.047        | 0.015        | 0.509        |
| Aspartic acid     | 0.2         | 0.2         | 0.01        | 0.1         | 0.2         | 0.2         | 0.01        | 0.458        | 0.048        | 0.029        |
| Asparagine        | 2.5         | 2.3         | 0.1         | 2.3         | 2.4         | 2.5         | 0.1         | 0.061        | 0.021        | 0.033        |
| Cysteine          | 1.3         | 1.3         | 0.05        | 1.3         | 1.5         | 1.2         | 0.0         | 0.268        | <0.001       | <0.001       |
| Glutamic acid     | 6.0         | 4.8         | 0.4         | 3.9         | 7.0         | 4.6         | 0.3         | 0.014        | <0.001       | 0.093        |
| Glutamine         | 11.6        | 13.3        | 0.5         | 11.3        | 13.7        | 12.8        | 0.6         | 0.008        | 0.030        | 0.005        |
| Glycine           | 19.7        | 16.1        | 0.9         | 19.5        | 16.1        | 16.8        | 1.1         | 0.004        | 0.072        | 0.563        |
| Proline           | 2.6         | 2.5         | 0.1         | 3.1         | 2.2         | 2.3         | 0.1         | 0.699        | <0.001       | 0.082        |
| Serine            | 5.2         | 5.6         | 0.3         | 4.6         | 4.9         | 6.8         | 0.3         | 0.731        | <0.001       | 0.247        |
| Taurine           | 0.8         | 1.0         | 0.05        | 1.0         | 1.0         | 0.7         | 0.1         | 0.010        | 0.001        | 0.203        |
| Tyrosine          | 1.8         | 2.0         | 0.1         | 1.7         | 1.9         | 2.0         | 0.1         | 0.003        | 0.669        | 0.455        |
| <b>Total NEAA</b> | <b>61.6</b> | <b>58.9</b> | <b>0.61</b> | <b>59.8</b> | <b>61.4</b> | <b>58.4</b> | <b>0.61</b> | <b>0.046</b> | <b>0.167</b> | <b>0.908</b> |

### 3.4.3 Nitrogen excretion

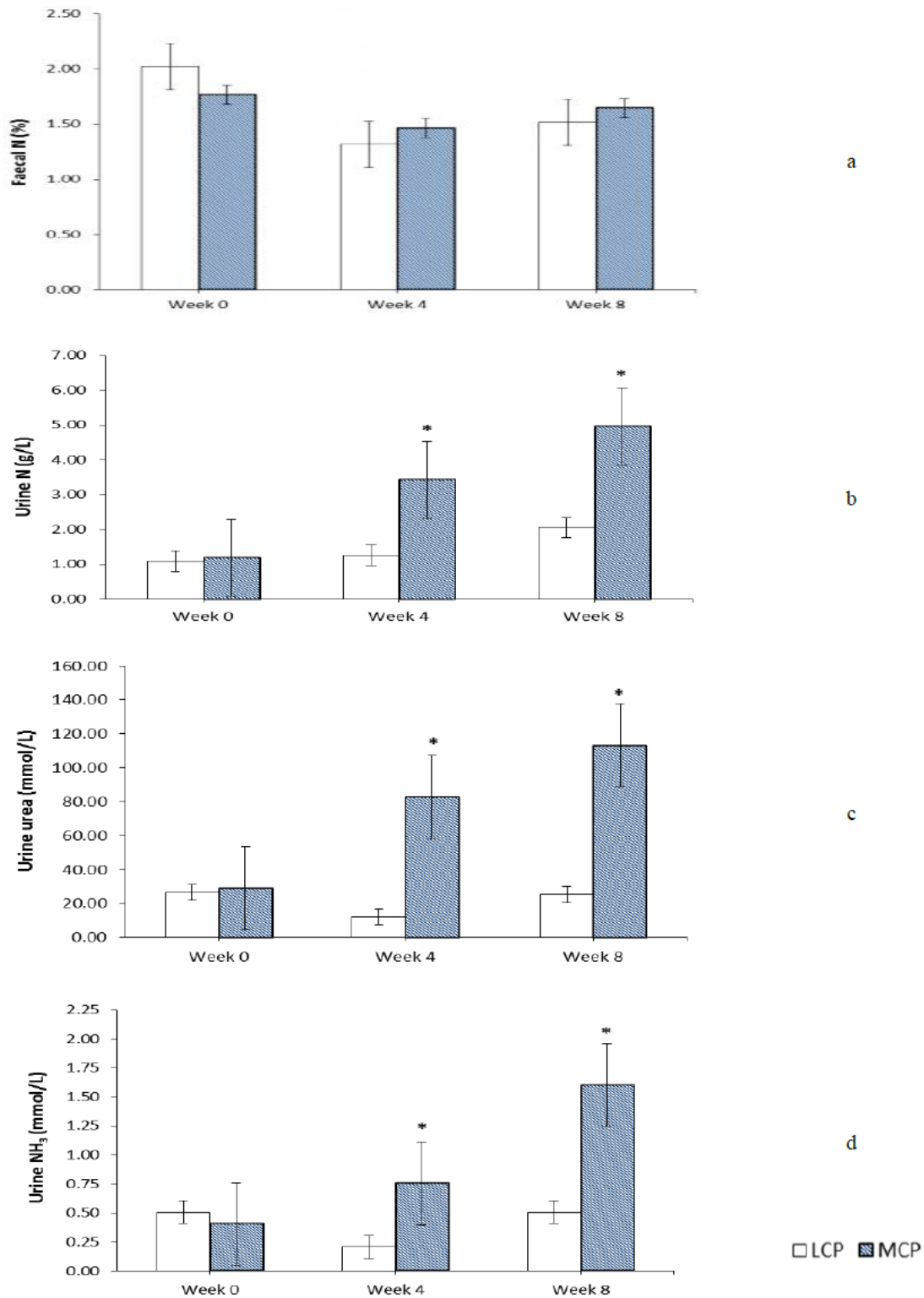
The apparent N intake of the two groups was 102 and 208 g N/cow/day, equivalent to 640 and 1,300 g protein/cow/day for LCP and MCP, respectively. The concentration of N in faeces and urine N were relatively low throughout the study and were altered by nutritional treatment (Figure 3.4). For fecal N% there was a significant interaction between treatment and time ( $P < 0.01$ ). Faecal N of both LCP and MCP decreased from the starting point to week 4, but the decline in N concentration was more pronounced for the LCP treatment.

The urine concentrations of N, ammonia and urea were similar for treatment groups at week 0 but over the course of the study the effect of feeding regime resulted in differences. Although, urine N increased throughout the study in both groups ( $P < 0.001$ , Figure 3.4 b), the MCP had twice the urine N% of LCP at week 4 ( $3.43 \pm 0.50$  vs  $1.26 \pm 0.22$  g/L, in MCP and LCP, respectively,  $P < 0.001$ ) and week 8 ( $4.96 \pm 0.40$  vs  $2.05 \pm 0.20$  g/L, in MCP and LCP, respectively,  $P < 0.001$ ). A similar trend was observed for urine urea and ammonia (Figure 3.4 c and d). Creatinine excretion was also elevated from the starting point throughout the study in both groups but did not differ between groups ( $P > 0.05$ ).

The cows in the MCP group excreted 64 g N/day more than cows in the LCP group (Table 3.8,  $P = 0.002$ ). This difference in urine N excretion arose from increased N concentration and N load per urination as there was no difference in mean urine volume per event, or total daily volume. The proportion of N intake excreted in urine was 0.39 and 0.50 for LCP and MCP respectively.

**Table 3.8 Urination parameters from cows fed a low crude protein (LCP) and moderate crude protein (MCP) diet**

|                                | LCP  | MCP  | Treatment difference<br>(high-low) | P-value |
|--------------------------------|------|------|------------------------------------|---------|
| Mean urine event size (L)      | 2.48 | 2.58 | $0.09 \pm 0.48$                    | 0.900   |
| Mean urine N conc. (g N/L)     | 2.0  | 4.2  | $0.22 \pm 3.0$                     | <0.001  |
| N load per event (g N/event)   | 4.8  | 11.3 | $6.5 \pm 2.1$                      | 0.003   |
| Time between urination events  | 0.12 | 0.11 | $-0.01 \pm 0.009$                  | 0.250   |
| Daily urine-N (g N/cow/day)    | 40   | 104  | $64 \pm 16$                        | 0.002   |
| Daily urine volume (L/cow/day) | 20.5 | 23.3 | $2.9 \pm 3.7$                      | 0.450   |



**Figure 3.4** Effect of feeding different crude protein levels (LCP; □ and MCP; ▨) in dairy cows during late gestation on (a) faecal N and urine (b) nitrogen (N), (c) urea and (d) ammonia (NH<sub>3</sub>) concentration. Error bars represent standard error, \* indicates significant differences ( $P < 0.05$ ) between means at a particular sampling point.

### 3.4.3 Milk production and animal health

#### *Animal health*

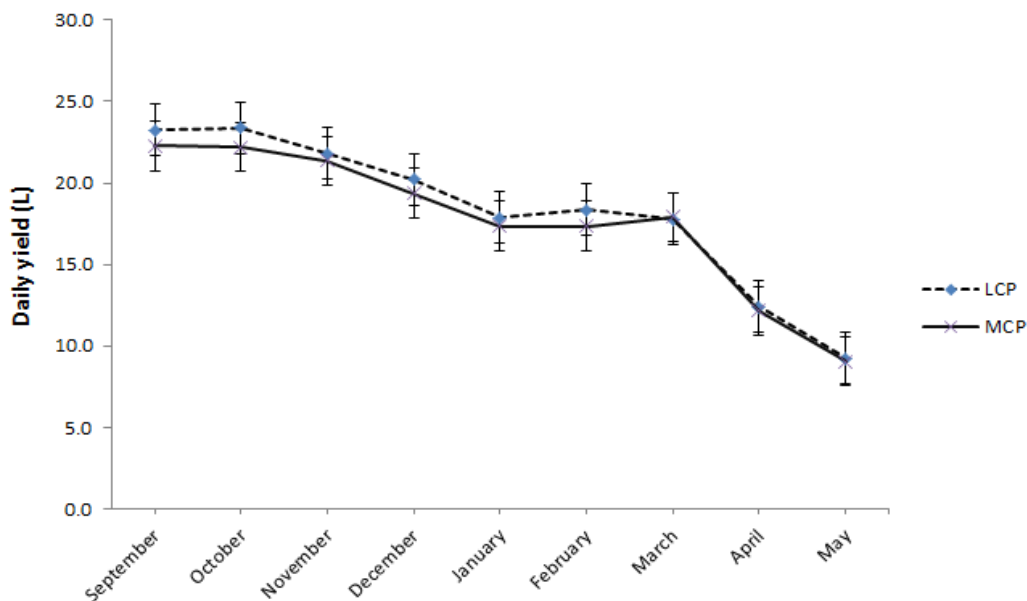
Although we were unable to statistically analyse the effect of winter feeding regime on animal health, the effect of the winter feeding regime on cows requiring treatment for various metabolic and health issues during the following lactation season is presented in Table 3.9. Generally there was a 28% higher incidence of health issues for cows in the LCP group compared with the MCP group, which was driven largely by more occurrences of mastitis. The conception rate of the cows from the LCP was 95.6%, compared with 90.0% of the MCP cows (from total 90 mating cows in each group, there were 4 and 9 non-pregnant cows in LCP and MCP, respectively).

**Table 3.9 Effect of winter feeding treatment on the occurrence of animals requiring treatment for health conditions**

| <b>Condition</b>               | <b>LCP<br/>(n = 95)</b> | <b>MCP<br/>(n = 95)</b> |
|--------------------------------|-------------------------|-------------------------|
| Breech calf                    | -                       | 1                       |
| Twisted gut                    | 1                       | -                       |
| Lame                           | 5                       | 6                       |
| Mastitis                       | 25                      | 19                      |
| Retained placenta              | 4                       | 1                       |
| Sick*                          | 6                       | 3                       |
| Staggers                       | -                       | 2                       |
| Milk fever                     | 2                       | -                       |
| Cows put up for mating         | 90                      | 90                      |
| Non-pregnant cows after mating | 4                       | 9                       |
| <b>Total treatments</b>        | <b>43</b>               | <b>32</b>               |

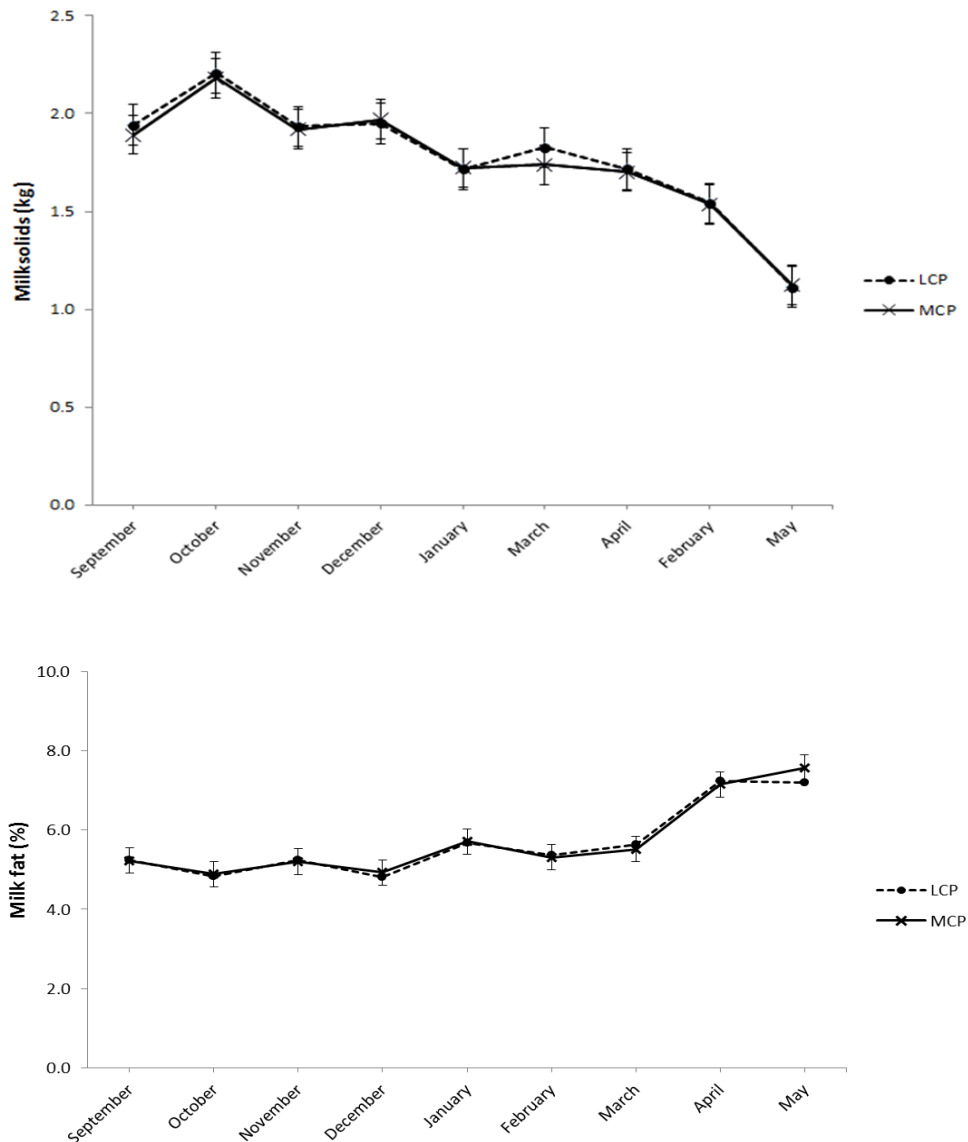
**Note:** \*Sick cows were reported to be related to respiratory symptom and inflammation

After calving, cows in the current study were separated into four farmlet groups (explained in section 3.2.2.3, lactation period management) and their milk yield and composition were monitored. The cows which were allocated to the spare herd (14 cows-with undefined stocking rate or forage type) tended to be the later calving cows and had lowest milk yield ( $3,923 \pm 152$  litres/lactation) and fewer days in milk ( $247 \pm 7.5$  DIM) when compared to cows in the other farmlets ( $P < 0.05$ ), these cows were subsequently excluded from the analysis. There were no main effects or interactions between winter treatment diets (LCP vs MCP) or farmlet systems on days in milk ( $278 \pm 1$  DIM) or average lactation yield. There was no effect of winter feeding on daily milk yield or composition; milksolids (kg), or percentage of fat and protein throughout nine herd test ( $P \geq 0.05$ ) as shown in Figure 3.7-3.9. The average yield was  $5,108 \pm 91$  vs  $5,187 \pm 83$  litres/lactation in LCP and MCP, respectively ( $P > 0.05$ ).

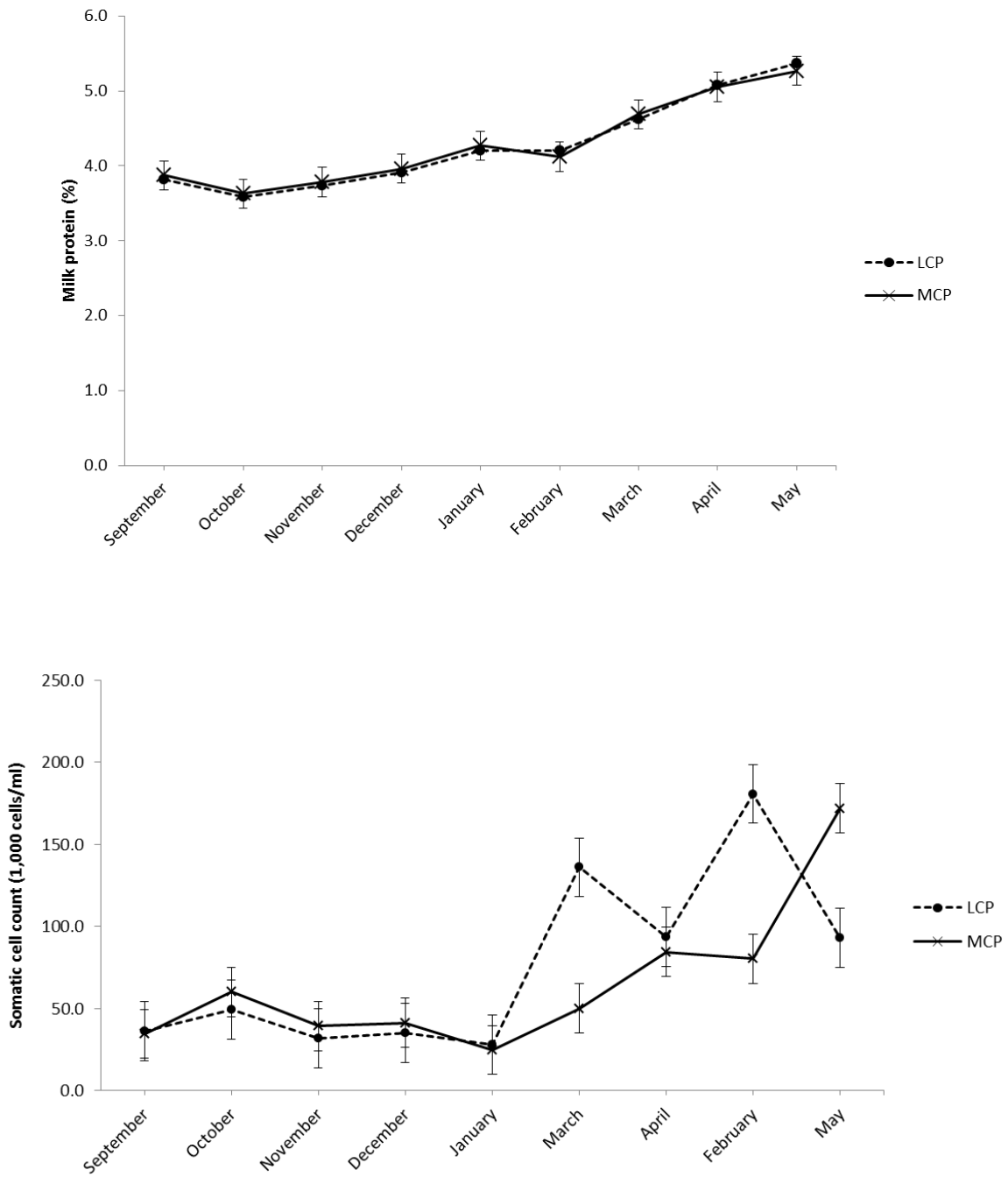


**Figure 3.5 Milk yield (L) of cows previously fed winter diets consisting of low crude protein (LCP) and moderate crude protein (MCP) diets. Error bars represent standard error of means**

Total milk solids (fat plus protein) was similar for MCP and LCP at  $484 \pm 11.1$  vs  $472 \pm 15.0$  kg/lactation ( $P>0.05$ ), respectively (Figure 3.6). Lactation milk fat and protein (kg) did not differ between groups at an average  $259.2 \pm 5.4$  and  $218.3 \pm 4.2$  kg, respectively. On average there was no significant difference in SCC but during late lactation, there was more incidence of high SCC in the LCP treatment cows which is also reflected in number of mastitis treatments (Table 3.9).



**Figure 3.6** Milk solids (kg) and milk fat (%) of cows previously fed winter diets consisting of low crude protein (LCP) and moderate crude protein (MCP) diets. Error bars represent standard error of means.



**Figure 3.7 Milk protein (%) and somatic cell count (1,000 cells/ml) of cows previously fed winter diets consisting of low crude protein (LCP) and moderate crude protein (MCP) diets. Error bars represent standard error of means.**

### 3.5 Discussion

In the current study, we found that a low crude protein diet (7.4% compared to 13.6% CP of DM) during late gestation resulted in substantially lower protein intake and as a result decreased urinary N excretion. In terms of lactation performance there did not appear to be any negative carry over effects of protein restriction during the non-lactation period. Questions remain regarding the impact of a low protein diets in late gestation on animal health.

#### 3.5.1 Energy and protein status

##### *Energy*

Cows from both groups had similar live weight gain and essentially maintained their BCS throughout the late gestation study. This result reflects similar apparent ME intake, which differed by less than 10% between the treatments (107 vs 100 MJ/d for MCP and LCP, respectively). From the literature, energy is generally the most limiting nutrient for prepartum cows (Grummer, 1995, Bell, 1995) and BCS is typically a good indication of a cow's energy reserve (Ferguson et al., 1994). Based on AFRC. (1993), ME requirement for pregnant, non-lactating cows in late gestation is recommended at 75 to 90 MJ ME/day. Of this energy, the ME requirements for gestation increases from 11 to 35 MJ ME/day between 220 and 280 days of conception. For both groups, it was assumed that the weight of the concepta increased by 17 kg with no gain in BCS. The average efficiency of ME for maintenance was approximately 0.6, calculated by AFRC. (1993) equation :  $ME_m = 0.35ME/GE + 0.503$ , where GE = gross energy, 18.4). This value is lower than the expected 0.7 based on feed quality. The difference is expected to be due to possible losses in digestibility associated with low protein (NRC, 2001, Santos et al., 2001), or more likely the cold conditions and increased energy demand for thermoregulation (Ketelaars and Tolcamp, 1992, Miaron and Christopherson, 1992). Using the ME requirement supplied by Roche et al. (2005), which accounts for thermoregulatory needs, resulted in suitable allocation of energy for cows in this study.

In the current study, starch content of maize silage appeared to be low at around 1.8% DM. This is normally found in early stage or low grain maize silage, resulted in lower DM intake (Khan et al., 2015). While other study reported that starch content in lucerne silage also low at average lower than 1% of DM (Melvin, 1965). Most carbohydrates were degraded by microorganism in the rumen to produce volatile fatty acids and absorbed through the rumen wall (Esther et al., 2003). Low starch content in high quality roughage resulted in only minimal quantity of glucose (less than 10% of requirement) obtain for absorption to the small intestine (Esther et al., 2003, Donkin and Armentano, 1995). Accordingly we found that the LCP and MCP diets had low starch content which likely to not impact on glucose supply or made change to energy balance in our experiment.

Although the cows in this study maintained their condition, there were some blood metabolites which indicated a negative energy balance in the cows around drying off (week 0 of the study) and towards 285 DCONC (week 8 of the study). The level of NEFA at week 0 in the experiment was high in both treatments which may indicate a relatively abrupt feed restriction as cows were drying off (Andersen et al., 2005, McArt et al., 2013a). According to McArt et al. (2013a), the serum BHBA  $\geq 1.4$  mmol/L observed at week 4 of the study indicated that both groups were exposed to hyperketonemia based on their threshold values of 1.2 mmol/L. Later at 1-2 weeks prepartum, BHBA concentration had decreased in both groups indicating increased prepartum lipolysis to support higher mammary demand for energy and AAs, which likely contributed to the negative nutrient balance around calving (Kesser et al., 2017).

In pasture based systems, a severe negative energy status of the cow is reflected through a combination of glucose ( $<3$  mmol/L), NEFA ( $>1$  mmol/L) and BHBA ( $>1.2$  mmol/L) in the blood, rather than BHBA alone (Phyn et al., 2017). Serum NEFA concentration of both groups was increased at week 8, but did not exceed 1 mmol/L. There were relative differences in NEFA between the groups with higher levels in the LCP cows (0.4 mmol/L) than MCP (0.2 mmol/L). However, these levels were still within the range of the recommended 0.3-0.5 mmol/L (McArt et al., 2013b). A rapid elevation in NEFA ( $\geq 0.5$  mmol/L) points to poor adaptation to negative energy balance (NEB) (Ospina et al., 2010) and increased risk of post-partum health problems (McArt et al., 2013b). Conversely, a slightly elevated serum NEFA ( $\leq 0.3$  mmol/L) before calving is commonly observed in near

term dairy cows and is related to metabolic adaptations to maintain energy balance (Ospina et al., 2010, Andersen et al., 2005). The higher elevation of NEFA at week 8 may indicate the chance of negative energy balance in near term LCP cows.

In this study, serum glucose concentration did not differ between treatments. At four weeks of the study, plasma glucose was slightly decreased, which is in line with previous studies (Andersen et al., 2004, Andersen et al., 2005, Santos et al., 2001). Increasing prepartum AA supply by supplementing CP above 12% was found to impact hepatic gluconeogenesis (Overton et al., 1998) but not plasma glucose concentration (Moorby et al., 1996, Van Saun et al., 1993, Putnam and Varga, 1998). However, plasma glucose may not be a good indicator of energy status as a result of being under substantial homeostatic control (Van Saun and Sniffen, 2014). High NEFA but low BHBA in the LCP dams points to more lipolysis from fat storage. The elevated pre-calving NEFA concentration in LCP ( $\geq 0.3$  mmol/L) indicates a risk factor of ketosis postpartum (Seifi, 2011) and retained placenta (Chapinal et al., 2011).

### *Protein*

Using the data collected in the current study the metabolisable protein requirements of the experimental cows in late gestation (220 to 260 DOC), who were not gaining weight, was between 280-320 g MP/day (AFRC., 1993). Based on apparent intake and book value assumptions around the degradability of the protein and organic matter, these MP requirements could be supplied by microbial protein if the crude protein content of the diet was 12% or more. To meet their protein requirements for the current diets cows needed to consume approximately 1.1 kg CP each day, which means that cows in LCP consuming only 0.6 kg CP daily were restricted by approximately 45% rather than the planned 20% restriction (Table 3.3). The cows in MCP consumed twice as much dietary protein as those in LCP, but as discussed in the following section the high proportion of N intake excreted in the urine (39 and 50% for LCP and MCP respectively, Table 3.7) indicates that dietary N and recycled urea to the gut was able to meet requirements.

The nitrogen use efficiency observed here reflects a balance of energy and protein suited to requirements. From a protein perspective, the cows in the current study did not

differ in terms of amino acid concentrations in the blood. Increasing protein or AA supply as with the MCP group likely supported the improved energy balance of this group compared to LCP (Putnam and Varga, 1998). The transfer of cows from crop to pasture prior to calving may also have contributed to alleviating any protein deficiencies which might have occurred. Indeed, Grummer (1995) indicated that feeding above 12% CP during the last three weeks pre-calving reduced the risk of primary ketosis and retained placenta.

### *Health*

The health indicators from the current research are difficult to interpret. Numerically, there appeared to be a higher risk of health issues around transition and mammary infections with the LCP group, but reproductive performance was numerically worse for the MCP group. Although cows in the winter feeding study were managed differently in farmlets during lactation it is useful to explore possible impacts of winter feeding regimes for future research. Longer-term impacts of diet on immune status and health of cows in this study may have been indicated by the evidenced variation in somatic cell count. In our study, we found the trend of fluctuating SCC in both treatments (Figure 3.7). During mid to late lactation, the LCP cows tended to have higher SCC than those in the MCP ( $P < 0.1$ ). Increased SCC in the LCP may reflect the higher incidence of mastitis compared to the MCP cows. The elevated SCC in late gestation is typically found as there is increased bacterial invasion of the teat canal which damages the glandular parenchyma tissue. This damage consequently increases SCC, the incidence of mastitis and reduces milk yield (Sharma et al., 2011). The lack of significant differences in SCC between treatments in our present study seems similar to the considerable variation that has been reported previously (Alhussien and Dang, 2018).

Cows in the LCP group had a lower intake of calcium, potassium and sulphur than cows in the MCP group because of lower concentrations of those minerals in the maize silage. While difficult to statistically compare, there were 2 cases of milk fever in LCP cows. Reinhardt et al. (2011) reported that cows those diagnosed as subclinical hypocalcemia (low serum calcium concentration at  $< 2.0$  mM) had markedly higher serum NEFA indicating inferior energy balance. Also, low calcium may be related to infections as it reduces the ability of immune cells to respond to stimuli (Kimura et al., 2006). In our study, there was

also a higher incidence of retained placenta in LCP than MCP cows. Low selenium is another factor influencing retained placenta, though we did not measure Se in this study. Zhang et al. (2002) reported that low intake of calcium during gestation might be related to the retained placenta or membranes, as calcium is vital for muscle contractions required for expulsion of the placenta. Furthermore, Martinez et al. (2012) reported a three times greater incidence of metritis in cows with low serum calcium. In our study, calcium intake in LCP was 80% lower than MCP, which may have resulted in increased health problems, including milk fever and retained placenta. However, the incidence of retained placenta in the current study was still within the normal range at 4-18% of the herd (Han and Kim, 2005). To fully realise the impact of late gestation protein nutrition regimes in a NZ pastoral setting, it would be useful to collect data on animals at a commercial level as the small numbers often used in experimentation make it difficult to determine cause and effect.

### **3.5.2 Nitrogen excretion**

Not surprisingly, higher nitrogen intake of MCP cows resulted in more urinary nitrogen excretion. In our study, the MCP diet (13.6% CP) resulted in double the urinary N excretion of the LCP cows (7.4% CP). Blood parameters (BUN) and faecal N also supported reduced N status of the cows in LCP. It is well established that low protein or N intake results in lower N excretion in dung and urine (Bryant et al., 2018a, Edwards et al., 2015, Edwards et al., 2014a). Exceptions do exist, however, as a comparable study reported by Ruppert et al. (2003) observed higher N intake (approx. 680 vs 620 g/d) in lactating cows fed lucerne compared with maize silage and found lucerne fed cows had higher faecal N (approx. 203.0 vs 177.0 g/d). Typically, 70% of nitrogen intake is excreted to the environment via urine and faeces (Phuong et al., 2013). As N intake increases beyond requirements, relatively more N is partitioned to urine compared with dung or milk (Kebreab et al., 2010). In this study, feeding late gestation cows low protein diets of 7.4% CP resulted in lower urinary N excretion, which is practical to reduce nitrate leaching due to lower build-up of N in the soil.

The results of this research presents novel information regarding the urinary N excretion behaviour of cows on high moisture, high energy and low protein crops such as fodder beet. Urinary N excretion was lower for LCP cows as a result of lower concentration of N in the urine rather than variation in urine volume. These findings are consistent with studies comparing urine N excretion in pasture based studies with lactating cows. For

example, Bryant et al. (2018b) showed that changes in urinary behaviour as a result of feed were due to urination frequency and N concentration rather than urine volume. Interestingly the total urinary N reported for LCP cows was much lower than previous studies in kale (Edwards et al., 2014, Edwards et al., 2015) and ryegrass (Mangwe et al., 2019). Cows in LCP were found to excrete just 40 g N/day which represents 42% of their N intake, which was lower than the 50% of N intake excreted in urine of MCP cows. In lactating cows N intake is greater, often at 400-700 g N/cow/day and up to 56% is excreted in urine (Bryant et al., 2014). In those late lactation studies, dietary N is high relative to requirements. In the current study there was little surplus N being offered to cows which resulted in a lower proportion of N being excreted in urine.

### **3.5.3 Milk production**

In our study, lactation yields and milk composition of both treatment groups did not differ, although we expected lasting effects of negative energy balance (NEB) in the LCP group. Other studies reported that elevated serum NEFA ( $\geq 0.33$  mmol/L) 2 weeks before calving was found to be associated with more than 600 kg milk loss in cows and heifers (Ospina et al., 2010). Chapinal et al. (2012) also reported that elevated serum NEFA and BHBA in the week before calving was related to milk loss in early lactation. The optimum threshold for NEFA and BHBA concentrations to predict milk loss were  $\geq 0.5$  mmol/L and  $\geq 0.6$  mmol/L, respectively (Chapinal et al., 2012). We found the inverse correlation between serum NEFA in week 8 and days in milk ( $r = -0.431$ ,  $P = 0.04$ ), suggesting that there was a possibility of less DIM in the LCP cows due to prepartum NEB.

In the present study, the last blood samples were collected 20 days - on average - before calving and likely did not capture changes in energy balance associated with moving cows to *ad-lib* pasture during the 7-10 days pre-calving period (range from 1-26 days). If the timing of NEB is critical in the last 7-14 days before parturition, our management of avoiding calves being born on crop areas may have disguised treatment differences. This result may have been exacerbated by the LCP cows being offered a pasture area of higher yield and quality than the MCP (Table 3.5). Increased energy and protein density in diets of cows in last 21 days pre-calving is one of the compelling methods to enhance energy intake and correct prepartum negative energy balance (Vandehaar et al., 1999). This management

practice might be the reason for the diminished effect of LCP on cow health, live weight gain and BCS. Long et al. (2009) and Kwon et al. (2004) also reported that a nutritional remedy after a short term nutrient restriction minimized the adverse effects on the conception, foetal growth and blood parameters such as amino acids profile of dams and the offspring.

There were no consistent effects of feeding regime on milk composition, though there was a tendency for increased mean milk fat percentage from MCP cows ( $P = 0.054$ ). Previous studies reported that increasing prepartum CP to above 12% DM might result in a deleterious effect on consequent milk performance (Greenfield et al., 2000) or fail to show a positive impact (Putnam and Varga, 1998, Putnam et al., 1999, Vandehaar et al., 1999). Santos et al. (2001) also found that increasing protein in the diet from 12.7% to 14.7 % CP declined milk yield, especially in multiparous cows in contrast to primiparous cows. High CP at more than 12% may disadvantage milk performance in multiparous cows, more than primiparous because younger animals which are still growing may need more protein (NRC, 2001, Santos et al., 2001). The reason for high CP in prepartum diets reducing milk yield is not clear, it might be that the surplus protein needs to be degraded to ammonia then metabolised to urea in the liver, which requires additional energy in the process (NRC, 2001).

### **3.6 Conclusion**

Under the conditions tested in the present study, we found that feeding low dietary CP (7.4% CP, 45% lower than requirements) to late gestation cows markedly reduced urinary N excretion to the environment. The LCP cows maintained their BCS and live weight throughout the experiment with no detectable deleterious impact on following lactation performance; milk yield or composition. However, questions remain with regards to the influence of low protein diet during late gestation on longer term animal health. Improving nutrient supply by transitioning from crop to higher protein pasture at near term (1-2 weeks before calving) may recover energy and/or protein balance and reduce detrimental effects on the dam's milk production and health. In the next chapter, the impact of maternal low protein on foetal growth and development during late gestation is studied.

## Chapter 4

# The effect of maternal diet on *in-utero* development and passive immunity transfer

Part of this chapter has been published:

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### 4.1 Introduction

The long term productivity of the national dairy herd relies on successful rearing of replacement stock, and the factors influencing this success begin *in-utero*. In this regard, nutritional management of pregnant cows has implications on replacement heifer production as it impacts reproductive system development of the *in-utero* foetus, and can alter the postnatal growth pathway, metabolism, and body composition of the offspring (Rhind, 2004). *In-utero* foetal growth is at its most rapid during late gestation, approximately 3-4 weeks prepartum in cattle (Dunlap et al., 2015, Moisés et al., 2015). Thus, cows have higher nutrient demand in late compared to the first half of the gestation. What happens to cows during late gestation also influences colostrum synthesis (Nowak et al., 2012).

An example of reduced nutrient supply (50% vs 100% of NRC requirement of all nutrients) for ewes and lambs was demonstrated by Kwon et al. (2004). They reported a decrease of total amino acid and some individual amino acids (AAs), including arginine in maternal and foetal plasma when ewes were restricted during mid or late gestation. Pacheco et al. (2016) reported that replacing high protein ryegrass with fodder beet, in lactating cows, decreased the proportion of arginine, citrulline and ornithine in total plasma amino acid (TA) ( $P < 0.05$ ). Arginine is essential for placenta angiogenesis, mammal growth and increased birth weight (BW) (Wu and Morris, 1998, McCoard et al., 2013).

A deficiency in protein during gestation has shown to result in failure of passive transfer (FPT) of immunity to offspring in addition to poor early lactation milk yield of the dam (Bell et al., 2000). Colostrum not only provides nutrients for the calf after birth but is also the primary source of immune components for the offspring (Quigley and Drewry, 1998). Nutritional deficiency in late gestation not only has potential negative effects on development of the *in-utero* foetus but carry over effects on the postnatal life of the calf due to reduced colostrum quality (Weaver et al., 2000). As newborn calves are agammaglobulinemia, passive immunity transfer from high quality colostrum is essential for their survival (Conneely et al., 2014, Gulliksen et al., 2009).

In Chapter 3, we investigated the impact of gestational protein restriction on dam milk performance and found no noticeable effect of maternal diet on her productivity. However, there was some evidence in blood parameters which indicated variation in energy and protein balance of the dams fed different protein allocation. While there was no apparent negative impact on the dam, the effect of variation in blood metabolites on calf *in-utero* development and immunology warranted investigation. Preliminary studies comparing skeletal development of calves born from cows fed kale or fodder beet (FB) in late gestation showed lower skeletal weight of calves from cows fed FB diets compared with kale fed cows (Bryant & Pirat 2014). One conclusion was that the higher protein in kale diet improved AA supply and more closely met foetal requirements. However, those results were confounded because both crops and supplements differed and the data represented only one season.

The objective of this study was to investigate the effects of feeding different protein levels (from cows fed the same FB crop with either a low or moderate protein supplement) during late gestation on 1) *in-utero* calf growth and development and 2) Passive immunity transfer.

## **4.2 Materials and methods**

The experiment was conducted between 1 June and 25 August 2017 at the Ashley Dene Research and Development Station (ADRDS), Lincoln University, with the approval of the Lincoln University Animal Ethics Committee (AEC No. 2017-8).

### 4.2.1 Management

This study is a continuation of that described in Chapter 3 with the aim here to observe the immediate impact of late gestation nutrition on calf birth size, colostrum quality and passive immunity transfer. The maternal trial was described in Chapter 3. Briefly, 190 pregnant, spring calving Friesian x Jersey dairy cows were stratified into two groups of fodder beet (FB) crop and supplemented with either low crude protein (CP) maize silage (equivalent to 7.4% CP, LCP) or moderate protein lucerne silage (equivalent to 13.6% CP, MCP). The maternal feeding trial was carried out from 1<sup>st</sup> June to 30<sup>th</sup> July 2017. Cows calved between 26<sup>th</sup> July and 25<sup>th</sup> August 2017. To avoid cows calving on crop where conditions were often wet and muddy, cows were transferred from crop to pasture approximately 7-10 days, before parturition. Transfer of cows to pasture was based on their expected calving date and visual identification of the udder and vulva changing in preparation for calving. A cow identified as approaching calving was referred to as a 'springer'. The number of days on pasture was counted from the day cows were removed from crop to parturition. Gestation length was calculated from the insemination day to calving day.

Once calving commenced, cows and calves were collected from the 'springer' paddock, once daily or more frequently in bad weather. Dam and calf pairing were recorded by visual assessment. We were unable to confirm exact parentage but because dams were kept separate in their treatment group we were able to confirm which treatment the calves came from. Birthdate, maternal treatment and calf gender were also recorded. Time of calving was noted wherever possible. Calves were removed from their dam around 10 am each day, usually within day 1 (12-24 hours of birth). At the time of collection, dams and calves were checked for suckling, by observing dam's teat characteristics (swelling size and cleanness) and calf appearance (stomach appearance and colostrum faeces presence). The suckling evidence was recorded as suckled colostrum (SC) or not suckled colostrum (NSC). Calves were weighed, stature measurements collected and blood sampling in calf rearing facility (details below). Immediately after weighing and stature measurements in the pen (see below for details) calves were bottle fed 2 L of first milking colostrum ie. bottle fed by 12 pm. Pooled colostrum, from day 2-4 milking, was available to calves *ad lib* in a calfateria feeder for the next four days, and calves taught to drink from the feeder the following morning. The quality of the gold colostrum was determined as described in section 4.2.2.2.

Day old calves received pooled (gold) colostrum from cows which had calved in the previous 24 hours. Calves 2 to 4 days of age were fed 2 x 2 L pooled colostrum from cows which had calved within 4 days – otherwise termed the ‘colostrum mob’.

**Table 4.1 Summary of the animal assignment and data collection in Chapter 4**

|   |  |                                   |  |
|---|--|-----------------------------------|--|
| <b>Chapter 4</b><br>Effect of maternal nutrition on <i>in utero</i> development and passive immunity transfer | 157 cows had calving during 26/07/2017 - 25/08/2017  |                                   |  |
|   | Colostrum from 152 cows<br>76 LCP and 76 MCP (80% of each group)<br>(excluded colostrum from 2 paralysis, 1 dystocia and 2 twins cows)                           |                                   | Collected data including of<br>1. Colostrum IgG, amino acid profile, total protein |
|   | 159 calves born to those 157 cows:<br><u>Collected data of 139 calves</u> (excluded 10 Wagyu, 2 twins and 6 dead calves due to bad weather)                      |                                   |  |
|   | 72 bull calves<br>LCP 37 MCP 35  | 67 heifer calves<br>LCP 34 MCP 33 | 2. Liveweight, stature measuring   |
|   | Random sampling 20 samples from heifers in each group (total 40 samples)*  |                                   |  |
|   | 40 heifer serum<br>LCP 20 MCP 20   |                                   | 3. Serum IgG, protein and AA profile at 1 <sup>st</sup> day.                       |
|   | 4 days old heifers (might or might not be the same calf of 1 <sup>st</sup> day sampling) were random sampling before assign to the preweaning trial (Chapter 5)* |                                   |  |
|   | LCP 15   | MCP 18                            | 4. Serum IgG, protein and AA profile at 4 day.                                     |

## 4.2.2 Sampling and measurement

In this chapter, we studied the impact of maternal protein nutrition in late gestation on *in-utero* calf development and colostrum quality. The summary of animal assignment in Chapter 4 is presented in both Figure 1.2 (page 6) and in Table 4.1.

### 4.2.2.1 Calf development

To determine the effects of the maternal diet on calf development *in-utero*, calves born in the first four weeks of calving (n=139) between 30<sup>th</sup> July and 25<sup>th</sup> August 2017 were weighed and stature recorded. Measurements were carried out in the calf rearing facilities between 8 am and 10 am each morning. Live weight was determined using a manual weigh crate (Prattley Industries Ltd. Temuka). After weighing, calves were manually posed into a

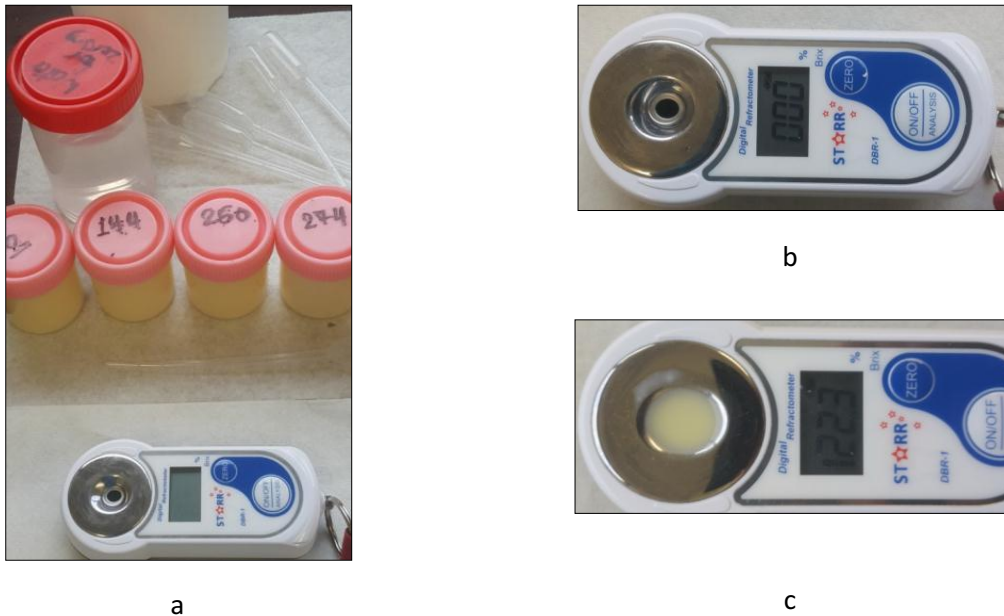
straight standing position, with head upright and forward. Heart girth, distance from crown to shoulder, shoulder to tail head, wither height, hip height and waist circumference were measured using a standard 150 cm tape. All measurements were made in duplicate or triplicate to reassure. Data from Wagyu calves, twins or dead calves were not included (total 20 calves). On completion of live weight and stature measurements, heifer and bull calves were allocated to their appropriate pens which separated the two genders.

#### **4.2.2.2 Colostrum**

The impact of maternal nutrition on colostrum quality was determined on 152 cows (n = 76/treatment) which calved between 30<sup>th</sup> July and 25<sup>th</sup> August. Colostrum from cows suffering from dystocia (assisted calving) or which had produced twins were not included as they may have been associated with low serum immunoglobulin levels (Waldner and Rosengren, 2009). Colostrum samples were collected at the first machine milking postpartum (once a day, c. 2.30 p.m.) by collection of milk from all quarters into a test bucket.

Milk in the test bucket was mixed and approximately 50 ml sub sample was collected into a 100 ml polypropylene bottle. The remaining milk was decanted into a drum for subsequent feeding to calves. The 50 ml sample of colostrum was split into two subsamples. The first 30 ml subsample was frozen immediately and later sent to LIC for analysis of determining milk fat, protein, lactose, total solid and somatic cell count (SCC). The second 20 ml subsample was evaluated for colostral immunoglobulin G (IgG) concentration using a digital refractometer (adapted from Morrill et al. (2012)). In brief, approximately 0.3 ml of whole fresh colostrum was pipetted on a refractometer prism (DBR-1, Starr Instruments), and % Brix (sugar content) was recorded (Picture 4.1) and repeated four times for each sample, and the average value recorded. The refractometer was cleaned then tared with distilled water at the start of each set of analyses. To validate the refractometer for IgG 20 colostrum samples (10 per group) were collected between 18<sup>th</sup> – 25<sup>th</sup> August 2017. The colostrum was defatted by centrifugation at 2200 g for 5 min at room temperature and a 2ml aliquot of the defatted colostrum sample was frozen immediately. Frozen colostrum samples were sent to New Zealand Veterinary Pathology Laboratory in Hamilton for IgG

validation by a Turbidimetric Immunoassay (Besser et al., 1988). Reagents for analysis were supplied by Midland BioProducts (Boone, IA, USA).



**Picture 4.1 The digital refractometer (a) zero calibration with distilled water (b) and % Brix verified from a colostrum sample (c)**

#### **4.2.2.3 Passive immunity transfer**

Failure of passive immunity transfer was determined by analysis of IgG in blood serum of 40 random, day old heifer calves (20 LCP and 20 HCP). Approximately 6 ml of blood was sampled via jugular venepuncture within 24 hours of birth. The blood was collected into an evacuated blood tube with no anticoagulant and allowed to clot. Serum was separated by centrifugation at  $3,300 \times g$  for 20 min at  $25^{\circ}\text{C}$ . Approximately 0.3 ml of serum was used to evaluate % Brix by refractometer (DBR-1, Starr Instruments) in 4 replicates of each sample and IgG was estimated following the approach of Morrill et al. (2013). Again validation of the refractometer for IgG was determined from 20 random serum samples (10 per maternal group) using the Turbidimetric immunoassay (TIA) method (Besser et al., 1988) (New Zealand Veterinary Pathology Laboratory, Hamilton).

The remaining serum was divided into three vials and stored at  $-20^{\circ}\text{C}$  until analysed for serum protein, albumin and amino acids. The protein was analysed using the Variomax CN analyzer. Albumin concentration was determined by Daytona (Cat. No. AB 3800). Serum amino acid profile of the calf was analysed by HPLC analysis (Agilent 1100 series).

### **4.3 Statistical analysis**

Data were analysed using Genstat (version 18.1, VSN, Hemel, Hempstead, UK). To compare the effect of wintering treatments on calf BW and skeletal development, model terms were maternal protein treatment and calf gender as fixed terms, while gestation length and days on pasture were included as covariates and individual calf as a random term in the ANOVA model.

Pearson's correlation coefficients for colostrum IgG concentration and serum IgG concentration were regressed against Brix Refractometer % using the general linear regression model. The coefficients were then applied to all Brix samples to calculate IgG in colostrum or serum. Means for colostrum IgG were analysed using maternal protein treatment and parity (primiparous and multiparous) as fixed effects of testing the main effects and interactions. For means of calf serum IgG, serum protein content and amino acid profile, the maternal effect, colostrum suckling (SC = suckled colostrum and NSC = not suckled colostrum) and time of sampling (day of age) were used as fixed terms and individual calf as a random term. Means separation ( $P < 0.05$ ) was performed using Fishers protected LSD test. Values show as means  $\pm$  SEM.

## 4.4 Results

### 4.4.1 Climatic condition

Although the climate conditions over the experiment have previously been presented in Chapter 3, it is worth noting variation in rainfall and temperature during the calving period (26/07/2017 to 25/8/2017) as there were likely large differences in challenges to thermoregulation of calves. The overall temperature was similar at average range of 2-13 degree for minimum and maximum, respectively. The poorest conditions occurred during 13/08/2017 to 25/8/2017 with the highest average rainfall of 3.6 mm/day compared to the previous period (26/07/2017 to 12/08/2017) at an average 2.2 mm/day. Wind speed (km per hour; km/h) was also higher during 13/08/2017 to 25/8/2017 at approximately 15.8 km/h compared to 7.8 km/h previously.

### 4.4.2 Impact of protein in maternal diet on calf birth weight and stature measurements

There was no effect of maternal diet on calf BW or stature parameters, (Table 4.2). Calf BW was on average  $33.2 \pm 0.6$  kg LW. For the stature measurements; the average heart girth, neck length, spine length, wither height, hip height and waist circumference were on average  $74 \pm 0.4$ ,  $34 \pm 0.6$ ,  $54 \pm 0.4$ ,  $71 \pm 0.4$ ,  $73 \pm 0.4$  and  $73 \pm 0.6$  cm, respectively ( $P > 0.05$ ). Date of birth and the individual animal had no effect on calf BW and stature measurements in this study ( $P > 0.05$ ). There was a strong positive correlation between BW and heart girth measurements ( $r = 0.89$ ,  $P < 0.001$  Figure 4.1).

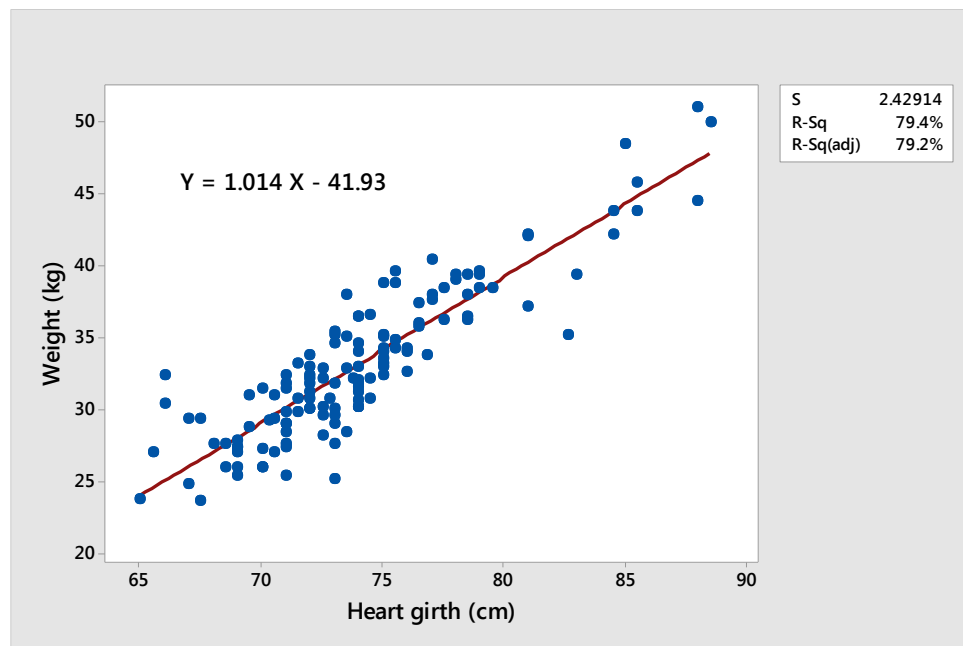
Gestation length of the cows was similar among treatments at an average of  $281 \pm 0.38$  days. Days on pasture pre-calving was similar for both groups as described in Chapter 3 (section 3.4.3.1, LCP =  $8 \pm 0.9$  vs MCP =  $10 \pm 1.1$  days,  $P > 0.05$ ). Days on pasture pre-calving and gestation length were both positively associated with calf BW ( $r = 0.352$ ,  $P = 0.007$  and  $r = 0.184$ ,  $P = 0.040$ , respectively).

As expected, bull calves were larger than heifer calves in nearly all of the stature measurements with the exception of neck length (Table 4.2). The suckling data showed that 80% of calves from both maternal treatments had suckled colostrum before collection.

**Table 4.2 Effect of low crude protein (LCP) and moderate crude protein (MCP) in dam diet during late gestation on birth weight (kg) and stature measurements (cm) of 1-day old calves (n = 136)**

| Parameter                       | Maternal treatment |         | Gender         |                |                  | P-value |            |
|---------------------------------|--------------------|---------|----------------|----------------|------------------|---------|------------|
|                                 | LCP                | MCP     | M <sup>1</sup> | F <sup>2</sup> | Trt <sup>3</sup> | Gender  | Trt*Gender |
| Birth weight (kg)               | 33±0.70            | 33±0.63 | 35±0.7         | 32±0.6         | 0.530            | 0.002   | 0.390      |
| <b>Stature measurement (cm)</b> |                    |         |                |                |                  |         |            |
| heart girth                     | 74±0.57            | 74±0.58 | 75±0.6         | 73±0.5         | 0.990            | 0.002   | 0.591      |
| neck length                     | 33±0.94            | 34±0.81 | 34±0.9         | 34±0.8         | 0.396            | 0.884   | 0.075      |
| spine length                    | 55±0.65            | 54±0.60 | 55±0.5         | 54±0.7         | 0.825            | 0.206   | 0.089      |
| withers height                  | 71±0.62            | 70±0.55 | 72±0.5         | 69±0.5         | 0.530            | <0.001  | 0.384      |
| hip height                      | 73±0.65            | 73±0.60 | 74±0.6         | 72±0.6         | 0.562            | 0.011   | 0.096      |
| waist circumference             | 74±0.76            | 73±0.83 | 75±0.4         | 72±0.7         | 0.284            | 0.003   | 0.912      |

**Note** M<sup>1</sup> = bull calf (male), F<sup>2</sup> = heifer calf (female), Trt<sup>3</sup> = maternal treatment

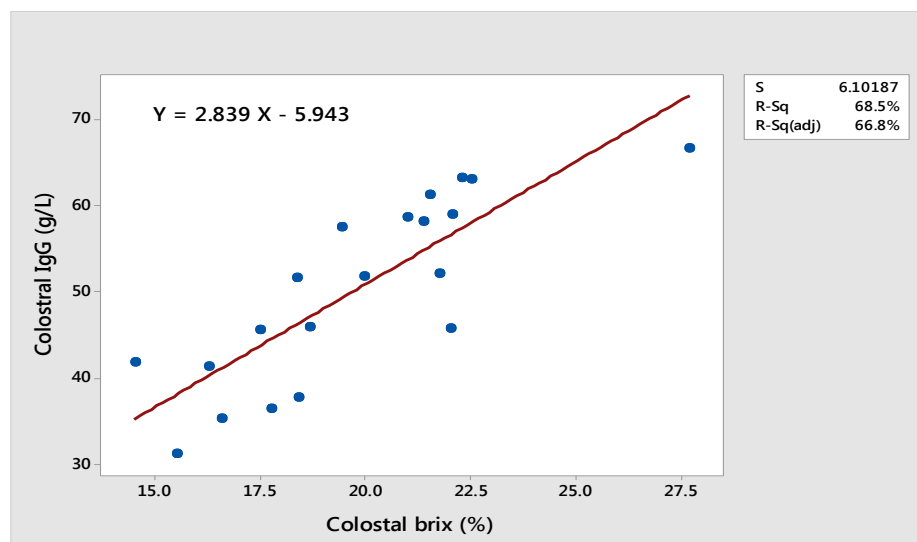


**Figure 4.1 Relationship between calf birth weight (kg) and heart girth (cm). The regression equation to predict birth weight was  $Y = 1.014 X - 41.93$ , where  $Y = BW$  and  $X =$  heart girth ( $r = 0.89$ ,  $R^2 = 79.4$ ,  $P < 0.001$ )**

### 4.4.3 Colostrum quality

In the present study, there was no effect of maternal diet on colostrum composition ( $P>0.05$ , Table 4.3). Colostrum contained approximately two times more solid content ( $20.1\pm 0.52\%$ ) than normal milk ( $9.7\pm 0.05\%$  as previously presented in Chapter 3). Similarly, colostrum protein content was three times higher than normal milk ( $12.9\pm 0.44$  vs  $4.1\%$ ). Fat percentage tended to be higher than normal milk at  $6.7\%$  compared to  $5.6\%$ , respectively. Lactose content in colostrum tended to be lower at  $3.5\%$  compared to  $5.0\%$  in normal milk. Somatic cell count in colostrum was very high at approximately  $1,020\pm 188$  (unit 1,000 cell/ml).

There was a positive relationship between Brix and measured IgG in colostrum (Figure 4.2). Colostral IgG concentration was not different between treatments ( $P>0.05$ ) as presented in Table 4.3. Percentage of colostrum samples with IgG concentration  $\geq 50$  g/L was slightly higher in LCP (37 vs 30% of samples in LCP and MCP, respectively). Parity of the cow significantly affected colostrum quality, with multiparous cows having higher colostral protein and IgG concentrations than primiparous cows. The colostrum composition was not impacted by an interaction between treatments and parity ( $P> 0.05$ ).



**Figure 4.2 Relationship between Brix (%) and measured (turbidimetric immunoassay) immunoglobulin G (IgG) concentration (g/L) in colostrum. Pearson's correlation regression equation of IgG (g/L) =  $2.839 X - 5.943$  ( $R^2 = 68.5$ ,  $P < 0.001$ ), where X is Brix%**

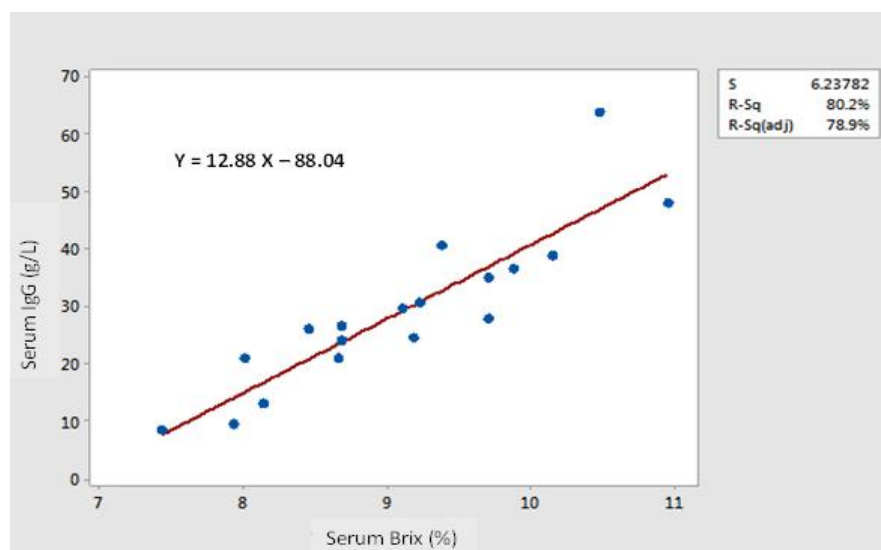
**Table 4.3 Effect of feeding low crude protein (LCP) and moderate crude protein (MCP) during late gestation and parity of the cows between primiparous (Pri) and multiparous (Multi) during late gestation on colostrum composition and Immunoglobulin G (IgG) concentration**

| Parameter                               | Treatment |           | Parity     |           | <i>P-value</i> |        |
|---|-----------|-----------|------------|-----------|----------------|--------|
|   | LCP       | MCP       | Pri        | Multi     | Trt            | Parity |
| Fat (%)                                 | 6.8±0.30  | 7.0±0.31  | 7.2±0.30   | 6.3±0.25  | 0.979          | 0.002  |
| Protein (%)                             | 11.2±0.57 | 10.6±0.50 | 10.8±0.52  | 13.2±0.58 | 0.390          | 0.009  |
| Lactose (%)                             | 3.5±0.10  | 3.5±0.09  | 3.7±0.07   | 3.4±0.10  | 0.984          | 0.016  |
| Total solid (%)                         | 20.5±0.63 | 19.7±0.65 | 19.5±0.52  | 20.5±0.64 | 0.393          | 0.291  |
| Somatic cell count<br>(1, 000 cells/ml) | 1,830±605 | 1,346±463 | 780±214    | 2,073±588 | 0.545          | 0.117  |
| IgG (g/L)                               | 45.6±1.81 | 44.1±1.79 | 41.0 ±1.60 | 47.6±1.69 | 0.481          | 0.013  |

#### 4.4.4 Transfer of passive immunity

##### Serum protein and IgG

There was a close relationship between serum Brix % and IgG as determined by TIA (Figure 4.3,  $r = 0.864$ ,  $P < 0.001$ ). Early colostrum consumption, based on visual assessment of suckling, had a significant effect on the concentration of calf serum IgG and most of the serum proteins. Calves which had suckled early (SC) had higher serum IgG concentration than calves which had not suckled (NSC) on both day 1 ( $P < 0.001$ ) and day 4 ( $P = 0.038$ , Table 4.4). There was no effect of maternal treatment on serum IgG concentration ( $P > 0.05$ ). Concentration of IgG was positively correlated to protein and globulin concentration ( $r = 0.40$  and  $0.41$ ,  $P = 0.01$ ) but negatively correlated to albumin concentration and A/G ratio ( $r = 0.42$  and  $0.44$ ,  $P = 0.001$ ). In the current study, serum IgG concentration in day 4 was related to concentration of day 1 ( $r = 0.560$ ,  $P = 0.016$ ).



**Figure 4.3** The regression equation for serum IgG estimation. The regression equation to predict calf serum IgG concentration  $Y = 12.88 X - 88.04$  was the best fit with  $R^2 = 80.2$ ,  $P < 0.001$ , where  $Y =$  serum IgG and  $X =$  average % Brix

There were no effects of maternal diet on calf serum protein concentrations at one or four days after birth (Table 4.4). At day one, the NSC calves had lower total protein concentration than SC calves ( $P < 0.05$ ). By day four the serum protein fractions of the NSC calves were increased by 25% but were still lower than the SC calves ( $P < 0.001$ ).

**Table 4.4** Effect of feeding low crude protein (LCP) and moderate crude protein (MCP) in dairy cows during late gestation on calf serum protein contents, and IgG (values are means  $\pm$ SEM) ) in calves which have (SC) or have not (NSC) suckled colostrum in the first 24 hours after birth

| Parameter     | Age | Treatment       |                 | Colostrum consumption |                 | P-value |        |       |
|---------------|-----|-----------------|-----------------|-----------------------|-----------------|---------|--------|-------|
|               |     | LCP             | MCP             | SC                    | NSC             | TRT     | COL    | INT   |
| Total protein | 1   | 58.7 $\pm$ 2.21 | 62.4 $\pm$ 2.60 | 69.0 $\pm$ 1.94       | 41.3 $\pm$ 0.47 | 0.281   | <0.001 | 0.355 |
| (g/L)         | 4   | 58.7 $\pm$ 2.84 | 61.8 $\pm$ 3.83 | 66.4 $\pm$ 2.31       | 51.1 $\pm$ 3.80 | 0.521   | <0.001 | 0.510 |
| Albumin       | 1   | 23.3 $\pm$ 0.74 | 23.0 $\pm$ 0.40 | 22.1 $\pm$ 0.34       | 25.7 $\pm$ 0.61 | 0.399   | <0.001 | 0.195 |
| (g/L)         | 4   | 23.1 $\pm$ 0.41 | 22.5 $\pm$ 0.45 | 22.8 $\pm$ 0.43       | 22.7 $\pm$ 0.41 | 0.344   | 0.729  | 0.284 |
| Globulin      | 1   | 35.5 $\pm$ 4.12 | 39.7 $\pm$ 3.84 | 46.9 $\pm$ 2.10       | 15.6 $\pm$ 1.14 | 0.261   | <0.001 | 0.567 |
| (g/L)         | 4   | 35.7 $\pm$ 3.02 | 39.3 $\pm$ 3.95 | 43.6 $\pm$ 2.52       | 28.4 $\pm$ 3.91 | 0.632   | 0.011  | 0.633 |
| A/G ratio     | 1   | 0.9 $\pm$ 0.20  | 0.8 $\pm$ 0.12  | 0.5 $\pm$ 0.04        | 1.7 $\pm$ 0.15  | 0.121   | <0.001 | 0.199 |
|               | 4   | 0.7 $\pm$ 0.11  | 0.7 $\pm$ 0.10  | 0.5 $\pm$ 0.03        | 1.0 $\pm$ 0.14  | 0.381   | 0.011  | 0.723 |
| IgG (g/L)     | 1   | 18.2 $\pm$ 3.23 | 21.4 $\pm$ 3.01 | 23.0 $\pm$ 2.34       | 8.5 $\pm$ 2.73  | 0.904   | 0.005  | 0.810 |
|               | 4   | 26.7 $\pm$ 3.10 | 29.9 $\pm$ 4.42 | 31.8 $\pm$ 2.60       | 15.2 $\pm$ 5.21 | 0.326   | 0.012  | 0.684 |

**Note** SC = suckling colostrum, NSC = no suckling colostrum (observation as described in materials and methods, part 4.2.1), A/G ratio = Albumin/Globulin ratio. Age is shown as a time of sampling (day 1 and 4 of life). P-value shows the significance of means by the effect of maternal treatment (Trt), colostrum suckling (Col), interaction between maternal treatment\*colostrum suckling (INT).

The globulin fraction of SC calves was twice that of the NSC calves (68% vs 37%,  $P < 0.001$ ) in both maternal treatments. At day four, the concentration of globulin in NSC calves was increased by 80%, but still lower than SC calves. In contrast, albumin concentration was dominant in the NSC calves at more than 62% of total protein in day one, twice the concentration in SC calves ( $P < 0.001$ ). The ratio of albumin/globulin was highest in NSC calves at day one compared to SC calves of the same age (average 1.8 vs 0.51,  $P < 0.001$ ). No interaction effects between maternal treatment and colostrum suckling (Trt\*Col) or maternal treatment, colostrum suckling and age (Trt\*Col\*Age) on protein fractions were found.

#### **4.4.5 Serum amino acid profile**

The influence of protein in the maternal diet during late gestation on the profile of calf serum AA is presented in Table 4.5. Of the 22 AA, five accounted for 52% of circulating AA and these included Alanine, glycine, valine, proline and leucine on both the first and fourth day of age. In general, lower crude protein (LCP) in the maternal diet had no carryover effects on circulating serum total AA ( $\mu\text{M/L}$ ) or any of the essential AA (%). However, calves born from dams in the LCP diet had lower serum serine concentration ( $P < 0.001$ ) and higher taurine concentration ( $P = 0.038$ ) compared to calves from dams in MCP.

The age of the calf and whether they had suckled in the first hours of life were important factors which altered calf serum AA profile. Most AA concentrations were lower in NSC calves compared to SC calves, excluding some NEAAs; glycine, taurine glutamine and serine, which were higher ( $P \leq 0.001$ , Table 4.5). The concentration of total AAs and most EAAs (included leucine, isoleucine, lysine valine and arginine) on day 4 were 21 – 86 % higher than day 1. In contrast, the concentration of several NEAAs including taurine, glycine, alanine and proline were 20 to 54% lower than day 1.

There was an interaction between maternal treatment and age of the calves (sampling time) on concentration of several AAs; tryptophan ( $P = 0.035$ ), serine ( $P = 0.022$ ) and taurine ( $P = 0.017$ ).

**Table 4.5 Effect of feeding low crude protein (LCP) and moderate crude protein (MCP) in maternal diet during late gestation and colostrum suckling (suckling, SC and no suckling, NSC) on total amino acid (AA, uM/L) and percentage of calf serum amino acids (AAs) at day 1 and 4 of life**

| Amino acid<br>(%)      | Treatment   |             |             | Colostrum   |             |             | Age         |             |             | P-value      |              |              |              |              |
|------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|--------------|--------------|--------------|--------------|
|                        | LCP         | MCP         | SEM         | SC          | NSC         | SEM         | 1 day       | 4 days      | SEM         | Trt          | Col          | Age          | Trt*Col      | Col*Age      |
| <b>Total AA (uM/L)</b> | 1,432.6     | 1,533.7     | 105.9       | 1462.0      | 1597.1      | 120.8       | 1,333.3     | 1,658.3     | 91.58       | 0.843        | 0.217        | 0.003        | 0.298        | 0.022        |
| Arginine               | 4.73        | 4.86        | 0.33        | 4.96        | 4.38        | 0.37        | 4.02        | 5.61        | 0.26        | 0.863        | 0.242        | 0.003        | 0.784        | 0.854        |
| Histidine              | 4.19        | 3.88        | 0.29        | 4.17        | 3.48        | 0.31        | 4.29        | 3.71        | 0.31        | 0.390        | 0.428        | 0.838        | 0.237        | 0.037        |
| Isoleucine             | 5.04        | 6.02        | 0.51        | 6.10        | 4.33        | 0.64        | 3.96        | 7.35        | 0.31        | 0.238        | <0.001       | <0.001       | 0.048        | 0.004        |
| Leucine                | 8.41        | 9.90        | 0.88        | 10.02       | 7.31        | 1.10        | 6.53        | 12.15       | 0.52        | 0.361        | 0.002        | <0.001       | 0.161        | 0.008        |
| Lysine                 | 2.01        | 2.33        | 0.21        | 2.29        | 1.98        | 0.27        | 1.57        | 2.86        | 0.13        | 0.140        | 0.039        | <0.001       | 0.347        | 0.396        |
| Methionine             | 1.67        | 1.69        | 0.10        | 1.79        | 1.35        | 0.14        | 1.51        | 1.86        | 0.10        | 0.754        | 0.033        | 0.007        | 0.238        | 0.044        |
| Phenylalanine          | 3.29        | 3.46        | 0.18        | 3.52        | 3.04        | 0.19        | 3.08        | 3.72        | 0.17        | 0.213        | 0.047        | 0.045        | 0.267        | 0.666        |
| Threonine              | 5.87        | 5.42        | 0.19        | 5.77        | 5.03        | 0.18        | 5.63        | 5.54        | 0.19        | 0.110        | 0.114        | 0.447        | 0.278        | 0.094        |
| Tryptophan             | 2.64        | 2.37        | 0.14        | 2.70        | 1.81        | 0.14        | 2.49        | 2.46        | 0.13        | 0.592        | <0.001       | 0.414        | 0.712        | 0.006        |
| Valine                 | 12.15       | 12.50       | 0.95        | 13.37       | 9.37        | 1.17        | 10.03       | 14.70       | 0.68        | 0.928        | 0.001        | <0.001       | 0.303        | 0.001        |
| <b>Total EAA</b>       | <b>53.5</b> | <b>51.6</b> | <b>2.52</b> | <b>54.3</b> | <b>40.5</b> | <b>2.52</b> | <b>46.5</b> | <b>58.6</b> | <b>2.52</b> | <b>0.337</b> | <b>0.421</b> | <b>0.348</b> | <b>0.211</b> | <b>0.047</b> |
| Alanine                | 14.15       | 11.14       | 1.11        | 11.73       | 13.89       | 1.34        | 15.09       | 9.45        | 0.76        | 0.088        | 0.061        | <0.001       | 0.379        | 0.012        |
| Asparagine             | 2.08        | 2.44        | 0.17        | 2.20        | 2.59        | 0.21        | 2.07        | 2.53        | 0.16        | 0.387        | 0.106        | <0.001       | 0.516        | 0.001        |
| Aspartic acid          | 0.38        | 0.32        | 0.03        | 0.38        | 0.26        | 0.03        | 0.34        | 0.35        | 0.03        | 0.710        | 0.006        | 0.375        | 0.291        | 0.209        |
| Cysteine               | 0.27        | 0.37        | 0.07        | 0.32        | 0.38        | 0.07        | 0.23        | 0.44        | 0.08        | 0.719        | 0.745        | 0.553        | 0.899        | 0.318        |
| Glycine                | 9.72        | 9.88        | 1.19        | 8.39        | 14.14       | 1.73        | 13.05       | 6.59        | 0.92        | 0.076        | 0.001        | <0.001       | 0.004        | 0.003        |
| Glutamic acid          | 3.91        | 3.60        | 0.41        | 4.33        | 1.87        | 0.35        | 4.06        | 3.38        | 0.38        | 0.981        | <0.001       | 0.434        | 0.403        | <0.001       |
| Glutamine              | 3.12        | 3.97        | 1.05        | 2.01        | 8.51        | 1.10        | 3.99        | 3.32        | 0.87        | 0.099        | <0.001       | <0.001       | 0.130        | <0.001       |
| Proline                | 7.20        | 6.97        | 0.61        | 7.42        | 5.94        | 0.52        | 7.90        | 6.21        | 0.50        | 0.990        | 0.115        | 0.495        | 0.476        | 0.012        |
| Taurine                | 3.26        | 2.67        | 0.50        | 2.54        | 3.94        | 0.62        | 3.97        | 1.81        | 0.31        | 0.038        | 0.001        | <0.001       | 0.109        | <0.001       |
| Tyrosine               | 3.37        | 3.14        | 0.24        | 3.49        | 2.43        | 0.26        | 3.03        | 3.42        | 0.21        | 0.610        | 0.001        | 0.009        | 0.202        | 0.008        |
| Serine                 | 2.53        | 3.06        | 0.17        | 2.49        | 3.96        | 0.30        | 3.17        | 2.55        | 0.23        | <0.001       | <0.001       | 0.004        | <0.001       | 0.040        |
| <b>Total NEAA</b>      | <b>46.5</b> | <b>48.4</b> | <b>2.52</b> | <b>45.7</b> | <b>59.5</b> | <b>2.52</b> | <b>53.5</b> | <b>41.4</b> | <b>2.52</b> | <b>0.345</b> | <b>0.954</b> | <b>0.443</b> | <b>0.683</b> | <b>0.141</b> |

## 4.5 Discussion

The effects of dietary protein restriction during late gestation on dairy cows were reported in Chapter 3. The research presented in this chapter considers whether there was any carry over effects of maternal nutrition during late gestation on calf development and transfer of passive immunity.

### 4.5.1 Effect of maternal nutrition on *in-utero* calf development

In the present study, to ascertain the growth and development of the *in-utero* foetus, stature measurements were used along with BW. Restricted protein in the dams diet during late gestation did not affect the stature development or weight of newborn calves. Birth weights were in the normal range of  $32.2 \pm 0.7$  kg LW for NZ Friesian x Jersey calf (Hickson et al., 2015). Carstens et al. (1987) also showed that feeding low CP diets of 7 or 11% (equivalent to 55 and 91% of CP requirements) to late gestation cows did not affect calf BW. Likewise, Martin et al. (1997) found no significant effect of restricted protein (6.8 vs 10.4% CP DM basis) during the last 140 days of gestation on calf BW or most visceral organs weight (except for the lungs). Later studies also reported that protein supplementation of cows during the last third of gestation had little influence on calf BW (Martin et al., 2007, Larson et al., 2009). Although the concentration of crude protein in the diet from our research and others is below recommended levels, it is probable that the foetus is acquiring sufficient nutrients due to total metabolisable protein needs being met either through increased intake of lower protein feeds or mobilisation of body tissues.

In Chapter 3, cows from LCP had a higher level of serum non-esterified fatty acid (NEFA) at week 38 of gestation. The non-esterified fatty acid is referred to as one of the metabolic stress indicators in dams (Chapinal et al., 2011, McArt et al., 2013b) and has been associated with decreased BW of calves (Ling et al., 2018). Although elevated NEFA was reported in this study, we did not recognize any adverse effect on calf BW here. The possible reason might be that although NEFA serum levels in LCP cows was higher than in MCP cows, the serum concentrations were still lower than the cut –off level at  $\geq 5$  mmol/L (McArt et al., 2013b, Ling et al., 2018). However, exposure to high maternal NEFA during late gestation has the potential to restrict calf growth postpartum and is recognised as

requiring further study (Ling et al., 2018), a question we hope to address in the subsequent chapter.

One concern of this study was the transfer of cows from crop to pasture the week before calving and that this may disguise treatment differences. However, Kleemann et al. (1993) reported that increasing protein and energy in the short term pre-parturition (1-2 weeks before calving) did not improve BW or survival rate of lambs. Martín et al. (2012) also reported no effect of mid-late gestation nutrition on ewe foetal weight at day 140, but in a previous study (Kenyon et al., 2011) reported the impact of the same period nutrition on BW and body length in twin lambs. Those previous studies indicated that the influence of late gestation nutrition might not happen until the last few days pre-calving.

In addition, the lack of difference in growth response of the *in-utero* foetus may occur due to the large variation in growth during 1-2 weeks prior calving. Although the foetus has accelerated growth during late gestation, growth rate then declines after 230 days (Eley et al., 1978, Prior and Laster, 1979). Further, the growth rate of the *in-utero* foetus during the last three weeks prepartum is low, and the foetal nutrient requirement is decreased (Bell, 1995). In this study, the foetus of the MCP cows may have reduced growth earlier as reached the maximum capacity of the placenta faster than the foetus of LCP cows, resulting in calves of similar size.

#### **4.5.2 Effect of maternal nutrition on passive immune transfer**

Colostrum composition of samples taken 12 to 24 hours after calving in the present study was not altered by the effect of maternal treatment (Table 4.3). Though values here differ slightly to previous reports, they are still in the range of  $14.9 \pm 3.3$  % CP,  $6.7 \pm 4.2$  % fat,  $2.5 \pm 0.7$  % lactose (Kehoe et al., 2007b, Holmes et al., 2007). Variation here is likely due to differences in the timing of sampling after calving, and the large variation in components and physicochemical properties of colostrum which has previously been reported by McGrath et al. (2016).

There was a very high concentration of SCC in colostrum ( $> 1 \times 10^6$  cell/ml), which is in the normal range of  $1-2 \times 10^6$  cells/ml for colostrum (Holmes et al., 2007), but

considerably greater than in standard whole milk ( $<0.2 \times 10^6$  cells/ml). McGrath et al. (2016) explained that high SCC in colostrum is typically not related to any infection but caused by physiological factors and penetration of cells through leaky tight junctions between the mammary epithelial cells.

With respect to the calf, one of the most important compounds in colostrum is immunoglobulins which provides passive immunity to the calf when enough colostrum is consumed shortly after birth. In our present study, colostral IgG levels at approximately 45 g/L, did not appear to be affected by maternal treatment feeding regimes. We did find that the cow's parity altered protein content and colostral IgG levels, which is consistent with previous studies (Aydogdu and Guzelbektes, 2018, Quigley and Drewry, 1998). Multiparous cows had 22% higher colostral IgG than primiparous cows ( $P=0.03$ ), in agreement with Gomes et al. (2011a) and Muller and Ellinger (1981) who found the concentration of IgG in multiparous cows was higher than the primiparous. The average IgG concentration in the first milking colostrum of the present study was slightly lower than the international benchmark of  $>50$  g/L, but still in the range of NZ report at 30-150 g/L (Holmes et al., 2007). Parrish et al. (1948) and Gomes et al. (2011a) reported that colostral protein and globulins decrease rapidly with each milking after calving. In our study environment, the first milking was 12-24 hours after parturition during which period suckling of cows by her calf likely impacted on colostral protein and immunoglobulins content.

The lack of effect of maternal protein levels during late gestation on colostrum composition is supported by previous studies. Kehoe et al. (2007b) described that diet during gestation does not directly influence protein and fat content in colostrum. Nowak et al. (2012) reported no effect of diet energy concentration during late gestation on colostrum composition and serum immunoglobulins. Parrish et al. (1948) indicated no significant difference between high protein (alfalfa hay supplemented with 25% CP concentrate) and low protein diet (hay supplemented with corn) during late gestation on colostral components.

Even though there was a lack of evidence to support any impact of maternal diet on colostral IgG concentration, Quigley and Drewry (1998) stated that prepartum low protein (lower than 9%) might result in a reduction of IgG absorption in newborn calves. Low absorption of IgG may result in failure of passive immune transfer which is classified as

serum IgG lower than 10 g/L between 24 and 48 h of age (Godden, 2008, Weaver et al., 2000). However, we found no evidence of an effect of maternal treatment on IgG absorption by calves as serum total protein, albumin, globulin, the ratio of albumin/globulin and IgG content were all similar between treatments of 1 and 4 day old calves (Table 4.3), and were in NZ normal range (Holmes et al., 2007).

The lack of difference in serum IgG suggests no effect of maternal treatment on both the quality of colostrum or the calf's ability to suckle and absorb nutrients. Cuttance et al. (2017) reported that the failure of passive immunity transfer occurred when calves failed to suckle. This disability might be the result of calves being too small and weak or too large and weak from a difficult birth. In this study, calves were collected around 12-24 hours after calving, and it was observed that more than 70% of the calves were considered to have suckled colostrum. Thus we assumed no difference between treatments for the calf's ability to suckle, which we assume was due to calves having similar growth and size development. We found that colostrum consumption had a significant effect on calf serum total protein and protein fractions. Not surprisingly, calves which had not suckled (NSC) had significantly lower serum total protein compare to suckled (SC) calves. Previously, studies indicated that albumin was the largest component in the serum of neonates, at more than 58% of the total serum protein (Tóthová et al., 2016). Albumin content is variable between species (Nagyová et al., 2017) but typically decreases in 24 hours after colostrum consumption, then gradually increases from the first 2-30 days of life (Nagyová et al., 2017, Tóthová et al., 2016).

Interestingly the serum IgG levels of calves in this study increased between day 1 and 4 from the baseline of 23.0 and 8.5 g/L in day 1 to 32.0 and 15.2 g/L in day 4, in SC and NSC calves, respectively. This result contrasted with the previous study of Wilm et al. (2018), who showed a reduction in serum IgG in dairy calves over the first ten days of life. In their study, the calves were not allowed voluntary suckling from their dams but were manually fed 4 L of colostrum only once after collection from the dams (around 3 hours after calving). After that, the calves were delivered 4 L of whole milk two times/day. In our study, the calves voluntary suckled from their dams before collection and fed 4 L of first milking colostrum during the first three days and pooled colostrum (ie colostrum from milkings between day 2 and 4 after calving) on day four. This continued feeding of high-quality colostrum post separation of calf and dam may explain differences between our results of

those and Wilm et al. (2018) but still does not explain why serum IgG increased after 24 hours when absorption of IgG into the bloodstream typically stops (Stott et al., 1979). We did not perform a hematocrit test and it might simply reflect dehydration of calves showing an elevation of IgG in the blood serum. Villarroel et al. (2013) reported that the highest concentration of IgG in calf serum was at 2-3 days of age. The peak represented that it need some lag time for the passive immunity to present in the serum after absorption from the bloodstream. Other studies have also reported an increase in serum IgG of free suckling animals from day 0 to day 30 of life (Tóthová et al., 2016, Nagyová et al., 2017).

#### **4.6 Conclusion**

The results from the maternal trial (Chapter 3 and this chapter) indicated that an eight week dietary protein restriction of 45% recommended requirements wasn't so severe, prolonged or occurred at a time as to result in compromise of dam or calf. During late gestation the dam was able to buffer nutritional deficiencies to maintain normal growth and development of the *in-utero* calf. Maternal protein supplement had no impact on calf serum IgG and protein fractions concentration and did not influence transfer of passive immunity in this study.

## Chapter 5

# The effect of maternal nutrition and milk allowance on heifer progeny growth and lactation performance

### 5.1 Introduction

Prenatal nutrition is crucial for foetal growth and development. Poor intrauterine conditions have been identified as driving factor leading to postnatal growth retardation (Greenwood and Bell, 2003, Gao et al., 2013a), and subsequent impairment of lactation (Paten et al., 2013), growth (Greenwood and Bell, 2003, Gao et al., 2013a) and fertility (Mossa et al., 2013, Rinaldi et al., 2013). In dairy production systems it is believed that without adequate nutrition, severe intrauterine growth retardation may result in long term slower growth of heifers, extend the age at which animals become productive, thereby reducing the overall lifespan production (Garg and Bhanderi, 2016).

In pastoral dairy system countries such as New Zealand, where environmental regulations increase the pressure on farmers to reduce nitrogen surpluses by feeding low N diets, the resulting mitigation actions may cause long term negative flow-on effects to livestock. The risk of inadequate intrauterine nutrition may be increased if farmers continue to shift towards lower protein diets, particularly in late gestation dairy cows, to prevent N pollution. In the previous study (Chapter 4) we tested the null hypothesis that feeding late gestation dairy cows a diet lower in crude protein content than recommended by ruminant feeding guidelines would have no effect on *in-utero* calf development as measured within hours of birth. The results of that study supported the null hypothesis showing no immediate adverse effect on calf birth weight (BW) or stature as a result of maternal feeding and protein restriction.

Our results suggested that the dam was able to buffer suspected nutritional shortcomings in her diet to protect the normal development of the calf *in-utero*. However, the longer-term goals of this project were to compare the impacts of maternal feeding on not just the development of the calf *in-utero* but throughout its early life to weaning. There may be other *in-utero* deficiencies which do not become apparent in the progeny until later

in their development. Questions remained regarding the impact of *in-utero* nutrition on pre-weaning growth, which previous research indicated foetal programming alteration by long term effects on heifer production by improving health, growth and feed efficiency (Khan et al., 2011, Obeidat et al., 2013, Diaz et al., 2001).

Previous research has already demonstrated that growth during early life (i.e. pre-weaning stage) can impact the post-weaning growth rate (Greenwood and Cafe, 2007, Roche et al., 2015). Improved pre-weaning growth has been associated with improved reproductive performance (Roche et al., 2015) and greater first lactation milk production (Chester-Jones et al., 2017). Further, Soberon et al. (2012) found that every 1 kg increase in pre-weaning average daily gain (ADG) resulted in an increase in 850 kg of milk yield in the first lactation. In New Zealand dairy calf rearing, using the conventional milk allocation regime calves are offered a 'restricted' milk or milk replacer diet at 10% of body weight (Garcia-Muniz et al., 1998) or approximately 4 L/calf/day (Margerison et al., 2013, Thomson et al., 2018). This feeding below optimal requirements is designed to encourage solid feed intake, stimulate rumen development and reduce rearing costs (Muir et al., 2002). While pre-weaning growth rates may be lower by adopting this restricted rearing regime, adequate post-weaning nutrition over the duration to calving at 22 months will enable target live weight to be met (MacDonald et al. 2010). However, a survey of NZ dairy heifers found a large percentage fail to reach the target live weight at mating and pre-calving (Handcock et al., 2016).

It may be possible to use nutritional interventions to recover the effects of poor *in-utero* nutrition or post-partum feeding and provide benefits pre-weaning (Greenwood and Cafe, 2007). In the view of intrauterine nutrition, we sought to investigate the carryover effect of maternal protein limitation on pre-weaning calf growth when calves were offered either a low milk allowance (10% of BW) or a high milk allowance (20% of BW). We hypothesized that feed conversion efficiency (FCE) of high allowance calves would be greater than low allowance calves. Secondary to this we speculated that unforeseen impacts of *in-utero* protein-restriction from the maternal diet, may impact post-natal FCE.

The objective of this study was to determine whether there are carryover effects of maternal feeding regime on the health and growth and development of the calf from dams

which had received different crude protein (CP) content diets and whether the response of the calf to restricted or high milk allowance alters feed efficiency and growth.

## **5.2 Materials & methods**

### **5.2.1 Experimental site and design**

This research was a continuation of the experiment described in Chapters 3 and 4 (see section 4.2.1) and the calf nutrition intervention phase was conducted between 2 August and 22 September 2017. The location of the experiment continued at the Ashley Dene Research and Development Station (ADRDS), Lincoln University, with the approval of the Lincoln University Animal Ethics Committee (AEC 2017-13).

The experimental design was a factorial 2 x 2 design with three temporal blocks (classified as a batch). The first factors were the two *in-utero* treatment groups from dams which were on an either moderate (13.6% crude protein, MCP) or low protein (7.8% crude protein, LCP) diet. The second factor is the neonatal intervention using high level of milk feeding (high allowance, HA, 20% of BW) versus standard (conventional, CON, 10% of BW) calf rearing practice pre-weaning. A total of 60 female Friesian x Jersey dairy calves from experimental dams were allocated to one of two feeding regimes based on their maternal treatment and date of birth.

### **5.2.2 Management**

Initial management and handling of newborn calves are described in Chapters 3 and 4. Briefly, all experimental calves were measured within 24 hours and, following measurement, calves were placed in a 'colostrum pen' for four days and received two x 2L bulk colostrum (first milking colostrum mixed from cows on all treatments, Chapter 4) per calf per day. At the end of the colostrum feeding period (four days of age), heifer calves from each maternal nutrition group were randomly assigned to one of the two feeding regimes (HA or CON). Although dams in the LCP and MCP groups were balanced for calving, we were unable to control the exact birth date of calves in maternal treatments. To account

for variation in the birth date, and pen size of the rearing facilities, calves were delegated to batches based on maternal group and birthdate. Groups of 20 calves were reared in three batches of approximately 5 calves/treatment/batch. During the first week of calving, more calves were born from LCP compared with MCP and vice versa in the third week. Table 5.1 show the allocation of calves to each treatment within each batch.

**Table 5.1 Number of heifers in each group**

| Batch | Calving date   | Maternal treatment |    |                  |    | N  |
|-------|----------------|--------------------|----|------------------|----|----|
|       |                | Low protein        |    | Moderate protein |    |    |
|       |                | Pre-weaning regime |    |                  |    |    |
|       |                | CON                | HA | CON              | HA |    |
| 1     | 30 Jul - 5 Aug | 7                  | 7  | 3                | 3  | 20 |
| 2     | 6 - 12 Aug     | 5                  | 5  | 5                | 5  | 20 |
| 3     | 13 - 21 Aug    | 3                  | 3  | 7                | 7  | 20 |
| N     |                | 15                 | 15 | 15               | 15 | 60 |

**Note:** CON = Conventional (10% BW or 3-4 L/calf/d), HA = High allowance (20% BW or 6-8 L/calf/d)

### 5.2.3 Rearing facilities

The rearing facility was arranged into six indoor calf pens for six groups of 10 calves (3.0m x 5.5m, minimum of 1.65 m<sup>2</sup> per calf). All pens were bedded with straw, and more bedding was added as required to keep the area clean. The facility was naturally ventilated, and freshwater was provided for each pen (Picture 5.1). From day 4 to day 20 of age calves had no access to pasture but had *ad lib* access to a formulated calf pellet. The heifer calves were moved from the shed to the grass paddock when they reached approximately 3 weeks of age. Once calves were transferred to the paddock, windbreaks and calf shelters were provided for shelter (Picture 5.2 and 5.3).



**Picture 5.1 Calf rearing pens**



**Picture 5.2 Windbreaks**



**Picture 5.3 Calf shelter**

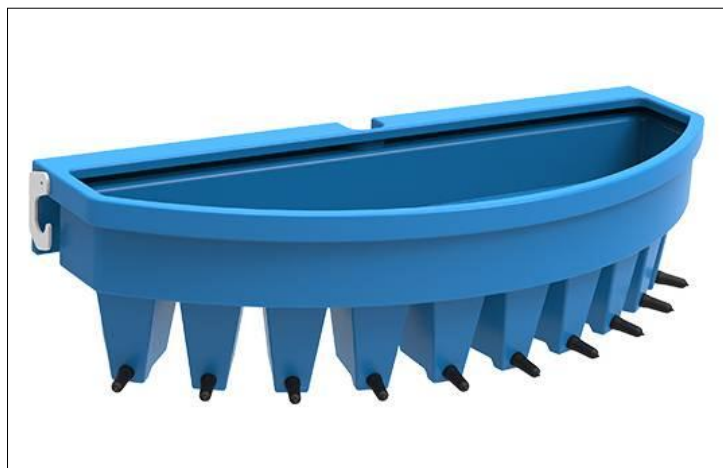
#### **5.2.4 Feeding**

Calf feeding was carried out by two staff at approximately 0800 am and 0300 pm each day. The formula of commercial calf milk replacer (CMR) was made from whey proteins and animal fat. For the first 20 days the starter CMR formula contained a coccidiostat (Ancalf™, Batch no. B0172, Fonterra, New Zealand) while a standard whole milk finisher CMR was offered from 21 days to weaning (Ancalf™ Finisher, Batch no. B0395, Fonterra, New Zealand). The transition from colostrum to CMR was made over four days by combining CMR with colostrum at increasing proportion of 25% per day.

Both starter and finisher CMR formulas were mixed at 150 g of CMR/L with 4 days transition time. The composition of starter CMR contained 3.4% protein, 2.6% fat, 5.7% lactose and 0.9% mineral on an as fed basis. In the finisher CMR, the protein, fat, lactose and mineral component were 2.9, 2.6, 6.3 and 0.9 % (as fed basis), respectively.

The calves received either CON (10% BW or 3-5 L/calf/d) versus HA (20% BW or 6-8 L/calf/day) split into two equal feeds per day for 5 weeks (39 days of age). The formulation of CMR for the CON group was based on the energy and protein requirements to meet live weight (LW) gain targets of at least 400 g/d, for calves at approximately 30-55 kgLW (NRC, 2001). The milk was fed in a plastic 10 teat compartment calf feeder (Picture 5.4) where each compartment had a 3 L capacity. During feeding, two staff monitored drinking

behaviour and ensured that each calf had equal access to milk. A feeder bottle was used for any slow drinkers to ensure they acquired the correct amount of CMR.



Picture 5.4 10-teat compartment calf feeder

Table 5.2 Nutrient composition (% of DM) of calf milk replacers (CMR) and calf starter pellets used in the experiment

| Nutrient component      | CMR1                    | CMR2<br>(finisher)      | Calf starter<br>pellets |
|-------------------------|-------------------------|-------------------------|-------------------------|
| Dry matter              | 95.4±0.19               | 95.1±0.11               | 88.2±0.11               |
| Crude protein           | 26.9±0.11               | 22.4±0.08               | 20.8±0.18               |
| Fat                     | 20.7±0.05               | 20.6±0.05               | 3.6±0.26                |
| Lactose                 | 45.1±0.12               | 48.4±0.10               | -                       |
| Neutral detergent fibre | -                       |                         | 13.8±0.26               |
| Acid detergent fibre    | -                       |                         | 6.4±0.28                |
| Minerals                | -                       |                         | 6.8±0.11                |
| Organic matter          | -                       |                         | 93.2±0.11               |
| ME (MJME/kgDM)          | 20.73±0.38 <sup>1</sup> | 20.32±0.51 <sup>1</sup> | 14.4±0.04 <sup>2</sup>  |

**Note:** ME<sup>1</sup> (CMR) was calculated according to NRC (2001) and ME<sup>2</sup> (starter) was calculated according to CSIRO (2007)

## 5.2.5 Measurements

### 5.2.5.1 Feed measurement

The intake of milk replacer for individual calves was determined by two staff who recorded any refusals after 30 minutes of feeding. Refusals of CMR (if any) were measured by a 1 L plastic volumetric beaker and recorded for individual calves at the time of feeding.

A sample of milk replacer was collected on two consecutive days on four occasions (n = 8 samples), and milk powder was sampled each week (n = 3 samples) and were stored at -20°C until analysis of nutrient composition (CP, fat and lactose by LIC). Metabolisable energy (ME, MJ/L) of CMR was calculated followed the equations of GE (Mcal/kg) = 0.057 CP% + 0.092 fat% + 0.0395 lactose%, while DE = 0.97\*GE and ME = 0.96\*DE (NRC, 2001).

Refusals of calf starter pellets were weighed and recorded every day. The group average intake was calculated by dividing the weight of consumed pellets by the number of calves in the group. Calf starter pellets were sampled on three occasions and separated into two subsamples. The first subsample was analysed for DM content in forced air oven at 60°C for 48 h. The second subsample was freeze-dried and ground to 1 mm sieve (ZM200, Retsch, Haan, Germany) and kept for chemical analysis (crude protein; CP, ether extract; EE, acid detergent fibre; ADF, neutral detergent fibre; NDF and organic matter) by wet chemistry methods (AOAC, 1990). Metabolisable energy (ME) was calculated as MJME/kgDM = 14.55 - 0.0155 MADF (CSIRO, 2007).

### 5.2.5.2 Heifer measurements

To compare the effect of treatments on growth and development, pre-weaned calves were weighed at the start of the study (day 4) and again at week 5 when the nutritional regimes ceased and at 8 weeks when calves were weaned from milk. Skeletal measurements; body length (length of crown to shoulder, shoulder to tail head), heart girth, wither height, hip height, and waist circumference was measured (using a standard measuring tape) at day 4 and the end of week 5 and week 8.

Health assessment of calves was adapted from Hill et al. (2016a). Every day, prior to and during feeding a scorer observed each calf for general health and scored visible faeces.

Faeces score was assigned using a scale of 1 to 5; with 1 being normal, but thick in consistency, 2 being normal but less thick, 3 being abnormally thin, 4 being watery and 5 being watery with abnormal colour. Faeces with blood or mucus were scored at 5, and the faeces were sampled for infective microbial identification. Calves with an abnormal faecal score of  $\geq 4$  received a 50% reduction in their CMR allocation and were then gradually increased to their normal treatment feeding in 2-3 days. No electrolytes were given to the calves. Antibiotic or medical treatments were recorded daily and administered based on diarrhoea, lethargy, coughing and nasal discharges. Those treated calves were in the same pen with the remainder of their batch.

At the end of week 5, calves were weighed using a manual weigh crate (Prattley Industries Ltd. Temuka) and stature measurements were carried out before their morning meal. Then they were commingled into a single mob and received the same feeding management of 5 L/calf/day until weaning - at 8 weeks of age. At the end of week 8, weaning weight and stature were measured in a vet race in the cattle yards where the heifers were held and measured individually. From there on all calves were weighed regularly using walk-over scales, from weaning through to the end of their first lactation. Average LW and weight gain at 4, 6, 9, 15, 18 and 21 months (pre-calving) was recorded using the Livestock Improvement Corporation's MINDA™ Live herd-recording software (MINDA).

### **5.3 Statistical Analysis**

The effect of treatment was compared at three age categories: colostrum stage of 4 days old, intervention stage to 5 weeks $\pm$ 3 days and weaning stage at 8 weeks $\pm$ 3 days of age. Calf variables including feed intake, live weight, live weight gain and stature measurement (hip height, waist circumference, heart girth, withers height and body length). The general linear model procedure of Genstat (v. 12, VSN International) was used. Fixed terms included maternal treatment, milk allowance and their interaction, covariates included BW and colostrum stage skeletal measurement for weight gains and body developments; batch was treated as a block and individual calf as replicate. Means for post-weaning growth response and first lactation performance were analysed using Genstat ANOVA, with maternal

treatment and milk allowance used as fixed effects and batch as a block and individual calf as replicate. Statistical significance between treatment diets was established as  $P < 0.05$ .

## **5.4 Results**

### **5.4.1 Impact of maternal nutrition and allowance on calf health**

Two calves (both were from the LCP maternal diet, HA group) in the third batch died during the experiment (Table 5.3). Both calves had watery faeces (without mucus or bloody scouring) for a few days. No antibiotic treatment was applied, but the feeding rate of these calves was reduced to 2.0-2.5 L/meal. The first calf died on day 7 during the feeding trial, and another one died after the end of the trial before weaning. Thus only 58 heifers remained for the final 8 weeks of live weight and body skeletal measuring.

There was no effect of maternal or feeding treatment on faeces score or the number of abnormal faecal days (Table 5.3). Out of interest, a significant blocking effect showed that calves in Batch 3 (i.e. born during a week of poor weather conditions) had worse faecal scores than the previous two batches with an average 5-week faecal score of 1.25 compared to 1.09 and 1.06 for batch 1 and 2, respectively. Batch 3 also had almost 3 times more abnormal faecal days compared with batch 1 or 2 (1.4 compared to 0.5 days in both batches 1 and 2, not showed in Table 5.3). After 3 weeks of age, when the calves were moved to the grass paddock, the faecal scores of the calves in the third batch was still slightly higher than other batches with an average 1.15 compared to 1.00 and 1.08 of the calves in batch 1 and 2, respectively. Further, 24 calves (from both maternal and allowance groups) showed signs of bloody faeces. The highest prevalence was found during 18-23/08/2017 (batch 3). The faeces samples which were collected for microbial testing, did not show any presence of pathogenic organisms tested.

**Table 5.3 Effect of maternal diet and high (20% of live weight) or low (10% of Live weight) allowance on faecal score and the number of the abnormal faecal days (discolours or blood appearance)**

| Dam treatment  | LCP       |      | MCP  |      | SEM  | P-value |          |           |
|--|-----------|------|------|------|------|---------|----------|-----------|
|  | Allowance | CON  | HA   | CON  |      | HA      | Maternal | Allowance |
| Mortality<br>(no of calves)                                  |           | 2    |      |      |      |         |          |           |
| No of calves with<br>abnormal faecal<br>score <sup>1,2</sup> | 5         | 11   | 4    | 9    |      |         |          |           |
| Average faecal<br>score                                      | 1.16      | 1.10 | 1.09 | 1.19 | 0.05 | 0.727   | 0.575    | 0.093     |
| Persistence of<br>scours (days)                              | 1.00      | 1.60 | 1.75 | 2.11 | 0.38 | 0.166   | 0.256    | 0.751     |

**Note:** <sup>1</sup>Abnormal faecal score was determined at score of  $\geq 3.0$ . <sup>2</sup>No of calves with abnormal score was counted for individual calf (no repeat counting). Different of means were analysed by maternal diet treatment and calf CMR allowance as fixed effects and batch as a block. M\*A = interaction between maternal diet treatment and calf allowance.

#### 5.4.2 Composition and intake of feeds

Milk replacer intake of CON calves was constant from day 4 until weaning at 4 litres/calf/day. CMR intake of the HA group was slowly increased to reach a maximum at 8 litres in the first three weeks. The date to reach peak allocation was due to some HA heifers experiencing abnormal faecal score and requiring reduced CMR intake for few days. After 21 days, CMR intake decreased and fluctuated at around 7-8 litres/calf/day ( $P < 0.001$ ) (Figure 5.1 and Table 5.4). The final nutrient intake of HA calves from milk replacer was two- fold compared to calves in CON (Table 5.4).

There was no impact of previous maternal treatment (LCP or MCP) or its interaction with feeding regime on calf intake. The CON groups consumed two times more starter pellet than the HA groups (Table 5.4,  $P = 0.03$ ). Accordingly, nutrient intake from starter pellets was higher in CON than HA calves. However, the increased intake of starter pellets from the CON group was not enough to compensate for the higher ME intake from CMR of the HA calves. Overall, calves in HA consumed approximately 36% more protein and ME than the

conventional group, but 47% less NDF. Although pasture intake was not known, the nutritive value of the pasture for pre-weaning calves was  $11.5 \pm 1.03$  % CP,  $37.5 \pm 1.37$  % NDF and  $12.6 \pm 0.17$  MJME/kgDM.

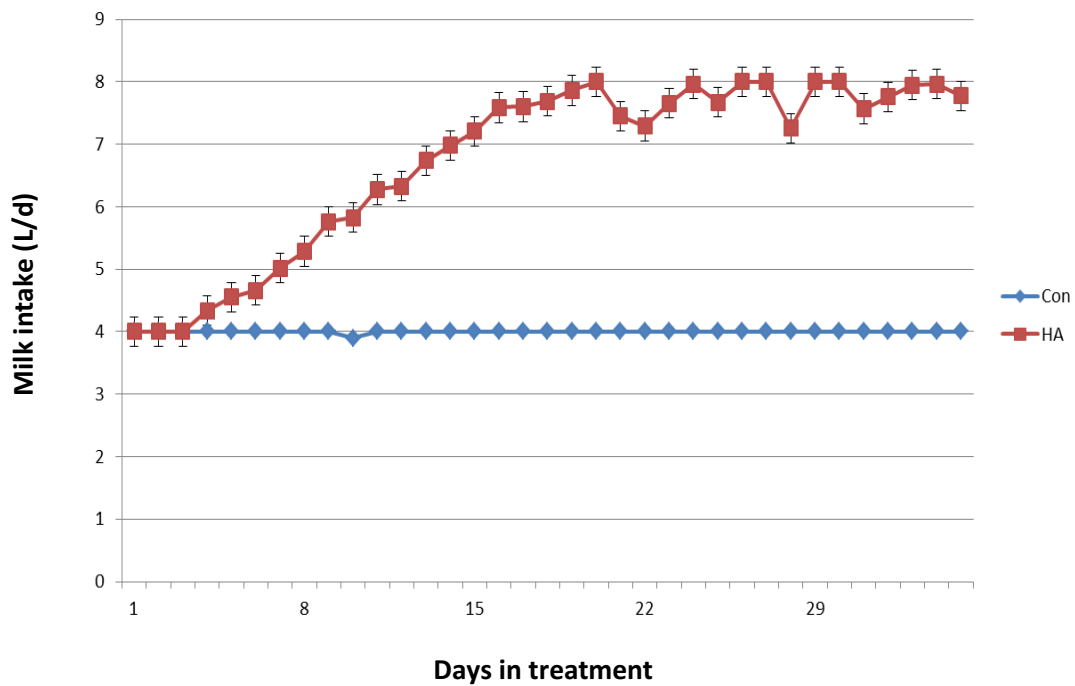


Figure 5.1 Daily intake (L/d; mean  $\pm$  SE) of milk replacer of heifers in high allowance (HA) and conventional (CON) milk replacer in the preweaning experiment

**Table 5.4 Intake of calf milk replacer (CMR) and starter meal of heifer calves between 4 days and 5 weeks of age. Calves were born from dams which had been fed either a low (LCP) or moderate crude protein (MCP) diet in late gestation and were fed either 10% (CON) or 20% (HA) of their live weight after birth.**

| Maternal diet<br>Allowance                           | LCP          |              |             | MCP          |              |             | P-value      |                  |              |
|--|--------------|--------------|-------------|--------------|--------------|-------------|--------------|------------------|--------------|
|  | CON          | HA           | SEM         | CON          | HA           | SEM         | Maternal     | Allowance        | M*A          |
| <b>Total intake</b>                                  |              |              |             |              |              |             |              |                  |              |
| CMR intake (L/calf) <sup>1</sup>                     | 126.5        | 219.8        | 3.52        | 127.1        | 222.1        | 2.84        | 0.650        | <0.001           | 0.800        |
| CMR powder (kg/calf) <sup>1</sup>                    | 19.0         | 33.0         | 0.55        | 19.1         | 33.3         | 0.43        | 0.650        | <0.001           | 0.800        |
| Starter intake (kg/calf) <sup>2</sup>                | 4.1          | 2.2          | 0.45        | 4.1          | 2.8          | 0.41        | -            | 0.036            | -            |
| <b>Daily intake</b>                                  |              |              |             |              |              |             |              |                  |              |
| CMR powder (g/calf/d) <sup>1</sup>                   | 596.6        | 1,040.3      | 5.72        | 597.4        | 1,045.4      | 4.92        | 0.645        | <0.001           | 0.735        |
| Starter intake (g/calf/d) <sup>2</sup>               | 139.3        | 74.1         | 17.70       | 139.3        | 74.1         | 17.72       | -            | 0.064            | -            |
| <b>Average daily nutrient intake (g/d, DM basis)</b> |              |              |             |              |              |             |              |                  |              |
| CMR protein  | 160.5        | 279.8        | 1.53        | 160.7        | 281.2        | 1.34        | 0.645        | <0.001           | 0.735        |
| Starter protein                                      | 28.9         | 15.4         |             | 28.9         | 15.4         |             | -            | -                | -            |
| <b>Total protein</b>                                 | <b>189.4</b> | <b>295.2</b> | <b>1.50</b> | <b>189.6</b> | <b>296.6</b> | <b>1.33</b> | <b>0.645</b> | <b>&lt;0.001</b> | <b>0.735</b> |
| CMR ME (MJME/d)                                      | 12.1         | 21.2         | 0.35        | 12.2         | 21.3         | 0.14        | 0.645        | <0.001           | 0.735        |
| Starter ME (MJME/d)                                  | 2.0          | 1.1          |             | 2.0          | 1.1          |             | -            | -                | -            |
| <b>Total ME (MJME/d)</b>                             | <b>14.1</b>  | <b>22.2</b>  | <b>0.14</b> | <b>14.2</b>  | <b>22.3</b>  | <b>0.11</b> | <b>0.645</b> | <b>&lt;0.001</b> | <b>0.735</b> |
| Starter NDF (g/d, DM basis)                          | 19.2         | 10.2         |             | 19.2         | 10.2         |             | -            | -                | -            |

**Note** <sup>1</sup> value is calculated from the exact day in the trial of each calf. <sup>2</sup> values are calculated as average intake of calves in CON or HA group, without maternal treatment classification (n = 30 for CON group and n = 29 for HA group). ME = metabolisable energy, NDF = neutral detergent fibre.

### 5.4.3 Impact of maternal nutrition and allowance on growth responses of heifers

#### 5.4.3.1 Prewaning

Live weight parameters are presented in Table 5.5 and means are adjusted for BW as a covariate. There was no effect of maternal treatment or its interaction with feeding treatment on calf live weight. The average four-day live weights were similar among treatments at  $37.1 \pm 0.62$  kg. Between day 4 and day 35, the high allowance (HA) heifers grew faster and were 11% (9 kg LW) heavier than the CON calves by 5 weeks of age (Table 5.5,  $P < 0.001$ ).

During the first 5 weeks of the feeding regime, the overall ADG of all treatments was  $0.38 \pm 0.02$  kg/d, with a range from 0.12 to 0.80 kg/d. Mean ADG of the CON group was lower than the HA at 0.29 vs 0.47 kg/d ( $P < 0.001$ ). Although the HA heifers were receiving 5 L/calf/day of milk as CON between week 5 and 8, the effect of high allowance on live weight was still evident at weaning (8 weeks) where the HA heifers were approximately 12% (10 kg LW) heavier than the CON heifers ( $P < 0.001$ ). However, between weeks 5 and 8 when calves received similar milk allocation there was no difference in growth rate.

The feed conversion efficiency of DM intake during the trial period (kg weight gain/kg DM intake, from d 4 to 39) was not different between maternal treatments averaging 0.46 vs  $0.42 \pm 0.03$  kg/kg DM, for LCP and MCP, respectively ( $P > 0.05$ , data not shown in a table). Correspondingly, there was no effect of maternal treatment on FCE of ME intake 24.0 vs  $21.0 \pm 1.0$  g LWG/MJ ME, for LCP and MCP respectively ( $P > 0.05$ ).

There was no effect of maternal treatment on body development of the heifers through the weaning stage (Table 5.6). Stature measurements of heifers in the HA group at the end of the pre-weaning trial (5 weeks) were greater than those in the CON group ( $P < 0.05$ , Table 5.6). Several differences were still evident until weaning (Picture 5.5). There were also no interactions between maternal treatments and allowance, FCE for the CON and HA group was similar for dry matter ( $0.43$  vs  $0.46 \pm 0.03$  kg/kg, respectively,  $P > 0.05$ ) and ME ( $22$  vs  $23 \pm 0.1$  g LWG/MJ ME, respectively,  $P > 0.05$ ) intake.

**Table 5.5 Effect of maternal treatments of low crude protein (LCP) and moderate crude protein (MCP) diets and allowance on live weight, growth responses of heifers in the preweaning experiment (values are means across 3 batches). Milk replacer intake over 5 week milk replacer trial and daily nutrient intake between calves from 2 maternal treatments: with 2 pre-weaning treatments: CON (conventional regime, 10% BW), HA (high allowance, 20% BW). Number of heifers in each group is in the brackets.**

| Maternal diet                | LCP       |             | MCP        |             | SEM  | P-value    |          |           |
|------------------------------|-----------|-------------|------------|-------------|------|------------|----------|-----------|
|                              | Allowance | CON<br>(15) | HA<br>(14) | CON<br>(15) |      | HA<br>(15) | Maternal | Allowance |
| <b>Live weight (kg)</b>      |           |             |            |             |      |            |          |           |
| 4 days                       |           | 37.3        | 37.5       | 36.1        | 37.4 | 0.62       | 0.116    |           |
| 5 weeks                      |           | 47.2        | 54.1       | 46.2        | 53.6 | 1.01       | 0.939    | <0.001    |
| 8 weeks                      |           | 79.5        | 87.9       | 78.8        | 87.5 | 1.43       | 0.447    | <0.001    |
| (Weaning)                    |           |             |            |             |      |            |          |           |
| <b>Live weight gain (kg)</b> |           |             |            |             |      |            |          |           |
| 4 days - 5 week              |           | 9.9         | 16.6       | 10.1        | 16.2 | 0.70       | 0.576    | <0.001    |
| 4 days - 8 week              |           | 42.2        | 50.3       | 42.7        | 50.1 | 1.04       | 0.250    | <0.001    |
| 5 – 8 week                   |           | 32.3        | 33.5       | 32.6        | 33.9 | 0.58       | 0.228    | 0.368     |
| <b>Growth rate (kg/day)</b>  |           |             |            |             |      |            |          |           |
| 4 days - 5 week              |           | 0.3         | 0.5        | 0.3         | 0.5  | 0.02       | 0.576    | <0.001    |
| 4 days - 8 week              |           | 0.7         | 0.9        | 0.8         | 0.9  | 0.02       | 0.250    | <0.001    |
| 5 – 8 week                   |           | 1.5         | 1.6        | 1.5         | 1.6  | 0.03       | 0.228    | 0.368     |

**Note:** Maternal treatment and pre-weaning allowance were fixed effects. M x A\* = Interaction between maternal treatment and calf allowance.



**Picture 5.5 Altered growth impact of different milk allowances in twin heifer calves from same maternal diet treatment, twin heifers from MCP dam were allocated to different allowance group, showing different growth response of pre-weaning feeding. Heifer number 30 in the CON group was smaller than number 31, her twin whose was in the HA group.**

**Table 5.6 Effect of protein in maternal treatment (low crude protein, LCP and moderate crude protein, MCP) and allowance (conventional regime, CON and high allowance, HA) on stature measurements of heifers at 4 days, 5 weeks in the experiment and weaning at 8 weeks (values are mean across 3 batches). Number of heifers in each group in parenthesis**

| Maternal diet                            | LCP               |                   | MCP               |                   | SEM  | P-value |          |           |
|--|-------------------|-------------------|-------------------|-------------------|------|---------|----------|-----------|
|  | Allowance         | CON               | HA                | CON               |      | HA      | Maternal | Allowance |
|  | (15) <sup>1</sup> | (14) <sup>1</sup> | (15) <sup>1</sup> | (15) <sup>1</sup> |      |         |          |           |
| <b>Body measurement (at 4 days, cm)</b>  |                   |                   |                   |                   |      |         |          |           |
| Heart girth                              | 75.0              | 75.4              | 74.3              | 75.2              | 0.52 | 0.315   | -        | -         |
| Neck length                              | 31.4              | 31.1              | 31.7              | 32.3              | 0.46 | 0.084   | -        | -         |
| Body length                              | 53.1              | 52.9              | 53.0              | 52.4              | 0.61 | 0.770   | -        | -         |
| Wither height                            | 71.6              | 70.3              | 70.6              | 71.2              | 0.52 | 0.203   | -        | -         |
| Hip height                               | 73.4              | 73.9              | 73.3              | 72.9              | 0.53 | 0.114   | -        | -         |
| Hip circumference                        | 73.6              | 75.1              | 75.1              | 73.6              | 0.68 | 0.596   | -        | -         |
| <b>Body measurement (at 5 weeks, cm)</b> |                   |                   |                   |                   |      |         |          |           |
| Heart girth                              | 86.4              | 89.1              | 86.2              | 89.9              | 0.60 | 0.471   | 0.001    | 0.764     |
| Neck length                              | 37.4              | 38.7              | 37.9              | 40.6              | 0.55 | 0.954   | 0.011    | 0.339     |
| Body length                              | 64.7              | 68.3              | 65.9              | 68.2              | 0.60 | 0.619   | 0.013    | 0.335     |
| Wither height                            | 77.8              | 78.7              | 76.6              | 78.7              | 0.42 | 0.632   | 0.046    | 0.917     |
| Hip height                               | 80.1              | 80.5              | 79.3              | 82.1              | 0.50 | 0.312   | 0.064    | 0.467     |
| Hip circumference                        | 86.0              | 89.5              | 86.2              | 91.4              | 0.90 | 0.359   | 0.003    | 0.963     |
| <b>Body measurement (at 8 weeks, cm)</b> |                   |                   |                   |                   |      |         |          |           |
| Heart girth                              | 94.4              | 98.2              | 93.1              | 96.5              | 0.62 | 0.397   | <0.001   | 0.183     |
| Neck length                              | 42.7              | 46.8              | 42.1              | 41.8              | 1.03 | 0.142   | 0.395    | 0.318     |
| Body length                              | 70.1              | 72.5              | 70.2              | 74.6              | 0.78 | 0.123   | 0.051    | 0.609     |
| Wither height                            | 83.8              | 82.9              | 81.5              | 82.8              | 0.53 | 0.408   | 0.896    | 0.582     |
| Hip height                               | 86.3              | 86.7              | 84.3              | 85.7              | 0.48 | 0.155   | 0.426    | 0.831     |
| Hip circumference                        | 93.7              | 98.7              | 94.4              | 97.3              | 0.77 | 0.409   | 0.003    | 0.089     |

**Note:** Maternal treatment and pre-weaning allowance were fixed effects. M x A\* = Interaction between maternal treatment and calf allowance.

#### 5.4.4 Post-weaning growth and lactation

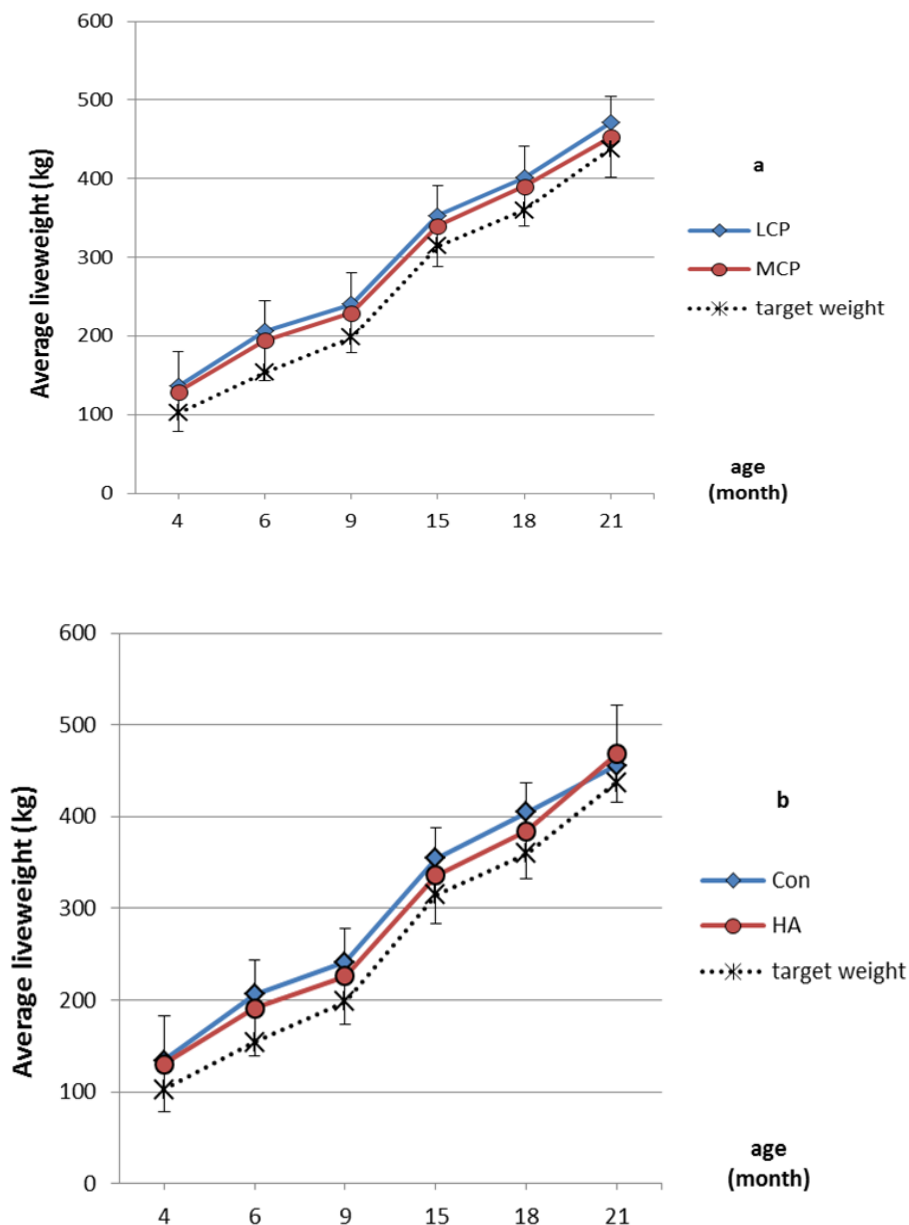
The MINDA database was used to track the post-weaning growth response of the heifers. Not all heifer calves from the pre-weaning study were retained in the replacement group to continue to the end of the first lactation. Of the calves in the study, data from 10 heifers from the CON and 10 heifers from the HA group, survived to 21 months of age (9 from LCP and 11 from MCP maternal experiment, see Figure 1.2).

There were no interactions between maternal nutrition and milk allowance for growth. There was no difference in post-weaning growth response of the heifers as a result of maternal treatments (LCP or MCP, Figure 5.2a) or due to milk allowance regime (CON or HA, Figure 5.2b) ( $P>0.05$ ). By the time heifer calves reached four months of age, differences in pre-weaning live weights had disappeared. Average live weights at 4, 6, 9, 15, 18 and 21 months (pre-calving) were  $132\pm3.0$ ,  $199\pm4.1$ ,  $234\pm4.1$ ,  $345\pm6.0$ ,  $395\pm6.0$  and  $462\pm11.6$  kg LW, respectively. Weight gain was also similar among the groups at approximately 1.0, 0.87, 0.53, 0.83, 0.76 and 0.65 kg/day, at age of 4, 6, 9, 15, 18 months and pre-calving, respectively. The post-weaning LW of heifers in this study were all above the target industry LW through 21 months of age (Figure 5.2 a and b).

Of the 20 heifers which survived to calving, 15 heifers (7 from LCP and 8 from MCP), survived their first lactation for which MINDA data was collected (7 from CON and 8 from HA group). These heifers were managed together in a single mob after weaning and reached their first lactation in the 2019 season. Our results showed that levels of CP in maternal diet during late gestation had no impact on milk performance of the progeny in their first lactation (Table 5.7). There were no interactions between maternal nutrition and milk allowance for milk yield or milk composition. However, in spite of low numbers of animals, pre-weaning nutrition did appear to affect milk yield ( $P=0.053$ ).

Pre-weaning allowance resulted in 29 more days in milk for HA heifers ( $P = 0.038$ ) and increased milk yield by 27% ( $P<0.10$ ) compared with the CON heifers. No difference in other milk parameters was found. Percentage of fat and protein were not different at respective averages of  $5.1\pm0.1$  and  $4.1\pm0.1$  %. The yield of milksolids, fat and protein was similar at respective averages of  $348\pm11.4$ ,  $193\pm6.6$  and  $155\pm5.0$  kg/lactation. The variation in days in milk was due to later drying off as calving dates were not different at average 27/07/2019  $\pm$  4.8 vs 26/07/2019  $\pm$  4.2 day for CON and HA heifer, respectively. Breeding

worth (BW) and production worth (PW) were not different between allowance groups at onaverage of  $139 \pm 7.4$  and  $102 \pm 28.4$  vs  $144 \pm 12.0$  and  $176 \pm 36.9$  for CON and HA, respectively.



**Figure 5.2** Average of post-weaning live weight (kg) of the heifers from (a) maternal treatments (low crude protein, LCP (n=29) and moderate crude protein, MCP (n=30)) and (b) allowance; Con (conventional regime, n=(30) ) and HA (high allowance, (n=29))

**Table 5.7 Average milk yield and composition of progeny born in 2017 from dams in 2 different maternal treatments (low crude protein, LCP and moderate crude protein, MCP) and allowance (conventional regime, CON and high allowance, HA). Number of heifers in each group is in the brackets.**

| Maternal diet            | Maternal treatment |                      | Allowance            |                      | SEM     | P - value           |          |           |
|--------------------------|--------------------|----------------------|----------------------|----------------------|---------|---------------------|----------|-----------|
|                          | Allowance          | LCP (7) <sup>1</sup> | MCP (8) <sup>1</sup> | CON (7) <sup>1</sup> |         | HA (8) <sup>1</sup> | Maternal | Allowance |
| Days in milk (day)       |                    | 235.8                | 237.57               | 222.3                | 251.1   | 8.35                | 1.000    | 0.038     |
| Milk yield (L/lactation) |                    | 3,419.0              | 3,816.0              | 3,318.0              | 4,001.0 | 260.34              | 0.393    | 0.053     |
| Milksolids (kg)          |                    | 314.3                | 348.8                | 305.3                | 360.5   | 24.99               | 0.583    | 0.109     |
| Fat (%)                  |                    | 4.8                  | 5.1                  | 4.8                  | 5.0     | 0.25                | 0.818    | 0.559     |
| Fat (kg)                 |                    | 172.1                | 193.0                | 167.2                | 200.9   | 14.44               | 0.540    | 0.074     |
| Protein (%)              |                    | 4.0                  | 4.1                  | 4.2                  | 3.9     | 0.15                | 0.973    | 0.623     |
| Protein (kg)             |                    | 142.1                | 155.9                | 138.2                | 162.0   | 10.71               | 0.453    | 0.089     |
| SCC (1,000 cell/ml)      |                    | 31.6                 | 29.9                 | 38.4                 | 25.3    | 6.31                | 0.573    | 0.345     |

**Note:** 1) Values are averaged from 3 herd tests (23/9/2019, 04/12/2019 and 11/03/2020). Values in brackets are number of heifers. Maternal treatment and allowance as fixed effects, the batch of birth as a block.

## 5.5 Discussion

In Chapter 4 we found that maternal protein restriction during late gestation did not cause any obvious immediate risk to neonates. In Chapter 5, the combined effect of maternal diet and postnatal feeding regime on the growth, development of heifer calves pre-weaning and post weaning to end of 1<sup>st</sup> lactation was studied.

### 5.5.1 Effect of the maternal protein restriction on pre-weaning calf health

The pre-weaning growth stage is an essential period in the life of young stock as inadequate nutrition can influence the milk production of replacement heifers (Soberon et al., 2012). We expected calves from LCP dams to struggle more with health issues because they were expected to be smaller at birth as had occurred in previous studies (Zhang et al., 2002, Bell, 2005, Gao et al., 2013b). The smaller calves might be prevented from suckling and acquiring the necessary colostrum intake to avoid failure of passive immune transfer (Vasseur et al., 2009). However, based on the evidence collected in this study there were no carry over effects of maternal nutrition on calf health using measures such as average faecal score or scours persistence (Table 5.3).

Although animal numbers here are small, there are a number of reasons that contributed to similar health and development of calves from protein restricted-dams. Firstly, calf BW was similar as was the percentage of calves which suckled and acquired passive immunity (see Chapter 4). A previous study which compared nutritional restriction in late gestation cows (1.2 times of maintenance level vs *ad libitum*) also did not find a response in altered body weight or length of the foetus (Duarte et al., 2013). However, those authors did note that the small intestine mass and length were greater in the foetus from the restricted dams. This evidence indicates perhaps some compensatory development which will improve acquisition of nutrients post-partum though alteration of small intestine surface and increasing nutrient and immunoglobulin absorption ability (Duarte et al., 2013, Meyer and Caton, 2016).

Although we did not measure the fat depth or adipose tissue, we suspected that calves from LCP dams would be more vulnerable to cold conditions than MCP calves due to

having more limitations in thermoregulation. This theory arose from links between FB feeding, serum arginine and brown adipose tissue (BAT). McCoard et al. (2013) using lambs showed maternal arginine supplementation during mid to late gestation increased BAT mass which is essential to thermoregulation and survival of neonates (McCoard et al., 2014). Wallace et al. (2015), using lambs also showed that poor maternal nutrition influenced the amount of BAT. In Chapter 3 we recorded lower serum arginine in LCP cows compared to the MCP calves. We speculated that low BAT as a result of low arginine supply may contribute to weaker calves in the LCP group, subsequent increased risk of not suckling or succumbing more quickly to pathogens than those calves from MCP. While the numerical trends favoured the hypothesis that LCP calves would have worse health outcomes (as reflected by higher mortality and numbers of LCP calves with an abnormal fecal score) we lack sufficient replication and repeatability from this single cohort of calves to draw strong conclusions.

There are limited studies showing the effect of maternal nutrition on BAT for cattle. Martin et al. (1997) reported no effect of restricted protein (7% compared with 10% CP DM basis, in isocaloric diets) during late gestation on calf BW or brown adipose tissue mass and composition. The reason for lack of difference may relate to the impact of timing of undernutrition on development of vital organs. Because the genes that regulate foetal adipose tissue are developed through mid-gestation (Wallace et al., 2015), these tissues are already established by late gestation so undernutrition in late gestation are unlikely to alter these tissues.

### **5.5.2 Effect of maternal nutrition on heifer growth and development**

There was no evident carryover effect of different protein levels in the maternal diet on pre-weaning heifer growth performances. Heifer calves from LCP or MCP dams in the same feeding group had similar live weight, ADG and FCE. The lack of maternal effect here is perhaps not surprising, given the lack of maternal effect on calf BW or morphological development (Chapter 4).

As discussed previously the likely reasons for the lack of response in our present study compared with previous studies primarily relate to the severity of nutrient deficiency

(ND), the duration of ND and the timing of ND (stage of gestation). Notably, the severity and duration of ND in the present study were less than previous reports where offspring impact was observed. Cafe et al. (2006), who studied beef cows, found that a low plane of nutrition (low pasture yield with less than 6% CP) had a negative impact on both calf BW and weaning weight. Their study, however, observed the effect of severe protein deficiency (half that recommended by NRC for maintenance during gestation cow) over a more extended period of ND than our present study (i.e. 6-8 months pre-calving, to the end of early gestation). Early to mid-gestation is the stage of gestation where significant development of vital organs and tissues occur but minimal development with regards to foetal growth (Funston et al., 2010a, Gopalakrishnan et al., 2005). A long duration of extreme maternal ND during this critical stage has consequently shown to result in low BW and altered foetal development along with dysfunction of vital mechanisms which persisted through postnatal life of the calf (Eley et al., 1978). Accordingly, high pre-weaning morbidity and mortality rate have also been reported due to low BW (Greenwood et al., 1998). Further, in Cafe et al. (2006) the cows faced with a low plane of nutrition lost 47 kgLW at parturition compared to the 55 kgLW gained in the high nutrition plane treatment. Low nutrition cows then produced less milk during lactation resulting in lower pre-weaning growth responses of their calves which were naturally reared with the dams (Cafe et al., 2006).

The CP levels in those studies were lower than in the present study, and the duration of nutritional deficit continued for a longer period. Further, the lack of observed adverse impacts of late gestation maternal protein restriction on pre-weaned calves in the current study was also probably because cows in both groups maintained their LW and BCS through calving (Chapter 3, Table 3.6). No subsequent effects on BW and body development of the calves from LCP or MCP were found (Chapter 4, Table 4.2) and passive immunity transfer and serum AA profiles were similar for calves from both maternal treatments (Table 4.3 and Table 4.4). However, numerical differences in expression of calf health issues (Table 5.3) would highlight this as an area for continued research.

### **5.5.3 Effect of pre-weaning feeding regime on health responses of calves**

While maternal nutrition appeared to have little impact on health and growth of calves, the milk allowance effect was more pronounced. The heifers in the HA group did show some abnormal faecal scores, which was attributed to nutritional scours. This evidence agreed with previous studies (Drackley, 2007, Bartlett et al., 2006, Muir et al., 2002, Jasper and Weary, 2002) where considerably softer faeces were generally found in calves fed higher quantities of milk replacer and increased days of elevated faeces score compared to the conventional allowance or whole milk feeding. The rapid intake of milk in two daily feeds may have upset the gut of some calves, but did not have negative effects on growth or intake at the next feed. Similarly, Ballou et al. (2015) studied the impact of high (HPN) or low plane of nutrition (LPN) (at 5-6 L/d of high CP and fat CMR vs 4 L/d of standard CP and fat CMR) on growth response and resistance to an oral drench of *Salmonella enterica* in dairy calves. The HPN calves had evidence of higher faecal score than the LPN, but calves fed HPN also had increased growth rate, FCE and were more resistant to *S. enterica* after weaning.

### **5.5.4 Effect of feeding regime on growth response of heifers**

Pre-weaning nutrition was the most significant factor impacting on heifer development. Offering 20% versus 10% of a calf's BW and live weight as milk resulted in significant differences in live weight gain. It was not surprising that calves offered more milk had higher energy intake and subsequently greater growth, a response to increased feeding that is previously well documented (Rosenberger et al., 2017, Hill et al., 2008, MacPherson et al., 2016). Growth response and protein requirements of the calf depend on energy intake, as noted previously if energy or protein intake is too low then this fails to meet optimum growth (Akins, 2016, Bartlett et al., 2006). In our present study, total metabolisable energy (MJME/d) and crude protein (g/d) for either CON or HA were above maintenance for the pre-weaning calf as estimated using standard feeding equations (NRC, 2001). The average daily nutrient intake of the HA calves was 36% higher than the CON group (Table 5.4).

At the outset of the study, we were concerned that intense large suckling bouts with our twice-daily feeding regime would reduce FCE, especially when we noted variation in

faecal scores. However, Kmicikewycz et al. (2013) studied dairy calves fed either 2 or 4 meals and found that while feeding frequency altered starter intake of calves on the lower protein diets, they concluded that increased feeding frequency did not alter overall growth or FCE. Similarly, Kehoe et al. (2007a) reported no differences in growth response between calves fed 1 or 2 times per day at 10% BW after 2 weeks.

As we were unable to determine intake from pasture through herbage measurements we attempt to estimate pasture requirements from energy models to meet the observed level of growth (Table 5.8). On that basis CON calves are likely to have consumed 250-300 g DM/d as pasture to meet energy needs for growth (3.6 MJME/day). These equations don't take into account energy for activity or heat loss being outside so are likely to be conservative estimates, particularly considering that the HA calves needed to consume less ME than estimated to meet their growth requirements.

**Table 5.8 Metabolisable energy requirement (MJ ME/head/d) and metabolisable energy intake for live weight gain (LWG) of calves from two milk replacer feeding treatments; conventional (CON, n=30) and high allowance (HA, n= 29).**

|                                 | CON<br>(30) <sup>1</sup> | HA<br>(29) <sup>1</sup> |
|---------------------------------|--------------------------|-------------------------|
| <b>ME requirement (MJ/d)</b>    |                          |                         |
| ME <sub>m</sub>                 | 14.8                     | 15.8                    |
| ME <sub>g</sub>                 | 2.9                      | 4.6                     |
| Total ME <sub>req</sub>         | 17.7                     | 20.5                    |
| <b>ME intake (MJ/d)</b>         |                          |                         |
| ME estimated from CMR           | 12.1                     | 21.2                    |
| ME estimated from starter       | 2.0                      | 1.1                     |
| ME estimated from CMR + starter | 14.1                     | 22.2                    |
| ME required from pasture        | 3.6                      | -1.8                    |

**Note:** ME<sub>m</sub> = metabolisable energy for maintenance, ME<sub>g</sub> = metabolisable for growth (live weight gain). Metabolisable energy intake from pasture was considered from the difference between total requirement for live weight gain and ME from calf milk replacer (CMR) plus starter meal. ME of pasture analysed by NIRs was 12.6 MJ ME/kgDM.

High levels of milk replacer intake reduced solid feed consumption as expected, which is in line with previous studies (Hill et al., 2006, Groenendijk et al., 2018, Hill et al., 2016b, Khan et al., 2011, Jasper and Weary, 2002). Although solid feed intake increased during the trial period in both treatments, the amount consumed was higher in the CON group. Some previous studies reported that low intake of solid feed might impede rumen development and result in a lower growth rate after weaning (Suarez-Mena et al., 2011). Low post-weaning growth rates increase the risk of heifers being underweight when they reach calving and impaired lactation. However, there have been studies showing that while calves with restricted feeding had lower pre-weaning growth, they also had faster rumen development, compensatory preweaning growth and no significant effect on post-weaning growth or first lactation performance (Kesser et al., 2017).

In the current study, the HA calves maintained higher LW than CON calves even after their milk allocation was reduced to that of the control group (5 to 8 weeks of age) but maintained the same growth rate. This finding is also in line with previous results of Kesser et al. (2017). Lack of difference between the two groups can also be explained by the fact that while milk intake was decreased from approximately 8 to 5 L/calf/d for HA calves. The underdeveloped rumen of the HA calves made it difficult for them to fully compensate lower milk energy intake with pasture energy intake (de Passillé et al., 2011, Rosenberger et al., 2017). Recommended weaning practices for calves on ad-lib diets suggest restricting milk allocation prior to weaning to ease weaning stress and support growth by encouraging solid intake and rumen development (Bach et al., 2013, Khan et al., 2016, Khan et al., 2011).

Average live weight at weaning (8 weeks of age) across the calves in the present study was 83.4 kgLW, more than double the BW as recommended in Soberon et al. (2012). By 3 months of age HF x Jersey crossbred calves are expected to reach 18% of their mature live weight or 90-95 kg LW (Burke et al., 2007) which means the calves in this study needed to gain 10 kg in four weeks or 360 g LWG/day. Immediately prior to weaning these calves were gaining over 1 kg/day. The treatments in this study did not disadvantage the growth of calves or ability to meet the target. Consequently, by 4 months of age, all calves were similar in LW (Figure 5.2 a and b) irrespective of maternal nutrition or pre-weaning feeding. In our study, post-weaning growth rate did not differ between allowances or maternal treatments. The lack of difference between groups for growth and live weight post-weaning,

despite growth differences preweaning, was also observed by Margerison et al. (2013). However, those authors did not explain as to why there were no obvious carryover effects of higher weaning weight. The lack of variation was likely the result of changes in rumen development immediately post-weaning, especially in HA calves which may have slowed their growth rate (Table 5.5).

The growth rate was lower at the age of 9 months (during the first winter of their life) and pre-calving (also during winter) which is a typical observation in pasture reared replacements (Handcock et al., 2019a). The average growth rate of heifer calves pre and post-weaning depends on management factors including breed (Handcock et al., 2019a), milk type, amount of feeding, weaning method and post-weaning group size (Brickell et al., 2009). Brickell et al. (2009) reported the ADG from 1 to 6 months of heifer fed milk replacer at average 0.73 vs 0.92 kg/day in restricted commercial feeding or *ad libitum* feeding, respectively. This result is close to our recent study, which found ADG from 4 day-8 weeks at average 0.75 vs 0.90 kg/day, in CON and HA heifer, respectively (Table 5.5). In addition, Handcock et al. (2019a) reported a growth rate of crossbred (HF x J) heifers during 3-5 month of age at 0.75 kg/day or 0.68 % /day. These previous results support the ability to gain the expected LW of the heifers in the current study.

#### **5.5.5 Effect of feeding regime on first lactation performance of the heifers**

The high allowance pre-weaning regime showed advantages over the control regime because of longer DIM in the 1<sup>st</sup> lactation. Increased DIM was due to extended lactation since the average calving date at the beginning of the lactation between the groups was not different. Milk yield was higher in HA heifers compared with the CON ( $P=0.053$ ). When adjusted for MS%, the difference was around 18% more milksolids yield from HA compared with CON heifers ( $P=0.109$ ). With our small number of animals we were unable to report this difference as significant. Though there is growing evidence that pre-weaning growth rate can significantly affect lactation performance of dairy cows.

Korst et al. (2017) reported a positive impact of *ad libitum* pre-weaning feeding on first lactation milk yield, even though they too reported a lack of statistical difference due to small sample size (28 heifers). Korst et al. (2017) stated that milk yield of the heifers from feeding groups only differed during the last two months of the lactation ( $P>0.05$ ). A similar

trend was found in the present study, with milk yield in the final herd test (11/03/2020) being higher in the HA compared to the CON at average 14.2 vs 11.2 L/day ( $P = 0.065$ ), while little differences were evident in early lactation. Our results suggest that early life nutrition improves longevity of milk yield in the first lactation, irrespective of live weight or growth rate between weaning and calving. Soberon and Van Amburgh (2013) using a large database of dairy cows and replacements in the US found a significant positive relationship between pre-weaning growth rate and first lactation milk yield. They calculated that every 1 kg of pre-weaning ADG would bring about 1,551 kg more milk in first lactation.

Margerison et al. (2013) studied increasing pre-weaning calf nutrition by supplementing a plant extract, with additional amino acids to whole milk. They presented similar trends, i.e. higher pre-weaning growth and LW, but no difference in LW from post-weaning to lactation. In the study of Margerison et al. (2013), first lactation milk yield and composition were greater in the enhanced nutritional groups compared to the normal whole milk. In their study, the difference in lactation was due to higher milk yield, not the result of more DIM as was the cause in the current study.

Previous studies reported that enhanced pre-weaning growth rate resulted in increased development of the principal tissue in mammary glands; mammary parenchyma (PAR) and mammary fat pad (MFP) (Knight and Peaker, 1982, Vailati-Riboni et al., 2018). More recently, PAR development is becoming a key focus in the research for maximizing future milk production (Vailati-Riboni et al., 2018). However, development of MFP and PAR are intertwined (Hovey et al., 1999, Vailati-Riboni et al., 2018) and these tissues had both revealed to respond differently to different types of nutrition (Brown et al., 2005a, Daniels et al., 2009, Piantoni et al., 2012).

Brown et al. (2005a) reported that increasing more protein to fat (30.3% CP and 15.9% fat) in pre-weaning calf diet resulted in rapid growth and increased rate of development of PAR. Daniels et al. (2009) found that high fat intake pre-weaning (27% CP and 20% fat or 27% CP and 28% fat compared with 20% CP and 21% fat) increased the MFP but did not alter PAR. Molenaar et al. (2020) also reported an increased growth rate of the preweaned calves fed high amounts of whole milk (8 vs 4 L/d). Milk performance was not reported in that study, but the high allowance whole milk calves showed 40% reduction in the ratio of PAR: MFP compare with the conventional group. They suspected that high fat content in whole milk encourages the development of MFP rather than PAR. In the study of

Molenaar et al. (2020) the calves were offered whole milk from pasture-fed cows which has a relatively high fat to protein of 1.25:1.0 (Margerison et al., 2013). This high fat to protein ratio was similar to the study of Daniels et al. (2009) and might be a reason for smaller PAR:MFP ratio.

In the present study, the calves were fed a CMR with a low content of fat to protein at 2.6 and 3.4 % as fed basis (detail in part 5.2.4 and Table 5.2), or equivalent to the fat to protein ratio of 0.76:1.0. Khan et al. (2011) stated that calves respond better to high allowance when CMRs contain higher protein and lower fat (up to 30% protein with 15-20% fat). The mechanism for improving milk performance was outside the scope of this project but may relate to the alteration of PAR. This is an interesting area for future research.

## **5.6 Conclusion**

In the present study, differences in maternal protein did not affect calf growth rate from birth to weaning or beyond. On the other hand, high allowance of calf milk replacer increased heifer weaning weight, growth rate and body development and milk yield. However, the benefit of high milk allowance pre-weaning on post-weaning ADG and weight gain disappears within a month of weaning due to compensatory growth. The impact of level and type of milk feeding on mammary gland development is an area of future research for calves reared in pastoral systems.

## Chapter 6

# The effect of maternal diet on long term performance of the offspring

### 6.1 Introduction

Optimal rearing of the replacement heifers is important for achieving successful dairy production outcomes. Throughout the life of the heifer, many factors can impact their performance. From a nutritional perspective, nutrient restrictions at specific stages of life can play an important role in growth and development and subsequent reproductive and lactation performance. There have been a number of research investigations on the effects of post-weaning, or pre and post-puberty growth rates on lactation performance. In contrast, there have been few studies investigating as early as *in-utero* on the impacts on lactation performance of dairy cows (Macdonald et al., 2005). There is growing evidence to suggest that maternal nutrition during gestation can influence the long term productivity of the progeny (Wu et al., 2006, Meyer and Caton, 2016, McCoard et al., 2017). However, there is little information on the effect of feeding regime in pastoral production systems on progeny outcomes for dairy.

Funston et al. (2010b) showed that protein supplementation of beef cows during late gestation increased progeny weaning live weight (LW) and improved the fertility of heifer offspring. In rodent models, Guzman et al. (2006) reported that protein restriction of pregnant rats resulted in delayed puberty of female pups and delayed time to the first estrus compared with control groups. In cattle, Martin et al. (2007) suggested that the ovarian folliculogenesis and the endometrial gland are still developing through gestation until completion near calving. Those authors highlighted the risks associated with metabolic disturbances *in-utero*, which could lead to metabolic disorders later in life. Further, nutrient restrictions of the dam may disrupt the development of these tissues, leading to carry over effects on the progeny. These disorders are expected to manifest in performance outcomes such as reduced growth rate, poor fertility, lower milk yield and compromised immune status (Greenwood and Cafe, 2007, Meyer and Caton, 2016).

The genesis and development of many crucial tissues take place during foetal development, including mammary tissue, gastrointestinal tract and liver, which are sensitive to prenatal nutrition (Funston et al., 2010a, Godfrey and Barker, 2000). For instance, Martin et al. (2007) reported protein restriction in late gestation dams did not alter birth weight (BW) but found that the heifers had lower pre-breeding weight and decreased pregnancy rate compared with the supplemented dams. Zhao et al. (2018) and Chadio et al. (2017) found long term physiological effects (smaller livers and altered gene expression) in male ruminants from undernourished dams. In addition, low maternal protein nutrition was reported to alter the structure and function of pancreatic islets of rodent offspring, which in turn resulted in permanent changes in insulin and glucose metabolism (Funston et al., 2010a). Although insulin and glucose might have a small impact in the grazing ruminant (Funston et al., 2010a), even small alterations in nutrient metabolism were proved sufficient to influence milk production in dairy cows (Murphy et al., 2000).

In the current research presented in this thesis we have investigated the carry over effects of maternal feeding over two groups of a small number of dams and an even smaller number of progeny. From those results we found that the low crude protein in maternal diet during late gestation did not show an immediate or carry over negative impact on the growth performance of the offspring. We compared late gestation feeding of fodder beet (FB) diets differing only in supplement type, with both diets supplying relatively low protein. Typically New Zealand farmers could also feed alternative diets such as pasture or green crops such as kale which are likely to supply a different nutrient profile to that of FB. To improve confidence in conclusions regarding late gestation feeding of spring calving dairy cows it is useful to evaluate a wider population of animals under a range of feeding conditions. To address this challenge the opportunity arose to collate data from a commercial demonstration farm (LURDF) which had previously adopted four different winter feeding regimes (three regimes being compared directly) as part of an investigation into productivity and environmental impacts of winter systems based on crop (Edwards et al., 2014b, Edwards et al., 2014a). That study was carried out over three years and enrolled 300 spring calving cows each year for a crop feeding study (kale or FB) with surplus animals wintered on pasture. Following the first year, calves which were born to cows on a low protein diet (11-12% CP) (i.e. fodder beet or high yielding kale) were smaller than calves

born from cows which had received a moderate protein diet (13% CP) (Bryant and Pirat, 2014). This outcome raised questions regarding whether lower BW of calves on fodder beet was typical or simply an anomaly of that particular season/set of management. If the lower BW on fodder beet was a true effect of feeding a low protein diet to late gestation cows – what then were the long term impacts on the progeny?

The objective of the study was to compare the long term effects of feeding different crops: kale, fodder beet or pasture to cows during late gestation on growth and milk production of offspring. The data of calves from a commercial herd over three consecutive winters (2012-2014) are analysed in terms of effects of maternal diets on growth after birth and milk production in first and second lactation of the progeny.

## **6.2 Materials & methods**

### **6.2.1 Management and feeding information**

In this study, cows from a commercial demonstration (LUDF) participated in a feeding study over three consecutive winters (June and July 2012, 2013 and 2014) and their progeny followed to their second lactation. Of the circa 500 cows available, 300 multiparous Friesian x Jersey non-lactating, pregnant multiparous dairy cows were blocked into two replicated mobs of 50 cows based on calving date, live weight, body condition score, age and breeding worth (Table 6.1 and 6.2). All cows were transported to the Lincoln University Ashley Dene research farm (43°39.05'S, 172°19.21'E, 38 m a.s.l) on 1<sup>st</sup> of June and offered 1 of 3 experimental diets (plus one group of 200 cows on pasture as a control group) according to three forage crops:

1) Early sown kale (EK): High kale allowance (14 kg DM/cow/day) plus barley straw (3 kg DM/cow/day) supplying approximately 180 MJ ME/cow/day, and 350 g N/cow/day (equivalent to 11.5% of crude protein, CP).

2) Late sown kale (LK): Low kale allowance (11 kg DM/cow/day) plus green crop oat silage (5 kg DM/cow/day) supplying approximately 181 MJ ME/cow/day, and 330 g N/cow/day (equivalent to 12.9 % CP).

3) Fodder beet (FB) (8 kg DM/cow/day) plus ryegrass baleage (6 kg DM/cow/day) supplying approximately 186 MJ ME/cow/day, and 240 g N/cow/day (equivalent to 11.5% CP).

4) Pasture (control) with a high stocking rate system (over 4 cows/ha) (Pangborn et al., 2011) (average to 18 % CP).

Management and feeding of the cows are described by Edwards et al. (2014b). Briefly, cows were fed in order to gain half a body condition score over winter. Supplements were fed in the morning prior to a fresh crop allocation which was supplied daily. Cows remained *in situ* in the crop paddock (i.e. no standoff area) until they returned to LUDF on 26-28 July (approximately 1 month prior to calving), where they were returned to pasture and silage diets and calved on the milking platform. The cows which did not participate in the trial were predominantly later calving mixed age cows and early calving heifers which were offered a pasture and silage diet through the winter. When the experimental cows were returned to the milking platform at LUDF, they were combined into a single dry and springer mobs and treated as per best commercial practice (Pangborn et al., 2011).

**Table 6.1 Blocking information of the 300 cows on crop in each year from 2012-2014**

| Blocking information | Cohort           |                  |                  |
|----------------------|------------------|------------------|------------------|
|                      | 2012             | 2013             | 2014             |
| Actual calving date  | 21/08/2012 ± 2.1 | 20/08/2013 ± 1.1 | 21/08/2014 ± 2.1 |
| Live weight          | 520.7 ± 5.30     | 500.5 ± 3.13     | 494.0 ± 0.03     |
| Body condition score | 4.5 ± 0.04       | 4.3 ± 0.02       | 4.0 ± 0.03       |
| Age                  | 4.1 ± 0.10       | 4.6 ± 0.24       | 4.4 ± 0.23       |
| Breeding worth       | 108.5 ± 2.20     | 136.3 ± 3.78     | 149.1 ± 5.42     |

Note: Values are means±SEM

During calving, calves were collected and brought to the calf rearing pens 1-2 times/day. At the rearing pens, the calves were provided 2 litres/calf of bulk first milking

colostrum twice daily in the morning and afternoon for 4 days. All heifers from different maternal diet treatments were raised on the same farm under the same management practise after birth between years 2012-2014. Maternal identification was carried out by DNA testing. Information of dams (i.e. number, feeding treatment, replication, calving date) was collected from the farm records.

The growth and milk yield information of heifers born in the year 2012, 2013 and 2014 were collected from Livestock Improvement Corporation's MINDA™ Live herd-recording software (MINDA) to analyse growth response adjusted by age at 3, 6, 12, 15 and 22 months (first pre-calving age). Information of the first and second lactation milk yield and composition of each cohort were also collected from Livestock Improvement Corporation Ltd (LIC) herd test record and MINDA.

### **6.3 Statistical analysis**

All data were processed using Microsoft Excel 2013. Means for growth rate, live weight gain and survival rate of the offspring were compared using one-way analysis of variance (ANOVA) in the GenStat 18.1 (VSN International, U.K.) with dam diet as a fixed effect, year born (cohort) as a block.

To compare the effect of maternal diet on milk performance of the offspring, means of 1<sup>st</sup> and 2<sup>nd</sup> and cumulative lactation yield and composition were analysed by ANOVA using maternal diet as a fixed effect, cow birth year as a block, days in milk as a covariate. Days in milk were analysed separately by ANOVA using maternal diet as a fixed effect, and cow birth year as a block. A *P*-value less than 0.05 were considered as statistically significant.

**Table 6.2 Details of the experimental cows assigned to winter crop feeding regimes consisting of early sown kale (EK), late sown kale (LK) and fodder beet (FB) between the years 2012-2014.**

| Blocking information | Cohort    |           |           |           |           |           |           |           |           |           |           |           |
|----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                      | 2012      |           |           |           | 2013      |           |           |           | 2014      |           |           |           |
|                      | EK        | LK        | FB        | Pasture   | EK        | LK        | FB        | Pasture   | EK        | LK        | FB        | Pasture   |
| Number               | 112       | 115       | 68        | 325       | 101       | 102       | 101       | 293       | 90        | 90        | 90        | 209       |
| Actual calving date  | 26/08/12  | 26/08/12  | 27/08/12  | 11/08/12  | 22/08/13  | 20/08/13  | 20/08/13  | 20/08/13  | 18/08/14  | 25/08/14  | 18/08/14  | 20/08/14  |
|                      | ±1.8      | ±1.8      | ±2.6      | ±0.8      | ±1.1      | ±1.3      | ±1.2      | ±1.0      | ±2.9      | ±2.8      | ±2.0      | ±2.1      |
| Live weight          | 522.2±4.9 | 519.6±5.5 | 521.6±5.8 | 519.5±4.8 | 503.1±2.8 | 500.0±3.4 | 497.5±3.1 | 501.5±3.2 | 477.4±2.2 | 506.3±4.1 | 497.6±2.7 | 494.7±3.1 |
| BCS                  | 4.5±0.04  | 4.5±0.04  | 4.5±0.05  | 4.0±0.04  | 4.3±0.04  | 4.3±0.03  | 4.3±0.03  | 4.3±0.02  | 4.0±0.04  | 4.1±0.03  | 4.0±0.03  | 4.0±0.02  |
| Age                  | 4.0±0.1   | 4.0±0.1   | 4.0±0.1   | 4.5±0.2   | 4.3±0.2   | 4.5±0.2   | 4.6±0.3   | 5.2±0.2   | 4.7±0.3   | 4.2±0.1   | 4.7±0.3   | 4.2±0.1   |
| Breeding worth       | 112.1±2.2 | 106.0±2.0 | 109.2±2.2 | 106.5±2.5 | 141.4±3.3 | 140.1±3.6 | 136.2±4.7 | 127.4±3.5 | 152.6±5.6 | 157.8±6.3 | 146.2±5.8 | 138.1±4.0 |

**Note:** cows in EK, LK and FB were blocked prior to receiving their winter crop treatments, the cows in the pasture group were not blocked and represent a mix of early calving heifers and late calving mixed age cows.

## 6.4 Results

### 6.4.1 Effects of maternal treatment on progeny growth response

During the 2012-2014 calving seasons, the cows in EK, LK and FB group had a similar average calving date, 90% of cows had calved between the 10<sup>th</sup> and 24<sup>th</sup> of October each year. Due to the high proportion of early calving heifers, the cows in the pasture group calved on average 5-9 days earlier than other groups over three calving seasons.

Commercial best practice grazing and regular monitoring of young stock resulted in mean LW at or above the industry target for all cohorts at each age category (Figure 6.1). There was no effect of maternal diet on LW or growth rate after weaning through to the pre-calving period (Figure 6.1 and Table 6.3). Calves born to cows wintered on pasture appeared to have a small LW advantage at 3 months of age ( $P = 0.09$ ), which had disappeared by the next weighing.

**Table 6.3 Growth responses of the progeny born in 2012-2014 from dams in 4 different diet treatments early kale (EK), late kale (LK), fodder beet (FB) and pasture**

| Parameter                      | EK    | LK    | FB    | Pasture | SEM  | <i>P</i> -value |
|--------------------------------|-------|-------|-------|---------|------|-----------------|
| <b>Live weight (kg)</b>        |       |       |       |         |      |                 |
| 3 month                        | 112.5 | 113.3 | 111.1 | 117.00  | 1.10 | 0.091           |
| 6 month                        | 175.1 | 175.7 | 171.7 | 180.4   | 1.27 | 0.129           |
| 12 month                       | 274.2 | 268.9 | 270.0 | 274.2   | 1.65 | 0.198           |
| 15 month                       | 384.8 | 381.9 | 376.4 | 382.1   | 2.04 | 0.463           |
| 22 month                       | 496.1 | 499.0 | 487.6 | 496.4   | 2.93 | 0.539           |
| <b>Live weight gain (kg/d)</b> |       |       |       |         |      |                 |
| 3-6 month                      | 0.87  | 0.87  | 0.83  | 0.87    | 0.01 | 0.687           |
| 6-12 month                     | 0.64  | 0.62  | 0.62  | 0.60    | 0.01 | 0.547           |
| 12-15 month                    | 0.89  | 0.89  | 0.88  | 0.88    | 0.01 | 0.898           |
| 15-22 month                    | 0.50  | 0.51  | 0.50  | 0.51    | 0.01 | 0.874           |
| Overall (3-22 Mo)              | 0.65  | 0.66  | 0.67  | 0.66    | 0.01 | 0.946           |

The 2014 cohort were the heaviest at 15 months ( $401\pm 3.1$  kgLW) but were lightest at 22 months ( $468\pm 3.9$  kgLW), compared to other cohorts. The LK and pasture group of 2013 cohort had the highest LW at 22 months at more than 500 kg. The 2014 cohort had higher ADG than those born in 2013 or 2012 at average 0.73, 0.63 and 0.61 kg/d, respectively ( $P < 0.001$ ) (Figure 6.1).

#### **6.4.2 Effect of maternal treatment on heifer survival**

Survival of the heifers at 6, 15, 22 months of age and the end of the first lactation is shown in Table 6.4. At 6 months of age survival was 100% followed by a gradual drop out of approximately 2.0, 6.0 and 7.0% at 15, 22 months and end of the first lactation, respectively. The highest drop-out (7.0%) of heifers occurred between mating and calving, presumably due to removal of heifers that did not get in calf or failed some other criteria for moving forward as a replacement animal.

There was no difference of the existence from the maternal treatment effect at any stage of life ( $P > 0.05$ ). Year of birth (cohort) had a high impact on heifer existence at 22 months and end of the 1st lactation ( $P < 0.01$ ).

#### **6.3.2 Effects of maternal treatment on progeny milk yield and composition**

Average milk yield was 4,500 kg/cow/year and 4,800 kg/cow/year in respective first and second lactations giving an average of 21.3 and 24.0 litres/cow/day. There was no effect of maternal treatment during late gestation on milk yield, milk composition or days in milk for the first or second lactation of the progeny (Table 6.5).

Milk yield and milksolids yield were greater in the second lactation. Days in milk as a covariate had significant effect on milk yield, milksolids and protein percentage in the first lactation, but only affected milk yield in the second lactation.

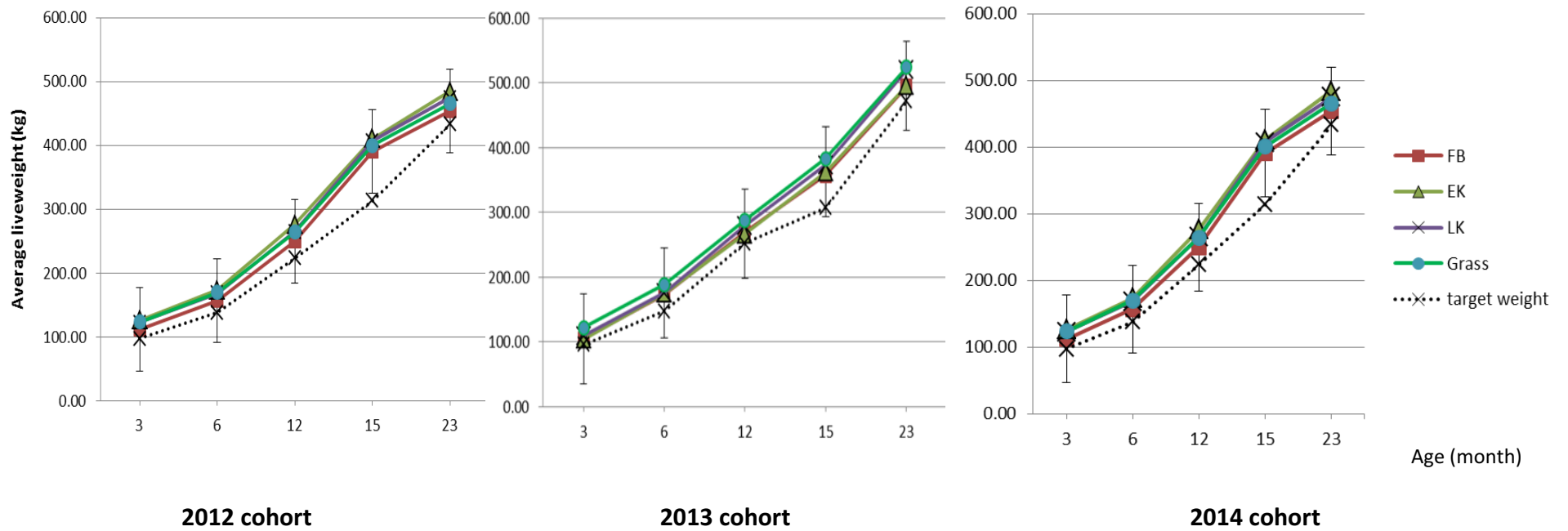


Figure 6.1 Mean live weight (kg) of heifers born in 2012, 2013 and 2014 from four maternal feeding groups compare to target weight at particular points of age (month)

**Table 6.4 Number of the heifers born to four maternal diets; early kale (EK), late kale (LK), fodder beet (FB) and pasture in three cohorts (2012-2014), (values are in means).**

| Cohort                                     | 2012 |    |    |    | 2013    |    |    |    | 2014    |    |    |    | SEM | P-value |       |        |
|--|------|----|----|----|---------|----|----|----|---------|----|----|----|-----|---------|-------|--------|
|  | TRT  | EK | LK | FB | Pasture | EK | LK | FB | Pasture | EK | LK | FB |     | Pasture | TRT   | Cohort |
| <b>N of heifer at weaning</b>              |      | 8  | 14 | 12 | 36      | 20 | 24 | 25 | 78      | 11 | 15 | 12 | 36  |         |       |        |
| <b>N of heifer at 6 m</b>                  |      | 8  | 14 | 12 | 36      | 20 | 24 | 25 | 78      | 11 | 15 | 12 | 36  |         |       |        |
| <b>N of heifer at 15 m</b>                 |      | 8  | 12 | 12 | 34      | 20 | 24 | 25 | 78      | 11 | 15 | 12 | 35  | 1.23    | 0.442 | 0.237  |
| <b>N of heifer at 22 m</b>                 |      | 7  | 10 | 10 | 32      | 20 | 22 | 23 | 70      | 11 | 15 | 12 | 35  | 4.33    | 0.796 | 0.001  |
| <b>N of heifer at end of 1st Lactation</b> |      | 7  | 6  | 7  | 24      | 13 | 16 | 15 | 45      | 11 | 15 | 12 | 33  | 5.97    | 0.443 | 0.007  |
| <b>N of heifer at end of 2nd Lactation</b> |      | 7  | 6  | 7  | 24      | 11 | 15 | 12 | 39      | 11 | 15 | 12 | 33  | 6.42    | 0.581 | 0.005  |

**Note:** the analysis of variance excluded the pasture group, which was not balanced for a comparison

**Table 6.5 Average of milk yield and composition of progeny born in 2012-2014 from dams in 4 different maternal treatments; early kale (EK), late kale (LK), fodder beet (FB) and pasture (values are means)**

| Parameter                 | Maternal treatment |        |        |         | SEM   | P-value |
|---------------------------|--------------------|--------|--------|---------|-------|---------|
|                           | EK                 | LK     | FB     | Pasture |       |         |
| 1 <sup>st</sup> lactation | n = 38             | n = 45 | n = 44 | n = 137 |       |         |
| Milk yield (L)            | 4,479              | 4,558  | 4,496  | 4,412   | 63.0  | 0.746   |
| Milksolids (kg)           | 404                | 415    | 408    | 404     | 5.3   | 0.766   |
| Milk protein (%)          | 4.2                | 4.2    | 4.2    | 4.2     | 0.02  | 0.751   |
| Milk fat (%)              | 4.9                | 4.9    | 5.0    | 5.0     | 0.04  | 0.948   |
| Days in milk              | 235                | 239    | 239    | 240     | 1.5   | 0.693   |
| 2 <sup>nd</sup> lactation | n = 33             | n = 41 | n = 40 | n = 120 |       |         |
| Milk yield (L)            | 4,842              | 4,959  | 4,805  | 4,729   | 68.1  | 0.424   |
| Milksolids (kg)           | 480                | 481    | 476    | 467     | 5.3   | 0.430   |
| Milk protein (%)          | 4.2                | 4.2    | 4.2    | 4.3     | 0.02  | 0.171   |
| Milk fat (%)              | 5.1                | 4.9    | 5.0    | 5.0     | 0.04  | 0.677   |
| Days in milk              | 235                | 232    | 235    | 237     | 2.5   | 0.817   |
| Cumulative productivity   |                    |        |        |         |       |         |
| Milk yield (L)            | 8,889              | 9,178  | 8,847  | 8,975   | 134.0 | 0.893   |
| Milksolids (kg)           | 851                | 879    | 850    | 856     | 10.5  | 0.818   |
| Milk protein (kg)         | 388                | 403    | 388    | 393     | 5.0   | 0.806   |
| Milk fat (kg)             | 463                | 477    | 462    | 464     | 5.7   | 0.842   |
| Days in milk              | 452                | 460    | 451    | 458     | 4.3   | 0.887   |

**Note:** To compare the statistical difference between groups, the dam's treatment was used as a fixed effect and day in milk as a covariate.

## 6.5 Discussion

### 6.5.1 The long term effects of feeding different crops on progeny growth

Our results showed no impact of dam's wintering crop type during late gestation on the performance of the progeny. These results likely reflect that while the nutrient profiles from the winter feeding regimes may have differed, the supply of nutrients was not limiting with cows receiving over 100 MJ ME/day and with dietary CP content which were at or only slightly below recommendation of 12%. Furthermore, cows were all on pasture diets on average three weeks prior to calving so potentially any differences in nutrient supply from crop diets diminished prior to calving.

Growth post-weaning also plays an important role on first lactation milk yield (Soberon and Van Amburgh, 2013) and in the current study there was no effect of maternal diet on post-weaning growth and development. The heifers in the current study achieved LW's above the industry targets at all points from 6 -22 months of age. The New Zealand dairy industry has set guidelines and benchmarks for the rearing of replacement heifers to ensure productivity. Heifers at 3, 6, 15 and 22 months of age are targeted to reach 18%, 30%, 60% and 90% of their mature LW (Burke et al., 2007). These targets are generated to ensure heifers reach puberty and are ready to be mated at 15 months and join the milking herd at 22 months of age. Our results showed that wintering cows on a generous allocation of fodder beet or kale during late gestation is unlikely to negatively impact the ability of their progeny to reach LW targets to 22 months of age under best practice management.

In line with other reports, we also found that growth rate of the heifer was seasonally variable through 22 months of age as is typical in NZ pasture-based rearing (Handcock et al., 2019a, Holmes et al., 2007). The average LW gain (ADG,  $0.66 \pm 0.006$  kg/day) of the heifers in the current study were higher than national statistics reported by Handcock et al. (2019a) who reported average ADG from 3-22 month of age at  $0.57 \pm 0.001$  kg/day. Furthermore, the growth rate during the pre-puberty phase (3-15 month) of the heifers in our study was also high at 0.75 kg/day. In our study, the heifer's growth rate decreased at the age of 6-12 and 15-22 months (during summer to autumn) this result is consistent with the national data base for pastoral reared heifers (Handcock et al., 2019a). This result might be due to feed quality which declines over summer, and which may also be restricted if pasture growth rates are limited during the summer season (de Clifford et al., 2014).

### 6.5.2 The long term effects of feeding different crops on progeny milk production

Previous research with sheep has shown that a high plane of nutrition during late gestation improves growth response and subsequent lactation performance of the progeny (Paten et al., 2013, Swanson et al., 2008, Blair et al., 2010). However, we did not find any significant effect of the maternal diet on progeny milk yield, milk composition or days in milk. As discussed in the previous section, it is unlikely that the dams nutrition was restricted leading to similar *in-utero* nutritional status and comparable growth response throughout postweaning. Ultimately the carry over effect of this was that the lactation performance of heifers was subsequently not altered by maternal feeding regime.

Nutrition during pre-puberty to gestation has been identified as the main factor influencing subsequent lactation performance (Sejrsen and Purup, 1997, Sejrsen, 2007). At prepuberty, the accelerated development of the reproduction system (i.e. ovary) decreases the mammary development. The severity of this negative effect depends on how early the nutrient restriction occurs (Rowson et al., 2012, Berry et al., 2003). Heifers which do not achieve their target weight at puberty may be at higher risk of dystocia, and consequently reduced milk production (McNaughton et al., 2002). While we did not collect information on difficult birth of the heifers in this study, the fact that they were well grown relative to target would suggest that any calving difficulties would not be due to missing pre puberty targets.

Capuco et al. (1995) reported that there can be a negative impact of rapid LW gain during pre-puberty on mammary development and subsequent lactation performance. Sejrsen et al. (2000) comparing TMR systems reported a reduction of milk yield at approximately 1.5 kg/day for each 100 g of ADG above the breed optimum, whereby oversized animals have a greater incidence of fatty udder. In contrast, Macdonald et al. (2005) indicated that the ability to reach or exceed 100% of mature target live weight at the first lactation had no impact on milk production or only a small increase was found.

Even though the pre-pubertal growth rate of the heifers in the current study was high at average of 0.75 kg/day, we did not notice any significant negative effect of a high growth rate on heifer milk yield. The first and second lactation milk yield and milk composition of the heifers from different maternal diets were approximately 24% greater compared to the previous study of Macdonald et al. (2005). Further, Bryant et al. (2004) reported no significant negative effect of pre-pubertal growth rate at 0.8 kg/day of HF heifer on

subsequent milk yield. Similarly, Carson et al. (2000) revealed no difference in pre-pubertal ADG between 0.70 and 0.95 kg/day on first lactation milk yield and composition.

## **6.6 Conclusion**

Adequate maternal nutrition during late gestation is an important factor for the replacement heifer production. Feeding high energy feeds consisting of maintenance crude protein content for 6-8 weeks of late gestation is unlikely to result in negative effects on the progeny. Even though difference existed in the type of prepartum diet, the progenies born to dams had no apparent nutrient restriction and subsequent good management contributed to their ability to reach their high levels of growth and lactation performance.

## Chapter 7

### General discussion and conclusion

#### 7.1 Introduction

In New Zealand, winter crops such as fodder beet (FB) and kale are commonly used to feed non lactating cows during late gestation because of their high yield and ability to maintain good feeding value at these high yields (Edwards et al., 2014a, Gibbs, 2014). The adoption of these crops has increased as dairy farmers intensified their farming systems and identified opportunities to preserve pasture on the milking platform by grazing cows off farm during winter. However, nutritionally these forages are substantially different from the pasture diets once commonly used as late gestation feed (Jenkinson et al., 2014). The additional drive to reduce nitrogen losses to soils has accentuated differences between novel and historic winter feeding practices and this change highlighted the need to evaluate the effect on cow performance and the subsequent growth, development and productivity of the offspring.

Late gestation is a vital period for the *in-utero* foetus as 80% of foetal growth happens in this stage (Redmer et al., 2004, Greenwood and Cafe, 2007). During late gestation, the maternal nutrient requirement is increasing in response to the rapid growth of the foetus (Bell et al., 1995), in addition to storing fat as body condition and preparing for the upcoming lactation (Caton et al., 2007, Bell et al., 2000). Birth weight (BW) of the calf is one important factor which impacts on the subsequent health of newborn calves and later growth response (Rooke et al., 2016, Greenwood and Cafe, 2007). Some studies have indicated that protein restriction during the late gestation period harms the development and strength of the calf via reduced activity of immune organs (He et al., 2012), or ineffective passive immune transfer (Quigley and Drewry, 1998). Further, it is also known that the near term cow typically reduces her dry matter intake (DMI) which might result in inadequate nutrient intake (Bell, 1995, Garnsworthy and Jones, 2010). This reduction in essential nutrients is thought to not only effect the foetal development but also the cow's post-partum health and production (Bell, 1995, Santos et al., 2001). Several studies reported that either low quality or quantity of nutrients might cause intrauterine growth restriction (IUGR) as shown in cattle (Zhang et al., 2002), sheep (Kwon et al., 2004, McCoard et al.,

2017) and rats (Gao et al., 2012b, Ramadan et al., 2013). Intrauterine growth restriction altered structure of essential organs (Maritz et al., 2004, He et al., 2013), increased perinatal and neonatal loss rate (Quigley and Drewry, 1998, Zhang et al., 2015), as well as altering growth performance in later life (Wu et al., 2006).

In addition to the *in-utero* phase, pre-weaning is another critical stage in heifer development since the relative growth rate is very high and has influences on later life growth performance (Greenwood and Cafe, 2007, Akins, 2016). However, measurable long term effects likely depend on subsequent nutrition both during the suckling period (Heinrichs and Lesmeister, 2007, Holmes et al., 2007) and post-weaning (Chester-Jones et al., 2017, Davis Rincker et al., 2011, Soberon et al., 2012). It was therefore argued that nutritional intervention might correct any adverse impacts of inadequate *in-utero* nutrition, though our knowledge and understanding of this area is still limited. Thus, the objective of the current study was to investigate the impacts of maternal nutrition on progeny development and immune status, specifically the effect of a widely adopted, low protein crop, fodder beet and associated supplements. These crops and supplements are at the minimum threshold of adequate protein nutrition for a late gestation dairy cow, which may suit lower urinary N excretion but uncertainty over foetal and dam requirements remained. A series of null hypotheses were established (Chapter 1) which related to questions which were addressed in this PhD research project:

Null hypothesis 1: Feeding a low protein supplement with a low protein crop to late gestation dairy cows will have no effect on dam performance or calf development and colostrum quality.

Null hypothesis 2: Increasing milk allowance to offspring of dams fed a protein-restricted diet will not increase pre-weaning growth rates.

Null hypothesis 3: The progeny of cows fed fodder beet in late gestation will have similar growth and performance as progeny from cows fed alternative late gestation diets.

The following discussion will review the extent to which these hypotheses are accepted or rejected.

## **7.2 Fodder beet feeding with a low protein supplement in late gestation does not alter dam performance or *in-utero* calf development**

We were unable to reject the first null hypothesis because we found no evidence that feeding a low protein supplement with a low protein crop to late gestation dairy cows affected dam performance or calf development and colostrum quality. The study did, however, identify other aspects of the effects of different protein intake on cow performance (Chapter 3), neonatal response and passive immune status (Chapter 4).

### *Impact on cow energy status body condition score and health*

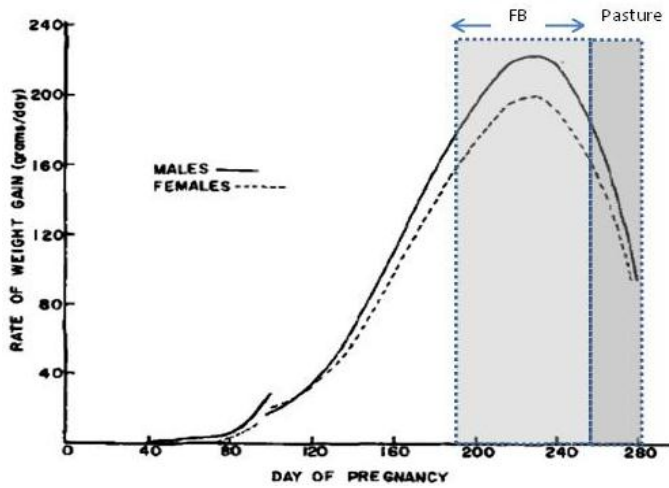
Low protein (7.8 vs 13.6% CP) during late gestation did not alter the physical responses of the cows. Body condition score (BCS) and live weight (LW) of the cows were similar for both groups reflecting the comparable metabolize energy intake. Cows in both treatments maintained a BCS of 4.5 and gained approximately 15 kg LW as conceptus by the end of the winter crop feeding period.

Late gestation is the period of the most rapid *in-utero* foetal growth along with pronounced placenta function placing a high nutrient demand on the dam (Bell et al., 2000). Inadequate supply of nutrients during this period results in a negative energy balance (NEB) which in turn leads to protein and fat mobilization (Veerkamp et al., 2003) to meet the requirement for energy and amino acids of the dams and *in-utero* foetus (Bell et al., 2000). In this study, apparent ME intake of cows in the low (LCP) and moderate (MCP) protein groups were 101 and 107 MJ/cow/d which is above the recommended requirement of 75-90 MJ according to AFRC but in line with recommended requirements according to Roche et al (2005). By week 8 of the study (roughly 2 weeks prepartum), compared to cows in MCP, cows in LCP had a relatively larger NEB as reflected by higher serum levels of non-esterified fatty acid (NEFA) indicating increased fat mobilization (Spiegler et al., 2014). Moreover, lower beta-hydroxybutyrate (BHBA) concentration in the LCP dams may also indicate increasing metabolism of ketone bodies as an energy source (Kesser et al., 2017). While these parameters reflect small energy differences between the two groups, on the whole these indicators did not raise concerns over general animal health as the concentration of serum NEFA and BHBA of cows in both groups were under critical level thresholds (Phyn et al., 2017).

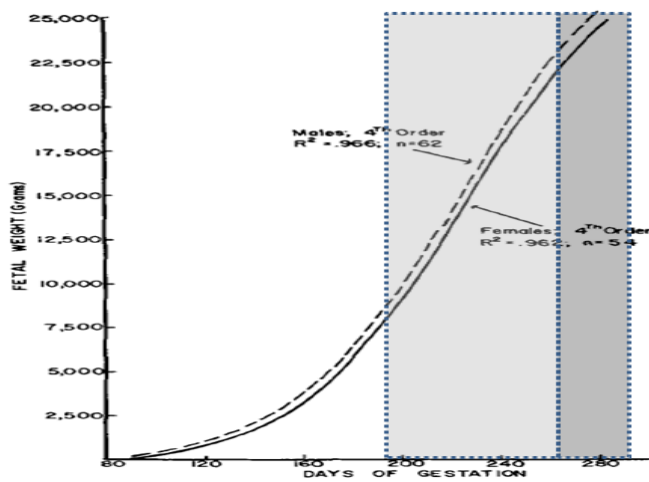
This study tested the effect of protein, not energy, restriction on foetal development and maternal productivity. According to Bell (2005), during late gestation, 55% of placental energy supply is provided by amino acids (AAs), 35% to 40% are from glucose and lactate, the rest 5-10% is from acetate. In Chapter 3 restricting protein by up to 45% of that recommended for requirements resulted in reduced serum urea compared to the MCP group but there was no treatment effect on blood amino acids. Lack of variation in body weight of either the dam or the calf would indicate a moderate restriction of protein. Even under the current protein restriction of the LCP group the dam's metabolic adaptation enabled her to cope with reduced dietary supply of amino acids. Likely, through a combination of greater conservation of MP, increased protein synthesis in the liver or increased tissue proteolysis for mobilisation of essential amino acids (EAAs) (Lopes et al., 2020), though some of these metabolic adaptations may have come at the cost of slightly higher NEB (Putnam and Varga, 1998, Grummer, 1995).

Worth noting also, that based on standard farm practice, the near term prepartum cows (approximately 8-10 days prepartum) received a high allocation of pasture which likely contributed to an elevated protein supply to both dam and foetus (Figure 7.1 a, b). This increase in protein nutrition may offset some of the previous deleterious effect of low protein intake on cow performance. The timing of diet restriction relative to various aspects of foetal development is important. In this study the beginning of the FB feeding period coincided with the period of accelerated foetal growth (Figure 7.1 a, b). Pasture allocation occurred roughly at the time the foetal growth rate was declining (230 days of gestation, Figure 7.1a, (Eley et al., 1978) and may have provided an opportunity to supply key limiting amino acids to complete the growth of specific tissues.

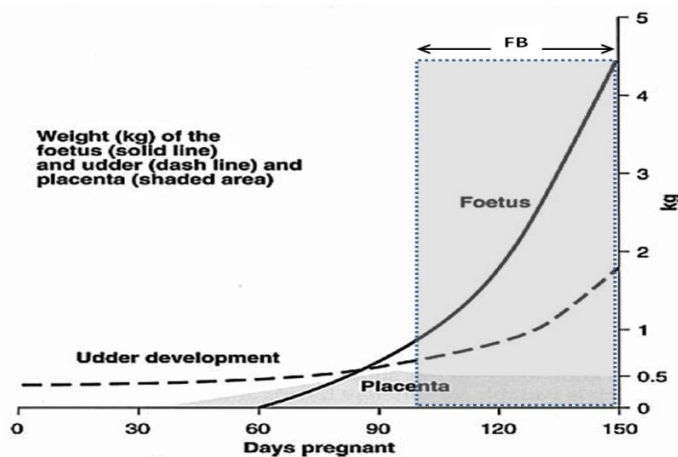
Several researches highlighting the negative effects of maternal nutrition on offspring in ruminants has used sheep models. While, the growth rate of the ovine foetus is also curvilinear (Figure 7.1c) the pattern of placenta development, particularly in the maternal tissue, appears to differ between cows and sheep; therefore, caution must be used when comparing the responses to altered nutrition during pregnancy between these 2 species (Funston et al., 2010a).



a. Fodder beet (FB) and pasture feeding during the stages of different rate of bovine foetal weight gain



b. Fodder beet and pasture feeding during the curve of bovine foetal weight (gram)



c. Normally fodder beet feeding from mid gestation to late gestation (i.e. lambing)

Figure 7.1 Period of fodder beet feeding (light gray area) followed by pasture grazing (dark gray area) in the present study (Figure 7.1 a and b; adapted from Eley et al. (1978)) and normally fodder beet feeding in ewes during mid-late gestation (Figure 7.1 c; adapted from [www.agric.wa.gov.au](http://www.agric.wa.gov.au))

Colostrum composition did not differ between treatments (Table 4.3). Colostral IgG levels was determined as adequate (>30 g/L) on NZ pasture based system (Coleman et al., 2015). This level might be not surprising in the present study, since the concentration of colostral IgG is known to decrease over time after calving to first milking (Coleman et al., 2015) as described in Chapter 4. The calves were allowed to be with the dams for between 12 to 24 hours before transfer to the rearing facility providing equal opportunity for suckling. Thus no difference between treatments on calf serum IgG level either day 1 or day 4 of life was found. Our results showed that colostrum consumption had a positive impact on protein and serum composition and IgG level in day 1 and 4 of life.

#### *Effect on in-utero calf development*

In our current study the goal was to end lactation with experimental cows at a condition score of 5.0 so that we were able to feed a maintenance + pregnancy allocation throughout the observation period. Even though in our recent study, the cows failed to reach the targeted BCS before dry off or thereafter (Chapter 3), we could not find the evidence of IUGR (Chapter 4). Roche et al. (2005) suggested that NRC recommendations for the requirements of dairy cows were not adequate for pastoral fed animals in ambient conditions below thermoneutral conditions. Consequently we adopted the more generous allocation of 107 MJ ME/cow/day plus 10% for utilization.

While there was tendency for more BCS gain in MCP ( $P=0.061$ ) compared with LCP, this could be explained by differences in apparent ME intake. Speijers et al. (2005) reported that calf BW was not related to ADG of the dams, finding no significant impact of maternal diet on calf BW in their study. The results of the current, and previous (van der Drift et al., 2012), studies support the idea that previously well-fed cows can compromise body reserves for deficiencies in dietary protein and/or energy in priority of the *in-utero* calf development in late gestation. Mechanistically it is believed that the reasons for this are related to the capacity of placental nutrient regulating mechanisms (Dunlap et al., 2015), maternal body adaptation (Lopes et al., 2020) and nutrient partitioning prioritization of the foetal growth and development (Bell, 2005). Given that there was little to no dietary ME restriction in the current study, differences in the NEB were not apparent until the end of gestation when it becomes increasingly difficult to meet requirements through feeding. For cows in the LCP

there was considerably less amino acids to spare for gluconeogenesis which would have contributed to lower NEB.

We also found lower serum concentrations of several amino acids, including arginine in the LCP cows than the MCP ( $P < 0.001$ ). Fairbairn et al. (1992) reported that total free AA in lucerne silage (as used in MCP) was approximately 28% higher than in whole plant maize silage (as used in LCP) which likely contributed to differences in serum AA concentrations in this study. However, serum arginine concentrations increased from the initial level in both groups (Table 3.5) which may be because arginine is synthesised from other AAs, i.e. glutamine, glutamic acid, proline and citrulline (Wu and Morris, 1998). These AAs had been reported in slightly higher concentrations in maize silage than the lucerne silage (Fairbairn et al., 1992).

#### *Impact on cow production during lactation*

Irrespective of farmlet allocation post calving, we were not able to determine differences in milk yield and composition associated with winter feeding regime.

There was no difference in lactation yields or milk composition, even though the LCP cows had shown signs of negative energy balance (higher NEFA) two weeks before calving. The lack of effect is probably a combination of limited treatment effect on live weight, and the use of *ad libitum* pasture feeding in the days preceding calving. Adjusting the nutrition to pasture immediately pre and post-calving may have diminished the negative energy balance and consequently minimized the adverse effect of late gestation treatment (Vandehaar et al., 1999). Though increased nutrient availability for 7-10 days before calving is unlikely to have made an appreciable difference to BCS. Roche et al (2009) found that cows calving at BCS of less than 5.0 were more likely to have poorer reproductive performance. Interestingly, in spite of lower BCS at calving, the reproductive performance of LCP cows was better than that of MCP. We accept that a number of factors –not related to winter feeding - could have contributed to differences in lower 6 weeks incalf rate. But given that Dalley et al. (2020) found that cows fed FB during winter had improved reproductive performance over kale-fed cows there maybe aspects of these crop-supplement feeding regimes we are yet to elucidate.

### **7.3 *In-utero* nutrition did not alter heifer progeny responses to different milk feeding regimes**

To investigate the carryover effect of maternal treatment and the impact of the nutritional plane on pre-weaning growth, we compared the intervention (high allowance; HA) vs the conventional (CON) milk feeding regime in heifer calves from the late gestation experiment. When the experiment was designed, it was anticipated that calves born from LCP dams might be disadvantaged by their maternal nutrition and this raised questions regarding the role of nutritional intervention after birth. Our results revealed that, a short duration maternal protein restriction did not alter calf BW or subsequent growth response of calves, which included LW, LW gain, average daily growth rate (ADG) or pre-weaning body stature.

In the current study, we did not observe any significant effect of maternal nutrition on heifer health, survival or growth. More than 80% of neonates in our experiment were considered to have suckled colostrum before separation from their dam roughly 12 hours after birth. The serum IgG was confirmed the prevalent suckling behavior and explains the lack of difference in health and survival due to maternal treatment. Similarly, the lack of growth response is in line with a previous report from Martin et al. (1997) who found maternal restricted protein during late gestation did not alter BW or tissue composition of offspring. Similarly, Duarte et al. (2013) reported no difference in foetal weight from dams experiencing either a low or high plane of nutrition during gestation. Interestingly Duarte et al. (2013) observed greater intestine length and villi surface in the foetus from restricted cows which had the potential to improve absorption of nutrients and immunoglobulins post calving (Meyer and Caton, 2016, Duarte et al., 2013, Funston et al., 2010a).

While there were no carry over effects of dam feeding, increasing milk allowance to calves after birth enhanced pre-weaning growth rate. Supporting previous research of Brickell et al. (2009) we also found here that high CMR allowance improved other stature measures such as heart girth and body length but not wither height. The heifers in the intervention high allowance pre-weaning regime had significantly higher energy and protein intake. Even though the CON calves consumed more ME from the starter and pasture than the HA, solid feed intake was not enough to compensate leaving total ME intake below that of HA calves. Lorenz et al. (2011) indicated that intake of solid feed is negligible during early of life, i.e. the first 3 weeks age. Hence, calves on restricted feeding regimes might be only able to achieve 20-30% of their normal growth.

Soberon and Van Amburgh (2013) reported that increased feeding to 2 times maintenance requirement resulted in 400-500 kg more milk in first lactation. High allowance pre-weaning in the current study improved lactation performance largely due to more days in milk compared to the CON. This result agreed with previous studies which report that increased energy and protein intake during pre-weaning stage has a positive influence on the growth and development of both the body and mammary gland (Brown et al., 2005b, Brown et al., 2005a, Davis Rincker et al., 2011), and consequently improve milk production (Roche et al., 2015, Soberon et al., 2012). Esselburn et al. (2015) also indicated that higher energy intake from CMR supplemented with fat or fatty acid, resulting in increased weaning LW, feed efficiency and improved health-related scores. However, in their companion study, the impact of the improved pre-weaning growth did not affect the mass or composition of the mammary parenchyma through 8 weeks of age (Esselburn et al., 2013). The work of van Amburgh and Soberon suggest that growth and development of mammary secretory cells very early in life are as important, or more important than mammary parenchyma deposition in older calves. This is an area of future research with results of the present study indicating that the impacts of early life nutrition may influence the resilience of secretory tissues or maintenance of body weight or condition as observe by longer lactation in HA calves.

## **7.4 Type of winter crop in late gestation did not affect milk production of progeny**

In Chapter 4, we found that protein levels in the maternal diet did not significantly alter calf development and post-calving health based on their passive immunity status. In addition, in Chapter 5, we reported that heifers from LCP or MCP dams responded to pre-weaning milk allowance regime similarly (i.e. no interaction between maternal nutrition or pre wean nutrition) in terms of their growth and body development through 22 months and consequently similar performance in their first lactation performance.

Using a larger data set from the nearby commercial demonstration farm, our results comparing different crops fed to late gestation cows showed that when their offspring were reared under similar farm practice, no variation in lactation performance was detected (Chapter 6). The result revealed no impact of the maternal diet types on the average growth rate or milk production of the progeny. Likely the best practice management used to ensure ME requirements were met, even under minimal protein nutrition has led to practices which can minimize N loss (Edwards et al., 2014b) without compromising the foetus.

The previous studies have mostly been concerned with the pre-puberty LW gain (McNaughton and Lopdell, 2013, McNaughton et al., 2002), and reported both positive (Handcock et al., 2019b) and negative (Sejrsen et al., 2000) relationships with lactation performance. We found the pre-pubertal growth rate of the heifers at 0.75 kg/day was not detrimental to the first and second lactation yield and composition, which is in line with others (Carson et al., 2000, Bryant et al., 2004). In New Zealand's pastoral system, underfeeding heifer replacements to the extent that they do not reach target live weights by calving has the largest negative effect on first lactation milk yield. Under grown heifers partition more energy to growth and less to milk, impacting total milk yield. Poor animal health (eg chronic scours) pre weaning which substantially checks calf growth also reduces first lactation milk yield (Aghakeshmiri et al., 2017, Martin et al., 1962).

However, under best management rearing practice of the Lincoln University demonstration farm, the heifers were well managed and met growth targets. Therefore differences could not be detected between groups of maternal winter feeding, even among cohorts where calf BW might have differed.

## 7.5 Study limitations and future research

The majority of results presented in this research have been derived from a small, unreplicated population of animals so some of the conclusions drawn are done so cautiously. For example, the carry over effect of winter feeding and protein nutrition on dam health and reproductive performance is a subject worth pursuing in future research. The relationship between fodder beet and mineral nutrition especially Ca, may be related to higher health problem i.e. retained membrane (Zhang et al., 2002) in LCP compare to the MCP group. A study to evaluate the concentration of blood minerals of fodder beet with low protein supplement diet would be worth conducting to confirm our preliminary results and we recommend more data collection on commercial level is needed.

Our study found little effect of relatively large treatment differences on the in utero development of the calf. While the parameters we investigated were limited to a small number of variables which shed some light on why differences were or weren't observed. However, measurements of adipose tissue type, and mammary tissue to document effects of nutrition on mammary parenchyma deposition would have been interesting to report. Equally the the limitation of uterine and placenta capacity may be another factor which explained lack of response to nutrition from diet changes during the last trimester *in-utero* fetus growth (Buczinski et al., 2007, Dunlap et al., 2015). Experiments to clarify this concept may worth conducting to demonstrate on pasture based NZ dairy production system.

## 7.6 Conclusions

In the current study, offering high energy, restricted protein diets for 50 days in late gestation, did not have detrimental effects cow performance during parturition and lactation. Maternal metabolic adaptation enabled the dam to regulate nutrient supply to the foetus removing any effect of maternal diet on the calf. There did not appear to be any hidden long term effects of maternal nutrition as the different protein diet during late gestation did not impact growth or lactation performance of the progeny.

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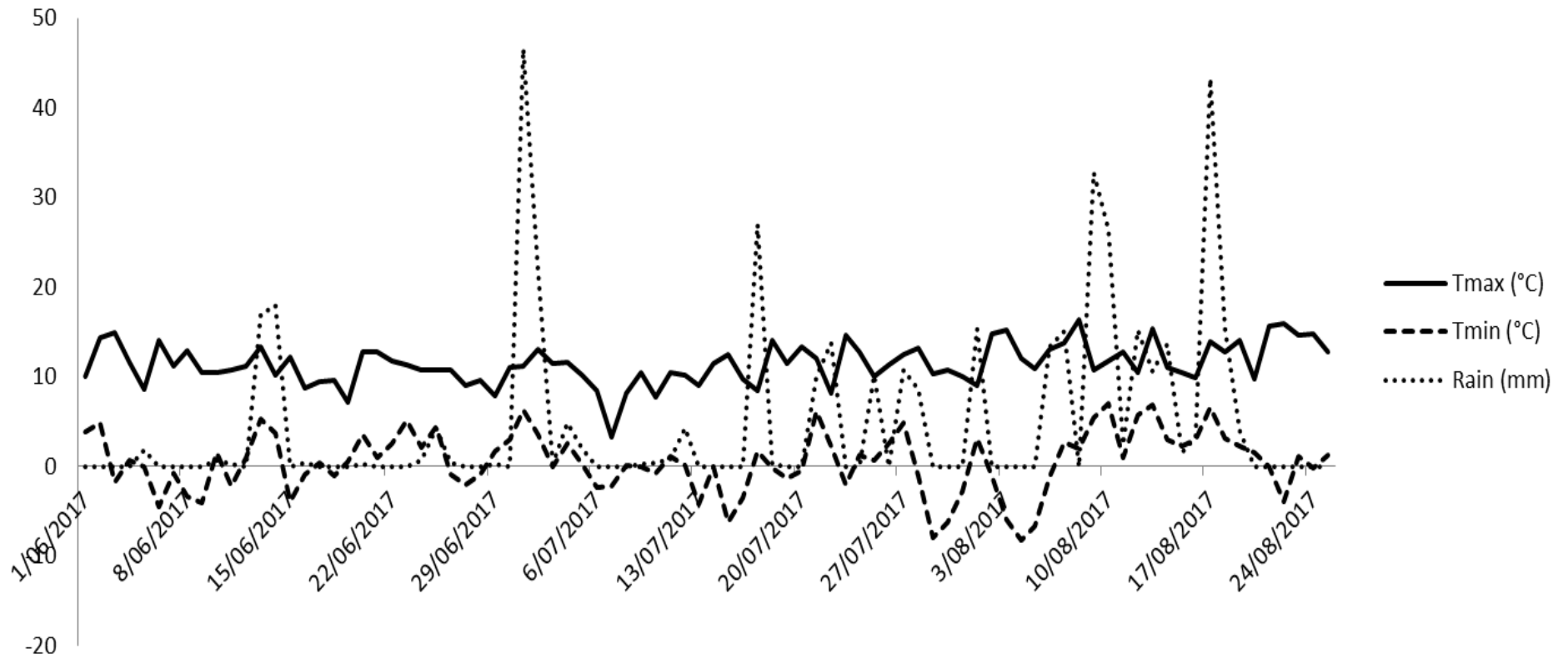
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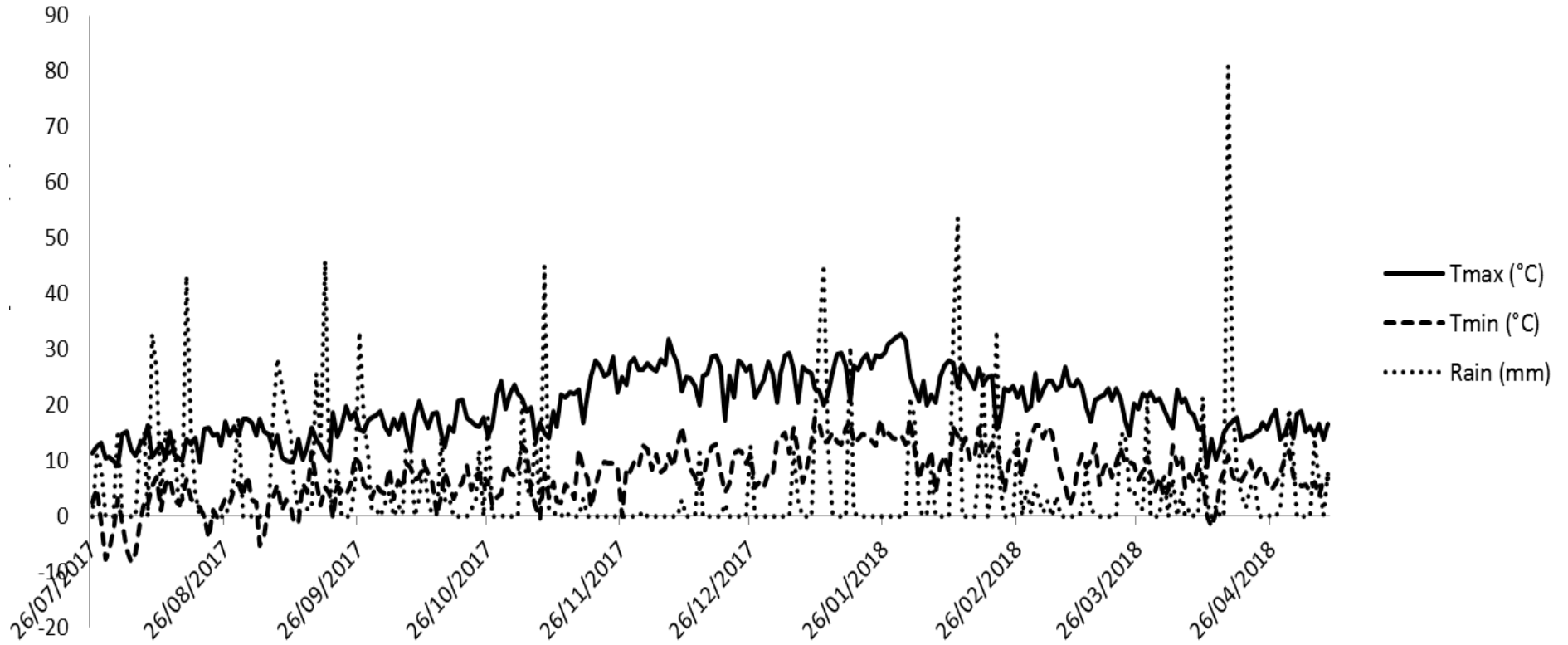
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## **Appendix**



**Appendix 1 Climate condition during wintering trial to calving period**



**Appendix 2 Climate condition during lactation period**

**Appendix 3 Individual calf serum IgG level (g/L) at day 1 and 4 of life sorted by suckling appearance; not suckled (NSC) and suckled (SC)**

| Calf ID | Suckling appearance | IgG level (g/L) |              |
|---------|---------------------|-----------------|--------------|
|         |                     | at 1 day        | at 4 day     |
| 2       | NSC                 | 30.52           | -            |
| 17      | NSC                 | 8.24            | 13.97        |
| 18      | NSC                 | 1.22            | -            |
| 22      | NSC                 | 7.59            | 16.93        |
| 35      | NSC                 | 2.76            | 12.55        |
| 46      | NSC                 | -               | 20.80        |
| 51      | NSC                 | 5.34            | 11.14        |
| 52      | NSC                 | 7.92            | -            |
| 54      | NSC                 | 1.93            | -            |
| 61      | NSC                 | 13.39           | 15.64        |
| 208     | NSC                 | 5.98            | -            |
| 3       | SC                  | 14.03           | -            |
| 6       | SC                  | 36.90           | -            |
| 10      | SC                  | 23.37           | -            |
| 12      | SC                  | 17.19           | -            |
| 13      | SC                  | 5.66            | 23.18        |
| 14      | SC                  | 20.47           | -            |
| 15      | SC                  | 11.14           | 22.66        |
| 16      | SC                  | 27.24           | -            |
| 19      | SC                  | 3.09            | -            |
| 25      | SC                  | 17.25           | 22.41        |
| 26      | SC                  | 22.73           | 36.12        |
| 27      | SC                  | 51.06           | -            |
| 28      | SC                  | 27.88           | 37.93        |
| 29      | SC                  | 53.00           | -            |
| 30      | SC                  | 6.95            | 35.61        |
| 31      | SC                  | 29.17           | 53.38        |
| 34      | SC                  | 52.35           | 17.58        |
| 36      | SC                  | 17.58           | 19.89        |
| 37      | SC                  | 26.27           | 31.55        |
| 38      | SC                  | 27.56           | 53.25        |
| 40      | SC                  | -               | 30.13        |
| 41      | SC                  | 18.22           | 42.69        |
| 42      | SC                  | 10.81           | 18.54        |
| 44      | SC                  | 15.32           | 22.41        |
| 45      | SC                  | 7.27            | -            |
| 47      | SC                  | -               | 11.78        |
| 48      | SC                  | 18.22           | 39.47        |
| 49      | SC                  | 23.69           | 39.79        |
| 50      | SC                  | 34.00           | 46.88        |
| 55      | SC                  | 30.78           | -            |
| 56      | SC                  | 7.59            | -            |
| 58      | SC                  | 14.68           | -            |
| 59      | SC                  | 20.80           | 29.81        |
| 60      | SC                  | 3.41            | 19.83        |
| 62      | SC                  | 18.86           | 23.05        |
| 63      | SC                  | -               | 20.80        |
| 64      | SC                  | -               | 16.48        |
| 65      | SC                  | 16.29           | 23.63        |
| 67      | SC                  | 53.64           | 63.94        |
| 68      | SC                  | 44.62           | 54.61        |
|         | <b>Mean NSC</b>     | <b>8.49</b>     | <b>15.17</b> |
|         | <b>Mean SC</b>      | <b>23.03</b>    | <b>31.76</b> |

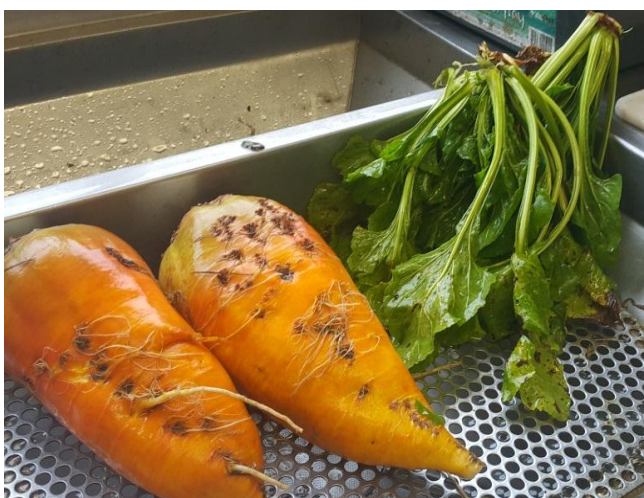
## Appendix 4 Fodder beet sampling and subsampling



1. Fresh weight measurement in the field used a tripod scale.



2. Separation of bulb and leaf to before weighted and recorded apart in the field.



3. Two FB bulb and leaves were collected and cleaned before subsampling.



4. Each bulb was cut into 3 parts (top, middle and tip), then cut into  $\frac{1}{4}$  longitudinal pieces. Randomly selected one piece from each part. Do the same process to another bulb.



5. Cut all selected pieces from both bulbs into small pieces.



6. Chopped all parts to make finely small pieces and mixed together.



7. Sampling approximately half of the sample for blending.



8. Fodder beet sample in a kitchen blender.



9. Subsampling 100-200 g. of fodder beet into a labelled plastic bag for freeze dry.



10. Subsampling another 100 g of fodder beet into a labelled paper bag for DM analysis by the force air oven.



11. Chopped all leaf parts from both samples to make finely small pieces and mixed together.



12. Subsampling 100-200 g of fodder beet leaves into a labelled plastic and paper bag. The first subsample was used for freeze drying, another subsample was used for DM analysis.