Intake and performance of dairy cattle on forages in winter

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The effect of crop type, crop allowance and feeding frequency on DM intake, body condition score (BCS) gain, grazing behaviour and rumen physiology of dairy cattle fed forage crops during winter was examined in three experiments. Experiment 1 examined the effect of wintering dairy cows outdoors on the industry standard allowance of kale (11 kg DM of kale + 3 kg DM of barley straw, K11), with the higher allowance of kale (14 kg DM of kale + 3 kg DM of barley straw, K14) and Italian ryegrass at low allowance (11 kg DM + 3 kg DM of barley straw, G11). BCS gain over the six week winter feeding period was higher for K14 and G11 (0.3 BCS units) than K11 (0.2 BCS units). The % DM utilisation of K11 (96%) and K14 (88%) were higher than G11 (70%), leading to higher DM intake in K14 (12.1 kg DM of kale/cow/day) and K11 (10.5 kg DM of kale/cow/day) than G11 (7.9 kg DM of grass/cow/day). Within 6 hours of being offered a fresh break, cows had consumed over 86% of their apparent daily intake with DM consumptions of 10.4, 10.5 and 7.3 kg DM/cow for the K11, K14 and G11 treatments, respectively. Grazing behaviour was altered by both forage type and allowance. Cows on the K14 treatment grazed for longer over the day than cows in K11 and G11. Rumen ammonia concentrations peaked 7 h after the morning allocation of feed and concentrations ranged from 108 to 212 mg NH_3/l for K14, 91 to 306 mg NH_3/l for K11 and 57 to 269 mg NH_3/l for G11. Rumen pH fell to reach its lowest values of 5.7 for G11, 6.0 for K11 and 6.2 for K14 between 7-10 h after feeding forage in all treatments. However, the rumen pH of K11 and K14 remained high (> 6.0) throughout the day. Further, from bout counts of rumen pH, G11 had more frequent and longer bouts of rumen pH under each threshold except the threshold 6.4 compared with K11 or K14. Hence, there was little evidence of sub-acute rumen acidosis (SARA) in kale-fed cows. Urinary N% was higher for K11 (0.58%) than K14 (0.43%) and G11 (0.52%). Faecal and urine N output per cow was higher for K14 (335 g N/cow/day) and K11 (289 g N/cow/day) than G11 (227 g N/cow/day). Based on stocking density, total faecal and urine N output per hectare was higher for K11 (413 kg N/ha) and K14 (355.9 kg N/ha) than G11 (82.3 g N/cow/day).

Experiment 2 examined the effect on rumen physiology of feeding cattle either once (1.5 kg DM/day barley straw at 0800 h plus 7 kg DM/day of kale at 0900 h, K1) or twice (0.75 kg DM barley straw 0800 h plus 3.5 kg DM kale 0900 h and 0.75 kg DM barley straw at 1400 h plus 3.5 kg DM of kale at 1500 h, K2) per day over a 7 day period in individual metabolism crates. No significant differences were observed between the two treatment groups in the rumen concentration of acetic acid, propionic acid, butyric acid, ammonia and rumen pH. There was a distinct diurnal pattern of rumen ammonia concentration which was characterized by a peak 2 h post feeding for both K1 and K2. Rumen ammonia concentration ranged from 27.5 mg NH_3/l to 170 mg NH_3/l for K1 and from 43.7 mg NH_3/l to 158 mg NH_3/l for K2. Rumen pH remained high (>5.8) on both treatment groups.

Experiment 3 examined the effect of wintering dairy cows outdoors on either kale or grass fed in one (11 kg DM kale + 3 kg DM of baled barley straw offered in the morning) or two allocations (5.5 kg DM of kale grazed + 1.5 kg DM barley straw offered morning and afternoon) per day. BCS gain over the six week winter feeding period was higher for grass-fed cows (0.5 BCS units) than kale-fed cows (0.2 BCS units), but unaffected by feeding frequency. % DM utilization was higher for kale-fed (97%) than grass-fed cows (76%) leading to higher apparent DM intake of forage in kale-fed (9.7 kg DM/cow/day) than grass-fed cows (7.7 kg DM/cow/day). % DM utilization and apparent DM intake were not affected by feeding frequency. Prehension bite rate was greater for grass-fed (37.3 bites/min) than kale-fed cows (7.6 bites/min), but more mastication bites were required for kale-fed cows. Cumulative DM intake after 2, 3 and 6 h was greater in cows fed once than twice a day and for kale than grass after 3 and 6 h. Mean eating time was greater on cows offered forage once (477 min) than twice (414 min) per day. Rumen ammonia concentration rose rapidly after the first meal, with all four treatments reaching a peak value within 3 hours of grazing time. From the bout counts, cows which were fed grass recorded the most frequent rumen pH of <5.8 with cows fed grass twice a day recording the most frequent rumen pH of <5.5. Hence, there was no evidence of SARA in kale-fed cows.

Overall, the results show BCS gain over the winter feeding period was higher in grass than kale-fed cows when forage was offered at 11 kg DM/cow/day. This was despite % DM utilization and apparent DM intake being lower for grass-fed cows. Increasing kale allowance to 14 kg DM/cow/day increased DM intake and BCS gain. Increasing feeding frequency from once to twice per day decreased the intake rate within the first 6 hours after allocation but did not affect total daily DM intake, % DM utilization or BCS gain. Rumen ammonia concentration for both kale and grass-fed cows peaked 3 h and 7 h after the morning

allocation, but the concentration stayed within the range of 20-800 mg $NH_{3/}$l for maximum rumen bacterial growth. Rumen pH remained high (>5.8) for kale-fed cows, hence indicating no evidence of SARA. The calculated faecal and urine N output/ha were higher for kale than grass-fed cows reflecting greater yield of kale leading to increased stocking density.

Keywords: grass, kale, winter, DM intake, utilization, body condition score, rumen pH, rumen ammonia, acetic acid, propionic acid, butyric acid, nitrogen losses.

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Chapter 1
Introduction

New Zealand dairy production is pasture based (Holmes *et al.* 2002), and thus matching the dairy cow reproductive cycle with pasture production is desirable. Peak feed demand periods during the reproductive cycle are experienced during late pregnancy when foetal growth peaks and in early to mid-lactation. In order to ensure that lactation peak coincides with peak spring pasture growth, approximately 365-day calving interval is maintained (McNaughton *et al.* 2003; Sanders 2004). However, this 365 day interval is disrupted by extended post-partum anoestrus interval (PPAI), which is a common form of infertility identified in New Zealand (NZ) dairy herds (Burke *et al.* 2005; McNaughton *et al.* 2003). Animals with short PPAI (average interval from calving to first postpartum oestrus is between 35 and 50 days) have an advantage since they have higher conception rates and calve in time to ensure 365 day intervals (Sanders *et al*. 2004). Prolonged PPAI is associated with low body condition score (BCS) and body weight at calving (Burke *et al.* 2005; Mwaanga & Janowski 2000; Sanders 2004).

Prolonged PPAI delays first insemination date and conception thus extending calving interval (CI) which in turn reduces the length of subsequent lactation (Sanders *et al*. 2004). Hence the farmer's financial returns from milk sales are reduced as a result of a reduced number of pregnancies, withdrawal periods following treatment and the cost of managing the prolonged anoestrous period (Mwaanga & Janowski 2000). To reduce these negative impacts on dairy cow productivity, a target body condition of 5.0 on the NZ 1-10 condition score scale at calving under the NZ dairy system is recommended for mixed age cows and 5.5 for first and second calvers (DairyNZ; Nichol *et al*. 2003). This has also shown to be important for milk production. Grainger *et al*. (1982) noted an increase in milk fat production of 7.5 ± 3.5 kg for each BCS increase between 2.0-3.5 (1-8 AUS scale) at calving. However, Macky (2010) and Nichol *et al*. (2003) reported that many cows fall below this recommended BCS at drying off. Judson & Edwards (2008) indicated that most cows are dried off at a body condition score of 4.5 or lower. To achieve high dairy productivity there is need to maintain desirable BCS through drying off in appropriate BCS; alternatively a scheme that ensures that over the winter dry cow feeding period there is a timely regain of body condition lost through lactation is desirable. This has become an important aspect amongst NZ dairy systems (Judson & Edwards 2008; Judson & Edwards

2010). In this context, cows are often dried off in May in BCS of 4.5, with aim of gaining 0.5 BCS units over winter feeding period.

Despite increased interest in NZ in housing cows indoors during winter (de Wolde 2008), most cows are wintered outdoors on forage crops or pasture supplemented with silage, hay or straw (Nichol *et al.* 2003; White *et al.* 1999). Nichol *et al*. (2003) identified forage brassicas, particularly kale (*Brassica oleracea*) and swedes (*Brassica napus*), as the main source of winter feed for dairy cows in the South Island. This is mainly because brassica crops are capable of growing at lower temperatures than perennial ryegrass (*Lolium perenne* L.), produce a large amount of high quality dry matter (DM) per unit area (e.g. up to 18 t DM/ha) (Judson & Edwards 2008) relative to perennial ryegrass at the time of feeding during winter. Also, they maintain high nutritive value (85-90% digestibility, 12-13 MJ ME/kg DM, 15-25% CP in leaves; Valentine & Kemp (2007) throughout the winter feeding period. Further, there are concerns over the low utilisation of perennial ryegrass during winter, when grazed on wet soils (Keogh *et al*. 2009a).

Despite these attributes, anecdotal evidence suggests that the BCS gain of dairy cows on kale over winter may be less than expected (Judson & Edwards 2008). From a survey of dairy herds in Canterbury, Judson & Edwards (2008) suggested that this may reflect low DM intake due to poor crop allocation, further constrained by high utilisation at the low allowances offered leading to poor diet quality. Furthermore, they suggested that higher DM allowance may be a method of increasing DM intake and diet quality. Despite those suggestions, there is little data using feeding regimes typical of NZ were kale is high proportion of the diet. For example, by allocating more kale crop, high residuals might be left behind after grazing.

Due to the perceived poor performance of dairy cows grazing kale, cows are often wintered on grass, despite its lower yield (Pangborn & Gibbs 2009). Keogh *et al*. (2009a), showed a decrease in BCS of cows grazed on perennial ryegrass pastures compared to those grazed on kale. However, there is no evidence from typical NZ systems to support the findings of Keogh *et al*. (2009a).

In outdoor wintering systems, the kale crop is typically fed as a single meal in the morning, with electric fencing used to control break widths and allocation. A typical diet offered is to achieve 11 kg DM *offered*/cow/day of kale and 2 to 3 kg DM *offered*/cow of grass silage or straw. Based on DM utilisation of 70% of forage crop and ME values for kale (12 MJ ME/kg DM) and straw (7.3 MJ ME/kg DM), this will give ME intake of 114 MJ ME/day; this is close to 112 MJ ME/day calculated to be required for a 500 kg dairy cow to put on 0.5 BCS units over a six week feeding period during winter (Nichol *et al*. 2003). This feed management is generally associated with high DM intake rates; for example after 6 hours of grazing cows had consumed 10.4 kg DM of kale (Rugoho *et al*. 2010). Previous work has reported on the effect of altering the feeding frequency of pasture on cow behaviour and DM intake (Dalley *et al.* 2001; Gregorini *et al.* 2009; Kennedy *et al.* 2009), but little is known of the effect of feeding frequency for cows grazing kale. Although a proportion of graziers shift cows to fresh break of kale more than once daily with a view to improve DM utilisation (Gibbs 2009), there is a lack of published data on the effect of variation in the frequency of allocation on cow behaviour plus rumen function, DM intake and gain in BCS. There is also little experimental data on DM utilisation, DM intake foraging behaviour and rumen function of cows grazing kale compared to other forages such as grass (Gazzola *et al*. 2008).

A further factor that may influence performance of cows grazing kale is the forage composition of the kale. The nutritive composition of kale crop is of a low concentration of neutral detergent fiber (NDF) (<300 g/kg DM) and high concentration of water soluble carbohydrates (WSC) (>200 g/kg DM (Barry & Manley 1985). The combination of low NDF and high WSC concentrations has been suggested to lead to problems with rumen acidosis in total mixed ration diets (Krause & Oetzel 2006). However, there is limited data for cows grazing kale.

This thesis presents a series of experiments which measure utilisation, intake, foraging behaviour of cows grazing kale and rumen function of cows out-wintered on kale and grass forages.

1.1 Research aim and objectives

The aim of this study was to develop feeding and forage crop allocation strategies to ensure cows reach the BCS of 5.0 (1-10 NZ scale) at calving and to ensure cows gain greater than 0.5 BCS during the winter feeding period. The specific objectives of the thesis were to:

(1) To quantify the effect on DM intake, DM utilisation, N excretion and body condition score of cows wintered outside on kale at the industry standard (11 kg DM of kale + 3 kg DM of barley straw) with the higher allowance of 14 kg DM of

kale + 3 kg DM of straw) and perennial ryegrass at low allowance (11 kg + 3 kg DM of barley straw).

(2) To quantify the effect on the foraging behaviour, rumen pH, rumen ammonia and concentration volatile fatty acid production of cows wintered outside on kale at the industry standard (11 kg DM of kale + 3 kg DM of barley straw) with the higher allowance of 14 kg DM of kale + 3 kg DM of straw or grass at low allowance (11 kg + 3 kg DM of barley straw)

(3) To evaluate the effect of feeding kale once versus twice per day on the rumen function of Friesian steers in an indoor feeding trial.

(4) To examine the effect of once versus twice a day allocation of kale and grass on BCS gain, cow behaviour, forage intake, DM utilisation and urinary nitrogen excretion of dry cows during the winter feeding period and their subsequent milk production during early lactation.

(5) To evaluate the effect of feeding kale and grass once versus twice per day on grazing behaviour, rumen ammonia levels, VFA concentrations and rumen pH of , non-lactating New Zealand crossbred dairy cows in winter.

1.2 Thesis structure

This thesis consists of eight Chapters. Chapter 2 reviews the literature with particular reference to wintering systems and reasons for poor performance on brassicas. The remaining Chapters reports on 3 experiments (two outdoor and one indoor) considering effects of crop type, allowance and feeding frequency on body condition score and rumen physiology. More detailed information about the outline of this thesis is described Figure 1.1:

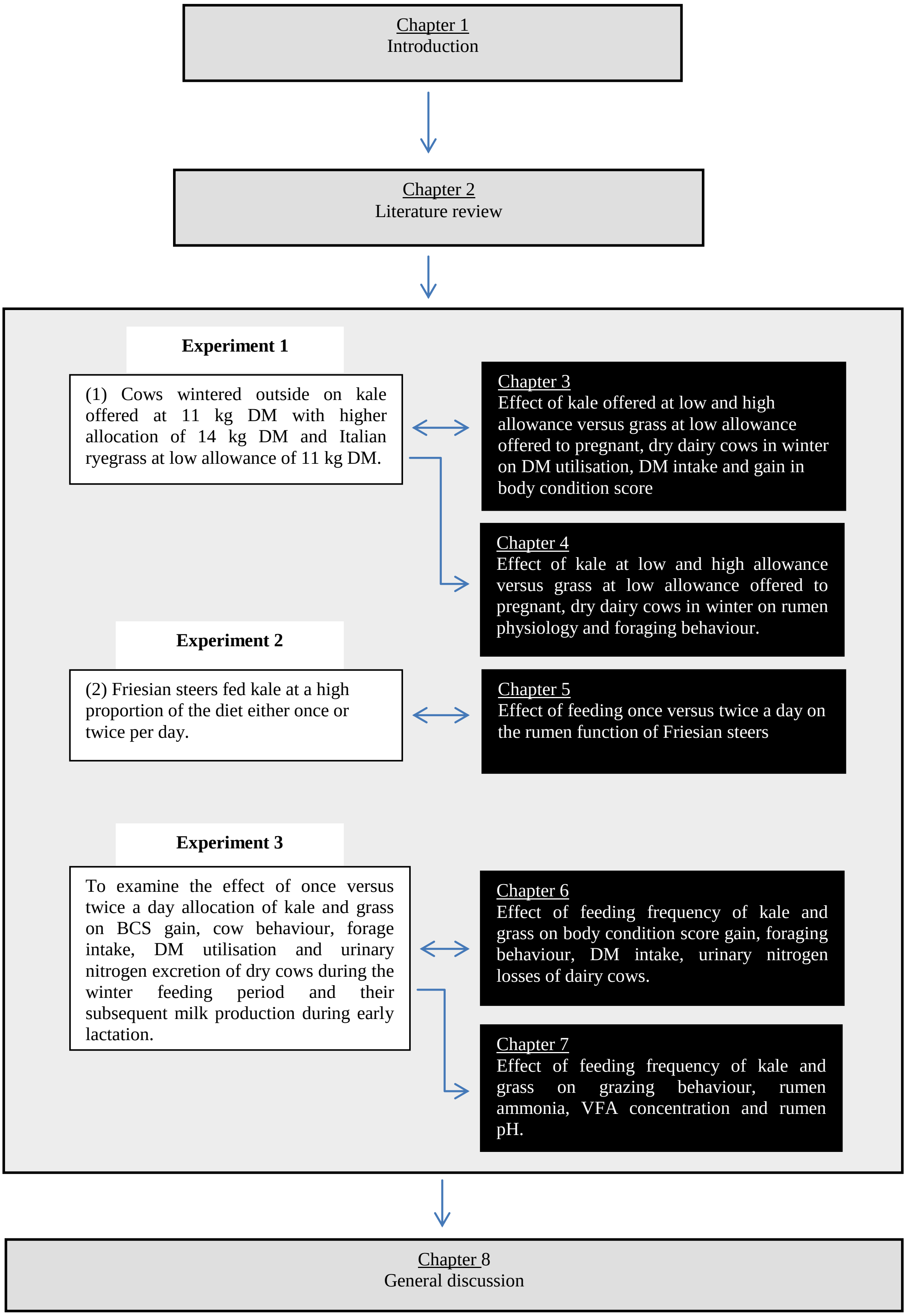


Figure 1.1: Flow diagram showing thesis structure.

Chapter 2
Literature review

2.1 Wintering systems and forage types

For the last 15-20 years, the dairy industry has been expanding rapidly in the South Island, New Zealand (Pangborn & Gibbs 2009), with the proportion of New Zealand's milk produced in the South Island rising from 2 % in 1995 to 39 % of New Zealand's milksolids in 2011. In South Island dairy systems, the use of land resources for milk production is split into two key parts. First, a milking platform area, where dairy cows are located during lactation. Second, a dairy support land area, where a majority of cows are moved to during the winter, the dry cow feeding period. This contrasts with the North Island, where herds are out-wintered on pasture on the milking platform with supplements (Pangborn & Gibbs 2009). This may not be the case in South Island mainly because pasture growth in winter period is limited (<3-4 t DM/ha) (Paramenter & Boswell 1983). As a result most South Island dairy cows are wintered off the farm on specialised crops such as forage brassicas, (particularly kale (*B. oleracea*) and swedes (*B. napus*) (Nichol *et al.* 2003; Pangborn & Gibbs 2009; White *et al.* 1999).Wintering of dairy cows in this situation provides two significant challenges (Dalley *et al*. 2011; Judson *et al*. 2010). First, the inability to feed cows to achieve calving BCS targets of 5.0 (NZ 1-10 scale). Cows are often dried off in autumn at BCS of 4.5, with targets of gaining 0.5 BCS units by calving. Typically, this gain needs to occur over a 6-8 weeks period from the end of May to the start of August. Second, environmental concerns, which largely revolve around nitrogen leaching losses associated with grazing cows at high stocking densities on free draining soils during winter.

2.1.1 Forage brassicas

The advantages of forage brassicas are that they are capable of growing at cooler temperatures than perennial ryegrass (*Lolium perenne L.*), and producing large amounts of high quality dry matter (DM) per unit area in winter. In survey of kale crops in Canterbury, DM yields in winter ranged from 5 t DM/ha for dry land crops to 17 t DM/ha for irrigated crops supported by high inputs of fertilizer (de Ruiter *et al*. 2002; Judson & Edwards 2008). Maximum yields at the same time of year would be around 5-8 t DM/ha for forage cereals (de Ruiter *et al*. 2002) and 3-4 t DM/ha for ryegrass (Paramenter & Boswell 1983).

Also, brassicas such as kale (*B. oleracea*) are of high nutritive value. For example, they typically have a digestibility of 85-90% (range represents different cultivars), metabolisable energy (ME) concentration of 11-13 MJ/kg DM and a protein content of between 15-25% (leaves) and 8-15% in bulbs (Valentine & Kemp 2007).

Despite brassicas having high nutritive value, there are also anti-nutritional factors associated with some brassica crops. For example the nitrate content of kale is often high and ranges between 1 to 2% of DM and they contain S-methyl cysteine sulphoxide (SMCO) (0.4 to 4.0 %) and thiocyanates (0.10 to 0.15%) (Smith 1980) that may limit nutritive value. Rumen fermentation of SMCO can cause haemolytic anaemia and is also associated with reduced DM intake in sheep in the study by Barry & Manley (1985).

The kale crop also contains low levels of fibre (neutral detergent fibre (NDF), 18.0-48.2% DM and acid detergent fibre (ADF), 14.3-34.1% DM), high levels of non-structural carbohydrates (WSC), (30.7-44.6% DM) and a relatively low dry matter percentage (DM%), (13-18%) (de Ruiter *et al*. 2007). As a result of a high WSC, low NDF content and relatively low DM% of forage, feeding of kale as the main component of the diet, has been suggested to promote low rumen pH in cattle (rumen acidosis) (Nichol *et al*. 2003).The normal rumen pH is in the range of 5.5-6.8, and depending on the diet, subacute rumen acidosis (SARA) is defined as an extended period where rumen pH falls below pH 5.5 (Marie Krause & Oetzel 2006). However, in an extended series of feeding trials using kale at NZ industry standard rates, Gibbs (2009) using indwelling rumen pH monitoring reported consistently high (>6.0) rumen pH values, concluding there was no risk of rumen acidosis with kale feeding in cattle. Nevertheless, on the basis of earlier reports, to eliminate rumen acidosis, most farmers feed their dry cows with feed rations containing 50-60% kale and 30-35% of high fibre supplements such as silage, hay or straw (Nichol *et al*. 2003).

2.2 Reasons for poor performance on brassicas

The high nutritive value and standing DM of forage brassicas relative to other forages during winter, would suggest they would be useful as winter forages for dairy cows. However, Judson & Edwards (2008) indicated that farmers struggle to meet the targeted body condition at calving by feeding kale to dry cows as a major component of their winter diet. For example, in winter feeding study (outdoor) at Lincoln university cows gained 0.45 BCS units over winter feeding period on diet of 11 kg DM of kale/cow/day and 5 kg DM of silage/cow/day (GR Edwards unpublished). This was despite Groves (2008) calculating the ME intake (kale + silage) to be 140 MJ ME/cow/day, which exceeded the 112 MJ ME/day calculated by Nichol *et al*. (2003), to be required to ensure cows gain 0.5 BCS units over the 8 week period in winter, dry cow feeding period. In the sections below, a number of the reasons for the poor performance and inability of cows to gain adequate BCS are discussed.

2.2.1 Crop type

One possible reason why cow body condition does not reach recommended targets of 0.5 BCS units over the winter period is crop type. Several Irish studies (Gazzola *et al*. 2008; Keogh *et al*. 2009 a; b) have compared wintering systems based on kale, swedes, fodder beet and grass silage. A recent study in Ireland (Keogh *et al.* 2009a) examined performance of cows in late pregnancy and early lactation out-wintered on kale, swedes or perennial ryegrass or fed grass silage indoors for 10 weeks. The study showed cows offered 8 kg DM kale/cow/day + 4 kg DM/cow/day of perennial ryegrass silage, 8 kg DM swedes/cow/day + 4 kg DM/cow/day of perennial ryegrass silage outdoors or perennial ryegrass silage *ad libitum* indoors had BCS of 3.27, 3.20 and 3.85 (Irish 1-5 scale) respectively at calving. In contrast, those offered 12 kg DM/cow/day perennial ryegrass *in situ* had the lowest BCS of 2.85 ($P<0.001$) at calving. These BSC values correspond to BCS of 6.15, 5.98, 6.93 and 5.10 respectively on NZ 1-10 scale at calving (Roche *et al*. 2004). There was no difference in milk, fat and protein yields among treatment groups in early lactation. No data was presented on DM intake or ME of forages fed in the field to ascertain whether forage crops differed in amount of ME consumed.

In a further study in Ireland, Keogh *et al*. (2009b) showed that the gain in BCS score was greater in cows fed grass silage (0.37) than fodder beet (0.22) and kale (0.09) (1-5 Irish scale) during the winter period . In agreement Gazzola *et al*. (2008), BCS of grass-fed cows was lower (2.89) compared to kale-fed cows (3.40) and swedes fed-cows (3.32) (Irish 1-5 scale).

Gazzola *et al*. (2008) further showed cows on perennial ryegrass spent a longer time grazing (585 min/day) than swedes (578 min/day) and kale (515 min/day) but no data was presented on forage nutritive value or the intake from the different forages to explain treatment effects. Despite these findings from Ireland, anecdotal evidence in the NZ dairy industry is that cows perform better (higher BCS gain) when wintered on grass than kale (Pangborn & Gibbs 2009).

Of note is that little NZ data is available comparing performance of cows on kale and grass. Anecdotal evidence from farmers in NZ suggests better performance on grass than kale, although this has been not tested in experiments were both grass and kale allowance is controlled.

2.2.2 Low DM intake due to slow adaptation to kale diets

A further explanation for the poor performance of cows grazing kale may be low DM intake due to slow adaptation to kale diets. Sheep take at least five weeks to fully adjust to eating brassica after transfer from grazed pasture (Barry 1978; Muir *et al.* 1995). During this period both intake and live weight gain may be depressed, probably due to rumen organism adaptation to a large intake of a low moisture crop; shift in ratio of non-structural carbohydrates (NSC) to crude protein (CP); low fibre intake; mineral imbalances; and other sub-clinical effects (Barry 1978; White *et al*. 1999). This initial period of depressed intake and live weight gain has also been observed in cattle (Barry 1978) and cows may take more than two weeks to adjust to maximum voluntary feed intake (Nichol *et al*. 2003). In an indoor feeding trial by Keogh *et al*. (2009c), increasing the proportion of kale in the diet from 0 to 100% was associated with reduction of DM intake (7.3 versus 8.9 kg DM/cow/day, respectively).

To limit the effects of this diet change, Barry (1978) emphasised the need for adapting the stock gradually to the new brassica crop by the use of a runoff paddocks of pasture or ensuring that diet is only partly brassica based. Further, animals should be fed supplement (grass silage and/or hay or straw) prior to introduction to the crop (Nichol *et al*. 2003).

2.2.3 Low DM intake due to poor crop utilisation

Judson & Edwards (2008) examined the hypothesis that poor performance on kale may be associated with low DM utilisation leading to low DM intakes. DM utilisation was surveyed in 49 dairy herds in Canterbury. Kale utilisation ranged from less than 40% to greater than 90%, with a mean of 80% on kale DM yields that ranged from 5-17 t DM/ha. In a further study Groves (2008), DM utilisation of kale was not affected by either sowing date or kale cultivar. During this study, DM utilisation averaged 88%, even under wet winter conditions. In an Australian study by Stefanski *et al*. (2010) dairy cows grazing forage rape (*Brassica napus* L.*cv*. 'Goliath') planted at different sown rates of 2, 3, 3.5 and 5 kg/ha, preferred grazing the forage rape sown at 2 kg/ha. However, this preference did not increase or decrease DM utilisation and under the conditions of trial utilisation was low (around 42%) (Stefanski *et al*. 2010).

Despite these high DM utilisation values, Judson & Edwards (2008) noted that two thirds of the herds consumed less than their targeted DM intake by more than 1 kg DM/cow/day and some by 8 kg DM/cow/day. Inaccurate crop allocation was considered by Judson & Edwards (2008) as the major factor contributing to poor outcomes in relation to cow condition as compared to other factors. Their reasoning was that DM utilisation was generally high and DM yield assessments accurate. Hence, low DM intakes were probably due to poor allocation (getting the break size wrong), a management factor which was also emphasised by Nichol *et al*. (2003).

DM utilisation may also be linked to forage allowance. Muir *et al*. (1995) noted that the level of DM utilisation decreased from 77% to 32% as the allowance increased from 3% BW (4.3 kg DM/day/bull) to 15% BW (21.3 kg DM/day/bull) when they fed bull calves with kale (leafy type cv. Winfred). Muir *et al*. (1995) also observed that bull calves tended to consume more of the leafy type brassica (90% of the total crop) compared to stemmy type (77% of the total crop) when offered at low allowance of 3% BW. Keogh *et al*. (2009b) in an outdoor study examined the effect of two forage allowances offered to pregnant dry dairy cows. In this study (Keogh *et al*. 2009b), cows on high allowance were offered 9 kg DM/cow/day of kale or fodder beet plus 5 kg DM/cow/day of baled grass silage and cows on low allowance were offered 6 kg DM of kale or fodder beet plus 3.5 kg DM baled grass silage daily. Cows on high allowance had a greater BCS (3.05) at calving than those at low allowance (2.73) (Irish 1-5 scale). This corresponds to BCS of 5.6 and 4.8 on 1-10 NZ scale (Roche *et al*. 2004). Judson & Edwards (2007) noted that in Canterbury, high apparent daily DM intakes of kale of 10 to 12 kg DM/cow/day were a result of high daily allocation of kale (13-15 kg DM/cow/day), and were associated with moderate DM utilization of around 70-80%. In contrast, high utilisation of greater than 90% was associated with lower levels of allocation (e.g. < 10 kg DM/cow/day). As total daily allowance increased from 7 to 21 kg DM/cow/day kale DM utilisation decreased from 87% to 55%.

Judson & Edwards (2009) also stressed high DM utilisation may lead to a poorer quality diet of kale. From kale samples, they showed that ME declined from 12.7 to 6.6 MJ ME/kg

DM from the top (leaf) to bottom of stem. Diet quality, estimated from utilisation and ME values was calculated to decline from 12.7 to 10.1 MJ ME/kg DM based on the relative proportions of stem and leaf as DM utilisation increased from 55% to 87%. Thus, high utilisation may lead to poor body condition score change outcomes due to the fact that cows will be forced to eat lower quality stems of kale (Judson & Edwards, 2008). Judson & Edwards (2008) recognised the importance of post-grazing residual grazing as a useful tool determining if dry cows fed on kale are getting their targeted daily intake.

2.2.4 Poor rumen function

In current kale feeding regimes, kale is generally fed as a single daily break in the morning. Based on DM yield and target allowances, break widths are calculated and fences are shifted daily. Silage, straw and hay supplements are fed prior to the new break being offered or cows are given continuous access to supplements. This feeding regime for kale potentially leads to high intake rates as cows quickly consume offered forages. Based on kale disappearance, Groves (2008), estimated DM intakes of 8 kg DM/cow over the 3 hour period following access to a new break. Rumen function of Friesian steers on this type of feeding regime was examined (Gibbs unpublished data). It was noted that offering steers kale once-a-day caused diurnal fluctuations in rumen concentrations of volatile fatty acids (VFA), ammonia and lactic acid. Specifically, ammonia levels rose sharply to >200 mg NH_3 /L within 2 hours after the 9 am meal of 9 kg DM kale/head, remained elevated for a period of 6 hours, before declining to levels <100 mg NH_3 /L for the remaining 16 hours of the day. The high levels of ammonia recorded in the study soon after feeding, and low levels later in the day, could potentially affect microbial growth and activity, and hence, microbial degradation of feed (Cabrita *et al.* 2006). Satter & Slyter (1974) suggested that rumen ammonia concentrations between 20 and 800 mg NH_3/l are enough to support rumen bacterial growth rates.

It is plausible that alternative feeding regimes may give improved rumen function. For example, twice a day feeding may give a more consistent intake pattern and lower fluctuations in key rumen functions. Alternatively, higher allowances in the field of kale or different quantities and allocation patterns of straw may improve rumen function. This will depend however, on how meal patterns in the field respond to changes of allocation regime

2.2.5 Animal health considerations on winter brassicas

Nitrate poisoning

Nitrate poisoning is one of the major health risks when feeding any winter forage crop to dry cows (Nichol *et al.* 2003). It occurs when nitrate is converted to nitrite by rumen microbes at a rate beyond the formation of ammonia (Nichol 2007). The use of nitrogenous fertilizers and periods of rapid growth after some period of drought or frosting are major factors that may increase the levels of nitrate in brassica plants (Nichol *et al.* 2003). Nitrate concentration less than 10.0 g $N0_3^{-1}$/kg DM are considered safe, concentrations greater than 20.0 g $N0_3^{-1}$/kg DM are toxic and levels between 10.0-20.0 g $N0_3^{-1}$/kg DM are considered harmful if they are brassica only diets (Nichol 2007).

Clinical signs of cows affected by nitrate poisoning include cows appear uncoordinated with an increased respiratory rate, cows going down like milk fever, dead cows or if they survive cows may abort their calves. Nitrate poisoning risk may be reduced by feeding cows hay or straw and filling up all hungry cows for not more than an hour per day before a meal of brassica crop and by gradual adaptation of cows into crop at the beginning of the dry period (Nichol *et al.* 2003; White *et al.* 1999). Based on this farmers are therefore recommended to accustom cows to crop slowly and feed low nitrate supplements for example straw or silage to slow DM intake and dilute nitrate concentrations in the diet.

Red water/kale anaemia/haemoglobinuria

A further problem associated with feeding kale is the presence of S-methyl cysteine sulphoxide (SMCO). S-methyl cysteine sulphoxide is the agent for a potentially fatal haemolytic anaemia caused by the production of dimethyl disulphide by the rumen microbes (de Ruiter *et al.* 2007; Gibbs 2009). Clinical signs include weakness, diarrhoea, increased heart rate, decreased appetite, and loss of BCS and live weight (Nichol *et al.* 2003).

Bloat and frosted brassica crops

Cows grazing frosted brassica crops are at risk of having bloat, for reasons not clear at the stage. Nichol *et al*. (2003) indicated that it may be due to frosted plant cell of frosted brassica crop being more fragile and breaking down more rapidly in the rumen. Hence, more methane gas and lactic acid produced as a result of rapid rate of fermentation in the rumen. Waiting for the frost to lift from the crop and feeding more fibre will reduce risk of frost bloat (Nichol *et al.* 2003)

2.3 Environmental challenges

New Zealand dairy production is predominantly pasture based (Holmes *et al.* 2002) where animals graze outdoor all year around. Traditionally cows have been fed on a mixture of ryegrass and clover, which are lower cost and generally considered more nitrogen efficient compared to other forages (Eckard *et al.* 2007). Due to high global food demand New Zealand dairy farmers were forced to increase production by producing more milk solids per hectare by using nitrogenous fertiliser to increase forage yields (Eckard *et al.* 2007; Pacheco & Waghorn 2008).

The dietary CP requirements for grazing animals is around 11% for maintenance, 14% for growing cattle and 18% for lactating cows (Pacheco & Waghorn 2008). This equates to 1.8%, 2.2% and 3% of DM respectively, when expressed in N concentrations. However, these values are lower than the CP content of many in temperate grasses (Ledgard *et al*. 2001) and kale (Barry & Manley 1985) of greater than 20% (3.2% N) of DM. Hence, as a result of this difference between N requirements by plants and N requirements by animals, N ingested by grazing animals far exceeds N off-take in products (De Klein *et al.* 2010; Pacheco & Waghorn 2008) and around 60-99% of the ingested are excreted through urine and dung (Haynes & Willians 1993). This has raised concerns about the environmental effects of nitrogen emitted from dairy farming systems i.e. nitrogen leaching losses to surface and ground waters and nitrous oxide (N_2O) emissions to the atmosphere. Most of the nitrate leaching in grazed pastures occurs under the urine patch areas, mainly due to high nitrogen loading rates (Di & Cameron 2002; Ledgard *et al.* 1999; Scholefield *et al.* 1993). For example the nitrogen loading rate under a dairy cow urine patch maybe around 1,000 kg N/ha (Di & Cameron 2007).

The CP content of kale is higher in leaf (20%) and lower in stems (12%) (White *et al*. 1999); however the weighted average of kale leaves and stems is similar to that of grass (18%) (Rugoho *et al*. 2010). Nitrogen excretion is related to nitrogen content of diet and nitrogen intake (Kebreab *et al*. 2001). However, there is no published data on the nitrogen concentration of urine when cows are winter fed on kale or on calculations of nitrogen excreted in urine. Despite these uncertainties, recent studies suggest that wintering of animals on forage crops is a major source of nitrogen losses from livestock systems (De Klein *et al.* 2010; Monaghan *et al.* 2007; Smith *et al.* 2008). For example, Monaghan *et al.* (2007) reported that wintering of dry dairy cows in South Island contribute 60% of the total dairy system nitrogen leaching despite representing only 15% of the whole dairy system area. This was attributed to grazing of forage crops at high stocking densities (on average 500 cows/ha/day) in wet soils during winter, (Houlebrook *et al.* 2009), hence leading to excessive excretal nitrogen onto grazed forage crop area, when plant uptake is low or nil (Monaghan *et al.* 2007). Also, crops are grazed and therefore do not regrow, this limits uptake of nitrogen from soil. Of note is that this is accentuated on high yielding crops grazed to low residuals as crops are confined on small areas for longer time periods.

2.4 Conclusions

Forage brassicas, such as kale are a major component of the winter diets of non-lactating, pregnant (dry) dairy cows in southern New Zealand. Kale is a useful winter crop because it is capable of producing high DM yields of better quality forage (ME and CP) compared to perennial ryegrass in the winter period. Performance of dairy cows on kale over winter may be less than expected. Possible reasons for poor performance on brassicas are: (1) crop type (2) low DM intake due to slow adaptation to kale diets (3) low DM intake due to poor crop utilisation (4) animal health issues associated by feeding brassicas. Wintering of animals on forage crops is a major source of nitrogen losses from livestock systems.

Chapter 3

Effect of kale offered at low and high allowance versus grass at low allowance offered to pregnant, dry dairy cows in winter on DM utilisation, DM intake and gain in body condition score

3.1 Introduction

Forage brassicas, such as kale (*Brassica oleracaea*) are often a major component of the winter diets of non-lactating, pregnant (dry) dairy cows in southern New Zealand (White *et al.* 1999). Kale is a useful winter crop because it is capable of producing high DM yields of better quality forage (ME and CP) relative to perennial ryegrass (*Lolium perenne* L.) in the winter feeding period (Brown *et al.* 2007; Gowers & Armstrong 1994). Despite these attributes, evidence suggests that cows often fail to gain 0.5 body condition (BCS) over dry cow period. Judson & Edwards (2008) considered a number of reasons for this including low DM intake due to poor allocation and poor diet quality.

One approach proposed to improve BCS gain on kale is to increase DM allowance. The standard allowance is often 11 kg DM/cow/day of kale plus 3 kg DM/cow/day of barley straw. However, this is generally associated with high DM utilisation (e.g. >80%) (Judson *et al.* 2010). Further, high DM utilisation of kale leads to lower diet quality (ME) as the ME of stem is lower than leaf and declines from top to bottom of stem (Judson & Edwards 2008). Increased allowance is associated with higher DM intake (Roche *et al.* 2005; Wales *et al*. 1999) and reduced utilisation (Cosgrove & Edwards 2007; Virkajarvi *et al.* 2002; Wales *et al*. 1999) in pastures, but limited information is available for cows grazing kale. Therefore increasing allowance may increase both DM intake and diet quality.

To date, only one study (Keogh *et al.* 2009b) has examined the effect of kale allowance during winter on metabolic status, calving performance and on milk production during early lactation. High allowance cows were offered 9 kg DM of kale/cow/day plus 5 kg DM/cow/day of baled grass silage and those offered low allowance were offered 6 kg DM of kale/cow/day plus 3.5 kg DM/cow/day of baled grass silage. Cows offered kale at high allowance (9 kg kale DM +5 kg DM grass silage/cow/day) gained 0.36 BCS whereas cows on low allowance (6 kg kale DM +3.5 kg DM grass silage/cow/day) gained 0.05 BCS (1-5

Irish condition score scale). However, the allowance of 9 kg DM/cow/day is still considered a low proportion of kale to standard diets of dairy cows grazing kale during winter (11 kg DM/cow/day of kale + 3 kg DM/cow/day of straw) (Nichol *et al*. 2003).

A further approach suggested to increase BCS gain during the winter period is the use of grass instead of kale (Matthews *et al.* 1999; Nichol *et al.* 2003). Despite quality being lower, due to high dead material and stem in a pasture sustained at high mass there is easier transition periods between milking plateform and wintering, less risk of nitrate poisoning and no secondary plant compounds such as SMCO (Nichol 2007; Smith 1980). In the study by Keogh *et al.* (2007) dry dairy cows which were out-wintered on grass allocated at 12 kg DM/cow/day had lower BCS (2.85) compared to those which were offered kale at 8 kg DM/cow/day plus 4 kg DM/cow/day of perennial ryegrass (3.27) at the end of winter. However in New Zealand there is little experimental data on DM utilisation, DM intake and foraging behaviour of cows grazing kale in response to changing allowance and crop type.

The excretion of nitrogen in urine is important contributor to nitrate leaching from winter dairy support land (Monaghan *et al.* 2007). Potentially, N excretion may be lower on kale than grass due to the lower CP content of diet. However, no data from outdoor grazing to confirm this.

The objective of this study was to quantify the effect on DM intake, DM utilisation, N excretion and body condition score of cows wintered outside on kale at the industry standard (11 kg DM/cow/day of kale + 3 kg DM/cow/day of barley straw) with the higher allowance of 14 kg DM/cow/day of kale + 3 kg DM/cow/day of barley straw) and Italian ryegrass at low allowance (11 kg/cow/day + 3 kg DM/cow/day of barley straw).

3.2 Materials and Method

Site

The experiment was carried at Lincoln University Field Service Centre research area, Canterbury, New Zealand (43˚38’S and 172˚27’E, 15 m.a.s.l). It was carried between June and August 2009. The soil that the trial took place on is a Wakanui silt loam, which is classified as integrated between gley soil, yellow earths and recent soils (Yeates *et al*. 2006). Following ploughing, and cultivation, kale 1.5 ha (*cv*. Regal) and of 3 ha Italian ryegrass (*Lolium multiflorum*; *cv*. Tabu) were sown at rates of 4.0 kg/ha and 25.0 kg/ha on 15 December 2008 and 18 December 2008, respectively. Grass was fertilised with 50 kg

N/ha as urea on 15 February 2008. Kale was fertilised with 108 kg N/ha as urea on each of 26 January 2009, 22 and 17 February 2009. Grass plots were grazed lightly with sheep in February (to promote tillering) and cut for silage in March, prior to being shut up for cow grazing.

3.2.1 Experimental design

On 1 June 2009, 45 pregnant, non-lactating, spring calving Friesian × Jersey dairy cows were blocked according to age (5.3 ± 3.0), body condition score (4.6 ± 0.6; 0-10 NZ scale), live weight (530 ± 9.5 kg) and calving date (22 August ± 20 days) and assigned at random to one of three outdoor feeding treatments:

K14: high kale allowance (14 kg DM kale + 3 kg DM barley straw/cow/day;

K11: low kale allowance (11 kg DM kale + 3 kg DM barley straw/cow/day;

G11: Italian ryegrass (11 kg DM grass + 3 kg DM barley straw/cow/day.

Each treatment had 15 cows. Four of the cows in each group were rumen fistulated cows. Each feeding treatment was assigned to 30 m wide paddock strips, with daily break widths determined by weekly allowance and DM yield of crop. Typical break widths were 3.5 ± 0.3 m, 4.7 ± 0.4 m, 13.8 ± 3.1 m for K11, K14 and G11, respectively. In order to minimise animal health disorders such as nitrate poisoning, kale-fed cows were adapted to the crop over a period of six days (30 May to 4 June), with full allocation given on 7 June 2009. In this period kale DM intake started at 2 kg DM/cow/day with the rest being pasture. The kale component was increased progressively by 2 kg DM/cow/day until the full allocation of 14 kg DM/cow/day was given on the 7th day for K14 cows. For K11 cows, kale crop was progressively increased by 2 kg DM/cow/day for the first 4 days and by 1 kg DM/cow/day up to the seventh day. Cows were fed straw at 0800 h in a wire mesh feeder at the paddock entrance and forages were offered in a single break at 0900 h. The paddocks for K14 and K11 were not back fenced at any time during grazing, but G11 paddocks were back fenced at the end of each week.

Justification of the kale allowances (11 and 14 kg DM/cow/day

The pasture + straw treatment allowance of 11 kg DM/day/cow is the industry standard and from industry related work (G. Edwards unpublished). The low allowance of kale treatment is based on 70% kale utilisation in winter and the predicted ME intake of 112 MJME/day by Nichol *et al*. (2003) required to ensure cows gain 0.5 BCS units over the 8 week period in winter. However, previous study by Groves (2008) showed that cows failed to gain 0.5 BCS units. According to Judson & Edwards (2008), intake increased by 0.8 kg DM kale per 1 kg increase in kale allowance; therefore expect kale intake to increase by 2.4 kg DM/day (approximately 28.8 MJ ME/day) when going from 11 to 14 kg DM/day. This is a significant increase in intake and will lead to ME intake increasing by approximately 28.8 MJ ME/day across the range from 8-15 kg DM/day/cow.

All procedures were approved by the Lincoln University Animal Ethics committee (AEC) and licensed in accordance to the Animal Welfare Act, 1999, section 100.

3.2.3 Measurements

Crop measurements

Pre-grazing kale DM yield was recorded on Monday of each week by cutting to ground level six randomly positioned 1 × 1 m quadrats within the area estimated to be grazed over the following week by each group of cows. Total fresh weight was recorded in the field and a sub-sample of 3 plants from each quadrat was returned to the lab. These plants were separated into leaf and stem, and oven dried at 65˚C to a constant weight to determine DM% and the leaf to stem ratio. Dried samples of leaf and stem were ground and analysed for crude protein (CP), water soluble carbohydrates (WSC), acid detergent fiber (ADF), neutral detergent fiber (NDF), metabolisable energy (ME) and digestible organic matter digestibility (DOMD) by near-infrared spectrophotometer (NIRSystems 5000, Foss, Maryland, USA). Prediction equations were based on separate calibrations for silage and green forage using AOAC (1990) approved wet chemistry procedures ($R^2 > 0.95$). Metabolisable energy (ME) was calculated according to the following equation: ME (MJ/kg DM) = Digestible organic matter content × 0.016 (g/kg DM) (McDonald *et al*. 2002).

Intake measurements

On Tuesday of each week, post-grazing DM yield was measured in the areas grazed the previous week on Tuesday, Thursday and Sunday. All remaining kale was removed in two 1 × 1 m quadrats per break on the allotted days. Samples were washed to remove any soil or excrement and oven dried at 65˚C to a constant weight to determine DM%.

Italian ryegrass DM yield was measured with a calibrated rising plate meter (RPM; Jenquip F150 Electronic Pasture Meter) pre-grazing and post-grazing on the same daily schedule as the kale measurements. A total of 50 RPM measurements were taken pre-grazing on Monday and 50 measurements post grazing on Tuesday, Thursday and Sunday.

A calibration was derived from a total of 42 quadrats each 0.2 m^2 cut to ground level (kg DM/ha = 104 RPM + 727, r^2 = 82.6%). The same equation was used for pre and post grazing measurements.

From the pre- and post-grazing measurements, DM yield (kg DM/ha), DM utilisation (%, pre-post DM yield/pre DM yield * 100) and estimated apparent intake of kale or grass (kg/cow/day) were calculated. Measurement of straw residuals in the mesh feeder indicated that > 95% of straw was consumed on a daily basis by the group of cows.

Individual cow daily DM intake was measured in the 4 rumen fistulated cows in each treatment in weeks 2 and 5 of the experiment using the n-alkane technique (Dove & Mayes 1991). The n-alkanes were dosed directly into the rumen each day over a 10 day period from 8 July to 17 July. The 10 days consisted of 5 days of preliminary dosing to equilibrate throughout the gut, followed by 5 days of faeces sampling (by grab sample) and herbage sampling. Samples collected were bulked across days and later used for n-alkanes analysis. Analysis of n-alkanes was done using a method described by Dove & Mayes (2006) and individual intake was calculated from the equation by Dove & Mayes (1991):

Daily herbage intake (Kg DM/day) $I_H = \left(\frac{F_i}{F_j} * \left(D_j + I_s * C_j\right) - I_s * C_i\right) / \left(H_i - \frac{F_i}{F_j} * H_j\right)$

Where:

I_H is the daily herbage intake (kg DM/day);

C_i is the concentration of old-chan alkane in straw (mg/kg DM);

C_j is the concentration of even-chain alkane in straw (mg/kg DM);

F_i is the concentration of old-chain alkane in faeces (mg/kg DM;

F_j is the concentration of even-chain alkane in faeces (mg/kg DM);

D_j is the weight of dosed even-chain alkane C_{32} (mg/d);

I_s is the intake of straw (kg DM/day);

H_j is the concentration of even-chain alkane C_{32} in the herbage (mg/kg DM);

H_i is the concentration of odd-chain alkane C_{29} in the herbage (mg/kg DM).

Old-chain alkane C_{29} was used instead of C_{31} or C_{33} in the calculations as been demonstrated by Dove & Mayes (1991), because the concentration of C_{31} and C_{33} were very low in the stems of kale crop.

Forage disappearance

Cumulative DM intake of kale or grass was determined on two days in week 5 by measuring disappearance of the crop. On both days in each kale paddock, two 1 × 1 m quadrats were cut to ground level pre-grazing and then at 1, 2, 3, 6 and 24 h after allocation. Samples were washed and DM yield determined as per pre- and post-grazing measurements. For grass, 50 RPM measurements were taken every one hour after allocation of grass. It was calibrated by two quadrats each of 0.2 m^2 which were cut to 1 cm above ground level in each grass paddock. The samples were weighed in the field to obtain FW and sub sampled to determine DM % and dried at 65˚C. This data set was used to calibrate the Rising Plate Meter in conjunction with the quadrats cuts, each hour commencing at 9 am (see the calibration equation above). Herbage intake was based on the disappearance of herbage DM between sequential measurements.

Body condition score and liveweight

Body condition score was measured by one skilled scorer and determined during the weeks 1, 3 and 6 of the trial period using the New Zealand scoring system (1-10) (Roche *et al.* 2009). Liveweight was measured concurrently using portable scales.

Faecal and urine sampling

Faecal and urine samples were collected from all 45 cows in week 2 and week 5. The time of sampling was 1200 h on all sampling days (3 h after forage allocation). Faecal samples were collected after stimulation to induce defecation. Urine was collected (approximately 60 ml) mid-stream after manual stimulation by rubbing vulva. One ml of concentrated 6M sulphuric acid was added into the urine to prevent volatilisation of ammonia. Both faecal and urine samples were kept in ice before storage at -20^{o}C until analyses. In all cases, total sampling time for all 15 cows (each treatment group) was less than 15 minutes, after which, cows were released back onto their daily pasture and kale allowance and left undisturbed till the next sampling period.

Faecal and urine analysis

Faecal samples were thawed overnight at 4^{o}C and thoroughly mixed to obtain a representative subsample. The subsample was weighed to obtain fresh weight and was oven dried at 100°C and reweighed to determine percentage dry matter (% DM). The second subsample was freeze dried for a period of 5 days at 0.5 mbar (supplier; Cuddon Limited, New Zealand Model E. D. 5.3). After freeze drying, the samples were ground through a 1mm sieve (ZM200, Retsch) and used for analysis of nitrogen percentage by combustion of sample under oxygen supply and high temperatures using Variomax CN Analyser; Elementar (supplier; Elementer Analysensysteme GmbH; Germany). Urine samples were thawed overnight at 4^{o}C and mixed to obtain a representative sub-sample. Subsamples were used for ammonia and urea analysis using the COBAS (Analyser: Roche Cobas Mira Plus CC., New Zealand). Urine ammonia concentration was determined by enzymatic UV method using radox ammonia kit and urea concentration was determined by kinetic UV assay (Roche/BUN kit).

3.3 Calculations and data analysis

3.3.1 Estimated nitrogen losses calculations

The nitrogen partition was calculated using the water balance. The equations used were derived from Feeding standards for Australian livestock (2000) and NRC (2000). The N intake (NI) (kg/cow/day) for cows in each treatment was calculated from the N concentration of kale and straw and apparent DM intake of each diet component (kale and straw).

Nitrogen (N) excreted per day (g N/cow/day) was calculated from the following equation:

N excreted (kg N/day) = $NI\ (kg\,\frac{N}{day} - N\ retained\ in\ foetus\ \left(kg\,\frac{N}{day}\right) - N\ retained\ in\ BCS\ gain\ (kg\,\frac{N}{day})$

Where N retained in foetus (g N/day) was calculated from the following equation:

N retained in foetus (kg N/day) = $\frac{7.9 \times \{0.0344 \times EXP\ (-0.00262 \times t)\}}{6.25}$

Where *t* is the days in pregnancy.

N retained in BCS gain (kg N/day) was calculated from the following equation:

N in BCS gain kg N/day = $\frac{[0.200886-(0.0066762\times CS1)]\times LW1-[0.200886-(0.066762\times CS2)]\times LW2}{6.25}$

Where:

CS1= current BCS plus BCS gain (BCS units);
CS2= current BCS (BCS units);
LW1= current liveweight plus liveweight gain (kg LW);
LW2= current liveweight (kg LW).

The total N excreted throughout the six weeks period was calculated from the following equation;

$$\text{Total N excreted kg N/ ha} = \frac{\text{N output per cow (kg N/cow)} \times \text{Number of cows} \times \text{Number of days}}{\text{Total area grazed (ha)}}$$

3.3.2 Statistical analyses

Statistical analysis used GenStat (v14, VSL, Hempstead, UK) and ANOVA procedures. Mean values of DM yield, % DM utilisation and estimated apparent DM intake average across the six weeks were analysed by ANOVA with forage treatment as a fixed effect and week as replicate. Individual intake, body condition score and cow live weights, urinary N%, faecal N%, urinary ammonia concentration and urinary urea concentrations were analysed by ANOVA with cow (block) as random effect and forage treatment as a fixed effect. Where ANOVA was significant, a least significant difference ($\alpha < 0.05$) was used to test which means were significantly different from each other. No statistical analysis was carried out on cumulative intake rate measurements as this was carried out on only two days and there were no replicates. Also no statistical analysis was done for urinary nitrogen losses as they were calculated values.

3.4 Results

3.4.1 Nutritive composition of forage

The nutritive composition of kale and grass are presented in Table 3.1. Kale plants were comprised of 40.5% leaf and 59.5% stems. Grass was higher in DM content ($P<0.001$) and fibre (NDF) ($P<0.001$) and lower in soluble sugars (WSC) ($P<0.001$) and metabolisable energy (ME) compared to kale. Though variation in protein (CP) for leaf and stem occurred in kale i.e. kale leaf had higher CP% (23.3%) and ME (13.1 MJ ME/kg/DM) but lower than kale stem (13.6% and 11.6 MJ ME/kg DM respectively), the weighted average of both diets was similar (Table 3.1).

Table 3.1: Forage composition (%DM) and metabolisable energy content (MJ ME/kg DM) of kale and grass plants offered to dairy cows from week 1 to 6 of the trial. Kale plants represent the weighted average of kale leaf and stem. LSD = least significant difference for kale plant versus grass plant (α <0.05).

	Kale					
Parameters	**Leaf**	**Stem**	**Plant**	**Grass**	**P value**	**LSD**
CP	23.3	13.6	17.5	18.7	0.01	0.9
WSC	21.6	38.8	31.9	21.4	<0.001	2.7
ADF	19.0	26.8	23.6	22.9	NS	1.3
NDF	20.8	36.2	30.0	43.1	0.001	1.7
DOMD%	83.5	73.2	77.3	72.5	<0.001	1.9
DM	10.9	11.4	11.3	15.8	<0.001	1.7
ME	13.1	11.6	12.2	11.9	0.022	0.2

CP: Crude protein; WSC: water soluble carbohydrates; ADF: acid detergent fibre; NDF: neutral detergent fibre; ME: metabolisable energy; DOMD: digestible organic matter digestibility and DM: dry matter. Sig. = Significance from ANOVA of kale plant versus grass plant. NS = means not significant.

3.4.2 Apparent intake, individual intake, forage utilization and Intake rate

Pre-grazing DM yields across the six weeks of the trial averaged 14.9 t DM/ha for kale and 4.4 t DM/ha for grass (Table 3.2). Post-grazing DM was greatest for K14, intermediate at G11 and lowest for K11. DM utilisation was greatest for K11, intermediate at K14 and lowest for G11. Apparent DM intake was greatest for K14, intermediate at K11 and lowest for G11.

Table 3.2: Pre and post-grazing DM, percentage utilization of pre-grazing dry matter (DM) and apparent DM intake of kale offered at 11(K11) or 14 (K14) kg DM/cow/day and grass offered at 11 (G11) kg DM/cow/day to dairy cows in winter over six weeks. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA (α < 0.05).

	K14	**K11**	**G11**	**P value**	**LSD**
Pre- grazing (t DM/ha)	14.9^{a}	14.9^{a}	4.4^{b}	<0.001	0.3
Post-grazing (t DM/ha)	2.0^{a}	0.7^{c}	1.3^{b}	<0.001	0.2
Utilisation (%)	86.8^{b}	95.4^{a}	71.8^{c}	<0.001	1.9
DM Intake (kg/cow/day)	12.1^{a}	10.5^{b}	7.9^{c}	<0.001	0.2

Table 3.3 shows individual DM intake in week 5 of the trial calculated from alkane analyses compared to apparent intake in the same week calculated from pre- and post-grazing measurements. Individual cow DM intake was greater for K14 than G11 and K11. The DM intake from individual cow intakes differed from apparent intake by +1.0, - 1.5 and +0.6 for K14, K11 and G11, respectively.

Table 3.3: Yield (t DM/ha), apparent and individual DM intake of cows offered kale at 11 (K11) or 14 (K14) kg DM/cow/day and grass offered at 11 (G11) kg DM/cow/day in week 5 of the trial. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA (α<0.05).

	K14	K11	G11	P value	LSD
Yield (t DM/ha)	16.1[a]	15.8[b]	4.4[c]	P<0.001	0.004
Apparent intake (kg/cow/day)	12.5[a]	10.9[b]	7.8[c]	P<0.001	0.3
Individual intake (kg/cow/day)	13.5[a]	9.4[b]	8.4[c]	0.008	3.1
Difference	1.0	-1.5	0.6	NS	3.1

Individual intake = intake for kale or grass; Difference = individual intake minus apparent intake; NS = means not significant at 5% level.

Differences in cumulative DM intake between treatments, following a 0900 h allocation of forage are shown in Table 3.4. Intake rates were high in all three treatments. Within 1 hour of allocation, it was estimated that cows had consumed greater than 3 kg DM in all three treatments, with an indication of higher DM intake in grass. Within 3 hours of being offered a fresh break, it was estimated that cows had consumed over 75% of their daily intake, and after 6 hours cows offered 11 kg DM/cow/day of grass or kale had nearly consumed their daily total DM intake (> 95%). In contrast, cows offered 14 kg DM/cow/day continued to consume forage, eating a further 1.6 kg DM/cow, while those in other two treatments ate only 0.4 kg DM/cow (Table 3.4).

Table 3.4: Daily DM forage intake, percentage utilization of pre grazing DM and cumulative forage disappearance (% cumulative DM intake of daily total in parentheses) of kale offered at 11 (K11) or 14 (K14) kg DM/cow/day and grass offered at 11 (G11) kg DM/cow/day to dairy cows in winter.

	K14	K11	G11
DM Intake (kg/cow/day)	12.1	10.7	7.6
Utilisation %	86.7	97.1	69.2
Forage disappearance (kg DM/cow)			
Hour			
At 1 h	3.0 (24.9)	4.1 (38.4)	5.1 (66.6)
At 2 h	7.5 (61.4)	6.0 (56.3)	6.3 (83.2)
At 3 h	9.4 (77.4)	8.4 (78.4)	6.8 (89.8)
At 6 h	10.5 (86.1)	10.4 (97.6)	7.3 (95.7)
At 24 h	12.1 (100.0)	10.7 (100)	7.6 (100)

3.4.3 Body condition score and live weight

Body condition score and liveweight are shown in Table 3.5. After 3 weeks of the trial, BSC and liveweight were similar across treatments. After 6 weeks of trial, BSC was higher in G11 and K14 than K11. Cows in K14 and G11 gained 0.3 units of BCS and those in K11 gained 0.2 units of BCS. Liveweight after 6 weeks was greater in G11 than K11 and K14. Weight gain was greatest at G11 (+46.8 kg LW), intermediate for K14 (+45.4 kg LW) and lowest at K11 (+30.1 kg LW).

Table 3.5: Body condition score and live weights of dairy cows measured in week 1, 3 and 6 offered kale at 11 (K11) or 14 (K14) kg DM/cow/day and grass offered at 11 (G11) kg DM/cow/day in winter. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA (α<0.05).

	K14	K11	G11	P value	LSD
Week 1					
Body condition score (BCS)	4.7	4.6	4.7	NS	0.2
Liveweight (LW)	559	541	566.	NS	40.1
Week 3					
Body condition score	5.0	4.8	5.0	NS	0.1
Liveweight	587	560	597	NS	36.7
Week 6					
Body condition score	5.0[a]	4.8[b]	5.0[a]	0.009	0.1
Liveweight	605[b]	572[c]	613[a]	0.04	34.5
Difference (BCS) (week 1-6)	+0.3[a]	+0.2[b]	+0.3[a]	NS	0.2
Difference (LW) (week 1-6)	+45.4	+30.1	+46.8	NS	24

Difference is the difference between BCS of week 1 and week 6; NS = means not significant at 5% level.

3.4.4 Estimated nitrogen losses

Urine N%, faecal N%, urine urea and ammonia levels and estimated total nitrogen losses are shown in Table 3.6. The percentage of nitrogen in faeces was higher for G11 followed by K14 and K11 which had similar values. Urinary N% was highest for K11, intermediate for G11 and lowest for K14 (significant at P = 0.075) There was also no significant difference in the concentration of ammonia or urea in urine. Each cow consumed about 359.3 g N/cow/day from K14 treatment, 314.5 g N/cow/day from K11 treatment and around 256.9 g N/cow/day for G11 treatment. From calculated values N output was higher for kale-fed cows (311.7 g N/cow/day) and lower for grass-fed cows (227.2 g N/cow/day). Losses per hectare were also higher for kale-fed cows by 65%.

Table 3.6: Faecal nitrogen percentage (N%), Urine percentage (N%), urine ammonia (NH3), urine urea (mmol/l), N intake (g/day/cow), N output (g N/cow/day) and estimated total nitrogen loses per hectare (kg N/ha) of dairy cows which were offered kale at 11 (K11) or (K14) DM/cow/day and grass offered at 11 (G11) kg DM/cow in winter. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA.

	K14	K11	G11	P-value	LSD
Urine NH3 mmol/l	0.78	2.24	2.68	NS	2.7
Urine Urea mmol/l	7.64	7.24	5.53	NS	3.5
Faecal nitrogen %	1.96[b]	1.80[c]	2.46[a]	<0.001	0.2
Urine nitrogen %	0.43	0.58	0.52	NS	0.1
N intake in kale (g/cow/day)	338.8	294.0	236.4	-	-
N intake in straw (g/cow/day)	20.5	20.5	20.5	-	-
Total N intake of diet (g/cow/day)	359.3	314.5	256.9	-	-
N excreted in dung (g N/day)	139.1	121.8	95.1	-	-
N excreted in urine (g N/day)	195.5	167.0	132.0	-	-
Total N output per cow (g N/cow/day)	334.6	288.8	227.2	-	-
Total N excreted (6 weeks) (kg N/cow)	14.1	12.1	9.5	-	-
Total N excreted per hectare (kg N/ha)	355.9	412.6	82.3	-	-

LSD = Least significant difference; α < 0.05. NS = not significant.

3.1 Discussion

3.5.1 Apparent intake, individual intake and utilization

The utilisation of kale crop at both high and low allowance was high, with greater than 87 % of pre-grazing forage DM removed on average. The high level of DM utilisation of kale is consistent with the study of Judson & Edwards (2008). In a survey of 49 kale paddocks grazed by dairy cows in Canterbury, utilisation averaged 80% of pre-grazing forage DM allocated, with more than 90% being utilised in 40% of the cases. The result is also consistent with the study of Greenwood *et al*. (2011) where cows offered kale at 10 kg DM/cow/day utilised greater than 95% of pre-grazing kale available.

Increasing allowance from 11 to 14 kg DM/cow/day resulted in average DM utilisation declining from 96 to 88%. However, this reduced DM utilisation was not sufficient to offset the increased allowance and apparent DM intake from pre- and post-grazing measurements was on average 1.8 kg DM/cow/day higher at 14 than 11 kg DM/cow/day of kale crop. These differences were greater when individual cow intakes was measured by alkane analysis (13.5 v 9.4 kg DM cow/day), although both estimates of intake confirm that utilisation of kale will be high. As crop quality (ME and CP %) declines from top to bottom stem (Judson & Edwards 2008), diet quality would also have been greater for the 14 kg DM/cow/day allowance. However, these effects might be expected to be small in the current study as differences in utilisation between K11 and K14 were small on the leaf cultivar Regal used in this study. More pronounced differences in utilisation might be expected on stemmy cultivars such as Gruner (Gowers & Armstrong 1994). Despite, limited effect on utilisation, the current data indicate that modifying allowance may be useful tool to differentially feed cows with varying requirements for both DM intake and diet quality (e.g. young versus mature, low versus high body condition score) over the winter feeding period.

Cows grazing grass had lower DM utilisation and lower DM intake than cows grazing kale. The lower DM utilisation most probably reflects the existence of a grazeable horizon at the base of the sward in grass (Edwards *et al*. 1995), combined with wet soil conditions causing trampling of grass into the soil surface when break fed. The practical implication of this result is that in order to achieve the same daily DM intake of grass as for kale (offered at 11 kg DM/cow/day), the allowance of grass would have to be approximately 15.1 kg grass DM/cow/day. Taking into account the lower winter yields of grass to kale, the increased area required to achieve the same intake on grass will put further pressure on wintering costs.

3.5.2 Intake rate

Though the grazing behaviour disappearance had limited replication, the large differences in rate of intake between the three forage systems allow some basis for speculation. Within 3 hours of being offered a new allocation (0900-1200h), cows were estimated to have consumed >78% of their total daily intake in all forage treatments. These corresponded to DM intakes of 8.4, 9.4, and 6.4 kg DM for K11, K14 and G11 respectively. These high values are consistent with previous work. Dobos *et al*. (2009) reported that >70% of daily intake of perennial ryegrass by dairy cows was achieved in the first 4 hours after offering a fresh break. The implications of these patterns of intake for kale and grass on rumen physiology are considered in Chapter 4. By 3 pm in the afternoon, six hours after being offered a fresh break, cows offered grass and kale at 11 kg DM/cow/day had consumed close to their total daily intake. Cows offered kale at 14 kg DM/cow/day allowance grazed for longer and consumed a further 1.6 kg DM. Based on these results, monitoring kale grazing residuals mid-afternoon following a morning allocation of forage, and modifying break widths may be a useful management tool to manage intake and diet quality. High residuals (in this study for 2 t DM/ha for 15 t DM/ha crop) will be indicative of cows achieving better diet quality and higher DM intake.

3.5.3 BCS and Live weight

The effects of allowance and crop type of BCS gain and LW gain were relatively small in this study. Over 6 weeks LW gain was 15 kg more at K14 than K11 and K14 cows gained 0.1 BCS units more than K11. The results of the current study are consistent with the study by Greenwood *et al*. (2011), who noted that cows which were offered high allowance of kale (14 kg DM/cow/day) gained 0.8 BCS units more than those offered kale at low allowance (11 kg DM/cow/day) over the end of winter period. Also in agreement, an Irish study by Keogh *et al*. (2009b) reported that cows offered high allocation of kale (9 kg DM kale/cow/day) gained 0.3 BCS units (1-5 Irish scale) more than those offered low allocation of kale (6 kg DM/cow/day). Grass-fed cows compared similar to K14 cows. This, result is not consistent with the studies by Gazzola *et al*. (2008) and Keogh *et al*. (2007), which reported a lower gain in BCS of cows fed on perennial ryegrass pastures over the winter period compared to those offered kale. However, another Irish study, Keogh *et al*. (2009b) showed that grass silage-fed cows had a greater gain in BCS during winter than cows offered kale.

However, it should be noted that in the present study, no cows gained greater than 0.5 BCS over 6 weeks. This is perhaps surprising given calculated ME intake for each treatment. Based on apparent DM intake of 12.2 kg DM/cow/day for K14 and 10.5 kg DM/cow/day for K11 plus 3 kg DM/cow/day of straw at ME values of 12.2 MJ ME/kg DM for kale and 7.3 MJ ME/kg DM for straw calculated ME intakes were 171 MJ/cow/day for K14 and 150 MJ/cow/day for K11. These are in excess of 112 MJ ME/day calculated by Nichol *et al.* (2003) and 115 MJ ME/day calculated by (Nicol & Brooks 2007) to gain 0.5 BCS units over the winter period. Further, G11 cows had greater gain in BCS units than K11 cows (0.3 versus 0.2 BCS units respectively) and similar to K14 cows, even though calculated ME intake of G11 was 115 MJ ME/cow/day.

The reason for the inequality is not clear. Greenwood *et al.* (2011) suggested the difference may reflect the inability to sustain ME intake during transition from pasture to kale at the start of winter feeding period. ME intake could be restricted in this time period, when cows are adapted to kale crop. However, management during transition period of a gradual increase of kale in diet with remainder grass should have ensured that ME requirements were met.

A further factor that may cause disparity between BCS gain and ME intake is the method of prediction of ME. The ME of 12.2 MJ ME/kg DM for kale used in the present study was calculated from the equation for roughages (MJ ME/kg DM) = Digestible organic matter content × 0.016 (g/kg DM) by Mcdonald *et al.* (2002). However, due to low fiber and lipid content of kale, the coefficient in the above equation derived using more fibrous feeds may be inadequate for determination of energy in brassica crops (Greenwood *et al*. 2011).

A further possible reason may be due to the effect of anti-nutritional factors such as nitrates and SMCO that are suspected to limit performance on kale, even when fed at high allowance (Judson *et al.* 2010). Rumen fermentation of SMCO can cause haemolytic anaemia (Smith 1980; Barry & Manley 1985), associated with reduced intake in sheep and reduce animal performance (Barry & Manley 1985). However, this did not appear to be the case in this study as DM utilisation did not vary throughout the six weeks of trial. DM utilisation was 88.6, 87.0, 84.9, 87.4, 89.3, 83.4 and 86.8% for K14 and 91.9, 93.7, 94. 5, 96.6, 98.8 and 97.1% for K11 cows over the six weeks of the trial. Also in support of this, in the present study there were no clinical abnormalities such as cow weakness, diarrhoea and increased heart rate was observed. This was also noted by Keogh *et al*. (2009c) for dairy cows fed on 100% kale diet.

3.5.4 Estimated faecal and urinary nitrogen loss

Grassland-based systems of ruminant production are characterised by the biological inefficiency of converting plant materials which are relatively low in protein to animal products which are high in protein (Kingston-Smith & Theodorou 2000). As a result a large proportion of dietary nitrogen is lost to the environment through urine and faeces (De Klein *et al.* 2010; Pacheco & Waghorn 2008). Nitrogen excretion is directly proportional to the intake of nitrogen (Jonker *et al*. 1998). K14 cows had greater N intake (359.3 g N/cow/day) compared to K11 cows (314.5 g N/cow/day) and would have been expected to consume a diet with a higher CP content as the content of CP decreases down the stem (Judson and Edwards 2008). Based on this it would appear reasonable for K14 cows to have high N% in their urine. However, the N% of urine was higher in K11, for a reason which is not clear at this stage. However, may be due to high water content of kale (11.3 % DM) leading to dilution of urine (Ledgard *et al*. 2007).

From calculated values, kale fed cows consumed 13.5% more nitrogen than grass-fed cows. This was mainly because of low DM intake by grass-fed cows. As the nitrogen excretion is directly proportional to the intake of nitrogen (Jonker *et al*. 1998; Kebreab *et al*.2001), kale-fed cows in the present study excreted more nitrogen through faeces and urine (311.7 g N/cow/day) compared to grass-fed cows (227.2 g N/cow/day). The calculated average value for N excreted through faeces and urine from cows grazing kale in winter in the present study is 5% higher than the one reported in the recent study by Miller *et al*. (2012) of 284 g N/cow/day, which was calculated using different methods i.e. using creatinine excretion as an indicator of urine volume. Total N excreted per hectare was also higher for kale-fed cows (384 kg N/ha) and lower for grass-fed cows (82.3 kg N/ha). These values are higher compared to those reported by Stefasnki *et al*. (2010) (27.9–30 kg N/ha) when dairy cows were fed on forage rape with crop yield of 8-10 t DM/ha and % DM utilisation of 50%. These results demonstrate that crop yield and % DM utilisation will be important determinants of N losses/ha, thus, difficult to assign a single value of N losses/ha to wintering system.

3.6 Conclusion

- The utilisation of kale DM exceeded 87% of pre-grazing forage DM when offered at both 11 and 14 kg DM/cow/day.
- Increasing allowance from 11 to 14 kg DM/cow/day resulted in average utilisation declining from 96 to 88%. However this reduced utilisation was not sufficient to offset the increased allocation and apparent DM intake was on average 1.8 kg DM/cow/day higher at 14 than at 11 kg DM/cow/day.
- Body condition score gain over the 6 weeks period was 0.1 units higher for K14 than K11 cows. G11 cows compared similar to K14 cows.
- Nitrogen intake and output was high for kale-fed cows compared to grass-fed cows.

Chapter 4
Effect of kale at low and high allowance versus grass at low allowance offered to pregnant, dry dairy cows in winter on rumen physiology and foraging behaviour

4.1 Introduction

Despite increased interest in New Zealand in housing cows indoors during winter (de Wolde 2008), most cows are wintered outdoors on forage crops such as kale and grass supplemented with silage, hay or straw (Nichol *et al.* 2003; White *et al.* 1999). Nichol *et al* (2003) identified forage brassicas, particularly kale and swede, as the main source of winter feed for dairy cows in the South Island of New Zealand. This is mainly because brassica crops are capable of growing at lower temperatures than perennial ryegrass, and produce a large amount of high quality dry matter (DM) per unit area at time it is required in mid-winter (Valentine & Kemp 2007).

A potential limitation of the feeding value of forage brassicas is that the concentration of neutral detergent fibre (NDF) is low, often less than 300 g /kg DM (Barry & Manley 1985; NRC 2001); this is in addition to a high DM digestibility (>800 g/kg) (Valentine & Kemp 2007). A NDF value of at least 330 g/ kg DM (NRC 2001) has been considered optimum for rumen function. Further, forage brassicas contain high concentrations of non-structural carbohydrates (NSC) (e.g. > 200 g/kg DM (Barry & Manley 1985). The combination of low NDF and high NSC levels has been suggested to lead to problems with rumen acidosis (pH <6) in total mixed ration diets (Krause & Oetzel 2006) and in kale (de Ruiter *et al.* 2007; Nichol 2007; Nichol *et al.* 2003) and may lead to altered volatile fatty acid (VFA) production (Keogh *et al.* 2009c).

While several studies (e.g. Van Soest *et al*. 1991; Mertens 1997) have outlined the effects of different NDF and NSC contents within various forages (i.e. perennial ryegrass and whole crop cereals) on rumen function, there is scarcity of literature on the effect of feeding diets of high proportion of kale. Keogh *et al* (2009c) in an indoor study examined the effect of dietary proportion of kale and grass silage on rumen pH and VFA concentrations in dry dairy cows during winter period. They noted that increasing the dietary proportion of kale decreased dry matter (DM) intake with minimal effects on rumen pH, total VFA concentration and individual VFA proportions. Of note is that this was an indoor feeding trial and it is plausible that outdoor grazing will create a pattern of intake that will affect rumen function.

Results from Chapter 3 indicate that intake rates of kale (1.7 kg DM/h) and grass (1.2 kg DM/h) are high in cows in the immediate period after a fresh break is offered. Several authors (e.g. Dalley *et al*. 2001; Jamieson & Hodgson 1979; Wales *et al*. 1999) have measured the effects on grazing behaviour of cows grazing perennial ryegrass and how this is affected by forage allowance. However, there is little data on the foraging behaviour of cows grazing kale, with only one Irish study (Gazzola *et al.* 2008) on the foraging behaviour of dry cows out-wintered on kale. This study showed that grass-fed cows spent more time eating (585 min/day) compared to kale-fed cows (515 min/day) and ruminating time was higher for kale-fed cows (338 min/day) than grass-fed cows (270 min/day). However, this study was conducted with low proportions of kale in their diet (8 kg DM/cow/day) compared to the New Zealand industry standard of 11 kg DM/cow/day.

The objective of this study was to quantify the effect on foraging behaviour, rumen pH, rumen ammonia and volatile fatty acid production in the rumen of cows wintered outside on kale at the industry standard kale allowance of 11 kg DM/cow/day with the higher kale allowance of 14 kg DM/cow/day and Italian ryegrass at the low allowance of 11 kg DM/cow/day.

4.2 Materials and Methods

4.2.1 Experimental design

The experiment was carried out within the allowance study described in Chapter 3. Details of experimental design are described in Section 3.2.1.

4.2.2 Measurements

4.2.2.1 Crop measurements

All crop measurements have been carried out as been described in Section 3.2.3.

4.2.2.2 Animal measurements

Foraging behaviour

The behaviour of all 45 cows was visually observed over a one 24 h period from 0800 h during both weeks 2 and 5 of the experiment period. All cows were observed in the same day. Every 15 minutes individual cows were recorded as either: eating forage, eating straw, ruminating or idling. From these data, times in each activity were calculated by multiplying each behaviour activity frequency by a 15-min interval.

Rumen sampling

Rumen sampling was carried out over one 24 h period during both weeks 2 and 5 of experimental period. Four rumen-fistulated cows in each of the K11, K14 and G11 treatments were fitted with backpacks which contained data loggers for temperature and pH (Delta Log, Milan, Italy). These data loggers were linked to pH and temperature probes (IJ44: Ionode Pty Ltd, Brisbane, Australia) weighted with a 1500 g steel paddle and inserted to the ventral sac floor. The pH and temperature were recorded every 15 seconds for 24 h. Rumen sampling was done on separate days to the days when foraging behaviour was recorded i.e. on a day before foraging behaviour measurements.

Spot samples of rumen fluid were collected over a 24 h period starting immediately following the completion of the rumen pH and temperature measurements for 24 h. Sampling periods were conducted every four hours from 0800 h. The four fistulated cows in each treatment group were brought together from the forage into a set of yards for the collection. All rumen samples were taken via a hand grab method from the bottom of the ventral sac region of the rumen. Samples for VFA and NH_4 analysis were removed and squeezed through two layers of cheese cloth. Samples for VFA were immediately placed on ice. A total of 1ml of 6M sulphuric acid was added to samples for NH_4 analysis (to prevent volatilisation by ensuring pH remained < 4). In all cases, the total sampling time for the four fistulated cows was less than 15 minutes, after which, cows were released back onto their daily pasture and kale allowance and left undisturbed until the next sampling period. After sampling, all samples were placed into a blast freezer set at -20^oC until required for analysis.

Rumen contents sample analysis

The concentration of VFA in the sampled rumen fluid was measured by thawing the frozen vials then vortexing and inverting this sample and removing a 2ml sub-sample for analysis. The sub-samples were centrifuged at 13000 rpm for 30 minutes at 4^oC. From this sample, 500µl of the supernatant was placed into a 1.5 ml centrifuge tube and 100µl of the internal standard, 200µl of metaphosphoric acid and 200µl of deionised water were added. Samples were then vortexed and placed at 4^{o}C for 30 minutes, before being centrifuge at 13000 rpm for 15 minutes and filtered through a 0.45 µm nylon syringe filter. Samples were then placed into tubes ready for injection into the high performance liquid chromatography (HPLC) machine (Hewlett Packard 1100 Series HPLC system) (Chen & Lifschlth 1989).

The frozen acidified samples for NH_4 analysis were thawed out over night at 4^{o}C and maintained at this temperature or below throughout the extraction protocol. Samples were vortexed and inverted to ensure homogeneity and a sub-sample was removed into a 2ml centrifuge tube. The sub-samples were centrifuged at 13000 rpm in a refrigerated bench top centrifuge set a 4^{o}C for 30 minutes. After centrifugation, samples were filtered through a 0.45µl syringe top filter placed into a 2 ml syringe. A 1000µl aliquot was then added to 9 ml of deionised sterilised water. All samples were then analysed for NH_4 concentration via Flow Injection Analyser (FIA) analysis immediately following the dilution process as been described by Blakemore *et al*. (1987).

4.3 Calculations and data analysis

4.3.1 Rumen pH

The rumen pH measurements recorded every 15 seconds were averaged for consecutive 10 minutes for each treatment group, giving 160 pH records (4 per minute for 10 minutes for 4 cows per treatment). In addition, the recorded pH of all cows in each treatment group were used for each 10 minute block to calculate the proportion of total records within six pH bands : < 5.0; 5.0-5.5; 5.5-5.8; 5.8-6.0; 6.0-6.4 and > 6.4. A rumen pH of 5.0 is a threshold for clinical acidosis, pH 5.5 is a threshold for subacute acidosis, pH 5.8 is the threshold below which NDF degradation is compromised (de Veth & Kolver 1999), pH 6.0 is the threshold above which no NDF impact is observed and pH 6.4 is generally considered the upper pH limits in active rumens.'Bout counts' were also calculated using the recorded pH values of each cow in each treatment group. If the recorded pH was less than each of the above thresholds for 2 min, it was counted as a single bout and the time in minutes of that bout was calculated. The means for bout counts plus bout times for all cows in each treatment were then calculated.

4.3.2 Acetic acid, propionic acid and butyric acid means

From the VFA values measured at each time point, the mean for acetic acid, propionic acid and butyric acid was calculated from the area under the curve (AUC). The AUC approach was carried out to avoid type II errors associated with repeat sampling due to a lack of independence. The area under the curve was derived from the trapezoid formula as explained by Pruessner *et al.* (2003), and the calculated values were then used for subsequent statistical analysis. The mean from AUC values was analysed in two time periods: am (from 0800 h to 1600 h) and pm (from 2000 h to 0400 h) so as to assess relations with grazing events.

4.3.3 Statistical analysis

Statistical analysis was carried out using GenStat (v14, VSL, Hempstead, UK). The minutes in each foraging behaviour over each 24 h period and in 4 h periods throughout the day were analysed by the ANOVA model including cow (block) as random effect and forage treatment as a fixed effect. Foraging behaviour data was averaged over the two sampling days prior to analysis. Where ANOVA was significant, a least significant difference ($\alpha < 0.05$) was used to test which means were significantly different.

4.4 Results

4.4.1 Foraging behaviour

The total time spent in each foraging behavior over 24 h period averaged across week 2 and week 5 is shown in Table 4.1. Forage treatment did not affect the number of minutes eating straw. The time eating forage was greatest for K14, intermediate for K11 and lowest for G11. Grass-fed cows ruminated for longer than either K11 or K14. Cows in K11 spent the greatest time idling, with G1 intermediate, and K14 the least.

Table 4.1: Foraging behavior (minutes per day) of cows offered kale at 11 (K11) or 14 (K14) kg DM/cow/day and grass at 11 (G11) kg DM/cow. Data are average of sampling period in weeks 2 and 5. Means within row are significantly different according to LSD test (P<0.05) following significant ANOVA.

	K14	K11	G11	P-value	LSD
Eating straw	75^a	66^a	65^a	NS	15.1
Eating forage	441^a	378^b	326^c	<0.001	35.1
Ruminating	334^a	326^a	421^b	<0.001	34.2
Idling	590^a	670^b	628^b	0.004	44.2

LSD = least significance different ($\alpha < 0.05$). NS = not significant at 5% level.

The foraging behavior of cows in 4 h blocks over a 24 hour period is shown in Table 4.2. The first 4 h after allocation of break was dominated by time spent eating forage. Time eating forage was greatest for K11 and G11 than K14 . Cows in K14 ruminated for longer than cows in K11 and G11, although there was little rumination during this time period.

From 4 to 8 h, eating time was greater for K11 and K14 than G11. G11 cows ruminated for longer than K11 and K14 cows. From 8 to 12 h, eating time was greater and ruminating time lower for K14 than K11 and G11. From 12 to 16 h, K14 cows continued to spend more time eating than K11 and G11 cows, although foraging behaviour of this time period was dominated by idling. From 6 to 20 h, ruminating time was greater for G11 than K11 and K14. During the 20-24 h period, there was little eating behaviour in any treatment.

Table 4.2: Foraging behavior (minutes accumulated in 4 h periods) of cows offered kale at 11 (K11) or 14 (K14) kg DM/cow/day and grass at 11 (G11) kg DM/cow measured in weeks 2 and 5 of the trial period. Means within row are significantly different according to LSD test (α<0.05) following significant ANOVA.

Time	Hours	Behaviour	K14	K11	G11	P-value	LSD
8:00 a.m-12:00 p.m	0 - 4	Eating	170[a]	193[b]	196[b]	<0.001	12.6
		Ruminating	20[a]	7[b]	7[b]	<0.001	6.1
		Idling	50[a]	40[b]	37[b]	0.05	11.2
12:00 p.m-4:00 p.m	4 - 8	Eating	169[a]	175[a]	127[b]	<0.001	21.4
		Ruminating	22[a]	15[a]	35[b]	0.028	14.1
		Idling	49[a]	50[a]	78[b]	0.003	18.0
4:00 p.m-8:00 p.m	8 - 12	Eating	102[a]	57[b]	46[b]	<0.001	17.9
		Ruminating	52[a]	91[b]	86[b]	<0.001	5.3
		Idling	86	92	108	NS	21.1
8:00 p.m-12:00 a.m	12 - 16	Eating	52[a]	16[b]	8[b]	<0.001	15.2
		Ruminating	64[a]	77[a]	106[b]	<0.001	14.8
		Idling	124[a]	147[b]	126[a]	0.010	16.3
12:00 a.m- 4:00 a.m	16 - 20	Eating	20[a]	5[b]	7[b]	0.008	10.0
		Ruminating	76[a]	58[b]	97[c]	<0.001	17.9
		Idling	144[a]	177[b]	136[a]	<0.001	14.5
4:00 a.m- 8:00 a.m	20 - 24	Eating	3[a]	0[a]	8[b]	<0.001	3.2
		Ruminating	100[a]	78[b]	90[b]	0.005	13.1
		Idling	137[a]	162[b]	142[a]	0.001	13.4

NS = not significant at 5% level.

4.4.2 Rumen VFA, pH and ammonia concentration

Rumen VFA concentrations from the area under curve (AUC) analysis are shown in Table 4.3. The concentration of acetic acid in morning and evening samples was higher in K14 and G11 than K11. The concentration of propionic acid was higher for K14 and G11 than K11. Although the concentration of butyric acid was higher for kale-fed cows, forage treatment had no significant effect on butyric acid concentration in the rumen from AUC values.

Table 4.3: The concentration of rumen acetic acid, propionic acid and butyric acid and ammonia (AUC units) for cows offered kale at 11 (K11) or 14 (K14) kg DM/cow/day and grass at 11 (G11) kg DM/cow measured in weeks 2 and 5 of the trial period. Means within row are significantly different according to LSD test (α<0.05) following significant ANOVA.

	K14	K11	G11	P-value	LSD
Acetic acid a.m	817[a]	634[b]	814[a]	0.028	142.9
Acetic acid p.m	866[a]	661[b]	790[a]	0.004	99.3
Propionic acid a.m	230.9[a]	148.8[b]	225.7[a]	<0.001	34.4
Propionic acid p.m	234.8[a]	153.6[b]	194.1[c]	0.002	22.7
Butyric acid a.m	136.5	99.5	102.2	NS	43.5
Butyric acid p.m	139.5	97.7	84.8	NS	53.0
Ammonia a.m	128.8	152.4	140.4	NS	36.3
Ammonia p.m	90.5[a]	70.8[a]	47.5[b]	0.018	27.2

Mean values obtained from area under the curve (AUC) of VFAs and ammonia concentration time curve; a.m (0800 hr to 1600 hr) and pm (2000 hr to 0400 hr). NS = not significant at 5% level.

No significant forage treatment effects were detected for ammonia concentration in the rumen during the morning. During the afternoon rumen ammonia concenatration was greater in K11 and K14 than G11 (Table 4.3).

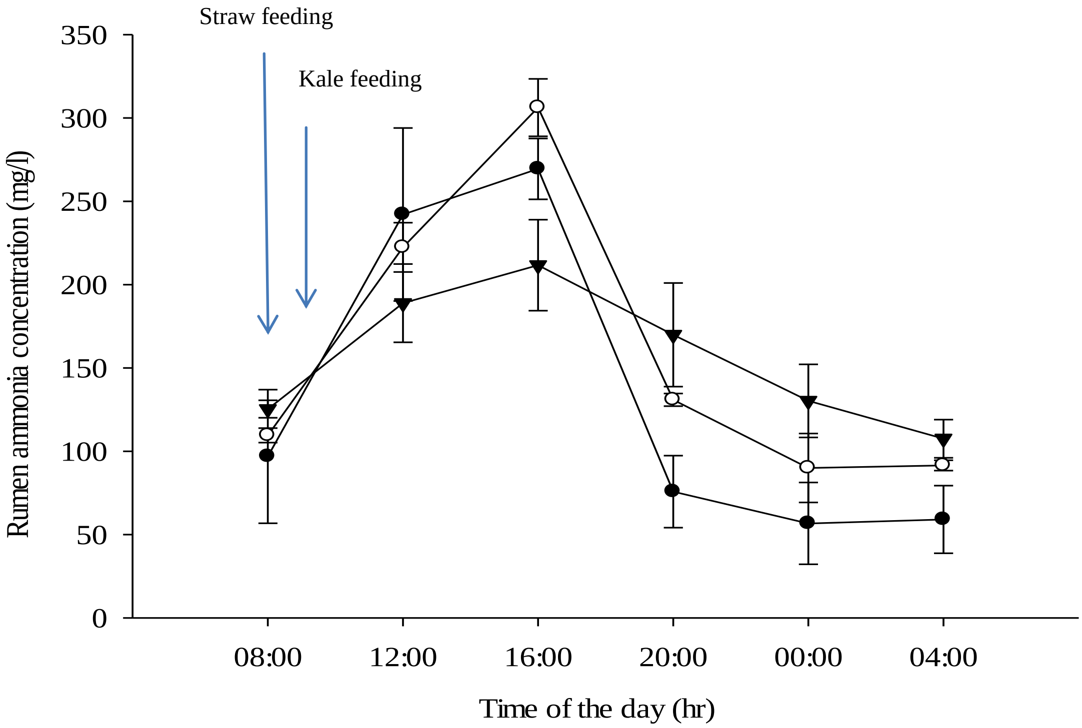


Figure 4.1: Rumen ammonia concentration (mg/l) cows offered kale at 11 (K11, ○) or 14 (K14, ▼) kg DM/cow/day and grass at 11 (G11, ●) kg DM/cow. Values are means ± SEM.

The rumen fermentation characteristics following barley straw and kale feeding are shown in Figures 4.1 to 4.2. The peak ammonia concentration in all three treatments was at 1600 h. 7 h post-feeding of forage (0900 h) (Figure 4.1), the peak concentration was greatest in K11, intermediate in G11 and lowest in K14. The ammonia concentration declined sharply in all treatments from between 1600 h and 2000 h. The ammonia concentration was lowset from 2000 h until straw feeding in the G11.

The concentration of VFAs differed over time relative to feeding. The peak acetic acid concentration occurred 11, 7 and 3 h after offering forage for K14, K11 and G11, respectively. The peak propionic acid value was at 11 h post feeding of forage for K14, K11 and at 3 h post feeding for G11. The peak butyric acid value was at 11 h after offering forage for K14 and K11 and 3 h post feeding for G11 (Figure 4.2a, b and c respectively). The peak concentration of acetic acid was greater for K14 (84.4 mmol/l) than K11 (62.4 mmol/l) and G11 (78.0 mmol/l). The peak concentration of propionic acid was the same for K14 (24 mmol/l) and G11 (24 mmol/l) and smaller for K11 (15 mmol/l). The concentration for butyric acid was greater for K14 (16 mmol/l) than K11 (11.3 mmol/l) and G11 (10 mmol/l)

(a) (b) (c)

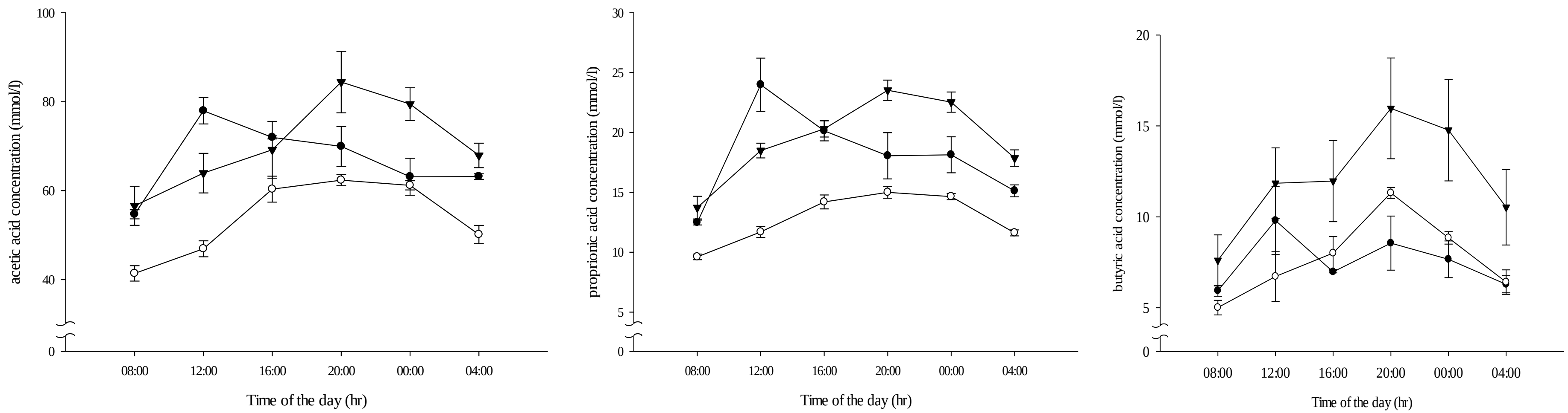


Figure 4.2: Diurnal pattern of acetic acid **(a)**, propionic acid **(b)** and butyric acid **(c)** concentration of cows offered kale at 14 (K14;▼) or 11 (K11;○) kg DM/cow/day and grass at 11 (G11; ●) kg DM/cow. Values are means ± SEM.

The diurnal pattern of rumen pH, as shown by the average of 10 min time periods, for all diet treatments is displayed in Figure 4.3. Rumen pH was in general higher in K11 and K14 than G11. Soon after feeding (0900 h), the decline in pH was greatest in G11, reaching pH of around 5.7. In contrast, rumen pH in K11 and K14 remained high after feeding before declining to 6.2 for K14 and 6.0 for K11 and rising in a similar pattern.

The mean proportion of recorded pH values in each threshold band (< 5.0, 5.0-5.5, 5.5-5.8, 5.8-6.0, 6.0-6.4, and >6.4) are displayed in Figure 4.4. G11 had a greater proportion of recorded pH below 5.8 than K11 or K14, while K11 had a greater proportion of recorded pH values below 6.0 compared to K14. Rumen pH was more frequently lower in all treatment groups 4 to 6 h after morning feeding.

The summary of mean pH bouts under thresholds of 5.0, 5.5, 5.8, 6.0 and 6.4 is displayed in Table 4.4. G11 had more frequent and longer bouts of rumen pH under each threshold except 6.4 compared with K11 or K14. K11 had more frequent and longer bouts of rumen pH below each threshold except 6.4 compared with K14 cows.

Figure 4.3: Diurnal rumen pH (10 min intervals) after cows were offered kale at 11 (K11) or 14 (K14) kg DM/cow/day and grass at 11 (G11) kg DM/cow.Values are means ± SEM.

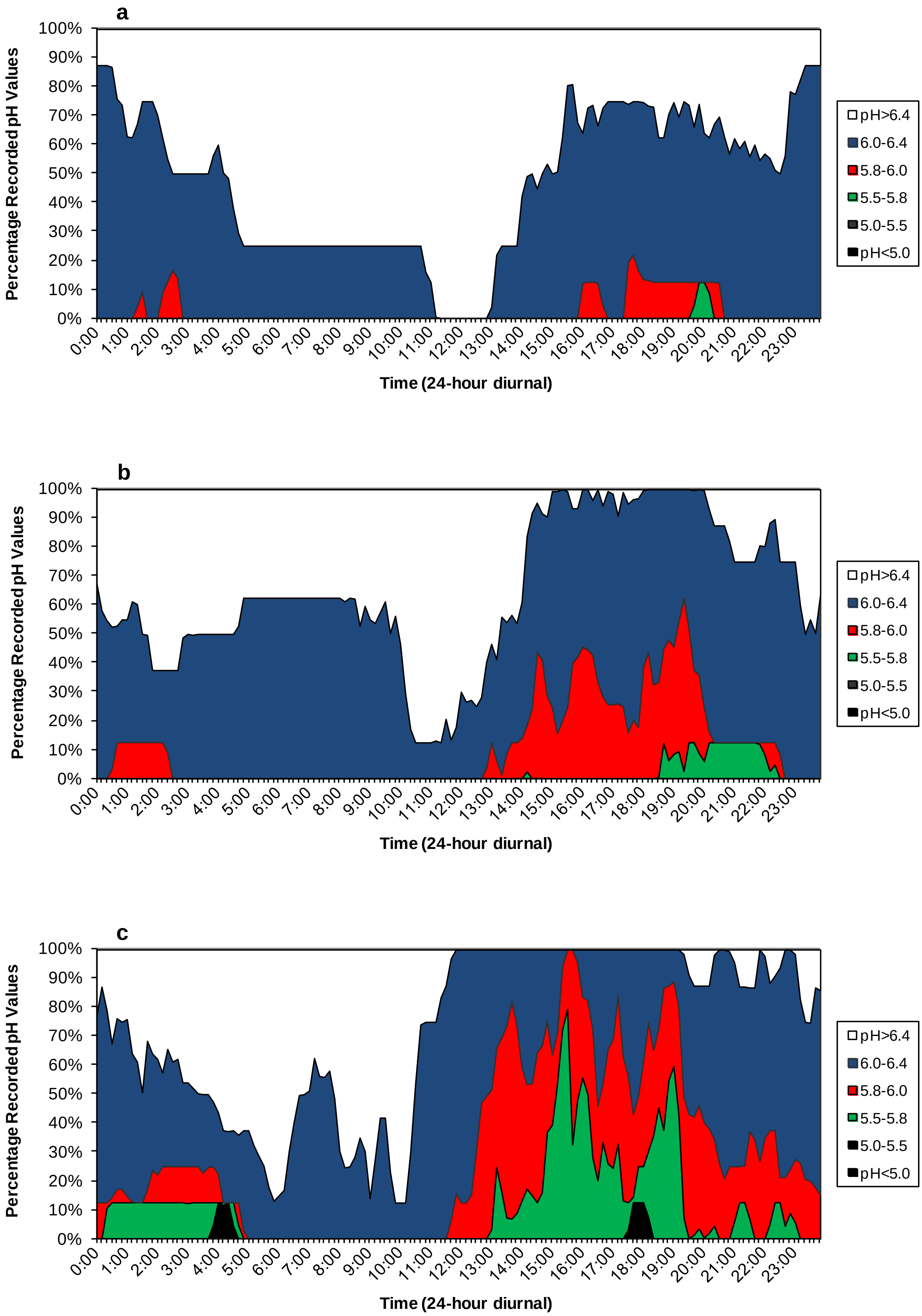


Figure 4.4: Proportion of recorded rumen pH values in each 10 min block across the diurnal period within each treatment group of cows offered kale at 14 (K14; **a**) or 11 (K11; **b**) kg DM/cow/day and grass at 11 (G11, **c**) kg DM/cow.

Table 4.4: Rumen bout summary of dairy cows offered kale at 14 (K14) or 11 (K11) kg DM/cow/day and grass at 11 (G11) kg DM/cow.

	Bouts <5.0:				Bouts <5.5:				Bouts <5.8:				Bouts <6.0:				Bouts <6.4:			
Treatment:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:
G11	0.0	0.0	0.0	0.0	2.0	0.3	37.6	0.6	29.0	3.7	37.2	10.2	58.0	7.4	45.8	8.7	36.0	4.6	151.1	48.6
K14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.1	29.8	0.0	7.0	0.9	40.4	23.0	30.0	3.8	152.6	43.7
K11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0	0.9	26.3	15.2	25.0	3.2	48.4	14.2	42.0	5.3	102.8	36.0

4.5 Discussion

4.5.1 Foraging behaviour

The foraging behaviour of the cows was affected by forage type, with cows grazing kale spending more time eating forage than cows grazing grass. This was even the case when forages where offered at the same allowance; cows in K11 grazed on average 52 min longer than cows in G11. This result is in contrast to the study of Gazzola *et al*. (2008) who reported no significant effect on the total time spent eating forage between kale and grass. The reason for the difference is unclear but may reflect higher allowances used in the current study (11 and 14 kg DM/ cow/day for kale) compared to the study of Gazzola *et al*. (2008) (8 kg DM cow/day for kale). The higher allowances used in this study may have provided a greater opportunity to graze for longer on kale where foraging is not restricted to an ungrazeable horizon at the base of the sward (Edwards *et al*. 1995). In contrast, for grass, an ungrazeable horizon at base of sward may restrict intake and grazing time; this may be further restricted by wet soil conditions leading to trampling of forage and cows stopping foraging.

A feature of the temporal pattern of foraging was that during the first 4 hours of grazing, cows offered a low allowance (G11 and K11) spent more time eating forage and less time ruminating compared to cows offered kale at high allowance (K14). This occurred despite all cows being offered straw one hour prior to forage allocation. Due to the lower apparent daily DM intake of K11 (10.5 kg DM/cow/day) and G11 (7.9 kg DM/cow/day), and that they had grazed little overnight, it is reasonable to assume that the cows on these treatments were hungry and their high hunger levels forced them to increase their eating time during the next available time for grazing (Cosgrove & Edwards 2007; Gregorini *et al.* 2009). Cows in K14 grazed for longer in the afternoon. This is probably reflects that feed was still available as shown by residuals around 5.2 t DM/ha remaining at 3 h post feeding in the afternoon (Chapter 3).

Gazzola *et al*. (2008) reported that dairy cows spent more time ruminating when fed grass than kale, due to lower utilization and DM intake of grass forage. However, the results of this study show that ruminating time was greater for grass-fed cows than for kale-fed cows despite lower utilization of grass (71.8) compared to kale (> 80%). As kale plants have a high percentage of non-structural carbohydrates (NSC; 31.9%), they may require less rumination (Gazzola *et al.* 2008). Further the grass had higher NDF and ADF than kale, and this may have contributed to an increased rumination time for grass (Gazzola *et al.* 2008; Beauchemin & Yang 2005; Woodford *et al*. 1986).

Within the kale treatments, there was more time grazing and less time ruminating in K14 than K11. The cows offered the low allowance of kale (11 kg DM/cow/day) did not increase their grazing time in response to low feed availability (Cosgrove & Edwards 2007), perhaps due to the rapidly diminishing feed availability, with little kale remaining after 8 h (0.8 t DM/ha). Alternatively, the cows on the low allowance may have harvested the kale tops quickly and then reduced grazing time when only the stems were available. Hence, the amount of work in harvesting kale becomes more important than for other feeds, since, as the feed becomes scarce, intake may be reduced not by limited grazing time but by the reduced cost-benefit ratio of feeding behaviour (Ketelaars & Tolkamp 1996).

The increase in ruminating time of cows in K11 relative to K14 may reflect that K11 cows has higher DM utilisation, and hence would have consumed a diet of a higher proportion of stem and fibre. However, Gibbs & Roche (2010) suggest that rumination may also be a function of increased non grazing time, which would also explain these observed differences.

4.5.2 Rumen fermentation

VFA production

The concentrations of rumen acetic acid and propionic acid were greater for K14 than K11 This study contrasts with that of Keogh *et al*. (2009c) who found that offering greater dietary kale proportions (i.e. 85-100%) did not have an effect on rumen VFA concentration. However, it should be noted that cows were offered a total of 10 kg DM of kale daily in the study of Keogh *et al*. (2009c) and this is considered a low allocation according to New Zealand standards. Whereas, in the present study cows in high allocation were offered 14 kg DM/cow per day and their intake was higher compared to low allocation cows.

The peaks of acetic, propionic and butyric acid occurred later during the day after offering forage for K14 (at 11 h) than K11 (at 7 h) and G11 (at 3 h). This may be because K14 cows kept on eating for longer (346 min) after the first 4 h of grazing compared to K11 (253 min) and G11 cows (196 min).

The concentration of each of the VFA was lower for K11 than K14. This most probably reflects lower DM intakes, as there is a close relationship between rumen VFA and DM intakes (Friggens *et al*. 1998; Sun *et al*. 2012). Generally, butyric acid was higher for kale-fed cows than grass-fed cows. One of the diet components that can promote high rumen butyric acid is WSC (Chamberlain *et al*. 1983) and this was 11% higher in kale than grass crop (Table 3.1). Similar results were also obtained in the study by Sun *et al*. (2012) in terms of butyric acid concentration. During this study (Sun *et al*. 2012), the concentration of butyric acid was high in sheep fed fresh chicory (high WSC) compared to those offered fresh ryegrass pasture (low WSC).

Rumen ammonia

In the present study feeding allowance did not affect rumen ammonia concentration both am and pm samples as calculated from AUC values. Similar results were obtained in sheep offered chicory (*Cichorium intybus* cv. Choice) or perennial ryegrass (*Lolium perenne* cv. Quartet) (Sun *et al*. 2012). Sun *et al*. (2012) showed that feeding level (1.3× or 2.2× maintenance ME) and its interaction with forage species did not have an effect on the concentration of rumen ammonia. However, it should be noted that in the present study kale-fed cows (K11 or K14) had high levels of rumen ammonia during the afternoon compared to grass-fed cows (G11).

Measurements of DM intake showed high intake rates of up to 1.8 kg DM/h for K14, 1.7 kg DM/h for K11 and 1.2 kg DM/h for G11 cows in the present study in the first 6 hours after offering forage (Chapter 3; section 3.3.1.2). Reflecting this, ammonia concentration for all forage treatments peaked 7 h after the morning allocation of feed. In comparative studies, rumen ammonia concentrations reached a maximum 7 h post feeding in cows fed total mixed diets (Dewhurst *et al*. 2000) or pasture (Trevaskis *et al*. 2004; Soriano *et al*. 2000). In the present study ammonia concentrations declined in all treatments, but this was less pronounced in K14. This most likely reflects that K14 cows grazing for longer into the evening.

Satter & Slyter (1974) suggested that rumen ammonia concentrations between 20 and 800 mg NH_3/l are enough to support maximum rumen bacterial growth rates. Rumen ammonia concentrations for K14 (108-212 mg NH_3/l), K11 (91-306 mg NH_3/l) and G11 (57-269 mg NH_3/l) in this study are within this range. However, it should be noted that G11 and K11 spent significant time close to lower concentrations. As these concentrations appeared to be related to feeding events, it provides scope for manipulating rumen ammonia concentration through feeding frequency.

Rumen pH

Subacute rumen acidosis (SARA) is the term used to describe the periods of moderately depressed rumen pH (between 5.5-5.0) (Marie Krause & Oetzel 2006). In dairy cows, SARA is mainly due to inadequate rumen buffering which is caused by low fibre intake or excessive intake of rapidly fermentable carbohydrates and lastly, inadequate rumen adaptation to a highly fermentable diet (Marie Krause & Oetzel 2006; Nichol *et al.* 2003). Kale contains low level of NDF and ADF compared to the level of non-structural carbohydrates (NSC) (Barry & Manley 1985; Nichol *et al.* 2003) (Table 3.1). High DM intake of diets with rapidly fermentable carbohydrates may potentially cause rumen acidosis (Keogh *et al*. 2009c). In the current study, rumen pH fell to reach its lowest values of 5.7 for G11, 6.0 for K11 and 6.2 for K14 between 7-10 h from time of feeding forage in all treatments. This pattern was also observed by Trevaskis *et al*. (2004) in response to cows being fed a fresh pasture break. However, the rumen pH of K11 and K14 remained high (> 6.0). Further, from bout counts G11 had more frequent and longer bouts of rumen pH under each threshold except 6.4 compared with K11 or K14. Hence, there was little evidence of rumen acidosis shown in cows fed kale compared to grass, in contrast to Nichol *et al*. (2003), and similar to that reported by Gibbs (2009). This might suggest a greater buffering effect of kale compared to grass (Keogh *et al.* 2009c). High mastication rates have been observed in cows grazing kale compared to grass (Gazzola *et al.* 2008), thus increasing saliva production, which is rich in potassium, bicarbonates and phosphates (Van Soest 1994). Hence, there may be greater buffering effect on rumen pH in cows fed on kale than on grass.

From the rumen pH bout counts (Table 4.5) cows offered kale at low allocation recorded a greater number of bouts of pH <6.0 compared to K14 . As K14 cows had a better diet quality compared to K11 cows (consumed more CP), it has been suggested that this reflects a higher buffering effect from the degradation of proteins to ammonia (Jasaitis *et al*. 1987), but given the increased CP content of the G11 diet (18.7%), where the rumen pH was significantly lower, this seems unlikely.

4.6 Conclusion

- Cow behaviour was significantly affected by forage type and allowance. Cows on kale spent more time eating forage than those on grass. Cows on K14 spent more time grazing and less time ruminating than cows on K11.
- There was an identifiable rumen pH response after the daily break (0900 h). Kale cows had high rumen pH than grass cows.
- Dry dairy cows eat more kale if offered at high allowance and there was no evidence of acidosis on cows out-wintered on kale.

Chapter 5

Effect of feeding kale once versus twice a day on the rumen function of Friesian steers.

5.1 Introduction

Kale (*Brassica oleracaea*) is often a major component of the winter diets in southern New Zealand due to its high nutritive value and standing DM relative to other forages available at the same time (Brown *et al.* 2007; de Ruiter *et al.* 2007; Nichol *et al.* 2003). In current kale feeding regimes, kale is generally fed as a single daily break. Based on DM yield and target allowances, break widths are calculated and fences are shifted daily, typically in the morning. This feeding regime for kale potentially leads to high intake rates as cows quickly consume offered forages (Rugoho *et al.* 2010). For example intake rates of 1.8 kg DM/h for cows offered 14 kg DM/cow/day of kale plus 3 kg DM/cow of barley straw and 1.7 kg DM/h for cows offered 11 kg DM/cow/day of kale plus 3 kg DM/cow of barley straw after 6 h of grazing were calculated by Rugoho *et al*. (2010). Kale typically has relatively low neutral detergent fibre (NDF) (<300 g/kg DM) (Barry and Manley 1985) and high DM digestibility (>800 g/kg DM) (Valentine & Kemp 2007), which may result in reduced rumen pH and altered volatile fatty acid (VFA) production (Keogh *et al*. 2009c). Several authors have outlined the effects of forages with different NDF and intake patterns on rumen physiology (Mould and Orskov 1993; Mulligan *et al*. 2002). However, there is a scarcity of data on the effects of feeding diets containing a high proportion of kale on rumen pH, VFA and ammonia.

Keogh *et al*. (2009c) measured the effect of dietary proportions of kale and grass silage on rumen pH and VFA of dairy cows. They showed that increasing dietary proportions of kale did not reduce rumen pH and did not affect the rumen concentration of VFA. Further, pen fed studies with cattle (Gibbs, unpublished data) simulating high rates of kale intake indicated a marked diurnal pattern to rumen metabolite concentration with once daily feeding. Rumen ammonia levels (NH_3) rose sharply to >200 mg NH_3/L within 2 h after the 9 am meal of 9 kg DM kale/head, stayed elevated for a period of 6 h before declining to levels <100 mg NH_3/L for the remaining 16 h of the day. This pattern may affect rumen function, as low ammonia concentrations closer to 20 mg NH3/L is a threshold limiting concentration which affect microbial growth and activity (Satter & Roffler 1975; Satter &

Slyter 1974), hence, microbial degradation of feed (Cabrita *et al.* 2006). It is plausible that alternative feeding regimes may give improved rumen function. In other studies with concentrate diets showed that increasing the feeding frequency from two to four (Shabi *et al*. 1999), two to twelve (French & Kennelly 1990) and two to hourly (for 24 hours) (Sutton *et al*. 1986) meals per day reduced the diurnal variation in rumen pH. This was thought to be due to more even distribution of feeding time over the day (Devries *et al*. 2005). Further, in the studies by Sutton *et al*. (1986) and Shabi *et al*. (1999) feeding dairy cows more than two times per day on concentrate diets tended to decrease rumen pH. This was thought to be due to increased DMI and rumen digestion of NSC (Shabi *et al*. 1999). Also, feeding fresh chicory (*Cichorium intybus* cv. Choice) and perennial ryegrass hourly for 24 h versus twice per day reduced the diurnal variation in rumen pH of sheep (Sun *et al*. 2012). Experiment 1 shows that the concentration of rumen ammonia of dry cows offered kale at low allowance (11 kg DM/cow/day) and grass also at 11 kg DM/cow/day spent significant time close to lower concentrations, twice a day feeding may give a more consistent intake pattern and lower fluctuations in key rumen metabolites such as VFA concentration, rumen ammonia levels and pH.

Analysis from brassica crops shows *in vitro* Digestible Organic in Dry Matter percentage (%DOMD) of kale crop ranges from 70 to 87% (Fraser *et al*.2001; Wilson *et al*. 1989). However, these *in vitro* values need to be validated *in vivo*. There are few *in vivo* digestibility studies for animals grazing brassica crops. Studies with sheep grazing kale show that *in vivo* digestibility are around 88% for kale leaf and 87% for kale stems and 88% for whole kale plant (Barry *et al*. 1984).

The objective of this study was to evaluate the effect of feeding kale at a high proportion of the diet either once versus twice per day on rumen pH, VFA and ammonia concentration of Friesian steers.

5.2 Materials and methods

Site

The experiment was carried at the Johnstone Memorial Laboratory Research Farm, Lincoln University, Canterbury, New Zealand.

5.2.1 Experimental design

The trial was carried out with four rumen fistulated Friesian steers (LWT: 405+/- 14 kg). The experimental design was a two treatment, two-period crossover study. The steers were fed once a day on kale (7 kg DM/day) and straw (1.5 kg DM/day) for a period of 14 days. After 14 days, steers were housed separately in pens for the trial period. Two steers were fed once a day (K1) with 1.5 kg DM/day straw at 0800 h and 7 kg DM/day of kale at 0900 h. The other two steers were fed twice a day (K2) with 0.75 kg DM straw at 0800 h and 3.5 kg DM/day kale at 0900 h, followed by 0.75 kg DM straw at 1400 h and 3.5 kg DM of kale at 1500 h. Cows were fed these diets for 12 days. After this 12 day feeding period, the steers were loaded into individual metabolism crates for measurements over 7 days. In this period, steers were offered the same dietary treatment as the previous 12 days.

The steers were released after the 7 day period from the metabolism crates and fed kale and straw (1.5 kg DM/day straw at 0800 h and 7 kg DM/day of kale at 0900 h) outdoors for 14 days. After the 14 day period the steers were housed again and were put under the same treatment for 12 days in pens followed by 7 day period in metabolism crates, with the two treatment groups reversed.

Kale was cut fresh each day during the trial period from the paddock located at the Field Service Centre, Lincoln University (Section 3.2.3). The kale was cut to ground level, with individual plants left intact, and was transported to laboratory. Each day during the trial period, two representative plants were removed, weighed fresh and then broken down into stem and leaf components, weighed once again and oven dried in an air forced oven (65˚C) to a constant weight to determine DM%.

5.2.2 Measurements

During the 7 day measurement period, daily feed refusals were separated into kale and straw, and dried to a constant weight at 65^{o}C in a fan forced oven to determine DM of feed refused. For each steer, total 24 h collection of faeces and urine were collected daily and weighed. A 500 g faecal sample was obtained each day from the 24 h collection after thorough mixing, and from this duplicate samples were used for DM assessment by drying for 14 d at 65^{o}C in a fan forced oven. A further sample (approximately 10% of total 24 h collection) was bulked each day from each steer and kept frozen at -20^{o}C. For analysis, bulked samples were thawed and mixed and a 500 g subsample freeze dried for subsequent N content analysis. A subsample of urine (approximately 5 % of total 24 h collection) was bulked across days and kept frozen at -20^{o}C. Samples were thawed, and a sample 100 ml subsample from each steer was then prepared for N content analysis. Nitrogen concentration was obtained by the Dumas combustion method for all faecal and urine samples (combustion of sample under Oxygen supply and high temperatures using Variomax CN Analyser; Elementar).

Rumen VFA and NH_3 were sampled from the ventral sac of each steer every 2 h from 0830 h for 24 h on day 5 of the total faecal and urine collection period, according to the techniques described in section 4.2.2.2. Rumen pH was measured continuously by indwelling sensor for 24 h in each steer on day 4 of the total faecal and urine collection periods described in section 4.2.2.2.

5.3 Calculations and data analysis

5.3.1 Digestible Organic Matter in Dry Matter (%DOMD)

%DOMD was calculated using the equations described by Rymer (2000):

(1) Dry Matter Digestibility (%DMD) was calculated using the following equation:

DMD = ([Total DM Intake] - [Total DM Faeces]) / [Total DM Intake]

Where: [Total DM Intake] = [Total DM Feed] - [Total DM Refusals]

Organic Matter Digestibility (%OMD) was calculated using the following equation:

(2) OMD = ([Total OM Intake] - [Total OM Faeces]) / [Total OM Intake]
where: [Total OM Intake] = (1 - [Feed ASH])*[Total DM Feed] - (1 - [Refusals ASH])*[Total DM Refusals]
and: [Total OM Faeces] = (1 - [Faeces ASH])*[Total DM Faeces]

(3) Digestable Organic Matter in Dry Matter, %DOMD:
DOMD = ([Total OM Intake] - [Total OM Faeces]) / [Total DM Intake]

5.3.2 Rumen pH

The calculations to produce a mean (10 min interval) diurnal pattern, the proportions of recorded rumen pH in each threshold band (< 5.0, between 5.0 and 5.5, between 5.5 and 5.8, between 5.8 and 6.0, between 6.0-6.4, and >6.4), and mean bout count in those threshold bands, are detailed in 4.3.1.

5.3.3 Acetic acid, propionic acid butyric acid and rumen ammonia means

Area under a curve values for acetic acid, propionic acid and butyric acid and ammonia for both am and pm were calculated as described in section 4.3.3.

5.3.4 Statistical analysis

Data was analysed by ANOVA of a cross over design with two replicates. In all cases (plant/animal) data group (over days) was used as the response variable and week as replicate. Least significant difference test ($\alpha < 0.05$) were used to compare means were ANOVA was significant.

5.4 Results

5.4.1 True DM intake, % utilisation and nitrogen output in faeces and urine

The true DM intake, % DM utilization, N intake and faceal and urine output of K1 and K2 treatments are shown Table 5.1.

Table 5.1: DM intake, Dry matter digestibility % (%DMD), Apparent Digestible Organic in Dry Matter % (%DOMD), %N in faeces and urine and N excretion in faeces of steers offered kale once (K1) or twice (K2) per day. LSD = least significance different (α <0.05).

	Frequency			
	K1	**K2**	**P value**	**LSD**
Forage offered (kale + straw) kg DM/steer/day)	8.94	8.94	NS	0.43
Forage refusals (kale + straw) kg DM/steer/day	0.24	0.22	NS	0.12
DM Utilisation (kale + straw) (%)	97.3	97.5	NS	1.41
DM Intake (kale + straw) (kg/steer/day)	8.7	8.7	NS	0.46
%DMD	72.6	73.8	NS	0.04
%DOMD	67.1	67.9	NS	0.03
N% Faeces	2.21	2.22	NS	0.057
N % urine	0.33	0.37	<0.001	0.018
Faeces N output g//steer/day	51.9	49.6	NS	5.74
Urine N output g/steer/day	91.1	95.7	NS	13.53

NS = not significant at 5% level.

The nitrogen concentration in urine was higher (P<0.001) for K2 than K1, although the urinary nitrogen output was not significantly different between the two treatments. Feeding frequency did not affect apparent intake, DM utilisation, nitrogen concentration or output in faeces. Apparent %DOMD of kale plus barley straw diet averaged 67.5% and was not different between treatments.

5.4.2 Rumen VFAs, pH, and ammonia concentration

The areas under the curve values for rumen VFA concentration are presented in Table 5.2. There was no significant effect of feeding frequency in both am and pm on the concentration of acetic acid, propionic acid and butyric acid.

Table 5.2: Effects of feeding kale once (K1) versus twice (K2) a day on the proportions (relative to total volatile fatty acids) of acetic, propionic and butyric acids in morning (am, 0800 h to 1600 h) and afternoon (pm, 2000 h to 0400 h). Values obtained from area under a curve (AUC units). LSD = least significance different (α <0.05).

	Kale			
	K1	**K2**	**P value**	**LSD**
Acetic acid a.m	631.1	645.4	NS	47.94
Acetic acid p.m	694.0	694.3	NS	101.00
Propionic acid a.m	157.7	159.7	NS	3.78
Propionic acid p.m	192.5	183.9	NS	16.82
Butyric acid a.m	121.4	128.2	NS	14.98
Butyric acid p.m	153.9	156.8	NS	3.39
Ammonia a.m	63.9	76.7	NS	16.34
Ammonia p.m	153.9	156.8	NS	3. 12.

NS = not significant at 5% level.

However, there were noticeable differences at different diurnal periods (times of the day) for the concentrations of acetic acid propionic acid and butyric acid (Figure 5.1a, b and c respectively). The mean concentration of acetic acid, propionic acid and butyric acid increased overnight for both treatment groups. The peak value for acetic acid, propionic acid and butyric acid occurred at approximately 11 h after feeding the morning meal for K1 and approximately 6 h after feeding the second meal for K2 (Figure 5.1a, b and c respectively).

(a) (b) (c)

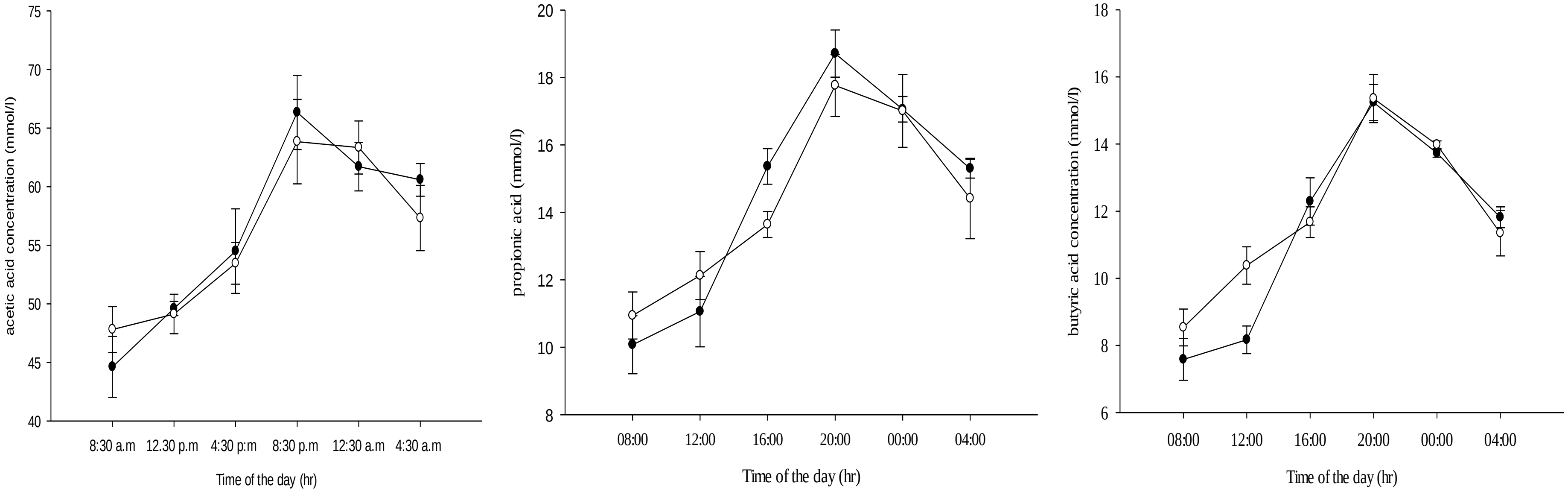


Figure 5.1: Diurnal pattern of acetic acid **(a)**, propionic acid **(b)** and butyric acid **(c)** of steers offered kale, once (●) or twice a day (○). Values are means ± SEM.

The mean am and pm rumen ammonia concentrations for K1 and K2 are shown in Table 5.2. Feeding frequency had no significant effect on the concentration of ammonia in the rumen both am and pm. The diurnal patterns of rumen ammonia concentration are shown in Figure 5.2. There was diurnal variation in the concentration of rumen ammonia. Peak rumen concentration occurred 2 h after feeding in the morning. Steers offered kale in two meals had a second small peak of rumen ammonia overnight (9 h after feeding the second meal).

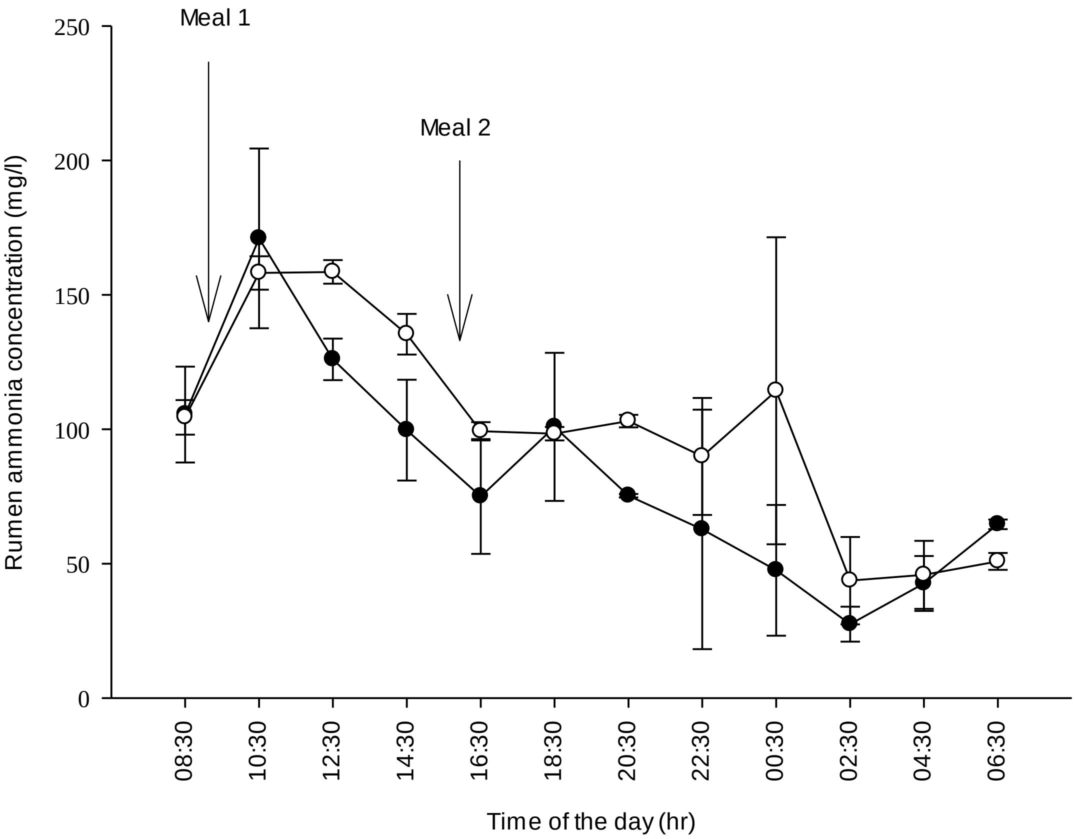


Figure 5.2: Diurnal pattern of rumen ammonia concentration of steers offered kale, once (K1,●) or twice a day (K2,○). Values are means ± SEM.

The effect of feeding frequency on rumen pH is shown in Figures 5.3 and 5.4 and Table 5.3. There was a broadly similar diurnal variation of pH between K1 and K2, with a decline after the morning meal at 0900 h. However, in K2, steers maintained a lower pH from approximately 1200 h until feeding at 1500 h (Figure 5.3). The rumen pH for both kale treatments was consistently greater than 6 (Figure 5.3). K2 steers have more frequent and longer bouts of rumen pH below each threshold than K1 steers (Table 5.3).

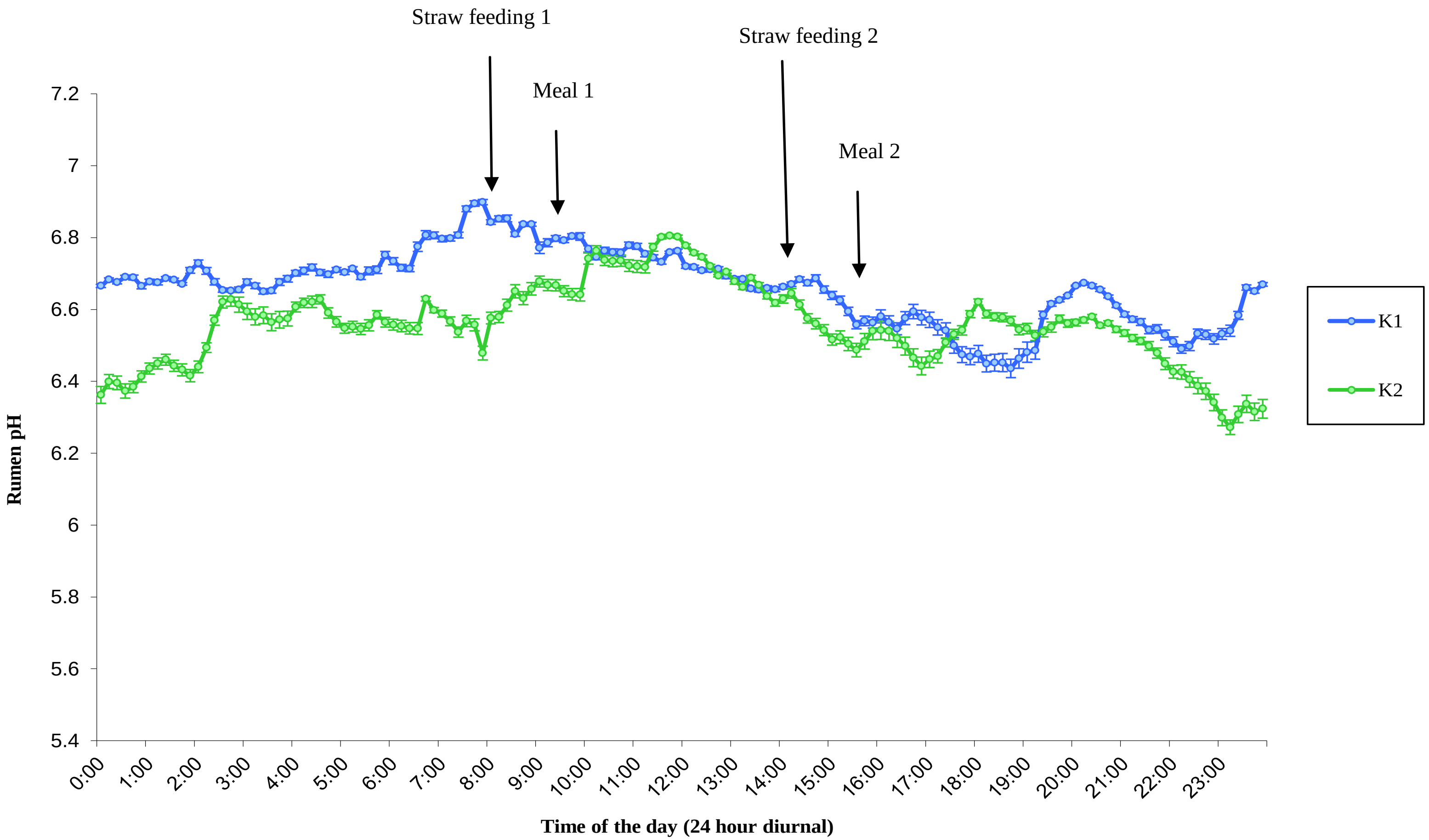
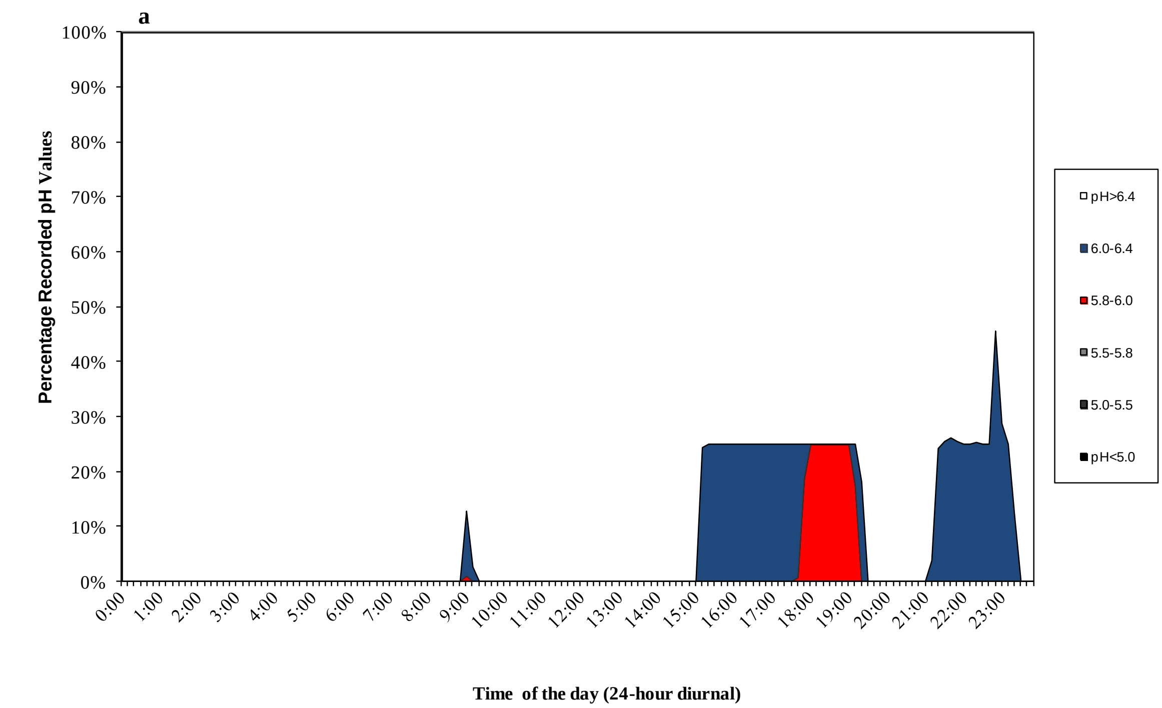


Figure 5.3: Diurnal rumen pH (10 min intervals) after feeding kale once (K1) or twice (K2) a day. Values are means ± SEM.



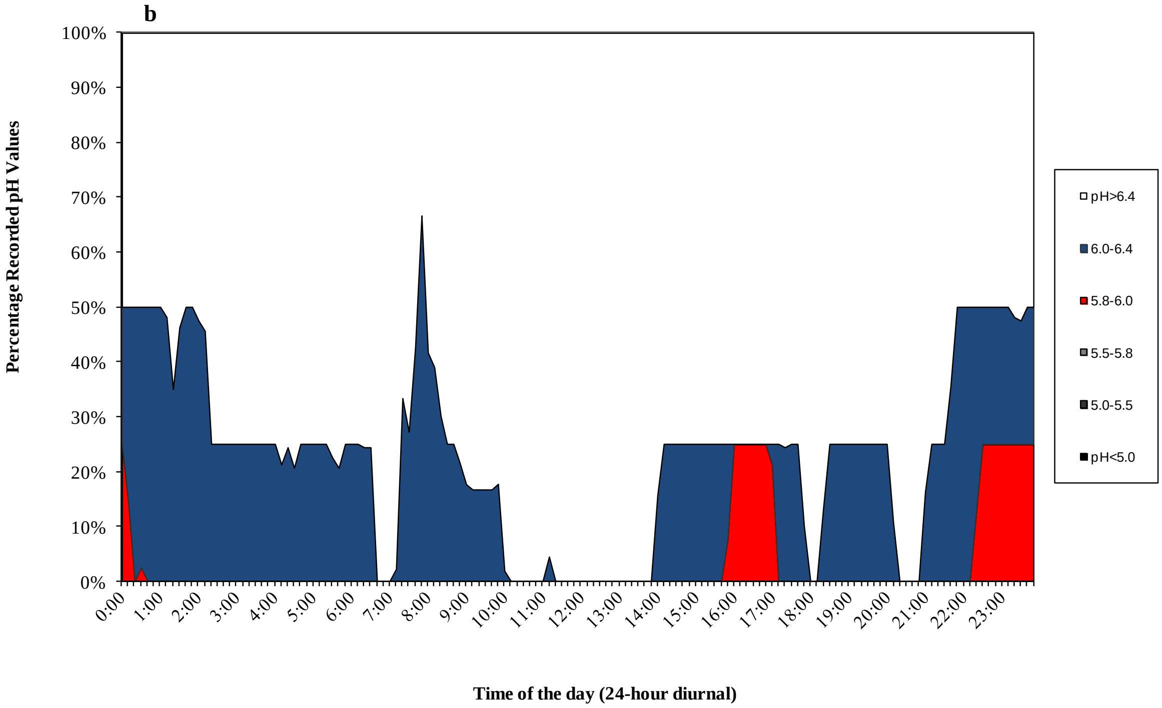


Figure 5.4: Percentage recorded rumen pH values in six rumen pH bands of Friesian steers after feeding kale (**a**) once (K1) or (**b**) twice (K2) a day.

Table 5.3: Bout count summary of Friesian steers after feeding kale at 7 kg DM/steer/day offered either once a day (K1) or twice a day (K2) indoor during winter period.

	Bouts <5.0:				Bouts <5.5:				Bouts <5.8:				Bouts <6.0:				Bouts <6.4:			
Treatment:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:	Bout Count:	Avg bouts per day:	Avg mins per bout:	Mins per bout SE:
K1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.2	84.3	0.0	4.0	1.0	98.6	59.0
K2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.5	90.6	20.1	9.0	2.2	134.3	33.7

5.5. Discussion

5.5.1 True intake and % utilisation

There was no effect of feeding frequency on DM intake and utilisation of kale. This result agrees with previous studies with dairy cows grazing perennial ryegrass-white clover pastures where feed was increased from one feed to six feeds per day (Dalley *et al.* 2001) and from two feeds per day to four feeds per day (Gregorini *et al.* 2009). However, it is noted that the experimental design was that feeding was restricted (1.4% of BW) and not *ad libitum*. Thus, the lack of an effect on DM intake may reflect a restricted feeding approach, with little opportunity for DM intake or diet quality to be enhanced with more frequent feeding.

5.5.2 Nitrogen output in faeces and urine

The nitrogen concentration of urine was greater in cows fed twice (0.37%) than once per day (0.33%). A possible reason for this is unclear at this stage; however, it may reflect their eating pattern whereby steers in once a day may have spent a longer period of time not eating due to lack of feed whereas those fed twice a day were provided with fresh kale during the afternoon. Despite, a higher N concentration in urine in K2, there was no difference in total N excretion in urine, indicating that total N loss in livestock wintered on kale may be similar when offered at the same allowance.

5.5.3 Apparent Digestibility

The average digestible organic matter in dry matter percentage (DOMD %) for kale plus straw diet calculated from apparent digestibility values was 67.5 % and was unaffected by feeding frequency. The value is lower to previous studies of sheep grazing kale (88%), sheep grazing autumn pasture (83%) (Barry et al. 1984), studies of sheep grazing young leafy pasture (86%) (Waghorn & Barry 1987) and studies of sheep (74%) fed on rape (Kaur et al. 2009; Kaur et al. 2010). The averaged %DOMD value of 67.5% is lower than obtained from *in vitro* studies of 70 to 87% (Fraser *et al*. 2001; Wilson *et al*. 1989). However, there is no data at the present of *in vivo* digestibility of dairy cows grazing kale and for the *in vitro* digestibility values to be used in any strategy they have to be confirmed *in vivo.*

5.5.4 Rumen fermentation

Rumen pH

While many studies have examined the effects of brassica forages on sheep metabolism and performance (e.g.Cassida *et al*. 1994; Lambert *et al*. 1987), there is little information on effects on rumen physiology in cattle (e.g. Keogh *et al*. 2009). Kale contains high concentrations of readily fermentable carbohydrate (Chapter 3) and low concentrations of structural carbohydrates. One potential problem with increasing the proportion of rapidly digestible material such as kale is that microbial activity within the rumen may be limited as rumen pH falls due to rapid VFA production (Keogh *et al*. 2009c).

However, in this study were kale sustained a high proportion of the diet (70%), rumen pH remained high (>5.8) and there was little evidence of acute or sub-acute acidosis. This result is in agreement with the study of Keogh *et al.* (2009c), who reported no evidence of sub-acute acidosis in cows fed kale solely in the diet, and with Gibbs (2009) who similarly concluded rumen acidosis is not a risk with kale fed cattle. The reasons for the relatively high rumen pH observed with kale are not clear, given the high WSC content, low NDF content and rapid intake rates associated with kale. However, it may reflect the greater buffering capacity of kale compared to other crops. Brassicas contain a high proportion of water (80-95%) and Gazzola *et al.* (2007) noted high mastication in cows grazing kale. This may lead to high saliva production thereby contributing to no marked decline in rumen pH. However, the clear observations of relatively high rumen pH suggest there is little value in precautions against rumen acidosis in kale feeding systems in NZ.

In concentrate diets studies, benefits have been observed when feed was offered more than twice per day (Cabrita *et al*. 2006; Gibson *et al*. 1984). For example, increasing the feeding frequency from two to four (Shabi *et al*. 1999), two to twelve (French & Kennelly 1990) and two to hourly (for 24 hours) (Sutton *et al*. 1986) meals per day reduced the diurnal variation in rumen pH. This was thought to be due to more even distribution of feeding time over the day (Devries *et al*. 2005). Further, in the studies by Sutton *et al*. (1986) and Shabi *et al*. (1999) feeding dairy cows more than two times per day on concentrate diets tended to decrease rumen pH. This was thought to be due to increased DMI and rumen digestion of NSC (Shabi *et al*. 1999). Also, feeding fresh chicory (*Cichorium intybus* cv. Choice) and perennial ryegrass hourly for 24 h versus twice per day reduced the diurnal variation in rumen pH of sheep (Sun *et al*. 2012). However in the same study (Sun *et al*.

2012), like the present study feeding animals twice daily forage did not alter rumen pH. This may be due to no difference in DMI between K1 and K2 in the present study.

VFA production

The effects of frequency of feeding on the concentration of rumen VFA are inconsistent (Cabrita *et al.* 2006). In the study by Sutton *et al*. (1986), increasing feeding frequency from twice daily to hourly for 24 h had smaller effects on the concentration of rumen VFA and the difference was insignificant, although there was a general tendency for the proportion of acetic acid to increase and that of propionic acid to fall. Whereas, in the study by Shabi *et al.* (1999), more frequent feeding (from twice daily to four times daily) increased the proportion of propionic acid to acetic acid. In the present study offering kale to Friesian steers either once or twice a day did not have an effect the mean acetic acid, propionic acid and butyric acid concentrations. Similar results were reported by Sun *et al*. (2012), who noted no significant effect on the concentration of individual VFA by offering fresh chicory or ryegrass to sheep twice daily.

For both treatments the peak value for acetic acid, propionic acid and butyric acid occurred at approximately 11 h post-feeding the morning meal and approximately 6 h after the second meal for K2 steers (Figure 5.1 and 5.2). Like the study of Sun *et al*. (2012), the rumen VFA profiles were not affected by the feeding frequency and followed the same pattern during the day.

Rumen ammonia

Rumen ammonia concentration ranged from 27.5 mg NH_3/ to 170.9 mg NH_3/l for K1 and from 43.7 mg NH_3/ l to 158.6 mg NH_3/l for K2. These values are within the range indicated by Satter & Styler (1974). However, these values are smaller than stock fed grass (Trevaskis *et al*. 2004).

There was a distinct diurnal pattern which was characterised by a spike 2 h post feeding for both treatment groups. Similar results were reported in the study by Sun *et al*. (2012), who noted that offering sheep chicory or ryegrass twice daily rumen ammonia concentration reached a peak 2 h after feeding. However, this result differs to studies with concentrate diets (Dewhurst *et al*. 2000) and when ryegrass is offered to dairy cows (Trevaskis *et al*. 2004) once daily. Both studies (Dewhurst *et al*. 2000; Trevaskis *et al*. 2004) noted the highest concentration of rumen ammonia 7 h after feeding.

In general, K2 steers had higher rumen ammonia levels across the diurnal period, but there was no significant difference between the two treatments from AUC means. In agreement, Sun *et al*. (2012) noted no difference in the concentration of rumen ammonia by offering sheep fresh forage twice-daily.

Unlike in the study by Dewhurst *et al.* (2000), who noted a second peak from cows which were offered concentrate diet once daily, in the present study, the animals which were fed kale twice a day had a second peak of rumen ammonia concentration overnight, which may reflect the effect of a second meal during the afternoon. However, it should be noted that the second peak was smaller than the one after the first meal. This may show the different of time between meals i.e. cows were deprived of feed for longer after the second meal (about 18 h) were as the duration from the first meal to the second meal was only 6 h, hence, cows had some nibbling on kale and on leftover of straw from the morning meal.

While this trend suggests there may be an advantage in rumen nitrogen supply with two feeding periods daily, further work is required to assess the rumen microbial protein supply effect of this practice.

5.6 Conclusion and implications

- Offering Friesian steers kale either as a single meal or two meals (one in the morning and the other afternoon) did not have an effect on the mean acetic acid, propionic acid, and butyric acid and rumen ammonia levels. There was relatively minor diurnal variation of post-feeding rumen pH during the present study between the two treatments, and rumen pH was observed to be relatively high in both treatment groups. There was no evidence of rumen acidosis.

Chapter 6

Effect of feeding frequency of kale and grass on body condition score gain, foraging behaviour, DM intake, urinary nitrogen losses of dairy cows.

6.1 Introduction

Regaining body condition score (BCS) lost through lactation is an important goal for feeding of dry, pregnant dairy cows. The positive effects of achieving a body condition score of 5.0 (0-10 NZ scale) at calving was associated with an increase in milk yield and milk fat percentage (Graiger *et al*. 1982; Roche *et al*. 2007a) and a short period between calving and planned start of mating (Roche *et al*. 2007b).

Cows in the South Island of New Zealand are often wintered on kale with smaller numbers wintered on grass (Judson *et al*. 2010) and fodder beet (Gibbs 2012). Data from Chapter 3 (Section 3.3) and from the study by Greenwood *et al.* (2011) suggests that BCS gain over the winter dry cow feeding period on the industry standard allocation of 11 kg DM/cow/day of kale and 3 kg of high fibre supplement such as straw may be less than the expected gain of 0.5 BCS units, and method to improve performance are sought. Previous work indicates that allowance may affect performance (Judson *et al.* 2010; Judson & Edwards 2008). For example, high utilisation of kale crops due to low allocation promotes lower DM intakes and also results in reduced diet quality. Results from Chapter 3 show that cows offered kale at the high allowance of 14 kg DM/cow/day + 3 kg DM/cow/day of barley straw gained 0.3 BCS units over the winter period while those offered 11 kg DM/cow/day + 3 kg DM/cow/day of barley straw gained only 0.2 BCS units over the winter period. Further, Greenwood *et al.* (2011) showed dry dairy cows gained 1.0 BCS units at an allocation of 14 kg DM of kale/day/cow but only 0.23 BCS units at 10 kg DM of kale/day.

A further possibility to improve BCS gain may be to alter the frequency of feeding. Typically breaks of winter forage crop are offered once a day in the morning and fed with hay or silage prior to attempt minimising gorging (Nichol *et al.* 2003). From this feeding regime, intake rates are very high. For example, Rugoho *et al.* (2010) cows offered kale once a day (high or low allocation) consumed an average of 8.4 kg of kale over the first 3

h. Further, results from Chapter 4 (section 4.4) show that in response to this high intake rates, rumen ammonia levels rose rapidly within two hours from the time of feeding and reached a peak approximately 7 h after feeding. The rumen ammonia levels observed during this study are within the range of 20-800 mg NH_3/l suggested by Satter & Slyter (1974). However, it should be noted that cows which were offered kale and grass at 11 kg DM/day spent significant time close to lower levels; hence, given close link to feeding events, it was necessary to explore other feeding frequency options. Also, offering forage twice per day may potentially improve performance as less trampling and improved crop utilisation may occur. This may be particularly important when grass is offered, as utilisation and so DM intake of this crop is low with once a day allocation (Chapter 3, section 4.4.2).

Previous work has reported on the effect of altering the feeding frequency of pasture on cow behaviour, DM intake and milk production in early lactation (Dalley *et al.* 2001; Gregorini *et al.* 2009; Kennedy *et al.* 2009). Dalley *et al.* (2001) and Kennedy *et al.* (2009) showed no benefit in daily herbage intake or milk yield by altering feeding frequency from one feed per day to six feeds per day. Bite mass was smaller when feeding frequency was increased from two feeds per day to four feeds per day (Gregorini *et al.* 2009). However, there is no published literature on the effect of feeding frequency of winter forage crops on BCS gain, cow behaviour, forage intake and milk production in early lactation. Indoor trials (Chapter 5) noted no difference in kale digestibility with increased frequency but did increase in N concentration in urine. The reason for urine N concentration is unclear, but if confirmed at field scale will have important implication for N losses.

The objective of this study was to examine the effect of once versus twice a day allocation of kale and grass on BCS gain, cow behaviour, forage intake, DM utilisation and urinary nitrogen excretion of dry cows during the winter feeding period and their subsequent milk production during early lactation.

6.2 Materials and Methods

Site description

The experiment was conducted from 30 May to 18 August 2010 with the forage crop conducted at the Lincoln University dryland research farm (Ashley Dene), located on the Canterbury plains, New Zealand (43˚39’S, 172˚19’E). The site is approximately 35 m above sea level, with a Eyre shallow stony loam soil (Yeates *et al*. 2006). Following ploughing and cultivation, the kale crop for the experiment was established on 15 December 2009. Kale cultivar 'Regal' was sown at 4 kg/ha. The cultivar Regal is characterised as having a high leaf: stem ratio relative to other kale cultivars. Kale plots were fertilised with 300 kg/ha DAP (Di-ammonium phosphate) prior to sowing and 100 kg N/ha as urea on 27 January 2010. Italian ryegrass cultivar 'Feast' was sown at a sown at 25 kg seed/ha on 15 February 2010 and fertilised with 109 kg N/ha as urea on 22 March 2010.

All procedures were approved by the Lincoln University Animal Ethics committee (AEC) and licensed in accordance to the Animal Welfare Act, 1999, section 100.

Meteorogical data

Air and soil temperatures (˚C) for the winter feeding recorded at Lincoln meteorological station, 13 km north east of the trial during the months of June and July. Over the winter forage crop trial period, the mean air temperature was 6.0˚C between a maximum of 17.1˚C and a minimum of -4.4˚C. The total rainfall over the trial period was 277.8 mm which occurred on 27 rainfall days. This was approximately 40% of the expected annual rainfall (680 mm) for Canterbury. The number of ground frosts was also high at 29 days.

6.2.1 Experimental design

The experiment design was two replicate groups of 2×2 factorial, with treatments being forage type (kale or grass) and feeding frequency (once or twice per day allocation).
A total of 48 multiparous, pregnant, non-lactating Friesian x Jersey dairy cows from Lincoln University Research Farm were assembled in late May 2010 immediately after drying off and blocked into six blocks of eight cows based on mean calving date (20 August ± 20 days), body condition score (4.5±0.5), liveweight (530±9.5 kg) and age (5.3±4.7). Cows from each block were randomly allocated to two replicates of four treatment groups.

Treatments

The four treatment groups were:

- K1: 11 kg DM of kale grazed in situ + 3 kg DM of barley straw offered once per day.
- K2: 5.5 kg DM of kale grazed *in situ* + 1.5 kg DM of barley straw offered twice per day.
- G1: 11 kg DM of grass grazed *in situ* + 3 kg DM of barley straw offered once per day.
- G2: 5.5 kg DM of kale grazed *in situ* + 1.5 kg DM of barley straw offered twice per day.

There were two fistulated cows in one of the two replicates groups of each treatment.
In the once per day treatments, straw was offered at 0800 h and kale or grass at 0900 h. In twice per day treatments, straw was offered at 0800 h and 1400 h and kale or grass at 0900 h and 1500 h. Cows grazed treatments for a total of 60 days from 30 May 2010 to 17 July 2010. In order to minimise animal health disorders such as nitrate poisoning, kale cows were adapted to crops over a period of six days (30 May to 4 June), (as been described in Chapter 3, section 3.2.1) with full allocation given on 7 June 2010. Straw was fed in a wire mesh feeder at the paddock entrance. Both kale and grass were strip grazed in 15 m wide paddocks, with daily breaks width determined by allowance and DM yield. Typical daily break widths were 4.8 ± 0.7 for kale cows and 6.8 ± 2.0 for grass cows, with values half of this for twice per day allocation. Kale paddocks were not back-fenced during grazing period but cows on grass were back fenced at the end of each week.

At the conclusion of the winter feeding period (16 August 2010) all cows were returned to the Lincoln University Dairy Farm and maintained on perennial ryegrass white clover pasture (14-18 kg DM/cow/day) until 60 days of lactation. The mean calving date was 20 August ± 20 days.

6.2.2 Measurements

6.2.2.1 Crop measurements

Forage yield

Pre-grazing kale dry matter yield (DM yield) was measured on Monday of each week in each paddock by cutting to ground level three randomly allocated 1×1 m quadrats within the area estimated to be grazed over next week. Total fresh weight (FW) was recorded in the field and five kale plants were returned into the laboratory and separated into leaf and stem components. These were then weighed for their FW and oven dried at 65˚C to a constant weight to determine DM% and the leaf to stem ratio. The DM% obtained from the previous week's samples was applied to the FW of the current week's samples for calculation of break size. Grass pre-grazing DM yield was measured in each paddock using a calibrated rising plate meter (PP Jenquip F150 Electronic Pasture Meter) on the same daily schedule as kale. A total of 50 measurements were taken. A single calibration was derived from a total of twelve 0.2 m^2 quadrats (Kg DM/ha = 78.119 RPM + 480.1, r^2 = 80.7 %).

Each week three whole fresh kale plants were collected from each grazing block and sent to the lab for the analysis of S-methylcysteine sulphoxide (SMCO). In the lab the kale plants were separated into leaf and stem and SMCO analysis was done using the method described by Gustine (1985).

On Tuesday of each week, post grazing DM yield was measured in the areas grazed the previous week on Tuesday, Thursday and Sunday. For this, all remaining kale was removed in two 1 x 1 m quadrats per break on the allotted days. Samples were washed to remove any soil or excrement and oven dried at 65˚C to a constant weight to determine DM%. For grass a total of 50 measurements post grazing were taken on Tuesday, Thursday and Sunday.

From pre and post grazing measurements, DM yield (kg DM/ha), DM utilisation (%, pre-post DM yield/pre DM yield * 100) and estimated apparent intake of kale or grass (kg/cow/day) were calculated. Measurement of straw residuals in the mesh feeder indicated that > 95% of straw was consumed on a daily basis by the group of cows.

Dried samples of grass, kale (leaf and stem) were ground and analysed for forage composition by near-infrared spectrophotometer (NIRS). Metabolisable energy (ME) was calculated according to the following equation: ME (MJ/kg DM) = Digestible organic matter content × 0.016 (g/kg DM) (McDonald *et al*. 2002).

Forage disappearance

Forage disappearance in the first 3 hours of crop allocation was estimated on 30 June (week 4) and 4 July (week 6). In each kale paddock, two 0.5 m^2 quadrats were cut to 1 cm above ground level pre-grazing and then at intervals of 1, 2, 3, 6 and 24 hour after allocation. The samples were weighed in the field to obtain FW and sub sampled for DM % determination as per pre and post grazing measurements. Sub samples were separated into leaf and stem components and dried at 65˚C and DM % calculated. Grass DM yield was measured using a calibrated rising plate meter. A total of 50 RPM measurements were taken per break every 30 minutes after allocation of grass until at the 6 h and the following morning at 24 h. A single calibration curve was derived from a total of ten quadrats each 0.2 m^2 quadrats cut to ground level throughout the grazing down period (kg DM/ha = 99.6 RPM + 230, r^2 = 74.9). Herbage intake was based on the disappearance of herbage DM between two sequential measurements.

6.2.2.2 Animal measurements

Cow behaviour

Cow behaviour was observed over the four hour period following the allocation of straw to each group on 1 July (week 4) and 15 July (week 6). Every 5 min, cows were recorded in six categories by visual scan as either: grazing forage, eating straw, idling and ruminating. Cow biting behaviour was observed over the 3 h period following the allocation of a new break (0900 h) to each group in the morning and following the afternoon feed at 1500 h for cows fed forage twice a day. At 30 min intervals, three cows from each paddock (of six) were timed for sixty seconds and the number of bites recorded. For cows on kale both prehension bites (ripping herbage from the ground) and mastication bites (jaw/grinding action) were recorded. For grass, only prehending was recorded opposed to kale were both prehension and mastication were recorded. Biting rate was calculated by number of bites per 60 seconds

Faecal and urine sampling

Faecal and urine samples were collected from 48 cows at 1200 h in week 1, 2, 3 and 4. Faecal samples were collected after stimulation to induce defecation. Urine was collected (approximately 60 ml) mid-stream after manual stimulation by rubbing vulva. One ml of concentrated 6M sulphuric acid was added into the urine to prevent volatilisation of ammonia. Both faecal and urine samples were kept in ice before storage at -20^{o}C until analysis. In all cases total sampling time for all 6 cows (each treatment group) was less than 15 minutes, after which, cows were released back onto their daily pasture and kale allowance and left undisturbed till the next sampling period.

Faecal and urine analysis

Faecal and urine analysis were done using the methods described in Chapter 3 (section 3.2.3).

Body condition score and liveweight

Body condition score was measured by an experienced scorer and determined during the week 1, 3 and 6 of the trial period using the New Zealand (0-10) scoring system (Roche *et al.* 2009). Liveweight was measured concurrently using portable scales. Measurements were done from 1200 h.

Milk yield

Milk yield was recorded daily for the first 60 days of the subsequent lactation using an automatic recording system. Milk samples were collected once per week on consecutive morning and evening milkings. Milk fat, protein and lactose percentage were measured (LIC Ltd., Christchurch). Milk solids was considered to be the milk fat plus protein (kg/day) determined by the component percentage and total milk yield at each individual milking session for each animal.

6.3 Calculations and data analysis

6.3.1 Nitrogen intake calculations

The N intake (NI) (g N/cow/day) for cows in each treatment was calculated from the N concentration and apparent DM intake of each diet component (kale and straw).

6.3.2 Statistical analysis

All the data apart from bitting rate data was analysed by ANOVA of a 2×2 factorial design GenStat (v14, VSL, Hempstead, UK) with two replicates. For all response variables (plant and animal) the data analysed was the mean of each of the plot. For animal data, the unit of replicate was group of animals. Forage composition, DM yield and utilisation data were averaged for a treatment across the 6 weeks of winter feeding prior to analysis. Forage behaviour and forage disappearance data was analysed separately for each week. Bitting rate data was analysed by repeated measurements with group of animals as replicate and repeated measures for time since offering break. Where ANOVA was significant a LSD test ($\alpha < 0.05$) was used to determine differences among treatment means.

6.3 Results

6.3.1 Diet chemical composition, forage apparent intake and utilization.

Nutritive composition is presented in Table 6.1. Kale plants were comprised of 60.7% leaf and 39.3% stem. Water soluble carbohydrates (WSC) and metabolisable energy (ME) were higher and fibre (NDF), dry matter percentage (DM %) was lower in kale than grass (Table 6.1). Though variation in protein (CP) for leaf (25.3 %) and stem (17.59 %) occurred in kale, the weighted average of both kale and grass was similar (Table 6.1). The SMCO were high in kale leaves than kale stems.

Table 6.1: Forage composition (%DM) and metabolisable energy content (MJ ME/kg DM) of kale and grass plants offered to dairy cows from week 1 to 6 of the trial. Kale plants represent the weighted average of kale leaf and stem. Data are averaged across weeks. LSD = least significance different (α <0.05).

	Kale					
Parameters	**Leaf**	**Stem**	**Plant**	**Grass**	**P value**	**LSD**
CP	25.34	17.59	22.42	24.00	NS	2.153
WSC	20.28	37.42	26.22	17.41	0.001	4.884
ADF	17.45	22.39	19.58	20.39	NS	1.866
NDF	24.58	32.63	28.08	40.38	0.001	3.322
SMCO	0.20	0.13	0.17	-	0.04	0.06
DM	10.36	11.57	10.79	11.62	—	—
ME	12.96	12.51	12.74	11.82	0.001	0.366

CP: Crude protein; WSC: water soluble carbohydrates; ADF: acid detergent fibre; NDF: neutral detergent fibre; ME: metabolisable energy; sulphur methylcysteine sulphoxide: SMCO; and DM: dry matter. Sig. = significance from ANOVA of kale plant versus grass plant. For SMCO, Sig = significant from ANOVA of kale leaves and kale stems.

Pre-grazing DM yields averaged across week 1 to 6 of the experiment averaged 7.0 t DM/ha for kale and 3.0 t DM/ha of grass. Post-grazing forage DM yield was greater for kale than grass over the six week period. DM utilisation was greater on the kale than grass. Apparent DM intake was higher in kale than for grass by 2.1 kg DM.

Table 6.2: Pre- and post-grazing DM, percentage DM utilization of pre-grazing dry matter (DM) and apparent DM intake of kale or grass fed once (1) or twice (2) per day from week 1 to 6 of the experiment. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA (α<0.05).

	Kale		Grass		Level of significance			
	1	**2**	**1**	**2**	**Species (S)**	**Frequency (F)**	**S×F)**	**LSD**
Pre-grazing (t DM/ha)	6.59[a]	5.97[b]	2.62[c]	2.64[c]	<0.001	<0.001	<0.001	0.16
Post-grazing (t DM/ha)	0.17	0.22	0.60	0.64	<0.001	<0.001	NS	0.04
Utilisation (%)	97.4	96.2	76.0	75.1	<0.001	NS	NS	1.56
DM Intake (kg/cow/day)	9.7	9.6	7.6	7.5	<0.001	NS	NS	0.17

NS = not significant

Frequency of feeding had no effect on the apparent intake and forage utilisation of both kale and grass (Table 6.2).

6.3.2 Forage disappearance

The disappearance of kale and grass forage over the 24 h period is shown in Tables 6.3 (week 4) and 6.4 (week 6). Over the first hour of grazing in the morning, there was no significant treatment effects (Tables 6.3 and 6.4). In both weeks, cumulative DM intake after 2, 3 and 6 h was greater (P<0.001) in cows fed once than twice per day and for kale than grass after 3 and 6 h (P<0.001) (Tables 6.3 and 6.4).

Table 6.3: Cumulative forage DM intake (kg DM) of dry dairy cows offered kale or grass fed once (1) or twice (2) per day from week 4 of the experiment. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA (α<0.05).

	Kale		Grass		Level of significance			
Hour	**1**	**2**	**1**	**2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
1	4.49	3.16	5.32	3.62	NS	NS	NS	2.63
2	6.00	4.27	6.44	3.70	NS	0.004	NS	1.21
3	7.78	4.84	6.89	3.88	0.004	<0.001	NS	0.50
6	9.96[a]	5.24[c]	7.20[b]	4.10[d]	<0.001	<0.001	0.009	0.61
7	–	8.74	–	7.94	NS	–	–	1.36
8	–	9.48	–	8.07	NS	–	–	1.40
9	–	9.72[a]	–	8.10[b]	0.07	–	–	1.96
24	10.72	10.57	8.61	8.67	<0.001	NS	NS	0.10

NS = not significant at 5% level.

After 6 h, cumulative DM intake was greater for kale-fed cows than grass-fed cows and for cows fed forage once a day (kale or grass) than cows fed forage twice a day. After 24 h, cumulative DM intake was greater in kale than grass and for once than twice per day allocation. From 7 h to 9 h no measurements were done for once a day treatments for both kale and grass. Measurements were done for twice a day treatments and analysis during this period compared kale (K2) and grass (G2). Only for 9 h was intake greater for kale-fed cows compared to grass-fed cows in week 4.

Table 6.4: Cumulative forage DM intake (kg DM/day) of kale or grass fed once (1) or twice (2) per day from week 6 of the experiment. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA (α<0.05).

	Kale		Grass		Level of significance			
Hour	**1**	**2**	**1**	**2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
1	7.21	3.59	6.38	3.63	NS	0.007	NS	2.47
2	8.54	4.38	7.36	4.00	0.031	<0.001	NS	0.91
3	9.13	4.95	7.84	4.09	0.004	<0.001	NS	0.72
6	10.02[a]	5.01[c]	8.01[b]	4.17[d]	0.001	<0.001	0.033	0.71
7	–	8.95	–	7.94	NS	–	–	1.84
8	–	9.56	–	8.12	NS	–	–	3.01
9	–	10.03	–	8.21	NS	–	–	1.37
24	10.71	10.20	9.63	8.82	NS	0.004	NS	0.93

NS = Not significant.

Prehension bite rates and mastication rates are shown in Table 6.5. Feeding frequency did not affect the prehension bite rate in either week. Prehension bite rate was lower in kale than grass in both weeks by 30 bites/min (week 4) and 36 bites/min (week 6). Mastication chew rate for kale-fed cows did not differ with feeding frequency on both weeks. A comparison of within twice a day feeding showed no significant difference in bite rate between morning and afternoon in any time period i.e. the bite rate did not decline through time.

Table 6.5: Prehension biting rate (bites/minute) of kale or grass and mastication chew rate (chews/minute) of kale fed at once (1) or twice (2) per day from week 4 and 6 of the experiment. LSD = Least significant difference (α<0.05). LSD for comparison of prehension bites AM for time = 5.58 and P-value = 0.024 and LSD for comparison of prehension bites PM for time = 4.00 and P-value = NS.

	Kale		**Grass**		**Level of significance**			**kale**	**Grass**	**Level of significant**		**Kale**		**Level of significance**	
	1	**2**	**1**	**2**	**Species**	**Frequency**	**LSD**	**2**	**2**	**Species**	**LSD**	**1**	**2**	**Frequency**	**LSD**
	Prehension bites AM		Prehension bites AM					Prehension bites PM				Mastications AM			
Week 4															
Minutes															
0	9.9	8.5	35.7	38.2	<0.001	NS	5.14	5.0	39.2	<0.004	4.12	39.7	28.7	NS	51.13
30	9.1	7.5	44.1	40.5				5.9	45.0			41.6	39.0		
60	8.8	8.3	37.6	44.6				6.3	41.2			34.4	35.7		
90	7.2	7.3	40.9	39.0				4.6	38.3			39.6	38.8		
120	6.2	4.1	31.3	35.5				6.6	33.5			34.6	33.4		
150	4.1	7.5	34.1	40.3				3.2	35.0			26.7	27.3		
Week 6	**kale**		**Grass**		**Level of significance**			**kale**	**Grass**	**Level of significant**		**Kale**		**Level of significance**	
	1	**2**	**1**	**2**	**Species**	**Frequency**	**LSD**	**2**	**2**	**Species**	**LSD**	**1**	**2**	**Frequency**	**LSD**
	Prehension bites AM		Prehension bites AM					Prehension bites PM				Mastications AM			
Minutes															
0	8.2	7.5	52.8	34.3	0.017	NS	20.42	5.0	50.5	0.011	19.01	38.4	33.4	NS	59.10
30	5.9	4.4	48.9	34.0				4.2	51.3			39.2	42.1		
60	6.8	3.1	46.1	30.7				3.4	48.2			24.0	29.7		
90	5.1	3.2	33.3	32.9				3.8	38.2			21.8	26.0		
120	3.7	5.0	30.5	19.6				12.3	46.0			24.7	24.9		
150	3.2	4.2	35.5	30.2				4.8	48.4			22.2	20.9		

NS = Not significant.

6.3.3 Body condition score (BCS), milk yield and composition.

Body condition score and liveweight are shown in Table 6.6. Feeding frequency had no significant effect on the BCS or liveweight. The change in BCS and liveweight over six weeks was greater in grass than kale by + 0.2 BCS units and + 31 kg LW, respectively (Table 6.6).

Table 6.6: Body condition score, liveweight of dairy cows offered kale or grass once (1) or twice (2) per day. LSD = least significance different (α <0.05).

	Kale		Grass		Level of significance			
	1	**2**	**1**	**2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
Week 1								
Body condition score	4.8	5.0	4.8	4.8	NS	NS	NS	0.29
Liveweight	526	545	556	522	NS	NS	NS	67.9
Week 6								
Body condition score	5.1	5.0	5.4	5.1	NS	NS	NS	0.24
Liveweight	546	555	606	563	NS	NS	NS	67.9
Difference BCS	+0.3	0.0	+0.5	+0.4	0.01	NS	NS	0.31
Difference LW	+20	+10	+50	+41	<0.001	NS	NS	14.05

NS = not significant at 5% level.

Milk yield and composition over the first 60 days of lactation was not affected by forage type or feeding frequency during the pre-lactation period (Table 6.7).

Table 6.7: Milk yield (L), milk fat (kg), milk protein (kg), milk solids (kg) per cow per day and cell count averaged over first 60 days of lactation for cows fed kale or grass once (1) or twice (2) per day. LSD = least significance different (α <0.05).

	Kale		Grass		Level of significance			
	K1	**K2**	**G1**	**G2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
Yield (L)	22.03	22.42	22.87	24.93	NS	NS	NS	2.96
Protein (kg)	3.85	4.23	3.96	4.04	NS	NS	NS	0.38
Fat (kg)	5.27	5.92	5.60	5.65	NS	NS	NS	0.51
Lactose (kg)	5.0	4.9	4.9	5.0	NS	NS	NS	0.10
SCC_× 1000	207.6	230.3	212.4	223.8	NS	NS	NS	198.31

NS = not significant at 5% level.

6.3.4 Grazing behaviour

The total minutes eating was greater in cows fed once than twice per day in both week 4 and 6 by 31 min for kale-fed cows and 33 min for grass-fed cows (Table 6.8). The duration of the first meal was greater in cows fed once per day than twice per day (Table 6.8). Time eating straw prior to 0900 h was lower for cows fed once per day than twice per day and kale than grass in week 4 (Table 6.8). Time spent eating straw after 0900 h was independent of treatment but was greater for kale than grass in week 4 (Table 6.8).

Table 6.8: The effect of species and frequency of forage on cow behaviour (minutes) for the duration of the 4 hours post offering straw in the morning in week 4 and 6 of the trial period. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA.

	Kale		Grass		Level of significance			
	1	**2**	**1**	**2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
Week 4								
Total eating forage	147.1	120.8	159.2	118.8	NS	<0.001	NS	10.01
Duration of first meal	116.3[a]	66.3[c]	110.4[a]	90.0[b]	NS	<0.001	0.096	17.58
Number of meals	2.1	3.4	2.8	3.1	NS	NS	NS	0.66
Eating straw < 9 am	57.1	51.3	41.7	37.1	< 0.001	0.005	NS	3.51
Eating straw > 9am	5.8	5.8	0.0	1.67	0.004	NS	NS	3.32
Ruminating	0.4	12.1	5.4	16.7	NS	<0.001	NS	6.29
Idling	29.2	48.3	32.1	57.1	NS	<0.001	NS	10.59
Week 6								
Total eating forage	150.4	115.4	140.8	116.0	NS	<0.001	NS	12.28
Duration of first meal	112.1	77 9	111.2	89.6	NS	0.002	NS	17.39
Number of meals	2.25[b]	3.67[a]	2.67[b]	3.17[a]	NS	<0.001	0.088	0.53
Eating straw < 9 am	57.5[a]	52.5[a]	45.0[b]	56.7[a]	NS	NS	0.005	5.62
Eating straw > 9am	6.67[a]	0.0[c]	0.0[c]	1.25[b]	NS	NS	0.003	0.89
Ruminating	2.5	8.3	7.9	5.0	NS	NS	NS	4.93
Idling	19.6[c]	53.3[a]	44.6[b]	54.8[a]	NS	<0.001	0.050	11.75

NS = not significant at 5% level.

Cows fed twice per day spent more time ruminating and idling than cows fed twice per day. Time spent ruminating in week 6 was not affected by forage type or feeding frequency. Cows spent more time resting when fed grass than kale (P<0.001) in week 6 only (Table 6.8). The number of meals did not differ between treatments in week 4. Cows fed once per day had fewer meals than those fed twice per day.

6.3.5 Estimated nitrogen losses

Faecal N%, urinary N%, and estimated N intake and output per cow are shown in Table 6.9. Faecal N% was greater in grass than kale-fed cows while urinary N% was greater for kale than grass-fed cows. From calculations, nitrogen intake (N intake) was higher in kale fed-cows (397 g N/cow/day) and low in grass-fed cows (226 g N/cow/day).

Table 6.9: Urinary urea, ammonia, nitrogen percentage, faecal nitrogen percentage, N intake (g N/cow/day and estimated urinary nitrogen output (g N/day) of dry dairy cows offered kale or grass fed once (1) or twice (2) per day from week 1 to 6 of the experiment. Means within a row followed by a different letter are significantly different according to LSD test following a significant ANOVA. LSD = least significance different (α <0.05).

	Kale		Grass		Level of significance			
	1	**2**	**1**	**2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
Urine Urea (mmol/l)	227.8[a]	155.2[b]	131.3[c]	158.9[c]	0.004	NS	0.002	43.80
Urine ammonia (mmol/l)	1.8[a]	1.0[a]	0.6[b]	0.5[b]	<0.001	0.004	0.022	0.44
Faecal nitrogen (%)	1.56	1.76	2.53	2.6	<0.001	NS	NS	0.21
Urine nitrogen (%)	0.74[a]	0.53[b]	0.48[b]	0.54[b]	<0.01	NS	0.005	0.13
N intake from kale (g/day/cow)	378.7	374.8	291.8	288.0	-	-	-	-
N intake from straw (g/day/cow)	20.5	20.5	20.5	20.5	-	-	-	-
Total N intake from diet (g/day/cow)	399.2	395.3	312.4	308.5	-	-	-	-

NS = not significant at 5% level.

6.4 Discussion

6.4.1 Dry matter intake and forage utilisation

Dry matter intake calculated from pre and post grazing measurements was greater in kale than grass by 2.2 kg DM. This was due to the greater utilisation of the crop in kale (97%) versus grass (76%). The high DM utilisation of kale is consistent to that obtained in Chapter 3 (section 3.4.2), but above the mean of 80% found in a survey of dairy cows herds in Canterbury (Judson & Edwards 2008). The lower DM utilisation of grass probably reflects an ungrazeable barrier of grass sheath and stem material at the base of the canopy. Also, in wet conditions grass is likely to have been trampled into the soil surface, which will have further restricted utilisation (Judson *et al.* 2010; Keogh *et al.* 2009a). As the soil became wet grass herbage became exceedingly trampled and pugging buried herbage into the soil.

Previous studies have noted that feeding more frequently does not affect on utilisation or DM intake (Abrahamse *et al*. 2008; Dalley *et al*. 2001). Similarly, in this study, there was no detectable effect of feeding frequency on DM utilisation or apparent DM intake of either grass or kale. As a result, there is no evidence that offering kale or grass twice a day leads to greater utilisation as a result of wide and shallow breaks which are thought to reduce trampling under wet soil conditions during winter.

Judson & Edwards (2008) suggested that the high utilisation of kale would potentially result in a reduced diet quality as stem of lower ME content is included into the diet. This would particularly be the case for high yielding crops of giant type cultivars such as Gruner. However, for the lower yielding, leafy cultivar (Regal) grown in this study, the proportion of leaf was high, and based on the NIRS calibrations used there was a small difference (0.5 MJ ME/ kg DM) in ME between leaf and stem. Thus the predicated diet quality at high utilisation of kale would have remained high and in excess of that of grass, even though utilisation of grass was lower.

6.4.2 Intake rate

Analysis of cumulative DM intake data showed that intake rate over the first 2 hours was high for both kale (3.6 kg DM/h for K1 and 2.1 kg DM/h for K2) and grass (3.5 kg DM/h for G1 and 2.0 kg DM for G2). These levels of DM intake in the initial period are low relative to previous studies in pasture (Dobos *et al*. 2009). Dobos *et al*. (2009) reported intake rate of 9 kg DM/h after 2 hours of grazing when lactating dairy cows were fed on kikuyu pasture (*Pennisetum clandestinum*) during summer period. In keeping with data from Chapter 3, cows fed once per day had consumed a very high proportion of their total daily intake (>95% in kale in grass) after 6 h. Over, the remaining 24 h only a further 0.3 kg DM/cow were consumed for grass and kale. This study indicates that at the industry standard allowances of kale and grass, intake rates are high; further it is likely that monitoring residuals approximately 6 h after forage allocation as being an appropriate method of assessing DM intake of cow.

Cumulative intake over the first 3 h of feeding after offering the break was greater in cows offered forage once per day than twice per day. Similar results were reported by Gregorini *et al*. (2008), with lactating dairy cows. During their study, cows which were offered pasture once a day had consumed 10 kg DM/cow and those offered pasture twice a day had consumed 8 kg DM/cow after 3 h of the grazing period. The difference in cumulative intake appears to reflect two main processes. First, both the allowance offered to cows fed once per day in the first break being twice high, so that physical barriers restricting intake (e.g. availability of feed and stem at base of grass sward) were approached more rapidly in cows fed twice per day. Second, although prehension bite rates did not change with feeding frequency there appeared to be a greater drive to eat with once per day feeding as the duration of the first meal was longer with once per day feeding.

6.4.3 Foraging behaviour

Gregorini *et al*. (2009) reported that the duration of the first meal after offering a fresh allocation was greater than when cows were offered their feed in two allocations than one. In the current study, the duration of the first meal was greater in cows fed once than twice per day, with cows grazing for longer over the first 2.5 hours. These results probably reflect two processes. First, a greater hunger drive in cows fed once per day as most (> 75%) of their daily intake had been achieved by 3 pm the previous day. Second, a rapid decline in forage availability in cows fed twice per day, which may restricted grazing and reduced the motivation to eat.

Total bite rate per minute averaged 42.3 bites per minute for kale (prehension plus mastication) and 38.5 bites per minute for grass (only prehension bites). These bite rates are similar to the values reported by Gregorini *et al*. (2009) for lactating dairy cows grazing pasture. Of note was the very low prehension bite rate in kale (7.4 bites/min) compared to grass (38.5 bites/min). In the current study, kale was observed to often be harvested in large (c. > 30 cm) sections of stem and leaf, which were then gradually draw into the mouth over series of mastication bites; in this concept mastications were more in line with prehension. This observation agrees with the general finding that bites of large mass take require more time for manipulation and processing (Cosgrove & Edwards 2007).

There were negligible effects of feeding frequency on prehension bite rate for cows grazing grass or kale over the first 2.5 h after a fresh break was offered. This result occurred despite forage availability declining more rapidly over the 2.5 h period when forages were allocated twice per day. This result is not consistent with the study of Gregorini *et al*. (2009) who reported cows fed allocation of pasture in two (i.e. 4 h after each milking; 2 × 4 h) rather than one allocation (i.e. 8 h between milkings; 1× 8h) had higher bite rates over the grazing session. However, Kennedy *et al.* (2009) also noted that restricting pasture DM had no effect on bite rate. It seems that in this study, the cows attempted to increase their intake rate over the grazing session by grazing for longer rather than biting faster.

6.4.4 BCS, milk yield and composition

Over the six week winter feeding period, the gain in BCS was greater (0.5 units) for grass than kale (0.2 units). The result contrasts with the study of Keogh *et al.* (2009a), which reported greater gain in BSC over winter for cows fed kale than perennial ryegrass. Keogh *et al*. (2009a) assigned this effect to lower DM intake reflecting lower utilisation in grass, a result that was also found in this study. The greater BCS gain in this study is surprising given the ME intake of grass relative to kale. Based on ME for grass 11.8 MJ ME/kg DM, 12.7 MJ ME/kg DM for kale and 7.3 MJ ME/kg DM for straw, the calculated ME intakes were 112 MJ ME/cow/day and 144 MJ ME/cow/day for grass and kale respectively. Some of the possible reasons for this include low ME intake during the transition period, inability to accurately predict ME of kale crop, and anti-nutritional factors (Section 3.4). Considering the average apparent DM intake of kale (once or twice) over six weeks of 9.7 kg DM/cow and SMCO concentration in kale crop (0.16%), the SMCO intake would be 16 g SMCO. This is lower than the threshold values of 10-15 g/100 kg reported by Smith (1980) to cause an acute haemolytic anaemia. This might explain why DM intake was not depressed, as utilisation of the kale crop was consistent across the weeks of the trial (96 ± 3%).

Previous studies (e.g. Dalley *et al*. 2001) have reported little effect of feeding frequency on the performance of lactating dairy cows, relative to effects of forage allowance. In this study with dry dairy cows, feeding frequency had no effect on the BCS change during the pre-partum period. This result is not a surprising result since there was no significant difference in DM intake and so ME intake between cows fed once and twice per day. Indeed, there was tentative evidence that on grass at least DM intake was lower when fed twice per day due to lower DM utilisation.

Milk solids production over the first 60 days of lactation was unaffected by feeding frequency or crop type cows were wintered on. The lack of an effect of feeding frequency is in line with the small differences in BCS at the end of winter feeding period. However, cows had higher BCS on grass, and so might have been expected to have higher milk solid production. Previous studies have noted that dry dairy cows which are in better condition at calving produced more milk in the following lactation (Domecq *et al.* 1997; Roche *et al.* 2009; Stockdale 2001). Roche *et al*. (2007b) reported that for every one unit in BCS score at calving, milk solids over the first 60 days of lactation were 32.5 kg/cow higher. However, it should be noted that the difference in BCS between kale-fed cows and grass-fed cows was small (0.2 BCS units) and may not have affected the milk production during the early lactation period. Further, the result of the current study supports the study by Keogh *et al.* (2009a), who noted no difference in both milk yield and composition between grass-fed cows and kale-fed cows, despite greater BCS at calving, a result that was attributed to the difference in DM intake between thin and fat cows after calving.

6.4.5 Estimated nitrogen losses

Unlike in the indoor study (Chapter 5), where steers which were fed kale once a day had less N% in urine (0.33%) compared to those fed kale twice a day (0.37%) due to unknown reasons, in the present study feeding frequency had no effect on N% in urine. Urinary N% was greater for kale (0.63%) than for grass (0.51%). This might be due to high N intake by kale-fed cows (397 g N/day/cow) compared to grass-fed cows (310 g N/day/cow), as high N intake leads to high N excretion through urine (Jonker *et al*. 1998; Kebreab *et al*. 2001). The average N% in urine during this study was low around 0.55% for both kale and grass-fed cows. This N% concentration in urine equates to 5.5 g N/l, lower than 11.5 g N/l reported by Haynes and Williams (1993) for dairy cows grazing pasture.

6.5 Conclusion and implications

- There is no positive result on the DM intake, utilisation during winter and production of dairy cows during lactation by more frequent allocations of feed in winter period.
- Therefore, because more frequent allocations of forage are likely to provide additional challenges for farm staff since each break has to be cut in the crop and the need to shift fences more often, they are not justified.

Chapter 7
Effect of altering feeding frequency of kale and grass fed to dairy cows in winter on rumen function and foraging behaviour

7.1 Introduction

A vital part of improving wintering outcome is to allocate forages such as kale or grass accurately (Judson *et al.* 2010; Judson & Edwards 2008). Crop allocation methods may limit ability of cows achieving their winter BCS targets (Judson *et al.* 2010). For example making the break width less by only 1 m can decrease allocation by 2 kg DM/cow/day depending on crop yield (Judson & Edwards 2008). Traditionally new breaks of forage crops are offered once a day in the morning and fed with hay or silage fed prior to the forage to attempt to minimise gorging and managing intake rate. For crops such as kale, intake rates are high from this feeding regime, with greater than 87% of total intake in the first 3 hours of grazing (Rugoho *et al*. 2010). Further, as the kale diet has low NDF, ADF and high WSC, there has been the suggestion of a rapid increase in rumen volatile fatty acids (VFAs) concentration leading to a reduction of the rumen pH, and subsequent effects of acidosis (Keogh *et al.* 2009c; Nichol *et al.* 2003). Such effects may be less pronounced if the diet is grass due to higher NDF and ADF content and lower intake rates. Further, with potential issues of rumen acidosis, it is proposed that the high intake rates with single meals may lead to metabolisable protein limitations later in the day (Dalley *et al*. 2011). Cabrita *et al.* (2006) indicated that offering the diet once daily can cause diurnal fluctuations in rumen concentrations of ammonia, VFA and lactic acid i.e. the rumen concentrations are high during the first ours of feeding and decreases to lower levels as the day progresses.

There have been numerous studies (see review by Cabrita *et al.* (2006) considering the effect of feeding frequency of total mixed ration diets on rumen physiology with housed animals. These show that when feed was offered more than twice per day there was reduction in the diurnal variation of rumen metabolites and pH. However, there is little comparable data from kale fed animals. Results in Chapter 5 (Section 5.4.1.2) from pen fed cattle indicated that the cattle fed kale twice a day had a second peak of rumen ammonia concentration overnight, which may reflect the effect of a second meal during the afternoon. This trend may suggest that there may be an advantage in rumen nitrogen supply with two feeding periods daily. Apart from these results, there is no data on the effect of offering winter forages such as kale more than once a day on the concentration of ammonia, VFA and rumen pH.

The objective of this was to evaluate the effect of feeding kale and grass once versus twice per day on grazing behaviour, rumen ammonia levels, VFA concentrations and rumen pH of non-lactating New Zealand crossbred dairy cows in winter. The effect of these treatments on BCS gain, cow behaviour, forage intake, DM utilisation and urinary nitrogen excretion of dry cows during the winter feeding period and their subsequent milk production during early lactation are described in Chapter 6.

7.2 Materials and Methods

7.2.1 Experimental design

The experiment was carried out within the allowance study described in Chapter 6. In brief, the four treatment groups were:

K1: 11 kg DM of kale grazed *in situ* + 3 kg DM of barley straw offered once per day.

K2: 5.5 kg DM of kale grazed *in situ* + 1.5 kg DM of barley straw offered twice per day.

G1: 11 kg DM of grass grazed *in situ* + 3 kg DM of barley straw offered once per day.

G2: 5.5 kg DM of kale grazed *in situ* + 1.5 kg DM of barley straw offered twice per day.

There were 48 dry dairy cows assigned to each replicate group with two fistulated cows in one of the two replicates groups of each treatment. More details of experimental design are described in Section 6.2.1.

7.2.2 Measurements

7.2.2.1 Crop measurements

Crop measurements were carried out as been described in section 6.2.2.1

7.2.2.2 Animal measurements

The behaviour of all 48 cows was visually observed over a one 24 h period from 0800 h during both weeks 2 and 5 of the experiment period. All cows were observed in the same day. Every 15 minutes cows were recorded as either: eating forage, eating straw, ruminating or idling. From the behaviour data, times in each activity were calculated by multiplying each behaviour activity frequency by a 15 min interval. The data was averaged across measurement days.

Rumen sampling

Rumen sampling was carried out over one 24 h period during both weeks 2 and 5 of experimental period. Two rumen-fistulated cows in one of the two replicates groups were fitted with backpacks which contained data loggers for temperature and pH (Delta Log, Milan, Italy). These data loggers were linked to pH and temperature probes (IJ44: Ionode Pty Ltd, Brisbane, Australia) weighted with a 1500 g steel paddle and inserted to the ventral sac floor. The pH and temperature were recorded every 15 seconds for 24 h. Rumen sampling was done on separate days to the days when foraging behaviour was recorded i.e. on a day before foraging behaviour measurements.

Spot samples of rumen fluid were collected over a 24 h period starting immediately following the completion of the rumen pH and temperature measurements for 24 h. Sampling periods was conducted every four hours from 0800 h. The two fistulated cows in each treatment group were brought together from the forage into a set of yards for the collection. All rumen samples were taken via a hand grab method from the bottom of the ventral sac region of the rumen. Samples for VFA and NH_4 analysis were removed and squeezed through two layers of cheese cloth. Samples for VFA were immediately placed on ice. A total of 1ml of 6M sulphuric acid was added to samples for NH_4 analysis (to prevent volatilisation by ensuring pH remained < 4). In all cases, the total sampling time for the two fistulated cows was less than 15 minutes, after which, cows were released back onto their daily pasture and kale allowance and left undisturbed until the next sampling period. After sampling, all samples were placed into a blast freezer set at -20^{o}C until required for analysis.

Rumen contents sample analysis

Rumen contents sample analysis was done as been described in Chapter 4 (section 4.2.2.2).

7.3 Calculations and data analysis

7.3.1 Rumen pH

The rumen pH measurements recorded every 15 seconds were averaged for consecutive 10 minutes for each treatment group, giving 80 pH records (4 per minute for 10 minutes for 2 cows per treatment). In addition, the recorded pH of all cows in each treatment group were used for each 10 minute block to calculate the proportion of total records within six pH bands : < 5.0; 5.0-5.5; 5.5-5.8; 5.8-6.0; 6.0-6.4 and > 6.4. Further details of rumen pH are described in Chapter 4 (section 4.3.1)

7.3.2 Acetic acid, propionic acid and butyric acid means

Area under a curve values for acetic acid, propionic acid and butyric acid and ammonia for both am and pm were calculated as been described in Chapter 4 (section 4.3.3). The mean from AUC values was analysed in two time periods: am (from 0800 h to 1600 h) and pm (from 2000 h to 0400 h) so as to assess relations with grazing events.

7.3.1 Statistical analysis

Data was analysed by ANOVA of a 2×2 factorial design GenStat (v14, VSL, Hempstead, UK) with two replicates. For behaviour data, the unit of replication was group of animals and for rumen function data, the individual cow was the unit of replicate. Where the interaction between forage and feeding frequency was significant a Fishers protected LSD test ($\alpha < 0.05$) was used to determine differences among treatment.

7.4 Results

7.4.1 Foraging behaviour

Foraging behaviour over the total day for each treatment is displayed in Table 7.1. Total time spent eating was higher on cows offered forage once a day (473 min/day for kale and 481 min/day for grass) and lower on cows offered forage twice a day (394 min/day for kale and 435 min/day for grass). Cows grazing grass ruminated for longer (299 min) than cows grazing kale (248 min). Cows grazing kale spent a longer time idling than grazing grass by 77 min and cows fed twice a day spent more time idling compared than cows fed once a day.

Table 7.1: Effects of forage type and feeding frequency on time spent eating, ruminating and idling over 24 h of non-lactating, spring calving Friesian × Jersey dairy fed once a day (K1 and G1) or twice a day (K2 and G2). Means with a different subscript are significantly different according to LSD teats follwing significant ANOVA (α<0.05).

	Kale		Grass		Level of significance			
	K1	**K2**	**G1**	**G2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
Eating	473	394	481	435	NS	<0.001	NS	44.81
Ruminating	243	253	300	298	0.002	NS	NS	43.22
Idling	725	794	659	708	<0.001	0.002	NS	56.79

NS = Not significant.

The total daily time of foraging behaviour between treatments arranged in 4 h blocks is displayed in Table 7.2. From 0800 h to 1200 h eating time and ruminating time were higher and idling time lower on kale than grass, and ruminating time was greater in cows fed once than twice per day. From 0800 h to 1200 h, there was significant interaction for eating and idling time. Eating time was greater in grass than kale for cows fed twice per day, but did not differ between kale and grass cows fed once per day. Idling time was less in grass than kale for cows fed twice per day, but did not differ between kale and grass in cows fed once per day. From 1600 h to 2000 h, there was significant interaction for eating and idling time. Eating time was greater in cows fed twice per day in kale fed cows only. Idling time was less in cows fed twice per day in kale fed cows only. From 2000 h to 0000 h, eating time and ruminating time were lower, and idling time higher in kale than grass, eating time was greater in cows fed twice per day. From 0000 h to 0400 h, cows spent more time idling for kale than grass. From 0400 h to 0800 h, cows spent more time ruminating and less time idling on kale than grass.

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Table 7.2: Foraging behavior of dry dairy cows offered kale at 11 kg DM/cow/day either once a day (K1) or twice a day feeding (K2) and grass offered at 11 kg DM/cow either once a day (G1) or twice a day (G2) measured every four hours. Means followed by a different letter are significantly different according to Fishers protected LSD test following a significant interaction from ANOVA (α<0.05).

			Kale		Grass		Level of significance			
Time	**Hours**	**Behaviour**	**1**	**2**	**1**	**2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
08:00 -12:00	0 - 4	Eating	191	171	136	136	<0.001	NS	NS	16.54
		Ruminating	20	29	4	14	<0.001	0.002	NS	8.06
		Idling	29	40	100	90	<0.001	NS	NS	17.28
12:00 -16:00	4 - 8	Eating	201^{a}	70^{c}	204^{a}	116^{b}	0.002	<0.001	0.005	21.92
		Ruminating	0	20	4	13.8	NS	<0.001	NS	9.08
		Idling	39^{c}	150^{a}	32^{c}	110^{b}	0.001	<0.001	0.017	20.15
16:00 -20:00	8 - 12	Eating	76^{b}	143^{a}	119^{a}	134^{a}	0.047	<0.001	0.006	25.08
		Ruminating	13	8	40	26	<0.001	NS	NS	15.21
		Idling	151^{a}	89^{b}	81^{b}	80^{b}	<0.001	<0.001	<0.001	24.15
20:00 -0:00	12 - 16	Eating	3^{c}	6^{c}	20^{b}	40^{a}	<0.001	0.010	NS	12.41
		Ruminating	34	38	101	83	<0.001	NS	NS	21.25
		Idling	203	196	119	117	<0.001	NS	NS	21.09
00:00 - 04:00	16 - 20	Eating	0	4	3	8	NS	0.032	NS	5.57
		Ruminating	80	65	83	92	NS	NS	NS	25.10
		Idling	160	171	154	140	0.042	NS	NS	24.32
04:00 - 08:00	20 - 24	Eating	0	0	0	1	NS	NS	NS	1.78
		Ruminating	96	94	69	69	0.007	NS	NS	26.38
		Idling	144	146	171	170	0.010	NS	NS	26.89

NS = not significant at 5% level.

7.4.2 Rumen fermentation

The area under a curve values for rumen VFA and ammonia concentrations of all treatment groups are presented in Table 7.3. In the am period, the mean area under a curve for acetic acid and propionic acid was greater for cows fed grass than kale. Acetic acid concentrations in the am were higher in cows fed twice than once per day. During the pm, propionic acid concentration was higher for cows fed grass than kale and cows fed twice than once per day. The mean area under a curve for butyric acid levels was not affected by forage type or feeding frequency during the am period. In the pm period, the mean area under a curve for butyric acid was greater for cows fed kale than grass and cows fed twice than once a day.

Table 7.3: The effects of feeding kale or grass once (1) or twice (2) per day on area under a curve (AUC) of acetic acid, propionic acid and butyric acid and ammonia concentrations (AUC units) in the rumen. Means followed by a different letter are significantly different according to Fishers protected LSd test following a significant interaction from ANOVA (α<0.05).

	Kale		Grass		Level of significance			
	K1	**K2**	**G1**	**G2**	**Species (S)**	**Frequency (F)**	**S×F**	**LSD**
Acetic acid a.m	900	1011	1150	1371	<0.001	0.027	NS	203.1
Acetic acid p.m	995	1090	973	1298	NS	0.002	NS	154.8
Propionic acid a.m	192	218	301	308	<0.001	NS	NS	32.7
Propionic acid p.m	217	232	234	286	0.026	0.022	NS	41.5
Butyric acid a.m	214	236	238	237	NS	NS	NS	91.8
Butyric acid p.m	200	262	150	174	<0.001	0.003	NS	35.5
Ammonia a.m	179	159	153	302	<0.001	NS	NS	63.4
Ammonia p.m	96[a]	106[a]	103[a]	184[b]	0.002	<0.001	0.005	32.2

NS = not significant; am (0800 h to 1600 h) and pm (2000 h to 0400 h).

The concentration of acetic acid, propionic acid and butyric acid varied throughout the day. (Figure 7.1a, b and c respectively). For kale-fed cows the highest acetic acid, propionic acid and butyric acid proportion occurred at around 2000 hours, 11 h post feeding the first meal for once a day cows and 11 h post feeding the morning meal and 5 h post feeding the second meal for twice a day cows (Figure 7.1a, b and c, respectively). The peak concentration value for acetic acid for grass fed cows was 7 h post-feeding the morning meal for once a day cows and 5 h post feeding the afternoon meal for twice a day cows (Figure 7.1a).

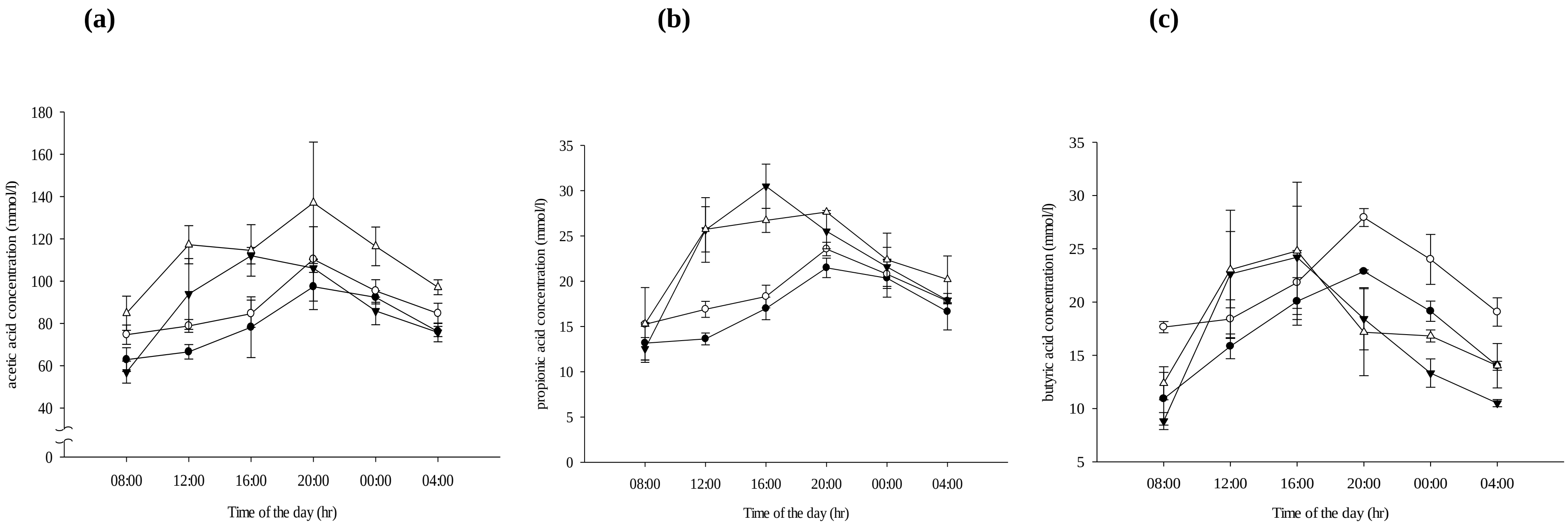


Figure 7.1 Diurnal pattern of acetic acid **(a)**, propionic acid **(b)** and butyric acid **(c)** of dairy cows offered kale, once (●) or twice (○) and grass, once (▼) or twice (△) a day. Values are means ± SEM.

The area under a curve for ammonia was greater for grass cows than kale cows during the am (Table 7.3). During the pm grass cows continued to have high levels of ammonia in their rumen compared to kale cows and cows fed twice a day had high levels of ammonia than those fed once a day forage. There was interaction between forage and frequency during the pm period; ammonia levels were higher for K2, G1 and G2 and lower for K1 cows. The effect of feeding frequency and forage type on the rumen ammonia concentration is shown in Figure 7.2. Cows fed on grass had a higher rumen ammonia concentration compared to those fed kale. The peak value for all treatments was observed 3 hours after the first meal, with no evidence of a second peak.

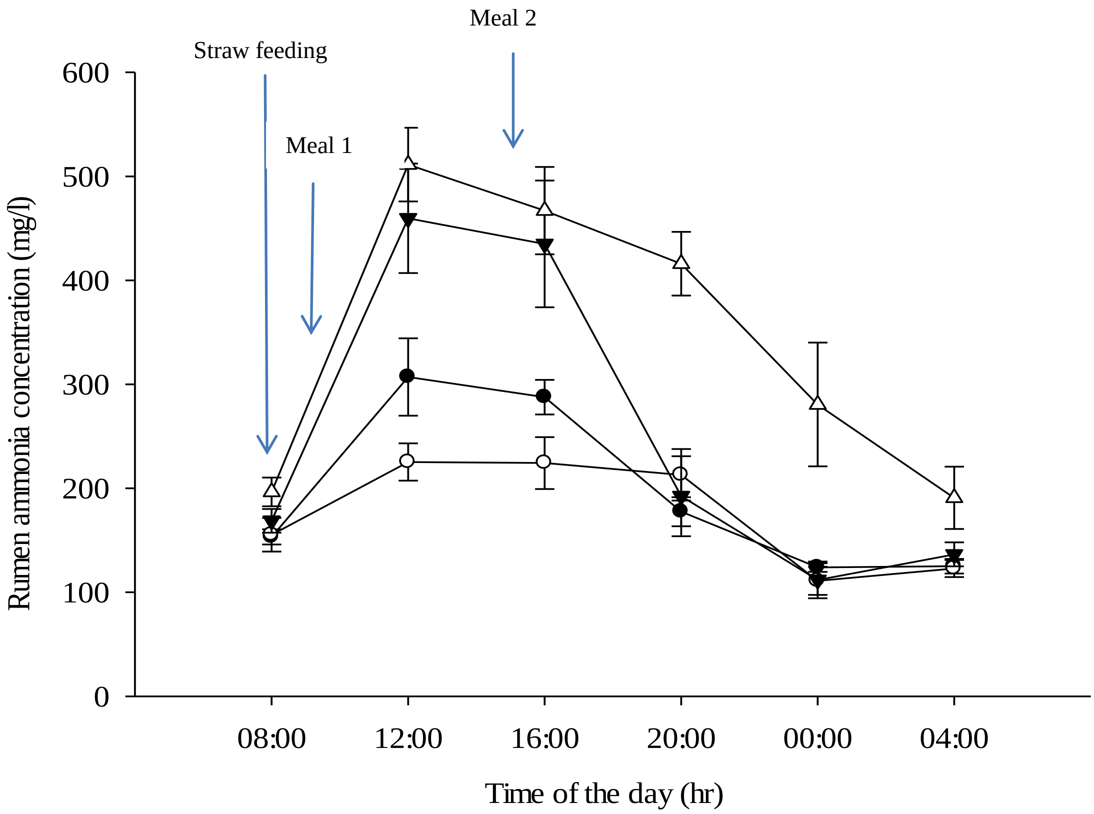


Figure 7.2: Diurnal pattern of rumen ammonia concentration of dairy cows offered grass once (▼) or twice (△) and kale once (●) or twice (○) a day. Values are means ± SEM.

Cows which were fed grass once a day had the highest pH from 2300 h until 0900 h, with other treatment groups (G2, K1 and K2) having similar rumen pH (Figure 7.3). Cows fed kale either once a day or twice a day followed the same pH pattern after feeding the first meal. In comparison grass fed cows, which were fed once a day or twice a day had lower pH from 1100 h (2 h after feeding the first meal) till 2200 h compared to their kale-fed counterparts but similar pattern of pH variation was also observed in grass treatments

(Figure 7.3). The proportion of bout counts < 5.5 and 5.5 - 5.8 was greater in grass fed cows than kale-fed cow. Further, cows which were fed grass twice a day recorded the least pH of <5.5 (Table 7.4) and it was 6 h after feeding the second meal around 1500 h.

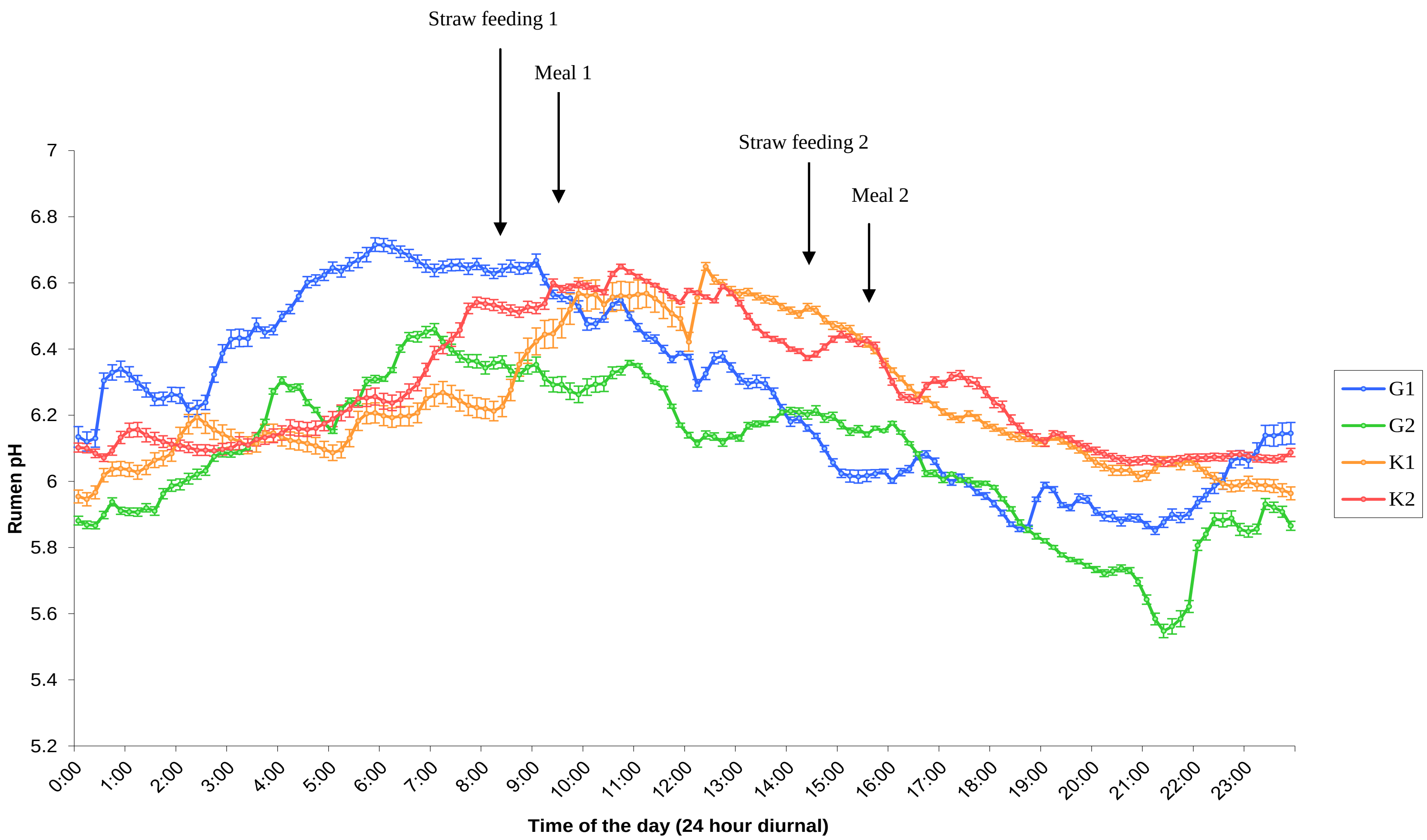


Figure 7.3: Diurnal rumen pH of dairy cows after feeding kale at 11 kg DM/cow/day once a day (K1), 11 kg DM/cow/day twice a day (K2), grass at 11 kg DM/cow/day once a day (G1) and grass at 11 kg DM/cow/day once a day (G2) during winter period. Values are means ± SEM.

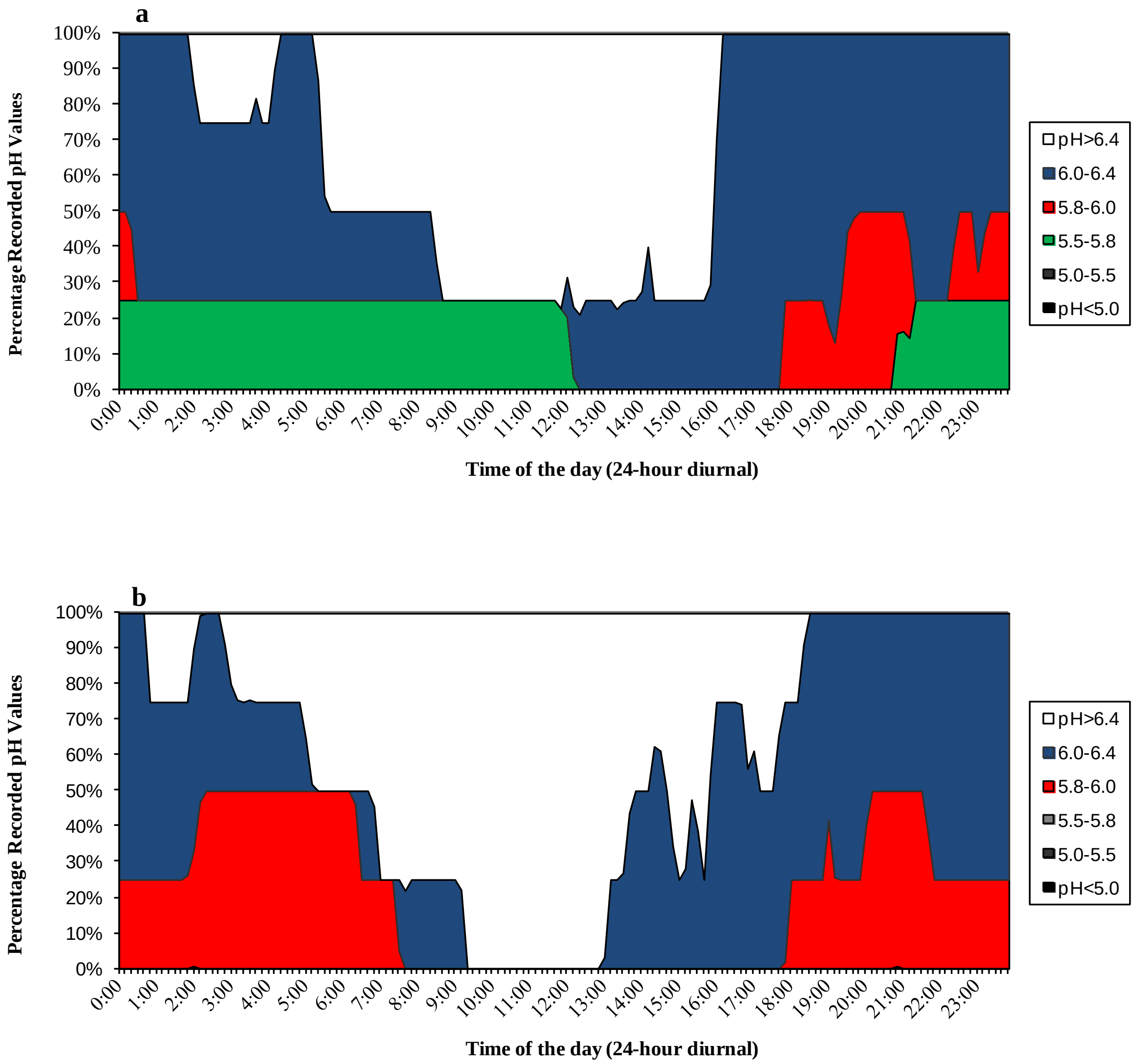
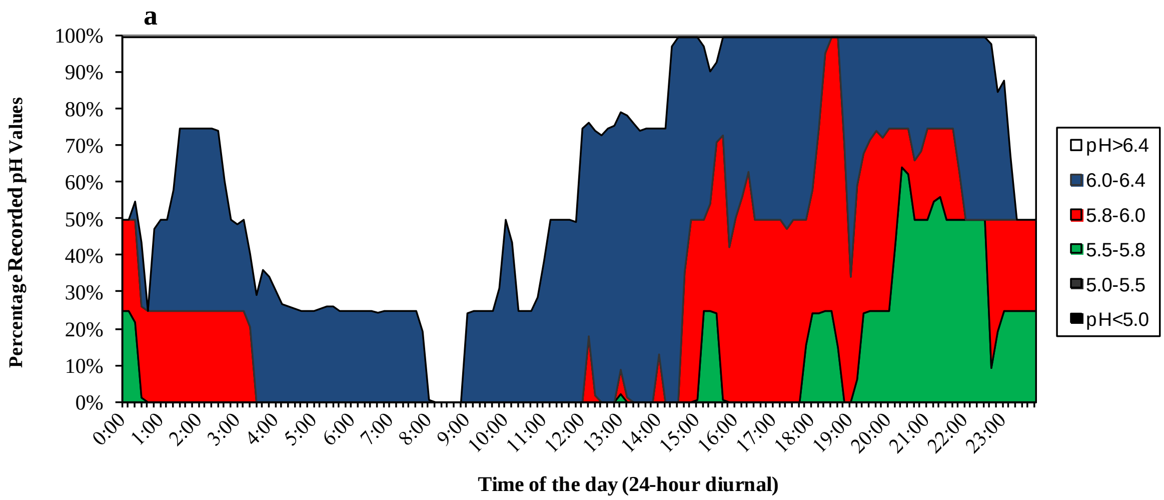


Figure 7.4: Rumen percentage recorded pH values for dairy cows after feeding kale offered at 11 kg DM/cow/day **(a)** once (K1) and **(b)** twice (K2) a day.



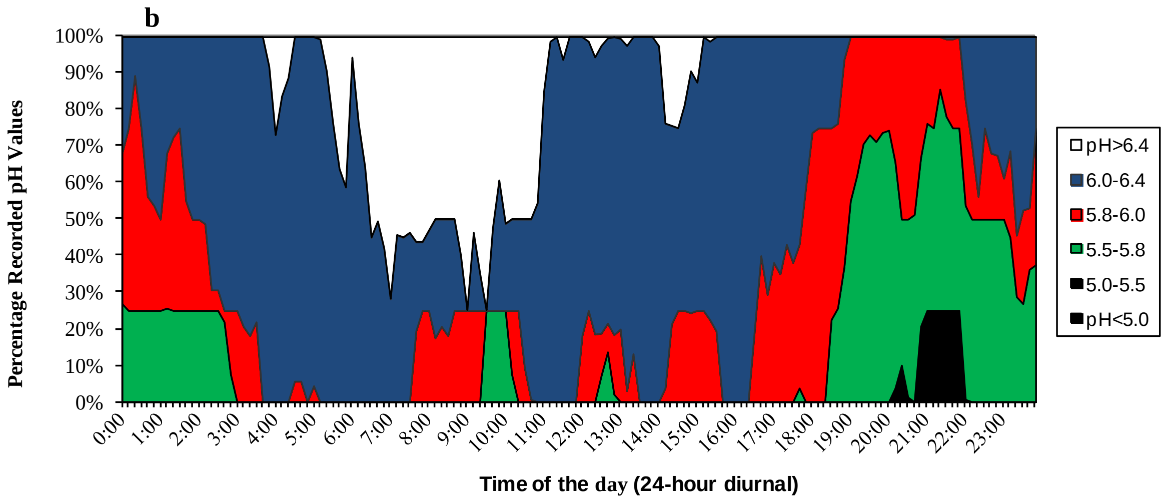


Figure 7.5: Rumen percentage recorded pH values for dairy cows after feeding grass offered at 11 kg DM/cow/day **(a)** once (G1) and **(b)** twice (G2) a day during winter.

Table 7.4: Rumen bout summary of dairy cows after feeding kale either once (K1) or twice K2) or grass either once (G1) or twice (G2) a day.

	Bouts < 5.5:				**Bouts < 5.8:**				**Bouts < 6.0:**				**Bouts < 6.4:**			
Treatment:	**Bout Count:**	**Avg bouts per day:**	**Avg mins per bout:**	**Mins per bout SE:**	**Bout Count:**	**Avg bouts per day:**	**Avg mins per bout:**	**Mins per bout SE:**	**Bout Count:**	**Avg bouts per day:**	**Avg mins per bout:**	**Mins per bout SE:**	**Bout Count:**	**Avg bouts per day:**	**Avg mins per bout:**	**Mins per bout SE:**
G1	0.0	0.0	0.0	0.0	7.0	1.7	78.1	26.8	15.0	3.7	105.5	44.6	9.0	2.2	138.1	54.5
G2	2.0	0.5	36.0	32.5	10.0	2.4	90.6	45.7	32.0	7.8	62.8	22.3	18.0	4.4	92.6	52.3
K1	0.0	0.0	0.0	0.0	1.0	0.2	10.8	0.0	5.0	1.2	58.8	15.8	6.0	1.5	412.9	180.2
K2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	1.0	294.1	156.9	9.0	2.3	245.1	105.9

7.5 Discussion

7.5.1 Crop type

7.5.1.1 Foraging behaviour

The total time spent eating did not differ between kale and grass. This is consistent with the results of Gazzola *et al*. (2008) who reported no significant difference in total time spent eating for cows offered grass, kale and swedes. One possible explanation for the lack of difference is the low allowance of 11 kg DM/cow/day which was used in this study. This would have been below the *ad lib* intake for both groups of cows, encouraging strong appetites in all cows (Gregorini *et al.* 2009). Previous studies noted that grazing time may increase in response to decreasing pasture mass, as animals seek to compensate for low bite masses; however, the resource was quickly depleted in this study, limiting the ability of cows to do this.

Forage type did however, affect ruminating and idling time with cows fed grass spending more time ruminating and less time idling than cows fed kale. As previously discussed in section 4.4.1.1, grass has higher NDF (Keogh *et al.* 2009c; Nichol *et al.* 2003, Table 6.1) compared with kale and this is likely to contribute to an increased rumination time shown for grass. This finding does not support the results by Gazzola *et al.* (2008) who reported lower rumination time in cows fed grass to cows fed kale. According to Waghorn *et al*. (2007) rates of NDF digestion ranges from 4 % to 29 %/hour depending on the type of forage and the rate slows down from about 3 to 16 %/hour as ryegrass matures (Chaves *et al.* 2006). As the pasture used was carried over from autumn, it may have been more mature compared to the pasture used in the later study by Gazzola *et al*. (2008), although no pasture quality was reported. This may explain the increased rumination observed in grass-fed cows in the present study.

Despite, little difference in total grazing time, it should be noted that there was a distinct eating and ruminating pattern in response to feeding time. Cows ate rapidly during the first 4 h of grazing and on average grazing time was longer for the first 12 h of grazing. Ruminating time was longer from 12 h of grazing until 24 h of grazing. These patterns have not been shown before and they reflect pattern of offering forage to dry dairy cows during the winter period.

7.5.1.2 Rumen Fermentation

VFA production

Brassica crops such as kale have higher levels of non-structural carbohydrates (NSC) than grass (Keogh *et al.* 2009c; Nichol *et al.* 2003). As these are readily fermentable it would be expected to observe high concentrations of VFAs in the rumen of cows fed kale. However, in the present study, higher concentrations of acetic acid and propionic acid were observed in cows fed grass. The higher acetic acid concentration in grass-fed cows was also reported by Khalili & Sairanen (2000) and may be due to high fibre in grass compared to kale crop, as diets in high fibre content give rise to acetic acid (Van Soest 1994). As fermentation of sugars results in an increase in butyric acid production (Chamberlain *et al*. 1983; Friggens *et al*. 1998; Van Soest 1994), this may also explain the higher butyric acid for cows fed kale compared to cows fed grass during the afternoon.

Rumen ammonia

Rumen ammonia concentrations were higher in cows fed grass than cows fed kale, particularly in the afternoon. High concentrations of rumen ammonia in dairy cows fed on grass pasture and low concentrations in diets with high sugar content diets were also reported in the studies by Khalili & Sairanen (2000) and Trevaskis *et al*. (2004). Of note is the ammonia concentration was greater for grass-fed cows than kale-fed cows in the present study. This was despite total N intake being 30% higher for kale than grass-fed cows. This may be due to the difference in physical structure between kale crop and grass. Kale was observed to be in large sections and grass in small particle sizes in the rumen during sampling times. As a result grass was broken down at a faster rate compared to kale crop in the rumen causing ammonia to be released at a faster rate in grass-fed cows than kale-fed cows. The rumen ammonia in grass-fed cows might have caused the slowing of the rumen epithelium blood flow (Kristensen *et al*. 2010) which in turn slowed the VFA absorption from the rumen. This might also explains why a lower rumen pH was observed in grass-fed cows, since; the depression of rumen pH is also associated with high concentration of VFA acids in the rumen (Forbes, 1995; Allen, 2000).

Rumen pH

The SARA pH threshold in TMR fed cows is generally accepted as 5.5 (Marie Krause & Oetzel 2006), and in the present study the evidence from bout counts did not suggest SARA in cows fed kale. Since high NSC intake would be expected to reduce pH, and high NDF intake to increase pH, lower pH values should be present in kale fed cows. However, in the present study cows fed grass recorded lower pH than kale-fed cows (Table 7.4). This response is reinforced by the results of Gibbs (2009), who reported no evidence of acidosis as the industry standard kale and straw ration produced a relatively high rumen pH compared to grass fed at the same allowance. Although grass had high levels of NDF (Table 6.1; section 6.3) compared to kale crop in the present study, the utilisation of grass was low (76%) compared to kale crop (96%) (Table 6.2; section 6.3), and hence, fibre consumption may have been lower in grass-fed cows. Also, since both kale and grass forages contained high levels of crude protein (22 % and 24 % of DM respectively), it would appear reasonable to expect relatively high pH on both treatments. Jasaitis *et al.* (1987) and Wadhwa *et al.* (2001) noted a high pH and associated with high buffering capacity of high protein feeds (15 to > 35% CP of DM) compared to high energy feeds. The high buffering capacity was thought to be as a result of protein degradation to ammonia. Plant proteins contribute between 10 and 20% of total buffering capacity (Playne & McDonald 1966).

7.5.2 Feeding frequency

7.5.2.1 Foraging behaviour

With concentrate diets, and unrestricted feeding, increasing the feeding frequency from once to twice per day and from two to four times a day increased the daily feeding time of dairy cows (Devries *et al*. 2005). In contrast, in the current study, eating time was greater in cows fed once per day. The results from the current study may reflect that cows fed once a day had greater eating motivation (Gregorini *et al*. (2009) or the likely effect of rapid depletion in herbage available for the cows fed forage twice a day. Another explanation is that cows in twice a day breaks might have stopped grazing in anticipation of moving to a new strip at 1500 h before forage availability becomes a limiting factor (Jamieson & Hodgson 1979) or they wanted to save energy by not grazing very low (minimizing net energy expenditure during grazing) (Cosgrove & Edwards 2007; Ketelaars & Tolkamp 1996).

Incremental increases in grazing time are often at the expense of ruminating and idling time (Abrahamse *et al.* 2008). In the present study feeding frequency had no effect on ruminating time, however, the time spent idling was greater in cows fed forage twice a day compared to those fed forage once a day, which is likely due to decreased total grazing time (Table 7.1).

7.5.2.2 Rumen Fermentation

VFA production

Despite high concentrations of NSC in kale compared to levels of fibre (NDF and ADF), and high intake rates of cows in once a day feeding of kale, offering kale and straw to dry dairy cows either once a day or twice a day using industry standard allocations of 11 kg DM kale did not affect acetic acid, propionic acid (both am and pm) and butyric acid concentrations in the am period (Table 7.5). There was also minimal benefit in terms of acetic acid and propionic acid when grass was offered at 11 kg DM plus 3 kg DM of straw either as a single meal at 0900 h or two equal meals one at 0900 h and the other at 1500 h (Table 7.6). Rumen concentration of acetic acid and propionic acid were high in cows which were offered grass twice a day compared to cows offered grass once a day.

Rumen ammonia

The daily average ammonia levels during the morning were higher for grass-fed cows than kale-fed cows (Table 7.3). Feeding frequency had an effect during the afternoon period. Rumen ammonia levels were higher for cows which were fed forage twice a day (kale or grass). There was also forage × feeding frequency interaction, with cows fed kale once per day having lower values than all other treatments. Rumen ammonia concentration rose rapidly after the first meal during the morning, with all four treatments reaching a peak value within 3 hours of grazing time (Figure 7.3). The data for kale and grass cows fed once a day fluctuated less over the day. This may be due to higher intake rates by cows fed once per day compared to cows fed twice per day, For example, after 6 h of grazing cows offered kale or grass once a day had consumed 10.1 and 7.7 kg DM/cow respectively and those offered kale or grass twice a day had consumed 5.1 and 4.1 kg DM/cow respectively.

Rumen pH

Krause *et al.* (2009) reported that the meal size and total DMI could be factors affecting the time after feeding of the daily minimum pH. Variation of pH throughout the day is largely influenced by the consumption of meals. This is when the time between each meal is extended causing the ruminant to consume the available feed quickly, which can cause acidosis to occur. The amount of feed available can be reduced to avoid this rapid consumption theory, along with multiple feedings per day (Krause *et al.* 2009); results have shown a reduced change in pH (Cabrita *et al.* 2006; Marie Krause & Oetzel 2006). However, diurnal variation in pH in both kale treatments and grass treatments followed the same pattern in the present study.

7.6 Conclusion

- Offering kale and grass at industry standard allocations did not affect mean grazing time between treatments but increased mean ruminating time in grass fed cows.
- Cows fed kale and grass once a day grazed for longer than cows offered forage twice a day.
- There was no evidence of rumen acidosis on any treatment.
- On average the concentration of acetic acid in the rumen was higher in grass-fed cows and lower in kale-fed cows. The concentration of propionic acid and butyric acid in the rumen was higher in kale-fed cows than in grass-fed cows.

Chapter 8
General discussion and conclusions

8.1 General Discussion

Forage availability (Greenwood *et al.* 2011), providing adequate forage (Dewhurst *et al.* 2000) and regaining BCS lost through lactation (Judson *et al.* 2010; Judson & Edwards 2008) are the main goals during winter feeding. Unlike in the North Island of New Zealand were winter feeding is mainly on grass forage, due to the cool weather conditions during winter season in South Island the traditional grass wintering systems have been replaced by feeding brassica crops like kale (Judson *et al.* 2010; Pangborn & Gibbs 2009) outdoors and off the milk platform (Beare *et al.* 2006). There is little experimental data on the use of kale in wintering systems for example, utilisation, intake and foraging behaviour of cows grazing kale in response to changing allowance, crop types (Gazzola *et al.* 2008) and feeding frequency. (Rugoho *et al.* 2010) Further, kale crop contains high levels of NSC, low NDF and a relatively low DM (Table 6.1), as a result is widely suggested to promote low rumen pH in dairy cows. However, research demonstrating this or investigating other aspects of rumen function like VFA production when dry dairy cows are winter-fed on kale crop is very scarce (Gibbs 2009). As a result this study sought to examine performance of cows grazing kale versus grass when offered at different allowance and frequency.

8.1.1. BCS gain

Forage allowance had an effect on the body condition score during the dry period. This was shown by the results of Chapter 3 where cows offered 14 kg kale/cow/day gained more BCS compared to cows offered 11 kg kale/cow/day. These results are in agreement with Greenwood *et al*. (2011) who found that cows which were offered high allocation of kale gained 1.0 BCS unit and those offered low allocation of kale gained 0.2 BCS unit over winter period. This probably reflects both a combination of greater DM intake (12.1 versus 10.3 kg DM/cow/day, respectively) and improved diet quality with more of the low quality stem left behind.

These results confirm that to gain 0.5 BCS units over a 6-8 winter feeding period, allowance approaching 14 kg kale DM/cow/day is required. These allowance values are higher given the calculated ME intake of cows on this allocation may approach 170 MJ ME/cow/day. This and the calculated value for cows fed 11 kg kale/cow/day (150 MJ ME/cow/day) is well in excess of the 112 MJ ME/day given by Nichol *et al.* (2003) and 115 MJ ME/day by Nicol & Brooks (2007) to gain 0.5 BCS units during winter period. The reason for the inequality is not clear, but may show the inability to sustain ME intake during the transition to pasture from kale (Greenwood *et al.* 2011). The other reason for lack of gaining 0.5 BCS may be due to the effect of anti-nutritional factors such as nitrates and SMCO that are suspected to limit performance on kale, even when fed at high allowance (Judson *et al*. 2010). Some evidence to support this comes from the results from Chapters 3 and 4 (experiment 1 and 3) showing that cows offered grass at 11 kg DM/cow/day gained more BCS compared cows fed kale at the same allowance, with grass lacking the amounts of secondary plant compounds. However, SMCO remained low in the present study (0.2 %) and no clinical signs of haemolytic anaemia were observed in the present study and the study by Keogh *et al*. (2009c). A further reason may be that maintenance energy requirement may have been underestimated. In a recent analyses, (Mandok *et al*. 2012) suggested there may be much greater requirements for energy to maintain a pregnant dry cow in NZ winters than had been previously recommended. These authors concluded that adjusting for pregnancy and activity, estimated maintenance energy requirements were 0.94 MJ ME/kg $Lwt^{0.75}$, thus underestimating the maintenance energy requirements of New Zealand dairy cows by more than 40 % from the previous recommendations of 0.55 MJ ME/kg $Lwt^{0.75}$. Reason for this may be due to higher genetic selection towards higher production as a result an increase in metabolic rate (Mandok *et al*. 2012). Based on this ME values for maintenance would be around 104 MJ ME/day for a 530 kg cow used in this study. If ME for gain not changed (24 MJ ME/day), then the total would be around 152 MJ ME/day, which is in line with the present study. As a result the ME values used in the present study are broadly correct.

A further reason is that ME of crops may have been overestimated; Clark *et al.* (1987) reported that the metabolisable energy (ME) of forage crops with high water soluble carbohydrates was overestimated with typical digestibility and methane yield estimates, as the gross energy of the plant is significantly lower when sugars are a substantial fraction of the carbohydrate present. Further, it is also possible that the ME value used during this study of (mean 12.18 MJ ME) may be overestimated from NIRS. The ME used in the present study was derived from the equation ME (MJ ME/kg DM) = Digestible organic matter content × 0.016 (g/kg DM) by McDonald *et al*. (2002). However due to low fibre and lipid content of kale, the coefficient in the above equation derived using more fibrous may be inadequate for determination of energy in brassica crops (Greenwood *et al.* 2011). Hence there is a need to determine the ME content of kale using *in vivo* measurements as been described by Clark *et al*. (1987).

Results from Chapters 3 and 4 (experiment 1 and 3) also suggested that cows offered grass at 11 kg cow/day gained the same BCS units as cows offered 14 kg kale/cow/day and more compared to cows offered 14 kg kale/cow/day. This is surprising given the ME intake of grass (114 MJ ME/cow/day) was lower than kale (145 MJ ME/cow/day) in both experiments. A possible reason for this is that kale-fed cows grazed kale stems which are low in ME (11.6%) and CP (13.6%) (Judson & Edwards 2008; Rugoho *et al.* 2010). However, this may be unlikely as protein content of grass plus straw diet (3.7% N) and of kale plus straw diet (3.5% N) was greater than the requirement of 1.8 % DM for maintenance (de Klein *et al*. 2010).

This data points towards grass being a more desirable crop to winter dairy cows on than kale. However, based on comparative crop yields, (15 t DM/ha kale compared to 4 t DM/ha of grass in winter), would mean the area required would be four to five times greater with grass compared to kale. This may not be feasible for many farmers on a cost and land access basis.

Feeding cows twice compared to once per day had very limited effect of DM intake, utilisation, or gain in body condition score over the winter feeding period. Further, feeding more frequently did not appear to improve any aspect of rumen function in way that would enhance cow performance. This occurred in both kale and grass, and provides further evidence (see Dalley *et al*. 1999) that shifting cows more frequently would have any significant advantage; indeed as it would increase labour requirements, it would thereby creating a more challenging wintering management than feeding once per day.

An alternative strategy to feeding more frequently may be to allocate forage crops less frequently, with view to reducing labour requirements. In an unpublished study at Lincoln University (Mathibiswana unpublished) the performance of cows fed kale once every day or every four days at the equivalent allowances of 14 kg DM kale/cow/day. The 8 week studied showed no differences in DM utilisation, apparent intake or BCS gain between the two treatments. Further, cow health problems were not more evident in cows fed every four days. This indicates that feeding cows every 4 days may be an effective strategy to reduce labour requirements in winter feeding systems without impacting on BCS gain.

8.1.2 Milk solids

Milk yield and composition during the early lactation was measured in Chapter 6 (Experiment 3). Despite the difference in BCS (0.2 BCS units) between grass-fed cows and kale-fed cows at end of winter feeding period, milk yield and composition did not differ during the first 60 d of lactation. This result does not support the reports by Roche *et al.* (2009) and Stockdale (2001) who noted the improvements of milk yield due to achieving BCS targets at calving (BCS of 5.0; 1-10 NZ scale). However, the difference in BCS at calving (Section 6.4.1.3) between kale-fed cows and grass-fed cows was small and may not have affected the milk yield production during early lactation, were cows were offered the same diet during the post-calving period. Keogh *et al*. (2009a) also noted no difference in milk yield and composition of dairy cows in early lactation between cows fed on kale and grass during winter despite the two treatments having different BCS at calving.

This study focussed on forage crop and forage allocation methods to increase DM intake, improve diet quality and increase BCS gain over the 6 to 8 winter, dry cow feeding period. The study confirmed that a gain of BCS units could be achieved if cows were fed at a higher allowance of 14 than 11 kg DM kale/cow/day. Recent analyses by Greenwood *et al*. (2011) examined strategies to increase body condition score gain on the milking platform prior to drying off. They showed that feeding 3 kg of concentrate/cow/day during lactation period increased BCS prior to grazing off, with treatment effect persisting throughout the winter feeding period when fed at low allowance of 11 kg DM kale/cow/day. Further in the first 60 d of lactation, milk solids was 0.1 kg/cow/day higher in those fed supplement than those not fed supplement. Cows that were not fed supplement required feeding levels of 14 kg DM kale/cow/day to gain body condition score. However, it was notable that this had no effect on lactation performance of cows. This indicates complex effects of feeding on body condition score and tentative evidence that providing additional energy in late lactation may be more beneficial in terms of MS production in following lactation than increasing kale availability in the winter dry cow feeding period. This pattern requires further research.

8.1.3 DM utilisation and residual management

Cows out-wintered on kale either at high or low allocation had greater DM utilisation of crop compared to cows out-wintered on grass. For all kale treatments in the present study utilisation was above 80% of pre-grazing forage DM. The high level of utilisation is consistent with the NZ studies (Greenwood *et al.* 2011; Judson & Edwards 2008) and Irish study by Keogh *et al*. (2009a).

High utilisation of kale crop is associated with lower DM intakes (Judson *et al.* 2010; Judson & Edwards 2008). For example in the present study increasing allowance from 11 to 14 kg DM/cow/day resulted in average utilisation declining from 96 to 88%. However, it should be noted that high utilisation of kale crop is not beneficial since it may also result in reduced diet quality (Judson *et al.* 2010; Judson & Edwards 2008; Rugoho *et al.* 2010) as the ME of kale crops declined from 12.7 MJ ME/kg DM for lower stem material (Judson & Edwards 2008). This data indicate that modifying allowance is a useful tool to differentially feed cows with varying requirements (e.g. young versus mature, low versus high BCS) over the winter period. High allocation of kale will benefit young cows since less bullying (more grazing space per cow) and also low BCS cows because of more feed availability compared to low allocation of kale. However, with higher allocation there is more wastage of feed and also higher cost, but if kale left behind could be used by other classes of stock for example bulls or carry over cows.

Within 3 hours of being offered a new allocation (0900-1200h), cows which were fed low allocation of kale and grass-fed cows were estimated to have consumed >78% of their total daily intakes. Also by 3 pm in the afternoon, 6 h after being offered a fresh break, cows offered grass and kale at 11 kg DM/cow/day had consumed close to their total daily intake. Cows offered kale at 14 kg DM/cow/day allowance grazed for longer and consumed a further 1.6 kg DM (Table 3.4).

Based on these results monitoring kale grazing residuals mid-afternoon following a morning allocation of forage and modifying break widths may be a useful management tool to manage intake and diet quality. Post grazing residuals have been used effectively in pasture based systems as a method of controlling pasture quality and cow intake. The use of post grazing residuals is less widely accepted in brassica crops subject to single grazing with little regrowth. This study indicates that viewing residuals at 3 pm in the afternoon following a 9 am feed may be an effective way of estimating DM intake and identifying whether cows are being fed sufficiently to gain 0.5 BCS units. As cows can consume around 10 kg DM in the six hour period, viewing that a small proportion of the crop (20-35%, 10-15 cm stems) is left behind, with the expectation that some of this consumed over the remainder of the day, is a good indicator of intake potential. High residuals (in this study around 4 t DM/ha for 15 t DM/ha crop) will be indicative of cows achieving better diet quality and higher DM intake. If, however, little remains at this point it is unlikely that cows will be consuming sufficient kale to meet requirements for body condition score gain due to allocation being too low. Examining residuals may therefore provide an effective visual tool for identifying whether cows are being fed adequately that complements measurements of crop yields.

8.1.4 Rumen function

Both Experiment 2 and 3 showed that there was relatively minor diurnal variation of post-feeding rumen pH (Figure 5.4 and 7.5) between the two kale treatments and also between the grass treatments but rumen pH was higher in kale than grass as been reported in the study by Gibbs (2009). As already been discussed in Section 5.5.1.2 the reasons for the relatively high rumen pH observed with kale are not clear, given the high WSC content, low NDF content and rapid intakes associated with kale. However, the clear observations of relatively high rumen pH suggest there is little value in precautions against acidosis in kale feeding systems in NZ. Thus farmers can offer their livestock kale without any restrictions.

Experiment 3 showed that offering kale to dry dairy cows either once a day or twice a day outdoor using industry standard allocations of 11 kg DM kale did not have an effect on the concentration of acetic acid, propionic acid and had minimal effect on the concentration of butyric acid. This result is consistent with that in Experiment 2 when Friesian steers were offered kale either as a single meal or two meals (one in the morning and another afternoon). However, in Experiment 3 there was minimal benefit in terms of acetic acid and propionic acid when grass was offered at the same allowance either once a day or twice a day. Increasing the feeding frequency from once a day to twice a day was associated with high rumen concentration of acetic acid and propionic acid.

Both experiments showed that there was a clear diurnal variation in the rumen concentration of rumen ammonia but there was no significant difference between the two treatments from AUC means. However, the cows which were fed kale twice a day had a second peak of rumen ammonia concentration overnight, which may reflect the effect of a second meal during the afternoon. Despite high CP content of kale crop and grass (Table 6.1), it should be noted that the levels of rumen ammonia stayed well above the limiting concentration of microbial growth of around 20 mg NH3/l and well below excessively high levels of ammonia of 800 mg NH3/l (Satter & Roffler 1975; Satter & Slyter 1974).

8.1.4 Supplement use

The study used barely straw as a supplement to be fed with both kale and grass. This was chosen to be in line with standard industry practice whereby barley straw is commonly used. In this context, it is worth considering the requirement of supplement. Keogh *et al*.(2009c) showed increasing the dietary proportion of kale up to 100% kale was associated with a progressive decrease in dry matter intake but with minimal effects on rumen pH, total VFA concentration and individual CFA proportions. In this study, rumen pH declined little when kale was fed relatively to grass. This indicates that may not be requirement for fibre supplement such as straw in diet. However, the straw supplement may serve other useful purposes, including lowering intake rate and dilution of intake of secondary compounds in kale such as nitrates and SMCO. Further, the straw may be useful in controlling movement patterns of cows in wet, cold conditions, and act to dilute dietary N, with subsequent reductions in urinary N excretion. A recent study by Miller *et al*. (2012) showed that urinary N excretion was lower in cows grazing kale supplemented with barley straw or whole crop cereal silage than cows supplemented with the higher quality *Lotus pedunculatus* silage.

8.1.5 Kale cultivar effects

This study was conducted using the kale cultivar Regal. This cultivar has been bred and marketed as a leafy cultivar having a high leaf to stem ratio. This high leaf to stem ratio (e.g. 61% for leaf and 39% for stem) has been confirmed in grazing studies (GR Edwards, unpublished). Differences in leaf to stem ratio are known to affect preference of livestock. For example, Gowers & Armstrong (1994) noted that sheep preferred stemmy cultivars compared to leafy cultivars in preference tests were animals were offered kale at high allowance. Further, differences in leaf to stem ratio may affect diet quality. Judson & Edwards (2008) calculated that when grazing to the same residual, cows grazing giant (stemmy) cultivars such as Grunner would have a lower diet quality (ME) than those grazing intermediate stem (leafy) cultivars such as Regal, reflecting the higher proportion of leaf in the total diet. From this, it was suggested that cultivar specific grazing residual recommendations may be required to achieve desired cow body condition targets. In this context, giant type cultivars may be more suited to a system where maintenance is the expectation, particularly where the aim is to leave little behind after grazing. Alternatively, if high allowances are used to improve body condition score gain on giant type cultivars, the cows may be followed in a leader follower system by stock with lower requirements (e.g. store livestock). Intermediate stem leafy cultivars may be better suited to situations where an increase in body condition score is the objective.

8.1.6 Grass cultivar effects

The study was conducted using Italian ryegrass (*L. multiflorum*) rather than perennial ryegrass (*L. perenne*). Italian type ryegrasses often have greater quality than perennial ryegrasses (Cosgrove *et al*. 2007), raising the possibility that gain in BCS may be higher for Italian ryegrasses. However, this advantage is likely to be small in autumn saved pasture that is fed in winter at 3-4 t DM/ha; pasture in this trial was all > 8 weeks since the last grazing and had begun to senesce. It is therefore less likely that the body condition score gain results would differ markedly between perennial and Italian ryegrass. However, due to greater cool season growth after the crop has been grazed in Italian than perennial ryegrasses, Italian ryegrasses may provide more additional feed on support land in the early part of spring; this may be useful for transitioning cows, prior to returning to the milking platform. Further, a recent study by Moir *et al*. (2012) showed that due to the more extensive rooting system of Italian ryegrasses, combined with greater cool season growth, Italian ryegrasses were more effective at mopping up excess nitrogen in the soil, and so reducing losses from urine patches as nitrate leaching.

8.1.7 Nitrogen losses

One concern with wintering cows in the South Island is the potential for high nitrogen losses as nitrate to soil water. This reflects the high stocking densities (on average 500 cows/ha/day) used in these feeding systems in combination with urine excretion on wet soils prone to nitrate leaching (De Klein *et al.* 2010; Houlebrook *et al.* 2009; Monaghan *et al.* 2007; Smith *et al.* 2008; Judson et al. 2010). Monagan *et al*. (2007) noted that wintering systems contributed a disproportionately high amount of the total N leaching losses in dairy systems. Thus, there is a need to identify forage system approaches that reduce N excretion in urine.

Results from experiment 1 showed that N output per cow was greater for kale than grass (311.7 g N/cow/day versus 227.2 g N/cow/day). This was mainly because total N intake for kale-fed cows was higher than grass-fed cows (337 g N/cow/day versus 257 g N/cow/day). On average the total N output per cow during this study of 312 g N/cow/day) is 5 % higher than the N output reported by Miller *et al*. (2012) of 284 g N/cow/day.

On average calculated N losses per hectare of urine and faeces for kale fed cows was 384 kg N/ha and this value was higher than for grass-fed cows (82 kg N/ha). These values are higher than for dairy cows grazing rape (28 to 30 kg N/ha) (Stefanski *et al*. 2010). Differences in N losses/ha will reflect crop yield which determine stocking density. Stocking densities during grazing were 1429 cows/ha for kale and 441 cows/ha for grass-fed cows. Differences in N loss/ha may further reflect differences in % DM utilisation which was also higher in kale (87-95%) than grass (72-75%). Lower values in rape study (Stefanski *et al*. 2010) may reflect overall low utilisation (50%), with cows moving across paddock more quickly, so leading to increased stocking density. In addition, differences will reflect whether supplement is fed with crop, so concentrating cows in small area. These results highlights that crop yield, utilisation and level of supplementation will be important determinant of N losses/ha.

The average N% in urine during this study was low around 0.5% for both kale and grass-fed cows. This N% concentration in urine equates to 5 g N/l, which is the same reported by Pacheco *et al*. (2010), when lactating dairy cows were fed on Italian ryegrass pasture during autumn period but lower than 11.5 g N/l reported by Haynes and Williams (1993) for dairy cows grazing pasture. Therefore this might mean that the concentration of N in urine patch is lower than 750 kg N/ha which is often assumed for urine patch. For example, using 10 litres of urine per m^2 as the rate of application (Hogg 1981) and urine N% of 0.5% the N concentration will be around 500 kg N/ha. This data needs careful consideration when calculating N leaching from winter feeding systems.

8.2 Reasons for methodological change

Experiment 1 consisted of three treatments and there was no replication of treatments and in experiment 3, treatments were replicated. This was based on the land available for experiment 1 and labour shortage. Yields of experiment 1 were around 15 t DM/ha (section 3.4.2) compared to 8 t DM/ha of kale in experiment 3 (section 6.3.1). There was need to cut kale breaks in experiment 1 compared to experiment 3. The first experiment used cow as replicate (Keogh *et al*. 2009b) while second experiment used group as replicate, as the interest was in accurate measurement of DM intake.

8.3 Future research

There is need for determining the ME content of kale using *in vivo* digestibility and methane yield measurements and effects of offering high allowance of kale 14 kg DM/cow/day and low allowance of kale (11 kg DM/cow/day) on the urinary nitrogen concentration and output. Further research is also needed to investigate the effects of offering additional energy during late lactation on the milk yield and concentration during early lactation. Also need for assessing the rumen microbial protein supply effect of feeding kale once versus twice per day. Lastly, the need to accurately measure the amount of nitrogen leached through wintering cows on grass versus kale. Work in all these areas will help contribute significantly to the development of solutions to overcome winter feeding challenges.

8.4 Conclusions

- Offering dry dairy cows high allocation of kale (14 kg DM/cow/day + 3 kg DM of straw) reduced crop utilisation compared to offering industrial allowance of 11 kg DM kale plus 3 kg DM of straw per day. Despite low utilisation on high allocation, dry matter intake was high compared to low allocation. Higher dry matter intakes and crop utilisation were observed for kale-fed cows compared to grass-fed cows and intake rates were high in both forage treatments.

- Cows which were offered high allocation of kale (K14) gained more BCS during winter compared to those offered low allocation (K11), which may reflect greater ME intake and greater nitrogen intake.
- Rumen pH is higher in kale-fed cows than in grass-fed cows and no evidence of SARA in kale-fed cows despite high WSC and high intake rates in kale.
- Feeding frequency had no effect on forage intake, utilisation, BCS and rumen pH. Therefore, more frequent allocations of forage are likely to provide additional challenges for farm staff since each break has to be cut in the crop and the need to shift fences more often thus not justified.
- The calculated faecal and urine N output/ha were higher for kale than grass-fed cows reflecting greater yield of kale leading to increased stocking density.
- Kale is a better crop when wintering cows due to its greater yields, higher ME value, high CP and better DM utilisation.

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Based on Chapter 3.

Dalley, D.E; Edwards, G; Rugoho, I; Stevens, D. 2011. Factors to consider when evaluating the success of your wintering system. *In*: Proceedings of the South Island Dairy Event conference 27th-29th June 2011, Lincoln University, Canterbury: 4-16.

Summary data presented from Chapter 6.

Intake and feeding behaviour of dairy cows grazing kale and grass at low and high allowances during winter

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ABSTRACT

Intake and foraging behaviour of dairy cows grazing kale offered at 11 (K-11) or 14 (K-14) kg DM/cow/day, and grass offered at 11 (G-11) kg DM/cow/day were measured during week 2 and 5 of a 7-week winter feeding period. The % dry matter (DM) utilisation of K-11 (96%) and K-14 (88%) were higher than G-11 (70%). Apparent DM intake (kg DM/day) was higher for K-14 (12.3) than K-11 (10.6). Apparent DM intake of G-11 was low (7.8 kg DM/day) due to the lower % DM utilisation. Grazing behaviour was altered by both forage type and allowance. Within 6 hours of being offered a fresh break, cows had consumed over 86% of their apparent daily intake with DM consumptions of 10.4, 10.5 and 7.3 kg DM/cow for the K-11, K-14 and G-11 treatments, respectively. Cows on the K-14 treatment grazed for longer over the day and consumed a further 1.6 kg DM. Based on these results, monitoring kale grazing residuals mid afternoon following a morning allocation of forage, and modifying break widths may be a useful management tool to manage intake and diet quality of cows with different nutritional requirements.

Keywords: grass; kale; winter; DM intake; utilisation.

INTRODUCTION

Forage brassicas, such as kale (*Brassica oleracaea*), are often a major component of the winter diets of dairy cows in southern New Zealand. Kale is a useful winter crop because it will produce higher DM yields of better quality in winter relative to perennial ryegrass (Brown *et al*., 2007; Gowers and Armstrong, 1994). Despite these attributes, anecdotal evidence suggests that performance of dairy cows on kale over winter may be less than expected. From a survey of dairy herds in Canterbury, Judson & Edwards (2008) suggested that this may reflect low DM intake due to poor allocation; furthermore they suggested that higher DM allowance may be a method of increasing DM intake and diet quality. However, there is little experimental data on utilisation, intake and foraging behaviour of cows grazing kale in response to changing allowance or crop types (Gazzola *et al.*, 2008). The objective of this study was to compare utilisation, intake and grazing behaviour on cows wintered on kale at low (11 kg/cow/day) and high (14 kg/cow/day) allowance compared to grass at low allowance (11 kg/cow/day).

MATERIALS AND METHODS

The experiment was conducted at Lincoln University, Canterbury New Zealand, between June and August 2009. Following cultivation, kale (cv. Regal, 1.5 ha) and Italian ryegrass (cv. Tabu, 3 ha) were sown at rates of 4.0 kg/ha and 25.0 kg/ha on 15 December 2008 and 18 December 2008, respectively.

On 1 June 2009, dry, spring calving Friesian × Jersey dairy cows (n = 45) were blocked according to age (5.3 ± 3), body condition score (4.6 ± 0.6), liveweight (530 ± 95 kg) and calving date (22 August ± 20 days) and assigned to one of three outdoor feeding treatments:

1. K-14, high kale allowance (14 kg DM kale + 3 kg DM barley straw/cow/day);
2. K-11, low kale allowance (11 kg DM kale + 3 kg DM barley straw/cow/day);
3. G-11, grass (11 kg DM grass + 3 kg DM barley straw/cow/day).

Both kale and grass crops were strip grazed in 30 m wide paddocks, with daily break width determined by allowance and DM yield. Typical break widths were 3.5 ± 0.3 m, 4.7 ± 0.4 m, 13.8 ± 3.1 m for K-11, K-14 and G-11, respectively. Cows were fed straw at 0800 h in a wire mesh feeder at the paddock entrance and forages were offered in a single break at 0900 h. Kale paddocks were not back–fenced during grazing, but cows on grass were back-fenced at the end of each week. At the end of the 7 week experimental period, cows were returned to grass pasture for calving. Results for DM intake, utilisation and behaviour of cows are reported for weeks 2 and 5 of study.

Pre-grazing kale DM yield was recorded on Monday of each week by cutting to ground level six randomly positioned 1 m^2 quadrats within the area estimated to be grazed over the following week. Total fresh weight was recorded in the field and a sub-sample of 3 plants from each quadrat was returned to the lab. These were separated into leaf and stem, and oven-dried at 65ºC to a constant weight to determine DM% and leaf to stem ratio. In week 5, dried samples were ground and analysed for forage composition by near-infrared spectrophotometer (NIRS). On Tuesday of each week, post grazing DM yield was measured in the areas grazed the previous week on Tuesday, Thursday and Sunday. All remaining kale was removed from three 1 m^2 quadrats per break on allotted days. Samples were washed to remove any soil or excrement, and oven-dried at 65ºC to a constant weight to determine DM%. Grass DM yield was measured with a calibrated rising plate meter pre and post grazing on the same daily schedule as the kale measurements. A total of 50 measurements were taken pre-grazing on Monday and 50 measurements post grazing on Tuesday, Thursday and Sunday. A single calibration was derived from a total of 42 quadrats each 0.2 m^2 (kg DM/ha = 104 RPM + 727, r^2 = 82.6%). From these measurements, DM yield (kg DM/ha), DM utilisation (%, pre-post DM yield/pre DM yield * 100) and estimated apparent intake of kale or grass (kg/cow/day, % DM utilisation * allowance) were calculated. Measurement of straw residuals in the mesh feeder indicated that > 95% of straw was consumed on a daily basis.

Cumulative DM intake was measured on two days in week 5. On both days in each kale paddock two 1 m^2 quadrats were cut to ground level pre grazing and then at: 1, 2, 3, 6 and 24 h after allocation. Samples were washed and DM yield determined as per pre and post grazing measurement. On an additional day in week 5, the behaviour of all cows were observed over a 24 h period from 0800 h. Every 15 minutes cows were recorded as either: eating forage, eating straw, ruminating or idling (not engaged in other activities). From these data, times in each activity were calculated by multiplying each behaviour activity frequency by a 15-min interval.

Treatment effects on DM yield, % DM utilisation and estimated apparent DM intake were compared by ANOVA in each week using sampling day as replicate. Foraging behaviour over 24 h period was analyzed by ANOVA with cow (block) as random effect and forage treatment as a fixed effect. No statistical analysis was carried out on cumulative intake rate measurements as this was carried out on only on two days

RESULTS

Nutritive composition is presented in Table 1. Kale plants were comprised of 40.5% stem and 59.5% leaf. Grass was higher in DM content and fibre (NDF) and lower in soluble sugars (WSC) and ME compared to kale. Though variation in protein (CP) for leaf and stem occurred in kale, the weighted average of both diets was similar (Table 1). Pre-grazing DM yields across weeks 2 and 5 of the trial averaged 15.0 t DM/ha for kale and 4.4 t DM/ha for grass (Table 2). Post grazing kale DM yield was greater for K-14 than K-11 in both weeks (Table 2). Utilisation of pre grazing DM was greatest for K-11, intermediate at K-14 and lowest for G-11 (Table 2). Apparent DM intake was greatest for K-14, intermediate at K-11 and lowest for G-11 (Table 2). Differences in cumulative DM intake between treatments, following a 0900 h allocation of forage, are shown in Table 3. Intake rates were high in all 3 treatments. Within 1 hour of allocation, it was estimated that cows had consumed >3 kg DM in all three treatments, with an indication of higher DM intake in grass. Within 3 hours of being offered a fresh break, it was estimated that cows had consumed over 75% of their daily intake and after 6 hours, cows offered 11 kg DM/cow/day of grass or kale had nearly consumed their daily total DM intake (>95%). In contrast, cows offered 14 kg kale DM/cow/day continued to consume forage, eating a further 1.6 kg DM/cow, while those on other two treatments ate only 0.4 kg DM/cow. Behaviour was significantly affected by forage type and allowance (Table 4). Cows on kale spent more time eating forage than those on grass. Cows on K-14 spent more time grazing less time ruminating than cows on K-11 (Table 4).

TABLE 1: Forage composition (%DM) and metabolisable energy content (MJ ME/kg DM) of kale and grass plants offered to dairy cows in week 5 of trial. Kale plants represent the weighted average of kale leaf and stem.

Parameters	Kale			Grass	Sig.
	Leaf	Stem	Plant		
CP	27.8	13.3	19.1	18.2	NS
WSC	14.2	38.5	28.8	22.5	**
ADF	17.7	25.7	22.5	23.2	NS
NDF	19.6	34.6	28.6	43.1	**
DM	10.7	11.4	11.1	15.8	**
ME	13.0	11.8	12.3	11.9	*

CP: Crude protein; WSC: water soluble carbohydrates; ADF: acid detergent fibre; NDF: neutral detergent fibre; ME; metabolisable energy and DM: dry matter. Sig. = Significance from ANOVA of kale plant versus grass plant, * $P < 0.05$, ** $P < 0.01$

TABLE 2: Pre and post grazing DM, percentage utilisation of pre grazing dry matter (DM) and apparent DM intake of kale offered at 11 (K-11) or 14 (K-14) kg DM/cow/day and grass offered at 11 (G-11) kg DM/cow/day to dairy cows in winter.

	Week 2					Week 5				
	K-14	K-11	G-11	LSD	Sig.	K-14	K-11	G-11	LSD	Sig.
Pre-grazing (t DM/ha)	14.2	13.9	4.4	-	-	16.1	15.8	4.4		
Post-grazing (t DM/ha)	1.85	0.88	1.33	0.608	**	1.73	0.20	1.30	0.188	**
Utilisation (%)	87.1	93.7	70.0	4.4	**	89.3	98.8	70.5	1.9	**
DM Intake (kg/cow/day)	12.1	10.3	7.7	0.57	**	12.5	10.8	7.8	0.23	**

LSD = least significance different (P = 0.05); Sig. = Significance from ANOVA, * P <0.05, ** P <0.01

TABLE 3: Daily DM forage intake, percentage utilisation of pre grazing DM and cumulative forage disappearance (% cumulative DM intake of daily total in parentheses) of kale offered at 11 (K-11) or 14 (K-14) kg DM/cow/day and grass offered at 11 (G-11) kg DM/cow/day to dairy cows in winter.

	K-14	K-11	G-11
DM Intake (kg/cow/day)	12.1	10.7	7.6
Utilisation %	86.7	97.1	69.2
Forage disappearance (kg DM/cow)			
At 1 hour	3.0 (24.9)	4.1 (38.4)	5.1 (66.6)
At 2 hours	7.5 (61.4)	6.0 (56.3)	6.3 (83.2)
At 3 hours	9.4 (77.4)	8.4 (78.4)	6.8 (89.8)
At 6 hours	10.5 (86.1)	10.4 (97.6)	7.3 (95.7)
At 24 hours	12.1 (100.0)	10.7 (100)	7.6 (100)

TABLE 4: Foraging behaviour (minutes per day) kale offered at 11 (K-11) or 14 (K-14) kg DM/cow/day and grass offered at 11 (G-11) kg DM/cow/day to dairy cows in winter.

	K-14	K-11	G-11	LSD	Sig
Eating straw	72	57	56	20.9	NS
Eating forage	493	387	318	50.2	**
Ruminating	236	313	375	37.4	**
Idling	639	683	691	52.3	NS

LSD = least significance different (P =0.05); Sig. = Significance from ANOVA , * P <0.05, ** P <0.01

DISCUSSION

The utilisation of kale DM exceeded 87% of pre-grazing forage DM when offered at both 11 and 14 kg DM/cow/day. The high level of utilisation is consistent with the study of Judson & Edwards (2008). In a survey of 49 kale paddocks grazed by dairy cows in Canterbury, utilisation averaged 80% of pre-grazing forage DM allocated, with more than 90% being utilized in 40% of the cases. Increasing allowance from 11 to 14 kg DM/cow/day resulted in average utilisation declining from 96 to 88%. However, this reduced utilisation was not sufficient to offset the increased allocation, and apparent DM intake was on average 1.8 kg DM/cow/day higher at 14 than 11 kg DM/cow/day. As crop quality (ME and CP%) declines from the top to the bottom stem (Judson & Edwards 2008), diet quality would also have been greater for the 14 kg DM/cow/day allowance. These data indicate that modifying allowance is a useful tool to differentially feed cows with varying requirements (e.g. young versus mature, low versus high body condition score) over the winter feeding period.

The practical implication of this result is that in order to achieve the same daily DM intake of grass as for kale (offered at 11 kg cow/day), the allowance of grass would have to be approximately 15.1 kg grass DM/cow/day. Taking into account the lower winter DM yields of grass relative to kale, the increased area required to achieve the same intake on grass will place further pressure on wintering costs.

Though the grazing behaviour assessment was not fully replicated the large differences in rate of intake between the three forage systems allow some basis for speculation. Within 3 hours of being offered a new allocation (0900-1200h), cows were estimated to have consumed >78% of their total daily intake in all forage treatments. These corresponded to DM intakes of 8.4, 9.4, and 6.4 for 11 kg DM kale, 14 kg DM/kale and 11, kg DM grass, respectively. The lower DM intake on grass reflected the lower total utilisation of grass than kale. The implications of the high intake rate of kale for rumen function and cow performance are unclear at this stage. Indoor studies with cattle (Gibbs SJ, unpublished data) simulating high rates of kale intake indicated that rumen ammonia levels rose sharply to >200 mg NH_3/L within 2 hours after the 9 am meal of 9 kg DM kale/head, stayed elevated for a period of 6 hours, before declining to levels <100 mg NH_3/L for the remaining 16 hours of the day. Experiments with altered frequency of allocation of crops are currently underway to explore potential problems associated with high intake rates and rumen function.

By 3 pm in the afternoon, six hours after being offered a fresh break, cows offered grass and kale at 11 kg DM/cow/day had consumed close to their total daily intake. Cows offered kale at 14 kg DM/cow/day allowance grazed for longer and consumed a further 1.6 kg DM. Based on these results, monitoring kale grazing residuals mid afternoon following a morning allocation of forage, and modifying break widths may be a useful management tool to manage intake an diet quality. High residuals (in this study around 4 t DM/ha for 15 t DM/ha crop) will be indicative of cows achieving better diet quality and higher DM intake.

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FACTORS TO CONSIDER WHEN EVALUATING THE SUCCESS OF YOUR WINTERING SYSTEM

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Summary

To be successfully implemented, all wintering systems require specific attention in the following areas:

- Feed budgeting and planning
- Stock management
- Risk management
- Staff skill development and relationship management and
- Environmental protection.
- Key performance indicators of successful wintering are:
- 90% of the herd achieving body condition score (BCS) targets at calving of 5 for mature cows and 5.5 for 2 and 3 year olds
- Less than 2% deaths between drying off and the end of calving
- No breaches of environmental rules or consent conditions
- Staff refreshed and prepared for calving
- Financial targets have been met.
- Recent research has indicated that
- High levels of crop utilisation (85 to 98%) can be achieved across a wide range of crop allocation and crop type
- Rate of intake is higher for kale than swedes but with both crops at least 85% of the daily intake can be consumed within the first 6 hours of feeding
- Both utilisation and rate of intake are lower when cows graze autumn saved pasture
- Allocating crop as two equal feeds 5 hours apart results in a slower intake rate but cows are still able to consume 98% of the daily intake within 3 hours of the second feeding
- Dry cows are capable of consuming up to 13 kg DM kale/day at a high level of utilisation
- Body condition score gain is unlikely when cows are offered 11 kg DM/cow/day kale and 2-3 kg straw during the dry period.
- The energy and protein content of kale decreases progressively down the stem – forcing high rates of utilisation will decrease the energy concentration of the diet especially in medium and giant kale cultivars.

Introduction

The South Island, New Zealand has seen significant growth in dairy farming over the last 15-20 years. For many South Island farmers, animal wintering provides a number of challenges not encountered in other dairy regions of New Zealand. The main animal wintering issue is the inability to grow sufficient pasture during winter to meet the daily feed energy requirements of current dairy farms. Winter pasture growth is usually less than 10 kg DM/ha/day (D Dalley unpublished; www.siddc.org.nz), which makes pasture based wintering an impractical option for many, due to the area of land required to accumulate sufficient pasture to meet herd feed requirements.

As a consequence alternative wintering systems such as grazing forages and off pasture systems, such as stand-off and housed systems have been adopted. The particular system a farmer selects will be determined by their individual circumstances, and all systems have an equal opportunity for success or failure (Dalley, 2010).

Regardless of the wintering system chosen, the system must maintain or improve the profitability of the farm business at the same time as achieving environmental, animal or social goals (Riemersma, 2007).

DairyNZ, in conjunction with MAF Sustainable Farming Fund and Environment Southland, have embarked on a 3 year research programme, called the Southern Wintering Systems Initiative, to provide key performance indicators (KPI) for a range of wintering systems against which farmers can compare the performance of their own systems or evaluate alternative wintering systems. While the project is based in Southland, the principles are directly transferable to other regions of the South Island.

The monitor farm project

Six commercial dairy farms across Southland and South Otago have been selected to participate in the study. Monitoring on the farms commenced in June 2010 and the information collected is being used, together with research at Telford and Lincoln University, to develop key performance indicators for feed, environment, animal health and welfare, people and finances.

The project aims to demonstrate that wintering decisions need to take into account:

Notes:

Profitability and risks

The profitability of forage crop systems is highly dependent on crop yields which vary greatly depending on crop management, soil condition, climate and the amount of supplement required. Housed systems can have the advantage of lower feed requirements during the winter but come with a higher capital cost, although some of this cost can be offset by increased days in milk (Beukes *et al*. 2010).

Do you know how much your wintering system is costing you, what additional income it is generating (increased days in milk, better BCS at calving) and what savings it is providing (replacement of fertiliser nutrients with manure and effluent)?

It is important to identify the risks associated with each wintering system and put plans in place to minimise these. A wintering system risk assessment has been undertaken for each component of the farm system on each monitor farm. An example template is provided below:

Risk	Importance	Likelihood	Why/Mitigation
Insufficient feed for winter	High	Low	Two long-term contracts for the provision of standing pasture for silage
Insufficient manure/effluent storage	Medium	Medium	Homework done on establishing size of storage requirement; provision to irrigate effluent via low rate during winter if conditions are suitable
Suitable bedding material not available	High	Medium	A range of sources available, good relationships developed for supply
Mastitis outbreak	Medium	Low	Blanket dry cow therapy/cows checked regularly through winter
Failure to achieve body condition score targets	Medium	Low	Ad libitum silage feeding through the winter
Farm owner has an extended period off the farm	Low	High	Simplicity of the system and low time commitment means staff members can ably implement the wintering system successfully.

Figure 1. Example wintering system risk assessment

System flexibility is important when considering risks. How easy is it to deal with insufficient feed, or a surplus of feed? Many farms in Southland/Otago operate a hybrid wintering system where several options are used for wintering cows on the farm to provide increased flexibility e.g. some cows on crop on the milking platform and some with a grazier. This does not appear to be as common in the Canterbury region. The level of flexibility required will depend on the system being operated and the region. For example, the reliability of consistent crop yields on non irrigated support blocks will be lower than irrigated land where soil moisture is unlikely to be a limiting factor. Therefore, having contingencies for poor crop yields in un-irrigated regions will be a high priority.

Feed supply, utilisation and quality

All systems require a robust feed budget and the provision of sufficient high quality feed to achieve calving BCS targets. By monitoring crop yield on a monthly basis from early autumn, feed budgets can be revised and decisions made early on how to deal with shortfalls.

The results from a number of crop monitoring projects suggest that there is a wide range in dry matter content of crops and that leaf bulb ratios also vary across farms and regions. For plants like fodder beet where there is a significant difference in protein levels between leaf and bulb it is beneficial knowing the proportion of each in the crop to ensure the protein requirements of the animals are met.

While high utilisation of kale crops generally promotes lower DM intakes, the conditions in which the crop is offered, the daily allocation and the crop yield can influence this. Recent research at Lincoln University and Telford Rural Polytechnic has investigated crop utilisation, rates of intake and body condition or liveweight change in cows wintered on kale, swedes or pasture (Table 1). On light stony Lismore soils at Ashley Dene, utilisation of 7.5 tonne kale crops was 88-97% with no difference in utilisation between once and twice daily allocation of kale (Table 1; Rugoho unpublished). In contrast, utilisation of kale and swedes on heavier soils at Telford were in the range of 80 to 95% (Table 1). Utilisation of pasture in the two experiments conducted by Rugoho (Rugoho *et al.* 2010; 2010 unpub.) was less than that achieved with kale. The utilisation observed in recent research is similar to that reported by Judson and Edwards (2008) for kale crops across Canterbury dairy farms.

Whether cows achieve BCS gains at these levels of utilisation will be dependent on the amount and quality of supplement being offered, therefore, it is also important to monitor cow behaviour. If cows on crops have consumed all the available feed within 4 hours of allocation, they will not be meeting their energy requirements for maintenance, pregnancy and BCS gain. In contrast, cows in stand-off and housed systems have an increased ability to gain condition

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Notes:

with a lower feed allowance due to higher feed utilisation, lower activity and lower energy requirements to stay warm. In cold and wet conditions, cows may need another 5 to 25 MJ ME/cow/day (0.5 to 2.5 extra kg DM) to keep warm (Verkerk 2011).

The body condition and liveweight change results from recent research differ between experiments, possibly reflecting the differences in energy intake. Greenwood *et al.* (2011) reported an increase in body condition score (0.8 BCS units) when cows were offered 14 kg DM kale and 3 kg DM straw but no increase when cows were offered 11 kg DM kale and 3 kg DM straw (Table 1). Greenwood estimated the energy intake of the two treatments to be 133 and 173 MJ ME/cow/day for 5 weeks for the low and high kale yields respectively. Pangborn and Gibbs (2009) were unable to detect changes in condition score when cows were offered 10 kg DM kale and 2 kg DM wheat straw. In contrast Stevens (unpub.) reported an increase in body condition score of 0.2 and 0.4 BCS units respectively, in one month, when cows consumed either kale (7.3 kg DM plus 3.4 kg baleage) or swedes (7.0 kg DM plus 5.0 kg pasture baleage).

Recent research (Greenwood et al. 2011) has demonstrated that high intake of kale can be achieved with once daily offering of crop, providing a generous allowance is given (Table 1). Offering kale or pasture as either a single feed or two equal breaks 5 hours apart did not increase dry matter intake (Table 1; Rugoho et al. unpub).

High crop utilisation may result in reduced diet quality (Judson & Edwards 2008; Rugoho *et al*. 2010) The ME of kale crops declined from 12.7 MJ ME/kg DM for leaf material to 6.6 MJ ME/kg DM for lower stem material (Judson & Edwards 2008). Cows eating 84% of an intermediate-stemmed kale cultivar (leaving just the lower stem) would have a diet quality (12 MJ ME/kg DM) greater than an equivalent cow grazing 80% (leaving just the lower stem) of a giant type cultivar (11 MJ ME/kg DM).

Recent research has demonstrated that dry cows are able to achieve very high rates of intake with both kale (Table 2) and swedes (Table 3). Rugoho offered 11 kg kale and 3 kg DM of straw either as a single feed at 10 am or two feeds split 50:50 at 10 am and 3 pm. Cows offered kale once per day had consumed 94% of their daily intake within 6 hours of feeding. For the two times per day treatment 98% of the daily intake had been consumed within 3 hours of the second feed (Table 2). Rate of intake was slower when pasture was offered with 83% of the daily intake consumed within 6 hours on once daily feeding and 93% consumed within 3 hours of the afternoon allocation with twice daily feeding (Table 2). In a second experiment the rate of intake of kale was increased when the kale allowance was lower (Table 2). Cows offered 11 kg DM kale/day consumed 98% of their daily kale intake in the first 6 hours of the day. At the higher allowance of 14 kg DM kale/day only 86% of the daily intake was consumed in the first 6 hours.

Table 1. Crop allocation (kg DM/cow./day), utilisation (%), intake (kg DM/cow/day) and body condition score change for recently completed research at Lincoln University and Telford Rural Polytechnic.

	Crop/Grass Allocation	Supplement Allocation	Supplement Type	Crop Yield	Crop Intake	Total Intake	Crop Utilisation	BW Change	CS Change	Reference
Kale High	14	3	Barley straw	15.2	12.1	14.8	88	+44	+0.4	Rugoho *et al.* 2010
Kale Low	11	3	Barley straw	14.9	10.7	13.4	96	+23	+0.2	Rugoho *et al.* 2010
Grass	11	3	Barley straw	4.4	7.6	9.3	70	+59	+0.2	Rugoho *et al.* 2010
Kale 1x	11	3	Barley straw	7.5	10.7	10.7	97	-	-	Rugoho (2010) unpub
Kale 2x	11	3	Barley straw	7.5	10.2	10.2	92	-	-	Rugoho (2010) unpub
Grass 1x	11	3	Barley straw	2.7	9.6	9.6	87	-	-	Rugoho (2010) unpub
Grass 2x	11	3	Barley straw	2.7	8.8	8.8	80	-	-	Rugoho (2010) unpub
Kale	8.4	3.4	Baleage	10.0	7.3	10.7	86	-	+0.2	Stevens (2010) unpub
Swede	8.6	5.1	Baleage	12.7	7.0	12.0	82	-	+0.4	Stevens (2010) unpub
Swede high	8	4.0	Baleage	12.7	7.6	13.0	86	-	-	Stevens (2010) unpub
Swede low	6	5.5	Baleage	12.7	5.1	8.9	95	-	-	Stevens (2010) unpub
Kale	10	2.0	Wheat straw	12.0 Est	8.5 Est	119 MJ ME	85% assumed	+2	-0.1	Pangborn & Gibbs (2009)
Pasture	6.3	7.0	Baleage	3.5	5.4 Est	117 MJ ME	85% assumed	+26	+0.1	Pangborn & Gibbs (2009)
Kale High	14	3	Barley straw	8.4	13.1	16.0	94	81	+0.8	Greenwood *et al.* (2011)
Kale Low	10	3	Barley straw	8.4	9.7	12.7	97	63	+0.1	Greenwood *et al.* (2011)

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Similar observations were made by Stevens (unpublished). When 8.6 kg DM kale were offered once per day 95% of the daily intake was consumed within the first 6 hours. By comparison only 70% of the daily swede intake was consumed during the same period. When two allocations of swede were compared in the last 4 weeks of the dry period, there was no difference in the rate of intake. Approximately 86% of the daily intake was consumed within 6 hours of crop allocation (Table 3). The pattern of supplement intake was similar to crop intake in the research of Stevens (unpub.).

Correct crop allocation is critical. Judson and Edwards (2008) reported that cows on 66% of farms surveyed, consumed at least 1 kg DM kale/cow/day less than target intake with one herd being short by 8 kg DM/cow/day. Correct allocation requires accurate estimation of crop yield, paddock size, break width and available supplement (Dalley *et al*. 2008).

Undertaking a supplementary feed inventory to determine the quality and quantity of supplement on hand will increase the likelihood of winter feed targets being met. Weigh a few silage bales and get a DM test done rather than relying on book values. Cereal silage contains insufficient protein to meet cow requirements through the winter, so should not be fed as a sole diet or as the only fibre source with fodder beet.

Table 2. Effect of feeding frequency (once vs twice daily) or kale allocation 11 vs 14 DM/cow/day allocation) on cumulative kale intake (kg DM/cow/day) determined by measuring crop DM disappearance (Rugoho unpub; Rugoho *et al.* 2010)

	Kale		**Grass**		**Kale 14**	**Kale 11**	**Grass 11**
	1	**2[1]**	**1**	**2**			
At 1 hour	7.21	3.59	6.38	3.63	3.0	4.1	5.1
At 2 hours	8.54	4.38	7.36	4.00	7.5	6.0	6.3
At 3 hours	9.13	4.95	7.84	4.09	9.4	8.4	6.8
At 6 hours	10.02	5.01	8.01	4.17	10.5	10.4	7.3
At 7 hours	-	8.95	-	7.94			
At 8 hours	-	9.56	-	8.12			
At 9 hours	-	10.03	-	8.21			
At 24 hours	10.71	10.20	9.63	8.82	12.1	10.7	7.6

[1]Twice (2) a day allocation cows received fresh allocation in the afternoon.

Table 3: Effect of crop type (kale or swedes) or swede allocation (high vs low) on cumulative DM intake of cows (kg DM/cow/day) offered crop once per day. Estimated intake determined by measuring crop DM disappearance (Stevens, unpub.)

	Kale	**Swede**	**Swede**	**Swede**
			8 kg DM	**6 kg DM**
At 1 hour	5.6	4.3	4.4	3.9
At 3 hours	6.2	4.7	5.9	4.2
At 6 hours	6.9	4.9	6.1	4.5
At 24 hours	7.3	7.0	7.2	5.1

Environmental impact

Most nitrogen (N) losses from forage crops come from leaching, rather than overland flow. Improved management practices such as minimal or no-tillage to establish crops (ex grass pasture) and the use of nitrification inhibitors can help to reduce these N losses (Monaghan *et al.* 2009). In contrast, most phosphorous (P) losses from forage crop paddocks occur through surface runoff, which also includes sediment and faecal matter.

Appropriate paddock selection and a well thought through grazing plan can minimise surface runoff and therefore the loss of nutrients and sediment into waterways. Below are some simple tips to consider when selecting and setting up paddocks for winter crops:

- Avoid paddocks with heavy soils and sufficient contour and slope to facilitate overland flow of water during periods when soils are waterlogged
- Graze towards waterways and roads. Ungrazed crop provides a buffer for runoff from grazed areas and a visual screen from road traffic
- Place bales away from the fenceline to allow good access and do not place them alongside drains and waterways where stock are likely to camp
- Move the back fence up regularly to reduce unnecessary walking and minimise soil structural damage

Notes:

- Fence off a riparian strip to form a vegetative filter near ephemeral streams and swales close to where they enter permanent waterways to reduce sediment and nutrient movement.
- Consider 3-4 rows of supplement to minimise walking distance when grazing
- Place the water trough centrally in the paddock, again to reduce walking.

Housed and standoff systems eliminate soil damage but require a good nutrient management plan and facilities for collecting, storing and spreading effluent/slurry/sludges. Significant savings in fertilizer costs can be made through the efficient use of the nutrients in the manure and effluent, however, this requires knowledge of the amount of material generated and the nutrient content.

On the monitor farm used in the Southland study that winters cows on stand-off pads with self feed silage, artificial fertilizer is now only applied to one third of the farm as one third receives the farm dairy effluent and the other third the effluent and slurry from the feed pads. This equates to an approximate savings of $36,000 per annum on fertiliser for a 120 ha effective milking platform, wintering 370 cows.

Animal health and welfare

The primary objective of all wintering systems is to prepare cows for the subsequent lactation. A critical KPI is BCS. Having 90% of the herd achieve BCS targets at calving of 5 for mature cows and 5.5 for 2 and 3 year olds is a prerequisite of all wintering systems. Any wintering system that fails to achieve these targets cannot be deemed successful.

The monitor farm project has identified 4 critical times during the year when whole herd BCS assessments are undertaken. These times have been chosen to provide information to inform key decisions on the farm at that time. The 4 times are:

- Prior to mating to identify at risk animals that may require intervention
- Early autumn as part of developing the autumn feed budget
- Prior to dry off for allocation of animals to mobs for wintering
- Late winter to determine the success of the wintering system in achieving the BCS targets

By identifying individual cows within each BCS range, groups of animals can be tracked and decisions made on the appropriate plan of action.

Recent research has indicated that cows fed to gain condition are less susceptible to cold stress. In contrast, cows in poor condition are more susceptible to cold stress and are more likely to get ill in cold conditions (Verkerk, 2011). Therefore plan your autumn and winter feeding to reach condition score targets early.

The second important KPI relates to the incidence of disease and the number of deaths during the winter and spring. Wintering systems should be achieving less than 2% mortalities from the start of winter to the end of calving. This equates to less than 10 deaths in a 500 cow herd.

To achieve both the BCS and mortality targets requires a smooth transition on and off the wintering system is required. This is particularly important when significant changes are being made in the diet such as when cows are being wintered on forage crops. Failure to transition cows onto crop correctly will reduce the amount of time available for BCS gain and increase the incidence of animal health issues.

Realistically, in a 10 week dry period, cows only have 6 weeks in which to gain condition as no condition is gained during the last 4 weeks of pregnancy (Roche, 2007). Poor transitioning onto crop can result in no condition gain for the first 2 weeks of the dry period, resulting in only 4 weeks in which to achieve calving BCS targets. Under these circumstances it is unrealistic to expect anything more than a 0.5 BCS unit gain over the winter period. In reality many herds only maintain condition during the dry period, therefore cows need to be dried off in the condition you are expecting to calve them in.

Incorporating a forage crop into the system on the milking platform can provide dual benefits and increase the flexibility of the overall system. Firstly, it allows cows to be transitioned onto the crop prior to drying off by allowing them access to the crop for 2-3 hours per day during the final 2 weeks of lactation and it also provides an area for late calving cows to come back onto in spring, reducing the demand for pasture and provides an area to stand cows off in wet conditions. Secondly, it can be used as a break crop in the regrassing programme or as part of the development process for new land.

In wintering systems based on pasture and silage, there is increased potential to achieve BCS targets that afford more flexibility with decisions during late lactation. However, consideration needs to be given to animal management in these systems. Any facility or system that decreases lying time is likely to have a negative impact on cow welfare (Fisher *et al.* 2002). If standoff and housed systems are being used permanently during winter with no on-off grazing then a minimum of 1 m^2/100 kg cow liveweight is required.

Whichever system is adopted, keep in mind that some cows will just not adapt to being kept off pasture. These cows do not lie down for long enough, may not eat well, and often

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become lame. Make sure the farm staff can identify these cows and have a plan in place to deal with such problems as they arise.

People

The type of wintering system utilised will dictate the knowledge and skill base required by farm staff, and it also creates a working environment which may be deemed desirable or undesirable by future employees.

All grass wintering systems are generally considered 'people friendly' utilising similar feed and labour management skills to those that are used during the lactation period.

Brassica systems require sufficient technical knowledge and skill to produce consistently high yielding crops and the work associated with the feeding of this is perceived as hard on people and machinery. In contrast, housed systems appear to have a lower labour requirement compared to other wintering systems and they create a more favourable working environment for staff.

In considering the performance of your wintering system from a labour perspective you need to consider:

- The total hours worked each day
- The continuity of the work i.e. can all the tasks be completed in a 2-4 hour block at the beginning of the day or is there a requirement for someone to be around throughout the day to offer more feed?
- The skill requirement of the system – in more complex systems more training will be required to ensure all staff can implement the system successfully or alternatively, the skilled labour will have less flexibility in regards to annual leave.
- The physical conditions in which the staff are working. While little can be done about the weather, good planning when setting up a crop for feeding can reduce the effort required by staff. For example, leaving an ungrazed strip along the length of the paddock will make it easier to access the feeding face to move the fence each day, compared with the alternative of slogging through mud.

Infrastructure

The final consideration for wintering is the infrastructure needs of the system. Individual farms have unique resource bases and there is a range of avenues for securing land, infrastructure, and machinery, at varying costs to farm businesses. Successful wintering systems require the efficient use of existing capital, infrastructure, land resources, and the use of investment principles to drive profitable decision making.

Conclusions

A successful wintering system will:

- Be profitable
- Meet the demands on safeguarding the environment
- Have healthy cows achieving body condition score targets of 5.0 for mixed age cows and 5.5 for first and second calvers and
- Ensure staff are happy and working sustainable hours

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