THE EFFECTS OF THE APPLICATION OF LARGE AMOUNTS OF NITROGEN FERTILIZER ON MILK YIELD, REPRODUCTIVE PERFORMANCE, AND ENERGY BALANCE OF SPRING-CALVED HIGH GENETIC MERIT COWS

A thesis presented in partial fulfilment of

the requirements for the degree of

Master of Applied Sciences (MApplSc)

in

Animal Production

Institute of Veterinary

And Biomedical Sciences

Massey University

Palmerston North, New Zealand

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2004

ABSTRACT

The intake of crude protein (CP) in amounts considerably above the cow's requirements has been associated by several overseas (OS) reports with reduced cow performance. In New Zealand (NZ), the CP content of pasture during spring is usually above the cow's requirements. During this time, application of nitrogen (N) fertilizer is a common practice which further increases the CP content of pasture. Despite this fact, few experiments have studied the effect of high CP intake on cow performance in NZ, and the existing results are not conclusive. Therefore, the objective of this experiment was to measure the effect of two levels of CP concentration in pasture caused by application of different amounts of N fertilizer, on milk yield, reproductive performance, and energy balance of grazing, spring-calved, high genetic merit cows in NZ.

Two groups, each of 20 high genetic merit cows, were offered a common pasture allowance (46 kg DM/cow/day) over the first 101 days postcalving, but from pasture which contained either a lower (low CP group) or a higher concentration (high CP group) of crude protein. The difference in CP concentration was created by applying either no N fertilizer or 35 kg of N fertilizer after every grazing.

Herbage masses were measured on every paddock weekly, and every day pregrazing and postgrazing, using a rising plate meter. These values were used to calculate pasture growth, and apparent dry matter intake per group. Pasture composition was measured by near infrared spectroscopy.

For each cow, milk yield and composition was measured by weekly herd tests. Reproductive performance was assessed by scanning of follicles and corpora luteal from day one until day 40 postcalving, and from 10 days before the predicted artificial insemination (AI) until 35 days post AI. Progesterone concentrations were measured in milk samples taken every second day.

Liveweight and condition score of each cow were measured weekly, and concentrations of NEFA and IGF1 were measured in blood samples taken

once every week. Energy balance was calculated as theoretical energy requirements minus estimated energy intake, and feed conversion efficiency was also calculated.

When compared with the low CP paddocks, the high CP paddocks produced more pasture (+1,260 kg DM/ha in 101 days) and it was of higher quality (11.8 vs. 11.4 MJ ME/kg DM; 83.4% vs. 81.2% digestibility; 24.5 vs. 21.6% CP). The apparent daily intakes of DM and ME of the two groups were not significantly different (16.2 and 15.9 kg DM/cow; 195 and 184 MJ ME/ cow). However, the high CP group ate significantly more CP than the low CP group (4.2 vs. 3.5 kg CP/cow/day, and had a higher blood urea concentration (7.8 vs. 5.3 mmol/l).

There were no significant or consistent differences between the two groups in milksolids yield (1.84 vs. 1.91 kg MS/cow/day for the high and low CP groups), reproductive measurements, liveweight, condition score or IGF1 concentrations. However, the high CP group had significantly lower NEFA concentrations than the low CP group (0.45 vs. 0.56 mmol/l). The high CP group had lower apparent feed conversion efficiency than the low CP group, which may have been partially due to the calculated value of 3.2 MJ ME extra required per day to excrete the extra urea from the high CP group.

In conclusion, even though the high CP group had higher blood urea concentrations, no significant differences in cow performance were detected between the two groups. The application of 130 kg N/ha produced an extra 1,264 kg DM/ha, which would have been expected to produce an extra 140 kg MS/ha, although this increase in MS yield may have been reduced slightly (by 9%) by the lower calculated feed conversion efficiency of the high CP group.

Subject for further research include attempts to increase the feed conversion efficiency of animals grazing pastures with high CP content, through the use of sources of extra rumen degradable energy; the effect of diets high in CP on embryo quality and viability; and the ecological impact of using large amounts of N fertilizers.

ACKNOWLEDGEMENTS

This thesis is dedicated to

My grandfather Dr. Francisco Ordóñez Ordóñez

My grandmother Ernestina Medina Ramirez

DVM Rafael Carvajal Aguilera

DVM Rafael Ordóñez Medina

Dr. Mario Rivera Ortiz,

for their invaluable contributions in my life.

I want to thank the Ministry of Trade and Foreign Affairs of New Zealand for the scholarship and especially to the society of New Zealand who, in the end, is the one who funded my scholarship.

I want to thank my supervisors Professors Colin Holmes, Tim Parkinson, Cory Matthew, Nicolas Lopez-Villalobos, Ian Brookes, and Jennifer Burke for their support, guidance, and trust in me which made possible this project a reality and a success. I want to offer a special acknowledgment to all staff and technicians, especially to Liz Gillespie and Geoff Purchas, who participated in this project.

I desire to thank all my family for their love, support, and guidance. Thanks to all my friends here in New Zealand, especially Mei, RuiLi, David and Felix, whom I owe their great support and comfort during many difficult times.

The success of this thesis has been the result of a shared effort of all the above mentioned.

Now, it is my part to apply this new knowledge and to

cooperate in the improvement of the society.

A better world is possible

LIST OF ABBREVIATIONS

ADF	acid detergent fibre
CP	Crude protein
DMI	dry matter intake
DPP	days postpartum
EIA	enzyme immunoassay
FSH	follicle stimulating hormone
GH	growth hormone
HGMC	high genetic merit cow
IGF1	insulin-like growth factor 1
LGMC	low genetic merit cow
ME	metabolizable energy
ME	metabolizable energy intake
NDF	neutral detergent fibre
NEB	negative energy balance
NEFA	non-esterified fatty acids
NZ	New Zealand
OMD	organic matter digestibility
OS	overseas
P4	progesterone
RDP	rumen digestible protein

RUP	rumen undigestible protein
SSS	soluble sugars and starch
TMR	total mixed ration

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1 INTRODUCTION

In order to promote the development and success of a grazing system it is important to understand its components and the way they interact, to augment the role of the positive factors, and to identify and overcome the possible negative ones. One of the main problems of dairy grazing systems is the imbalance between the nutrient content of pasture and the nutrient requirements of the cattle. Many studies indicate that the crude protein (CP) content of the pasture, especially in mixed pastures containing grass and clover, in temperate climates during spring can be much higher than the protein requirements of the cow (Wilson *et al.*, 1995b; Moller *et al.*, 1996b; Jacobs *et al.*, 1999; Stevenson *et al.*, 2003). Several authors have linked high CP intake with negative energy balance (NEB) (Butler, 1998; Chapa *et al.*, 2001), and low reproductive performance (Butler, 1998; Sinclair *et al.*, 2000; Chapa *et al.*, 2001). Since New Zealand (NZ) dairy cows are fed with pasture containing CP in quantities above their requirements during spring, it is important to know if there is a negative effect of the excessive CP intake on animal performance and if so how strong its impact is. The objective of this research is to test the effect of high CP intake from pasture during spring on the milk yield, reproductive performance, and energy balance of spring-calved dairy cows in NZ.

2 DESCRIPTION OF THE NZ DAIRY SYSTEM

2.1.1 Seasonal changes in pasture growth and nutritional characteristics

The NZ dairy system has a seasonal pattern of production that enables it to match the herd's nutrient requirements with the fluctuations in pasture growth and nutrient content. In order to match pasture production with pasture demand, more than 90% of the dairy farms have adopted a spring seasonal system of calving. In this system, cows are in a non-lactating state when pasture growth is at its lowest from May to July (winter), and calving occurs from July to September to match the highest cow dry matter requirement with the peak of pasture growth rate and pasture quality occurring from the end of September to the end of December (Holmes, 2001).

It is important to recognize the changes in pasture growth and composition through the year, in order to manage the balance between the pasture nutrient contents and the herd's nutrient demands. The changes in NZ pasture growth have been studied by several authors (Radcliffe, 1974a; Radcliffe, 1974b; Baars, 1975; Baars, 1976; Stevenson, 2003). Pasture growth starts increasing during spring reaching its highest rate between 50 and 80 kg DM/ha/day during October and December (AGMARDT, 2001a; Holmes, 2002; Stevenson, 2003) (Figure 2-1). For a spring-calved herd at a moderate to high stocking rate, the peak of herd DMI and the peak of pasture growth are matched in October (AGMARDT, 2001a; Holmes, 2001). After a peak in December, pasture growth generally decreases until June. Depending on the moisture availability, summer pasture growth can range from 10 to 50 kg DM/ha/day. These trends are mirrored by decreasing milk production at the end of lactation in spring-calved cows, unless supplementary feeds are added (Holmes, 2002). During the winter, pasture growth in NZ can decrease to between 5 and 30 kg DM/ha/day depending on the region (AGMARDT, 2001b; Holmes *et al.*, 2002a).

Nutritive parameters of pasture also show annual variations. Pasture digestibility is typically high (74-80%) in spring and autumn (Moller *et al.*, 1996b), but lower (60-70%) in summer (Nicol, 1987) (Figure 2-2). Digestibility is negatively related to the concentration of structural carbohydrates (acid detergent fibre (ADF) and neutral detergent fibre (NDF)) (Hodgson, 1990; Forbes, 1995a; Wilson *et al.*, 1995a). NDF and ADF tend to be lower around August and reach their highest values around January and February (Wilson *et al.*, 1995a; Stevenson, 2003). Low pasture digestibility can limit feed intake and animal performance (Nicol, 1987; Hodgson, 1990; Forbes, 1995b) by reducing DMI, rate of digestion, the speed of nutrient absorption, and the rate at which the residues pass through the tract (Hodgson, 1990). Therefore, dairy cows calving in early spring can be fed on spring pasture which is highly digestible and should encourage high nutrient intakes, as required for their period of highest milk yields.

Figure 2-1 Seasonal pattern of calving, breeding, and drying off and the synchrony between feed requirements and pasture growth (Holmes *et al.*, 2002a)

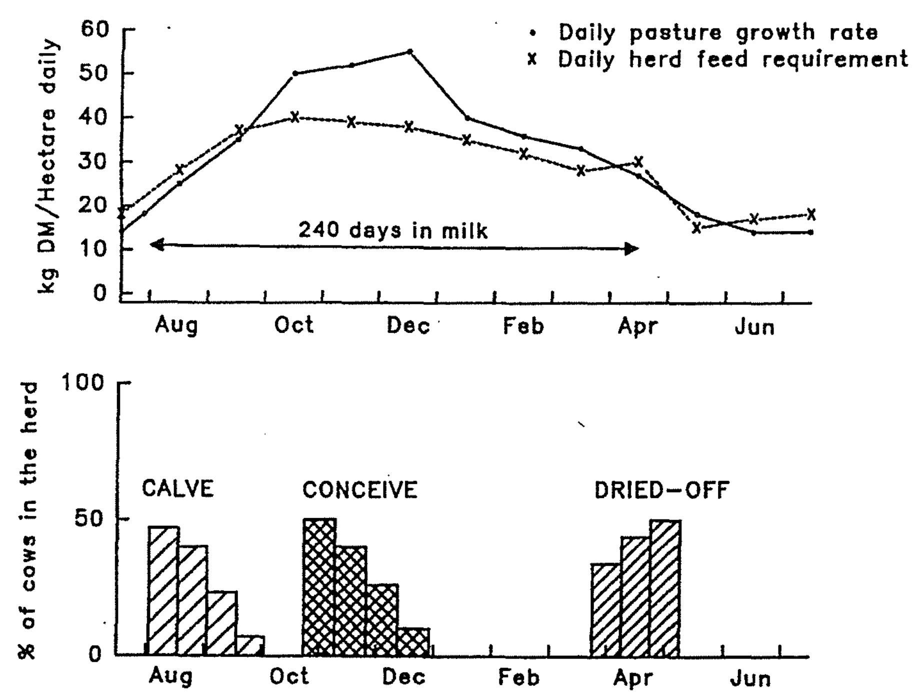
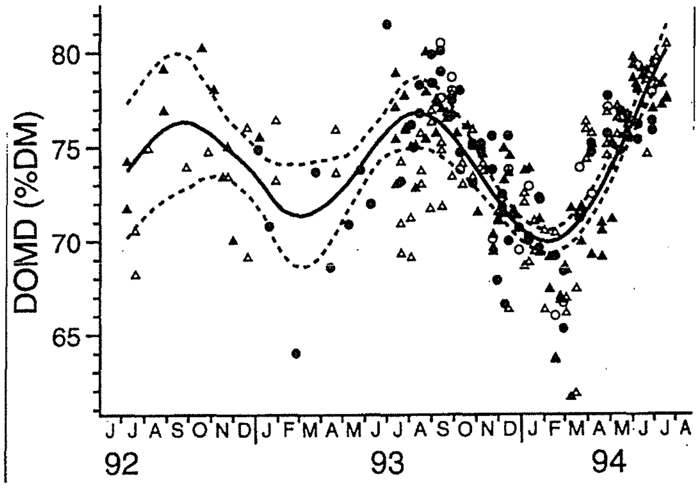


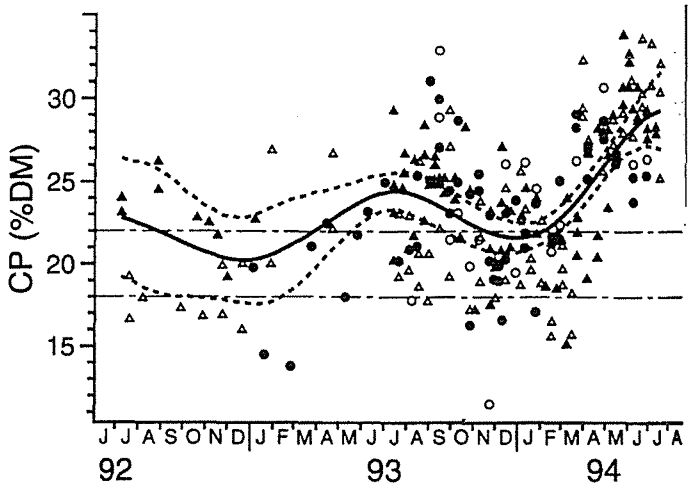
Figure 2-2 Seasonal variations in digestible organic matter in the dry matter (DOMD) of pasture samples collected from four dairy farms. The 95% confidence interval is shown by a dotted line about the mean concentrations (Moller *et al.*, 1996b)



The concentration of CP also displays a seasonal trend (Figure 2-3), and can be influenced by the fertilizer applications (Stevenson, 2003). The highest CP concentration in pasture occurs during late winter-early spring, which can also be associated with an increase in pasture growth and with applications of urea fertilizer (Stevenson, 2003). The lowest CP content occurs between November and January (Wilson *et al.*, 1995a; Stevenson, 2003). However, CP content of the ryegrass-clover sward grown in NZ is rarely less than the recommended dietary requirement of about 17% (Stevenson, 2003). In fact, many studies indicate that the CP content of the pasture, especially of mixed pastures containing clover, during spring can be higher than the protein requirements of the cow (Wilson *et al.*, 1995b; Moller *et al.*, 1996b; Jacobs *et al.*, 1999).

The metabolizable energy (ME) content of pasture (MJ ME/kg DM) follows a pattern which is similar to that of its CP percentage. Its lowest concentration occurs around January (9.0 MJ ME/kg DM) and its highest value occurs around July (11.3 MJ ME/kg DM) (Wilson *et al.*, 1995b). According to Stevenson (2003), the ME content of pasture is somewhat lower than cow requirements (11.4 MJ ME/kg DM) between August and February. Stevenson *et al.* (2003) also showed that soluble sugar and starch (SSS) content of pasture can also be lower than cow requirements during all the year. This deficit is bigger at the beginning of lactation and gets smaller during the dry weather in summer (Moller *et al.*, 1996b).

Figure 2-3 Seasonal variations in crude protein (CP) in the dry matter of pasture samples collected from four dairy farms. The 95% confidence interval is shown by a dotted line about the mean concentrations (Moller *et al.*, 1996b)



Summarizing, in the grazing dairy system in NZ the calving season mainly occurs during spring in order to match the cow's feed requirements with the pasture characteristics and availability. During this season, pasture availability is large, due to the high herbage accumulation rates and large intakes of dry matter are promoted due to the high digestibility of the pasture. During this time the SSS content in pasture can be somewhat lower than the cow's requirements, the ME content in pasture is close to the cow's requirements, and the CP content on pasture is usually above the cow's requirements. It is important to recognize the characteristics of the cow to understand how the availability of nutrients in pasture can affect its performance and in general how the cow fits into the system.

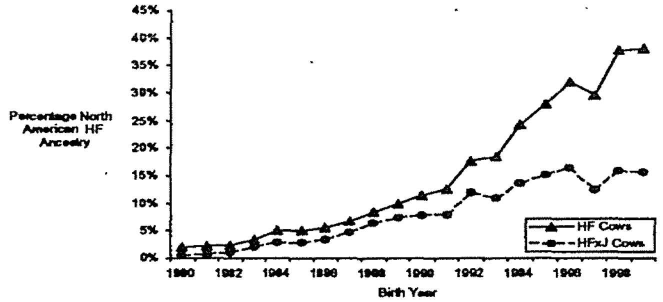
2.1.2 High genetic merit cows in 2000; their characteristics and implications for the NZ dairy system

Genetic improvements in NZ cows have been very important to gains in the efficiency of dairy farms (Holmes *et al.*, 2002b). Without such improvements, it would have been impossible to attain the current lactation and milk yields per cow (Lopez-Villalobos *et al.*, 2000a; Harris, 2001). The genetic improvement and the subsequent adaptation of the cow to its system have occurred in many ways. Firstly, since the NZ dairy system works on a seasonal basis, cows that have not been able to calve and lactate every year are culled (Xu & Burton, 2000; Holmes, 2001). By adopting this practice, farmers cull the animals that have not been able to adapt their metabolism and performance to the annual dairy system (Harris & Kolver, 2001). Another significant factor for genetic improvement during the last 20 years has been the incorporation of overseas (OS) genetics into the NZ genetics. During this period (1981-2001), the percentage of North American genetics in NZ Holstein Friesian has increased from 2% to 38% (Harris, 2001) (Figure 2-4). Incorporation of OS genetics into the national herd has increased yields of milk protein and lactose and also the genetic pool of the herd (Harris & Kolver, 2001; Bryant *et al.*, 2003). Crossbreeding (Holstein Friesian/Jersey) has also been used to increase the genetic diversity of the herd and exploit the effects of heterosis (Lopez-Villalobos *et al.*, 2000b). The result of such genetic selection is a High Genetic Merit Cow (HGMC) which is capable of producing large amount of milksolids but that also has, as detailed next, a higher incidence of metabolic diseases and lower reproductive performance (Holmes, 1999b; Fulkerson, 2000; Harris & Kolver, 2001). It is therefore important to recognize the productive and physiological characteristics of HGMC, since some of them maybe of relevance to understanding how high CP intakes reduce the animal's performance.

HGMCs have higher total DMIs (18.5 kg DM/cow/day) than medium genetic merit cows (17.5 kg DM/cow/day) (Holmes, 1999a; Buckley *et al.*, 2000). As a result of a higher DMI, HGMCs have had to modify their grazing behaviour. It has been found that the total grazing time is not affected by genetic merit

(O'Connell *et al.*, 2000). Therefore, to achieve a higher DMI the HGMC have had to develop higher biting rates whilst grazing than LGMC (58.4 *vs.* 51.4 bites/minute). According to previous studies, the upper limit for biting rate is about 60 to 70 bites/minute (Phillips & Leaver, 1985) over a total grazing time of about 10 hours/day (Stobbs, 1973). According to such parameters, the biting rate of the HGMC (37,000 bites/day) could be close to the upper limit of bites/day (40,000 bites/day). This could reinforce the view of Holmes (1999a) and Kolver (2002) in the sense that HGMC are not able to consume enough dry matter by grazing, when compared to their DMI from total mixed rations, to cover their daily requirements.

Figure 2-4 Percentage of North American Holstein ancestry present in New Zealand Holstein Fresian (HF) and HF-Jersey crossbreed cows by birth year (Harris & Kolver, 2001).



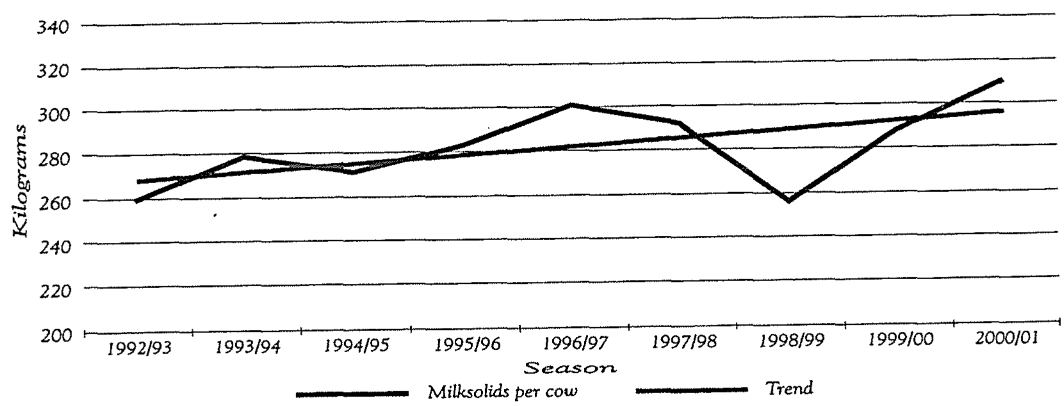
Genetic selection for milk yield has modified nutrient partitioning towards milk production. Metabolic studies of cows of high and low genetic merit have shown that there are no measurable metabolic differences between the two strains in terms of their ability to digest feeds, nor in their net efficiency for milk synthesis (Agnew *et al.*, 1998; Bryant *et al.*, 2003; Veerkamp *et al.*, 2003). Consequently, HGMCs must sustain their higher levels of milk production by higher intake, increasing mobilization of body reserves, reduction of tissue deposition or changing nutrient partitioning between milk synthesis and other metabolic functions (Agnew *et al.*, 1998; Bryant *et al.*, 2003; Veerkamp *et al.*, 2003) (Table 2-1).

Table 2-1 Metabolizable energy intakes (MEI), and proportion of energy partitioned to lactation (EP_l), maintenance (EP_m), and liveweight change (EP_g) of high genetic merit (HGM) and low genetic merit (LGM) Jersey cows (Bryant *et al.*, 2003)

	HGM	LGM	SED	Significance
Early lactation				
MEI (MJ/cow/day)	157	151	2.7	*
EP_m	0.38	0.38	0.008	NS
EP_l	0.67	0.63	0.013	**
EP_g	-0.05	-0.01	0.019	*
Peak lactation				
MEI (MJ/cow/day)	166	155	1.9	***
EP_m	0.36	0.38	0.005	**
EP_l	0.59	0.57	0.008	*
EP_g	0.05	0.05	0.011	NS
Mid lactation				
MEI (MJ/cow/day)	166	152	1.8	***
EP_m	0.37	0.40	0.005	***
EP_l	0.58	0.56	0.007	**
EP_g	0.05	0.04	0.009	NS
Late lactation				
MEI (MJ/cow/day)	139	127	1.9	***
EP_m	0.46	0.49	0.008	***
EP_l	0.54	0.51	0.009	**
EP_g	0.00	0.00	0.013	NS

Milksolid yields per cow have increased from 190 kg in 1950 (Holmes, 1999b) to 315 kg in 2003 (Livestock improvement, 2003) (Figure 2-5). It has been estimated that genetic improvement contributed about 70% of this increased yield over this period (Holmes, 1999a). Kolver (2000) compared the performance of cows in two different genetic strains under the same feeding system and found that current HGMCs produced more milksolids per cow per lactation (416 kg) than the low genetic strain (360 kg), which were daughters of 1970's proven bulls.

Figure 2-5 Trend in milksolids production per cow since 1992/1993 (LIC, 2003)

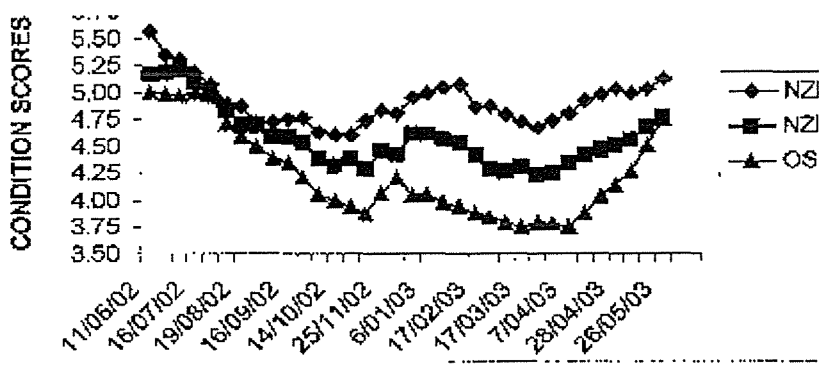


As a result of less energy proportionally available for other metabolic processes than milk synthesis, genetic selection for milk has reduced the condition score of the NZ cow across its lactation (O'Connell *et al.*, 2000; Kennedy *et al.*, 2003a; Kennedy *et al.*, 2003b). HGMCs lose more condition score (0.52 units) from Week 1 to 8 after calving, than medium genetic merit cows (0.32 units) during the same period (Buckley *et al.*, 2000). These differences were significant (P=0.05). Kennedy *et al.* (2003b) found that HGMC had lower condition score at calving (3.36 vs. 3.48 respectively) and at first insemination (2.93 *vs.* 3.2 respectively) than low genetic merit cows (LGMC). Also important is the fact that HGMCs were still losing condition score even 90 days after breeding, than LGMCs which by that time were already recovering condition score. Condition score at drying-off day was higher (5.4) for the NZ LGMCs than for the NZ HGMCs (4.9) (Macdonald, 2004, unpublished data). It is possible that the OS genetics had contributed to the reduction in condition score and liveweight during lactation of the NZ HGMC. This hypothesis is supported by the findings of several authors who, offering the same pasture allowances under grazing conditions, have shown that OS cows have lower condition score and liveweight at the beginning of lactation (Kolver *et al.*, 2000; Macdonald, 2004, unpublished data) and end of lactation (Macdonald, 2004, unpublished data) (Figure 2-6). The lower condition score of the NZ HGMC, compared to NZ LGMC, is due to differences in energy partitioning between milk production and body reserves (Kolver *et al.*, 2000).

It is reasonable to expect that such a reduction in ability to maintain or gain liveweight could be further increased as the percentage of OS genes and the pressure of genetic selection for milk production increase (Kolver *et al.*, 2000; Harris & Kolver, 2001).

Summarizing, the process of genetic selection has been successful in generating a HGMC which produces large yields of milksolids per lactation which are possible because of the higher DMI and higher energy partitioning towards milk. However, this process has also been accompanied by some negative changes including a reduction in the energy available for gain or maintenance of liveweight, together with an inability of the HGMC to achieve its maximum energy consumption from solely pasture-based diets. The lower energy availability for functions other than MS production could be further reduced by the extra energy required to excrete the urea originated from the extra intake of CP, potentially further depressing the liveweight, condition score, and even reproductive performance.

Figure 2-6 Condition score of the strain cows in the second year of the trial (Macdonald, 2004, unpublished data)



NZL= New Zealand low genetic merit, NZH= New Zealand high genetic merit, and OSH= overseas high genetic merit

2.1.3 Some reproductive parameters of the HGMC

Fertility of dairy cows is a very important factor in seasonal calving systems in order to maintain an average of 365-day calving interval (McNaughton *et al.*, 2003). This seasonal calving interval is planned to match the time when the maximum DMI of the herd occurs with the maximum herbage growth rates. Therefore, a successful breeding period, resulting from good herd fertility, will enable the herd to maintain a seasonal calving period required to maximize milk yield and feed utilization (Xu & Burton, 2000). Increases in genetic merit for milk yield (Jonsson *et al.*, 1999; Snijders *et al.*, 2000; Snijders *et al.*, 2001; Westwood *et al.*, 2002; Veerkamp *et al.*, 2003), has been linked to decreased reproductive performance in the NZ dairy herd (Xu & Burton, 2000; Harris & Kolver, 2001). Thus, it is important to consider the reproductive problems of the current HGMC since they may have been exacerbated by the consumption of diets containing CP in quantities above theoretical requirements, for example from grass/clover pasture in spring.

Many authors have found that HGMC cows are more likely to have impaired expression of oestrus behaviour than LGMCs (Harrison *et al.*, 1990; Snijders *et al.*, 2001; Westwood *et al.*, 2002). In HGMC, oestrus behaviour and milk production are negatively correlated (Harrison *et al.*, 1989; Westwood *et al.*, 2002). Failure in the display of the behavioural signs of oestrus have been related to low DMI, low ratios of plasma glucose to β-hydoxybutyrate, and low plasma cholesterol concentrations (Lean *et al.*, 1992; Westwood *et al.*, 2002). Absence of clear signs of oestrus can affect reproductive performance adversely, by causing failure of oestrus detection and potentially extending the calving interval. Consequently, early and clear signs of oestrus ensure acceptable submission rates during the breeding season (Harrison *et al.*, 1990).

Prolonged postpartum anoestrous intervals, the period following calving before the ovulatory cycles are re-established (McDougall, 1994), is a major cause of infertility in NZ HGMC (McNaughton *et al.*, 2003; Thiengtham, 2003). Many authors have found that genetic selection for milk yield has resulted in longer postpartum anoestrous intervals in OS (Jonsson *et al.*, 1999) and NZ HGMCs, compared with LGMCs (Verkerk *et al.*, 2000; McNaughton *et al.*, 2003) (Table 2-7). However, other studies suggest that post-partum ovarian functions and intervals between calving and first and latter ovulations, have not been affected in the HGMC cow as a result of genetic selection (Harrison *et al.*, 1990; Westwood *et al.*, 2000; Snijders *et al.*, 2001; Kennedy *et al.*, 2003b). Thus, Harrison *et al.* (1990) and Westwood *et al.* (2000) found that cows of high and low genetic merit have similar periods of time from calving to first ovulation (29 and 31 days, respectively) and length of first luteal phase (11 and 14 days). Other authors, such as Diskin *et al.* (2000) have postulated that the resumption of oestrous cycles is negatively influenced more strongly by the level of milk yield, than by the genetic merit of the cow. Thus, according to Diskin *et al.* (2000) high producing cows are more likely to have more severe Negative Energy Balance (NEB) and longer postpartum anoestrous intervals, as a result of having lower body condition scores' at calving (McNaughton *et al.*, 2003) and greater body condition score loss in early lactation (MacMillan, 1997). It is therefore possible to establish that genetic selection for milk yield has increased the postpartum anoestrus interval of the NZ HGMC when compared to OS HGMC and NZ LGMC.

Table 2-2 Body condition score and reproductive outcomes in oversears (OS) 1990's animals, presented as value for OS90's – value for New Zealand (NZ) 90'1 (McNaughton *et al.*, 2003)

	BCS at calving	BCS change from calving to 4 weeks	Postpartum anovulatory interval	Treated for anoestrus	12-week pregnancy rate
Year 1	-0.23 units	0.08 units	-20 days	-36%	-14%
Year 2	-0.20 units	0.06 units	-15 days	-11%	Not yet available

The generally lower reproductive performance of the HGMC has also been related with the quality of the oocyte more importantly than with the follicular physiology. According to Snijders *et al.* (2000), oocytes from HGMCs have formed fewer blastocysts and had lower cleavage and blastocyst formation rates than those from medium genetic merit cows. Snijders *et al.* (2000) also found a positive relationship between body condition score and the quality of the oocyte, i.e. greater cleavage and blastocyst formation rates, but could not find any link between oocyte quality and milk yield.

Changes in the pattern of secretion of reproductive hormones as a result of genetic improvement could also impair the reproductive performance. Progesterone (P4) is important since it maintains pregnancy in dairy cows (Veerkamp *et al.*, 2003) and pregnant cows have higher circulating concentrations of P4 within the first ten days after insemination than cows which did not conceive (Butler, 1998; Mann *et al.*, 1999). Few studies have investigated the effect of selection for milk on gestational P4 concentrations (Veerkamp *et al.*, 2003). However, Lucy (2001) found that cows of low genetic merit (from 1964) had plasma P4 concentrations that were significantly higher than in HGMCs. In another study using current high and low gemetic merit cows, it was also observed that P4 concentrations during the luteal phase of the first and third postpartum cycle were lower in high than low yielding cows (Reksen *et al.*, 2002).

Summarizing, genetic selection for milk yield has been accompanied by an impairment of the reproductive performance of the dairy cow which is mirrored by longer postpartum anoestrus intervals, impaired display of oestrus behaviour, lower oocyte quality, and lower circulating P4 concentrations. It is important to consider the factors that contribute to these changes in the NZ HGMC since they can potentially be negatively affected by the consumption of diets high in CP.

2.1.4 Effects of high CP intake on reproduction in the high genetic merit cows

Diets with a high content of CP are commonly used to support high milk yields per cow (Laven & Drew, 1999; Butler, 2000; Sinclair *et al.*, 2000). In NZ, for the reasons mentioned in section 2.1.1, grazing dairy cows usually consume lush spring pasture with high soluble CP content during spring (Moller *et al.*, 1996b; Smith *et al.*, 2001). The effect of high CP intakes on the reproductive performance of cows has been found to be negative by some (Butler, 1998; Butler, 2000; Sinclair *et al.*, 2000; Westwood *et al.*, 2002) but not all studies (Chapa *et al.*, 2001; Smith *et al.*, 2001; Kenny *et al.*, 2002).

Interval to first ovulation is an important reproductive parameter since cows must start cycling before the breeding season (Lucy, 2001). Resumption of ovarian activity has been shown to be affected by high CP intakes after parturition by some (Staples *et al.*, 1993; Barton *et al.*, 1996) but not all (Kaim *et al.*, 1983; Carroll *et al.*, 1988; Westwood *et al.*, 1998) authors. Diets containing 20% of CP prolonged the interval to first ovulation when the rumen digestible protein (RDP) was particularly excessive (72.5% CP) (Staples *et al.*, 1993) or when reproductive diseases were included in the analysis (Barton *et al.*, 1996). Other authors (Kaim *et al.*, 1983; Carroll *et al.*, 1988) have not found an effect of CP intake on reproduction when the CP% of the diet was between 19 and 21%. No negative effects of high protein intake have been observed on follicle development and ovulation (Blanchard *et al.*, 1990; Garcia-Bojalil *et al.*, 1994; Butler, 1998; Westwood *et al.*, 1998).

Cows fed with diets containing high concentrations of RDP had a negative relationship between urea concentrations and the likelihood of showing oestrus at the first ovulation; the high RDP group also had the largest liveweight loss at the beginning of lactation. Conversely, the high protein group had a higher likelihood of showing oestrus during the second ovulation when their liveweight gain was larger than for the control group (Westwood *et al.*, 2002), which suggests a possible link between the effect of high protein intakes and of energy balance on reproduction.

Progesterone is an ovarian steroid that adjusts and reflects the functioning of the hypothalamic-pituitary-ovary axis, and maintains pregnancy (Veerkamp *et al.*, 2003). As detailed next, the effect of high protein intakes on milk yield or serum P4 concentrations differs among authors and depends on the productive and energetic status of the cow, as well as on the quantity and type of the protein.

High CP intakes (Butler, 1998) and more specifically RDP (Staples *et al.*, 1993) have been related with low concentrations of P4. Westwood *et al.* (1998) reported that high dietary protein intakes in lactating dairy cows may increase the metabolic clearance rate of P4. According to Westwood *et al.* (1998) the higher clearance rate may be due to a perturbation of the hypophyseal-pituitary-ovarian axis either directly or indirectly by altering the energy metabolism in the cow affecting the rate at which the hormone is produced or cleared. This negative effect of protein on P4 could be increased by the NEB, which has been linked to low P4 concentrations during early lactation (Staples *et al.*, 1993; Butler, 1998).

The proposed interaction between the effects of protein intake and energy balance on P4 synthesis (Butler, 2001) is reinforced by the review of Butler (1998) who found that three out of four experiments offering high protein diets to lactating cows reported low P4 concentrations. Conversely, zero out of three experiments reported effects of high protein intake on P4 concentrations when offered to non-lactating animals. Butler (1998) also found that after subsequent ovarian cycles the P4 concentrations increased and the degree of the increase was positively related to the energy balance. This effect also was observed when cows have been fed on diets with high concentrations of RDP (Staples *et al.*, 1993).

According to Staples *et al.* (1993) the decrease in the P4 concentration is related to enhancement of the NEB of the cow due to the cost of detoxifying the ammonia escaping from rumen.

A link between protein intake and changes in the uterine environment was reported (Butler, 1998). Protein intakes of 23% were found to produce plasma urea concentrations higher than 19 mg/dl and to alter the pH and ion concentration in the uterus during the luteal phase (Jordan *et al.*, 1983). Such characteristics in the uterine environment are suboptimal for the support of embryo development (Eley *et al.*, 1981; Westwood *et al.*, 1998; Butler, 2000).

Effects of protein intake on embryo quality and survival differ between authors depending basically on the energy balance of the cow. High intakes of RDP by lactating dairy cows have been linked to reduction in the percentages of ova recovered, and of transferable embryos after artificial superovulation (Blanchard *et al.*, 1990). Such relationships could not be proved with non-lactating cows (Garcia-Bojalil *et al.*, 1994) and heifers (Kenny *et al.*, 2002). Elrod & Butler (1993) found that embryo quality and survival was affected in heifers fed with an excess of RDP and restricted to 70% of their ME requirements. Similarly, oocytes recovered from heifers fed on high plasma ammonia-generating diets had lower cleavage and blastocyst production *in vitro* than heifers offered fed on low plasma ammonia-generating diets (Sinclair *et al.*, 2000). The reduced reproductive performance and embryo viability observed in lactating dairy cows is possibly caused by interactive effects between high protein intakes and NEB rather than the effects of dietary protein or high concentrations of urea or ammonia *per se* (Butler, 2001).

Another possible reason for the link between high protein intake and embryo mortality could be that cultures of endometrial cells increase their prostaglandin $F_{2\alpha}$ secretion when exposed to urea (Gilbert *et al.*, 1996).

In theory, the NZ Holstein Friesian HGMC should have extremely low conception rates during spring if the evidence for negative effects of high protein intake on reproduction from the Northern Hemisphere were directly applied to the cow in NZ. However, many authors have found that NZ cows on pasture have an outstanding reproductive performance when compared with OS cows fed on pasture or total mixed rations (Kolver *et al.*, 2002; Fahey *et al.*, 2003; McNaughton *et al.*, 2003). The reason for the better reproductive performance of the NZ HGMC could be that, as explained throughout the present section, the reproductive performance seems to be affected not only by the amount of protein eaten but also by a more complex interaction between protein intake, genetic adaptation of the cow, and energy status, which could negatively interact making the cow more vulnerable to the effect of large intakes of CP. It is therefore important to understand the energy balance of the cow during the postcalving period to understand how it could interact with the consumption of large amounts of CP.

2.1.5 Effects of high CP intake on energy balance and milk production in the fresh-calved HGMC

After calving, cows have to increase their DMI by 1.5 to 2 fold in order to meet the high nutrient demands of milk production. However, HGMC are not able to achieve this rapid increase (Bryant *et al.*, 2003), and their energy requirements for milk production and maintenance exceed the amount of energy ingested (Reist *et al.*, 2003b). Increases in DMI during early lactation cannot restore the energy balance since HGMC are genetically predisposed to use the extra energy intake into milk production rather than to reducing the energy gap (Veerkamp *et al.*, 2003). This incapability of the cow to cover its energy requirements during early lactation leads to a NEB (Roche *et al.*, 2000).

Under grazing conditions, HGMC are especially likely to experience NEB as a result of the feed characteristics. Grazed pasture imposes limits on nutrient intake because of its high fibre density and low energy density compared with total mixed ration (TMR) (Ferris *et al.*, 2002). Therefore, the DMI of NZ HGMC is significantly lower when grazing on pasture than when fed on TMR (17 *vs.* 20.3 kg DM/day) (Kolver *et al.*, 2000). Another important limiting factor for DMI is that it takes the cow 10 hours to graze 17 kg of DM and only 5 hours to eat 20 kg of DM of TMR (Holmes *et al.*, 2002a).

Reduction in energy balance has also been related to the intake of high RDP as a result of the energy cost of converting excessive ammonia, resulting from high protein intakes, to urea for excretion in the urine (Butler, 1998; Chapa *et al.*, 2001). This could be particularly relevant under the grazing conditions of NZ where the cow's CP requirements after calving range from 190 (Stevenson *et al.*, 2003) to 220 g CP/kg DM (Moller *et al.*, 1996b) and the CP content of ryegrass during spring can be as high as 265 g CP/kg DM (Moller *et al.*, 1996b; Stevenson *et al.*, 2003). The cost of metabolizing and excreting urea can be so high in dairy cattle that it can increase the NEB and reduce the energy available for milk synthesis (Oldham, 1984; Twigge & Gils, 1988); according to Danfaer *et al.*(1980), a decrease in fat-corrected milk from 24.5 to 23.1 kg/cow/day can occur by increasing the percentage of CP in the diet from 19 to 23%.

NEB has also been related with low reproductive performance. In dairy cows, NEB usually begins a few days before calving and reaches its nadir about two weeks later (Butler, 2000). The NEB during the first three to four weeks postcalving has been implicated in the timing to first ovulation, which occurs about 30 days postpartum (DPP) (range 17-42 days) in the OS HGMC (Beam & Butler, 1998) and between 15 to 20 days later in the NZ HGMC (McNaughton *et al.*, 2003). Low NEB decreases LH pulse frequency, which has been related to growth rate and diameter of the dominant follicle (DF) as well as the capacity of the DF to produce sufficient oestradiol to induce a gonadotropin surge, insulin-like growth factor (IGF1), insulin, and increases of GH in blood (Butler, 2000). NEB can also decrease the P4 synthesis by affecting the IGF-GH axis. It has been proved that during NEB status the IGF concentrations in blood are reduced (Butler, 2000) and that the IGF-GH axis is important in determining steroidogenesis in luteal tissue in ewes (Roche *et al.*, 2000).

Summarising, the HGMC has a high predisposition to suffer from NEB especially when fed on grazed pasture, because of restrictions in the intake of energy, inherent to the physical properties of the feed. This NEB can be further depressed by the intake of large amounts of CP which require the expenditure of extra energy to cover the cost of excreting urea. This could potentially reduce the energy available for milk synthesis and also affect the reproductive performance through depressing the NEB (Figure 1-9), through modifications in the IGF1- insulin- GH axis.

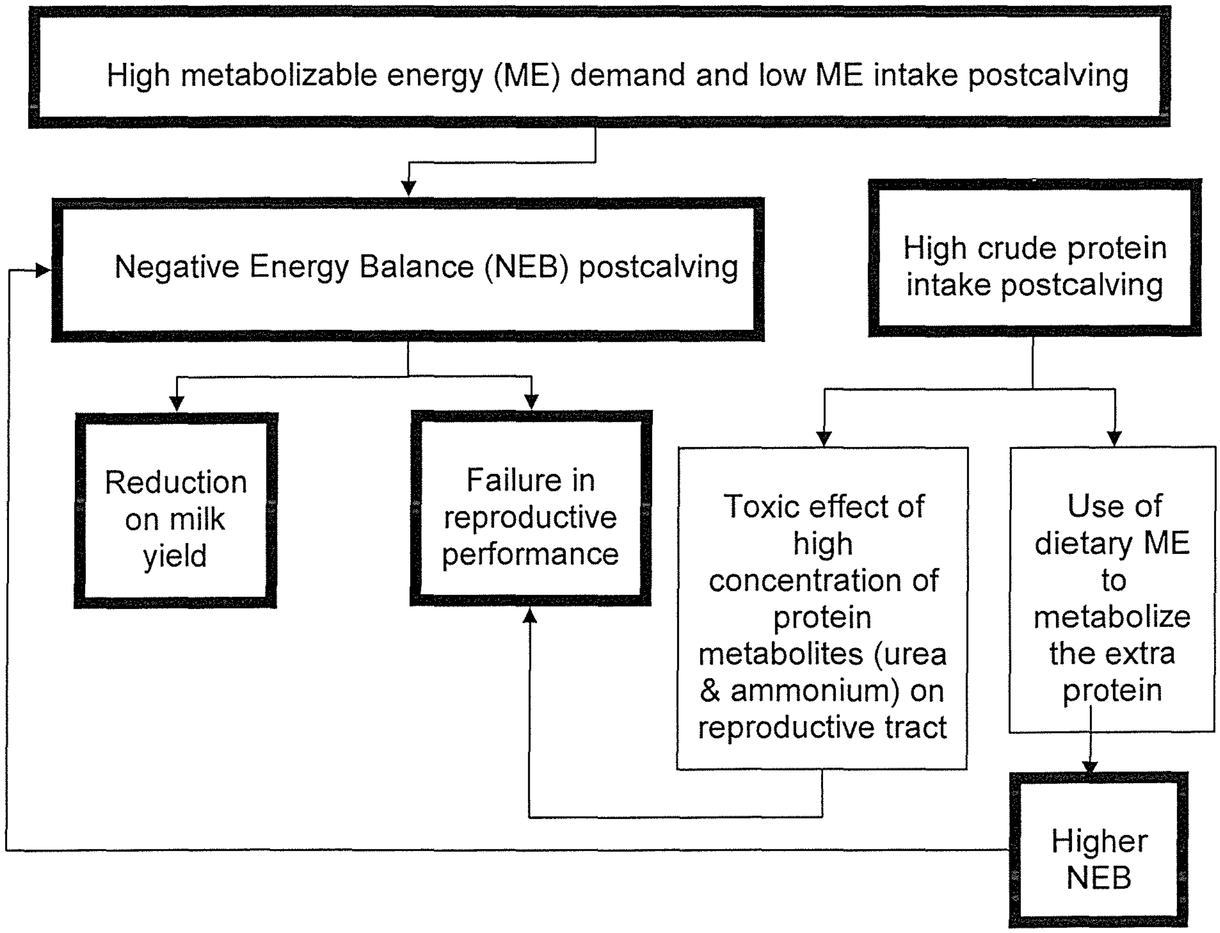


Figure 2-7 Interactions that occur in the dairy grazing system between crude protein intake and energy balance in the spring calved high genetic merit cow and their effects of cow performance.

2.2 THE IGF1 – INSULIN –GROWTH HORMONE AXIS IN THE HGMC

The NEB is particularly important for the dairy cow since it can affect the IGF1 –Insulin –Growth hormone (GH) axis (somatotropic axis) (Butler, 2000). The somatotropic axis strongly influences the reproductive performance (Spicer *et al.*, 1990; Yung *et al.*, 1996a; Roberts *et al.*, 1997; Butler, 2000; Roche *et al.*, 2000; Jorritsma *et al.*, 2003; Veerkamp *et al.*, 2003) and milk synthesis (Lucy, 2001). The somatotropic axis also has been proven to be a reliable index of the energy balance of the cow (Ferris *et al.*, 2002). Since the NZ HGMC has a high predisposition to present NEB postcalving, which potentially can affect the somatrotropic axis, it is important to understand the way the somatotropic axis regulates key productive functions of the cow.

IGF1 is an important metabolic and mitogenic factor promoting cell growth and metabolism (Griffin & Ojeda, 2000), increasing substrate uptake, and inhibiting apoptosis (Baxter, 2000). IGF1 is produced in different parts of the body including liver, bone cells, brain, muscle and mammary tissue and ovaries where they are considered to be responsible for tissue growth and differentiation. However, about 80% of circulating IGF1 is hepatic in origin (Griffin & Ojeda, 2000).

The highest IGF1 plasma concentrations take place 10 weeks prepartum (600 µg/l). From this point concentrations steadily decrease reaching a nadir (110 µg/l) two weeks postpartum (Schams *et al.*, 1991). From which it steadily increases over the whole lactation until achieve the starting concentration at 10 weeks prepartum (Roberts *et al.*, 1997; Walters *et al.*, 2002). The IGF1 activity is partially inhibited by the effect of six IGF binding proteins (IGFBP-1 to IGFBP-6), which have the ability to block the receptor activation of IGF1 and IGF1I (Baxter, 2000).

Growth hormone is produced in the anterior pituitary (Veerkamp *et al.*, 2003). The higher GH concentration in HGMCs occurs at parturition, before the pronounced decline in energy balance (Lukes *et al.*, 1989; Cohick, 1998). The mechanism that leads to rapid changes in the GH and IGF during early lactation are not completely understood (Lucy, 2000). Even though GH bioactivity has been reported to be controlled by serum binding proteins in humans (Bernard & Waters, 1997), such control has not been found in the bovine (Rose, 2002).

Insulin is released from the pancreas in response to rising glucose concentrations. Its action on glucose is antagonized by GH, glucagons, adrenalin, and glucocorticoids (Veerkamp *et al.*, 2003). Insulin exerts is effect mainly through energy availability for the cell (Bucholtz *et al.*, 1988) rather than via direct effects of the hormone itself (Veerkamp *et al.*, 2003).

2.2.1 The somatotropic axis and the energy balance

It has been proposed that energy balance during early lactation modifies directly and indirectly the somatotripic axis (Lucy, 2000). Postpartum NEB probably reduces the blood IGF concentrations since nutritional status partially controls the synthesis and secretion of IGF (Thissen *et al.*, 1994; Armstrong *et al.*, 2001). Armstrong *et al.* (2001) reported that heifers fed on high energy diets had higher mean plasma insulin and IGF1 concentrations than those fed on low energy diets. The intake of diets higher in energy also was accompanied by an increase on the expression of mRNA encoding components of the ovarian IGF system.

The effect of energy balance on the IGF concentrations has also been shown indirectly in several experiments. Spicer *et al.* (1990) and Walters *et al.* (2002) found a positive correlation between energy balance and IGF1 in dairy cows. Reestablishment of positive energy balance after calving was also accompanied with an increase of IGF1 concentration, meanwhile cows that remained in NEB and with lower condition score conserved low IGF1 concentrations (Spicer *et al.*, 1990; Ryan *et al.*, 1994; Walters *et al.*, 2002). A similar positive relationship between energy balance and IGF1 concentration has been found in Holstein heifers, which after being kept on positive energy balance during four oestrus cycles, had an IGF1 mean concentration 37% higher than heifers in NEB (Yung *et al.*, 1996b).

Recently, an interaction between circulating IGF concentration and feeding system and genetic strain has been described. Fahey *et al.* (2003) reported that plasma IGF1 concentration was higher for cows fed on a total mixed ratio compared to cows fed on grazed pasture. Fahey *et al.* (2003) also found an interaction with genetic strain since NZ cows had higher IGF1 concentrations than OS cows.

Conversely, GH is not negatively affected by NEB since GH concentrations in the cow increase during the prepartum period coinciding with the NEB (Gluckman *et al.*, 1987). NEB seems to exert an indirect positive effect on GH concentrations through reducing the concentration of IGF1, which has been considered as the primary negative feed back inhibitor for GH (Gluckman *et al.*, 1987; Lucy, 2000)

2.2.2 The somatotropic axis and reproductive performance

Follicle development in cattle is influenced by gonadotropic hormones and IGF1. In sheep and cattle for example, insulin and IGF1 and possibly IGF2 synergize with gonadotropins to stimulate cell proliferation and hormone production. An absolute requirement for IGF1 in normal ovarian function has been demonstrated recently using gene knock-out technologies (Baker *et al.*, 1996; Webb *et al.*, 1999).

Blood and follicular IGF1 concentrations have been correlated because the majority of the follicular IGF1 comes from the liver (Leeuwenberg *et al.*, 1996). However, the importance of liver (endocrine) IGF1 could be low for the ovary metabolism despite its local high concentration, because when the IGF1 gene in liver is knocked out the mice grew normally and had normal litter sizes (Sjogren *et al.*, 1999; Yakar *et al.*, 1999).

The effect of the IGF system on the ovary may ultimately depend more on the effect of the IGF binding proteins (IGFBPs) rather than on the absolute concentration of IGF (Lucy, 2000; Mihm *et al.*, 2000). According to Armstrong *et al.* (1998), dominant follicles posses the same amount of IGF1 as do subordinate follicles, but subordinate follicles have higher amounts of IGFBP than dominant follicles. The IGFBP may prevent the IGF from binding to receptors located within granulosa cells therefore avoiding growth and differentiation and leading the follicle to the atretic state (Mihm *et al.*, 2000).

Energy balance also has an indirect effect on the selection of the dominant follicle through modifying the concentration of follicular IGFBP. The concentration of mRNA of IGFBP2 and IGFBP4 in healthy follicles is reduced when high energy diets are offered. A reduction in the local concentration of IGFBP2 would increase the IGF bioactivity in the dominant follicle (Armstrong *et al.*, 2001).

According to Yung *et al.* (1996a), energy balance and IGF1 concentrations during the luteal phase have no negative effect on serum P4 concentrations. However, because animals with positive energy balance and high IGF1 concentrations have been associated with high P4 concentrations (Lussier *et al.*, 1987b), Yung *et al.* (1996b) suggested that circulating IGF1 has no direct effect on corpus luteum development but may have indirect effects through actions on granulosa follicular cells. If so, it could mean that the effect of higher IGF1 concentrations could take two oestrus cycles which is the time that follicles take to grow through the antral phase to the preovulatory size (Lussier *et al.*, 1987a). The results of Yung *et al.* (1996b) seem to contrast with those of Chakravorty *et al.* (1993) who found that addition of IGF1 increases DNA synthesis in bovine luteal cells *in vitro*. According to Yung *et al.* (1996a) and Mihm *et al.* (2000) the reason for the apparent inconsistencies between *in vitro* and *in vivo* data are not clear but may be related to the concentrations of IGF1 and its various binding proteins in the cell microenvironment. This theory is in agreement with Mihm *et al.* (2000).

Circulating concentrations of IGF1 are good indicators of the capacity of energy-restricted cattle to resume cycling after parturition (Roberts *et al.*, 1997). Beef cows that remained anoestrus for 20 weeks postpartum had low circulating IGF1 concentrations (<25 ng/ml) at weeks two and ten post partum. Conversely, cows that presented oestrus within the 20-week period showed higher circulating IGF1 concentrations since the second week postpartum (40 ng/ml) and this increasing trend was maintained until the 10th week when the hormone concentration reached 75 ng/ml.

Growth hormone is another component of the somatotropic axis that has been related with reproductive performance. Through indirect and direct actions, GH stimulates the later stages of folliculogenesis and luteinization and seems to be involved in follicle selection (Hull & Harvey, 2001). The action of GH on ovarian function may be direct since granulosa cells and oocytes contain GH receptor mRNA (Lucy *et al.*, 1998). There is probably no absolute requirement for GH in reproduction because mice with naturally occurring mutations in the GH gene and cattle with abnormal ST receptor expression (Chase *et al.*, 1998) can reproduce, even though their efficiency of reproduction is low (Lucy, 2000).

Summarising, the somatotropic axis is involved in many key productive aspects of the HGMC. The IGF1 is particularly sensitive to NEB experienced during the postpartum period, which makes from it a reliable parameter to assess the energy balance of the cow. In reproductive functions, the IGF1 is important to stimulate follicular cell proliferation, follicular selection, and hormone synthesis. However, blood IGF1 concentration may not be completely reliable to assess the reproductive ability of the cattle since other factors as their binding proteins and follicular IGF1 also regulate the ovary physiology. Finally, it is also important is that the blood IGF1 concentrations have been proven to be higher in NZ HGMC than in OS HGMC, which could be a reflection of the more positive energy balance postpartum and the better reproductive performance of the NZ than the OS HGMC.

2.3 FINAL INTEGRATION AND THE PRESENT EXPERIMENT

In conclusion, in NZ the calving season mainly occurs during spring in order to match the peak of the cows' requirements for feed with the time when pasture availability and nutrient content are high. During this time, the CP content of pasture is usually above the cows' requirements. The process of developing a HGMC for milk production has also been fundamental to increase the productivity of the dairy system. The NZ HGMC produces large yields of milksolids per lactation due to its high DMI and large energy partitioning towards milk synthesis. However, this characteristic has been accompanied by a reduction in the energy available for gain or maintenance of liveweight. This lower energy available for other metabolic functions could be further depressed by the extra energy used to excrete the urea originating from the extra intake of CP, potentially further decreasing liveweight, CS, and reproductive performance. Also important is the inability of the cow to satisfy its energy requirements during the postpartum period which lead to the NEB. The NEB can be further increased in the HGMC when fed on grazed pasture, because of the restriction in intake of energy inherent to the physical reducing the concentrations of IGF1, which is important to stimulate cell proliferation, follicular selection, and hormone synthesis properties of the feed and the extra energy required for excretion of urea. It is therefore important to know if the negative effect of consuming large amounts of CP, as described in OS reports, occurs on the NZ HGMC dairy system and, if so, at what extend.

In the current experiment, two groups of HGMC were offered a common pasture allowance over 100 days in early lactation but from pasture containing either a lower or higher concentration of CP. The difference in CP concentration was created by application of different amounts of urea fertilizer. The productive parameters to be assessed will be divided into three main areas, i.e. milk yield, energy balance, and reproductive performance.

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

The objective of this research is to test the effect of two different CP intakes from pasture during spring, caused by two different levels of N fertiliser applications, on the reproductive performance, energy balance, and milk yield of spring-calved dairy cows in NZ. Forty spring-calved Holstein-Friesian cows were divided between two groups of twenty cows each (High and Low Crude Protein) balanced by calving date, lactation number, liveweight, condition score, and production worth (Table 2-1). The high CP group was grazed on paddocks which were fertilized with urea from four weeks before the beginning of the experiment until the end of it. The low CP group was grazed on paddocks which did not receive any urea application from four weeks before the beginning of the experiment until the end of it. The experimental period began on 18th August and finished on 27th November.

Table 3-1 Mean values for the 20 cows in the two treatment groups measured before the start of the experiment

Group	Calving date	Lact. Number	LWt (kg)	CS*	PW **
HighN	7th August	2.7	466.6	4.3	114.65
No N	7th August	2.6	463.3	4.3	111.45

* Condition score (CS), based on 10 points scale as described by Macdonald & Roche (2004).

** Production worth (PW), net income per unit of feed (4.5 tonnes of dry matter) from milking cows (LIC, 2003)

3.2 FARM DESCRIPTION

The experiment was carried out from 18th August 2003 to 28th November 2003 at the Number 4 Dairy Farm Massey University, Palmerston North NZ. The farm is at an altitude of 80 m above sea level. The main soil type of the farm is Tokomaru silt loam which is a predominantly compact clay loam with compact subsoil, poor natural drainage, and with a tendency to dry out in summer. It has moderate natural fertility. Average soil temperature at 10 cm is 7°C during July and 18.5°C during January. The average annual precipitation is about 980 mm (Agricultural Services, 2003).

Ten paddocks were selected for the experiment (Table 3-2), because of their proximity to the milking shed. The total area allocated to the low CP treatment was 20% larger than for the high CP treatment in order to compensate for the lower pasture growth rates expected from the low CP paddocks.

Table 3-2 Paddock allocation on the experiment and areas

High CP paddocks		Low CP paddocks	
Paddock number	Paddock area (Ha)	Paddock number	Paddock area (Ha)
3	2.54	7	2.61
12	2.15	10	1.45
54	1.16	11	2.13
57	1.72	53	1.5
		55	1.29
		56	0.7
Total area	7.57	Total area	9.68

The predominant forage species on the paddocks were Perennial Ryegrass (*Lolium perenne*) and White clover (*Trifolium repens*) (Agricultural Services, 2003).

Urea was applied on the high CP paddocks at intervals of 4 to 6 weeks. This was based on the results of Moller *et al.* (1996a), who found that the difference in CP concentration of pasture between fertilized and non fertilized paddocks was still significant (P=0.01) in the sixth Week after application of N. The amount of fertiliser applied varied during the experiment to maintain a significant difference (P=0.05) in CP content between treatments. The maximum amount of N applied at one time was 50 kg N/ha and the minimum amount applied was 40 kg N/ha, based on the results of Moller *et al.* (1996a), who recorded a pasture CP content of 24.5% in the sixth Week after application of 40kg N/ha. Timing and amounts of N applications/ha are summarized in Table 3-3. Table 3-3 also summarizes the total amount of N fertilizer that each paddock received during the experiment.

3.3 PASTURE MANAGEMENT

Each time a new paddock was going to be grazed it was divided into breaks to facilitate grazing management. The area of each break, and therefore the number of breaks in each paddock, was calculated based on the herbage mass of the paddock, as detailed below, in order to ensure that the pasture allowance for both groups was similar (about 40 kg DM/cow/day) and to achieve maximum pasture intake (Holmes *et al.*, 2002a). Every day after morning milking a new break was offered for each group. The groups did not move to a new paddock unless they had grazed all the breaks in a paddock, or unless the herbage mass in the current paddock had increased to levels which could compromise pasture quality. The postgrazing herbage mass was maintained around 2,000 kg DM/ha to ensure that cows could achieve adequate bite sizes and daily intake of dry matter (Hodgson, 1990). Some high CP paddocks were grazed by extra cows that did not belong to the experiment in order to reduce the pasture accumulation and avoid a decrease in pasture quality resulting from high herbage masses.

3.4 SWARD MEASUREMENTS

3.4.1 Herbage mass

A rising plate meter was used to assess pasture yield on every paddock at weekly intervals. The following formulae were used (LIC, 2002):

From 18th of August to the 3rd of October 2003 the formula used to read the plate meter was, herbage mass (kg DM/ha) = 124 * average herbage height+ 150.

From the 5th of October to the 31st of October 2003 the formula used was, herbage mass (kg DM/ha) = 100 * average herbage height + 850.

From the 2nd of November to the 28th of November 2003 the formula used to read the plate meter was, herbage mass (kg DM/ha) = 110 * average herbage height + 1000.

Between the periods in which the three formulae were used there was always a two-day-transition period of adjustment during which the previous and the following formulae were alternated.

Pregrazing and postgrazing herbage mass was assessed daily by the plate meter. These measurements were done during the morning milking; therefore the pregrazing reading was obtained just before the cows entered into the new paddock and the postgrazing mass was obtained just after the cows left the grazed paddocks.

Three calibration cuts were used to test the accuracy of the plate meter (Figures 6-1 a, b, and c of the appendix). They were done on 18th September, 3rd and 30th October 2003.

Table 3-3 Total amount of N fertilizer applied to the High Crude Protein paddocks before and during the experimental period, 24th July to 28th November

Paddock	Paddock area	Date	Rate
3	2.5	24th July	50 kg N/ha
3		12th August	50 kg N/ha
3		1st September	25 kg N/ha
3		23rd September	30 kg N/ha
			155 kg N/ha
12	2.1	28th July	50 kg N/ha
12		12th August	50 kg N/ha
12		1st October	30 kg N/ha
			130 kg N/ha
54	1.1	24th July	50 kg N/ha
54		25th August	50 kg N/ha
			100 kg N/ha
57	1.7	24th July	50 kg N/ha
57		1st September	35 kg N/ha Breaks 1&2
57		6th September	35 kg N/ha Breaks 3 to 5
57		26th September	30 kg N/ha
	Total area 7.6		115 kg N/ha

3.4.2 Nutrient herbage analysis

In the first break to be grazed on each paddock, herbage samples for nutrient analysis were collected at grazing height. If the cows were going to stay for more than 5 days on the same paddock, a second pregrazing sample was taken about 4 to 6 days after the first sample was taken.

Samples were cleaned of mud by washing in running water. Samples were then oven-dried at 65° C for 24 hours, ground and taken to FeedTECH-Agresearch for Near Infrared Spectroscopy analysis (Corson *et al.*, 1999) for CP, ADF, NDF, soluble sugars and starch (SSS), organic dry matter digestibility, and ME.

3.5 ANIMAL MEASUREMENTS

3.5.1 Apparent intakes of dry matter and metabolizable energy

Dry matter intake was estimated as

DMI= (pre-grazing hm – post-grazing hm) * ha grazed/24 hrs

Number of cows

where hm is the herbage mass (kg DM/day) estimated by plate meter (section 3.4.1).

Estimated ME intake was calculated from the estimated DMI, multiplied by the measured ME concentration of the herbage (section 3.4.2) in the grazed horizon.

3.5.2 Liveweight and condition score

Liveweight and condition score of each cow were assessed once per week starting on 18th August until 27th October 2003. Condition score was determined by the same person on each occasion and was based on 10 points scale as described by Macdonald & Roche (2004).

3.5.3 Milk production and composition

Yields and composition of milk produced by each cow were measured by herd test once per week. Milk samples were taken during the afternoon and the next morning milking and submitted to be analyzed at Livestock Improvement Corporation, Hamilton. Concentration of fat and protein were analyzed using a Milkoscan 104 infrared analyzer (A/S N. Foss Electric, Denmark).

3.6 HORMONE ANALYSIS

3.6.1 Milk and blood sampling procedures

Milk samples were taken from all the cows of each group every second day before the morning milking. Samples were stored at -20°C until the end of the experiment to be analyzed for P4 concentrations. No preservative was added to milk samples.

Blood samples from all cows were taken at weekly intervals by venepuncture (coccygeal vein) using evacuated glass tubes with heparin anticoagulant from 18th August until 27th October, 2003. Blood samples were left to clot for one hour and centrifuged at 1,200 x g at 5°C for 20 min. Serum was separated into 2 aliquots and stored at -20 °C until analyzed.

3.6.2 Progesterone assay

Progesterone concentration in whole milk was measured using enzyme immunoassay (EIA) kits (Ridgeway Science LTD, UK). Detailed description of antibodies and enzymes used in this procedure have been reported by Sauer *et al.* (1982). Wells were emptied after being brought to room temperature and 10 µl of milk sample was added to each well. P4-enzyme label (200 µl) was added and left for 1.5 h at room temperature. After being washed three times with distilled water, 200 µl of substrate in buffer solution was added and left for 30 min. Optical densities were measured at 550 nm. Progesterone assays were developed by the masterate candidate at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

Assay sensitivities, intra-assay coefficients of variance, and inter-assay coefficients of variance for all the assays used in the present experiment are reported in Table 3-4.

3.6.3 Analysis of milk progesterone data

A luteal phase was considered to have occurred if milk P4 concentrations remained above 1 ng/ml for three consecutive milk samples (Harrison *et al.*, 1990). Irregular luteal phases were considered to be those where the P4 concentrations were lower than 1 ng/ml, or those that lasted for less than three consecutive blood samples and had been preceded by ovulation of a dominant follicle identified with the scanner (section 3.7.1). P4 profiles were prepared for each cow from parturition until the end of the experiment and were used to determine the area under the curve for all luteal phases (Reksen *et al.*, 2002). The area under the P4 curve was calculated using the trapezium rule (Altman, 1995).

3.6.4 Hormone assays from serum

Concentrations of IGF1, GH, and insulin were determined from serum to assess the energy status of both groups. Hormones were analyzed at the clinical laboratory of the Institute of Veterinary, Animal and Biomedical Sciences, Massey University and undertaken by Mrs. Sandra Fosyth. Only samples from the following days were used, 18th August -start of the experiment-, 26th August –near to the nadir of the energy balance (Butler, 2000; Ferris *et al.*, 2002; Reist *et al.*, 2003a)-, 1st September, 15th September –expected peak milk yield (Garcia & Holmes, 1999; Lopez-Villalobos *et al.*, 2001)- 28th September –energy balance equilibrium (Ferris *et al.*, 2002; Walters *et al.*, 2002) and 28th October 2003 starting of the breeding season.

IGF1 assay

Plasma concentrations of IGF1 were measured in duplicate by the chloramine-T radio immune assay method described by Gluckman *et al.* (1983). Interference by binding proteins was minimised by acid-ethanol cryoprecipitation method, validated for ruminants by Breier *et al.* (1991).

The IGF1 standard used was a recombinant hIGF-I (Amersham Australia, North Ryde, NSW) which was solubilised in 0.5M ammonium acetate, pH 5.5 at concentration of 1 mg/ml and stored at 20°C. After thawing, the IGF-I was serially diluted in assay buffer to the following concentrations: 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 ng/ml.

The antiserum used was rabbit antiserum to hIGF-1 (AFP4892898) supplied by NIDDK. The second antibody was obtained from donkey anti-rabbit serum (DARS; 1:20) and NRS (1:500), mixed to 1:5 DARS/NRS in assay buffer. Recombinant hIGF-I was iodinated using the chloramine T method.

Assay procedure

On Day 1, 100 µl of plasma and quality controls were extracted by mixing with 400µl acid-ethanol (1:7 HCL: absolute ethanol) in glass tubes. The tubes were then eddied and left to stand at 20°C for 30 minutes before centrifugation at 1,500 x g for 30 minutes at 4°C. The supernatant was then decanted into plastic assay tubes and 0.855N Tris (base) was added to neutralise the solution (e.g., 250µl supernatant + 60µl Tris) before it was left to stand overnight at -20°C. On Day 2 the samples were centrifuged at 1,500 x g for 30 minutes at 4°C (Beckman, J-6M/E, USA). The supernatant (100µl) was then added to 0.9 ml of assay buffer in new tubes. Then, 100 µl of the diluted samples were made to 300 µl with assay buffer and 100 µl of first antibody was added and incubated overnight at 4°C. On Day 3, 100 µl of tracer was added, followed by incubation overnight at 4°C, and on Day 4, 100 µl of second antibody/NRS mixture was added.

After incubation overnight at 4°C, 1 ml of 6% polyethylene glycol (PEG 6000) in assay buffer was added, the samples centrifuged for 30 minutes at 1,500 x g, and the supernatant aspirated and the activity of the precipitate was determined on a gamma counter (Packard Cobra-II, Auto Gamma).

Growth hormone

The GH standard (NIDDK oGH I-5) was kindly provided by NIADDK. The stock solution (250 ng/ml) was stored in 0.05M phosphate buffer + 0.25% BSA at -20°C. One aliquot was thawed and diluted serially to the following concentrations: 0.49, 0.98, 1.95, 3.9, 7.8, 15.61, 31.25, 62.5 and 125 ng/ml in 0.05M phosphate buffer + 0.5% BSA on the day of assay.

The antiserum (NIDDK-anti-oGH-2) was kindly provided by NIADDK. The hormone was iodinated with $Na^{125}I$ using Chloramine-T method.

Assay procedure

The assay inclded 6 replicates each of three quality control pools. On Day 1, plasma samples (100 µl), standards in 0.05M phosphate buffer + 0.25% BSA (100 µl) were diluted to 400 µl with 0.05M phosphate buffer and 50 µl of antiserum was added and incubated at 4°C overnight. On Day 2, 50 µl of tracer was added, vortexed and incubated for a further 48 hours, after which donkey anti-rabbit serum (50 µl: 1:7 in 0.05M phosphate buffer was added to all tubes (except TC's) before centrifugation at 1,500 x g for 25 minutes at 4°C. The supernatant was decanted off and the activity of the precipitate was determined on a gamma counter

Insulin

Insulin in plasma was measured in duplicate by the double-antibody radio immuno assay method of Hales & Randle (1963) as modified by Bassett & Wallace (1966) and described by Tindal *et al.* (1978).

The insulin standard used was highly purified crystalline ovine insulin (Sigma, Australia) dissolved in dilute HCl, pH 2.5 to 3.0 to a concentration of 2 mg/ml. It was then diluted in 0.05M phosphate to 800µg/ml and stored in 10 µl aliquots at -20°C to be used for iodination. A 10 µl aliquot was diluted in Buffer #1 + 0.25% BSA to 200µU/ml and frozen to be used as standards. After thawing, insulin was serially diluted to the following concentrations: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 µU/ml in Buffer #1 with 0.25% BSA on the day of the assay.

Insulin antiserum (GP2, 21/7/80), was kindly donated by Dr. Peter Wynn (CSIRO Division of Animal Production, NSW, Australia). The hormone was iodinated with NaI^{125} using chloramine T method.

Assay procedure

The assay included 6 replicates each of three quality control pools. On Day 1, duplicate 100 µl plasma samples or standards were diluted to 200 µl with Buffer # 1 + 0.25% BSA. Then 100µl antiserum was added and the tubes were incubated at 4°C overnight. On Day 2, 100 µl of the tracer was added, tubes were vortexed and incubation was continued for 48 h at 4°C. On Day 4, 100µl of goat anti-guinea pig serum (1:150 in Buffer #1 with 0.25% BSA) and 100µl of normal guinea pig serum was added and the tubes were vortexed. After incubation overnight at 4°C, 1.0 ml of 2% polyethylene glycol (PEG 6000) in Buffer # 1was added to the tubes (except TCs) before centrifugation at 1,500 x g for 25 min at 4°C (Beckman, J-6M/E, USA). The supernatant was decanted and the pellets were left to dry overnight before the activity of the precipitate was determined on a gamma counter (Packard Cobra-II, Auto Gamma).

Urea, non-esterified fatty acid (NEFA), and albumin were determined from serum at weekly intervals during the 16 weeks of the experiment. Blood was processed the same as explain above. The limits of sensitivity, inter- and intra-assay coefficients of variation are also shown in Table 2-5. Assay procedures were developed by the Masterate candidate at the Institute of Veterinary, Animal and Biomedical Sciences of Massey University with the kindy support of Sandra Forsyth.

NEFA

The NEFA's were assayed using a commercial kit manufactured by Wako Pure Chemicals Ltd, Osaka, Japan. The method relies on the acylation of coenzyme A by the fatty acids in the presence of CoA synthetase. The acyl-CoA is latter oxidised by acyl-CoA oxidase with the concomitant production of hydrogen peroxide. Peroxidase catalyses the condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline and 4-antiaminopyrine in the presence of hydrogen peroxide. A purple adduct is produced and there is a stoichiometric relationship between the amount of hydrogen peroxide produced and the NEFA concentration. The absorbance is measured at 550 nm. The kit was used in accordance with the manufacturer's recommendations. The assays were performed on a Cobas Fara II analyser manufactured by Hoffmann la Roche, Basel Switzerland. The NEFA's assays were developed by Dr Phil Pearce at the Institute of Food, Nutrition and Animal Health of Massey University.

Determinations of urea and albumin were done by colorimetric test using the equipment of Roche/Hitachi 914 ™.

Table 3-4 Limit of sensitivity, inter- and intra-assay coefficient of variation

Assay	Sensitivity	Intra-assay CV	Inter-assay CV
Progesterone	0.3 ng/ml	14.3%	13.4%
IGF1	0.04 ng/ml	4.6%	6.0%
Insulin	0.78 µu/ml	4.8%	7.2%
GH	0.86 µu/ml	6.0%	8.2%
Urea	5 mg/dl	0.8 %	3.4%
Albumin	0.2 g/dl	0.39%	1.71%
NEFA	0.025 mmol/l	3.5%	4.7%

3.7 REPRODUCTIVE ASSESSMENTS

3.7.1 Ovarian ultrasonography

Ovarian activity was assessed by transrectal ultrasound examination. Cows were drafted after morning milking and scanned in a herring bone pen. Scanning was undertaken in two groups of ten cows, to reduce the period that cows were kept out of the paddocks to less than 1.5 hours. The equipment used was a 100 Falco Vet (Pie Medical Equipment Bv, Netherlands) with a 7.5 MHz linear array transducer. The scanning periods were as follow:

The first scanning period was from the 20th August until the 21st September 2003, during which each cow was scanned every other day.

The second scanning period occurred from the 22nd of October, the start of mating, until the day of artificial insemination for each cow, and again each cow was scanned every other day.

The third scanning period started on the sixth day after artificial insemination of each cow, and was performed every fourth day until 35 days post artificial insemination of the last cow to be mated.

Images from the scanner were recorded and measured. Only follicles larger than 5 mm were recorded and plotted in relation to days after calving. Corpora luteum were also recorded.

3.7.2 Follicular dynamics

The dominant follicle was defined as the largest follicle present in the ovary at each particular scanning. Ovulation was defined as the disappearance of a large follicle followed by the presence of a corpus luteum, combined with milk P4 concentrations higher than 1 ng/ml (Reksen *et al.*, 2002)

3.7.3 Reproductive performance

A cow was considered to be in oestrus when she was observed to remain immobile while being mounted by herd mates (Harrison *et al.*, 1990). Tail paint was also applied by the middle of October to facilitate oestrus detection (Macmillan, 1998). If a cow had lost the tail paint it was closely observed for oestrus behaviour and was submitted to the technician for artificial insemination. The records of oestrus observations, milk P4, and follicular dynamics were reviewed and used for calculation of days from calving to first observed oestrus, calving to first ovulation, calving to first milk P4 >1ng/ml, days to conception, and services per conception.

3.8 ENERGY STATUS

3.8.1 Theoretical CP and ME requirements and estimated ME intake

The theoretical energy requirements were calculated for each group by averaging the cows energy requirements for lactation, liveweight, and maintenance, according to the equations given by (AFRC, 1993) and modified by Brookes, personal communication (2004) (Appendix, section 7.1.1). These were then subtracted from the mean estimated ME intake of each group (section 3.5.1).

The theoretical crude protein requirements were calculated for each group by averaging the cows protein requirements for lactation, liveweight, and maintenance, according to the equations given by AFRC, (1993) and modified by Brookes, personal communication (2004).

3.8.2 Calculated energy balance

This was calculated with the equations given by Ferris *et al.* (2002). ME intake (MJ ME/cow/day) was estimated as explained in section 3.5.1. The mean energy requirement for milk production (MJ/day) was determined from milk yield and milk composition data with milk energy concentrations being calculated using the equation of AFRC, (1993) and modified by Brookes, personal communication (2004). Similarly, the ME required for maintenance was calculated using the equation of AFRC, (1993) and modified by Brookes, personal communication (2004). Mean energy balance was calculated by subtracting the ME required for maintenance and milk production (energy for liveweight change was not included).

The values for liveweight, condition score (Yung *et al.*, 1996a; Ferris *et al.*, 2002), NEFA's (Jorritsma *et al.*, 2003), and IGF1 (Zulu *et al.*, 2002) were also used in assessment of the cows' energy balance.

3.9 COST OF METABOLIZING CP

Formulae given by AFRC (1993) were used to estimate, from the total amount of protein ingested, how much protein was catabolised and excreted in urine as urea by both groups. The amount of protein excreted in urine as urea was divided by 6.25 (AFRC 1993) to be transformed to nitrogen and multiplied by 5.45 (Blaxter, 1962) to obtain the cost (MJ ME) of excreting one gram of N as urea. The difference between treatments in the energy used to excrete urea was assumed to be utilized for excretion of the extra CP consumed by the high CP group.

3.10 STATISTICAL ANALYSIS

3.10.1 Pasture nutrient composition

Least-squares means and their standard errors for concentrations of ME (MJ/kg DM), CP (%), SSS (%), CP: SSS ratio, NDF (%), ADF (%), and OMD (%) were calculated for each treatment group using PROC MIXED of SAS (2001). The model included the fixed effect of treatment group (high CP and low CP) and the random effect of day.

3.10.2 Pasture management and estimated nutrient intake

The mean values for DMI, MEI, CPI, OMD, pregrazing herbage mass, postgrazing herbage mass, and pasture allowance were estimated for each treatment group using PROC MIXED of SAS (2001). The model included the fixed effects of treatment group, week postcalving, and the interaction of treatment group by week postcalving. Least-squares means and their standard errors were obtained for each group from Week 2 to Week 16 postcalving, considering repeated measurements in each cow through the experimental period.

3.10.3 Herd test, liveweight, and condition score

Least-squares means and their standard errors for yields of milk, milksolids, milk protein, and milk fat, were calculated for each treatment group in each week postcalving using PROC MIXED of SAS (2001). The model included the fixed effects of treatment group, week postcalving, interaction of treatment group by week postcalving; the effect of initial milk yield, production worth were included as covariables. Repeated measures for each cow within each treatment group were considered from Week 2 to 16.

3.10.4 IGF1, insulin and GH

Least-squares means and their standard errors for concentrations of IGF1, insulin, and GH were calculated for each treatment group using PROC MIXED of SAS (2001). The model included the fixed effects of treatment group, week postcalving, interaction of treatment group by week postcalving, and the effects of production worth as a covariable. Repeated measures for each cow within each treatment group were considered from Weeks 2, 3, 4, 6, 8 and 12.

3.10.5 Differences of theoretical ME requirements and observed MI intake between treatment groups

Least-squares means and their standard errors of the theoretical ME requirements and the observed ME intakes were obtained for each treatment group using PROC MIXED (2001) of SAS. The model included the fixed effect of treatment group (high CP and low CP) and the random effect of week postcalving.

3.10.6 Reproductive parameters

Least-squares means and their standard errors for the reproductive parameters evaluated were calculated for each treatment group using PROC MIXED of SAS (2001). The model included the fixed effect of treatment group and the effect of production worth as a covariable.

4 RESULTS

4.1 EFFECT OF NITROGEN APPLICATION ON PASTURE GROWTH AND NUTRIENT COMPOSITION

The experiment was carried out between 18th August and 27th November; during this period the application of nitrogen, as urea fertilizer, on the high CP paddocks resulted in a higher daily pasture growth compared to the low CP paddocks which did not receiv fertilizer (Table 4-1). Across the experiment the high CP paddocks produced 20% more dry matter than the low CP paddocks. The extra 1,264 kg/ha of dry matter resulted from the application of 130 kg N/ha. The pasture growth response to nitrogen was of 9.7 kg DM/kg N applied.

Table 4-1 Effect of N application on pasture growth

	High CP treatment	Low CP treatment
Average daily pasture growth (kg DM/ha)	61.8	49.2 ***
Pasture grown during the experiment (kg DM/ha)	6237	4972 ***
kg N applied/ha (from 24th July to 1st October)	130.3	-
Extra pasture grown from N (kg DM/ha)	1264	-
Pasture response to N (kg DM/kg N)	9.7	-

***P<0.001

The effects of nitrogen fertilizer on pasture nutrient characteristics are presented in the Table 4-2. ME was increased from 11.4 to 11.8 MJ ME/kg DM (P= 0.001) in the low and high CP paddocks respectively as a result of the nitrogen fertilizer. The percentages of soluble sugars were not different between the high and low CP paddocks. The increase in pasture production accounted for an extra 14,915 MJ ME/ha produced over the 101 days of the experimental period.

As a result of the N fertilizer application, pasture CP was higher on the high than on the low CP paddocks (25.4 vs. 21.6%; P= 0.01). The extra pasture growth accounted for 321 kg/ha of extra CP produced over the 101 days of the experimental period.

Nitrogen fertilizer reduced the fibre content on the high CP paddocks compared to the low CP paddocks. The ADF was reduced from 22.3 to 21.4% (P= 0.05) and the NDF was reduced from 41.3 to 40.3 % (P=0.01) for the high and low CP paddocks respectively. Organic dry matter digestibility was higher in high CP compared to the low CP paddocks probably as a result of lower fibre percentages (83.4 vs. 81.2%; P=0.01 respectively).

Table 4-2 Average pasture nutrient composition for the high and low Crude Protein paddocks and extra nutrient production from fertilizer

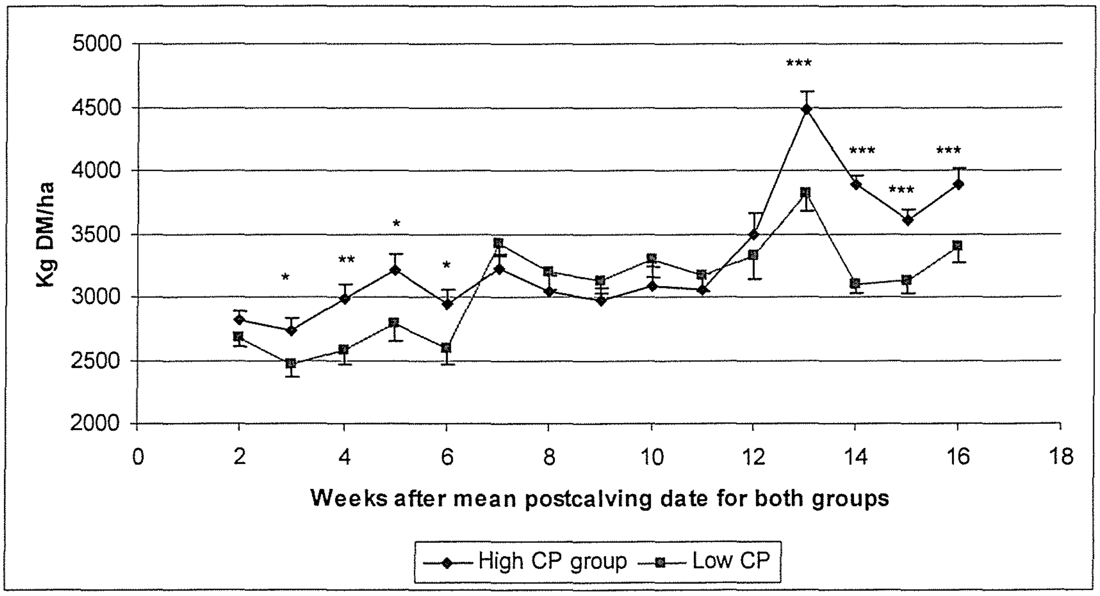
	High CP paddocks ±SE	Low CP paddocks ±SE
ME (MJ/kg DM)	11.8 ±0.03	11.4 ±0.03 ***
CP (% DM)	25.4 ±0.2	21.6 ±0.2 ***
SSS (% DM)	11.2 ±0.2	11.2 ±0.2 NS
CP to SSS ratio	2.4 ±0.7	2.0 ±0.7 ***
NDF (%)	40.2 ±0.2	41.2 ±0.2 **
ADF (%)	21.4 ±0.2	22.3 ±0.2 *
OMD (%)	83.4 ±0.2	81.2 ±0.2 ***
Extra CP from fertilizer (kg CP/ha)	321	-
Extra ME from fertilizer (MJ ME/ha)	14915	-

NS not significant, * P< 0.05, ** P< 0.01, *** P< 0.001

4.2 HERBAGE MASSES, PRE AND POSTGRAZING, AND APPARENT DRY MATER AND NUTRIENT INTAKES BY COWS IN THE TWO TREATMENTS

The average pregrazing herbage mass during the experiment was higher (P=0.001) for the high CP paddocks than for the low CP paddocks (3296 ±30 kg DM/ha and 3068 ±30 kg DM/ha, respectively). During the first 12 weeks of the experiment the pregrazing mass was not statistically different between the high group and the low CP group (3052 ±37 and 2964 ±37 kg DM/ha respectively, P=0.08) (Figure 4-1). After Week 12 the pregrazing mass increased in both groups, remaining significantly higher for the high than for the lower CP group for the last 4 weeks of the trial.

Figure 4-1 Pregrazing herbage mass of the two treatment groups during the experiment (Mean ± SE)

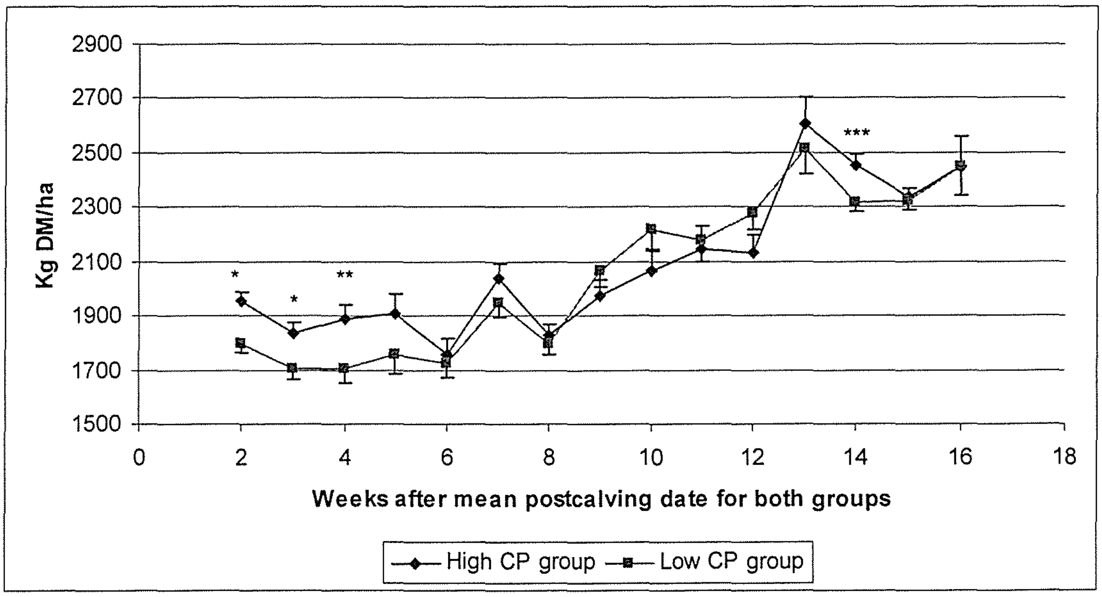


* P< 0.05, ** P< 0.01, *** P< 0.001

The average postgrazing herbage mass during the experiment was not found to be statistically different between the high and low CP groups (2091 ±17 kg DM/ha and 2050 ±17 kg DM/ha, respectively; P=0.08). Postgrazing masses were only significantly (P=0.05) different only during weeks 2, 3, 4 and 14 (Figure 4-2). Postgrazing residuals increased during the last half of the experiment for both treatments.

The average pasture allowance per cow during the 101 days of the experiment was not different between the high and the low CP groups 46.0 ±0.5 and 46.5 ±0.5 kg DM/cow/day; P=0.3). However, the pasture allowance for high CP group was significantly lower than the pasture allowance for the low CP group during Weeks 6 and 14 (40 and 45 kg DM/cow/day respectively). On the other hand, the pasture allowance of the low CP group was significantly lower during the 9th Week (46 kg DM/cow/day) compared to the high CP group (Figure 4-3).

Figure 4-2 Postgrazing residual herbage mass of the two treatment groups during the experiment (Mean ± SE)

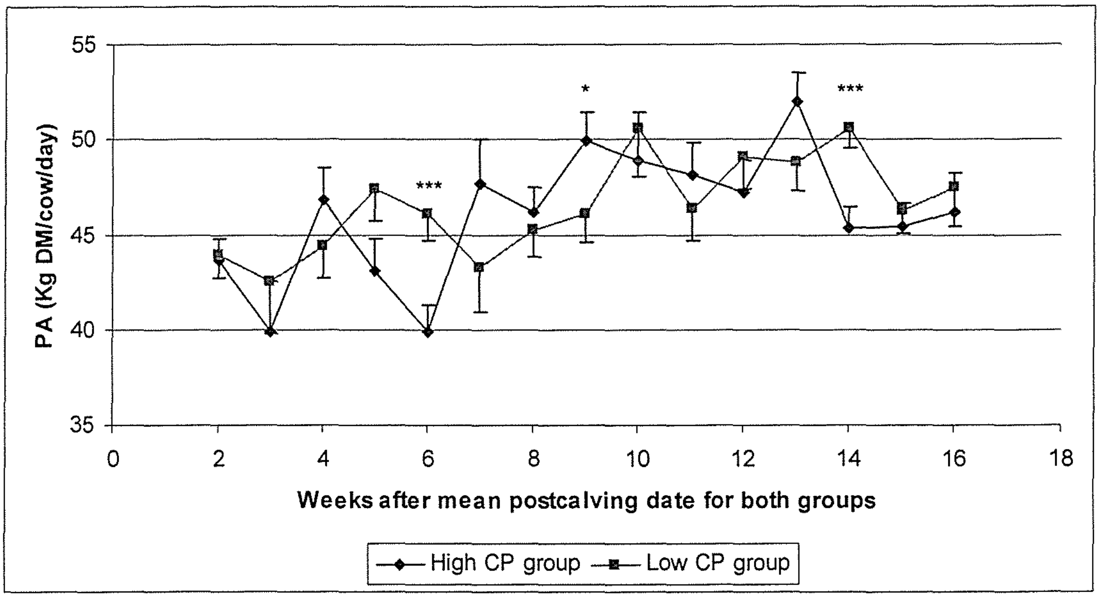


* P< 0.05, ** P< 0.01, *** P< 0.001

The average estimated DMI/cow was statistically (P=0.003) different between the high and the low CP groups (16.7±0.3 and 15.0 ±0.3 kg DM/cow/day, respectively). The high and the low CP groups started the experiment with similar DMIs (13.6 ± 1.0 and 14.5 ± 1.0 kg DM/cow/day, respectively; Figure 4-4). Both groups had three peaks in DMI which occurred at the same time (5th, 8th and 13th weeks) but at different extents for the high and low CP groups (17.2 vs. 17.8; 18.2 vs.19.7; and 21.5 vs. 16.3 DM/cow/day respectively). The DMI was not different (P=0.5) between groups during the first 12 weeks of the experiment. From Week 13 of the experiment, DMI was significantly (P= 0.001) higher for the high CP group than for the low CP group. This difference was present until the end of the experiment.

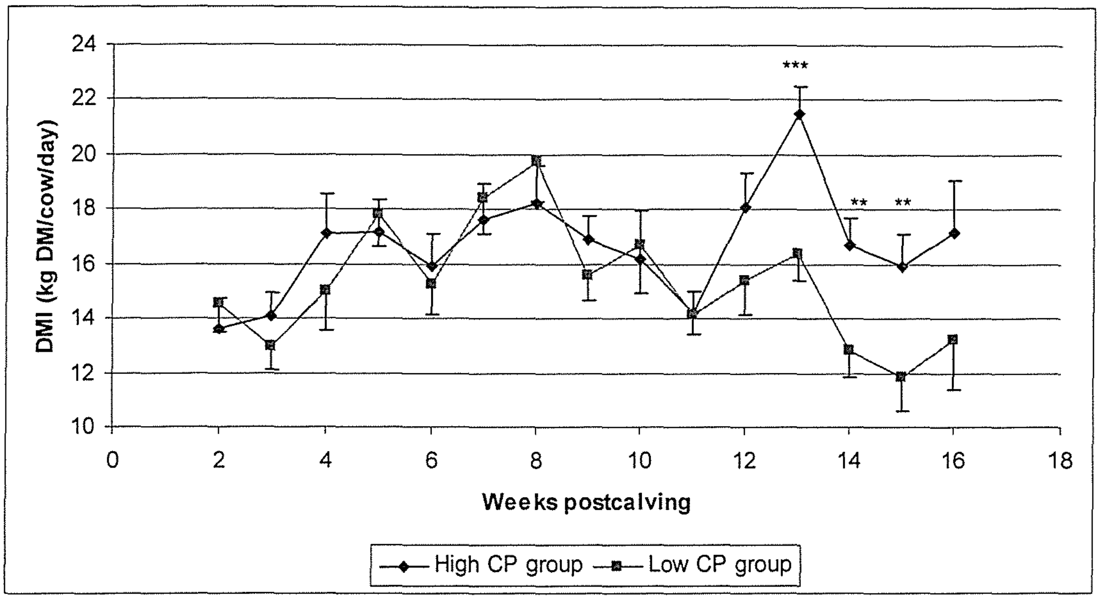
The average estimated MEI/cow/day was statistically higher for the high CP group than for the low CP group (197 and 175 MJ ME/cow/day respectively; P= 0.0001). The trend patterns of ME intakes of both groups (Figure 4-5) were similar to that described for DMI. The high and low CP groups started the experiment at similar ME intakes (162 ± 12 and 170 ± 12 MJ ME/cow/day respectively). The ME intake was not significantly (P=0.07) different between treatment groups during the first 12 weeks of the trial. This difference became significant since Week 13th.

Figure 4-3 Pasture allowances (PA) of the two treatment groups during the experiment (Mean ±SE)



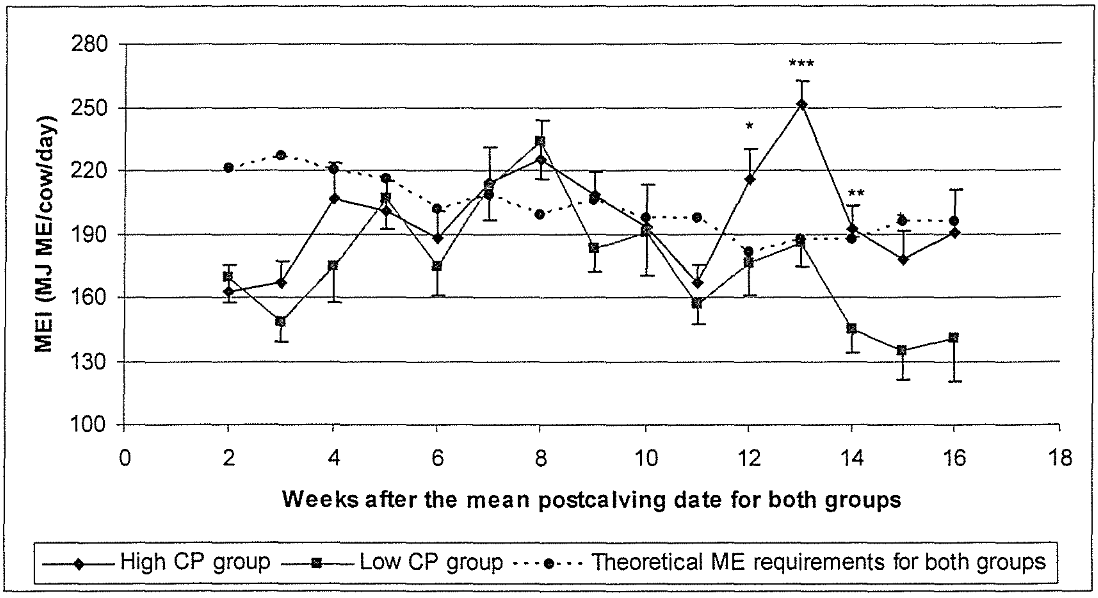
* P< 0.05, ** P< 0.01, *** P< 0.001

Figure 4-4 Estimated weekly dry matter intake (DMI) of the two treatment groups during the experiment (Mean ±SE)



* P< 0.05, ** P< 0.01, *** P< 0.001

Figure 4-5 Estimated weekly metabolizable energy intakes (MEI) of the two treatment groups during the experiment (Mean ±SE). Dotted line depicts the theoretical ME requirements AFRC (1993) and modified by Brookes, personal communication (2004).

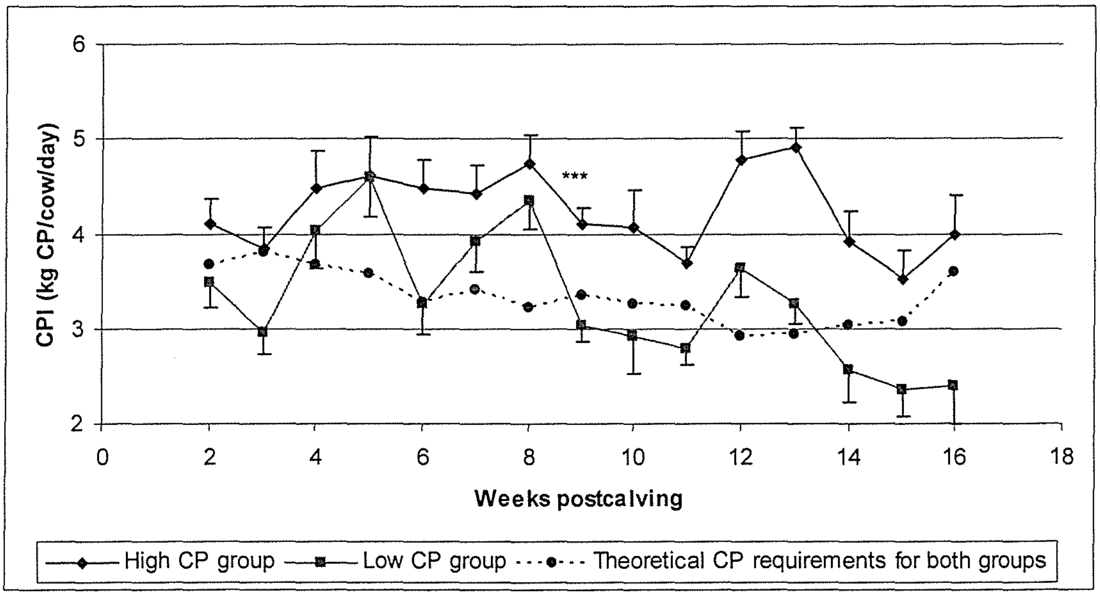


Intakes were calculated from DM intake and concentration of nutrients in the diet, ME and CP. * P< 0.05, ** P< 0.01, *** P< 0.001

Average of the estimated daily intakes of CP during the experiment were 4.2 ± 0.07 kg CP/cow/day and 3.3 ± 0.07 kg CP/cow/day for the high and low CP groups respectively, this difference was highly significant (P= 0.001). The CP intake of the high CP group was significantly higher during the whole experiment compared to the CP intake of the low CP group (Figure 4-6). The only exception to this difference occurred during the 5th Week when the low CP group had the same CPI as the high CP group (4.6kg CP/cow/day).

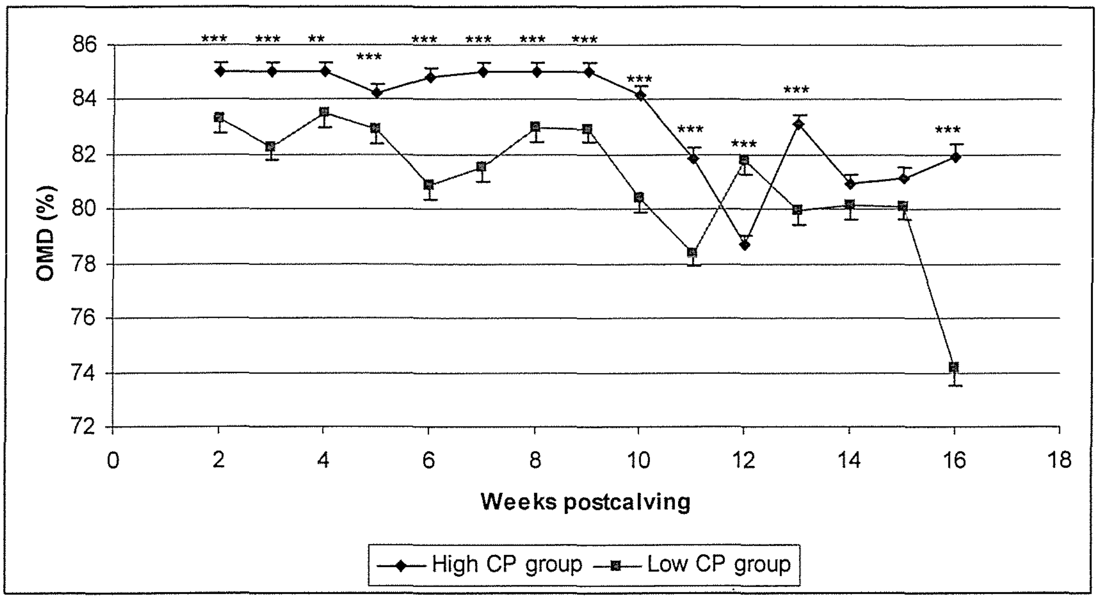
The OMD during the experiment was statistically higher for the high CP group than for the low CP group (83.3% and 81.0% respectively; P= 0.0001; Figure 4-7). Even though the OMD tended to decrease at an earlier date in the low CP group, both groups had a simultaneous decrease in pasture digestibility on the 10th Week.

Figure 4-6 Weekly average values for crude protein intakes (CPI) of the two treatment groups during the experiment (Mean ±SE). Dotted line depicts the theoretical ME requirements AFRC (1993) and modified by Brookes, personal communication (2004)



Intakes were calculated from DM intake and concentration of nutrients in the diet, ME and CP. * P< 0.05, ** P< 0.01, *** P< 0.001

Figure 4-7 Weekly average values for organic matter digestibility (OMD) of the two treatment groups during the experiment (Mean ±SE)



* P< 0.05, ** P< 0.01, *** P< 0.001

4.3 LIVEWEIGHT AND CONDITION SCORE OF COWS IN THE TWO TREATMENT GROUPS

Even though the average condition score of the high CP group tended to be lower than the average condition score of the low CP group (4.3 vs. 4.7 0 to 10 scale, respectively), the difference was not statistically significant (P= 0.3; Figure 4-8). The interaction between group and week postcalving was also not significant (P= 0.8).

Average liveweight for the high CP group was statistically similar to the low CP group (460 vs. 466 kg respectively; P= 0.4; Figure 4-9). Both groups started the lactating period at similar liveweight. The low CP group reached its liveweight nadir (457 kg) at the 4th Week postcalving and remained low until the 6th Week from when it started to gain liveweight. Conversely, liveweight nadir (443 kg) for the high CP group occurred in the 6th Week postcalving from which liveweight began to increase. Both groups finished the trial (16 weeks after calving) with the same liveweight.

Figure 4-8 Condition scores of the two treatment groups during the experiment (Mean ±SE)

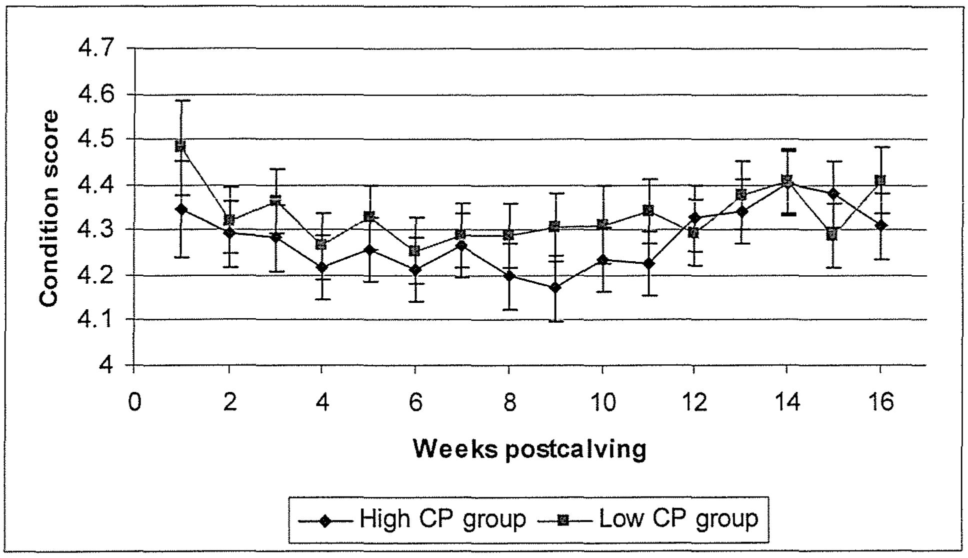
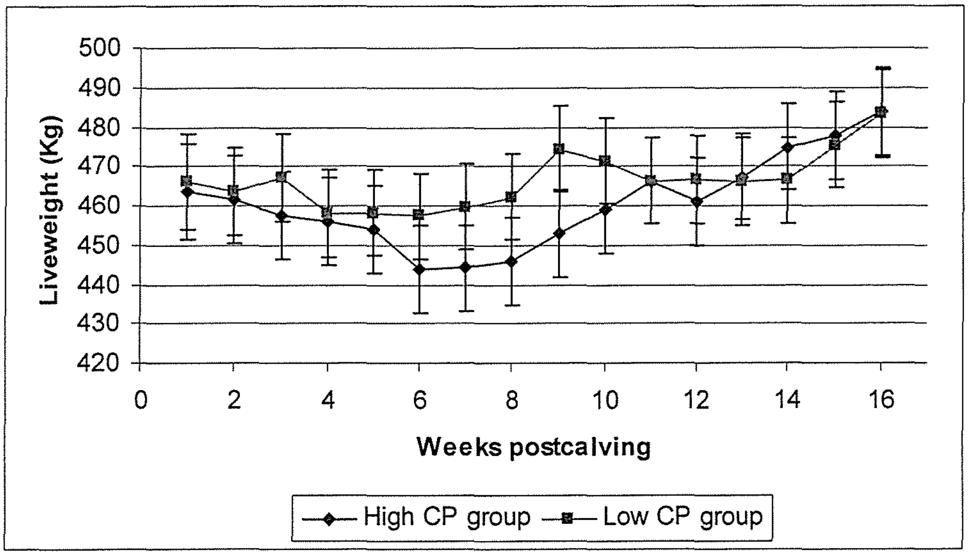
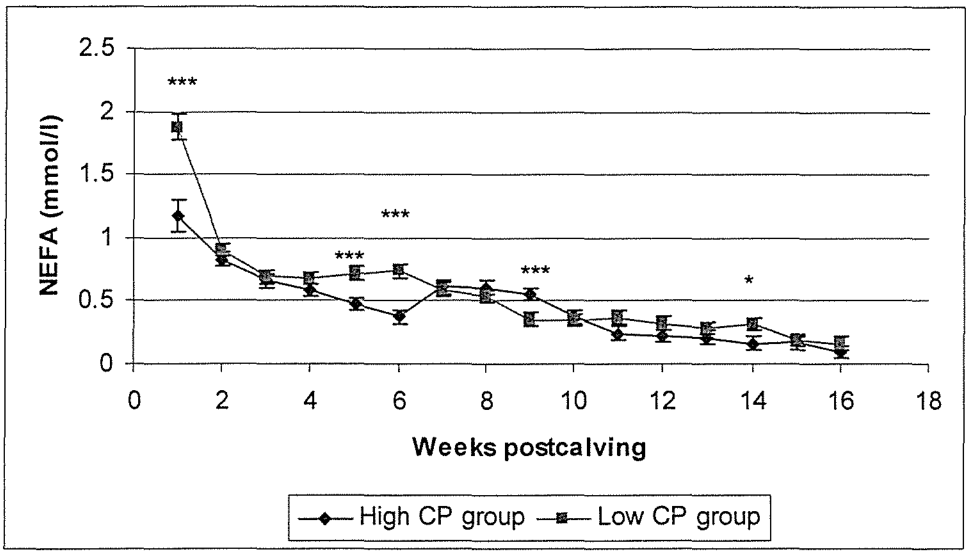


Figure 4-9 Liveweight of the two treatment groups during the experiment (Mean ±SE)



The blood NEFA concentrations were statistically lower for the high CP group compared to the low CP group (0.45 and 0.56 ±0.02 mmol/l, respectively; P=0.009; Figure 4-10). Week postcalving and the interaction between week postcalving and group also had significant (P= 0.0001) effects on concentrations of NEFA. Even though the NEFA concentrations were high for both groups at the beginning of the experiment, the low CP group had higher concentrations, and they also experienced the greater decrease in concentrations towards the second week post calving. The NEFA concentrations were also significantly (P=0.001) higher during Weeks 5, 6, 9 and 14 postcalving in the low group than the high CP group.

Figure 4-10 Blood NEFA concentrations (mmol/l) for both treatment groups during the experiment (Mean ±SE)

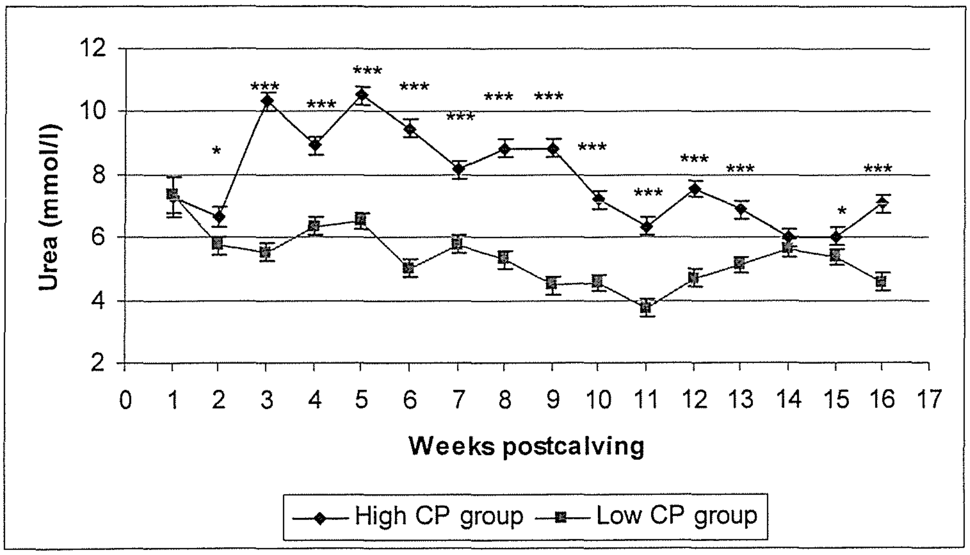


* P< 0.05, ** P< 0.01, *** P< 0.001

4.4 SERUM UREA CONCENTRATIONS IN THE TWO TREATMENT GROUPS

The high CP group had significantly (P= 0.001) higher serum urea concentration than the low CP group (7.8 and 5.3 mmol/l) (Figure 4-11). Week postcalving and the interaction between week postcalving and group had also significant (P= 0.001) effects on serum urea concentrations. Urea concentrations were the same for both groups at the beginning of the trial, but increased significantly (P=0.05) by Week 2 in the high CP group. After this point it started decreasing until Week 14 postcalving, when both groups again had similar urea concentrations.

Figure 4-11 Blood urea concentrations (mmol/l) for both treatment groups during the experiment (Mean ±SE)

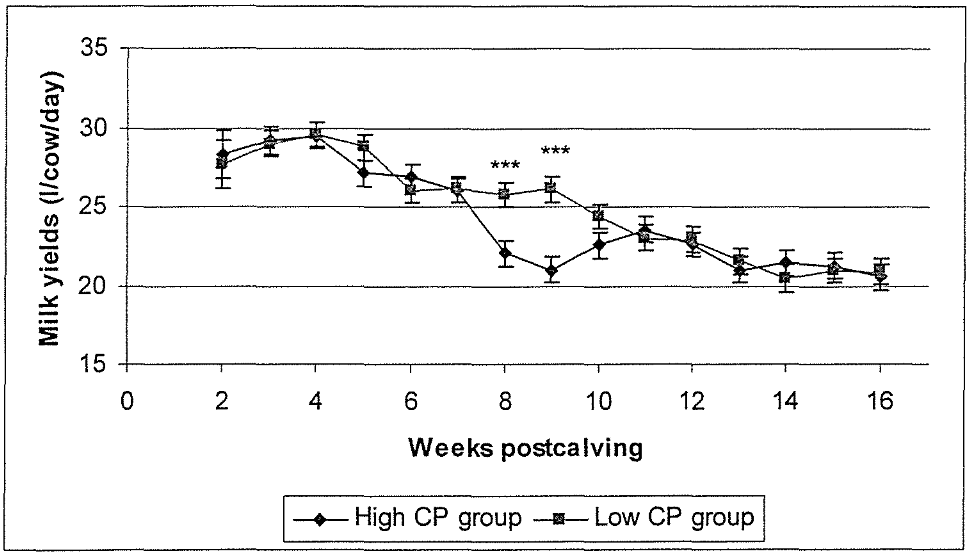


* P< 0.05, ** P< 0.01, *** P< 0.001

4.5 MILK PRODUCTION BY THE TWO TREATMENT GROUPS DURING THE EXPERIMENT

The average milk yield for both groups during the whole experiment was not statistically different between the high and the low CP group (24.1 and 24.8 l/day respectively). However, the interaction between treatment and week was significant (P= 0.0001; Figure 4-12. Both groups started their lactations at the same level of milk yield and reached their peak (29 litres/day) at Week 4 postcalving, after which yields started dropping. The lactation curve of the high CP group decreased more quickly reaching its milk yield nadir at Week 9 postcalving with 21 litres per day from which increased reaching a second peak of 23.5 litres at Week 11 postcalving. After the second peak the milk yield decreased slowly until Week 16 postcalving when both groups had again a similar milk yield. The decrease in milk yield for the low CP group occurred more steadily than in the high CP group. The milk yield nadir of the low CP group did not occur until Week 16 postcalving. There was a significant effect of the week postcalving and the interaction between group and week postcalving was also significant (P= 0.0001).

Figure 4-12 Milk yields (l/cow/day) for the two treatment groups during the experiment (Mean ±SE)

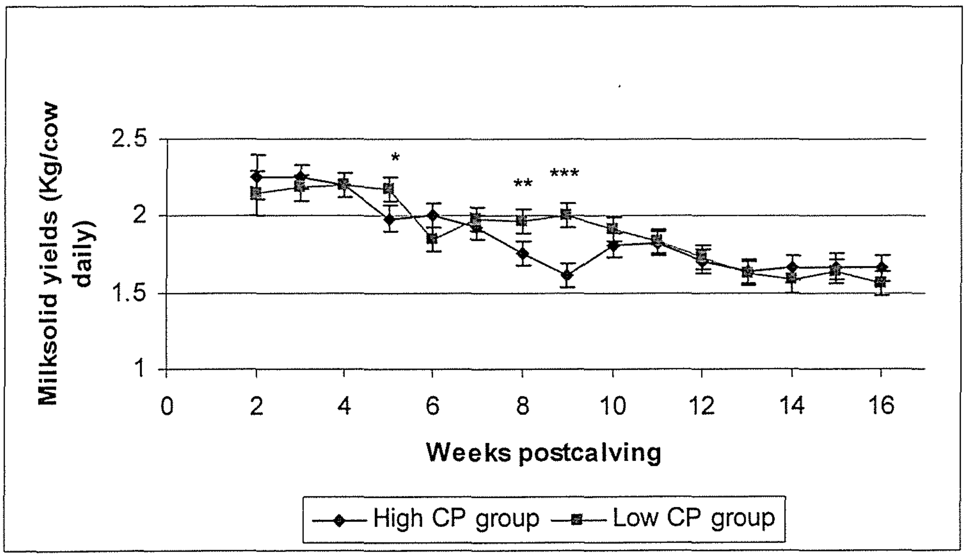


* P< 0.05, ** P< 0.01, *** P< 0.001

There was no significant effect of treatment on milksolids yield (1.8 and 1.9 kg MS/cow/day; P=0.3). However, milksolid yield was significantly (P= 0.0001) affected by week postcalving and by the interaction between week postcalving and group. The peak of milksolids yield of the high CP group lasted only three weeks compared with four weeks for the low CP group (Figure 4-13). The milksolid nadir for the high CP group occurred in the 9th Week postcalving with 1.5 kg MS/cow/day; by this time the milksolid yield of the low CP group was 2.0 kg DM/cow/day. The nadir of milksolids yield for the low CP group did not occur until the 16th Week postcalving with 1.5 kg MS/cow/day.

The difference in protein yield between the two groups was not significant (Figure 4-14). The average yield during the experiment for both groups was 0.80 kg/cow/day. There was a significant effect (Pr 0.0001) of week and the interaction between week and group was also significant. The trend of milk protein yield across the experiment for both groups was similar to the trend described for milksolids yield. The largest difference in protein yield between groups also occurred between the 8th and 9th Week postcalving.

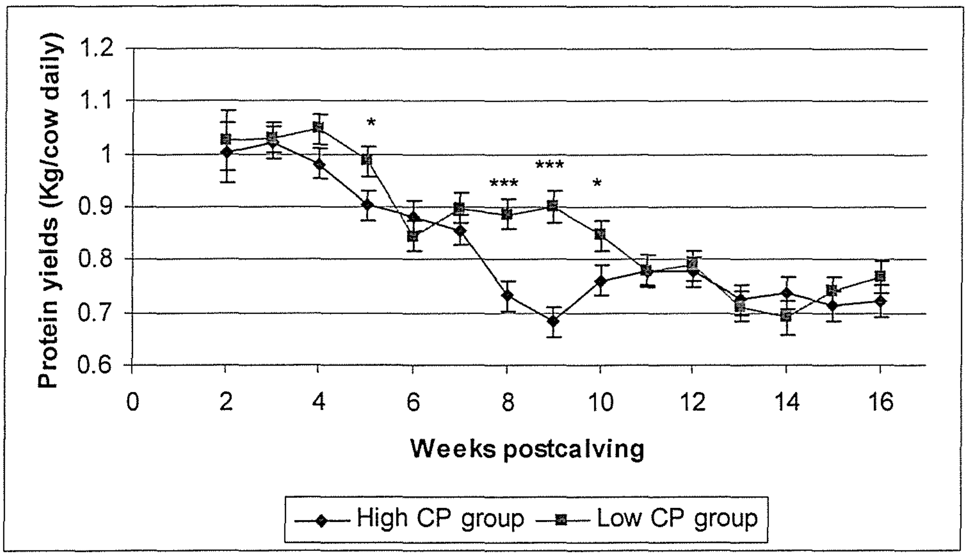
Figure 4-13 Average milksolid yields (kg/cow/day) for the two treatment groups during the experiment (Mean ±SE)



* P< 0.05, ** P< 0.01, *** P< 0.001

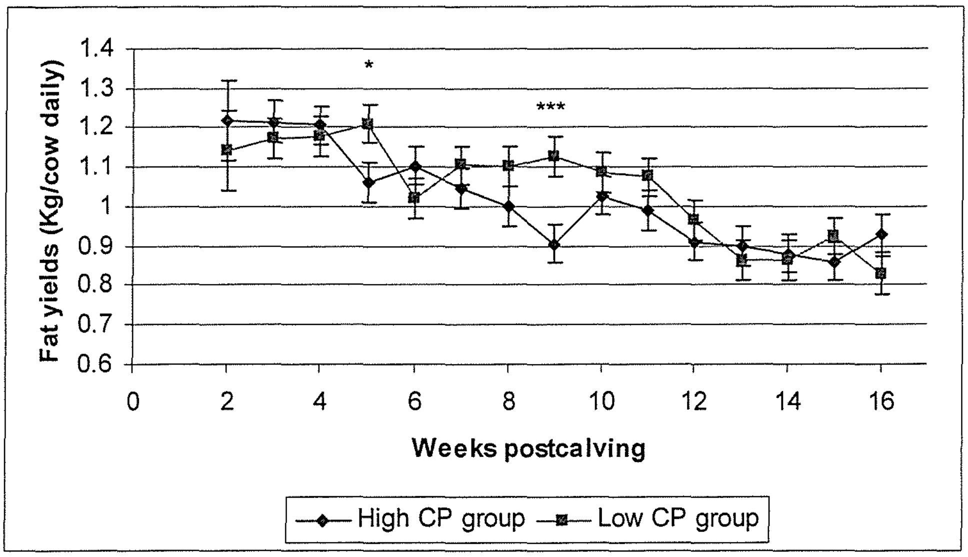
The difference in fat yield between the two groups was not significant (Figure 4-15). The average yield of milk fat during the experiment for both groups was 1.0 kg/cow/day. There was a significant effect (P= 0.01) of week and the interaction between week and group was also significant. The milk fat yield of the high CP group remained at its highest level up to the fourth week postcalving, meanwhile the fat yield for the low CP group kept rising until the 5th Week postcalving. During the 9th Week postcalving the difference in yield between both groups was high due to a sudden decrease in yield followed by a small increase of the high CP treatment group.

Figure 4-14 Average protein yields (kg/cow/day) for the two treatment groups during the experiment (Mean ±SE)



* P< 0.05, ** P< 0.01, *** P< 0.001

Figure 4-15 Average fat yields (kg/cow/day) for the two treatment groups during the experiment (Mean ±SE)

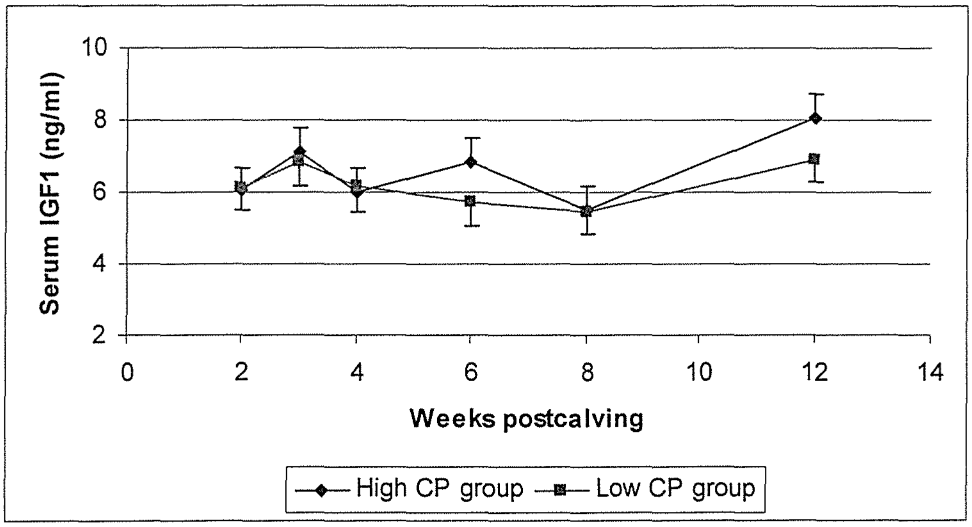


* P< 0.05, ** P< 0.01, *** P< 0.001

4.6 CONCENTRATIONS OF IGF1, INSULIN, AND GH IN COWS ON THE TWO TREATMENT GROUPS

There was no significant effect of treatment on IGF1 concentrations (Figure 4-16). The only significant effect (P= 0.001) on IGF-1 concentrations was due to week postcalving.

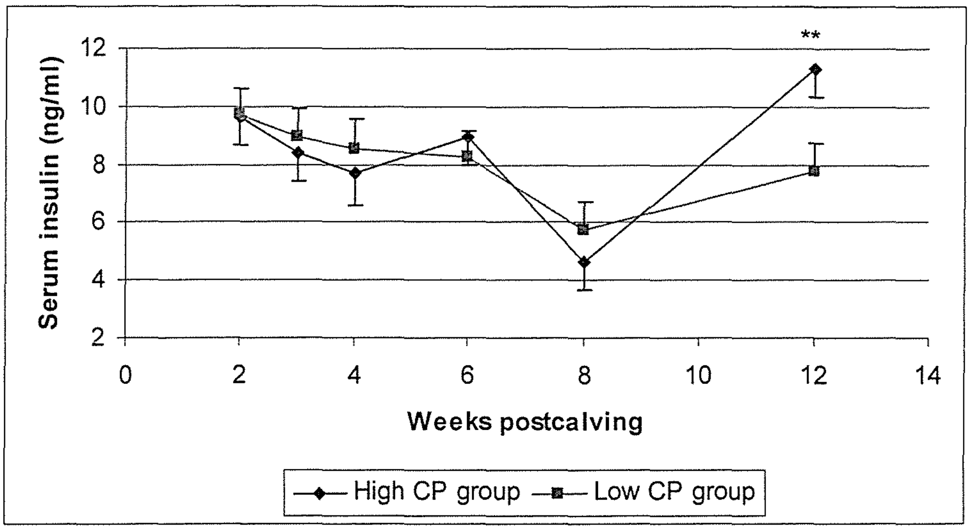
Figure 4-16 Mean (± SE) serum insuline-like growth factor 1 (IGF1) concentration for both treatment groups during the experiment



There were no significant differences in insulin concentrations between the two groups during the first 11 weeks of the experiment (Figure 4-18). However, in Week 12 postcalving the high CP group had a significantly higher (P= 0.01) insulin concentration than the low CP group.

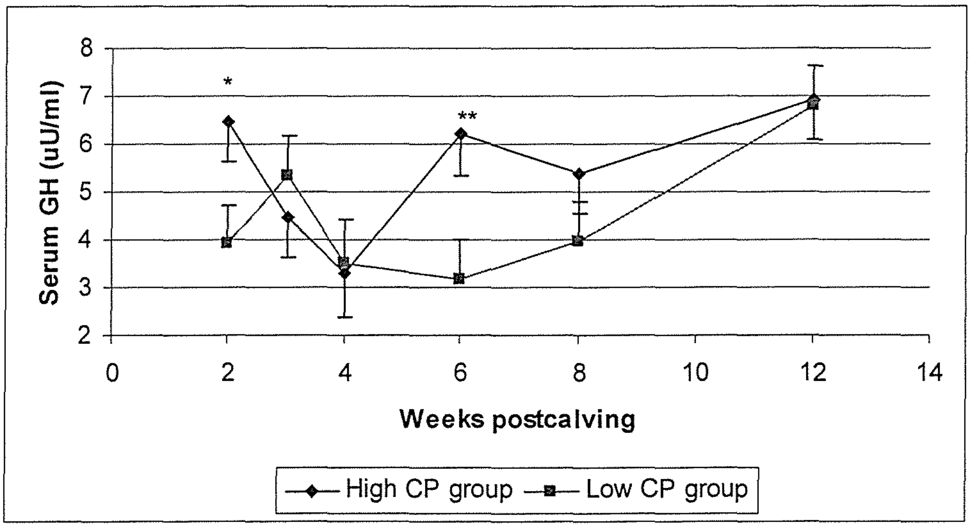
The concentration of GH was a significantly (P=0.03) affected by treatment and by the interaction between week postcalving and group (Figure 4-18). On weeks 2 and 6 postcalving the high CP group had a significantly (P= 0.05 and 0.01 respectively) higher GH concentration than the low CP group.

Figure 4-17 Mean (±SE) serum insulin concentrations for both treatment groups during the experiment



** P< 0.01

Figure 4-18 Mean (±SE) serum growth hormone (GH) concentrations for both treatment groups during the experiment



* P< 0.05, ** P< 0.01, *** P< 0.001

4.7 REPRODUCTIVE PARAMETERS IN THE TWO TREATMENT GROUPS

4.7.1 Days from calving to first oestrus, to first artificial insemination, to conception rate, and services per conception

Treatment did not have any significant effect on the days to first and second oestrus, days to first artificial insemination, days to conception, or services per conception (Table 4-3).

Table 4-3 Reproductive data of the two treatment groups (Mean ±SE)

Issue	High CP group ±SE	Low CP group ±SE	P
Days to first oestrus	53.9 ±4.3	58.9 ±4.3 a	0.483
Days to second oestrus	76.4 ±3.0	76.2 ±3.1 b	0.974
Days to third oestrus	88.0 ±2.7	87.7 ±3.0 b	0.963
Days to fourth oestrus	90 ±4.3	95.7 ±9.3 c	0.638
Days to first artificial insemination	82.6 ±1.7	85.1 ±1.7 d	0.327
Days to second artificial insemination	94.1 ±3.5	93.5 ±4.5 b	0.937
Days to conception	87.2 ±1.9	88.3 ±1.9 c	0.629
Services per conception	1.35 ±0.1	1.19 ±0.1 d	0.332

4.7.2 Luteal phases and progesterone synthesis in the two treatment groups

Treatment group did not significantly affect the evaluated parameters (Table 4-4). Cows from both groups had on average four luteal phases during the experiment. For both groups two out of four luteal phases were short (equal to or shorter than 10 days) (Table 4-4). Even though the accumulated P4 during the experiment for the "normal" luteal phases was not significantly different between treatments, the high CP group tended to have higher values for P4 accumulated across the trial. The mean P4 plateau across the experiment was also not statistically different between treatments. There were also no significant differences between treatment in the mean concentrations of the 1st, 2nd, 3rd, and 4th plateaux (data not shown).

Table 4-4 Effect of two different levels of crude protein (CP) intake on luteal phases and progesterone (P4) synthesis

	High CP group ±SE	Low CP group ±SE	P
Number of short luteal phases (≤ 10 days) per cow during the experiment	2	2	0.984
Number of "normal" luteal phases (> 10 days) per cow during the experiment	2	2	0.912
Accumulated P4 under the curve on "normal" luteal phases (ng/ml)	178.6 ±17.5	155.4 ±17.5 (P> 0.3)	0.328
Mean P4 plateau across the experiment (ng/ml)	2.9 ±0.13	3.1 ±0.14 (P>0.2)	0.204

4.7.3 Follicular dynamics in cows in the two treatment groups

The only significant (P= 0.02) difference between the groups in follicular dynamics was observed in the diameter of the dominant follicle in the period from 70 days postpartum (DPP) to conception; the high protein group had smaller (17 ±0.8 mm) DF's than the low protein group (20 ±0.8 mm). Other variables, including the time between the start of growing until the ovulation of the dominant follicle, the diameter of the dominant follicle, and the time of ovulation were not affected by treatment either during the first 40 DPP or from the period of 70 DPP to conception (Table 4-5).

Table 4-5 Effect of two different levels of crude protein (CP) intake on follicular dynamics in the two treatment groups (Mean ±SE)

	High CP group ±SE	Low CP group ±SE	P
Days from 0.5 mm to ovulation of the DF before 40 DPP	7 ±0.8	7 ±0.9	0.934
Days from 0.5 mm to ovulation of the DF from 40 DPP to conception	6.0 ±0.8	5.8 ±0.7	0.818
Diameter of DF (mm) before 40 DPP	20 ±0.1	19 ±0.1	0.694
Average day of 1st ovulation before 40 DPP	25.8 ±2.5	31.9 ±2.7	0.173
Diameter of DF (mm) from 70 DPP to conception	17 ±0.08	20 ±0.08	0.024
Average day of ovulation from 70 DPP to conception	82.2 ±2.5	80.7 ±2.4	0.683

5 DISCUSSION

5.1 EFFECT OF NITROGEN APPLICATION ON PASTURE GROWTH AND COMPOSITION

High CP intake by cows has been related to low reproductive performance, low milk yield, and increased NEB in dairy cows. To define whether this occurs in NZ pasture-fed cows, nitrogen fertilizer was applied in large quantities to increase the CP content of pasture. Differences in pasture growth and pasture nutrient composition were recorded, and pasture management was adjusted to offer similar pasture allowances to the cows on both types of pasture.

The effect of N fertilizer on pasture production has been widely studied. Fertilized paddocks have been reported to produce about 20% more pasture than non fertilized paddocks (Holmes, 1982; Harris *et al.*, 1994). In the present experiment, high CP paddocks produced 20% more pasture than the non fertilized paddocks, which is in agreement with the reports of the above mentioned authors. The different factors that can affect the pasture response to N fertilizers have been widely researched elsewhere, and reviewed by Ball & Field (1982), for example. Overseas (OS) reports (Stockdale & King, 1980; Gately *et al.*, 1984) cite a range of responses from 3 to 17 kg DM/kg N, whilst local reports (Holmes, 1982; Harris *et al.*, 1994; Moller *et al.*, 1996a) range from 7.4 to 12.5 kg DM/ kg N. In the present trial, a total of 1,264 kg of extra DM/ha resulted from the application of 130 kg N, which was a pasture growth response of 9.7 kg DM/kg N applied.

Nitrogen fertilizer improved the nutritive parameters recorded except for the soluble sugars which remained constant in both treatment groups. As a result of the application of N fertilizer, pasture CP was 15% higher ($P = 0.001$) in the high CP paddocks than in the low CP paddocks (25.4 vs. 21.6% respectively). This increase was similar to that reported by Moller *et al.* (1996a) who also found that CP of fertilized pasture increased by 15% after applying 40 kg N/ha (27% vs. 23% CP on non fertilized paddocks).

Reports of the effect of N fertilizer on the soluble sugar and starch content of pasture are not conclusive. Some (Jacobs *et al.*, 2002; McKenzie *et al.*, 2003) but not all authors (Ross *et al.*, 1978; Jacobs *et al.*, 1998) have reported that the contents of water soluble carbohydrates were negatively correlated (P= 0.001) with total and protein N concentration in pasture. It appears that the nitrogen application affects concentrations of soluble carbohydrates probably through stimulating new pasture growth and the consequent metabolism of carbohydrates (Donaghy & Fulkerson, 2002). In the present study, nitrogen fertilizer did not affect the measured SSS of the pasture, which was 11.2% for both treatments. More research is needed to understand the factors that influence the effects of N fertilizer on pasture sugar levels.

Nitrogen fertilizer reduced the fibre concentration of pasture. The ADF was reduced from 22.3 to 21.4% (P=0.05). This reduction (4%) was much less than the 11% reported by Moller *et al.* (1996a), who may have achieved a larger decrease in ADF content because the ADF in the non fertilized pastures was relatively higher than the value for the low CP paddocks of the present experiment (26.3 % vs. 22.3%, respectively), which could have allowed a smaller margin for improvement. The NDF was also reduced from 41.3 to 40.3% (P=0.01) in the present experiment, which represents a small reduction (1%) than the 7% reported by Moller *et al.* (1996a). Nitrogen fertilizer has also been observed to reduce the ADF and NDF percentages in annual ryegrass (Zhang *et al.*, 1995).

Digestibility of organic mater was increased by 3.5% (from 83.4 to 81.2%; P=0.001) in high CP paddocks, as a result of the lower fibre concentration. Similar results were reported by Zhang *et al.* (1995) who found that the use of urea increased the dry matter digestibility by 3.2% compared with non-nitrogen paddocks (82.1 *vs.* 84.9% respectively).

The effect of N fertilizer on ME content is not consistent. Some (McKenzie *et al.*, 2003) but not all (Jacobs *et al.*, 2002) authors have found a negative relationship between ME content in pasture and N fertilizer applications. A possible reason for such inconsistency is the fact that ME was calculated based on the OMD, and digestibility is negatively related to fibre content; i.e. the reports that show an increase in ME content, as a result of N fertilizer, also show a reduction in NDF. In the current experiment, the high CP paddocks showed a reduction in the ADF and NDF contents, which led to higher ME content in the high CP paddocks compared to the low CP paddocks (11.8 vs. 11.4 MJ ME/kg DM respectively).

5.2 REPRODUCTIVE PARAMETERS

5.2.1 Progesterone concentrations

Reduction of circulating P4 concentrations has been related to high CP intakes (Butler, 1998; Butler, 2001) and RDP intakes (Staples *et al.*, 1993). Exacerbation of NEB from ammonia and urea metabolism has been proposed as the main mechanism responsible for the reduction in blood P4 concentration (Yung *et al.*, 1996a; Walters *et al.*, 2002).

In a review of experiments, Butler (1998) found that high dietary CP intakes statistically reduced the plasma P4 concentrations in 3 out of 4 studies that used lactating dairy cows; on the other hand no statistical effect was recorded when heifers or non-lactating cows were used. Reduction of P4 blood concentrations have also been reported by other authors (Sonderman & Larson, 1989; Westwood *et al.*, 1998). According to Westwood *et al.* (1998), it is possible that the lower NEB of cows fed with high CP diets could reduce the availability of precursors for P4 synthesis and increase the metabolic clearance of P4. In contrast to the previously mentioned studies, the high CP group in the present experiment had P4 blood concentrations similar to the low CP group (P=0.2). Hence, it seems in the present experiment, that the amount of energy used to metabolize the extra urea of the high CP group was not large enough to increase the NEB and to statistically reduce the blood P4 concentrations.

High DMI has also been found to reduce the P4 blood concentration by increasing blood flow and passage rate of P4 through the liver, which is the primary site of P4 catabolism (Parr *et al.*, 1993). In the present experiment, the difference in DMI (1.7 kg DM) between groups was not big enough to cause a decrease in P4 concentrations. Some authors (Rabiee *et al.*, 2002) have found that a difference of 1.9 kg DM of DMI did not cause any difference in P4 plasma concentration; on the other hand, a significant (P=0.001) difference in P4 plasma concentrations were observed when the difference in DMI between the groups was 6.5 kg DM/cow/day.

In summary, the results of the present experiment suggest that the extra energy required to metabolize and excrete the urea from the high CP group (Section 5.6.2) was not large enough to increase the NEB and to reduce the blood concentrations of P4.

5.2.2 Postpartum restoration of ovulatory activity

The effect of CP intake on the restoration of follicular activity and the display of oestrus behaviour in the postpartum period has been studied by many authors (Kaim *et al.*, 1983; Carroll *et al.*, 1988; Staples *et al.*, 1993; Barton *et al.*, 1996; Westwood *et al.*, 1998); however, the results are not conclusive and the effects of CP intake on reproductive performance seems to be more closely related to the energy balance of the cow and to the occurrence of reproductive disorders (i.e. metritis) (Barton *et al.*, 1996) than to the amount of CP ingested per se.

As discussed in Section 2.1.4, reproductive physiology is closely related to the energy balance of the cow (Harrison *et al.*, 1990; Beam & Butler, 1998; Lucy, 2000; Lucy, 2001; Jorritsma *et al.*, 2003; Reist *et al.*, 2003a); therefore, one of the hypotheses of the present experiment was that the restarting of ovulatory activity would be delayed by the increase in NEB resulting from metabolism and excretion of the extra CP ingested.

However, the present experiment did not show any significant effect of CP intake on most of the reproductive parameters evaluated, despite the significant (P=0.0001) difference in CP intake between groups.

5.3 LIVEWEIGHT, CONDITION SCORE, NEFA AND IGF1

Liveweight, condition score, NEFA blood concentrations, and IGF1 blood concentrations were analyzed to assess energy balance. Assessment of energy balance was a major issue in the present experiment since one of the major hypotheses was that high CP intakes exert its negative effect on cow performance throughout reducing the energy balance.

Body condition score is a non-invasive, quick, and inexpensive method to estimate body reserves and energy balance (Suriyasathaporn *et al.*, 1998). Positive relationships between liveweight and energy balance have also been described (Yung *et al.*, 1996a; Ferris *et al.*, 2002). Results of the present experiment show that average condition score and cow liveweight were not significantly (P=0.8) different between treatments. In agreement with the results of the present experiment, it has been reported by several authors that there are no negative effects of high CP intake on condition score or liveweight change (Robinson *et al.*, 1991; Mackle *et al.*, 1995; McCormick *et al.*, 1999).

Energy balance is also reflected by the concentration of NEFAs in blood. NEFAs result from the hydrolysis of triglycerides (Veerkamp *et al.*, 2003), which typically occurs during NEB (Ferris *et al.*, 2002; Walters *et al.*, 2002). Blood NEFA concentrations have proved to be more sensitive for detecting the energy balance of the cow than other methods such as change in liveweight, condition score, and calculated energy balance (Jorritsma *et al.*, 2003). Similarly, Ferris *et al.* (2002) found that NEFA levels did identify animals with lower energy balance more effectively than use of information on condition score or liveweight. During the present experiment, the high CP group had significantly (P=0.009) lower blood NEFA concentrations compared to the low CP group. However, during the four weeks (1, 5, 6 and 14) when the NEFA were higher for the low CP group, only during Week 1 was NEFA concentration negatively correlated to condition score (P=0.003) and liveweight (P=0.005). The high NEFA blood concentration during the first week in the low CP group could have been due to low pasture quality. During this week the pasture had a brown-yellowish colour, possibly the lack of fertilizer affected the microorganism population in the soil and caused deficiency of nitrogen in the pastures. A higher NEFA blood concentration in the low CP group was unexpected and perhaps influenced by the lower but statistically non significant DMI and ME intake, as detailed in section 4.2, and with the higher but statistically not significant milksolids yield. No other significant correlations were found between NEFA concentrations and liveweight or liveweight change for the low CP group. Correlations were not calculated between NEFA and ME intake and DMI since, the values for intakes of individual animals were not available.

IGF1 has been found to be positively related to the energy status in dairy cows (Zulu *et al.*, 2002). However, little information exists in the literature on the relationship between the CP intake and IGF1. It was expected that the consumption of CP in amounts well above the cow requirements would increased the NEB and depressed the blood concentrations of IGF1. However, the IGF1 concentrations did not differ between treatment groups, although there was a tendency (P=0.1) for the low CP group, which had the higher NEFA blood concentrations, to have lower blood IGF1 concentrations than the high CP group (6.1 and 6.5 ng/ml, respectively). In agreement with the present experiment, Armstrong *et al.* (2001) did not find a significant effects of CP on blood IGF1 concentration; similar results were reported by Park *et al.* (2002). However, the results of Park *et al.* (2002) may not be a good benchmark for the present study since that study was done using dry cows, and factors that can interact with CP intakes in lactation were eliminated. Moreover the values of IGF1 blood concentrations presented in the current experiment must be interpreted with caution since they are much lower than other values reported from other NZ (Fahey *et al.*, 2003) and OS (Spicer *et al.*, 1990; Yung *et al.*, 1996a) reports.

The fact that condition score, liveweight, and IGF1 were not different between groups indicates that the effect of CP intake on energy balance of both groups possibly was not large enough at least to significantly affect the parameters studied in the present experiment. The difference in NEFA blood concentration between groups maybe was due to the higher sensitivity of the method to detect animals in NEB.

5.4 MILK PRODUCTION AND MILK COMPOSITION FOR THE COWS ON THE HIGH AND LOW CP TREATMENTS GROUPS

The effect of high CP diet on milk yield has been studied previously (Mackle *et al.*, 1996; Delaby *et al.*, 1998; McCormick *et al.*, 1999). It has been stated that intake of CP in amounts above the cow's requirements can lead to reduction in energy balance, which could possibly result in a reduction in the energy available for milk production (NRC, 1988; Twigge & Gils, 1988). High CP intake, with possible negative effects on milk yield, can result from application of large amounts of N-fertilizer to the pasture (Ross *et al.*, 1978) or imbalances of RDP and rumen undegradable (RUP) protein in the diet (NRC, 1988; Twigge & Gils, 1988; McCormick *et al.*, 1999).

Two main experimental designs have been carried out to test the effect of N fertilizer on cow production. Those in which the fertilizer is used to increase pasture accumulation or quality and those which, offering similar pasture allowances, assess the effect of different levels of N fertilizer applications directly on cow performance. Nitrogen fertilizer has been used successfully to increase milk yield per cow and per hectare when used to boost pasture availability at times when high DMI requirements of animals are not fully met by herbage accumulation rates during early spring (Garcia & Holmes, 1999) or early autumn (Penno *et al.*, 1995; Thomson *et al.*, 1997). Unlike the reports of Penno *et al.* (1995) Thomson *et al.* (1997), cows in both treatments of the present experiment were fed a generous and equal pasture allowance, which reduced the probability of a response in milk yield per cow to the availability of extra pasture. Production of extra herbage is not the only possible positive effect from fertilizer utilization. Increase in pasture quality can bring beneficial results after N fertilizer applications (Penno *et al.*, 1995). However, in the present experiment, lower pasture quality (OMD and ME content) resulting from nil N-fertilizer application, if did limit milk yield, must have been counterbalanced by other factors, since the low CP produced similar milk yields per cow to those of the high CP group.

In contrast to the experiments of Penno *et al.* (1995) and Thomson *et al.* (1997), discussed above, Mackle *et al.* (1996), Delaby *et al.* (1998), and Valk *et al.* (2000) used different levels of N fertilizer, leading to different CP intakes, to directly test the effects of the fertilizer on pasture composition, and consequently on milk yield per cow, when similar pasture allowances were offered to all treatments.

Valk *et al.* (2000) reported that milk yield per cow was not affected by reducing the use of fertilizer from 450 to 300 kg/ha/year. Furthermore, when the level of fertilizer was reduced to 150 kg/ha/year, the milk yield per cow was affected in only one out of three springs, which could merely have been due to limited CP intake (since the CP content of the pasture was only 16%). Delaby *et al.* (1998) used different levels of nitrogen fertilization and stocking rates (320 kg N/ha and 2.5 cows/ha and 0 kg N/ha and 1.7 cows/ha respectively) to offer the same pasture allowance per cow. The yields of milk and milk fat per cow, and protein contents did not differ between the treatments during the experiment. Such results are also in agreement with those reported by Peyraud *et al.* (1997) who undertook a cut and carry experiment with dairy cows fed on ryegrass at low and high N-fertilizer (0 k/ha and 80 kg/ha respectively) applications, which showed no significant differences in milk yields per cow between treatments. Hence, the present results mirror the findings of Peyraud *et al.* (1997), Delaby *et al.* (1998), and Valk *et al.* (2000) in that no differences in milk yield per cow were found between the high and low CP treatments.

On the other hand, Mackle *et al.* (1996) reported that use of N fertilizer at 150 kg N/ha led to lower yields of protein per cow in comparison to cows grazing on pasture fertilized with lower rates of N fertilizer (25 kg N/ha). This result is counterintuitive but, as protein concentration in milk is a reflection of the energy status of the cow (MacRae, 1987), the protein yields in the study of Mackle *et al.* (1996) could have been reduced by the high CP group having significantly (P=0.04) lower apparent intakes of DM and ME. Decreased protein concentration in milk also could have been due to a reduction in clover intake, since urea fertilizer application was followed by a reduction in the ratio of white clover to ryegrass, and intake of clover has been related to higher milk protein percentages, when compared to pasture alone (Grandison *et al.*, 1983). Results from the current experiment did not show any effect of N fertilizer upon yields of protein, or on protein percentages despite the fact that the high CP group had higher apparent DM and ME intakes during Weeks 13 to 15 of the experiment. Botanical composition analysis of the pasture was not done, therefore it is not known if the high CP paddocks had lower white clover contents than the low CP paddocks.

High RDP intakes have also been related to a reduction in energy balance, and potential decrease of milk yield. RDP in pasture during early spring exceeds both the theoretical cow requirements and the optimum RDP: rumen undigestible protein (RUP) ratio (AFRC, 1993; NRC, 2001; Holmes *et al.*, 2002a; Ridley, 2002), and N fertilizers also increase the RDP fraction in pasture (Ross *et al.*, 1978). If the RDP fraction is increased by the use of N fertilizers, as suggested by Ross *et al.* (1978) and Cuomo & Anderson (1996), it is possible that the RDP: RUP ratio could have been increased even further in the high CP paddocks compared to the low CP paddocks. Nonetheless, this did not lead to a significant difference in milk yield between groups. This would support the finding of McCormick *et al.* (1999) who also did not find any significant difference in milk yields after offering diets with different contents of RDP or different rates of degradability of the CP (Robinson *et al.*, 1991).

Though high CP intakes have been proposed to reduce the energy balance of the cow and consequently the availability of energy for milk production, in the present experiment, no major effect of N fertilizer, and its consequent increase in CP content of pasture, was observed for milk yields per cow. The only significant (P= 0.001) effect associated with the application of N fertilizer upon milk production was the reduction of the yields of milk, milksolids, fat and protein seen in the high CP group during Weeks 8 and 9 postcalving. Nevertheless, no significant difference was found in pasture allowance, DMI, ME intake, liveweight, and condition score which could have explained the results in these two weeks. Furthermore, there was no significant difference in CP intake between the treatments in Week 8.

Despite the various components of cow performance being non-significant in their own right, it is still interesting to calculate a feed conversion efficiency index which takes into account the various interactions between the parameters measured.

5.5 DRY MATTER INTAKE AND NUTRIENT INTAKE

Apparent DMI and ME intake, estimated from pasture disappearance as measured by the rising plate meter, were statistically (P=0.003 and P=0.001, respectively) higher for the high CP group than for the low CP group. However, as shown in Figures 4-4 and 4-5, both parameters started to be statistically different between groups only since Week 13 of the experiment. It is possible that a higher DMI and ME intake of the high CP group during the last four weeks of the experiment was not real since they were not reflected in the high CP group, when compared to the low CP group, as higher liveweight, condition score or milk yield.

This difference in DMI and ME intake was not expected since no limiting factors of DMI were apparent during the last 4 weeks of the experiment. Pasture allowance, which is the first limiting factor for DMI (Stockdale, 1985; Nicol, 1987; Holmes *et al.*, 2002a), was at least three times the estimated DMI for both treatments, which is the recommended to avoid restrictions in DMI (Holmes *et al.*, 2002a). In addition, it is likely that DMI would not have been restricted by pasture height since the minimum pregrazing herbage mass for the low CP groups during the interval from Week 13 to 15 was 3,000 kg DM/ha (Nicol, 1987; Hodgson, 1990; Holmes *et al.*, 2002a). Therefore, it is unlikely that limitations of pregrazing herbage mass and pasture allowance had been responsible for the difference in DMI between groups.

OMD, has been identified as one of the main limiting factors for DMI (Dalley *et al.*, 1999; McGilloway *et al.*, 1999), if pasture allowance is not restricted (Nicol, 1987); however, though there was a statistical difference (P=0.001) in OMD between treatments, it was consistent across the experiment and under practical conditions the size of such a difference has not been found to be responsible for different DMI (Peyraud *et al.*, 1997).

The differences between groups in apparent DMI and ME intake became larger by Week 13, when the pregrazing herbage mass increased. It is well known that readings from the rising plate meter must be interpreted with caution (Sanderson *et al.*, 2001; O'Donovan *et al.*, 2002). However, other authors (Lile *et al.*, 2001) have stated that readings from the rising plate meter are reliable if herbage mass ranges from 1,000 to 4,000 kg DM/ha. Possibly, in the present experiment readings from the plate meter became less accurate above "3,500" kg DM/ha (since Week 13) since, at this herbage mass, some of laying pasture occurred, which could have affected the reading of herbage height which is used to calculate the result from the rising plate meter. Therefore no larger DMI and ME intakes of the high CP group, compared to the low CP group, should laso have been due to errors in the estimation of herbage mass disappearance, and not due to any possible negative effect of OMD or limitations on pasture allowance.

A less likely explanation for the difference in DMI could be that it had been reduced in the low CP group due to a possible accumulation of low quality pasture in the base of the sward over the last weeks, which would not have been detected by sampling in the pasture at grazing height. Since the tillering density and the growth rate is lower in non-fertilized pastures, than in fertilized pastures (Ball & Field, 1982), it is possible that the quality of the pasture on offer could have been reduced, because low CP paddocks required a longer grazing interval than the high CP paddocks. Indeed, the association between long pregrazing intervals and low OMD has been well documented (Nicol, 1987; Dalley *et al.*, 1999; McGilloway *et al.*, 1999). As high CP paddocks had faster herbage accumulation rates, they also required of occasional extra grazing to remove the excess pasture to keep it leafy. This did not happen with the low CP paddocks which could have allowed the accumulation of more dead material and senescence, especially in the base of the sward, by the end of the experiment.

As shown in Figure 4-6, the higher CP intake of the high CP group occurred also from Week 13 to 16. Therefore the possibility exists that the higher DMI and ME intakes that occurred during this period were not reflected as a better animal performance because the extra nutrients ingested were used to excrete the high CP intake. Though this possibility exists is not likely since, as shown in Figure 4-2, the lowest differences in serum ammonia concentrations between groups occurred precisely during the last four weeks of the experiment, which indicates that the CP intake of the high CP group was at least not as large as during the rest of the experiment, and therefore not larger amount of energy was required to excrete urea. If the higher DMI, and consequently higher CP intake, of the high CP groups had been real it should also had been reflected in larger ammonia blood concentrations.

Though the averaged DMI and ME intake across the experiment were statistically higher (P=0.003 and P=0.0001, respectively), this difference was possible overestimated during the last four weeks of the experiment since no limiting factors for DMI were found, possible sources of error in the readings were present, and no sign of higher cow performance, from higher nutrient intake, were found. Therefore, for the following sections the DMI and ME intake will be considered only until Week 12, when the DMI and ME intake were still not significantly (P=0.5 and 0.07, respectively) different between treatments.

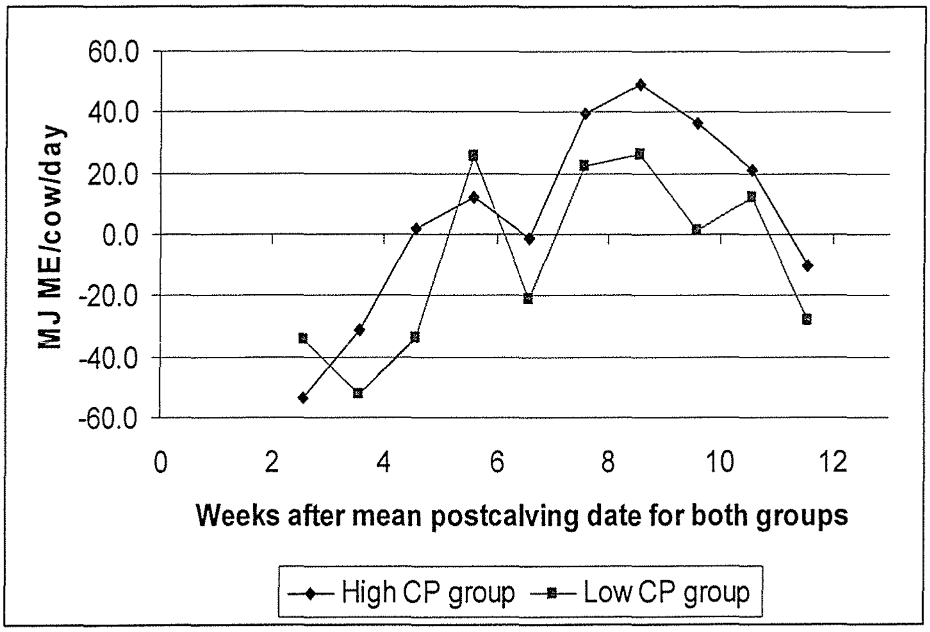
5.6 ENERGY BALANCE AND FEED CONVERSION EFFICIENCY INDEX OF THE COWS IN THE TWO TREATMENT GROUPS

Several authors have reported a negative interaction between high CP intakes and energy balance of dairy cattle (Butler, 1998; Chapa *et al.*, 2001). A hypothesis of the present experiment was that the NEB of spring-calved dairy cows could be exacerbated by the consumption of high-CP-spring pasture fertilized with large amounts of N-fertilizer.

From all the reviewed literature that studied the effect of CP on animal energy balance, only one report was found which assessed energy balance in the same way as in the present experiment, i.e. as energy input (MEI) minus the energy output (ME for milk and maintenance) (Ferris *et al.*, 2002). Average calculated energy balances during the study were 6.5 ±9.6 MJ ME/cow/day for the high CP group and -8.3 ±9.6 MJ ME/cow/day for the low CP group (Figure 5-1). The difference between these two means was 14.8 with and standard error of 5.7 MJ ME/cow/day and a probability of P=0.02. Therefore the average energy balance during Week 3 to 12 postcalving between the two groups was significantly different.

Since no other researchers have assessed the impact of CP intake in calculated energy balance, as in the present experiment, it is not possible to compare the present results with others. However, other experiments (Spicer *et al.*, 1990; Ferris *et al.*, 2002), not concerned with protein consumption, have also reported that changes in energy balance, at least to the extent that occurred in the present experiment, do not affect cow performance, condition score and liveweight.

Figure 5-1 "Energy balance" (metabolizable energy input minus metabolizable energy output) of the high and low crdue protein (CP) treatment groups



5.6.1 Theoretical and estimated ME requirements

The theoretical ME requirements (ME requirements for milk yield, maintenance, and liveweight change) and the estimated ME intake (MEI) (calculated from estimated DMI and pasture nutrient content) for both groups were averaged across the first 12 weeks of the experiment (Table 5-1). The theoretical ME requirements for the high and the low CP groups were similar (P=0.4) maybe because of their similar values for liveweight and yields of milk. In the calculations done no extra energy expense was considered in the high CP group for urea metabolism. The observed ME intakes tended (P=0.06) to be similar between treatments the high and the low CP treatments (192.3 ±7.2 and 183.4 ±7.2 MJ ME/cow/day respectively). Similarly, the high CP group tended (P=0.1) to have a larger intake of ME compared to their theoretical ME requirements, than the low CP group (2.8 MJ ME/cow/day vs. – 12.4 MJ ME/cow/day, respectively).

Since no other major parameters were statistically different between treatment groups (i.e. liveweight, condition score, milk yield), it is possible to infer that the high CP group appeared to require a higher energy intake (15.2 MJ ME/cow/day) and to be less efficient in its utilization to satisfy its energy requirements than the low CP group. It is possible that the extra energy apparently ingested by the high CP group, which was not used to increase milk yield or liveweight, could have been used for urea synthesis.

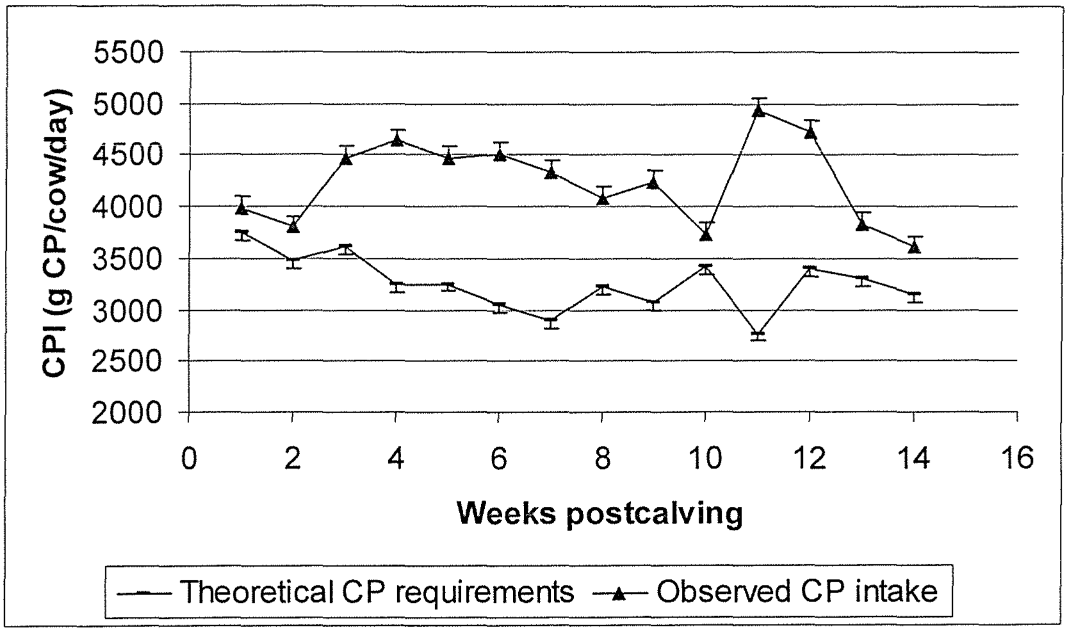
Table 5-1 Mean values for metabolizable energy (ME) theoretically required and apparently eaten by cows in the two treatment groups during the experiment

	High CP group (S.E.) First 12 weeks	Low CP group (S.E.) First 12 weeks
Observed ME intake (MJ ME/cow/day)	192.3 (±7.1)	183.4 (±7.1)
Theoretical ME requirements (MJ ME/cow/day)	189.5 (±6.1)	195.87 (±6.1)
Difference between theoretical and observed values	2.8 MJ ME/cow/day (±9.1)	-12.4 MJ ME/cow/day (±9.1)

5.7 PROTEIN INTAKE AND COST OF UREA EXCRETION

The high CP group had an average estimated CP intake of 4241 g CP/cow/day, which was higher (P=0.001) than the 3255 g CP/cow/day for the low CP group. Based on its theoretical requirement, the high CP group had a surplus intake of 986 g CP/cow/day, whereas the low CP group had a surplus intake of only 59 g CP/cow/day. Figure 5-2 shows the comparison between the estimated CP intake and the theoretical requirements for the high CP group.

Figure 5-2 Comparison between the theoretical requirements (CPR) and the observed CP intakes (CPI) throughout the experiment for the high and low crude protein groups



The high CP group used more ME to excrete N as urea, compared to the low CP group (9.8 MJ ME and 6.6 MJ ME respectively). The difference in ME between both groups (3.2 MJ ME/cow/day) could be attributed to the cost of excreting the extra urea from the high CP group. However, the calculated cost for urea excretion (3.2 MJ ME/cow/day) only partially explains the difference in ME intake between both groups (15.2 MJ ME/cow/day, Section 5.6.1). Several factors could have contributed to such imprecision. From formulae reviewed to determine the cost of excreting urea, no information was found detailing if the relationship between cost of excreting urea and the amount of urea excreted is linear or not. Therefore, it could be possible that the cost of metabolizing and excreting urea could be higher after certain limit. The energy used to excrete ammonia was not calculated based on the actual amount of urea excreted in urine; but was calculated based on the CP intake, which also was an estimated value. Also, factors such as the ratio of RDP: RUP and RDP degradability were not taken into account. Since no studies of the CP fractions were done during the experiment, the amount of ammonia generated by the extra RDP consumed could have been different from that assumed in the calculations.

In conclusion, though the extra CP intake of the high CP group was significantly (P=0.0001) higher than the CP intake of the low CP group, the calculated cost for excreting the extra CP ingested was minor and explained only a small part of the difference in ME intake between groups.

5.8 EFFECT OF N FERTILIZER ON THE DAIRY SYSTEM

Despite the large amount of literature that refer large CP intakes as possible reason for decreased cow performance, no major differences were found between treatments in yields of milk, reproductive performance, and energy balance. Furthermore, not only no negative effects were found on the high CP group but also significant and positive differences were found in favour of the high CP group.

The 130 kg N/ha resulted in an extra 1,264 kg/ha of dry matter

The increase in pasture production accounted for an extra 14,915 MJ ME/ha produced over the 101 days of the experimental period

The extra pasture growth accounted for 321 kg/ha of extra CP produced over the 101 days of the experimental period.

As a result of N fertilizer application, more pasture was grown (+1264 kg DM/ha), which allowed a higher stocking rate in the high CP group compared to the low CP group (2.6 vs. 2.0 respectively). Considering the milksolids yield/cow/day of the high CP group and the low CP group (1.8 and 1.9 kg MS respectively) the high CP group produced 4.8 kg MS/ha/day compared with only 3.8 kg MS/ha/day of the low CP group. During the 101 days of the experiment, this increase accounted for extra 101 kg milksolids extra/ha.

This extra 1264 kg DM of pasture grown from the N fertilizer could also had been conserved, at a stocking rate of 2 cows/ha, and fed later on during eventual times of pasture shortage or at the end of lactation to increase days in milk.

However, some issues should also be considered when applying high amounts of N fertilizer as the possibility of underground water pollution (Davies-Colley & Nagels, 2002; Francis *et al.*, 2003; McGechan & Topp, 2004), the financial constrains of fertilizer use (McGrath *et al.*, 1998), and ensure that the extra dry matter grown is consumed and converted into milk at the largest rate as possible.

6 CONCLUSION

In NZ, the CP content of pasture during spring is usually above the cow's requirements. During this time, application of N fertilizer is a common practice which is likely to further increase this CP content. However, the intake of CP in amounts well above the cow's requirements has been related by several reports from other countries with reduced energy balance, milk yield, and fertility of the cows. The present experiment was designed to measure any effects of the consumption of pasture with a lower or higher concentration of CP, on the performance of grazing cows during the first three months of lactation. The higher level of CP was created by the application of high levels of N fertilizer. Both groups of cows were offered the same daily herbage allowance

Overall, the application of 130 kg N/ha as fertilizer produced an extra 1,264 kg DM/ha as pasture growth and caused an increase in the CP concentration of the pasture (21.6% vs. 25.4%). If consumed, this extra pasture could have been expected to produce an extra 140 kg milksolids/ha, in the absence of any significant adverse effects on milk production, reproductive performance, condition score, and liveweight of the cow. The cows in the high CP group did show higher concentration of urea in their blood (7.8 and 5.3 mmol/l, respectively; P=0.001), but there were no consistent nor significant differences between the groups in milk production, liveweight, condition score or reproduction. The high CP group had lower calculated feed conversion efficiency. However, any adverse effect of the high CP pasture was not large enough to significantly depress the performance of each cow.

6.1 FURTHER RESEARCH

The following topics are suggested to be necessary:

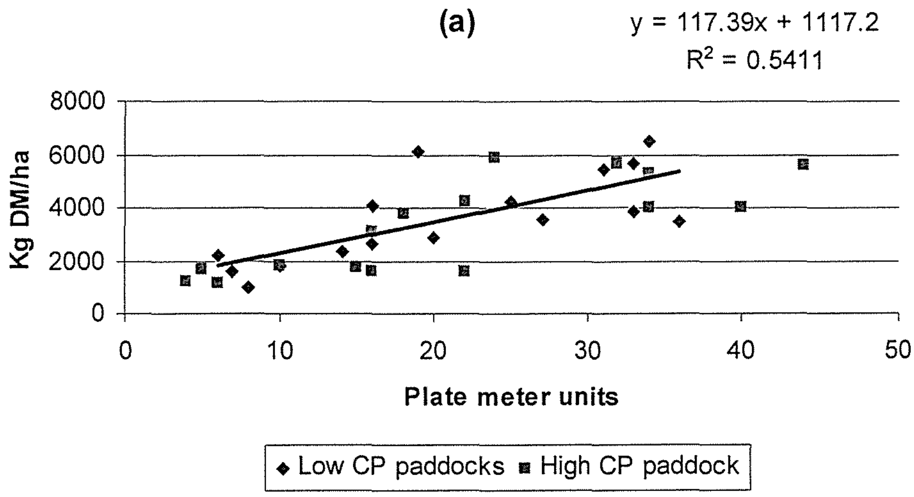
- To determine if the use of extra energy, i.e. maize silage, can improve the feed conversion and animal performance, of cows grazing on high CP pastures, by increasing the assimilation of the rumen degradable protein. It has been stated that the ability of the miroorganisms to metabolize the excessive ammonia, generated from the ruminal breakdown of diets containing large quantities of CP, could be improved by feeding the cow with energy sources of fast degradability by reducing ammonia escape from rumen and finally saving energy for urea synthesis (NRC, 2001).

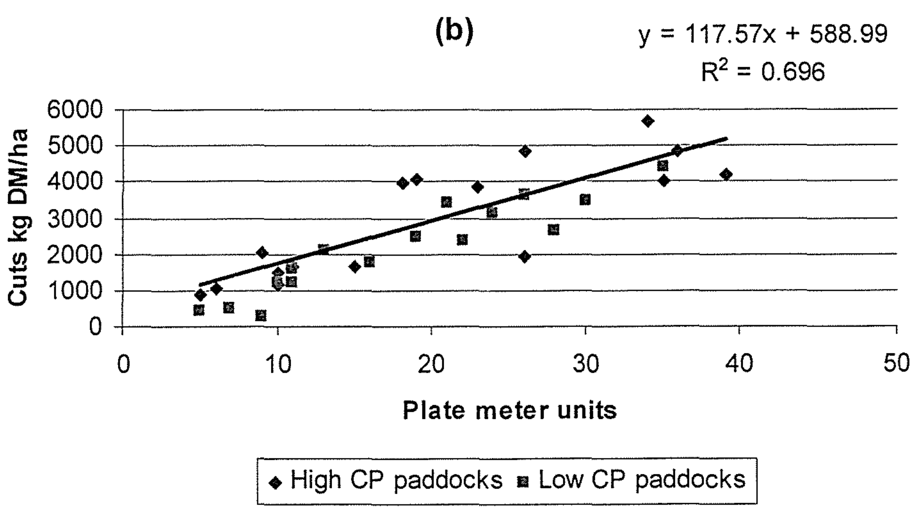
- To assess in detail the effect of large amounts of CP intake on uterine environment, embryo quality, and embryo viability. It has been proposed that high concentrations of ammonia in uterine and follicular fluid can lead to decreased embryo quality and viability (Armstrong *et al.*, 2001; Butler, 2001). The present experiment was not able to detect any lower reproductive performance in the high CP group; however, this study covered only the first 35 days post artificial insemination and was not able to assess the above mentioned factors.

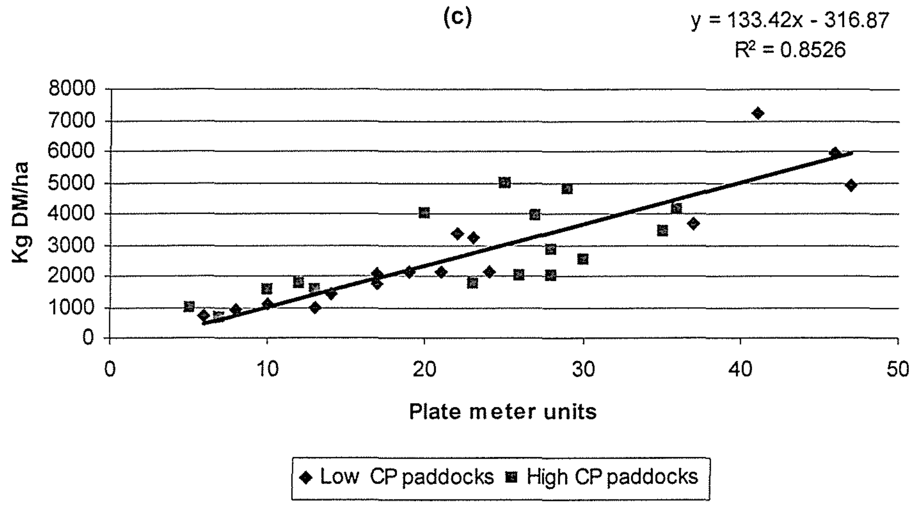
- To assess the ecologic impact of applying large amounts of fertilizer on underground water tables and atmospheric nitrous oxide. It has been proposed that use of high amounts of N fertilizer may result in high N losses to the environment, hence causing ammonia volatilization and nitrate leaching (Tamminga, 1992; Valk *et al.*, 2000). Though the application of N fertilizer at high rates showed to be beneficial in this experiment, it is of ecological importance to recognize the limits and environmental implications of urea application.

7 APPENDIX

Figure 7-1 Correlations between calibration cuts and readings from the plate meter on the 18th of September (a), 3rd of October (b), and 30th of October (c).







7.1.1 Calculations of ME requirements

The total ME requirements of dairy cattle as proposed by (AFRC, 1993) and modified by (Brookes, 2004) were calculated as:

Total ME requirements (MJME/day) = $M_m + M_l + M_{lw}$;

Where M_m, M_l, and M_{lw} represent the energy requirements for maintenance, lactation and liveweight gain respectively.

The ways of calculating the different efficiencies of energy were as follow: efficiency of energy use for maintenance K_m= 0.35q_m + 0.503, the efficiency of energy use for lactation K_l= 0.35q_m + 0.420, the efficiency of energy use for growth in lactating ruminants kg= K_l* 0.95 and the efficiency of the use of mobilised body reserves for lactation K_l= 0.84. q_m= ME of the pasture/ Gross Energy (18.4). ME of the pasture was calculated from NIRS.

ME for maintenance/ kg ^0.75 is calculated using:

M_m= FHP/K_m

where FHP= fasting heat production (0.43)

ME for lactation

Energy value (E_{Vl}) of milk is calculated using:

E_{Vl}= (0.376*MF%)+(0.209*MP%)+0.976

where: MF% is fat concentration and MP% is protein concentration, g/kg.

The energy required for lactation, M_l, is then calculated from:

M_l (MJ/day)= Y (E_{Vl})/k_l

where Y is the yield of milk in kg/day

ME for liveweight change

The energy retained in the body per kg of liveweight gain (M_g) is calculated as:

M_g= Eg/kg

Where Eg= 19 MJ/ kg L_w

The dietary energy spared per kg of liveweight lost (Mg_{-l}) by lactating animals is calculated as:

Mg_{-l}= Eg_{-l} /k_l

where Eg_{-l} = Eg/k_l

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