Supplementation of palm kernel expeller to grazing dairy farms in New Zealand

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Francisco Nogueira Dias

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Abstract

This thesis is composed of a series of studies to assess the nutritional value and characteristics of palm kernel expeller (PKE) for grazing ruminants. The chemical composition of PKE has shown high concentrations of neutral detergent fibre (NDF) (70.1±0.39 g/100g DM) and fat (9.0±0.37 g/100g DM), and a moderate content of crude protein (16.2±0.42 g/100g DM), with very low concentrations of starch and sugar (0.3%) and reasonable gross energy content (18.6±0.33MJ/kg DM). *In sacco* digestion kinetics of PKE and pasture showed that PKE presented lower values for the soluble fractions in dry matter (DM), crude protein (CP) (25-27% and 37-38%, respectively) than pasture (41 and 52%), and the majority of the NDF in PKE was also present in the slowly degradable fraction (58-59%). The values for mixtures of pasture plus PKE were generally intermediate to those of the two feeds alone and the rate of degradation (k) for DM and fibre fractions did not differ significantly between feeds. But the rate of degradation for CP in PKE was about three to four times lower than that in pasture (6.7-9.8%/h and 33.8-48.4%/h, respectively), and the addition of PKE with pasture reduced the rate of protein degradation for the mixture to a rate similar to that of PKE alone (around 10%/h). *In vitro* net ammonia (NH_3) production decreased as the amount of PKE increased in the mixture with pasture, while PKE was only able to maintain a surplus of NH_3 for a short period of time; in contrast volatile fatty acids (VFA) yields were increased, with butyrate percentage being higher at the expense of acetate. In the *in vivo* trial with lambs, daily pasture DM intake was reduced as the amount of PKE plus molasses (PKEM) was increased in both periods, but total DM intake was not increased with the addition of PKE, except when the values were converted to DM intake per kg of metabolic liveweight. Independent of the pasture quality offered, there was a linear decrease in the apparent digestibility of DM and CP with the addition of PKEM in the diet; however NDF digestibility of the diet was only decreased when PKEM was fed with good quality pasture (period 1). The apparent digestibilities of PKEM of DM, CP, and NDF for PKEM were around 63.0%, 52.0% and 68.5%, respectively, with the estimated concentration of digestible energy (DE) of PKEM being approximately 12.8 MJ /kg DM. Addition of PKEM caused a decrease in the diet's DE concentration with high quality pasture (period 1), but an increase with low quality pasture (period 2). Faecal nitrogen (N) increased and urine N decreased when increasing

amounts of PKEM were fed in the diet, however, N retention and VFA concentrations were only increased by the addition of PKEM to the low quality pasture (period 2). The supplementation of either 3 or 6 kg of PKE to cows in late lactation grazing a restricted pasture allowance (20 kg DM/cow/day) decreased pasture intake, but overall total DM intake increased. Cows actually consumed only 2.7 and 3.6 kg PKE/cow of the 3 and 6 kg PKE offered/cow and presented substitution rates of 0.30 kg/kg and 0.54 kg/kg, respectively. Supplemented cows produced 0.86 to 0.94 kg milksolids/cow daily, respectively, while cows offered restricted pasture allowance only produced 0.76 kg milksolids/day. Supplemented cows had higher concentrations of milk fat than cows offered pasture only, and marginal returns of 42 and 53 g of milksolids/kg of PKE to cows offered 3 and 6 kg of PKE, respectively.

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List of Abbreviations

A	soluble fraction
ADF	acid detergent fibre
AFRC	Agricultural and Food Research Council
AIC	Akaike information criteria
B	slowly degradable fraction
BCS	body condition score
BIC	Bayesian information criteria
BW	body weight
C.V	coefficient of variation
CHO	carbohydrates
CLA	conjugated linoleic acids
CNCPS	Cornell Net Carbohydrate and Protein System
CP	crude protein
dL	decilitre
DM	dry matter
DMD	dry matter digestibility
ED	effective degradability
EE	ether extract
eNDF	effective fibre
ERDP	effective rumen degradable protein
FA	fatty acids
FCM	fat-corrected milk
g	grams
GE	gross energy
GIT	gastro-intestinal tract
HA	herbage allowance
HG0P	*ad libitum* pasture allowance and no PKE
k	rate of digestion of the slowly degradable fraction
kg	kilograms
kp	fractional outflow rate
L	litre

LSmeans	least square means
LW	liveweight
$LW^{0.75}$	metabolic liveweight
m^2	square metre
MAF	Ministry of Agriculture & Forestry, New Zealand
ME	metabolizable energy
mg	milligram
mL	millilitre
MP	metabolizable protein
MPOC	Malaysian Palm Oil Council
MR	meter readings
MS	milk solids
MUN	milk urea nitrogen
N	nitrogen
NDF	neutral detergent fibre
NH_3	ammonia
NIRS	near infrared reflectance spectroscopy
ns	not significant
NZ	New Zealand
OMD	organic matter digestibility
P + PKE	pasture plus PKE
PD	potential degradability
PK	palm kernel
PKE	palm kernel expeller
PKEM	palm kernel expeller plus molasses
PKM	palm kernel meal
QDP	quickly degradable protein
R^2	coefficient of determination
RDP	rumen degradable protein
RG0P	restricted pasture allowance and no PKE
RGHP	restricted pasture allowance plus 3kg of fresh PKE
RGLP	restricted pasture allowance plus 6kg of fresh PKE
RMSPE	root mean square prediction error
RSMPE	root square mean prediction error

RUP	rumen undegradable protein
RW	ryegrass and white clover
Ryegrass	ryegrass-only
s.d	standard deviation
SBM	soybean meal
SDP	slowly degradable protein
SE	standard error
SR	substitution rate
SSS	soluble sugars and starch
t	time
TMR	total mixed ration
U	undegradable fraction
UK	United Kingdom
US	United States
VFA	volatile fatty acids
vs.	versus
Y	herbage mass

CHAPTER 1:
General Introduction

1.1. Introduction

Dairy production in New Zealand is characterized by the use of pasture, predominantly ryegrass and white clover, as the most important source of feed for dairy cows throughout the year. The majority of dairy farmers in New Zealand rely on a system where the balance between pasture supply and demand should be achieved during the whole year; however this system type can sometimes become vulnerable. Firstly, pasture growth is dependent on weather conditions which could lead to a negative feed balance (quantity and quality) during particular times of the year (droughts, wet conditions, long cold periods, etc) (Holmes et al., 2002). Secondly, for farmers to achieve a good balance between feed supply and feed demand using pasture as the main diet, the herd must calve in the spring and dry off in the autumn, which generally results in short lactation lengths, reduced yields and consequently can penalize farmers during periods of higher milk payout (McCall et al., 1999). Thirdly, it seems that high genetic merit cows do not reach their potential under solely grazing systems (Kolver and Muller, 1998; Kolver, 2003) which makes the use of supplementary feed necessary to increase their daily dry matter intake.

Since the 1980s, farmers using feed supplements have increased from 20 to 80% of the farms in 2007 (Holmes and Roche, 2007). The main factor behind this trend is the increased flexibility and decreased risk of feed deficits that supplements bring into the system. Therefore, today most farmers introduce supplements at some point and in different ways throughout the year. Less intensive systems have been using supplements mainly to cover short term feed deficits and sometimes increase lactation length. But, on the other hand, a few intensive dairy systems have been using supplements all year as part of the daily diet of dairy cows to increase milk production per cow and per hectare.

Supplements are more expensive than grazed pasture (Clark et al., 2007), therefore the right balance between the grazed pastures available and the supply of extra feed to the animals as supplements must be achieved (Holmes et al., 2002). Generally, the best economic return of the use of supplements is achieved when pasture is also fully utilized (Kellaway and Harrington, 2004).

Milk production by pasture based systems is generally limited by dietary energy supply (Kolver et al., 1998; Macdonald, 1999; Holmes, 2007); therefore, farmers have been looking for cheaper energy supplements to increase production without losing profitability. Consequently the use of agricultural and horticultural by-products as supplements has become more common lately (de Ruiter et al., 2007).

For the past five years, New Zealand farmers have paid more attention to a by-product from the palm oil industry called palm kernel expeller (PKE); because of its cost and energy content. However, there is little information about the true nutritive value of PKE for ruminants, and how to manage it in a pasture based system to achieve a better result when used as a supplementary feed in a grass system.

1.2. Objectives

Based on the considerations presented above, a project was initiated to study the nutritional aspects of PKE for dairy cattle. A series of experiments were carried out to characterize the nutritional value of PKE as a supplement for dairy cows in a pasture based system. The objectives of this research are:

- To determine the fermentation and degradation characteristics of PKE compared to pasture (ryegrass/white clover and ryegrass only) using *in sacco* (Chapter 3) and *in vitro* (Chapter 4) techniques.

- To measure the apparent digestibility of different combinations of PKE and freshly cut pasture (ryegrass/white clover) fed to young sheep, their nitrogen balance and estimate the energy content of PKE (Chapter 5).

- To evaluate the effect of different amounts of PKE on milk production and composition of dairy cows grazing temperate pastures (ryegrass/white clover) in late lactation (Chapter 6), and estimate dry matter intake and substitution rate of their diets.

CHAPTER 2:
Literature Review

2.1. Introduction

This literature review will give an overview of pasture-based systems and supplementation for dairy cows; showing results from scientific studies with a focus on milk production and composition. It will discuss the variation of pasture growth and allowance and how this affects pasture quality, and intake by cows. It will also present a general overview about the type of supplements, with special attention for PKE, and factors that affect the milk production response when supplements are being used in a pasture grazing dairy system. It will also review the use of different techniques including *in vitro*, *in sacco* and *in vivo* methods to measure digestive characteristics and digestibility of feeds and consequently evaluate the nutritional value of those feeds. The review ends with an overview about the Cornell Net Carbohydrate and Protein System model and its use with pasture based diets.

2.2. Characteristics of pasture-based systems

Today there is a wide range of dairy farming systems around the world, ranging from low yielding spring calving cows fed only pasture to confinement systems, where high yielding dairy cows calving all-year-round are fed formulated rations in a feedlot. Nevertheless, all systems follow a basic principle: feed is supplied and eaten by the cows and consequently milk is produced (Holmes, 2007). The main concept of pasture-based systems rely on the growth of pasture as the main source of feed, followed by grazing animals harvesting the grass and converting it into animal products (e.g. milk, meat) (Hodgson, 1990).

Pasture-based systems were predominant around the world before the early 1950s, although from that decade until the 1990s factors like the development of new technologies for feeding systems associated with advances in the yield of row crops (e.g. fertilizer, herbicides, cultivars) pushed dairy farmers towards more confined systems (Fontaneli et al., 2005). Eventually, this trend led to pasture-based systems being rated

by dairy producers as a low yielding milk production system and its main source of feed, grass, as a low milk producing feed.

However recently, the interest in pasture-based dairy systems has been revitalized due to economic, environmental, and animal welfare factors. The ongoing increase in the farm input costs (eg. feed, labour, equipments, and fertilizers), which are related to factors like the use of grains to generate energy and the removal of some subsidies, have challenged milk producers to look for systems with lower production costs. Figure 2.1 shows that production costs of confinement systems in the US can be more than two fold higher than the grass systems found in New Zealand and Australia (Dillon, 2006).

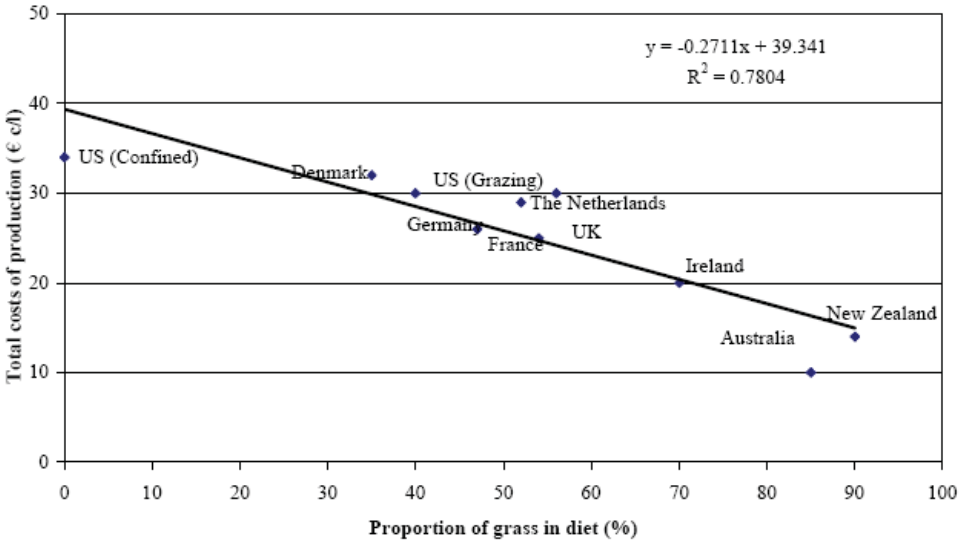


Figure 2.1. Relationship between total costs of production and proportion of grazed pasture cows ration. From Dillon (2006).

Some studies from different regions of the United States (US), which compared confinement with pasture-based systems, showed that overall pasture-based systems presented a lower cost of milk production and yielded a higher economic return despite the lower milk production (Soriano, et al., 2001; White et al., 2002; Fontaneli et al., 2005). However, economic studies comparing both systems need to be analysed with caution and several aspects need to be considered (type of cow, period of the lactation and quality of grass) (Tozer et al., 2003). Additionally, the choice for the feed system utilized in any dairy business should be the one that best achieves the targets of the farm and consequently guarantees the long-term sustainability of the business (Tozer et al., 2003).

Today, due to public concern, welfare and environmental issues are becoming important topics for dairy producers (Rutherford and Lodge, 2001). Consumers are pushing for dairy products produced by farm systems which preserve the landscape and at the same time also care about animal well being. Under this context of sustainability, it seems that pasture-based systems appeal to the consumers of dairy products with a more clean, green and friendly image than other production systems (Verkerk, 2003).

Despite particular regional differences in the environment, breed of the cow, type of plant or even management factors between those regions or countries, efficient pasture-based systems have always been characterized by high milk output per unit of land (Penno, 2002). Additionally, the biological principles characterized by the balances between pasture growth, senescence and quality associated with pasture consumption can be applied equally to the different type of pasture systems around the world (Holmes and Roche, 2007).

Over the years, pasture-based dairy systems have been based on high pasture utilization with high stocking rates to utilize pastures efficiently, and supplements, such as conserved forages, being used to cope with seasonality of pasture production caused by climatic constrains. However, this situation has been changing lately, as a consequence of rising world milk consumption in developing countries (Delgado, 2005), leading to higher world milk prices. Farmers are shifting to higher input grazing dairy systems where supplements are used to fill the energy deficit created by the use of cows with high genetic potential combined with high stocking rates and long lactations (Holmes and Roche, 2007), which are common practices in this type of system.

The genetic improvement achieved over the last few decades on milk production, has also resulted in a cow with greater nutrient requirements, and greater intake in order to produce their increased yields (Holmes, 2007). In New Zealand a study has shown that if the current trends in cow genetic selection persist, in 2014, the cows will have the genetic potential to produce at least an extra 35 kg milk solids per lactation and will be no more than 8 kg heavier than the animals born a decade earlier (Montgomerie, 2004). The extra milk production plus the heavier body size will lead to a greater feed demand, around 250 kg dry matter (DM) cow^{-1} of extra feed required by those animals.

Therefore if stocking rates are not adjusted to balance feed supply and demand, extra feed from pasture or supplements will have to be introduced into the pasture based system (Clark et al., 2007). More recently, several studies have shown that very high

genetic merit cows with potential for high milk yields might not be suited to grazing systems which require cows capable of large intakes of forage, and also restrict feed intake, when compared with confinement rations (Horan et al., 2005; Kolver et al., 2005; Macdonald et al., 2005).

High input systems are generally characterized by the use of more cows per hectare than the optimum stocking rate for pasture-only systems (Roche and Reid, 2002). As stocking rates increase pasture utilization also increases with milk yield per hectare (Macdonald et al., 2008); however, high stocking rates usually creates a feed deficit in early and late lactation, due to differences in feed demand by the cow and feed supply from pasture. Consequently, cows become underfed at the beginning of lactation, which can reduce fertility, and frequently have to be dried-off early because of low body condition score and pasture cover (Holmes and Roche, 2007). Therefore, the use of imported supplements allow farmers to increase pasture utilization with the use of more cows per hectare maintaining or increasing milk yield per cow and per hectare.

Supplements are also used in seasonal pasture dairy systems in attempt to increase lactation length and consequently increase annual cow production (Penno et al., 1999). However, body condition score and pasture cover at the end of the season cannot be compromised when extending the lactation length of the animals in the farm (Penno et al., 1999).

Therefore, supplementary feed has become an important factor in a pasture-based system around the world and today supplementary feeds are being used not just to prevent a feed deficit (Holmes, 2007), but also to increase individual animal performance when pasture alone is not adequate (McGilloway and Mayne, 2002).

2.3. Pasture as a feed for dairy cows

Grasslands are among one of the world's largest ecosystems, with approximately 26% of the total land area of the world classified as grazing lands (FAO, 2000). In New Zealand and Australia, more than 50% of the land area is covered with some type of grassland. One of the main reasons for the adoption of grass-based dairy systems is the fact that grazed grass is usually the cheapest source of nutrients for dairy cows (Figure 2.1; Dillon, 2006) when compared with other feeds used in more confined systems such as maize silage.

Pasture is the source of most of the nutrients consumed by grazing cows and a close match between the pasture production and the herd's feed requirements is a feature of all efficient grazing systems. However, pasture is not always available in the quantities and quality required to meet cows' requirements. Pasture is subjected to the effects of climate variations and environmental conditions (Porqueddu and Maltoni, 2005), affecting production and quality throughout the season. Generally, unrestricted quantities of pasture are available in spring. Additionally, even if pasture is available and of high quality, the process of grazing and some of the nutritive characteristics of pasture itself, restrict milk production to levels that can be achieved on total mix ration (TMR) diets (Kolver and Muller, 1998; Waghorn, 2002; Burke, 2003; Kolver, 2003). The following sections will review the main limitations of temperate pastures in New Zealand.

2.3.1. Constraints of pasture-based systems

In New Zealand (NZ) most of the pasture species are perennial, and predominantly composed by perennial ryegrass and white clover (Kemp et al., 1999). The production of pasture is seasonal with growth being limited by environmental resources (McKenzie et al., 1999). The main parameters affecting pasture growth in NZ are temperature in winter and moisture in summer (Holmes et al, 2002; Valentine and Kemp, 2007), with differences in soil fertility dictating the magnitude of the effect (Matthews et al., 1999). The pattern of seasonal pasture production in different dairy regions of NZ is shown in the Figure 2.2. Pasture growth rate increases to a peak during spring and reduces to a minimum during winter; with particular differences between regions (Figure 2.2). On average 14% of the annual pasture production occurs in the winter, while spring and summer are responsible for 32 and 33% of the production, respectively, and autumn 21% (Valentine and Kemp, 2007).

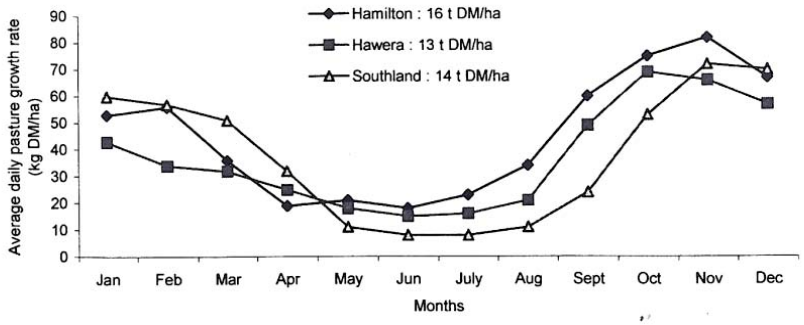


Figure 2.2.Yields of pasture growth in different dairy regions of New Zealand.Adapted from Holmes et al. (2002).

As pasture grows pasture chemical composition also changes throughout the season (Figure 2.3); however, the seasonal effect on pasture quality cannot be easily separated from the effect of plant maturity. During the different stages of pasture growth physical changes occur, which are reflected in the composition of the plant and consequently its digestibility. The effect of plant maturity is discussed in more details in 2.6.1.6 of this review.

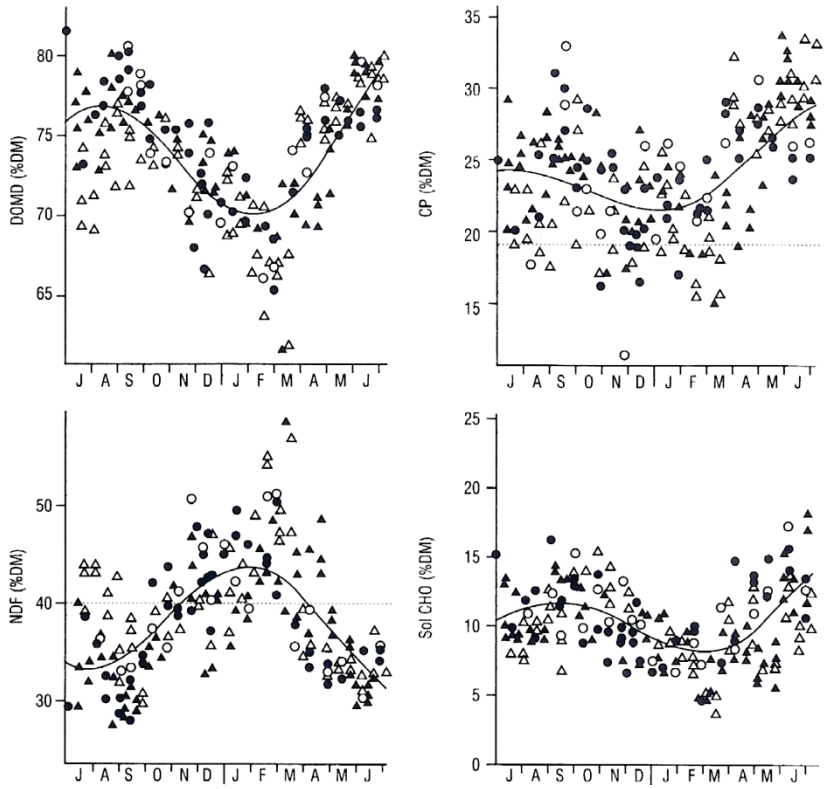


Figure 2.3. Variation of pasture composition throughout the year. Adapted from Hodgson and Brookes (1999).

In general, late autumn, winter and early spring are the times where leaf is predominant in the sward; fibre concentrations are relatively low and consequently digestibilities are high (Hodgson and Brookes, 1999; Waghorn and Clark, 2004). In contrast, with the reproduction growth in late spring and summer, stem and seedheads develop, and consequently digestibility decreases. During this time crude protein and digestibility reach their lowest values, while fibre concentrations markedly increase (Moller et al., 1996; Stevenson et al., 2003).

A good grazing management can have a direct effect in quantity and quality of pasture supplied to the cows throughout the season, and minimizes the limitations of pasture (Lambert et al., 2004). However, even when availability of pasture and quality is not a problem, the grazing process and some of the nutritive characteristics of pasture itself, restrict milk production to levels below what can be achieved on TMR type diets.

Low dry matter intake has been described as the most important factor limiting milk production in grazing dairy cows (Leaver, 1985; Kolver and Muller, 1998; Kolver, 2003); and several studies have shown that dairy cows grazing pasture have limited DM intake when compared with animals fed TMR (Ullyatt and Waghorn, 1993; Kolver and Muller, 1998; Bargo et al., 2002b; Waghorn, 2002). Kolver and Muller (1998) determined that lower intake obtained by cows consuming pasture was responsible for more than 60% of the difference in milk production when compared to cows consuming TMR.

Daily herbage intake can be calculated as the result of bite mass and bite rate, (same as rate of intake), and time spent grazing for 24 hours (h), which is the product of meal duration and number of meals per day (Hodgson, 1990). Research has shown that changes in sward conditions have direct impacts on bite dimensions, bite rate and grazing time; with importance for intake per bite driving overall herbage intake (Dillon, 2006). Intake per bite enhances with increases in the sward height (Hodgson and Brookes, 1999).

Herbage allowance (HA) is considered one of the major factors influencing pasture DM intake in grazing dairy systems (Leaver, 1985; Kellaway and Harrington, 2004; Dillon, 2006). Research results have shown a curvilinear relationship between HA and pasture DM intake (Figure 2.4a), so pasture DM intake increases at a progressively declining rate with increasing allowance (Peryaud et al., 1996; Wales et al., 1998; Bargo et al., 2002a; Taweel et al., 2005). Pasture DM intake usually increases until reaching a plateau equal to 10-12% of BW or 50 to 60 kg DM/cow per day for a

cow with 500 kg of body weight (Hodgson and Brookes, 1999). However, the rate which cows will increase DM intake with extra HA and the plateau value, when no extra DM intake is expected with extra HA, is variable. Peyraud et al. (1996) reported a plateau occurring at an allowance of 32.6 kg/cow per day when DM intake increased from 15.3 to 18.5 kg per day as HA increased from 20 to 54 kg DM per day. Conversely, data from Australia found a plateau of 55 kg/cow per day when DM intake increased from 11 to 18.5 kg/cow per day as HA increased from 20 to 70 kg/cow per day, with an average increase in the intake of 0.14 kg DM/kg DM increase in herbage allowance (Dalley et al., 1999). Also an increase in the DM intake with increases in HA from 7.1 to 16.2 kg DM/cow at low pasture mass, or from 9.9 to 19.3 kg DM/cow at high pasture mass was demonstrated by Wales et al. (1999). The DM intake increased at the ratio of 0.13 kg DM for every 1 kg DM increase in herbage allowance.

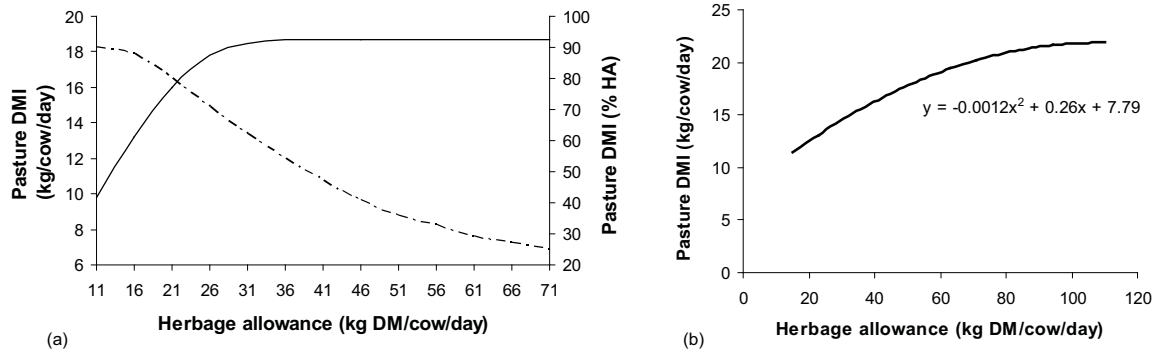


Figure 2.4. (a) Relationship between herbage allowance and pasture dry matter intake (DMI) expressed as kg/day (—) or in % of herbage allowance (HA) (---) (Taweel, 2006). (b) Equation reported by Bargo et al. (2003) to describe the relationship between pasture intake and herbage allowance, derived from the analysis of several studies.

Seven studies which measured intake of grazing cows fed pasture only at different allowances measured above ground level were reviewed by Bargo et al. (2003). Overall, they found that pasture DM intake increased 0.19 kg/kg of increased HA (range: 0.17 to 0.24 kg/kg), when pasture allowance ranged from 20 to 70 kg DM/cow per day. Additionally, they described the relationship between pasture DM intake and HA based on the studies reviewed and taking into account days in milk, milk production, and pasture offered and consumed (Figure 2.4b). The equation predicted a maximum pasture DMI of 21.9 kg/cow per day at a pasture allowance of 110 kg DM/cow per day, and that DMI increased at a rate of 0.26 kg/kg of increase pasture allowance.

Differences in the results obtained by the studies of the relationship between pasture DM intake and HA may be attributed to the different techniques employed to measure herbage intake (Demment et al., 1995). Additionally, the relationship between pasture DM intake and HA is dependent on several factors such as cutting height of the herbage and level of production of the animals (Stakelum et al., 2007).

Despite the fact that increasing herbage allowance increase pasture DM intake, the maximum pasture DM intake at the highest allowance did not reach the necessary level of intake to supply the amount of energy required by high genetic merit cows (approximately 4% of body weight, BW) to achieve their potential milk production (Kolver and Muller, 1998, Kolver et al., 2002). Moreover, at this high herbage allowance low pasture utilization is inevitable (Hodgson and Brookes, 1999). Additionally, Kolver and Muller (1998), using the Cornell Net Carbohydrate and Protein system reported that 61% of the difference in milk production between cows grazing *ad libitum* on good quality pasture and cows fed an *ad libitum* balanced total mixed ration diet are due to the lower intake, but variables such as grazing and walking, milk composition and liveweight differences, and converting the excess of nitrogen to urea account for the rest of the difference in milk production.

Carbohydrates (CHO) and protein are degraded and fermented in the rumen by microbes for their maintenance and growth, however, differences in the ratio of nitrogen (N) to CHO and their respective solubility and degradation rates may cause an imbalance between rumen degradable protein and energy (Nocek and Russel, 1988; Bach et al., 2005; Tas et al., 2006). Leafy pasture that is typical during early spring and autumn is characterized by high crude protein (CP) concentration with a high rate and extent of degradation in the rumen (Tamminga et al., 1990), which can lead to this imbalance and limit milk production (Burke, 2003).

Numerous studies have discussed the topic about the effect of N in the rumen when animals graze pasture with high amounts of CP concentration that is rapidly degraded by rumen microbes (Beever et al., 1986; Van Vuuren et al., 1990; Berzaghi et al., 1996; Westwood et al., 1998; Tas, 2006; Pacheco and Waghorn, 2008). The excess ammonia produced during the degradation of protein in the rumen is partially recycled via saliva and the remaining amount is converted into urea in the liver and excreted in the urine (Rearte, 2005; Pacheco and Waghorn, 2008), which contributes to a lower efficiency of N utilization by cows grazing high quality pasture. Tas (2006), citing a study of Van Vuuren (2003), reported that almost 80% of the CP above 15.5 g/100g

DM was lost in the rumen and excreted in urine. Several consequences of feeding diets with excess protein were listed by Pacheco and Waghorn (2008), but the energy cost of excreting surplus protein in the form of urea,and the environmental impact (Cameron et al., 2007) are two of the most important consequences.

2.4. Supplements

When pasture is used as the sole component of the diet in pasture-based dairy farming, this can introduce several constraints as discussed earlier, and supplements can be used as a tool to manage those constraints, and to buffer the system. Generally, the utilization of supplements aims either to improve overall animal intake and milk production or to maintain performance during periods of pasture shortage (McGilloway and Mayne, 2002; Dillon, 2006). However, supplements are more expensive than pasture (as DM basis) and their use is not profitable if pasture is replaced in attempts to improve the composition of the cow's diet (Holmes and Roche, 2007).

The utilization of supplements in grazing dairy systems, and responses in milk production have been reviewed by Kellaway and Porta (1993), Bargo et al. (2003), Kellaway and Harrington (2004), and more recently by Clark and Woodward (2007). The milk response to supplementation depends on factors related to the animal (cow genetic potential, body condition score, stage of lactation and energy intake) and to the feed (pasture and supplement quality, pasture allowance, supplement amount and substitution rate). This review will give an overview on the effect of supplementation on DM intake and the extent to which the cow partitions the extra energy into milk and not liveweight, and the effect of different supplements on milk production responses.

Additionally, this review will especially focus on a supplement called palm kernel expeller (PKE). This is a by-product from the palm oil industry that has gain great popularity between farmers in the last decade as a supplement for grazing dairy cows, however, very little information exists about the nutritional value of PKE and its effects on milk production when it is incorporated into the diet of pasture grazing ruminants, which will be discussed here.

2.4.1. Effect of supplements on dry matter intake

The use of supplementary feeds is normally undertaken to increase total energy intake and improve animal performance, as there are limits to the extent that a cow can increase DM intake when they graze fresh pasture alone. However, the efficiency in the use of supplements (kg increase in milk per kg increase in feed DM intake) is largely dependent upon the effect of supplementation on herbage intake (Peyraud et al., 1999).

Generally, when cows grazing on pasture are supplemented with concentrates, pasture intake will be reduced; this phenomenon is known as substitution. Substitution rate (SR) is the amount by which pasture DM intake is reduced (in kg DM) expressed per kg of supplement fed (Kellaway and Harrington, 2004). According to Penno (2002), and cited by Holmes and Roche (2007), SR is the most important factor explaining the variation in milk production responses to supplementation of grazing dairy cows; and usually, SR is negatively related with milk response, therefore, when SR is large, milk response is low (Bargo et al., 2003).

Numerous authors (Grainger and Matthews, 1989; Stockdale, 2000; Penno et al., 2006) have studied the relationship between SR and pasture intake prior to supplementation, and several equations have been proposed. Grainger and Matthews (1989) and Penno et al. (2006) reported similar equations, with lower substitution rates found for heavier cows (greater milk production), as shown in Equation 2.1:

$$SR = -0.495 + 0.314\ PDMI \qquad \text{(Equation 2.1)}$$

where PDMI is pasture dry matter intake of the unsupplemented cow per 100kg of live weight.

This relationship agrees with the concept of a cow's relative energy deficit, where SR decreases as the relative energy deficit of the cow increases (Holmes and Roche, 2007). However the relationship found by Stockdale (2000), showed a lower coefficient than those reported by Grainger and Matthews (1989) and Penno et al. (2006), and because that study involved a large number of experiments, it was concluded that other factors also affect SR in grazing animals.

Kellaway and Harrington (2004) suggested that the substitution rate effect is affected by pasture allowance, level of concentrate feeding, forage quality, supplement type and quality, duration of the change in the feeding level, and stage of lactation,

while Stockdale (2000) proposed that pasture species, pasture intake of the unsupplemented group, season and amount of supplement were the key variables affecting substitution. This review will focus on only the main factors affecting SR.

As expected, as pasture allowance increases, SR also increases, but total milk yield decreases. Bargo et al. (2003), in a review of several experiments, found a significant relationship between SR and pasture allowance, which was described by the following equation (Equation 2.2):

$SR = -0.55 + 0.05\ PA - 0.0006\ PA^2$ (coefficient of determination; $R^2 = 0.94$) (Equation 2.2)

As forage digestibility increases SR also increases (Kellaway and Harrington, 2004). Stockdale (2000) reviewed a series of grazing experiments conducted in Australia and found that at similar pasture and supplement intakes, SR was lower in the autumn and summer and greater in the spring (when pasture digestibility is usually lower).

Generally forage supplements result in higher substitution rates than concentrates (Mayne and Wright, 1988), which is agreement with a review of 39 data sets by Stockdale (2000) which reported that fibrous concentrates (eg. hay and maize silage) tend to have higher SR than concentrates at any level of pasture intake. Greater SR in forage supplements may be associated with a reduction in grazing time, probably due to the bulk characteristics of some forage supplements (Stockdale, 2000). However, Meijs (1986) reported that SR was much lower in fibre-based concentrates than starchy concentrates when fed at similar amounts to cows grazing ryegrass pasture. This can happen, occasionally, due to the negative effect of rapidly fermented starch on the rumen pH and fibre digestibility (acidosis), when large amounts of cereal grain supplements are fed to grazing cows (Stockdale, 2000). Kellaway and Harrington (2004) reported that lower SR are expected for concentrates digested more slowly (eg. protein meals and whole cereal grains) compared to rapidly fermented concentrates (eg. +processed grains).

Substitution rate increases as the amount of concentrate was increased (Faverdin et al, 1991; Wales et al., 1999). However, other studies have shown either no effect of concentrate amounts (Opatpatanakit et al., 1992), or an inconsistent effect of the amount of concentrates on SR (Peyraud and Delaby, 2001); and Bargo et al. (2003) concluded

that the lack of consistency between studies was the reason for the poor relationship found between SR and milk response.

2.4.2. Partitioning of nutrients

When supplements are fed to dairy cow's energy intake increases, and consequently, the net increase in energy is partitioned from milk production towards body tissue deposition as milk production approaches the cow's genetic potential (Moran, 2005). Additionally, the partition of nutrients in lactating dairy cows is directly linked with their stage of lactation. Generally, cows mobilize body tissue in early lactation and partition more nutrients to liveweigh gain in mid- to late-lactation (Holmes and Roche, 2007). This occurs not because of a modification in the nutritional status of the cow but as a consequence of changes in their physiological status (Friggens and Newbold, 2007; Roche et al., 2009).

In general, during the first third of the lactation cows have their highest drive to produce milk, as most of the extra feed is partitioned in to milk (Stockdale et al., 1987), resulting in larger marginal responses to additional supplement in short term experiments. Whereas in mid- and late- lactation the supplementation of grazing dairy cows with extra feed will result in the partition of energy towards body condition, which can result in lower milk responses when cows are supplemented for a short period of time (Holmes and Roche, 2007). However, the final fates of the extra liveweight (LW) gained and the substituted pasture should be taken into consideration when the full response (long-term) to supplements is calculated (Holmes and Roche, 2007).

Genetic selection is another important aspect of a cow's nutrient partition of extra energy when supplements are fed. Genetic selection for increased milk production has altered the characteristics of the "modern cow" (eg. overseas genetics), and today those animals mobilise more body tissue during early lactation and partition less energy towards liveweight gain in late lactation (Roche et al., 2006). A greater milk response per kg of concentrate for cows with overseas genetics than for NZ dairy cows has been reported by Kolver et al. (2005) in a two year study about the influence of dairy cow genotype on milksolids, body condition and reproduction response to concentrate supplementation. They also reported that overseas cows maintained a lower body condition score throughout lactation, which will demand extra feed, such as

supplements, during the dry period to achieve a suitable body condition score (BCS) before calving.

Feeding supplements in early lactation has been shown to be ineffective in reducing the rate of BCS loss, but reduced the duration of BCS loss (Roche et al., 2009), however in late lactation feeding level does affect BCS (Holmes and Roche, 2007).

2.4.3. Quality and type

Energy

Generally, in good quality ryegrass/clover pasture-based systems, energy intake is the first factor limiting milk production, as discussed in a previous section of this review (see section 2.3.1). Therefore, several studies have been conducted with grazing dairy cows supplemented with various energy sources. In Australia, the use of energy supplementation has been reviewed by Kellaway and Harrington (2004), and they showed that milk responses to energy supplements (eg. maize silage) in early lactation were greatest when pasture was restricted (0.6 kg milk/kg of supplement), however, no response or negative response to supplementation was reported when high quality pasture was available *ad libitum*. Moreover, in mid- and late-lactation the immediate responses to supplementation averaged 0.6 and 1.1 kg milk/kg supplement on good and poor quality pastures, respectively. Stockdale (1999) also reported that milk responses obtained from grazing dairy cows supplemented with grains is dependent on the characteristics of the pasture consumed with the supplement; and Kellaway and Harrington (2004) concluded that lower responses to supplementation will occur if the quality and quantity of pasture offered are high.

The type of energy supplementation can have an effect on the milk response of grazing animals. Cereal grains are commonly fed to grazing animals; however, there is evidence that rates of degradation in the rumen differ between them (Opatpatanakit et al., 1994), which can affect their digestion in the rumen and consequently their milk response. The addition of large amounts of rapidly fermentable starch in the rumen can increase the lactate production and reduce the rumen pH (Owens et al., 1998), causing depression of fibre digestibility (Mould et al., 1983). Therefore, grains with slow rates of degradation in the rumen (eg. sorghum and maize) are more likely to be beneficial (Doyle et al., 2005), as they have less effect on the fibre digestibility (Opatpatanakit et al., 1994) than grains with faster rates of degradation (eg. wheat, oat and barley). The

supplementation of cereal grains generally increases the production of propionate, and consequently milk protein content (Kellaway and Harrington, 2004). However, as the proportion of propionate increases in the rumen with starch supplementation, the ratio of acetate to propionate is reduced due to the negative effect of starch, and this can lead to a reduction in the milk fat concentration (Dixon and Stockdale, 1999). Grain processing (eg. grinding, steam-flaking) can also alter milk yield and concentration when supplemented to cattle, as processing generally increases the degradation characteristics of the different types of grains. Thus, it is increasingly important to know the characteristics of the grain, especially when more cereal concentrates are added to the diet of grazing dairy cows (Kellaway and Harrington, 2004).

The use of by-products and maize silage are alternatives to supply energy to cows grazing pasture with relatively high fibre concentration. In NZ, due to the high prices of traditional energy supplements (eg. maize grain), by-products such as brewers' grain, whey, molasses and PKE have become more popular among farmers, however, the use of most of these products is still localized (de Ruiter et al., 2007). Another issue with by-products is the great variation in their composition. Molasses, a residue from the sugar cane industry, is used during spring time in NZ, however, the addition of molasses to spring pasture diets has been reported to be have no beneficial effects, unless cows are under-fed (Kolver, 1998 cited by Clark and Woodward, 2007). The use of PKE has also increased in the last few years in NZ, and its use as a supplement feed for cattle will be discussed in more detail later in this review. Maize silage is the most common supplement used in NZ. Stockdale (1995) found higher milk responses when maize silage was fed to cows grazing low allowances of low quality pastures; however, when maize silage was fed with generous high quality spring pastures, the milk response to supplementation was negative. Bargo et al. (2003) showed that responses in milk production to maize silage supplementation depend on the pasture allowance; milk production may be increased at low allowances, but at high allowances milk yield may be similar to/or lower than the unsupplemented group. Moreover, milk fat percentage was not affected by maize silage supplementation and milk protein presented inconsistent results (Bargo et al., 2003).

Protein

Usually, temperate well managed pasture contains a crude protein content (20-30%), that is degraded rapidly in the rumen with up to 20% of the crude protein

escaping the rumen degradation and becoming available in the small intestine (McCormick et al., 2001). Therefore, the amount of metabolisable protein supplied by temperate high quality pasture rarely limits milk production (Holmes and Roche, 2007).

Nevertheless, some experiments have been carried out to verify if the supplementation of rumen undegradable protein (RUP) was necessary to high yield dairy cows grazing pasture. Bargo et al. (2003) reviewed eight studies where several sources of RUP were supplemented to high producing grazing dairy cows and effects on milk production and composition were measured. Only two studies showed a positive effect in milk production due to the supplementation of concentrates with high amounts of RUP. In terms of milk composition, only one study showed that milk fat percentage increased as RUP was added to the concentrate; and two studies showed contradictory results in relation to the effect of RUP on the milk protein percentage. They concluded that the protein supplied by the control diet, which in this case was pasture, has a major effect in the response to RUP supplementation.

Kolver (2003) reported that energy is the first-limiting nutrient in milk production of cows grazing high quality pasture, and that microbial protein supply will not limit milk production until it exceeds 38 kg/day. Therefore, amino acid supply, through the use of RUP supplements, should not produce any response in milk production until the energy deficiency of pastures is corrected (Kolver, et al., 2004), which is in agreement with the review of Kellaway and Harrington (2004). Additionally, Kellaway and Harrington (2004) concluded that cows in early lactation and with good body condition can present some protein deficiency as consequence of their relatively better supply of energy, resulting from body fat being mobilized faster than body protein.

In the summer pasture quality is low and crude protein values can be lower than 18% of the dry matter (Figure 2.3). Usually in this situation energy (eg. maize silage) or a supplement low in protein is supplied to the cows. As a result, protein supply from the diet can become deficient, and protein supplementation could be used to increase milk production. MacDonald et al. (1998), studying the use of different sources of protein to overcome protein deficiency caused by the supplementation of large amounts of maize silage to cows grazing summer and autumn pasture, found that soybean meal and fishmeal increased milk yield and body condition score. They concluded that once energy supplements (eg. maize silage) are introduced in the diet of grazing dairy cows in summer, protein supplements may be necessary to correct any protein deficiency and

increase milk yields, which is agreement with the conclusions of Kellaway and Harrington (2004). However, protein supplements are usually too expensive to be profitable in NZ, but PKE contains between 14 and 16% of CP, which could have an impact on the diet of animals grazing summer pasture, which contains low CP values (12-15% of CP)

Fat

The use of fat supplementation to grazing dairy cows and their main effects on milk production and composition has been recently review by Schroeder et al. (2004), therefore this review will briefly discuss the metabolism of fat in the ruminant and the effects on the yield and composition of milk caused by its use as a supplement for cows fed pasture.

The use of fat in the diets of ruminants has been advocated for the following reasons (Kellaway and Harrington, 2004; Schroeder et al., 2004):

- Increase the energy density of the diet and consequently increase energy intake, as fat has more net energy per kg for lactation than protein and carbohydrates;
- Improve energy efficiency due to the reduction of energy loss through heat, methane and urine and through the incorporation of dietary fatty acids directly into milk fat, which is more efficient than de novo synthesis in the udder;
- Avoid the use of large amounts of cereals and therefore reduce the risk of rumen acidosis and decreased milk fat percentage;
- Reduction in the mobilization of body fat and loss of liveweight due to reduction of energy deficit in early lactation.
- Increase the amount of fatty acids in the milk beneficial for health.

However, an excess of fat in the diet (more than 8-9% of DM) can cause adverse effects. According to several authors (Palmquist, 1984; Jenkins, 1993; Doreau and Chilliard, 1997) lipid supplementation can often causes reductions in the ruminal fibre digestion and in DM intake. These negative effects of fat supplementation have been associated with the inhibition of the microorganisms concerned with cellulose digestion and methane production, and a reduction in the palatability of the diet (Palmquist, 1984).

Usually, the fat content of forages ranges between 3 and 8% of DM, with temperate pastures showing a fatty acid (FA) content between 1 and 3%, with most of them being unsaturated (linoleic and linolenic) (Schroeder et al., 2004). Variation in the

pasture FA content is due to the season of the year, type of cultivar and pasture management. Cereal grains have lower fat content (2 and 4%) compared with forages, however oil seeds are high in fat (20-42%) (Schroeder et al., 2004). Therefore, a diet composed of temperate pasture and cereals, generally has a low fat content and the consequences of too much fat in the diet does not exist. However, the effects of dietary fat need to be clarified because PKE contains between 8 and 10% of fat, which could have an impact on the digestion metabolism.

In ruminants, fats are transformed in the rumen before they are absorbed in the intestine; therefore, the diet leaving the rumen is different from the diet consumed by the animal. Two major processes occur in the rumen: lypolysis and biohydrogenation (Doreau and Chilliard, 1997). In lypolysis, dietary lipids entering the rumen are hydrolysed by rumen bacteria, and fatty acids are released from the glycerol backbone. Glycerol and sugars released are fermented to volatile fatty acids and the free unsaturated fatty acids will pass through a second process, biohydrogenation, where unsaturated FA are hydrogenated to produce saturated FA for later absorption in the small intestine (Bauman et al., 2003). Fatty acids are reconverted into triglycerides for delivery to different organs and used in different ways (eg. burned to release energy, as fat tissue, or for the formation of milk fat in the mammary gland).

During the process of biohydrogenation several intermediate compounds are formed, and some of them are incorporated into body fat and milk fat of ruminants. In the case of pasture, during the biohydrogenation of linoleic (C18:2) and linolenic (C18:3) an intermediate compound is formed, vaccenic acid (trans-11 C18:1). This is absorbed postruminally and incorporated into adipose tissue and the mammary gland for later formation of conjugated linoleic acids (CLA), which are important for human health (Bauman et al., 2003).

The FA in milk are derived from two main sources, either direct from lipids in the circulating blood or from de novo synthesis within the mammary gland (Bauman and Griinari, 2003). In ruminants, around half of the milk FA comes from the de novo synthesis, which uses acetate and β-hydroxybutyrate (from butyrate) as its major carbon source for the FA synthesis. These are predominantly short- (4-8 carbons) and medium-chain (10-14 carbons) FA (Bauman and Griinari, 2003). The circulating FA taken up by the mammary gland is derived either from intestinal absorption of fat from the diet (90%), or from the mobilization of body fat, which forms mainly long chain fatty acids

(Bauman and Griinari, 2003). Fatty acids with 16 carbons can originate from both sources.

The studies of fat supplementation to grazing cows have been reviewed by Schroeder et al. (2004). They showed that fat supplementation (between 200 to 1000 g of fat cow/day) increased milk production in 80% of the experiments analysed, with an overall increase of 5.2% (1.05 kg/day) for fat-corrected milk (FCM), compared with cows grazing pasture only. They also showed that the fat's degree of saturation and the cow's stage of lactation influenced the milk response to fat supplementation, with supplemented saturated fats showing larger responses than unsaturated fat, and cows in mid-lactation showing greater milk responses than early-lactation cows. Furthermore, supplementation with saturated fat increased milk fat percentage from 3.5 to 3.7%, whereas supplementation with unsaturated fat reduced milk fat percentage from 3.5 to 3.2%. Overall milk protein percentage was slighted reduced, with a greater reduction when cows in mid-lactation were supplemented with unsaturated fat, but protein yield was not affected. They concluded that overall milk production can be increased by supplementation of fat to cows grazing pasture and the effects of fat supplementation on milk composition will depend on the degree of saturation.

2.5. Palm kernel expeller

During the last five years, the interest of farmers for a by-product from the palm oil industry called palm kernel expeller (PKE) has increased remarkably. The reason for that is because PKE can be used to reduce feed deficits and at the same time is relatively low price supplement when compared with other supplements. Despite the few studies found in the literature about the use of PKE in grazing systems, this review will discuss PKE origin and use in NZ, and some of its nutritive characteristics when use as a supplement for ruminants.

Palm kernel expeller is a solid by-product from the palm oil industry remaining after the palm kernel oil has been extracted. Its use in New Zealand has increased steadily in the past few years. The imports of PKE into NZ have increased from virtually nothing in 2000/2001, to almost 900,000 tonnes in 2007/08 (MAF, 2008). In some cases it is used mixed with other supplements, such as maize silage or grains, while in others it has been used as the sole supplement to pasture, in order to fill short term or emergency pasture deficits.

The fruit of the oil palm (*Elaeis guineensis* Jacq.) is composed of an outer mesocarp and an inner shelled nut containing the palm kernel. The oil palm is a native species of West Africa (Cornelius, 1977), but today it is also found in Southeast Asia and South America. The industry extracts the palm oil from the mesocarp, while palm kernel oil comes from the kernel of the nut. There are two processes commonly used to extract the palm kernel oil, screw pressing and solvent extraction. The solid residues generated from the expeller pressed palm kernel are known as palm kernel expeller (PKE) or cake, while the solvent extracted are known as palm kernel meal (PKM) (Figure 2.5). The main difference between the two types of palm kernel is the amount of residual oil, which is greater in the palm kernel extract produced by the expeller process. The term palm kernel (PK) will be used throughout this review to refer to both products.

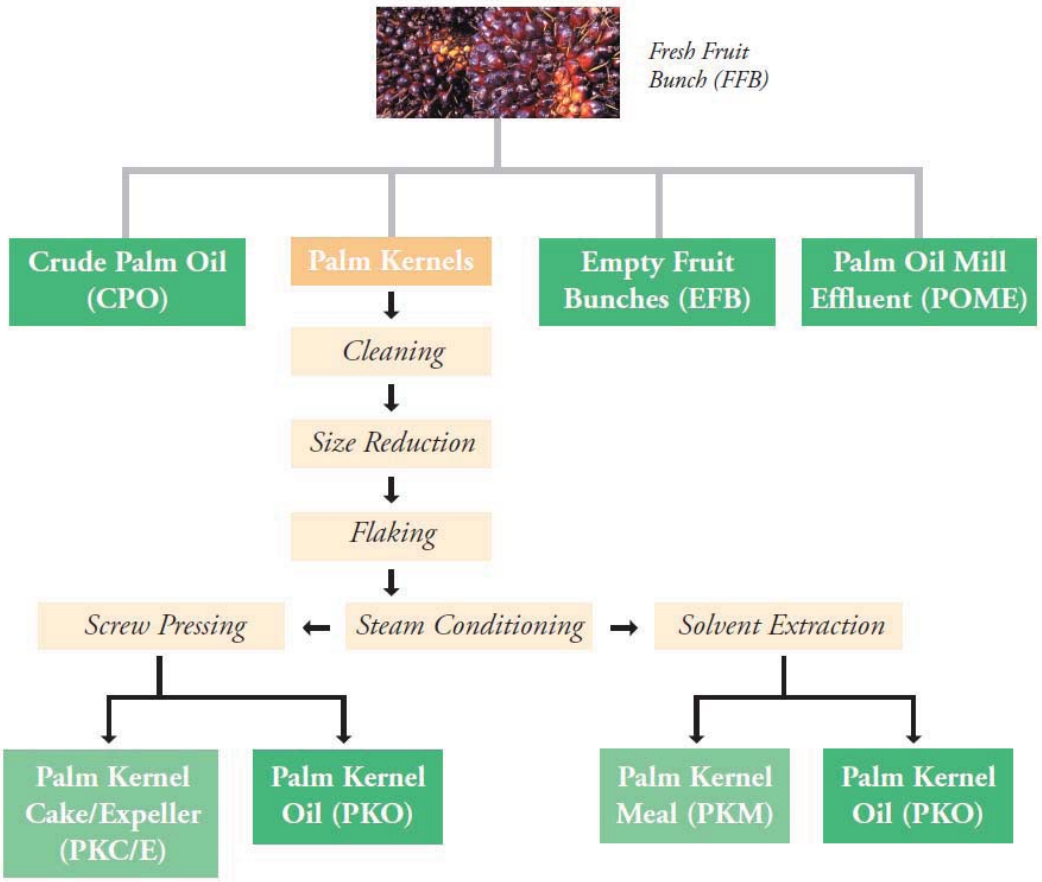


Figure 2.5. Process of oil extraction from palm kernel fruit (Source:MPOC, 2008).

During the last two decades global PK production has increased by 15% per year (FAO, 2002), as consequence of an increase in the palm oil extraction. Malaysia is the largest producer and exporter of palm oil and PK, with a estimated production of about

1.91 million tonnes of crude palm kernel oil and 2.16 million tonnes of PK (cake/expeller) in 2006 (MPOC, 2008). Currently, most of the PK produced is exported to Europe for use as cattle feed, however it is also used as feed for poultry (Sundu et al., 2006) and pigs (Babatunde et al., 1975).

In NZ, the use of PK was first reported in 2000 when a small quantity was imported into the country (Figure 2.6). Since then, imports of PK into the country have grown very rapidly, so that in 2008 approximately 800 thousand tonnes of PK have been imported into NZ (MAF, 2008), mainly to feed dairy cows. Most of NZ's PK comes from Indonesia and Malaysia, and according to de Ruiter et al. (2007), most of the product coming into NZ is from mechanical expelling. Therefore, this review will focus on the nutritive value of palm kernel expeller (PKE).

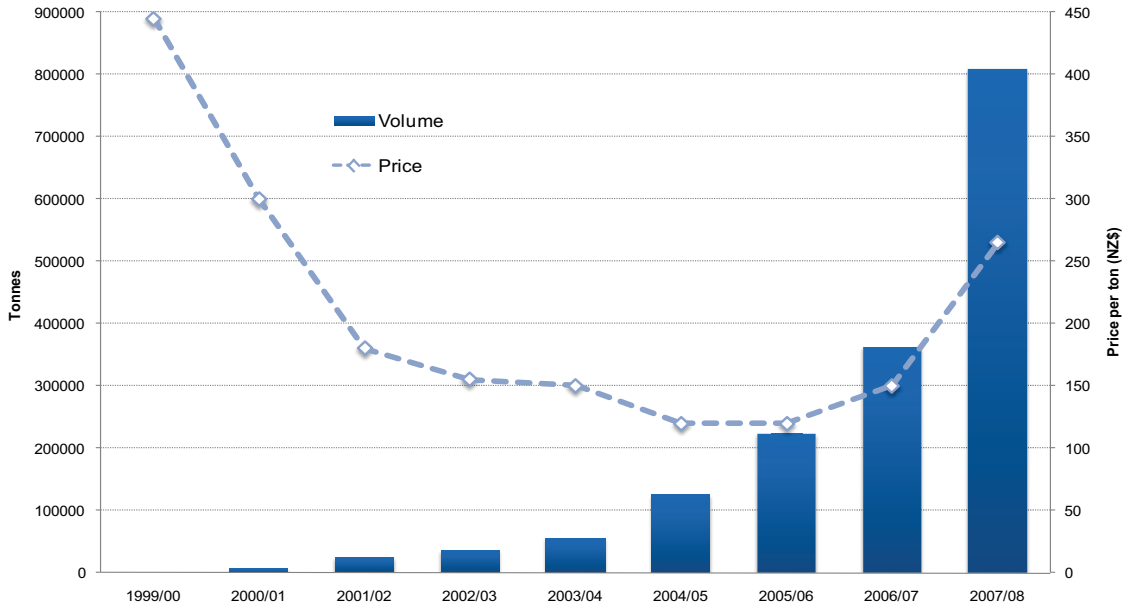


Figure 2.6. Price and volume of palm kernel imported into New Zealand since the year 2000. Adapted from MAF (2008).

Despite its growing importance as a feed for ruminants in NZ, very little information exists about the nutritional value of PKE, and practically none about its effects in pasture-based systems. The few scientific reports about PKE have shown that its chemical composition and digestibility can vary significantly (Table 2.1). In three studies where several PKE samples from different sources were analysed for chemical composition they showed that protein, fibre and fat are the main components, and their values ranged from 15.5 to 21.7 g/100g DM of CP, 66.5 to 81.8 g/100g DM of neutral

detergent fibre (NDF), and 4.5 to 12.8 g/100g DM of ether extract (EE) (Moss and Givens, 1994; Hindle et al., 1995; O'Mara et al., 1999). The wide variation in the PKE chemical composition are probably due to variations in the efficiency at the oil extraction during the expeller process, and to variations in the amount of shell content that remains in the by-product after the oil has been extracted. Additionally, differences between laboratories and methodologies used may account for some of the variation. The NDF content is very high when compared with pasture, but the OMD is higher than would be expected from a feed with such a high NDF content (Table 2.1).

Table 2.1. Average chemical composition (g/100g DM, unless stated) of palm kernel expeller (PKE) from different experiments.

Reference	DM	CP	EE	NDF	ADF	Lignin	Ash	Gross energy MJ/kg DM	OMD
Moss and Givens, 1994	92.2	17.2	10.8	69.5	44.1	-	4.5	20.9	75.0
Hindle et al., 1995	90.6	19.1	11.5	74.3	47.4	12.3	4.3	-	68.9*
O'Mara et al., 1999	88.2	16.4	7.8	80.1	54.3	18.1	4.9	20.6	65.3
Woods et al., 2003a	87.8	17.8	5.1	81.7	55.8	17.7	4.9	19.9	67.7
Carvalho et al., 2005	94.5	17.2	7.4	66.5	35.7	12.0	5.0	19.0	79.0
De Ruiter et al., 2007	91.5	17.2	9.2	69.2	42.1	-	-	-	-

**In vitro* organic matter digestibility.

The organic matter digestibility (OMD) of PKE varied widely between studies, ranging from 65.3 to 79.0 g/100g DM, measured either *in vitro* or *in vivo* (Table 2.1). O'Mara et al. (1999) measured *in vivo* digestibility in sheep of 8 samples of PKE from different sources, and also verified the accuracy of different *in vitro* methods (*in vitro* rumen fluid, neutral detergent cellulase with gammanase, and pepsin cellulase with gammanase) to predict OMD. They reported that none of the samples had an OMD greater than 70 g/100g DM and an average gross energy (GE) of 20.6 MJ/kg DM, and characterized PKE as a moderate quality feed for ruminants. They also found a low acid detergent fibre (ADF) and CP digestibility when compared with the other constituents, as a consequence of the low digestibility of the components concentrated in the ADF

fraction (cellulose and lignin), and also due to heat damage and the effect of the Maillard reaction on the protein fraction during the expeller process, respectively. These authors also concluded that the different *in vitro* methods underpredicted the OMD to a large extent, with no conclusive explanation for that.

In two separate studies where the OMD was also measured *in vivo* with sheep as the experimental unit, the OMD of PKE was much higher than *in vitro* studies, with values above 75 g/100g DM (Moss and Givens, 1994; Carvalho et al., 2005). The reasons for the differences between these studies and the results reported by O'Mara et al. (1999) are not clear, but the concentration of NDF and digestibility were greater in the samples analysed by O'Mara et al. (1999). Additionally a strong relationship between chemical composition and OMD was not found by O'Mara et al. (1999), but they did report a negative relationship between crude fibre and digestibility.

The ruminal degradability of DM and CP in PKE was measured by Woods et al. (2003a, 2003b, respectively) together with several other feeds commonly used in ruminant feeding, and these were incubated in the rumen of steers. PKE had the lowest soluble fraction for DM and CP, but the highest slowly degradable fraction for DM and CP, between the energy feeds tested. Additionally, they found a very slow rate of digestion of the slowly degradable fraction for DM and CP (0.03 and 0.02/h, respectively), and as a consequence they reported a very low effective degradable protein in the rumen, despite the large slowly degradable fraction of PKE. In contrast, in another study by Woods et al. (2003c) where the small intestinal digestibility of several feedstuffs were measured by *in vitro* or *in sacco* techniques in cows, PKE had the highest *in sacco* intestinal digestibility among all the energy feeds tested (77 g/100g DM). Additionally, they reported an increase in the rumen undegradable protein (from 39 to 65%, expressed as a proportion of the CP content), and in the amount of protein truly digested in the small intestine when the rumen outflow rate increased from 0.02 to 0.08/h.

Two studies reported the use of palm kernel in the diets of dairy cows and its effect on milk production (Davison et al., 1994; Carvalho et al., 2006). In the first, Davison et al. (1994) studied the use of increased amounts of a grain concentrate supplement composed of different proportions of PKE when fed to cows grazing a low allowance of pasture during the day, plus corn silage during the night. The results showed that milk fat percentage and fat corrected milk yield were increased with the addition of PKE into the grain concentrate supplement, and they concluded that PKE

could replace grain concentrate in rations for dairy cows at amounts up to 3 kg/day, with no deleterious effect on milk production. In the second experiment, Carvalho et al. (2006) also showed no significant effects on milk production when increasing amounts of PKM were fed to dairy cows eating corn silage based diet. However, the inclusion of PKM in the total mixed ration diet tended to increase the protein and lactose contents of milk (Carvalho et al., 2006). This suggests that differences in the type of palm kernel extraction, solvent or expeller, could cause different effects on milk composition.

These results indicate that PKE is a feed with unique characteristics, and based on the information reported above its use may be beneficial in the NZ pasture system as supplement to fill short-term feed deficits (eg. summer droughts), however more research is needed to clarify its nutritional value and its use as a supplement in grazing dairy systems.

2.6. Techniques to measure nutritional value of feeds

The food ingested by animals is the main source of energy required for their maintenance and production processes. Therefore, knowledge about feedstuffs is essential in order to supply, in quantity and quality, the amounts required by the different categories of farm animals. Feeds are evaluated by methods that describe their characteristics with respect to their ability to meet the animals' requirements.

Accurate information acquired about the ability of foods to supply required nutrients to the animals is essential for the development of efficient food evaluation systems and feeding strategies, and the maximization of animal production (Tamminga and Williams, 1998).

Laboratory analyses are the first steps for the determination of the feedstuff's nutritive value, whether using wet chemistry or near infrared reflectance spectroscopy (NIRS), and it provides the exact description of the feed composition. However, in this methodology there is no interaction between feed and animal, and it is unable to predict how much of these nutrients can be digested and used by the animal. Therefore, several hierarchical levels were developed for the different techniques to describe feed composition (Figure 2.7) (Mould, 2003).

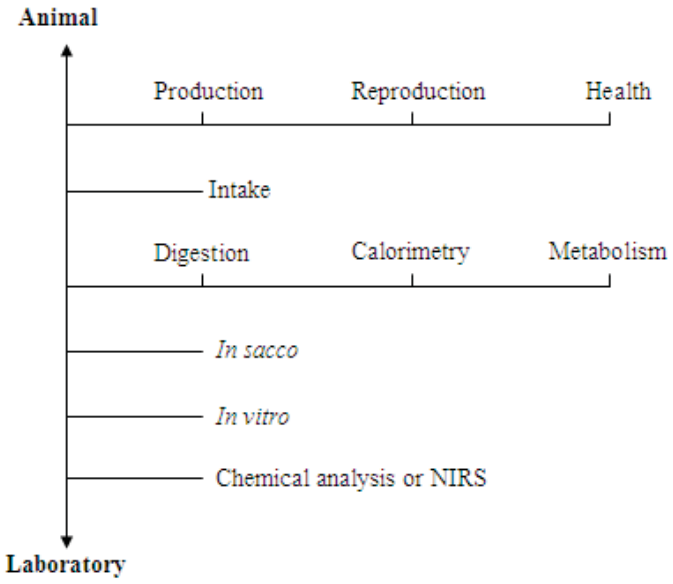


Figure 2.7. Hierarchy of feed evaluation methods, to measure the feed's value for animal production. Taken from Mould (2003).

Usually, the studies at the top of the hierarchy are the "ideal" methods to measure how much of the feed is contributing to the requirements of different classes of animals; however they are labour and time-consuming, expensive, and require large amounts of feed and animals (Mould, 2003). Therefore they are not used routinely, but they are undertaken to confirm the results obtained from methods at the lower hierarchical levels (Mould, 2003).

On the next level down, *in vivo* assessment is one of the most reliable methods to describe digestive and metabolic processes in the ruminant animal, because it measures the real response of the animal to different feeds and diets (López, 2005). However, the amount of time required plus the costs of the *in vivo* method, have led to the development and use of laboratory methods, such as *in sacco* and *in vitro*, which provide quicker and cheaper alternatives to predict feed digestibility (Kitessa et al. 1999).

2.6.1. In vivo digestibility methods

Digestibility can be defined as the amount or percentage of a feedstuff or dietary constituent ingested by the animal and not excreted in the faeces (Cochran and Galyean, 1994; Kitessa et al. 1999). The *in vivo* technique measures the nutrient losses in the

faeces, and consequently the estimates the digestible energy of feeds, has been described and used since the late 1800s (Schneider and Flatt, 1975).

In the traditional *in vivo* technique, the food under investigation is fed to the animals at maintenance level and faeces are collected for a period of seven to ten days after a period of adaptation to the diet. The amount of nutrient digested by the animal is conventionally called "apparent digestibility" and is equivalent to the quantity of nutrients consumed less the quantity excreted in the faeces (Equation 2.3).

$$\text{DM digestibility} = \left[\frac{\text{DM Intake} - \text{Faecal DM excreted}}{\text{DM Intake}}\right] \text{x } 100 \qquad \text{(Equation 2.3)}$$

The term apparent digestibility is used because the faeces contains feed not digested by the animal but also material of non-dietary origin (eg. microbial and endogenous matter), while true digestibility represents only the fraction of the feed available for digestion by the animal (Van Soest, 1994).

However, digestibility measured *in vivo* does not count for energy losses from methane or heat produced by the metabolism and presents no differentiation between feed degradation in the different sites of digestion or about degradation kinetics. Nevertheless, it does account for faecal energy losses which are the largest component (Schneider and Flatt, 1975). It is also an important technique to validate the *in vitro* procedures that have been developed (Rymer, 2000).

Previous reviews of techniques to evaluate the nutritional value of feeds have examined the sources of error in the digestion experiments (Schneider and Flatt, 1975; Kitessa et al. 1999; Cochran and Galyean, 2000). They described several factors which affected the outcome of *in vivo* digestibility experiments, and some of them will be discussed below.

2.6.1.1. Collection of faeces

Usually, most of the digestion trials use animals as the experimental unit and, therefore, measurement of daily feed intake and collection of faeces excreta by individual animals is necessary (Cochran and Galyean, 2000). Consequently, animals are kept in individual stalls, with the type of stall varying according to the excreta that will be collected. The most common stall used is the "metabolism" stalls, which permit

efficacious collection of the excreta and easy monitoring of intake. However, according to Schneider and Flatt (1975), the only concern about the use of metabolism stalls is that animals could become uncomfortable (sore feet and legs) and feed intake could be affected if they remain in the stalls for long periods.

When digestion trials are not conducted in optimal conditions (e.g grazing animals, buildings without environment control), it is important to recognize that environmental factors can influence intake, and consequently digestibility (Schneider and Flatt, 1975).

During the collection period, faeces must be collected without urinary contamination; therefore faecal bags are sometimes attached to the animals with a harness. Whenever possible, males should be used to measure digestibility, especially when urine and faeces must be collected without contamination.

2.6.1.2. Period and length of the experiment

Generally, the *in vivo* digestibility experiments are divided in two periods, the preliminary period and the collection period (Cochran and Galyean, 1994), however, three periods are also suggested, one for adjustment and transition, followed by the preliminary and collection time (Schneider and Flatt,1975).

The adjustment period is the time where the animals established the intake amounts proposed by the experiment and clears the digestive tract of residues from the previous diet (Van Soest, 1994). This time is also required by the animal to allow its ruminal microbial population to adapt to the experimental diet (Cochran and Galyean, 1994). The length of the preliminary period can vary, but usually is recommended between 10 and 14 days is recommended (Kauffman et al., 1980 cited by Cochran and Galyean, 1994).

The collection period, when feed, refusals, and excreta are collected quantitatively, begins after the animals have adapted to the experimental diet. Most authors agree with a length of between 5 and 10 days, with an average period of 7 days. They also emphasised that collection periods of less than 4 days are not recommended, except when markers are used (Cochran and Galyean, 1994).

2.6.1.3. Level of feeding

Many feed systems use the digestibility data obtained from studies where animals were offered feed at maintenance amounts; however such data often overestimates the digestibility of those feeds, which in farming practice are fed at amounts much higher than maintenance (eg. lactating dairy cows) (Church, 1993). This is due to decreases in digestive efficiency at higher levels of intake (Tyrrell and Moe, 1975; Colucci et al., 1982; Edionwe and Owen, 1989; Van Soest, 1994).

The increase in the level of feeding can lead to an increased rate of passage rate through the gastro-intestinal tract (GIT) (MacDonald, 1995; Kitessa et al., 1999). Consequently the food is exposed to the digestive processes for a shorter time, which can cause a decline in its digestibility. In addition, higher intakes not only reduce feed degradation, because of the lower retention time in the GIT, but are also likely to reduce the times of mastication and rumination of the food (Colucci et al., 1982).

Tyrrell and Moe (1975) concluded that digestion coefficients determined at maintenance level overestimate apparent digestibility when applied to animals with high feed demands (eg. lactating dairy cows), and digestibility is depressed by approximately 4% for each increase in intake equivalent to maintenance for lactating dairy cows fed mixed forage-concentrate diets.

The reduction in the coefficients of digestibility per unit of increase in feeding level (eg. from maintenance to twice that level) varies according to the feed group (concentrate or forages) and their mixes. The extent of depression in digestibility caused by an increase in the intake levels seems to be smaller for roughages (1 to 2%), with intermediate values for mixed diets of roughages and concentrates (2 to 3%), to larger values for some fibrous by-products (as high as 5%) (McDonald et al., 2002).

Studies have shown that the proportion of response in the coefficient of digestibility to different levels of intake may vary between all forage, all-grain, and forage grain mixtures. In the case of forages, several authors have found a minor decrease or even no effect in the digestibility of forages due to increasing feeding levels (Mbwile and Uden, 1997; Mulligan et al., 2001). Schneider and Flatt (1975) also reported that the detrimental effects due to the level of intake on the digestibility of forages are small, but variable.

The effect of intake on the digestibility of diets that contained different concentrates (copra meal, sunflower meal, gluten, soy-bean hulls and palm kernel meal)

has also been examined (Woods et al., 1999). Friesian steers were offered a diet with a ratio of 15:85, hay to concentrate, at maintenance level or two times maintenance level. These authors found that dry matter digestibility (DMD) decreased in all concentrates tested when the level of intake was increased. Soya hulls, gluten and palm kernel meal presented greater depressions than the other concentrates, with decreases of more than ten units of digestibility compared with that at maintenance levels of intake.

A linear decrease in the digestibility of distiller grains or soy-bean hulls was also found by another study when diets containing 50% corn silage and 50% concentrate were fed at maintenance, twice maintenance and *ad libitum* (Edionwe and Owen, 1989). However, the reduction in the DMD of soy-bean hulls was smaller than in the study of Woods et al. (1999).

The reduction in digestibility caused by increased feeding levels was measured in dry and lactating dairy cows fed diets containing different ratios of forage:concentrate (Colucci et al., 1982). Diets with large proportions of roughage showed smaller reduction in digestibility, with the size of reduction being proportional to the amount of grain in the diet. These results agree with Tyrrel and Moe (1975) who summarized data for diets containing different proportions of grain.

It has been reported that the concentration of cell walls in the diet is one of the main determinants of the depression in digestibility as level of feeding increases (Van Soest, 1994); and in order to be depressible, cell walls must be digestible; with cell wall concentration and rate of passage being proportional to the digestibility depression (Van Soest, 1994).

2.6.1.4. Associative effects

Interactions between feeds are common and when a food is fed in a mixture with other feedstuffs its digestibility is influenced not only by its composition but also by the chemical composition of the other feeds in the mixture. So, the apparent digestibility of the mixture is not equal to the average of the individual digestibilities. These differences are known as associative effects and can be positive or negative (Weiss et al. 1994; MacDonald, 1995; Kitessa et al. 1999; Doyle et al. 2005).

The introduction of concentrates into forage diets may increase the total digestibility of the mixture because they are usually higher in digestibility than forages (Bargo et al. 2002b). However, the interaction of concentrates and forages may cause

decreases in the fibre digestibility. These negative effects are most common when the digestibility of the forage is reduced by the addition of large amounts of fermentable carbohydrates in the diet, such as starch or glucose (Mould, 1988; Church, 1993; Dixon and Stockdale, 1999; Doyle et al. 2005).

Supplementing forage diets with rapidly fermentable carbohydrates can lead to reductions in the rumen pH, inhibition of cellulolytic microorganism's activity and consequently a depression in the rate of fibre digestion (Mould, 1988; Dixon and Stockdale, 1999; Bargo et al. 2002a). On the other hand, the depression in the digestibility of fibre in the rumen could also be caused by an increase in total dry matter intake (Bargo et al. 2002a), and the result is faster rates of passage from the rumen (Doyle et al. 2005).

An example of a negative associative effect can be found in the study of Thomas et al. (1988) where Friesian steers were fed diets of early and late cut perennial ryegrass silage with or without the supplementation of barley at either 280 or 560 g DM/kg of total DM. The inclusion of the barley (fermentable carbohydrate) in the diet of the late cut ryegrass silage decreased the apparent digestibility of the NDF from 653 to 562 g/kg. Additionally, these authors calculated that the proportion of the total metabolisable energy (ME) intake from cell walls was depressed as the amount of supplement increased in the diet. Reductions of 11 and 39% in the apparent digestibility of the ground hay offered to sheep in diets containing pelleted barley at ratios of 2:1 and 1:2 (hay:barley), respectively, was also calculated by Mould (1988).

It appears that negative effects on the digestibility of forage based diets with the inclusion of concentrates are more likely to occur when the diets are offered at levels of feeding above maintenance (Mould, 1988; Church, 1993). Furthermore, it seems that negative associative effects are expected to occur more often when larger amounts of concentrates are offered and pasture quality is low, and when carbohydrate supplements with high rates of degradation in the rumen are fed (Dixon and Stockdale, 1999; Doyle et al. 2005).

Positive effects (synergy) are less common when concentrates are added to forage diets and it seems that they occur in situations where the supplement will provide a nutrient which is limiting the development of certain rumen microorganisms, such as cellulolytic bacteria Mould (1988), or where there is an excess of protein or nitrogen compounds in the diet consumed by the animals Doyle et al. (2005). However, the most frequent positive associative effect is due to the alleviation of a nutrient deficiency with

the supplementation of a grain with high concentrations of that nutrient, which is not a true associative positive effect (Mould, 1988; Dixon and Stockdale, 1999).

2.6.1.5. Feed processing

Changes in the particle size of a feed during its processing (eg. pelleting or grinding) can affect its digestibility. The reduction of the particle size may either increase digestibility of feeds due to the exposure of more surface area for the enzymatic activity, or it can decrease digestibility by reducing the retention time of the feed and consequently diminishing the exposure of the feed digesta to the digestive system and its enzymes, or finally, it can have no effect at all (Kitessa et al., 1999).

In the case of forages, a depression on *in vivo* digestibility of DM and fibre digestibility was observed in a study where cows fed pelleted grass hay were compared with those fed chopped grass hay, at a maintenance level (Uden, 1988). The author also concluded that the decrease in fermentation rate and decrease in rumen digesta retention times were the main causes of the reduction in digestibility. Reducing particle size of forages through processing methods can also increase intake so that the amount of digestible energy consumed is similar or better than that of animals consuming un-processed forages (chopped forages or long hay) (Church 1993).

It appears that the increase in intake and consequently in the efficiency of digested energy by processed forages does vary, and generally medium-quality forages containing high amounts of cell wall show greater depression in digestibility than high-quality forages with lower cell wall content (Van Soest, 1994).

Processing can often increase digestibility, however it depends on the grain source and their inherent characteristics (Theurer, 1986; Owens et al. 1997; Owens and Zinn, 2005). The importance of the processing effect in the grain is direct related to the rate of starch disappearance in the rumen and total tract, with barley and wheat presenting greater values than corn and sorghum grain (Owens and Zinn, 2005). So, it appears that processing has more positive effects on the digestibility of sorghum and corn than barley or wheat, which contain starch that is less resistant to ruminal degradation (Church, 1993).

In the case of PKE, it seems that the process used during the oil extraction can have a impact on the digestibility of this by-product. According to O'Mara et al. (1999) the PKE derived from solvent extraction (chemical) has higher DM digestibility than the

PKE obtained from the expeller process (mechanical) (665 vs. 632 g/kg, respectively). This difference is probably due to the heat damage that occurs during the expeller process in the protein fraction, which resulted in a lower CP digestibility in the expeller samples when compared with samples extracted with a solvent (597 vs. 727 g/kg, respectively).

2.6.1.6. Forage maturity

Plant maturity is the most important factor affecting pasture quality with major influences on digestibility and consequently animal performance. The decrease of digestibility with increased plant maturity occurs mainly because of the changes in the proportions of leaf, stem and inflorescence and the differences in compositional characteristics between these three components (Waghorn and Barry, 1987; Chaves et al. 2006).

As pasture matures the proportion of plant components change (Figure 2.8). Studies with ryegrass have shown an increase in the ratio of stem to leaf as the plants progress to the reproductive stage (Wilman et al., 1976; Wilman and Agiegba, 1982). The flowering stem becomes quickly lignified reducing the quality of the grass (Valentine and Kemp, 2007).

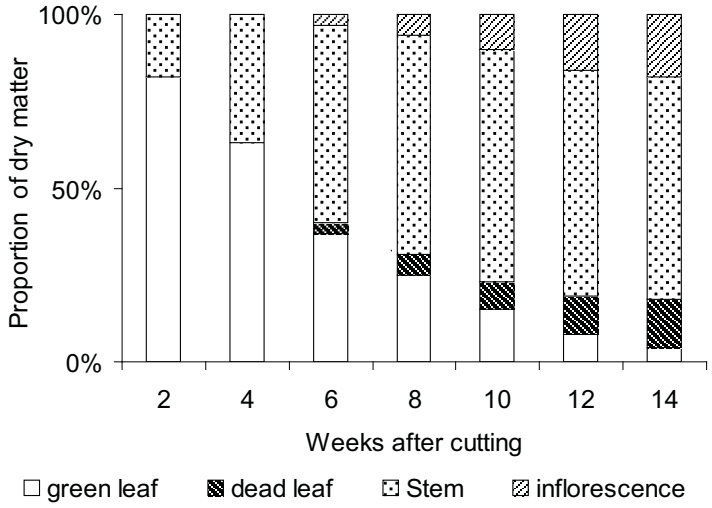


Figure 2.8. Changing in composition of Italian ryegrass components (% of plant DM) after the cut of the sward. Adapted from Wilman and Agiegba (1982).

At the same time that proportion of less digestible components increases in the plant with age, there are also changes in the chemical composition of those components (Table 2.2). According to Hodgson (1990) the digestibility of young leaf tissue fractions falls from 80 to 90% to around 70% in mature leaves due to the increase in the ratio of cell walls to cell contents and the scale of lignification of the cell walls.

Table 2.2. Composition and *in vivo* digestibility of perennial ryegrass harvested at 4 stages of maturity and fed to sheep at maintenance level[1].

	Young leaf	Mature leaf	Head emergence	Seed setting
Non-structural carbohydrates	14	12	11	10
Protein	15	12	11	6
Non-protein nitrogen	4	4	3	3
NDF	40	45	47	60
ADF	24	26	28	34
Lignin	3	4	4	7
Lipid	9	8	7	5
Digestibility of dry matter (%)	86	83	79	62

[1]Adapted from Waghorn and Barry (1987)

Living cells are mainly composed of cell contents and cell walls. Cell contents are composed of simple compounds of carbon and nitrogen and are digested rapidly, while cells walls have more complex compounds, cellulose and hemicellulose, which are less digestible (Hodgson, 1990). Reflecting the physical changes, the concentrations of more digestible fractions like soluble contents and protein decrease in the plant with ageing, whilst the concentration of fibre components (NDF, ADF and lignin) increase rapidly towards flowering, reducing the digestibility of the pasture (Table 2.2).

Several other studies have reported that an increase in pasture maturity reduces the protein and soluble carbohydrates components and increase cell wall contents (Cherney et al. 1993; Mambrini and Peryaud, 1994; Chaves et al. 2006). The most recent study showed that protein concentrations decreased from 23.7 g/100g DM in young material to only 5.0 g/100g DM in mature grass in spring time and while NDF fraction increased from 42.7 g/100g DM to more than 60.0 g/100g DM (Chaves et al., 2006). Similar results were found by Cherney et al. (1993) for NDF and protein concentrations when changes in the quality of five perennial grasses (tall fescue, foxtail,

timothy, reed canarygrass and meadow bromegrass) was measured after 42 days of re-growth.

Therefore the increase in the maturity of pasture can lead to a decrease in the physical (young leaves) and chemical components (eg. soluble carbohydrates, protein) that are highly digestible, reducing the nutritive value of the whole plant which becomes less digestible to the animals. In addition, the progressive decline in the pasture digestibility with maturity also generally decreases dry matter intake (Chaves et al., 2006). As shown above, plant maturity can have a large influence on the nutritive value of pasture, which is the main source of feed in this study, and therefore, of great relevance to the final results of the experiment realized in this study.

2.6.1.7. Differences in digestibility between sheep and cattle

The "ideal" situation for animal experiments is that the conditions and the animal species would be the same as the data obtained will be applied. However, most of the data obtained from "*in vivo*" digestibility experiments in ruminants were measured in sheep (O'Mara, 1999). Digestion studies with sheep are cheaper and simpler because sheep are small, easy to handle and the amounts of feed and excreta produced during the trials are smaller than for cattle (O'Mara et al. 1999, Woods et al. 1999). However, when digestibility data obtained with sheep are applied to cattle, differences in their digestive capacity must be considered (Colucci et al. 1989).

The difference in OMD between cattle and sheep was analysed, when several digestion studies involving different feeds were investigated (Cottyn et al., 1989). Maize silage was better digested by cattle than sheep, although the difference was small. For grass hay, grass silage and a mixed ration, quality was a very important characteristic, with sheep showing better digestion when the forage quality was higher, whereas cows showed better digestion when the forage quality was low. For moderate quality rations no difference in digestibility were noted between cattle or sheep. They also reported that sheep generally have a better protein digestibility than cows; however there was no difference between sheep and cow in the digestibility of protein in maize silage.

In a more recent study, differences between sheep and cattle in the digestibility of several concentrate feeds (barley, beet pulp, citrus pulp, maize gluten feed and grain screenings) fed at maintenance level were reported by O'Mara et al. (1999). They found differences between the two species only in the OMD of maize gluten feed, with cattle

having a better digestibility than sheep and concluded that according to these results, sheep do not have a better digestion of concentrates than cows, however there maybe differences in the protein and fibre digestibility.

Furthermore, no differences were found in the DMD, OMD, GE or ADF digestibilities between sheep and cattle when those animals were fed with seven different concentrates (copra meal, sunflower meal, gluten, soya hulls, palm kernel and soybean (Woods et al., 1999). However, these authors reported that cattle presented better digestion of the NDF fraction, while sheep showed a better digestion of CP, which is in agreement with other studies (Mertens and Ely, 1982; Cottyn et al., 1989).

The results of the correlation between cattle and sheep digestibility calculated from the analysis of several published studies by Mertens and Ely (1982) (Figure 2.9) showed that for low quality diets, cattle has better digestibility than sheep whereas sheep had higher digestibility than cattle when fed high quality diets. Lower metabolic losses and poorer ability to digest fibre components in sheep are the reasons for the difference of the digestive capacity between the two species (Van Soest, 1994).

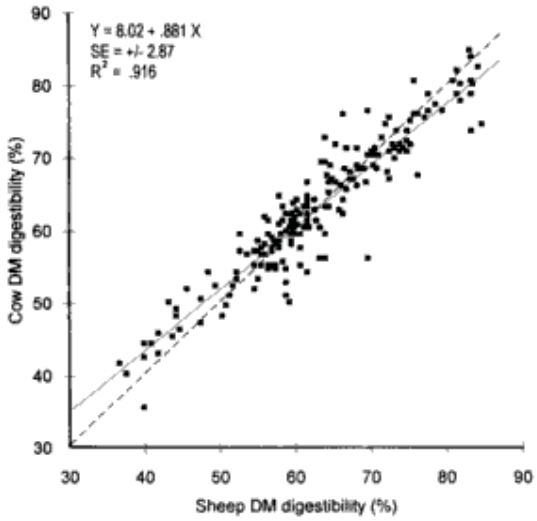


Figure 2.9. Relationship between dry matter digestibility of cattle and sheep fed the same diet. From Mertens and Ely (1982).

2.6.2. In vitro methods

In vitro fermentation techniques have been used since the 1940's, however, their use increased extensively with the advent of McDougall's buffer in 1948, which made it possible to enhance the length of *in vitro* incubations (Weiss, 1994). The utilization of

McDougall's buffer enables the system to be maintained at a pH around 6.7, which is ideal for the growth of cellulolytic microorganisms, and consequently the maximization of fibre digestion (Russell et al., 1979).

Since then, several different *in vitro* systems have been developed; however, all procedures followed a sequence consisted of an anaerobic fermentation of a sample substrate in the presence of a buffered rumen liquor, or sometimes enzymes, followed by an end point measurement (Van Soest, 1994; Weiss, 2004). *In vitro* systems present the possibility of analyzing both the residue after incubation and/or the metabolites of microbial digestion, such as gases. In this thesis the *in vitro* technique was used to measure the production of volatile fatty acids (VFA) and ammonia (NH_3) during the incubations. Due to the degree of complexity of the *in vitro* systems, this review will briefly describe and highlight some aspects of the most common methods.

2.6.2.1. Batch culture

This is the simplest and most commonly used *in vitro* fermentation system, and can be used to estimate digestibility or degradability in the rumen, VFA production and microbial synthesis when internal and external markers are used (López, 2005).

The single or two-stage method developed by Tilley and Terry (1963) is the method used most frequently to estimate DMD, and it has shown strong correlation between *in vivo* and *in vitro* digestibility results (Weiss, 1994; Kitessa et al., 1999). The first stage consists of the incubation of a finely ground sample for 48 h in a buffered rumen fluid, followed by another 48 h of digestion with pepsin in acid solution. Then, the insoluble residue is filtered, dried and combusted for the estimation of the OMD.

The microbial activity in the rumen fluid is a great source of variation in the *in vitro* method, and can vary due to the time of collection, species and diet of the donor animal, and variations between animal to animal within species (Kitessa et al., 1999). According to Weiss (1994) time of feeding should be kept constant, and he suggested an optimum time of collection around 8-12 h after feeding, which represents the time when fibrolytic enzymes activity peak. Another option to maintain good microbial activity in the rumen is to feed the animals several times a day to reduce variation due to rumen fluid collection (Weiss, 1994).

The effect of species of donor animal on the *in vitro* method has been inconsistent, with most of the results showing differences between animal species

(Horton et al., 1980; Waghorn and Stafford, 1993). Differences between animal species do not have large effects on the accuracy of the *in vitro* method, but the animal donor should be from same specie as the target animal (Weiss, 1994).

The diet fed to the donor animal has an impact on the microbial population and environment within the rumen, and consequently on the accuracy of the *in vitro* method. On the study of Calder et al. (1970) cited by Weiss (1994), he found that feeding donor animals with a relatively high concentrate diet (60:40 barley: alfafa hay) reduced *in vitro* DMD of alfalfa hay. Additionally, a greater cellulolytic fraction in the total bacterial population of cows receiving a high fibre diet compared with those receiving low fibre diets has been reported (Weimer et al.,1999).

Variations between animals within species may also decrease the precision of the method (Weiss, 1994). Differences between donor animals (sheep fed the same diet) in the activity of rumen fluid have been reported, and they affected significantly the *in vitro* organic matter disappearance (Ayres, 1991). Additionally, differences between individual cows in the microbial population of the rumen *inoculum* have been found to be larger than those related to the diet (Weimer et al., 1999).

A major disadvantage of the method is the requirement of fistulated animals to supply fresh rumen fluid, which entails having a donor animal (sheep or cattle) available at all times, and the costs (acquiring the animal, surgical cost, feed cost, labour to maintain the animal) and animal welfare issues involved with that (Weiss, 1994; Kitessa et al., 1999; Jones and Theodorou, 2000; Mohamed and Chaudhry, 2008). Those problems can be avoided by using enzymatic method, which uses cellulolytic enzymes as alternatives to rumen fluid (Jones and Theodorou, 2000). In addition, the long periods of time required to run the assay (Van Soest, 1994) and the fact that this method does not provide information regarding the degradation kinetics of the feeds incubated (Mould, 2003) are drawbacks of this method. These problems, however, can be overcome by using replicates sampled at different times to determine the rate of digestion (Getachew et al., 1998) and by shortening the pepsin digestion step when the residue after the *in vitro* fermentation is extracted with neutral-detergent (Weiss, 1994).

2.6.2.2. Continuous culture

Continuous culture systems have been developed to closely simulate ruminal fermentation conditions. Generally these systems are based on a regular addition of

buffer and nutrients, and a continual removal of fermentation products, until a steady state is established, which enables the microbial population to be maintained for long periods. The most commonly used continuous-culture systems are the dual-flow continuous-culture (Hoover et al., 1976) and the single-flow Rusitec (Czerkawski and Breckenridge, 1977). These systems can measure fermentation parameters, DM degradation, output of end-products and microbial protein synthesis, and provide digestibility values comparable with *in vivo* data (Calsamilglia et al., 2000). These systems have the ability to alter ruminal environment for study of conditions affecting microbial growth, which is a great advantage over batch culture systems (Michalet-Doreau and Ould-Bah, 1992). However, these systems are expensive and require some time to achieve the steady-state condition (López, 2005).

2.6.2.3. Products of fermentation

In vitro techniques can easily quantify the products of fermentation (eg. NH_3 and VFA) during the incubation of a substrate. The measurement of VFAs production during the fermentation process can be indicative of the nutritional value of feed tested, as VFA provides more than 50% of the digestible energy intake of the ruminants (France and Dijkstra, 2005). The predominant VFA in the rumen are acetic, propionic and butyric acids, with other minor acids (e.g. valerate, isobutyrate, caproate) present in relative small amounts. Propionic, acetic and butyric acids can all be used to generate adenosine triphosphate in the intermediary metabolism (Dijkstra, 1994), but they differ in their end uses and in their efficiencies of energy capture (Waghorn et al., 2007). Propionate is a major contributor of gluconeogenesis, whereas acetate and butyrate (converted to β-hydroxybutyrate) are lipogenic precursors (Harvatine et al., 2009). Additionally, the efficiency of energy capture from the oxidation of propionate is higher than from acetate or butyrate.

Another common use of the *in vitro* methods is to measure protein degradation by the concentration of NH_3 present during the *in vitro* incubation of a feed with rumen fluid (Broderick, 1978; Raab et al., 1983). The digestion of protein in ruminants starts with the degradation of dietary proteins by rumen microorganisms to amino acids and peptides, which are then degraded further to produce NH_3 and carbon “skeletons”, which can contribute to the VFA pool in the rumen (Waghorn et al., 2007). The majority of rumen bacteria use NH_3 as a sole N source for microbial synthesis (Russell,

1998). However, when ammonia is produced in excess, it is absorbed across the rumen wall and converted to urea for excretion (urine) or recycled to the rumen via saliva or directly from the blood (Pacheco and Waghorn, 2008).

The final NH_3 concentration in the batch culture incubations is the result of both protein degradation and NH_3 nitrogen utilization by ruminal microbes for growth, which can be as high as 10% of white clover N (Barrell et al., 2000). Moreover, the amount and nature of fermentable substrates affect ammonia utilization by rumen microbes, which can stimulate microbial NH_3 utilization to a greater extent than NH_3 release (Broderick, 1982; Burke et al., 2000). Nitrogen incorporation into bacterial protein during the incubations can be avoided either using an *in vitro* inhibitor procedure (Broderick, 1987), or using control samples with only rumen fluid during the incubations (Raab et al., 1983).

The measurements of NH_3 production will indicate relative ability to meet bacterial requirements of N; nonetheless, appropriate procedures need to be taken into account if net NH_3 release is measured. Additionally, parameters such as nature of the substrate, incubation time and buffer addition can affect the measurement of NH_3 during the incubation (Michalet-Doreau and Ould-Bah, 1992).

2.6.3. In sacco methods

The *in sacco* technique, also known as *in situ* incubation or the dacron bag technique, can measure the degradability of feedstuffs when undegradable porous bags containing a small amount of the feed is placed inside the rumen of a cannulated animal during a specified period of time (eg. 24h) (López, 2005). The *in sacco* technique has been used mainly to measure the rate and/or extent of ruminal digestion of different dietary constituents (Broderick and Cochran, 1999).

The *in sacco* technique has been shown to be a good predictor of forage digestibility and intake (Orskov, 2000), and is generally thought to be more accurate than *in vitro* methods (Kitessa et al. 1999; Gossilink, et al. 2004). It has also enhanced the understanding of N degradation in the rumen and has become the basis for describing nitrogen requirements of ruminants in several feed systems (eg. Cornell model). Despite its widespread use, the requirement for fistulated animals can limit its use as a routine method in commercial laboratories. In addition, information about the products of digestion cannot be obtained through the use of *in sacco* technique.

Lack of standardization in some methodological aspects of the technique seems to be a great source of variation of the *in sacco* technique. Several authors have extensively investigated the sources of variation in the results of this technique (Nocek, 1988; Michalet-Doureau and Ould-Bah, 1992; Vanzant et al. 1998; Broderick and Cochran, 2000; López, 2005; Mohamed and Chaudhry, 2008). An overview of the pertinent aspects that could affect the results obtained through the use of this technique is presented below.

2.6.3.1. Bag and sample characteristics

The bags used during incubation must be completely resistant to microbial degradation, and materials with this characteristic such as polyester, dacron and nylon are usually used today (López, 2005). However type of cloth, weave structure and pore size are some of the most important features of the bag. The use of bags with monofilamentous weave structure is recommended as it provides greater uniformity in pore size and a lower propensity to distort during incubation (Huntington and Givens, 1995). Also, the repeated reuse of bags can cause irregularities in the weave and, therefore should be avoided (Marinucci et al., 1992).

Pore size should allow free passage of fluids and microorganisms between the bag and rumen contents, but at the same time must prevent the loss of undigested material from the bag (Vanzant et al., 1998; Kitessa et al. 1999). Pores that are too small may be blocked during the incubation, causing accumulation of gas inside of the bags, acidification of the sample and result in bags floating above rumen contents (Huntington and Givens, 1995; Nozière and Michalet-Doreau, 2000). In addition, small pore size can potentially obstruct part of the microbial population present in the rumen content from reaching the sample inside of the bag, particularly the larger protozoans (Vanzant et al. 1998). Bags with pore sizes between 35-55 μm have been recommended (López, 2005).

The quantity of sample inside of the bag also affects the digestion kinetics. Bags should not be overfilled as this could result in a delay in the colonization of the bag and consequently cause an increase in the lag time and an underestimation of the sample degradability (Burke, 2004). However, the sample size must be large enough to provide sufficient residue for subsequent analysis (Nocek, 1988). The ratio of sample weight to bag surface area in the bag should be between 10-15 mg DM/cm^2 of bag surface area (Hvelplund and Weisbjerg, 2000).

Samples should be prepared in a way that facilitate handling, analysis, and reduce the particle size of the sample to a dimension similar to the particle distribution obtained after normal *in vivo* mastication and rumination processes (Broderick and Cochran, 2004; López, 2005). In addition, the preparation method must provide a homogeneous sample and not alter the composition of the feed (Burke, 2004). In most studies of digestion kinetics, samples were prepared by oven drying or freeze-drying prior to grinding through a specified screen size.

The literature describes many recommendations about the influence of particle size on *in sacco* degradation. Finely ground samples tend to have greater chances of mechanical losses from the bags, while materials with coarser particles tended to be associated with lower and more variable degradation rates (López, 2005). The establishment of the most suitable particle size for *in sacco* incubations is a very difficult subject, because of a significant interaction between particle size and feedstuff type (Michalet-Doureau and Ould-Bah, 1992).

Recommendations about the most adequate screen aperture have ranged from 1.5-3.0 mm for concentrate feeds and 1.5-5.0 mm for roughages (Vanzant at al. 1998; Broderick and Cochran, 2000), with variations between the type of feed. The idea of grinding roughages using a larger screen is to simulate the effect of chewing (López, 2005), while for concentrates the decrease of particle size during mastication is considered to be nil (Michalet-Doureau and Ould-Bah, 1992).

In the case of fresh feeds, such as grazed pasture, freeze drying and grinding or chopping into short lengths are not the best methods for achieving a particle distribution similar to that in chewed material (Waghorn and Caradus, 1994; Barrell et al., 2000). Forages have lignified structures that, unless ruptured, limited the access of bacteria and consequently the digestibility of cell walls (Burke, 2004). According to Barrel et al. (2000), no lag periods were found during the incubation of forages, and particle distribution was similar to that of chewed forage, when the forages were minced with a mechanized mincer, and concluded that mincing was a superior method to chopping or grinding.

2.6.3.2. Effects animals and their basal diet

Variations caused by differences between the host animals and their diets are one of the main sources of error in the *in sacco* procedure (Huntington and Givens,

1995; Kitessa et al., 1999). Research has shown that variation between animals is the largest source of variation (Mehrez and Orskov, 1977). Significant differences among cows were found in two studies where the *in sacco* disappearance of grains (Figroid et al., 1972) and white clover (Waghorn and Caradus, 1994) was measured. However, other reports found no significant variation between animals when the *in sacco* DM digestibility of cows was measured (Weakley et al., 1983; Nocek, 1985), and also that duration of incubation had a greater variation than differences between animals (Vanzant et al., 1996).

Despite this inconsistencies found in the literature most of the standard protocols suggested the use of 2-4 cannulated animals (Mehrez and Orskov, 1977; Nocek, 1988; Vanzant et al., 1998; Broderick and Cochran, 2000). However, according to Orskov (2000) if the purpose of the study is to rank the feed potential of forages, then only one animal is necessary for the incubations.

The basal diet fed to the host animal during *in sacco* incubations (as was also the case for the *inoculum* donor animal during *in vitro* incubations, 4.2.2) is another source of variation, and has a direct effect on the rate and extent of digestion of the dietary nutrients (Nocek, 1988). The diet composition has an influence on the rumen environment (Weimer et al., 1999), and consequently on the feed tested within synthetic bags as they are in intimate contact with the ruminal microbes (Vanzant et al., 1998). Generally, the increase in the concentrate to forage ratio fed to the host animal is associated with a decrease in the extent and rate of *in sacco* digestibility of forages (Weiss, 1994; Vanzant et al., 1998; Broderick and Cochran, 2000). Lindberg (1981) reported lower estimates of DM disappearance of forages and other feeds when those measurements were obtained from animals fed concentrate in their diets compared to measurements obtained in animals fed roughage-based diets only. Similar results were also obtained when soybean meal was incubated in the rumen of cows fed different hay to grain ratios (Weakley et al., 1983). The type of forage fed to the host animal can also have an effect on the rate and extent of *in sacco* disappearance, but the results are inconsistent (Weiss, 1994).

A standard recommendation is that *in sacco* trials should offer a basal diet composed of a mixed diet (e.g. 60% roughage and 40% concentrate) that meets animal's requirements, and should be fed at maintenance level during some time prior to the experiment (Broderick and Cochran, 2000).

2.6.3.3. Rumen pre and post-incubation

Some aspects of the pre and post-incubation routines that have been studied and shown to have some effect on the degradability results during the incubation process will be discussed below.

The rumen environment has a significant diurnal variation in its digestive activity, especially in animals fed only a few times per day (López, 2005). Thus, when the bags are incubated in the rumen relative to the time when the animal is fed can have an effect on the degradation rates of the feeds within the bags. Therefore, host animals should be fed and bags should be introduced into the rumen at constant times (Michalet-Doreau and Ould-Bah, 1992).

When bags are placed into the rumen they should be in contact with the liquid phase of the rumen contents, and squeezed during the muscular contractions to facilitate the exchange of the rumen liquor into and out of the bags (López, 2005). To assist the placement of the bags inside of the rumen a carrier weight is usually attached to a cord. The length of the cord is an important factor in the determination of the bag's position in the rumen (Kitessa et al., 1999), and the ratio of rumen depth to cord length (close to 1) is a useful guide (Huntington and Givens, 1995).

Rinsing *in sacco* bags with cold water after incubation is intended to stop microbial activity, and at the same time to remove the presence of any remaining rumen digesta and microbial matter (Huntington and Givens, 1995; Broderick and Cochran, 2000). But the washing process must also be careful to minimise DM loss due to the physical process of washing and squeezing/spinning (Kitessa et al., 1999). Several processes have been used to rinse bags after incubation, however, hand rising and machine rising (commercial washing machine) are the most common. Hand-rinsing consist of rinsing the bags under running cold water until the water is clear, with gentle manipulation of the bag. This method can be subjective (Weiss, 1994) and labour intense, therefore, the use of a washing machine, for a washing time of 10-15 minutes was recommended by Hvelplund and Weisbjerg (2000). Cherney et al. (1990) compared hand rinsing to machine rinsing and concluded that machine rinsing twice for two minutes and hand rising *in sacco* bags presented similar standard error (SE), however, machine rinsing twice for five minutes resulted in extreme DM losses.

2.6.3.4. Microbial contamination

During the ruminal *in sacco* incubation, bags containing the test feed are colonized by microbes, which must be removed by the washing procedure after incubation. However, despite washing, the bag residue can still contain some microbes adhered to the remaining particles, which can cause the underestimation of the extent and rate of digestion of the test feed. This contamination of the residues will have a small impact on the DM degradation, but as microbes contain high protein content, protein degradation can be underestimated (Hvelplund and Weisbjerg, 2000). The degree of underestimation due to this microbial contamination is variable, with smaller effects on the measurements with concentrates (Nocek, 1988) and greater underestimation in forages with low nitrogen contents (Michalet-Doreau and Ould-Bah, 1992). In a study where the microbial contamination of fourteen feeds using the *in sacco* method was determined, the concentrates evaluated had lower microbial contamination of the residual nitrogen (varied from 2.8% to approximately 25%) than forage, and cell wall-rich by-products (varied from approximately 6 to 81%) after 24 h in the rumen, with the highest numbers being found for barley straw, lentil straw and vetch-oat hay (Rodriguez and Gonzalez, 2006).

These effects of microbial contamination can influence the calculation of degradation kinetics, with reductions in lag times (Nocek and Grant, 1987) and an increase in the non-soluble degradable fraction and its degradation rate (Rodriguez and Gonzalez, 2006). A marker technique can be used to measure the bacterial contamination; however, the results may vary (Noziere and Michalet-Doreau, 2000). Additionally, the estimation of microbial contamination can be a laborious, and expensive procedure (Rodriguez and Gonzalez, 2006), and if the inappropriate marker is used, the correction can incur a greater error than failure to not correct for microbial contamination (Broderick and Merchen, 1992).

2.6.3.5. Modelling

Usually, the estimation of rumen degradation parameters is obtained by fitting the data obtained from the incubation of bags into the rumen over time into curves using mathematical models. López et al. (1999) reviewed the different mathematical models used to determine the ruminal degradation kinetics of feeds. The most common

approach used to describe degradation kinetics of incubated feeds is by the use of a first-order kinetics model (Orskov and McDonald, 1979) which includes a soluble fraction (A) in the rumen, a slowly degradable fraction (B) and an undegradable fraction (U). The model can also quantify the rate of digestion of the "B" fraction (k) and predict the potential degradability of the feed (PD) (Equation 2.4).

$$PD = A + B\,(1 - e^{-kt})\,,\ t \geq 0 \qquad \text{(Equation 2.4)}$$

McDonald (1981) revised the model and introduced a lag phase, which is the time (t) that microbes take to adhere to the substrate at the beginning of the incubation, when little or no digestion occurs (Equation 2.5, Figure 2.10). Lag periods are partly due to the delays, until microbial growth is sufficient to degrade the substrate inside of the rumen (López, 2005). Moreover, rate of hydration of the substrate within the bag and nutrient limitations also influence the lag period of the feed tested.

$$PD = A + B\,(1 - e^{-k(t-lag)})\,,\ t \geq 0 \qquad \text{(Equation 2.5)}$$

The use of the lag parameter within the McDonald model has been demonstrated to be advantageous when analysing feeds with low degradability and significant lag time (Dhanoa, 1988). Also in another study describing the N disappearance of aeschynomene hay and alfafa meal the introduction of the lag phase resulted in an improvement of the goodness-of-fit and the reduction of the residual standard deviation of the model (Denhan et al., 1989). The lag time is dependent on the nutrient losses during the first hours of incubation, and consequently the sample preparation and incubation procedure having a direct effect on it; therefore accurate and repeatable procedures are essential. The lag phase will be used in all digestion kinetics determinations described in this thesis.

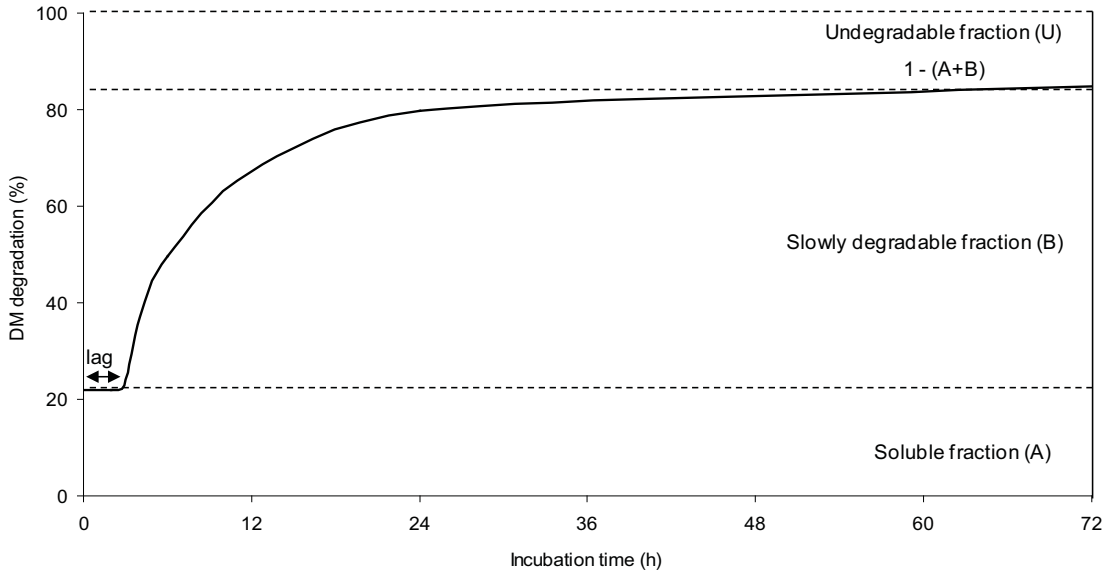


Figure 2.10. Degradation curve of typical roughage with a small lag phase. Adapted from Orskov (2000).

The combination of the *in sacco* degradation characteristics with the effect of rumen outflow rate to calculate the extent of ruminal degradation of the feeds, also known as effective degradability (ED), has been proposed by Orskov and McDonald (1979).

Generally, the ED is calculated without the lag period (Equation 2.6), however, the calculation of ED with the lag phase can be found in the literature (Sinclair et al., 1993) (Equation 2.7).

$$ED = A+((Bk)/k+kp))(1-e^{-(k+ kp)t}) \quad \text{(Equation 2.6)}$$

$$ED = A+((Bk)/k+kp))(1-e^{-(k+ kp)(t-lag)})(e^{-klag}) \quad \text{(Equation 2.7)}$$

Where A, B and k are defined in Equation 2.4 and kp is fractional outflow rate (%/h).

Fractional outflow rates (kp) of 5 and 6%/h have been used in several studies to evaluate either fresh forages (Hoffman et al., 1993; Kolver et al., 1998; Elizalde et al., 1999) or concentrates (Woods et al., 2003a). Additionally, outflow rates of 5 to 6 %/h

for cows with feeding levels twice the maintenance have been reported (AFRC, 1993). The ED calculations in this thesis will be done assuming an outflow rate of 5 and 6%/h for the feeds tested.

2.8. Conclusion

Grazing systems are affected by feed quality and availability, which consequently affects animal performance. Normally, supplements are used as a tool to either to improve overall animal intake and milk production, or to maintain performance during periods short pasture deficits. Supplements are more expensive than pasture, and the use of alternative feeds has gain importance lately. In recent years dairy farmers in New Zealand have fed increasing quantities of PKE, but with little supporting evidence for grazing cows. Overseas results about the nutritional value of PKE have shown that PKE is a unique feed with a great variability in chemical composition. Those characteristics can have a significant impact on animal performance when mixed with pasture. This thesis will study beyond the chemical composition of PKE, and will quantify the digestion and fermentation changes when PKE is incubated alone or mixed with pasture in different proportions, using *in vitro*, *in sacco* and *in vivo* methods, and also the impact on animal performance when PKE is fed to grazing dairy cows.

CHAPTER 3:
Digestion kinetics of palm kernel expeller measure by the *in sacco* method.

Partial results of this study have been published: Dias, F.N., J.L. Burke, D. Pacheco, and C. W. Holmes. 2008. *In sacco* digestion kinetics of palm kernel expeller (PKE). Proceedings of the New Zealand Grassland Association 70:259-264.

Abstract

Palm kernel expeller (PKE) is imported into New Zealand and fed to ruminants grazing pasture, but very little is known about its rumen digestion characteristics when fed in conjunction with pasture. The objective of this study was to use the *in sacco* method to determine the digestion kinetics of PKE, with and without pasture, and to compare these with those of pasture (~ 80% ryegrass and 20% white clover). In experiment 1, Dacron bags containing pasture, PKE or a 50:50 mixture of the two (on a dry matter basis; P+PKE) were incubated in two fistulated cows previously fed lucerne chaffage + PKE. In experiment 2, bags containing either pasture, or PKE only were incubated in the same two cows after being fed only lucerne chaffage. In both experiments the loss of dry matter, crude protein, neutral detergent fibre and acid detergent fibre were determined over 72 h. The results for experiment 1 and 2 showed that PKE contains significantly lower dry matter and crude protein soluble fraction (24.8% and 37.8%, respectively) than pasture (41.4% and 52.3%), but that PKE contains significant more slowly degradable protein fraction (61.4% and 57.5%) than pasture (54.7% and 45.9%). Most of the fibre was present in the slowly degradable fraction, with values above 58% and 51% for neutral detergent fibre and acid detergent fibre, respectively for all feeds. The degradation characteristics of the P+PKE mixture were generally intermediate to those of the two feeds alone. The lag phases of pasture, PKE and the P+PKE were all different from each other, and the diet fed to the host cow had a significant effect on the dry matter, crude protein and fibre degradation. The rate of degradation for dry matter and fibre fractions did not differ significantly between feeds in either experiment, but the rate of degradation for CP was significantly slower for PKE than pasture and

P+PKE, in both experiments. Overall, there were differences between pasture and PKE in the soluble and insoluble fractions; however their degradation rates were similar, except for crude protein. The addition of PKE with pasture reduced the rate of protein degradation for the mix P+PKE, which may result in a reduction in the excretion of nitrogen. Additionally, the differences in the lag phase, due to the type of diet provided to the cows, suggesting that adaptation to PKE is necessary to achieve its potential as a supplement for grazing cows.

Keywords: *in sacco*, palm kernel expeller, pasture, rumen, kinetics

3.1. Introduction

Dairy systems in New Zealand are based on grazed pastures; however during the last decade farmers have been looking for new feeds to supplement dairy cows in pasture based-system due to increases in genetic potential for milk yield, larger farms, higher land prices and increased operating costs (Clark et al., 2007).

During the last five years, there has been a rapid increase in the use of a by-product from the palm oil industry called palm kernel expeller (PKE) as a source of supplementary feed for ruminants. It has been used widely on dairy farms to reduce the feed deficits, because of its relatively low price compared with other supplements.

Despite its growing importance as a feed for ruminants in New Zealand, very little information exists about the nutritional value of PKE. The few scientific reports about PKE have shown that its chemical composition can vary significantly, with the DM reported to contain between 158 and 194 g/kg of CP, 685 and 801 g/kg of NDF and 78 and 128 g/kg of fat (Moss and Givens 1994; Hindle et al. 1995; O'Mara et al. 1999). These authors also reported that PKE OMD ranges between 650 to 750 g/kg DM when measured either *in vitro* or *in vivo*. However, there is some evidence that the rate of degradation in the rumen of the main PKE components, fibre and protein, is very slow (Hindle et al. 1995; Woods et al. 2003b).

Palm kernel expeller differs significantly from other supplements commonly used in New Zealand farms mainly because of its unique chemical composition; therefore a better understanding of its digestion in the rumen is required in order to make informed decisions about its inclusion as a supplement for dairy cows grazing temperate pastures. A variety of indirect techniques (e.g. *in sacco*, *in vitro*) have been

developed to measure the nutritive values of feeds, as the conventional method (*in vivo*) is expensive and labour intensive. The *in sacco* technique provides information about the kinetics of digestion and extent of ruminal degradation which is one important measurement in the case of such a unique feed as PKE.

Firstly, the objective of this study was to define the digestion kinetics of pasture and PKE when incubated alone or combined. We hypothesized that PKE would present lower rumen degradation values (soluble, A, and slowly degradable fractions, B, and degradation rate, k) than pasture, and the mix of the two feeds would decrease the soluble fraction and disappearance rate of pasture CP. The second objective was to verify the effect of PKE in the diet of the host animal on the digestion kinetics of PKE and pasture. The diet composition fed to the host animal has an influence on the rumen environment (Weimer et al., 1999) and consequently the digestion kinetics of the feeds incubated. Therefore, we further hypothesized that the presence of PKE would not affect pasture degradation in the rumen but would increase the degradation values of PKE.

3.2. Material and methods

The rumen digestion characteristics of PKE alone or in combination with pasture were measured using an *in sacco* technique. The pasture was composed of ryegrass (*Lolium perenne*; 80%) and white clover (*Trifolium repens*; 20%).

3.2.1. Feed collection and analyses

In all experiments, fresh spring pasture samples were cut in the morning with clippers to approximately 5 cm above the ground and immediately stored in a freezer (-20°C) until their preparation for incubation according to the methods described by Burke et al. (2000). The frozen pasture samples were first chopped and then minced to achieve a particle size distribution similar to that of pasture chewed by a ruminant (Waghorn et al., 1989).

The PKE samples used in each experiment were collected from a commercial feed mill (Premier Stock Feeds Limited, NZ) and stored in a dry place until incubation. The PKE and the frozen minced pasture samples were weighed (to provide a similar dry

weight) and sealed into Dacron bags for the *in sacco* incubation with each bag containing 5 or 6 grams (g) DM of the feed.

The DM content of the pasture and PKE samples were determined by drying the samples in a forced-air oven at 65°C for 48 h. Samples of the minced pasture were analysed for chemical composition by NIRS (FeedTech, Palmerston North). PKE samples were ground (1mm) and analysed by wet chemistry, due to the lack of a reliable calibration curve from NIRS, for ash (942.05), CP (968.06), fat (991.36) and starch (996.11) concentration (AOAC, 2005). Fibre content (NDF and ADF) were determined as described by Robertson and Van Soest (1981).

3.2.2. Host cows used for in sacco incubations

Two non-lactating Holstein-Friesian cows fitted with rumen cannulae were used as hosts for the *in sacco* incubations. In experiment 1, the host cows were fed a diet composed of lucerne chaffage (Fibre Fresh Feeds of New Zealand) and PKE (Premier Stock Feeds Limited– NZ). In experiment 2, the host cows were fed lucerne chaffage only (see Figure 3.1), to determine the digestion kinetics of PKE and pasture when the basal diet of the host animal does not contain PKE. In both experiments, the host cows were fed 24 hours prior to the incubations at maintenance level, with half of the diet being offered before 0900h and the other half offered after 1700h. Water was available *ad libitum*.

3.2.3. In sacco incubations

An overview of the *in sacco* experiments are presented below (Figure 3.1).

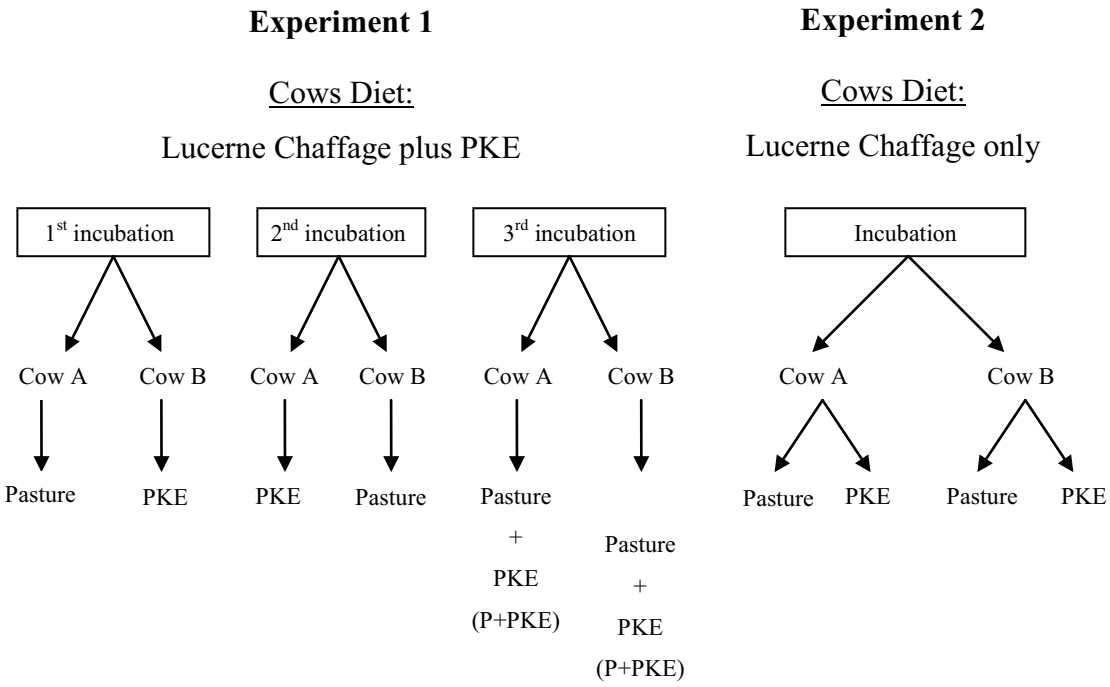


Figure 3.1. Description of the treatments evaluated in *in sacco* experiments.

In experiment 1, the cows received a diet composed of 75% lucerne chaffage and 25% PKE (on a fresh weight basis). Dacron bags (100 x 100 mm, 50µm pore size), each containing approximately 5 g DM of either pasture alone, PKE alone or a 50:50 mix (DM basis) of pasture plus PKE were incubated in three incubations that started every 48 h and last 7 days. In the first incubation, Dacron bags containing only pasture were incubated in Cow A whilst Dacron bags containing only PKE were incubated in Cow B, and this allocation was reversed between the cows during the second incubation. In the third incubation Dacron bags containing P+PKE were incubated in both cows. Triplicate bags of each feed were placed into the rumen and removed after 2, 4, 6, 8, 12, 24 and 72 h of incubation, in a total of 27 bags in each cow, including the standard. Lingerie bags were used for each set of seven bags to facilitate the removal of bags from the rumen, with one sample bag removed at each sampling time from each lingerie bag. After removal from the rumen, Dacron bags containing feed residues were hand-washed thoroughly with cold tap water until the rinse water was clear.

In experiment 2, cows received a diet composed of lucerne chaffage only. Dacron bags (100 x 100 mm, 50µm pore size) containing approximately 5 g DM of pasture or 6 g dry weight of PKE (on a DM basis) were incubated at the same time in both cows. In this experiment both feeds were incubated at the same time, and therefore, only duplicate bags of each feed were used, due to the limited number of bags that a single rumen can contain without affecting normal rumen activity and digestion (cannot exceed 30 bags). A total of 26 bags per cow were incubated. One lingerie bag containing 10 bags was used for each feed incubated, with two samples from each bag being removed after 2, 6, 12, 24 and 72 h. After that they were hand-washed as described above.

3.2.4. Assessment of repeatability and contamination

Soybean meal (SBM) was used as a standard feed to measure differences between cows and between incubations, and the bags were incubated in each cow and in each incubation and removed at 2, 6, 12 and 24 h. Three bags of each feed (0-h samples) were also hand-washed without incubation in the rumen.

Three blank Dacron bags were placed into the rumen of each cow during the first two incubations of experiment 1 and removed after 24 h to measure contamination by inflow of rumen DM into bags. There was no accumulation of DM into the bags.

Following removal from the rumen and after washing, all bags were dried in a forced–air oven at 65°C for 48 h and weighed to determine residual DM in the bags. The pasture residues were analysed for CP, NDF and ADF concentration by NIRS, while the PKE and P+PKE residues were analysed for CP (968.06, AOAC 2005), NDF and ADF (Robertson and Van Soest 1981) content using wet chemistry. The amount (g) of nutrients (CP, NDF and ADF) in the residues was calculated by multiplying the dry weight of each bag by the nutrient concentration of the feed residues. Residual amounts were expressed as a percentage of the initial weight of DM and nutrients incubated in each bag, and the degradation was calculated as 100 minus the residual percentage of DM, CP, NDF, and ADF.

3.2.5. Digestion kinetics

The ruminal kinetics of DM, CP, and fibre disappearance of each feed in each cow were estimated by fitting curves to the DM, CP, NDF, and ADF disappearance data using a non-linear least-square procedure (PROC NLIN; SAS, 2003). The curve selected for parameter estimation was the exponential decay curve with or without lag described by Lopez et al. (1999), which defines the PD (potential degradability) of the feeds at time t according to:

$$\text{PD DM, CP, NDF and ADF} = A + B\,(1-e^{-k\,(t-lag)}) \qquad \text{(Equation 3.1)}$$

where A is the measured soluble fraction (% of DM, CP, NDF, or ADF) from the 0-h hand-washed samples; B is the slowly degradable fraction (% of DM, CP, NDF and ADF); k is the fractional disappearance rate (%/h) of B; t is the time of incubation (hours) and the lag phase (hours) is the period of time during which no digestion occurs or digestion occurs at a slow rate (McDonald, 1981). For the no-lag models, lag=0.

Effective degradability (ED) was calculated according to Sinclair et al. (1993):

$$ED = A+((Bk)/k+kp))(1-e^{-(k+\,kp)(t-lag)})(e^{-kp*lag})) \qquad \text{(Equation 3.2)}$$

where A, B, k and lag are the parameters estimated from fitting Equation 3.1, and kp is the outflow rate.

In this experiment, ED of DM, CP, NDF and ADF were calculated using assumed rumen outflow rates of 5 and 6%/h kp, which approximate to the outflow rates in cows eating fresh forages (Hoffman et al., 1993; Kolver et al., 1998; Elizalde et al., 1999) or concentrates (Woods et al., 2003a), respectively. Also, the AFRC (1993) has reported outflow rates of 5 to 6 %/h for cows with feeding levels twice maintenance, which is equivalent to cows producing less than 15 litres (L) milk/day. This experiment was designed to simulate cows in late lactation, with production of 15 L milk/day. Effective rumen degradability of CP was calculated using outflow rates of 3, 6, 8%/h,

which are close to the outflow rates of dry cows, cows producing up to 15 L milk/day and cows producing more than 15 L milk/day (AFRC, 1993).

The metabolisable protein system (AFRC, 1993), was used to calculate protein degradability parameters:

- Quickly degradable protein (QDP, g/100g DM) = A * [CP]; where [CP] is the CP concentration (g CP/100g DM).
(Equation 3.3).

- Slowly degradable protein (SDP, g/100g DM) = [(B * k) / (k + kp)] * CP.
(Equation 3.4).

- Effective rumen degradable protein (ERDP, g /100g DM) = 0.8*QDP + SDP.
(Equation 3.5).

- Rumen degradable protein (RDP, g /100g DM) = QDP + SDP.
(Equation 3.6).

- Rumen undegradable protein (RUP, g /100g DM) = [CP] – RDP.
(Equation 3.7).

3.2.6. Statistical analyses

Kinetic parameters of DM, CP, NDF and ADF disappearance over time from *in sacco* incubations were predicted by fitting data from the bag residues to a non-linear equation (Orskov and McDonald, 1979) using a non-linear least square procedure (PROC NLIN; SAS, 2003) and were expressed as degradation curves. To evaluate the fit of each model (with or without a lag phase) to the DM, CP, NDF and ADF disappearance data measured during the incubations the root mean square prediction error (RMSPE), Bayesian information criteria (BIC) and Akaike's information criteria (AIC) were calculated and in all cases, the model including lag provided a better fit (smaller RMSPE, BIC and AIC values) to the measured disappearance data and therefore these analyses are presented here.

Recent literature (Hackman et al., 2008) suggests that fibre values should have a soluble fraction, A, that is 0. This assumption was applied to the data in this thesis

using the kinetic models described in Hackman et al. (2008), however based on statistical measures (AIC, BIC and RMSPE) the Orskov and McDonald (1979) kinetic model was considered more appropriate to derive degradation parameters and has been used here.

The parameters of the non-linear curve fitted for each feed were obtained using the observations from both cows (Table 3.2). The coefficient of determination for all fitted curves was greater than 0.97.

Variation between cows during incubations were assessed for SBM using the mixed procedure of SAS (Proc Mixed; SAS, 2003), with cow, incubation number and their interaction considered as fixed effects (see Appendix 3.1). Differences between cow, incubation number and their interactions were determined using the LSMeans statement with the PDIFF option and considered significant at $P<0.05$.

The ruminal disappearance characteristics of DM, CP, NDF and ADF in pasture, PKE and P+PKE were analysed using the Mixed model procedure of SAS (SAS, 2003) with cow considered as a random effect, and feed, diet and their interaction as a fixed effect. Differences between the feed, diet and their interactions were determined using the LSMeans statement with the PDIFF option and considered significant at $P<0.05$

The Mixed model procedure of SAS (SAS, 2003) was also used for the analysis of the CP degradability fractions of pasture, PKE and P+PKE, with feed, diet and their interactions considered as a fixed effect and cow as a random effect. Differences between the feed, diet and their interactions were determined using the LSMeans statement with the PDIFF option and considered significant at $P<0.05$.

3.3. Results

3.3.1. Chemical composition of feeds

The chemical composition of pasture and PKE are shown in Table 3.1. Pasture had a greater concentration of CP, soluble sugar and starch (SSS) and ash content, whereas PKE had a greater concentration of NDF, ADF and fat. The chemical composition of P+PKE used in the *in sacco* incubations was estimated as the arithmetic mean of the composition from pasture and PKE.

Table 3.1. Dry matter content and chemical composition (± standard deviation) of pasture, palm kernel expeller (PKE) and their mix (P+PKE; 50:50 on a DM basis) used during the *in sacco* incubations.

Item	Pasture[1]	PKE[2]	P+PKE[3]
Dry matter (%)	13.7	97.2	-
	---------- g/100 g DM ----------		
Crude protein	24.0 ± 0.65	16.0 ± 0.76	20.7
Neutral detergent fibre	51.9 ± 2.47	70.3 ± 4.78	63.8
Acid detergent fibre	25.9 ± 0.57	39.3 ± 2.24	33.6
Fat	4.0 ± 0.06	9.0 ± 0.52	6.9
Soluble sugars and starch	3.0 ± 1.02	0.3 ± 0.02[4]	-
Ash	10.8 ± 0.02	4.1 ± 0.67	7.6
Metabolic energy (MJ/kg DM)	11.4 ± 0.30		

[1] Analysed by NIRS
[2] Analysed by wet chemistry
[3] Calcuated as arithmetic mean of pasture and PKE
[4] Starch content only.

3.3.2. Assessment of repeatability between incubations

The results for DM loss of SBM (the standard feed) at each time showed no significant differences between incubations, and the rumen pH values were similar between incubations in experiment 1, but slightly higher in experiment 2, with average pH values around 7.0 (see Appendix 3.1). Furthermore, differences between the two cows used in all incubations of experiment 1 and 2 were not significant. A sample of the rumen fluid was taken from the cows at the beginning of each incubation and rumen parameters measured for the first three incubations of experiment 1. Rumen pH showed a stable rumen environment (low variation in the normal pH) between incubations with an average rumen NH_3 concentration value of 24.6 ±0.31 mmol/L, and total VFA concentration ranging from 98.2 to 116.5 mmol/L (Appendix 3.1). Based on these results no adjustment was made for alterations between experiments and with incubations at each experiment.

3.3.3. In sacco digestion kinetics

The DM, CP, NDF and ADF digestion fractions, degradation rates and effective degradability with lag times obtained in experiment 1 and 2 are presented in Tables 3.2 and 3.3, and Figures 3.2 and 3.3, respectively.

3.3.4. DM digestion kinetics of pasture and PKE

Table 3.2 presents kinetic results for *in sacco* digestion of pasture, PKE, and their mix when incubated in cows fed lucerne chaffage plus PKE (experiment 1), and the digestion kinetics of pasture and PKE when cows were fed only lucerne chaffage (experiment 2). The *in sacco* DM disappearance data for both experiments are illustrated in Figure 3.2a. The percentage of DM released during hand washing of non-incubated samples (i.e. soluble DM; fraction A) was significantly greater for pasture (41%) than for PKE (25 and 27%) in both experiments ($P<0.01$). When PKE was incubated with pasture in experiment 1 the soluble fraction increased considerably from 25 for PKE to 39%, but this value was still significantly lower than that of pasture ($P<0.01$). When only PKE was incubated there was very little DM disappearance during the first 5-9 h (Figure 3.2a) and the lag was greater in host cows that were fed only lucerne chaffage in experiment 2 ($P<0.01$). Combining pasture with PKE reduced the lag time from 5 h for PKE to 2.9 h for the mix, however this value was still significantly lower than the lag time of pasture ($P<0.01$). In experiment 1, pasture, PKE, and P+PKE had very similar degradation rates, with no significant differences between their values (around 12%/h, $P<0.53$). However, in experiment 2 when the host cows were fed only lucerne chaffage, the degradation rates were slower for PKE (10%/h) and faster for pasture (16%/h), but this difference was not significant.

After 72 h of incubation the U (undegradable fraction) value of the DM was much higher for PKE than for pasture. Between 13 and 14% of the PKE DM was undigested in the rumen, independent of the diet fed to the cows (experiment 1 vs. 2).

The ED for DM, which takes into account the effects of A and B pools, passage rate, assumed rumen outflow rate (5 and 6%/h) and lag time, showed greater values for pasture (79%) and 2) than for PKE (55%) in both experiments. The P+PKE mix presented intermediate values, between pasture and PKE. The different diets fed to the host cows (experiment 1 vs. 2) had a small effect on the ED of pasture; however, for

PKE the ED increased by more than 12% units in the host cows that were fed a diet containing PKE. Effective degradability was reduced by 3 to 7 % when the assumed rumen outflow rate was increased from 5 to 6%/h, respectively. This is relevant because the actual outflow rate of PKE from the rumen may be different from that of pasture.

3.3.5. CP digestion kinetics of pasture and PKE

The *in sacco* CP disappearance data are illustrated in Figure 3.2b and the estimated CP fractions and degradation parameters for experiment 1 and 2 are summarised in Tables 3.2 and 3.4. In both experiments 52% of the CP fraction of pasture was soluble and released from the bags, while PKE had approximately 37% of soluble CP, a difference that was significant in both experiments. The soluble fraction for P+PKE was intermediate between those of the two feeds, and significantly greater than that of PKE. The slowly degradable fraction of CP was significantly faster in PKE than pasture ($P<0.05$), with B values for P+PKE being intermediate. The diet fed to the host cows had no effect on the A and B fractions of either pasture or PKE.

The disappearance of CP from PKE was delayed for 8 and 11 h in experiment 1 and 2, respectively, but only 1 and 1.6 h for pasture and P+PKE, which were similar to the patterns of DM disappearance. In both experiments, the rates of CP degradation in pasture were about three and four times faster than those for DM degradation, respectively, whereas for PKE the rates of CP degradation were slower than those for DM degradation. Degradation rates of CP were significantly slower in PKE than pasture (7-10%/h vs. 34-48%/h) in both experiments ($P<0.05$).

Effective rumen degradability of CP and its different fractions were calculated for all feeds at three different assumed outflow rates (3, 6 and 8%/h) (Table 3.3). The ERDP of PKE ranged from 91 to 99 and 113 to 118 g/kg DM in experiment 1 and experiment 2, respectively, with significant differences between pasture and PKE in both experiments ($P<0.01$). The ERDP of pasture was almost twice that of PKE for all outflow rates in both experiments, but the differences were larger at the fastest assumed outflow rate of 8%/h. In both experiments, an increase in the assumed outflow rate from 3 to 8%/h caused a greater reduction in ERDP of PKE (18%) than pasture (5%). The ERDP of P+PKE was intermediate between those of pasture and PKE. Overall, the diet fed to the host cows had no effect on the ERDP of either pasture or PKE despite the numerical differences found in this study.

Rumen degradable protein can be calculated from Table 3.3 as the sum of quickly degradable protein plus slowly degradable protein. For PKE, at an assumed outflow rate of 6%/h, RDP averaged 117 and 109 g/kg DM (0.73 and 0.68 of total CP) in experiment 1 and 2, respectively, and there were significantly lower than those from pasture ($P<0.01$) that averaged 218 and 226 g/kg DM. When pasture was incubated with PKE in experiment 1 the RDP was intermediate between those of the two feeds.

Table 3.2. Mean values for dry matter (DM) and crude protein (CP) degradation characteristics of pasture, palm kernel expeller (PKE) and pasture plus PKE (P+PKE) defined by the soluble fraction (A), slowly degradable fraction (B), fractional disappearance rate (k), lag phase, undegradable residue (U=100-A-B), potential degradability (PD) and effective degradability (ED), which takes into account the effect of the assumed rumen outflow rate (kp).

Feeds incubated	Parameters							
	A (%)	k (%/h)	B (%)	lag (h)	U (%)	PD (%)	ED[1] (%)	ED[2] (%)
				DM kinetics				
Experiment 1- cows fed Lucerne chaffage with PKE								
Pasture	41.4	12.2	54.7	0.9	3.9	96.1	78.3	76.0
PKE	24.8	13.4	61.4	5.0	13.7	86.2	58.7	55.4
P+ PKE	38.8	11.6	53.1	2.9	8.1	91.9	70.6	67.9
Experiment 2 - cows fed Lucerne chaffage only								
Pasture	41.4	15.7	54.4	1.4	4.2	95.8	79.7	77.4
PKE	26.8	10.2	60.5	9.3	12.6	87.3	52.3	48.6
Standard Error	0.40	2.43	0.79	0.10	-	-	-	-
Feed effect	**	ns	**	**	-	-	-	-
Cow Diet effect	ns	ns	ns	**	-	-	-	-
Feed x Cow Diet effect	ns	ns	ns	**	-	-	-	-
				CP kinetics				
Experiment 1- cows fed Lucerne chaffage with PKE								
Pasture	52.3	33.8	45.9	1.0	1.8	98.2	90.0	88.6
PKE	37.8	9.8	57.5	7.7	4.7	95.1	64.0	60.6
P + PKE	45.4	10.8	52.3	1.1	2.3	97.7	79.2	76.9
Experiment 2- Cows fed Lucerne chaffage only								
Pasture	52.3	48.4	47.0	1.6	0.7	99.3	91.7	90.4
PKE	36.6	6.7	60.0	11.2	3.4	95.6	56.2	52.8
Standard Error	1.30	4.46	1.25	0.91	-	-	-	-
Feed effect	***	***	***	***	-	-	-	-
Cow Diet effect	ns	ns	ns	*	-	-	-	-
Feed x Cow Diet effect	ns	ns	ns	ns	-	-	-	-

[1, 2] $ED = a+((Bk)/k+kp))(1-e^{-(k+ kp)(t-lag)})(e^{-kp*lag})$.

[1] kp= assumed rumen outflow rate (kp = 0.05 h-1; 2 kp= 0.06 h-1)

Coefficient of determination for all fitted curves was > 97%.

*** P<0.01 and ** P<0.05, * P<0.1. ns, not significant.

Table 3.3. Effect of assumed rumen outflow rate per hour (kp) on the rumen crude protein (CP) degradability fractions of pasture, palm kernel expeller (PKE) and their mix (P+PKE) expressed in terms of quickly degradable protein (QDP), slowly degradable protein (SDP), effective rumen degradable protein (ERDP), and rumen undegradable protein (RUP) for both experiments

			kp = 3%/h			kp = 6%/h			K = 8%/h		
Feeds incubated	CP	QDP	SDP	ERDP	RUP	SDP	ERDP	RUP	SDP	ERDP	RUP
	g/Kg DM	g/kg DM	g/kg DM			g/kg DM			g/kg DM		
Experiment 1: Cows fed Lucerne chaffage + PKE											
Pasture	240	125.6	100.6	201.0	13.8	92.6	193.0	21.8	88.0	188.4	26.5
PKE	160	60.5	70.1	118.5	29.4	56.7	105.1	42.7	50.4	98.8	49.1
P + PKE	205	93.1	83.8	158.3	28.1	68.8	143.3	43.1	61.5	136.0	50.4
Experiment 2: Cows fed Lucerne chaffage only											
Pasture	240	125.6	106.2	206.7	8.2	100.3	200.8	14.1	96.8	197.2	17.7
PKE	160	58.6	66.4	113.2	35.0	50.8	97.6	50.7	43.9	90.7	57.6
Standard error	-	2.08	3.32	1.90	1.62	3.84	2.60	2.37	4.00	2.86	2.66
Feed Effect	-	***	***	***	***	***	***	***	***	***	***
Cow Diet effect	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Feed x Cow Diet effect	-	ns	ns	**	**	ns	**	**	ns	**	**

QDP = A * [CP],
SDP = [(B * k) / (k + kp)] * [CP],
ERDP = 0.8 [QDP] + [SDP],
RUP = CP - [QDP +SDP].
*** P<0.01 and ** P<0.05, * P<0.1. ns, not significant.

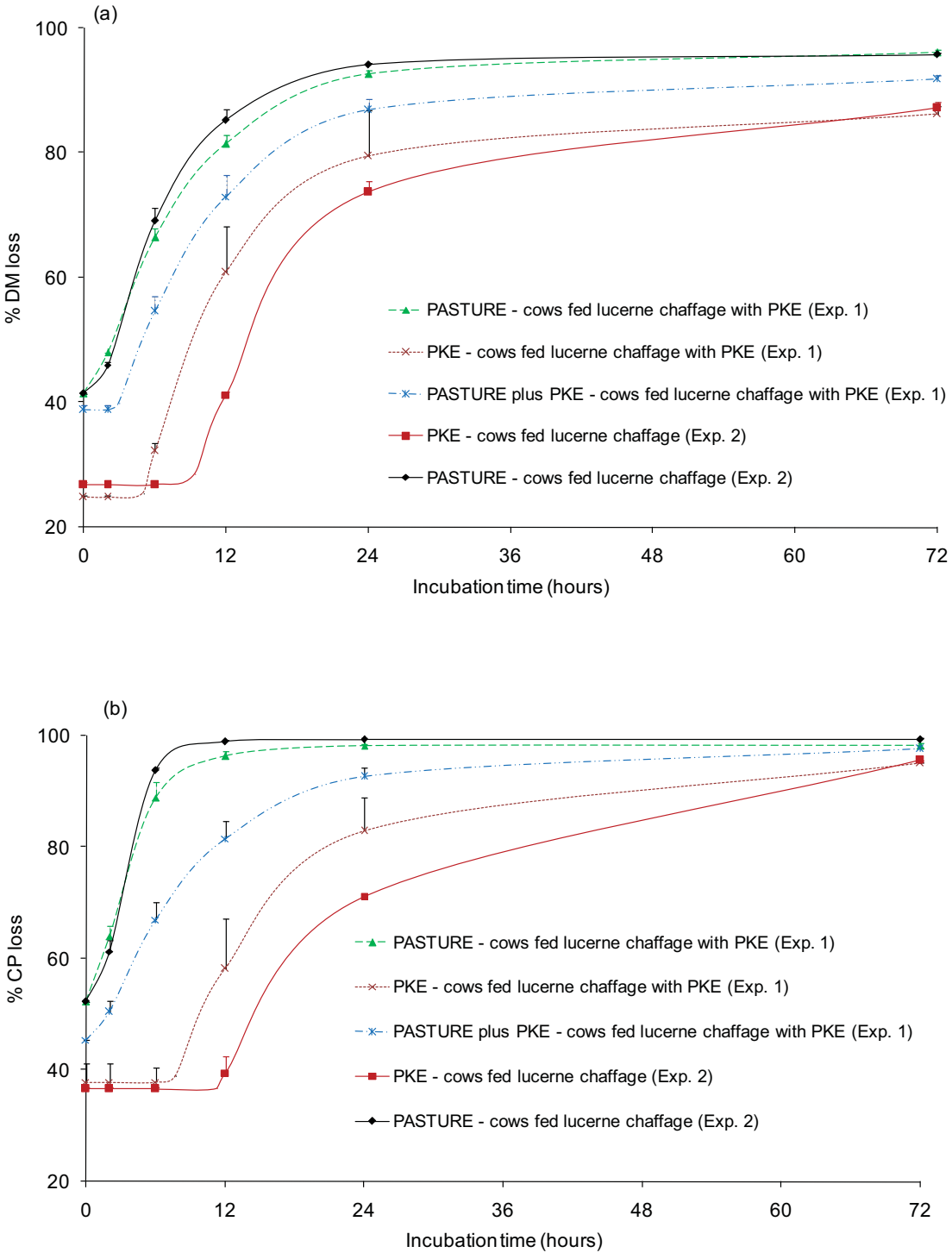


Figure 3.2. *In sacco* dry matter (DM) (a) and crude protein (CP) (b) fitted degradation curves of pasture, palm kernel expeller (PKE) and pasture plus PKE (P+PKE) in cows fed lucerne chaffage and PKE (experiment 1; Exp. 1) and cows fed lucerne chaffage only (experiment 2; Exp. 2). Error bars indicate standard deviations.

3.3.6. Fibre digestion kinetics of pasture and PKE

The results for NDF and ADF fractions are presented in Table 3.4 and Figures 3.3a and 3.3b. Both experiments showed that most of the NDF and ADF fractions of pasture, PKE and P+PKE were present mainly in the B pool, with values above 58% for NDF and 51% for ADF. About 27% and 35% of the NDF in PKE and pasture was released by the mincing preparation with 14% and 4% being undegradable residues, respectively. In experiment 1, the A fraction for P+PKE was similar to the average of both feeds; however, the B fraction of P+PKE was slightly lower than the average of both feeds.

The diet fed to the host cows had no effect on the fractions A, B and k for NDF or ADF, as was found also for DM and CP degradation results. However, a significant host cow diet effect ($P<0.01$) was found between NDF and ADF lag times of pasture and PKE, which also occurred in the degradation of the DM. The lag times were similar for both fibre fractions (NDF and ADF), and the degradation curves (Figure 3.3a and b) showed that during the first 10 h little or no digestion occurred with PKE fibre when it was incubated in the host cows fed lucerne chaffage only (experiment 1). However, in both fibre fractions, there was a large reduction in the lag phase of PKE when this feed was incubated in the host cows fed lucerne chaffage plus PKE (experiment 2).

The NDF and ADF digestion rates for pasture, PKE, and P+PKE were similar (10-14%/h) with no significant effect of feed, diet fed to the host cows or their interaction.

3.3.7. Effective degradability of pasture and PKE

The calculations of the ED for NDF and ADF at an assumed outflow rate of 5%/h showed an average of 73% (NDF and ADF) for pasture in both experiments and a range for PKE from 51% (NDF) in the host cows fed lucerne chaffage to only 61% (NDF) in the host cows fed lucerne chaffage plus PKE. Moreover, for PKE NDF the ED was greater than the DM ED when the host cows were fed lucerne chaffage plus PKE. An increase in the assumed outflow rate from 5 to 6%/h led to a decrease in the ED of ADF and NDF fractions in PKE, in both experiments.

Table 3.4. Mean values for neutral detergent fibre (NDF) and acid detergent fibre (ADF) degradation characteristics of pasture, palm kernel expeller (PKE) and pasture plus PKE (P+PKE) defined by the soluble fraction (A), slowly degradable fraction (B), fractional disappearance rate (k), lag phase, undegradable residue (U=100-A-B), potential degradability (PD) and effective degradability (ED), which takes into account the effect of the assumed rumen outflow rate (kp).

Feeds	Parameters							
	A (%)	k (%/h)	B (%)	lag (h)	U (%)	PD (%)	ED[1] (%)	ED[2] (%)
				NDF kinetics				
Experiment 1- cows fed Lucerne chaffage with PKE								
Pasture	35.5	10.5	60.6	2.1	3.9	96.0	72.5	69.6
PKE	27.8	10.7	58.8	3.2	13.4	86.4	61.3	58.2
P + PKE	32.7	10.2	57.6	1.4	9.7	90.2	68.7	66.0
Experiment 2 - cows fed Lucerne chaffage only								
Pasture	34.0	12.2	61.8	1.6	4.3	95.8	74.3	71.5
PKE	26.5	10.9	58.4	9.6	15.1	84.8	51.2	47.6
Standard error	0.81	1.5	0.79	0.44	-	-	-	-
Feed effect	**	ns	*	**	-	-	-	-
Diet effect	ns	ns	ns	**	-	-	-	-
Feed x Diet effect	ns	ns	ns	**	-	-	-	-
				ADF kinetics				
Experiment 1- cows fed Lucerne chaffage with PKE								
Pasture	37.0	10.0	59.0	2.1	4.0	96.0	72.3	69.4
PKE	27.0	11.1	51.5	3.4	21.6	78.3	56.3	53.6
P + PKE	34.2	11.6	50.6	2.9	15.2	84.8	64.8	62.2
Experiment 2- Cows fed Lucerne chaffage only								
Pasture	36.8	13.7	58.0	2.7	5.1	94.9	73.8	71.0
PKE	25.7	11.3	51.9	9.8	22.5	77.5	47.7	44.4
Standard error	0.64	2.0	0.93	0.35	-	-	-	-
Feed effect	***	ns	**	***	-	-	-	-
Diet effect	ns	ns	ns	***	-	-	-	-
Feed x Diet effect	ns	ns	ns	***	-	-	-	-

[1, 2] $ED = a+((Bk)/k+kp))(1-e^{-(k+ kp)(t-lag)})(e^{-kp*lag})$.

[1] kp= assumed rumen outflow rate (kp = 0.05 h-1; 2 kp= 0.06 h-1)

Coefficient of determination for all fitted curves was > 97%.

*** P<0.01 and ** P<0.05, * P<0.1. ns, not significant

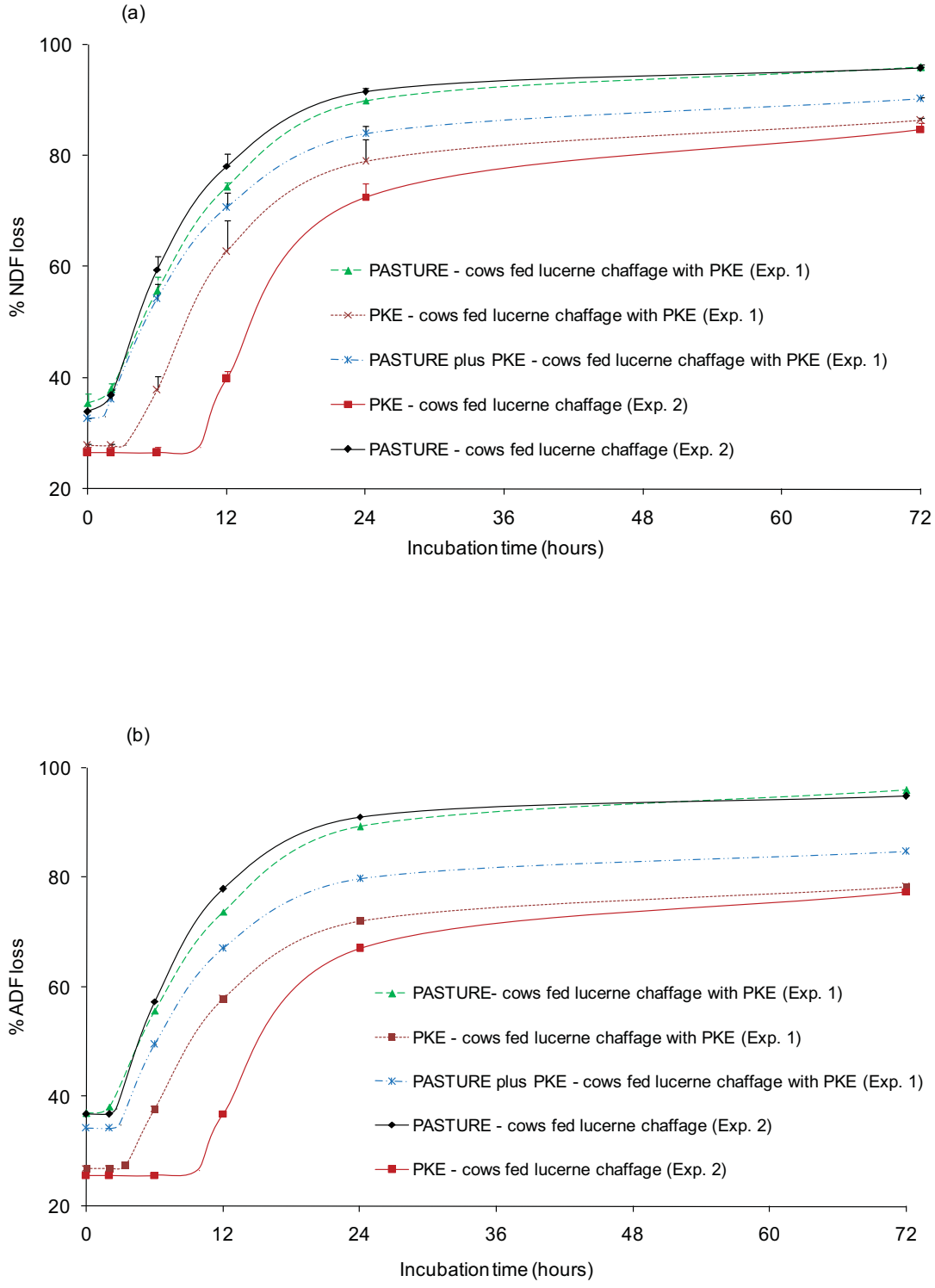


Figure 3.3. *In sacco* neutral detergent fibre (NDF) (a) and acid detergent fibre (ADF) (a) fitted degradation curves of pasture, palm kernel expeller (PKE) and pasture plus PKE (P+PKE) in cows fed lucerne chaffage and PKE (experiment 1; Exp. 1) and cows fed lucerne chaffage only (experiment 2; Exp. 2). Error bars indicate standard deviations.

3.4. Discussion

3.4.1. Chemical composition of feeds

The pasture (ryegrass and white clover) used in this experiment was of moderate quality, as determined from the values of 10.5 and 12.5 MJ/kg DM of metabolic energy for medium and high quality pasture reported by Waghorn et al. (2007). It had high values for NDF and CP and a low value for SSS concentration, suggesting that pasture samples had been harvested in a more mature stage (Chaves et al. 2006). The high CP concentration suggest pre-application of N fertilisation in the paddock (Pacheco and Waghorn, 2008) but this cannot be ascertained as the fertiliser history of the paddock from where the samples were collected was unknown. The low concentration of SSS (3 g/100g DM) obtained in this study could be related to the collection of samples in the morning after a cloudy and wet week, as sugar concentrations are totally dependent on the time of day and sunshine for re-synthesis of those components (Waghorn et al. 2007).

The CP concentration of PKE (Table 3.1) was similar to the values published by Dias et al. (2008a) (16.6 g/ 100g DM) and de Ruiter et al. (2007) (17.2 g/ 100g DM) in New Zealand, and was within the range of values (15.5 – 17.7 g/ 100g DM) obtained by Moss and Givens (1994), O'Mara et al. (1999) and Carvalho et al. (2005). However, for NDF, ADF and fat, the situation is less consistent. The concentrations of NDF and ADF obtained in this study (Table 3.1), and in the studies mentioned above, have shown wide variations in the fibre components of PKE, with values ranging between 67 to 82 and 37 to 57 g/ 100g DM for NDF and ADF, respectively. However, NDF and ADF concentrations of the PKE in this study were similar to that reported by Dias et al. (2008a), which were around 73 and 40%, respectively. The fat content of PKE has also varied between studies; however most of the values were above 7 g/100g of DM for mechanical extracted PKE, which could suggest that PKE used in this study (9 g/100g of DM of fat) was produced by mechanical extraction rather than by solvent extraction (O'Mara et al. 1999).

The variation in PKE composition between studies could be due to differences in the processing of the palm kernel used during oil extraction, at its different sources of origin. Also, according to O'Mara et al. (1999) differences in the laboratory procedures used to assess the chemical composition of PKE could cause some variation. In their

study great variation was found between the different enzymatic *in vitro* procedures used to predict the digestibility of palm kernel, and none of them were able to predict accurately the *in vivo* digestibility of PKE.

3.4.2. In sacco digestion kinetics

The results from this study have shown that the diet fed to the host cow had no effect on the values A, B or k, whereas other studies have reported a change in degradation rates when the diet of the cows was modified (Lindberg, 1981; Chaves, 2003; Salawu et al., 2001). Mertens et al. (1998) indicated that feeding cows Lucerne-based diets increased the *in sacco* degradation rates of both lucerne and maize silage, when compared with feeding diets containing maize silage only. Salawu et al. (2001) reported that degradability of perennial ryegrass silage incubated in cows fed on short straw spring pea was reduced; however the host cow's diet did not affect the degradability parameters in the pea/wheat bi-crops silages. According to López (2005) the type of feed incubated in the bag should also be included in the diet fed to the host animals for a more accurate measurement of ruminal degradation of the feed. In the present experiment the low number of repetitions could be one of the factors why numerical differences in the degradability values obtained for pasture and PKE from the different diets fed to the host cows were not significant.

Regardless of the diet fed to the host cows, this study shows that a large reduction of the lag period occurred when pasture was incubated with PKE samples. The lag time was reduced in all the fractions (DM, CP, NDF and ADF), but the greatest effect could be observed in the CP fraction. According to López (2005), the lag period is partly due to the inability of the rumen microbial population to degrade the substrate, until microbial growth is sufficient to saturate the substrate. Other factors, such as rate of hydration of the substrate, microbial attachment to feed particles and nutrient limitations, also influence the lag period. Therefore, the inclusion of pasture with a higher soluble CP fraction would be expected to increase the nitrogen availability within the PKE bags which aided rapid microbial growth and colonization.

The soluble and slowly degradable fractions of DM in pasture were in the range of values obtained by Burke et al. (2000) for perennial ryegrass and for white clover, and the CP values are similar to the values obtained by Chaves et al. (2006) for less immature ryegrass. Crude protein degradation rates of pasture were higher in this study

than those obtained by previous experiments (Barrell et al., 2000; Chaves et al., 2006), which used the same method for sample preparation. As mentioned before, the high CP protein concentration found in this study could suggest the fertilization of pasture with nitrogen; this can increase the concentration of CP and the percentage of non protein nitrogen, which is characterized by a high rate of rumen degradation (Van Vuuren et al., 1991; Peyraud et al., 1997). Another explanation for the high CP degradation rates could be related to the mathematical model chosen to fit the curves and estimate the rumen degradation parameters. Denham et al. (1989) showed an influence in the *in sacco* nitrogen disappearance of forages when a lag phase was included in the first-order kinetics model of Orskov and McDonald (1979). Degradation rates of the forages obtained by Denham (1989) using the lag phase were two fold higher than the values found in the model without lag phase; and they conclude that the inclusion of the lag period improved the goodness-of-fit of the model used to describe the nitrogen degradability of the forages tested.

Compared with pasture, the DM, CP, and fibre soluble fractions in PKE were smaller. However, the results obtained by Woods et al. (2003a, 2003b) in PKE DM and CP and AFRC (1993) in PKE CP for rumen degradability showed lower A and greater B values than the results of this experiment. Moreover, the present values for PKE CP and DM degradation rates were greater than the values obtained by Woods et al. (2003a, 2003b) and AFRC (1993), which were in the range of 2 to 7%/h. One of the reasons for those differences could be the source of PKE, as different sources of the same feed could have different nutritional values (Woods et al. 2003a). Additionally, the use of an exponential model without the lag period by the same authors could be another reason for the different degradability results of PKE. Dhanoa (1988) has shown that the use of a lag phase in the estimation of DM rumen degradation parameters of feed with low degradability, such as PKE, was advantageous. Nevertheless, both Woods et al. (2003a, 2003b) and in the present study show that PKE is a feed that is degraded slowly in the rumen.

Despite the high NDF content (Table 3.1), PKE has a very small particle size, low effective fibre concentration, and a fibre degradation rate similar to that of pasture (Table 3.4). Thus, PKE has a relatively higher digestion in and clearance of fibre from the rumen than pasture. The high percentage of NDF and ADF (13 and 22%, respectively) not degraded in the rumen is probably due to the high amount of lignin.

Knudsen (1997) reported that PKE lignin content is around 14%, which mainly comes from the nutshell contamination during the expeller process.

The percentages of PKE DM and CP that were not degraded in the rumen of cows fed a diet with PKE (U fraction; 14 and 5%, respectively) are greater than the values reported by Woods et al. (2003a, 2003b), which were 4.6 and 1.8% for PKE DM and CP, respectively. The chosen model used to fit the observed data may explain differences between the present results (lag included) and those of Woods et al. (2003a, 2003b, no lag included), as U is calculated by difference (U=100 – (A+B)). If instead of the model with the lag period, this study had applied the model without the lag period to the *in sacco* data, the values of PKE DM and CP not degraded in the rumen would have been lower, around 10 and 0%, respectively.

Factors such as sample preparation (Vanzant at al. 1998; Broderick and Cochran, 2000), bag surface area ratio (Hvelplund and Weisbjerg, 2000), sample size (Nocek, 1988), bag material and pore size (Huntington and Givens, 1995), host animal diet (Kitessa et al., 1999; Huntington and Givens, 1995) washing procedures (Cherney et al., 1990), and sampling sequence (Michalet-Doreau and Ould-Bah, 1992) could also affect the *in sacco* degradability and may in part explain differences in the results obtained by our study and others.

The differences between pasture and PKE in the rumen degradation characteristics are very clear when the ED is analysed. PKE has lower DM and CP soluble fractions and higher DM and CP slowly degradable fractions than pasture. Dry matter and fibre degradation rates are similar, except for PKE CP. The result of these differences is expressed in the effective degradability, which is an estimation of the *in vivo* digestibility. The ED calculated for PKE showed that this feed has lower degradability than pasture, and its digestion is more affected by higher outflow rates. The reason for this is probably due to the higher percentage of the slowly degradable fraction of PKE, which escapes from the rumen before being digested (Van Soest, 1994).

The addition of PKE into the pasture bags led to a dilution of the QDP and a great reduction of the CP degradation rates of the SDP (Table 3.2 and 3.4). Additionally, mixing the two feeds decreased the amount of rumen degradable protein and increased the undegradable portion, as a consequence of the low PKE rumen degradability of CP. Moreover, the amount of rumen undegradable protein of the mix increased as the outflow rates increased, reaching values of 25% at an assumed outflow rate of 8%/h. Those values are smaller than the data reported by Woods (2003b) and AFRC (1993),

which were 40%, at the same outflow rate. Despite the differences, it is certain that a portion of the CP in PKE reaches the lower intestines, with a small intestinal digestibility digestibility measured *in sacco* of 77% according to Woods et al. (2003c). The experiments cited in this chapter also showed that prior adaptation of the cow's rumen to PKE is necessary for better digestion, demonstrating the need to adapt cows to the supplement to achieve adequate digestion from its nutrients.

Digestion of fibre fractions in PKE were slightly faster than that for CP which demonstrate that microbial population was either able to degrade or colonise PKE fibre fractions. However, the lag period plays an important role in degradation rates, as rates of degradation after long lag periods are generally faster than rates of digestion obtained after slower lag periods.

3.5. Conclusions

Overall pasture and PKE differed in their soluble and insoluble fractions of DM, CP, NDF and ADF. However the degradation rates were similar for NDF and ADF, except for CP. PKE had slower CP degradation rates than pasture, and the addition of PKE to pasture reduced the high degradation rate of the pasture. As a result, the use of PKE as a supplement for dairy cows could have the additional benefit of reducing the total rumen degradability of protein in the diet. However, the use of large amounts of PKE, (>50% of the diet) as a sole supplement, could be limited by its maximum voluntary intake (Dias, et al., 2008a).

CHAPTER 4:
Fermentation products obtained from the *in vitro* incubation between palm kernel expeller (PKE) and pasture (ryegrass and white clover or ryegrass-only)

Abstract

The objective of this study was to measure rumen ammonia (NH_3) and volatile fatty acids (VFA) production of palm kernel expeller (PKE) alone or in combination with pasture composed of ryegrass-only (*Lolium perenne*; Ryegrass) or ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) (RW) using the *in vitro* technique. In the first experiment (experiment 1), pasture was incubated with PKE at different proportions and the rumen liquor for the incubations was obtained from cows fed lucerne chaffage plus PKE, while in the second (experiment 2), *in vitro* incubations were carried out with different proportions of Ryegrass and PKE when the rumen liquor was either obtained from cows fed lucerne chaffage plus PKE or lucerne chaffage only. In experiment 1 there was a decrease in the NH_3 net yield as the amount of PKE increased in the mix with PKE50% (50% of RW plus 50% of PKE), 75% (25% of RW plus 75% of PKE) and 100% (100% of PKE) resulting in a deficit of net NH_3 after 8, 6 and 4 hours (h) of incubating, respectively. The average net NH_3 yield for the entire 24 h of incubation was significantly lower ($P<0.05$) for the feeds containing 75 and 100% of PKE when compared with RW, PKE10% and PKE25%. In experiment 2, no significant differences were found between the first (rumen liquor obtained from cows fed lucerne chaffage plus PKE) and second incubations (rumen liquor obtained from cows fed lucerne chaffage only). But in the first incubation Ryegrass and PKE10% were the only feeds which presented a positive net NH_3 production for more than 12 h, while in the second incubation, Ryegrass, PKE10% and PKE25% sustained net NH_3 production for more than 12 h. Average net NH_3 yield calculated for the entire 24h of incubation for Ryegrass was significant ($P<0.05$) different from PKE100% in the first incubation, and from PKE75% and PKE100% in the second incubation. In terms of total VFA yield, in experiment 1, RW had the lowest total yield over 24 h of incubation (2.4 mMol/g DM), while feeds with PKE in their composition ranged from 2.6 to 3.5

mMol/g DM, with a peak for the PKE75% treatment. In contrast, in experiment 2, the lowest VFA yield was found for PKE100% in both incubations, with PKE10% showing the highest total VFA yield in the first incubation, and PKE50% in the second incubation. In both experiments, after 24 h of incubation, there was an increase in the proportion of butyrate at the expense of acetate, as the amount of PKE increased in the mix. In general, when PKE was mixed with pasture (RW or Ryegrass) net NH_3 production was reduced, with PKE only being able to maintain the net NH_3 production for the first 6 to 8 hours of incubation. The addition of moderate amounts of PKE with both pasture types (RW and Ryegrass) resulted in higher VFA productions than pasture alone, and higher butyrate percentages at the expense of acetate.

Keywords: *in vitro*; palm kernel expeller; pasture; volatile fatty acids, ammonia

4.1. Introduction

Palm kernel expeller (PKE) is a by-product from the palm oil industry after the mechanical extraction of the palm oil from the kernel of the fruit. It has been imported into New Zealand, mainly from South East Asia, since the year 2000 to be used as a supplement for ruminants, principally dairy cows. In 2007, more than 800 000 tonnes has been imported into NZ (MAF, 2008). The main use of PKE by dairy farmers is to fill short term feed deficits (eg. droughts) and extend lactation when the milk prices are high.

Despite its growth as a supplement for ruminants very little scientific information exists about the nutritional value of PKE in New Zealand. Palm kernel expeller chemical composition can vary significantly (Moss and Givens 1994; Hindle et al. 1995; O'Mara et al. 1999). Overseas studies have shown that PKE can be classified as an energy feed due to its oil concentration (8-12% of dry matter, DM) and high levels of neutral detergent fibre (NDF) (685 to 801 g/kg of DM). It contains medium levels of crude protein (CP) (158 to 194 g/kg of DM) and measured either *in vitro* or *in vivo*.

The knowledge about different feeds is essential in order to supply, in quantity and quality, the amount of nutrients required for maintenance and production of farm animals. Normally, feeds are evaluated by methods that describe their characteristics with respect to their ability to meet the animal requirements. The *in vivo* method is the most reliable to generate biological data about the characteristics of the feeds, however,

this type of study is laborious, time-consuming and expensive (López, 2005). Alternatively, laboratory methods, such as *in vitro* and *in sacco*, were developed to describe digestive and metabolic processes using less time and money.

In vitro fermentation techniques have been used since the 1940's, however, their use increased extensively with the advent of McDougall's buffer in 1948, which made possible to enhance the length of *in vitro* incubations (Weiss, 1994). Since that time, several different *in vitro* systems have been developed; however, all procedures followed a sequence that consisted of an anaerobic fermentation of a sample substrate in the presence of buffered rumen liquor, or sometimes enzymes, followed by an end point measurement (Van Soest, 1994; Weiss, 2004). In this study the *in vitro* technique was used to provide information about fermentation products (eg. NH_3 and VFA) of PKE alone or mixed with pasture, and clarify either if the CP content of PKE is enough to sustain microbial activity in the rumen or if the introduction of PKE to pasture would reduce the availability of ammonia to the rumen microbes. Furthermore, PKE VFAs production and profile can be indicative of the nutritional value of PKE and would also clarify if the introduction of great amounts of PKE to pasture can improve the VFA production when those feeds are mixed together.Therefore, the objective of this research was to define the fermentation products of pasture (ryegrass-only, or ryegrass and white clover), PKE and the various combinations of pasture and PKE using the *in vitro* technique.

4.2. Material and methods

The fermentation characteristics of pasture composed of ryegrass-only (*Lolium perenne*; Ryegrass), or 80% of ryegrass (*Lolium perenne*) and 20% of white clover (*Trifolium repens*) (RW) alone or in combination with varying amounts of PKE were measured using *in vitro* technique. In the first experiment (experiment 1), PKE was incubated with RW at different proportions and the rumen liquor for the incubations was obtained from cows fed lucerne chaffage plus PKE. Whereas in the second experiment (experiment 2), Ryegrass was used and a series of *in vitro* incubations were carried out with different proportions of Ryegrass and PKE, and the rumen liquor was either obtained from cows fed lucerne chaffage plus PKE or lucerne chaffage only (Figure 4.1).

During experiment 1 a low VFA concentration was observed during the first few hours of the incubation. It was thought this was due to the low concentration of soluble sugars and starch found in the RW samples or because of the PKE being incubated, as there appears to be no literature indicating the effect of PKE on the rumen microorganisms when cows are fed a diet containing PKE. Therefore, experiment 2 was designed to test the effects of incubating PKE either mixed with a pasture sample with a high concentration of soluble sugars and starch or incubated in the rumen liquor obtained from cows fed a diet without PKE.

The analytical procedure provided information on the NH_3 and VFA production during *in vitro* digestion of RW, PKE and its mix (experiment 1) using rumen liquor from cows fed lucerne chaffage plus PKE; and Ryegrass, PKE and its mix (experiment 2) using rumen liquor from cows fed either lucerne chaffage plus PKE or just lucerne chaffage .

4.2.1. Feed collection and analyses

Feed samples used in this experiment were collected, prepared and analysed as previously described in Chapter 3. Although, RW and Ryegrass samples were harvested at different times and from different paddocks due to the reasons explained previously.

In all experiments, the DM content of RW, Ryegrass and PKE samples was determined, and approximately 0.5 g of DM was used for the incubations.

4.2.2. Animals used for the rumen fluid source

Two non-lactating Holstein-Friesian cows fitted with rumen *cannulae* were used to provide rumen liquor for the incubations, and rumen liquor was used within 20 minutes of collection.

Cows were fed 75% of lucerne chaffage (Fibre Fresh Feeds of New Zealand) and 25% PKE (Premier Stock Feeds Limited– NZ), or lucerne chaffage only, as described in Figure 4.1. At all times, cows were fed at maintenance level twice a day, with half of the diet being offered at 0900h and the other half offered at 1700h. Water was available *ad libitum*.

4.2.3 In vitro incubations

The *in vitro* incubations were undertaken in an oscillating incubator (Orbital incubator, Gallenkamp, UK) with temperature control and a rack that can handle simultaneously up to 96 Schott bottles (50 mL) per incubation. An overview of the *in vitro* experiment is presented below (Figure 4.1).

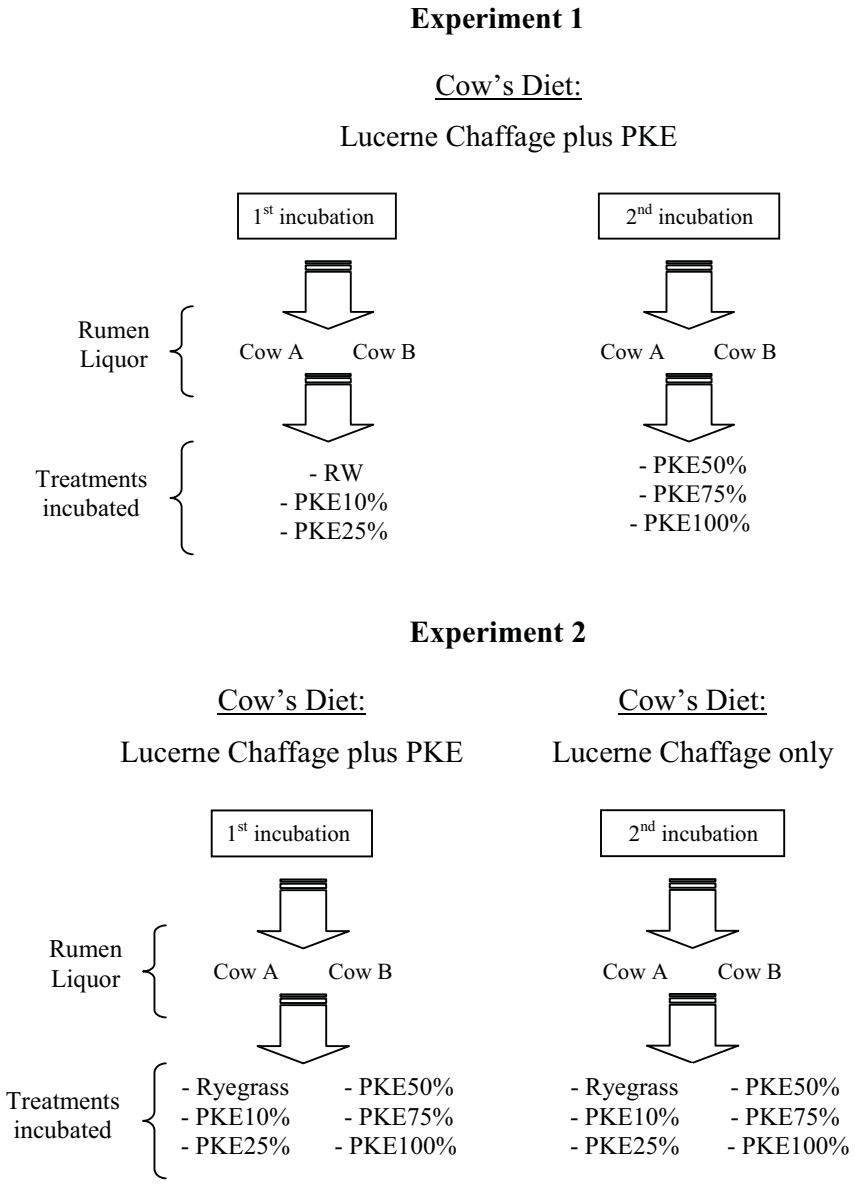


Figure 4.1. Description of the treatments used during *in vitro* experiments.

In experiment 1, schott bottles containing triplicate samples of RW, 90% of RW plus 10% of PKE (PKE 10%) and 75% of RW plus 25% of PKE (PKE 25%) were tested in the first incubation, while in the second incubation triplicate samples containing 50% of RW plus 50% of PKE (PKE 50%), 25% of RW plus 75% of PKE

(PKE 75%) and 100% of PKE (PKE 100%) were tested. The rumen liquor used in both incubations was collected from two cows fed lucerne chaffage plus PKE. Triplicate bottles from each treatment were removed for sampling and analysed at 0, 2, 4, 6, 8, 10, 12 and 24 h. Soybean meal was used as a standard with triplicate bottles being removed after 2 and 8 h of incubation to monitor variation between incubations.

In experiment 2, when Ryegrass was incubated, in the first incubation rumen liquor was obtained from two cows fed a diet of PKE plus lucerne chaffage, while in the second incubation both cows were fed lucerne chaffage only. Triplicate samples containing a mixture of Ryegrass and PKE at ratios of 0:100, 90:10, 75:25, 50:50, 25:75, and 0:100 were incubated simultaneously in both incubations and triplicate bottles were removed for analyses at 0, 2, 4, 6, 12 and 24 h. Frozen minced lucerne was used as a standard in both incubations with bottles being removed after 2 and 6 h of incubation.

The procedures were the same for all incubations and started with approximately 0.5 g DM of feed being weighed into 50 millilitres (mL) Schott bottles for incubation the following day. On the day of incubation, the bottles containing the samples were warmed to 39°C in the incubator for 60 minutes, gassed with CO_2 followed by the addition of 12 mL of artificial saliva (McDougall's buffer), 0.5 mL of reducing agent (cysteine sulphide) and 3mL of rumen liquor obtained from both cows, which were fed 2 h before collection of the rumen liquor, in all cases. Bottles were capped with lids fitted with valves to avoid fermentation gases escaping, and then they were returned to the incubator where the temperature was maintained at 39°C and the rack set at 90 oscillations per minute.

The pH of the rumen liquor was measured immediately after collection. Additionally, sub-samples were taken from rumen liquor and each *in vitro* bottle at the respective collection times for NH_3 and VFA determination. Ammonia sub-samples (1 mL) were acidified with 15 µL of hydrochloric acid, mixed and centrifuged (14,000 x g) for 15 minutes, after that the supernatant was frozen for NH_3 analyses by the glutamate dehydrogenaze enzymatic method (Neeley and Phillipson, 1988). Ammonia concentrations (µM NH_3 / mM feed N) presented in this chapter were corrected for concentrations in rumen inocula and have been expressed per gram of feed nitrogen incubated (Burke, 2004).

Rumen liquor and triplicate samples (1.5 mL each) from *in vitro* bottles were bulked at 0, 6, 12 and 24 h, centrifuged (14,000 x g) for 15 minutes and the supernatant frozen for later VFA determination according to the method described by Wronkowska

et al. (2006). VFA concentrations have been corrected for VFA concentrations in the rumen *inocula* and are expressed per kg of feed DM incubated.

4.2.4 Statistical analyses

In vitro incubations were carried out in two experiments with two incubations in each experiment and the variability between them was evaluated with the use of standard feeds (SBM in experiment 1, and lucerne in experiment 2). The differences in NH_3 production of SBM (experiment 1) and lucerne (experiment 2) between the two incubations were analysed using the Mixed model procedure (SAS, 2003), with incubation and time considered as fixed effects.

Ammonia concentrations in each bottle were used to calculate net ammonia production and analyses were based on net conversion of plant N to ammonia N. The Mixed procedure of SAS was used for the analyses, with feed and time being considered the fixed effects in experiment 1; and feed, time, diet and the feed x diet interaction considered the fixed effects in experiment 2. In both experiments differences between feeds were determined using the LSMeans statement with the PDIFF option and differences were considered significant at $P<0.05$.

For VFA, results were based on bulk samples (triplicate samples were bulked at 0, 6, 12 and 24 h) for each of the treatments tested. VFA production was expressed as net production (mmol/ g DM and mg/ g DM) incubated for different times.

4.3. Results

4.3.1. Chemical composition

The chemical composition of RW, Ryegrass and PKE samples are shown in Table 4.1. The RW and Ryegrass samples used in the *in vitro* experiments had similar ME content (around 11.5 MJ/kg DM) with a high CP content (around 24.0 - 22.7 g/100 g DM). However, the soluble sugars and starch content in the RW samples used in the first *in vitro* experiment were considerably lower than the Ryegrass samples used in the second *in vitro* experiment. Both NDF and ADF concentration were higher in RW than Ryegrass samples; however they had similar ash content.

The PKE sample used during the *in vitro* experiments contained lower CP values than RW and Ryegrass (16.0 vs. 24.0 and 22.7 g/100g DM of CP), with most of the energy coming from the fibre and fat fractions. Additionally, the ash concentration of PKE was approximately half of the value obtained for RW and Ryegrass and its starch content was minimum (0.03 g/100g DM).

Table 4.1. Dry matter content and chemical composition (± standard deviation) of ryegrass and white clover (RW), ryegrass-only (Ryegrass) and palm kernel expeller (PKE) used during the *in vitro* incubations.

Item	RW[1]	Ryegrass[1]	PKE[2]
Dry matter (%)	13.7	17.0	97.2
	----------g/ 100 g DM ----------		
Crude protein	24.0 ± 0.65	22.7	16.0 ± 0.76
Neutral detergent fibre	51.9 ± 2.47	44.3	70.3 ± 4.78
Acid detergent fibre	25.9 ± 0.57	22.8	39.3 ± 2.24
Fat	4.0 ± 0.06	4.0	9.0 ± 0.52
Soluble sugars and starch	3.0 ± 1.02	18.6	0.03 ± 0.02[3]
Ash	10.8 ± 0.02	10.5	4.1 ± 0.67

[1] Analysed by NIRS
[2] Analysed by wet chemistry
[3] Starch content only.

4.3.2. In vitro results

In vitro incubations enable the net yield of NH_3 and VFA to be measured over a period of 24 h. The contribution of rumen *inoculum* (Table 4.2) to ammonia and VFA pools was taken into account for the calculation of the net yields at each incubation time. Based on the parameters analysed, rumen *inoculum* appears to be similar between experiments (Table 4.2).

The standard feed used, soybean meal, presented similar pH and NH_3 concentrations (mMol/L) in both incubations of experiment 1. However, after 10 h of incubation soybean meal used in the second incubation of experiment 1 presented lower NH_3 concentration than the initial measurement at 2 h of incubation (Appendix 4.1).

The lucerne used as a standard feed in both incubations of experiment 2 had similar pH after 2 and 6 h of incubation (Appendix 4.1). However, NH_3 concentrations were greater in the second incubation than in the first.

Table 4.2. Rumen pH, ammonia (NH_3), volatile fatty acid (VFA) concentrations and ratio of acetate to propionate of cows used for the *in vitro* incubations and fed Lucerne chaffage plus PKE (Luc. chaff. + PKE) or Lucerne chaffage only (Luc. chaff.).

	Incubations			
	Experiment 1		Experiment 2	
Incubations	1st	2nd	1st	2nd
Animal diet	Luc. chaff. + PKE		Luc. chaff. + PKE	Luc. chaff.
Rumen pH	6.7	6.8	6.6	6.5
NH_3 concentration (mMol/L)	24.7	28.6	23.1	26.3
Total VFA concentration (mMol/L)	120.1	130.2	117.9	135.0
Acetate:propionate ratio	7.1	4.5	7.2	6.1

4.3.3. In vitro pH

During all *in vitro* incubations the McDougall's buffer was used to avoid abrupt changes in the pH values during the first few hours of incubation and also to simulate a rumen environment similar to ruminants fed forages (pH remain above 5.8). Therefore, *in vitro* pH was measured for all feed samples during the 24 h of incubation, and feed samples with pH below 5.8 were discarded. During experiment 1, all feeds presented a pH around 7.5 at 0 h of incubation and after 6 h of incubation almost all feeds declined to values below 7.0 (Figure 4.2). Feeds with a higher percentage of RW in the mix (first incubation) remained at the same pH or increased their pH up to 12 h of the incubation. The pH at 24 h of the incubation showed a subsequent decline for all feeds, except for PKE75% which had an increase in its pH. At the end of those incubations none of the feeds reached values below 6.0.

In experiment 2 incubations started with pH values above 7.3 for all feeds and after 2 h declined to 7.0. Between 4 and 12 h, most of the feeds had pH values around 6.8, with the exception of PKE25% and 50% which had decreased to 6.3 and 6.5, respectively (Figure 4.2). After 12 h of incubation most of the feeds reached pH values around 6.3, except for PKE75% and 100%, which remained at 6.8. In the second incubation of experiment 2, the pH of all treatments remained the same after 4 h of

incubation with values around 7.0. However, after 24 h of incubation for most of the feeds the pH was around 6.5, with further falls for PKE25% and 50%, but none of the feeds reached values below 6.0.

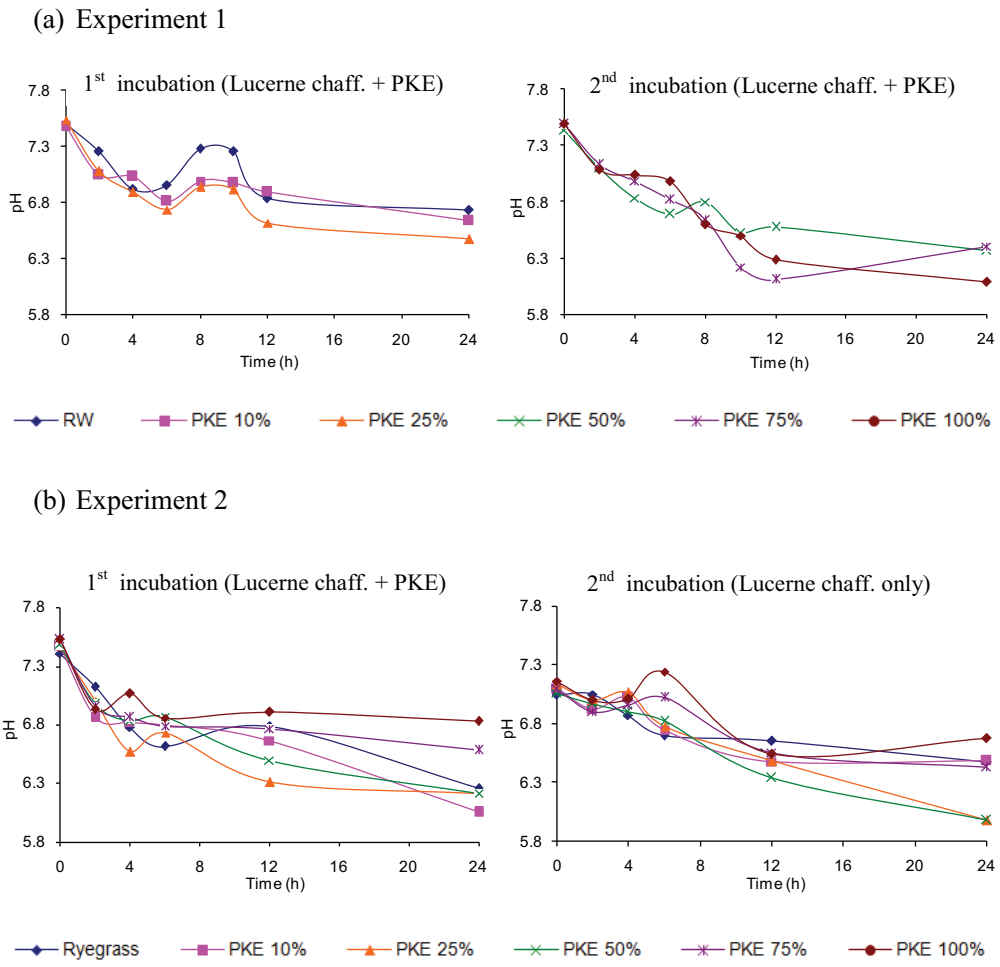


Figure 4.2. Changes in pH during *in vitro* incubations of (a) ryegrass and white clover (RW) and palm kernel expeller (PKE) (Experiment 1), and (b) ryegrass-only (Ryegrass) and PKE (Experiment 2). PKE contributes 10% (PKE10%), 25% (PKE25%), 50% (PKE50%), 75% (PKE75%) and 100% (PKE100%) of the mix with both RW and Ryegrass.

4.3.4. Net NH_3 yield

Net NH_3 yield indicates whether plant nitrogen is insufficient for microbial growth and the length of time which protein degradation exceeds the capacity for microbial utilization. Negative values suggest N incorporation into microbial biomass exceeded N release from plant protein degradation. In both incubations of experiment 1, when RW were incubated with different amounts of PKE, there was a decrease in the NH_3 net yield as the amount of PKE increased in the mix. Net NH_3 concentrations of bottles incubated with RW, PKE10% and PKE25% were greater than 0 during the entire 24 h of incubation; however bottles with 50, 75 and 100% of PKE resulted in a deficit of net NH_3 after 8, 6 and 4 h of incubation, respectively (Figure 4.3). Average NH_3 yield for the entire 24 h of incubation was significantly lower ($P<0.05$) for the feeds containing 75 and 100% of PKE in their composition than RW, PKE10% and PKE25% (Table 4.3).

In experiment 2, no significant differences were found in the overall net NH_3 production between the first and second incubation when the diets with and without PKE were fed to the cows before the rumen liquor collection (Table 4.3). However, the net NH_3 yields during the incubations in experiment 2 were lower than in experiment 1, as the pasture sample used in the second experiment contained ryegrass-only. Ryegrass and PKE10% were the only treatments which presented a positive net NH_3 production for more than 12 h, while PKE25% only produced NH_3 for around 6-7 h in the first incubation, and for 12 h in the second incubation (Figure 4.4). Furthermore, PKE50, 75 and 100% had peak net NH_3 concentrations after 2 h of incubation, and after that net yield decreased and reached the lowest value after 12 h of incubation. Regardless of the scale difference in the NH_3 net yields between experiment 1 and 2, the same trend was observed when the average NH_3 yield for the entire 24 h of incubation was calculated, with Ryegrass being significantly ($P<0.05$) different from PKE100% in the first incubation, and from PKE75% and PKE100% in the second incubation.

In experiment 1, mixes with more than 50% of PKE, appeared to be inadequate to sustain microbial growth for more than 6 h, whilst in experiment 2, in both incubations mixes with more than 25% of PKE were not able to release enough CP for microbial growth after 6 h of incubation, even with CP concentrations ranging between 160 to 210 g/kg DM in their composition.

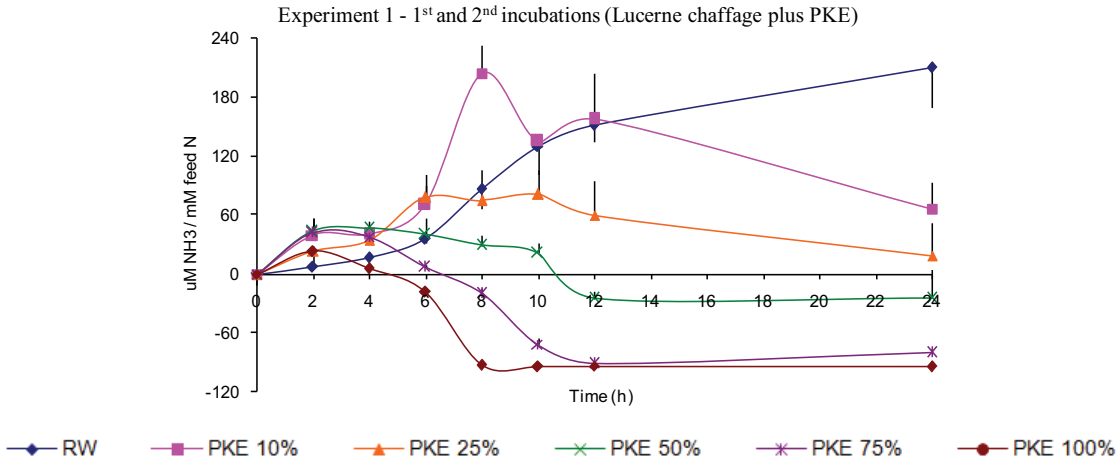


Figure 4.3. Average *in vitro* net ammonia production (± standard error) of ryegrass and white clover (RW) with palm kernel expeller (PKE) mixed at different proportions during 0, 2, 4, 6, 12 and 24 hours (h) of incubation. Cows fed lucerne chaffage plus PKE in both incubations.

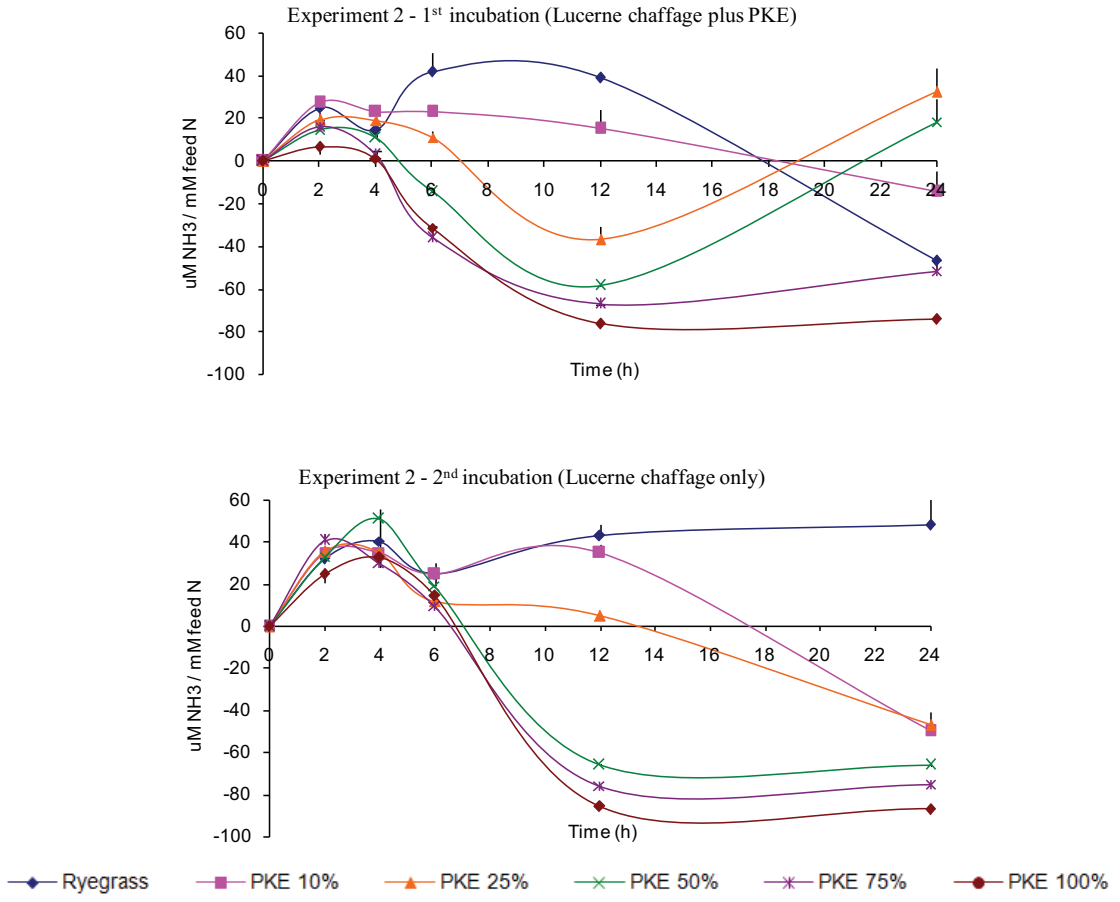


Figure 4.4. Average *in vitro* net ammonia production (± standard error) of ryegrass-only (Ryegrass) with palm kernel expeller (PKE) mixed at different proportions during 0, 2, 4, 6, 12 and 24 hours (h) of incubation. Cows fed lucerne chaffage plus PKE (a) and cows fed lucerne chaffage only (b).

Table 4.3. The average *in vitro* net ammonia produced (mMol of NH_3/mMol feed N) by each feed after 24 hours of incubation in Experiment 1, with ryegrass and white clover (RW) as the control treatment, and 2, with ryegrass-only (Ryegrass) as the control treatment. Each value is the average of all time points collected for each feed, and in each time triplicate bottles were collected for each feed.

	Experiment 1	Experiment 2	
Control	RW	Ryegrass	
Incubations	1st and 2nd	1st	2nd
Feed[2]	Cows fed Lucerne chaffage with PKE	Cows fed Lucerne chaffage with PKE	Cows fed Lucerne chaffage only
RW or Ryegrass	91.0 [a]	14.8 [a]	37.6 [a]
PKE 10%	102.3 [a]	14.9 [a]	16.1 [ab]
PKE 25%	53.2 [ab]	9.2 [a]	8.0 [ab]
PKE 50%	19.2 [bc]	-5.6 [ab]	-5.8 [ab]
PKE 75%	-25.1 [c]	-26.9 [ab]	-14.2 [b]
PKE 100%	-52.0 [c]	-34.7 [b]	-20.3 [b]
Time (P)	0.9867	0.0158	0.0002
Diet (P)	-	ns	
Feed*Diet (P)	-	ns	

[1] LSMeans for each feed with different superscript are significantly different (P <0.05).
(-) not measured.
[2] Ryegrass and white clover (RW) or ryegrass-only (Ryegrass) with 10% (PKE10%), 25% (PKE25%), 50% (PKE50%), 75% (PKE75%) and 100% (PKE100%) of PKE in the mix.

4.3.5. VFA yield

Concentrations of VFA per gram of DM incubated per time period have been summarized for experiment 1 (Figure 4.5), and experiment 2 for the two diets tested (Figure 4.6a – cows fed lucerne chaffage plus PKE; Figure 4.6b – cows fed lucerne chaffage only). In experiment 1, RW had the lowest total VFA yield after 24 h of incubation (2.4 mMol/g DM), while feeds with PKE in their composition ranged from 2.6 to 3.5 mMol/g DM (Figure 4.5). During the entire incubation acetate was the main VFA produced by all feeds, with smaller quantities of propionate and n-butyrate being produced (Table 4.4). The yields of propionate and n-butyrate increased in the next 6 h, but acetate still remained the main VFA being produced by all feeds.

After 24 h of incubation, there was a decrease in the proportion of acetate (almost 30%) as the amount of PKE increased in the mix. In contrast, the proportion of butyrate produced increased more than 50%, with PKE100% producing more butyrate

than propionate (Table 4.4). The proportion of propionate also increased with the addition of PKE in the mix; but the increase reached a plateau at 50 and 75% of PKE in the mix.

The ratio of acetate:propionate showed a substantial difference between the feeds after 24 h of incubation. As the amount PKE increased in the mix the ratio of acetate:propionate decreased from 3.7 to 2.3, while RW had a ratio of 4.0 (Table 4.4).

When the total proportion of DM degraded and released as VFA (mg/ g DM) was calculated, the data showed that all feeds had an increase in the conversion of DM into VFA as time of incubation increased (Table 4.7). RW presented the lowest conversion in the first 6 h (1.1%) and PKE100% in the first 12 h (7.6%). After 24 h of incubation the percentage of DM released as VFA increased with the addition of PKE into the mix (from 28 to 38%), except for the treatment with 100% of PKE which showed similar conversion to RW (24.0 vs. 25.0%, respectively).

Overall in experiment 2, the first incubation (cows fed lucerne chaffage plus PKE) resulted in higher VFA yields than the second incubation (cows fed lucerne chaffage only) in almost all feeds from 0-6 and 6-12 h (Table 4.5 and 4.6), with acetate being the main VFA produced during those first hours of incubation. After 24 h of incubation, the addition of PKE increased total VFA yields until 50% of PKE in the mix in the first incubation, while in the second incubation, VFA yield increased until 75% of PKE in the mix. In both incubations, the VFA yield of PKE100% was smaller than Ryegrass (2.9 vs. 1.8 and 2.5 vs. 2.4 mMol/g DM).

In experiment 2, acetate and n-butyrate percentages of both incubations followed similar responses observed in experiment 1; after 24 h of incubation. *n*-butyrate increased and acetate decreased with the addition of PKE in the mix. In the first incubation, propionate percentages decreased with the addition of PKE, however the same trend was not observed in the second incubation.

In the first incubation, the ratios of acetate:propionate increased with the addition of PKE in the mix, with values ranging from 1.6 for Ryegrass to 3.4 for 100%PKE, while in the second incubation no trend was observed with values ranging from 2.6 to 1.7, with Ryegrass showing the highest ratio.

Overall, the proportion of DM degraded and released as VFA was greater in experiment 2 than 1. However, when the first incubation is compared with the second the results showed that more DM was converted to VFA in the first incubation than in the second after 24 h of incubation for all feeds (Table 4.7). Mixes of Ryegrass and PKE

in the proportion of 25 and 50% and 50 and 75% had greater conversion of DM into VFA in the first and second incubations, respectively, in experiment 2

Experiment 1 – 1st and 2nd incubations (Lucerne chaffage plus PKE)

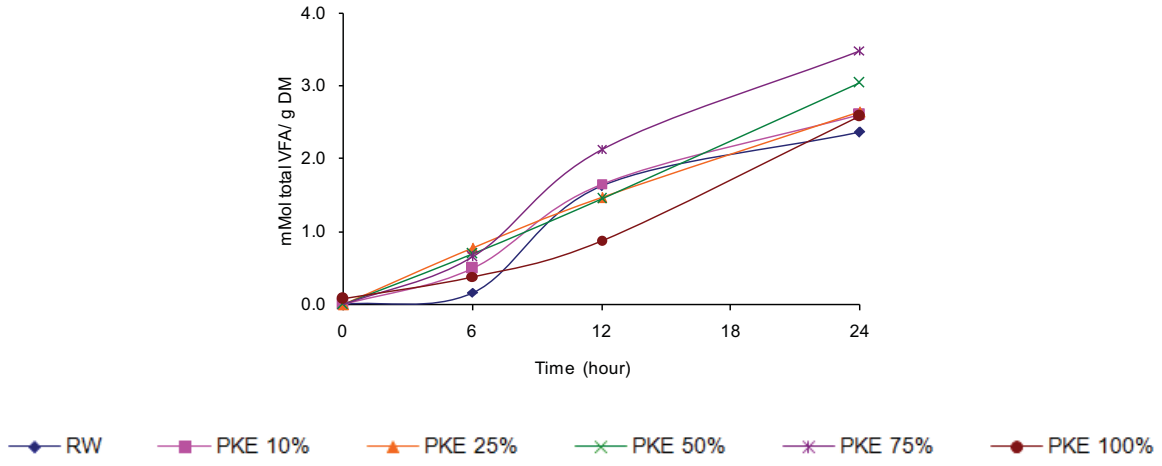
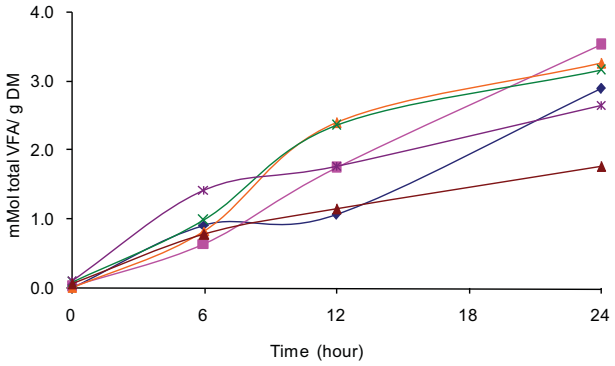


Figure 4.5. Total volatile fatty acids (VFA) yield (mMol/g DM) when different proportions of ryegrass plus white clover (RW) and palm kernel expeller (PKE) were evaluated *in vitro* (experiment 1). Cows fed lucerne chaffage plus PKE in both incubations.

(a) Experiment 2 – 1st incubation (Lucerne chaffage plus PKE)



(b) Experiment 2 – 2nd incubation (Lucerne chaffage only)

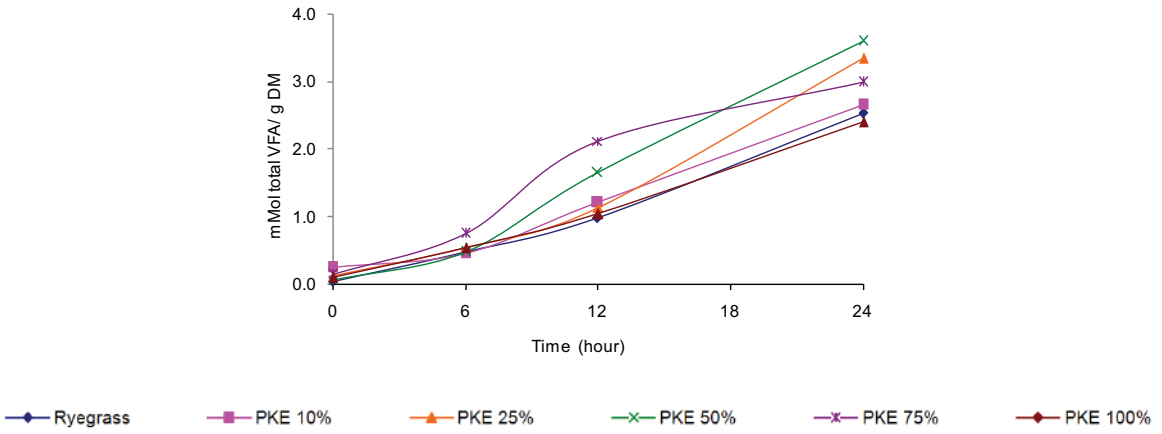


Figure 4.6 –Total volatile fatty acids (VFA) yield (mMol/g DM) when different proportions of ryegrass-only (Ryegrass) and palm kernel expeller (PKE) were evaluated *in vitro* (experiment 2). Cows fed lucerne chaffage plus PKE (a) and cows fed lucerne chaffage only (b).

Table 4.4. Experiment 1: cows fed lucerne chaffage plus palm kernel expeller (PKE). Total volatile fatty acids yield (mmol/g DM) of the different treatments after 6, 12 and 24 hours (h) of incubation; and molar percentages and ratios of the main volatile fatty acids. Each value is the average of triplicate bottles of each feed.

Feed[1]	Total mmol/g DM	Acetate (%)	Propionate (%)	*n*-Butyrate (%)	Minor (%)	A:P	(A+B):P
			6h				
RW	0.2	100.0	0.0	0.0	0.0	-	-
PKE10%	0.5	89.2	5.8	2.3	2.7	15.5	15.9
PKE25%	0.8	86.8	6.3	4.5	2.5	13.8	14.5
PKE50%	0.7	78.0	15.0	5.5	1.5	5.2	5.6
PKE75%	0.6	85.7	9.3	3.7	1.3	9.2	9.6
PKE100%	0.4	85.3	7.6	5.5	1.6	11.2	11.9
			12h				
RW	1.6	68.0	12.1	14.5	5.4	5.6	6.8
PKE10%	1.7	64.3	10.7	19.3	5.6	6.0	7.8
PKE25%	1.5	62.8	8.1	23.2	5.9	7.7	10.6
PKE50%	1.5	74.3	11.3	12.4	2.0	6.6	7.6
PKE75%	2.1	58.8	20.5	17.4	3.4	2.9	3.7
PKE100%	0.9	73.5	8.5	16.8	1.2	8.6	10.6
			24h				
RW	2.4	62.7	15.7	14.2	7.5	4.0	4.9
PKE10%	2.6	59.1	16.1	18.7	6.0	3.7	4.8
PKE25%	2.6	56.1	17.9	19.9	6.1	3.1	4.3
PKE50%	3.0	52.5	22.4	19.6	5.5	2.3	3.2
PKE75%	3.5	51.4	22.7	20.2	5.6	2.3	3.1
PKE100%	2.6	44.1	19.5	30.5	5.8	2.3	3.8

[1] Ryegrass and white clover (RW) with 10% (PKE10%), 25% (PKE25%), 50% (PKE50%), 75% (PKE75%) and 100% (PKE100%) of PKE in the mix.

Table 4.5. Experiment 2: cows fed lucerne chaffage plus palm kernel expeller (PKE): Total volatile fatty acids yield (mmol/g DM) of the different treatments after 6, 12 and 24 hours (h) of incubation; and molar percentages and ratios of the main volatile fatty acids. Each value is the average of triplicate bottles of each feed.

Feed[1]	Total mmol/g DM	Acetate (%)	Propionate (%)	*n*-Butyrate (%)	Minor (%)	A:P	(A+B):P
			6h				
Ryegrass	0.9	79.7	15.8	3.4	1.1	5.0	5.3
PKE10%	0.7	89.0	8.9	1.8	0.3	10.0	10.2
PKE25%	0.8	84.1	10.3	5.0	0.6	8.2	8.7
PKE50%	1.0	61.6	26.0	10.8	1.6	2.4	2.8
PKE75%	1.4	67.3	20.1	10.3	2.4	3.4	3.9
PKE100%	0.8	73.9	13.4	10.6	2.1	5.5	6.3
			12h				
Ryegrass	1.1	83.7	12.9	3.1	0.4	6.5	6.7
PKE10%	1.8	63.6	23.9	10.7	1.8	2.7	3.1
PKE25%	2.4	54.7	27.2	15.6	2.5	2.0	2.6
PKE50%	2.4	56.0	23.8	17.9	2.2	2.3	3.1
PKE75%	1.8	56.3	19.4	21.0	3.2	2.9	4.0
PKE100%	1.2	57.5	15.2	24.7	2.5	3.8	5.4
			24h				
Ryegrass	2.9	55.1	34.8	7.7	2.4	1.6	1.8
PKE10%	3.5	54.6	30.5	11.7	3.2	1.8	2.2
PKE25%	3.3	53.1	26.3	16.4	4.2	2.0	2.6
PKE50%	3.2	53.8	21.8	18.8	5.6	2.5	3.3
PKE75%	2.7	52.0	18.5	24.0	5.5	2.8	4.1
PKE100%	1.8	42.8	12.5	39.2	5.5	3.4	6.5

[1] Ryegrass-only (Ryegrass) with 10% (PKE10%), 25% (PKE25%), 50% (PKE50%), 75% (PKE75%) and 100% (PKE100%) of PKE in the mix.

Table 4.6. Experiment 2: cows fed lucerne chaffage only: Total volatile fatty acids yield (mmol/g DM) of the different treatments after 6, 12 and 24 hours (h) of incubation; and molar percentages and ratios of the main volatile fatty acids. Each value is the average of triplicate bottles of each feed.

Feed[1]	Total mmol/g DM	Acetate (%)	Propionate (%)	*n*-Butyrate (%)	Minor (%)	A:P	(A+B):P
			6h				
Ryegrass	0.5	84.9	11.8	1.5	1.7	7.2	7.3
PKE10%	0.5	91.7	6.7	0.7	0.9	13.6	13.7
PKE25%	0.6	86.3	10.3	1.6	1.7	8.4	8.5
PKE50%	0.5	78.5	13.2	5.4	2.8	5.9	6.3
PKE75%	0.8	69.6	19.4	7.1	4.0	3.6	4.0
PKE100%	0.5	69.1	20.9	6.5	3.6	3.3	3.6
			12h				
Ryegrass	1.0	87.4	9.7	2.2	0.8	9.0	9.3
PKE10%	1.2	84.7	11.5	2.8	0.3	7.3	7.6
PKE25%	1.1	76.6	15.2	6.9	0.8	5.0	5.5
PKE50%	1.7	67.2	19.8	10.3	0.8	3.4	3.9
PKE75%	2.1	54.3	24.9	16.7	1.4	2.2	2.9
PKE100%	1.0	59.8	21.8	14.7	1.9	2.7	3.4
			24h				
Ryegrass	2.5	62.4	24.3	9.3	0.3	2.6	3.0
PKE10%	2.7	57.4	31.3	9.7	0.2	1.8	2.1
PKE25%	3.4	53.1	31.1	12.9	0.3	1.7	2.1
PKE50%	3.6	52.8	26.5	16.9	0.4	2.0	2.6
PKE75%	3.0	47.7	28.7	18.3	1.0	1.7	2.3
PKE100%	2.4	47.0	27.7	21.8	0.8	1.7	2.5

[1] Ryegrass-only (Ryegrass) with 10% (PKE10%), 25% (PKE25%), 50% (PKE50%), 75% (PKE75%) and 100% (PKE100%) of PKE in the mix.

Table 4.7. Experiment 1 and 2: amounts (mg/g DM) of volatile fatty acids produced per *in vitro* incubation of the different treatments after 6, 12 and 24 hours. Approximately 0.5 g of dry matter was used during all incubations.

Feed[1]	6h	12h	24h
Experiment 1 - cows fed Lucerne chaffage plus PKE			
RW	11.4	108.8	250.7
PKE 10%	29.3	128.7	284.8
PKE 25%	46.4	134.4	292.6
PKE 50%	41.3	128.5	310.5
PKE 75%	38.8	166.6	375.9
PKE 100%	23.0	75.9	230.5
Experiment 2 - cows fed Lucerne chaffage plus PKE			
Ryegrass	55.3	119.6	293.7
PKE 10%	39.0	144.0	356.1
PKE 25%	50.1	194.2	390.7
PKE 50%	59.5	201.4	391.1
PKE 75%	85.8	192.8	352.5
PKE 100%	47.4	116.5	222.6
Experiment 2 - cows fed Lucerne chaffage only			
Ryegrass	28.8	88.0	240.1
PKE 10%	28.1	101.2	260.8
PKE 25%	33.5	102.1	303.2
PKE 50%	29.3	129.1	344.9
PKE 75%	45.7	173.3	353.3
PKE 100%	32.5	95.2	240.2

[1] Ryegrass and white clover (RW) or ryegrass-only (Ryegrass) with 10% (PKE10%), 25% (PKE25%), 50% (PKE50%), 75% (PKE75%) and 100% (PKE100%) of PKE in the mix.

4.4. Discussion

4.4.1. Chemical composition

Palm kernel expeller samples used in all *in vitro* incubations and the RW samples used in experiment 1 were the same as the PKE and RW used in the *in sacco* incubations described in Chapter 3.

The RW and Ryegrass samples used in this study presented similar characteristics, and can be characterized as good quality grasses (Waghorn et al. 2007). Ryegrass and white clover (RW) samples had higher values for NDF and CP, however, low SSS concentration than Ryegrass. Fibre was the main component in both grasses; however the lower NDF content of Ryegrass suggested that those samples were harvested in a more immature stage than RW samples (Chaves et al. 2006). High CP concentration suggested that both RW and Ryegrass samples had more leaf material than stem (Chaves et al., 2002), however higher CP values in the RW could be due to the presence of white clover (high CP content) in its composition (Burke et al., 2000) or the use of nitrogen fertilisation in the paddock (Pacheco and Waghorn, 2008), as mention previously in Chapter 3. The lower concentration of SSS (3 g/100g DM) of RW obtained in this study could be due to weather related factors, as sugar concentrations are totally dependent on the time of day and sunshine for re-synthesis of those components (Waghorn et al. 2007).

As discussed in Chapter 3, CP concentration of PKE (Table 4.1) is similar to the values reported by other publications in New Zealand and in the range of values obtained by overseas studies; however, fibre and fat fractions of PKE had greater variation between this experiment and other reports. NDF, ADF and fat concentrations of the PKE in this study were similar to that reported by Dias et al. (2008a), and they classified PKE in that study as being extracted by mechanical process due to its fat composition.

4.4.2.In vitro pH

During the *in vitro* incubations a buffer is used to reduce changes in pH, and the measurement of the pH is normally used to ensure that the *in vitro* system is simulating the *in vivo* rumen environment. Cows fed pasture diets normally have diurnal pH values

ranging from around 5.6 to 6.8 (Holden et al., 1994; Mackle et al., 1996; Carruthers et al., 1997; Wales et al., 2004), and values below 5.8 may be critical for digestion. According to de Veth and Kolver (2001a) microbial protein synthesis was optimized at pH 6.35 for high quality pasture, with pasture DM digestibility being reduced in great scale only when the pH was below 5.8 in an *in vitro* continuous culture system. In this study the increased amounts of PKE with either RW or Ryegrass resulted in some reduction of the pH; however, the results were inconsistent, but in all incubations pH values were maintained above 6.0 during the first 12 h of incubation.

4.4.3. Net NH_3 production

The estimation of the *in vitro* net NH_3 production of the different treatments showed greater net NH_3 production values in the *in vitro* incubations using RW than Ryegrass. Those differences are probably due to the presence of white clover in the RW samples of the first experiment. White clover is characterized as having higher crude protein concentrations with high degradation rates and NH_3 production (Burke et al., 2002).

The greater production of NH_3-N found in this study for the RW in experiment 1 and Ryegrass in experiment 2 is due to the quickly and extensively degradability of CP in the rumen (Van Vuuren et al., 1990). The NH_3-nitrogen produced in the rumen is either utilized by the rumen microorganisms for maintenance and growth or converted to urea in the liver, when the supply is higher than the requirements, and excreted in the urine (Tas, 2006). The steady increase in the net ammonia production for more than 24 hours observed in the RW and Ryegrass (second incubation) showed that there is a positive balance between supply and demand, and therefore an excess of ammonia is produced. This excess of ammonia can cause an impact in the metabolism (eliminate urea) (Pacheco and Waghorn, 2008) and also in the environment (nitrate leaching) (Cameron et al., 2007).

In contrast, the low CP degradability of PKE (Chapter 3) led to a low net production of NH_3. The incubation of PKE only was able to sustain NH_3 production for about 4 to 6 h, and as the amount of PKE increased in the mix with either RW or Ryegrass the net ammonia production was reduced. The reduction in the in the net NH_3 production and consequently the ruminal nitrogen losses is most likely due to the lower CP content and due to a reduction in the CP degradation of the mix, as PKE has lower

CP content than RW and Ryegrass, and lower degradation rates than RW (Chapter 3; Dias et al., 2008b). Additionally, the CP of PKE has lower rumen degradability than RW (see Chapter 3), which also decrease the availability of substrate for protein degradation and ammonia production. Therefore, rumen NH_3-N from RW and Ryegrass can be reduced with the introduction of PKE; however it is important to note that minimum ammonia concentration for microbial growth is necessary (3.5 mMol/L; Satter and Slyter, 1974), and the mixes with more than 50% of PKE were only able to be sustain the minimum concentration for about 4 to 6 h of incubation.

4.4.4. Volatile fatty acids

Volatile fatty acids are produced in the rumen as the end-products of microbial fermentation and they represent the major energy source absorbed by ruminants, accounting for 50 to 80% of total metabolisable energy supplied to ruminants (Merchen, 2002). Overall acetate was the main VFA produced during the incubations of this study, which is in agreement with France and Dijkstra (2005), who reported that during most of the times acetate is the most abundant VFA produced in the rumen when roughage based diets are fed.

Independent of the type of pasture used in this experiment the mixtures with PKE up to 75% improved *in vitro* VFA production (mmol/ g DM or mg/ g DM). This result is unexpected as the quality of both pasture samples was greater than PKE, and therefore a decrease in the VFA production should be expected. Additionally, the results of NH_3 production showed that after 6h of incubation N was restricted, which should affect VFA production, as carbon "skeletons" from the degradation of proteins contribute to the VFA pool in the rumen (Waghorn et al., 2007).

The *in vitro* incubations also showed that after 24 h of incubation the addition of PKE into the mixes increased the molar proportion of butyrate at the expense of acetate, independent of the grass type incubated with it. However, the response in the production of propionate was inconsistent when PKE was added to the different grasses throughout the incubations. According to Van Soest (1994) the distribution of the microbial population that digests feed nutrients in the rumen is usually determined by dietary composition. Therefore, the shift in favour of butyrate production due to the addition of PKE may suggest changes or interactions among microbial species in the rumen.

According to Rook (1964), the addition of concentrates to grass diets can lead to the development of either organisms that produce high proportions of propionate or high proportions of butyrate, and this result will depend on the type of supplement, rumen flora developed with the diet and physiological factors related to the animal. Additionally, the bottles with only PKE have shown lower VFA production than any other treatment tested; this result could be due to the fact that excess of lipids in the diet, more than 5-6% of DM, inhibit microbial activity and rumen fermentation (Jenkins, 1993). The average PKE fat content (9% of DM) is well above this limit.

The molar proportions of VFA produced in the rumen affected milk fat concentration (Sutton, 1989); therefore, the increased proportion of butyrate in the rumen, as a consequence of PKE supplementation, has a direct effect on milk fat concentration. Miettinen and Huhtanen (1996) reported that increased intraruminal infusions of butyrate resulted in an increase in the milk fat yield of dairy cows. After the absorption of butyrate by rumen epithelium, β-hydroxybutyrate is produced, and used for the synthesis of fatty acids in the mammary gland through de novo synthesis (Bauman and Griinari, 2003). The increase in the milk fat production that occurred due to PKE supplementation in Chapter 6 and was reported by Dias et al. (2008), was possibly a consequence of the high proportion of butyrate produced in the rumen and the higher fat content of the diet.

The lower amount of VFA produced from each kg of DM incubated in experiment 1 compared with 2, is probably due to the lack of rapidly degradable energy to accelerate the fermentation process in the incubations of experiment 1. The unusual small quantities of available soluble carbohydrates, such as soluble sugars and starch, found in RW samples and also from PKE (Table 4.1) may suggest that energy was limiting initial microbial growth, which is essential in the maintenance and development of the microbial biomass (Nocek and Russel, 1988).

The different diets fed to the cows, also had an effect on the VFA production of RW, Ryegrass and its mix with PKE. The greater VFA production from DM during the first 6 h of the first incubation suggested that the microorganisms were more adapted to that environment because the inocula was obtained from cows fed a diet containing PKE. Weimer et al. (1999) reported that the diet has an influence on the rumen environment (microbial population and chemical environment), and consequently on the VFA production. According to Huntington and Givens (1998) the *inoculums* used in the

in vitro incubations should be obtained from host animals fed diets similar to the test substrate.

4.5. Conclusion

In general, when PKE was mixed with pasture (ryegrass and white clover or ryegrass-only) net NH_3 production was reduced, with PKE only being able to maintain the net NH_3 production for just six to eight hours. In contrast, the addition of PKE with pasture resulted in greater VFA production, which is unexpected, and further studies are necessary. Acetate was the main VFA produced at all times, but the inclusion of PKE in the mix with pasture resulted in an increase of butyrate, with propionate production not changing during the incubations. In practice these results showed that PKE can affect nitrogen digestion of pasture and increased amounts supplemented in the diet can influence the availability of nitrogen in the rumen. Additionally, the mix between PKE and pasture can lead to a change in the VFA profile, which could affect the fat metabolism of the cow.

CHAPTER 5:

The effects of four levels of palm kernel expeller plus molasses (PKEM) supplementation on apparent digestibility, intake and nitrogen balance of sheep fed fresh pasture (ryegrass plus white clover)

Abstract

The effects of four levels of palm kernel expeller (PKE) plus molasses (PKEM) supplementation on apparent digestibility, intake and nitrogen balance were measured, when fed with fresh pasture (ryegrass and white clover) to lambs during two experimental periods. The pasture harvested in period 1 was of better quality than the pasture harvested during period 2, with a higher crude protein (CP; 20 vs. 13%) and lower neutral detergent fibre (NDF; 44 vs. 49%) content, respectively. Consequently, in period 1 the quality of pasture was higher than that of PKEM, but in period 2 pasture and PKEM were of similar qualities. In both periods no significant differences were found between treatments for total dry matter (DM) intake per lamb and per kg of metabolic liveweight ($LW^{0.75}$), except for total DM intake per kg of $LW^{0.75}$ at the *ad libitum* feeding level in period 1, which was significantly lower ($P<0.05$) than the other three diets containing PKEM. However, daily pasture DM intake was reduced as the amount of PKEM offered increased in both periods. Substitution rates ranged from 0.25 kg pasture DM/kg PKEM up to 1.05; these were lower at 15% of PKEM than at 45% of PKEM in the diet. At similar intakes, there was a linear decrease in the apparent digestibility of DM and CP with the addition of PKEM into the diet in both periods. However, NDF digestibility of pasture was significantly ($P<0.001$) decreased only in period 1 when PKEM was fed with good quality pasture, with similar values of NDF digestibility measured for PKEM and for the lower quality pasture fed in period 2. The estimated apparent digestibilities of DM, CP and NDF for PKEM were around 63%, 52% and 68.5%, respectively, measured in periods 1 and 2, and estimated by regression analyses from data for all lambs, fed at maintenance and *ad libitum*. The concentration of digestible energy (DE) of the diet decreased linearly when PKEM supplemented good quality pasture, whereas when PKEM was added to lower quality pasture, there was a linear increase in the DE concentration of the diet, with an estimated DE of

PKEM in both periods of approximately 12.8 MJ DE/kg DM. The intake of nitrogen (N) decreased linearly (P<0.001) as the amount of PKEM increased in the diet of good quality grass (period 1), while the opposite result was found when PKEM was fed with low quality pasture (period 2). In both periods, faecal N was significantly higher (P<0.001) when increasing amounts of PKEM were fed. In contrast, there was a gradual linear decrease in urinary N excretion in both periods when PKEM was fed in increasing amounts, with significant differences in period 1 (good quality pasture) (P<0.01). The results of N retention showed that PKEM supplementation to good quality pasture (period 1) led to a lower retention of N, although when supplemented to low quality pasture (period 2) there was a gradual increase in the retention of N. Volatile fatty acids (VFA) concentration in the rumen was reduced with PKEM supplementation to good quality pasture, but increased when fed to low quality pasture, either at maintenance or *ad libitum*. Palm kernel expeller supplementation can be used to improve diet quality of more mature pasture, despite the low DM and CP apparent digestibility. However, due to the large differences in digestibility between PKEM and high quality pasture, its supplementation decreased DE of the diet, retention of N and VFA concentration, but at the same reduced urine excretion.

Keywords: apparent digestibility, pasture, palm kernel expeller, nitrogen balance, intake, sheep.

5.1. Introduction

The incorporation of supplements into the diet of grazing dairy cows in NZ has increased in the last few years. Firstly, to fill short term feed deficits on the farm created by seasonal changes in pasture growth or by an increase in stocking rate; secondly to meet the feed requirements of high genetic merit dairy cows (Kolver et al., 2004); and thirdly to extend lactation. However due to the increase in the costs of feeds used in the farm (eg. pasture and supplements; Clark et al., 2007), interest in the feeding of lower-cost by-products from other industries has increased.

During the last eight years palm kernel expeller (PKE), which is a solid residue of the palm oil industry, has become a major feed used on NZ dairy farms, with imports of PKE increasing a thousand-fold between the year 2000 and 2008, with 800 000 tonnes being imported in 2008 (MAF, 2008). By-products, such as PKE, are generally

characterized by great variability in their chemical composition (de Ruiter et al., 2007), and overseas studies have shown that PKE quality can also vary significantly (Moss and Givens, 1994; Hindle et al., 1995; O'Mara et al.; 1999). According to O'Mara et al. (1999) PKE can be characterized as medium quality energy feed containing moderate concentrations of protein (around 16% of CP) and high fibre content (around 70% of NDF).

There is insufficient literature concerning the nutritive value of PKE for grazing dairy cattle. Based on the chemical composition of PKE coming into NZ, it can be concluded that PKE has reasonable amounts of protein (14-16% of CP) and energy (11 MJ ME/kg DM) (de Ruiter et al., 2007). Additionally the fat content in PKE (8% of DM) suggests that mechanical extraction is the main process used for its production. However chemical composition does not take into account possible interactions between PKE and the animal, and also between PKE and other feeds. Most of the published data has been measured with animals that have not been grazing on pasture.

Overseas studies have measured the *in vivo* organic matter digestibility (OMD) of PKE when mixed with conserved forages (eg. hay), giving estimated values of 650 g/kg (O'Mara et al., 1999), 750 g/kg (Moss and Givens, 1994), and 790 g/kg (Carvalho et al., 2005). According to Carvalho et al. (2005) these differences between digestibility values could be due to differences the NDF content of PKE, which was higher in the study of O'Mara et al. (1999) and similar to the other study. Moreover, O'Mara et al. (1999) found a negative relationship (increasing fibre content significantly reduced OMD) between crude fibre content and the digestibility of PKE, but the overall relationship between OMD and chemical composition was poor.

The amount of PKE in the diet could also have an effect on its digestibility. Carvalho et al. (2005) found a linear increase in the OMD with the addition of increasing amounts of PKE into a diet composed of dehydrated alfalfa. *In vitro* procedures can also be used to predict OMD of PKE; however most of the common enzymatic methods underestimate the digestibility of PKE (O'Mara et al., 1999).

Therefore, PKE is not like any other feed currently used on farm in NZ and an understanding of how it is digested by the ruminant in a pasture-based system is important. However, PKE cannot be fed as a sole feed to the ruminants and its own nutritional characteristics have to be estimated, when fed as supplement to other as the basal diet (e.g. pasture). We hypothesized that PKE would reduce the overall digestibility of the diet and digestibility would be decrease steadily as increasing

amounts of PKE were added to the diet. Based on the data from chapter 4, we would also expect a decrease in the concentration of VFA in the rumen with the addition of increasing amounts of PKE, with relatively more butyrate and acetate than propionate, and that the output of urinary N would also decrease.

Thus, the objective of this study was to measure the effects of four levels of PKE supplementation on apparent digestibility, intake and nitrogen balance when fed with fresh pasture to lambs.

5.2. Materials and methods

An *in vivo* experiment was conducted in two periods at Massey University, Palmerston North, New Zealand from late September until early December 2007. Twenty-four Romney-Suffolk ram lambs, 5-6 months of age, were used in the experiment. During both experimental periods 12 lambs (3 per treatment) were offered one of the diets, comprising pasture and increasing amounts of PKE (0, 15, 30 and 45% of the diet on an as fed basis) at either maintenance or 2 times maintenance (*ad libitum*).

The objective was for the diets to be composed of pasture and PKE only; however, during the pre-trial period most of the lambs experienced difficulties in consuming PKE probably as a consequence of its low palatability and small particle size. Therefore, the PKE was offered as a pellet blend composed of 90% PKE and 10% molasses (PKEM), and as a consequence the final diets were composed of pasture and PKEM at 4 different amounts (0, 15, 30 and 45% of the diet as feed basis).

5.2.1. Pre-trial period and animals

In September 2008 a pre-trial period was established to adapt the animals to a diet with PKE (Figure 5.1). Therefore, 35 lambs with similar liveweights (29.1kg ± 2.48), were fed a low allowance of pasture and supplemented with PKE (250g of PKE/lamb) twice a day for 20 days. Most of the lambs refused PKE, so a pellet blend of PKE with molasses (PKEM) was used in an attempt to increase the intake of PKE. Therefore, lambs went through another acclimatization period for 10 days where they were supplemented with 200g of PKEM per lamb twice a day, while grazing a low allowance of pasture.

Following the pre-trial period, 24 lambs were selected for the experiment, based on their consumption of PKEM. The same lambs were used in both periods, except for two lambs in the pasture treatment that were removed due to animal health reasons and replaced.

	Duration	Event
		Pre-trial period
	20 days	35 lambs grazing low allowance pasture in a paddock and supplemented with PKE
	10 days	35 lambs grazing low allowance pasture in a paddock and supplemented with a pellet blend of 90% PKE and 10% molasses (PKEM)
		Period 1
Adaptation period	7 days	24 lambs were selected, housed in individual metabolism crates indoor and allocated to diet treatment
Collection period	7 days	Pasture and PKEM intake was measured, and faeces and urine output were collected
	1 day	Rumen samples were collected using stomach tubing
		Inter-trial interval
	7 days	Lambs grazed "ad libitum" in a paddock with no PKEM
		Period 2
Adaptation period	7 days	24 lambs were selected, housed in individual metabolic crates indoor and allocated to diet treatment
Collection period	7 days	Pasture and PKEM intake was measured, and faeces and urine output were collected
	1 day	Rumen samples were collected using stomach tubing

Figure 5.1. Experimental schedule for period 1 and 2 with their respective adaptation and collection phases.

5.2.2. Feeds and sampling procedures

Following the pre-trial period, lambs were taken off pasture, housed in a large barn, and allocated randomly to individual metabolism crates and introduced to their diets over 7 days. Fresh pasture was cut daily between 0600 and 0730h with a sickle bar mower at a height of approximately 5 cm above ground level. The pasture fed to the lambs in the first and second period was taken from two different paddocks; even though period 2 began about three weeks after period 1, however at the time of harvesting they appeared to be at a similar stage of maturity. PKEM offered in both experimental periods was supplied in one batch by Denver Stock Feeds Ltd (Palmerston North).

Approximately 35% of the diet was offered in the morning (0900h) and 65% in the afternoon (1700h). The morning diet was fed to the lambs immediately after being weighed, while the remaining pasture was weighed in the morning according to each treatment and kept at 4°C until feeding time in the afternoon when PKEM was added. *Ad libitum* water was available at all times.

On the last day of the adaptation period harnesses were fitted for faecal collection. During the collection period, feed offered and refused, faeces, and urine volume were collected and recorded over a 7-day period, and lambs were weighed at the beginning and at the end of each digestibility period.

During both collection periods samples of pasture offered were taken daily and duplicate sub-samples used for dry matter determination, while only two samples of PKEM offered (one from each period) were taken during the experiment and duplicate sub-samples used for DM determination. The amounts of pasture and PKEM refused by each lamb were weighed daily, recorded and stored at -20°C, for later sub-sampling for DM content determination. Dry matter content was determined on samples of pasture and PKE by drying for 24 h at 100°C.

During each 7-day collection period the amount of faeces and urine were collected and recorded daily before the morning feed and a 5% and 10% sub-sample was taken from each lamb and stored at -20°C, respectively. Sulphuric acid (40-150 mL) was added daily to the urine buckets, according to the volume of urine produced individually by each lamb, to achieve a pH below 3 and avoid nitrogen losses. Prior to analysis, the daily sub-samples of faeces and urine were thawed, mixed proportionally,

bulked for each animal and frozen again for later analysis. Additionally, a sub-sample of faeces was taken for DM determination by drying in an oven for 72 hours at 100°C.

Lambs were maintained on the diet treatment for an additional day, after the 7-day collection period, for the collection of rumen samples for measurement of concentrations of ammonia and volatile fatty acids. In both periods, rumen contents were collected from each lamb by stomach tubing at 4, 8 and 12 hours after feeding in the morning. The pH of the rumen digesta was measured at the time of sampling, and the digesta were centrifuged (28,000 x g, for 15 minutes) to obtain a supernatant. One aliquot of the supernatant (1.5 mL) was collected and frozen for later VFA determination. A further 1 mL of the supernatant was acidified (15 μL of concentrated hydrochloric acid), micro-centrifuged (14,000 x g; for 15 minutes) and the supernatant was frozen for NH_3 determination. Before analysis, VFA samples were thawed, bulked within treatments and one sub-sample at each time (4, 8 and 12 hours) in each treatment/ period were analysed.

5.2.3. Analyses

Pasture offered and refused, and faeces were freeze-dried; the dried samples and samples of PKEM offered and refused were ground through a 1mm sieve. Feed offered was analysed by wet chemistry for ash (942.05), ether extract (991.36) (AOAC, 2005), and gross energy by adiabatic bomb calorimeter (Gallenkamp, UK). Neutral detergent fibre and ADF were determined for feed offered, refusals and faeces as described by Robertson and Van Soest (1981). The total N content of feed offered, feed refused, faeces and urine was analysed using a Leco analyser (968.06; AOAC, 2005), and results were expressed as CP (N x 6.25). Ammonia concentrations were measured using the glutamate dehydrogenase enzymatic method (Neeley and Phillipson, 1988) and VFA concentrations were determined according to the method described by Wronkowska et al. (2006).

5.2.4. Statistical analyses

Due to the differences in chemical composition of pasture between periods 1 and 2, the results for each period are presented individually. All data were analysed using procedures of Statistical Analysis System (SAS, 2003; version 9.1). Daily PKEM DM

intake, total daily DM intake (g and g/g $LW^{0.75}$) and liveweight gain per day (g) in each experimental period were analysed using the MIXED procedure with a linear model that included the fixed effects of percentage of PKE in the diet (0, 15, 30 and 45%). Daily PKEM DM intake, total daily DM intake (g and g/g $LW^{0.75}$) and liveweight gain per day (g) of each period is presented as the average of three lambs plus ± standard error.

The digestibility of all dietary components for individual lambs offered 0 to 45% PKEM in the diet were analysed using a random regression approach (MIXED procedure) to estimate by extrapolation the apparent digestibility of DM, CP, NDF and GE and concentration of DE of a diet consisting of 100% PKEM. Intake and proportions of PKEM in the diet were used as regressors, with animal as the random component. Linear and quadratic models were tested, however the linear model gave a better fit, based on the Bayesian information criteria (BIC) and Akaike's information criteria (AIC).

Nitrogen intake, faecal and urinary N excretion, and retention of N for individual lambs offered 0 to 45% PKEM in the diet were analysed using a random regression approach (MIXED procedure) to estimate by extrapolation the above variables of a diet consisting of 100% PKEM. Intake and proportions of PKEM in the diet were used as regressors, with animal as the random component. All the nitrogen data was adjusted for energy and DM intake effects in the analyses. Linear models gave a better fit than quadratic, based on the Bayesian information criteria and Akaike's information criteria.

Volatile fatty acid yields and NH_3 data are presented as least square means, derived from the samples bulked across time (4, 8 and 12 hours) within feeding levels in each period and for each diet (maintenance or *ad libitum*). Results were analysed by the MIXED procedure where multiple comparisons between the LSmeans of each treatment were made, a Bonferroni adjustment was applied to determine the effects of period, diet (maintenance or *ad libitum*), percentage of PKEM in the diet (feed) as well as interactions: period x diet; period x feed, diet x feed and period x diet x feed.

5.3. Results

5.3.1. Chemical composition

The chemical analyses of pasture and PKEM used in both periods are presented in Table 5.1. The pasture harvested in period 1 had greater content of CP, EE, ash and GE than the pasture harvested during period 2, and a lower concentration of NDF. The values of EE, ash and GE from PKEM were similar in both periods, with small differences in the CP and NDF content. Overall, in period 1 and 2, PKEM had higher values for NDF (by 22.7 and 14.8), EE (by 5.3 and 6.7) concentration and GE MJ/kg DM (by 1.3 in both periods) than pasture. However for CP content, PKEM had lower values than pasture in period 1, but higher values than pasture in period 2.

Table 5.1. Dry matter content, chemical composition and gross energy content of pasture and palm kernel expeller plus molasses (PKEM) offered during period 1 and 2.

Item	Period 1		Period 2	
	Pasture	PKEM	Pasture	PKEM
Dry matter (%)	15.4	91.1	20.7	91.1
	--------- g/100 g DM -------		-------- g/100 g DM ------	
Crude protein	20.0	15.8	12.9	16.7
Neutral detergent fibre	43.6	66.3	49.2	64.0
Ether extract	3.8	9.1	3.0	9.5
Starch and Sugars[1]	22.4	1.6	26.4	2.2
Ash	10.2	7.2	8.5	7.6
Gross Energy (MJ/kg)	18.2	19.5	18.0	19.3

[1] Calculated by difference (100 – crude protein – neutral detergent fibre – ether extract – ash)

5.3.2. Intake

The amount of PKEM offered initially to the animals in both periods (0, 15, 30 and 45% of the diet as fed basis) was based on a DM measurement of PKEM and pasture samples obtained at the beginning of the experiment (Table 5.2). However, the amount of PKEM offered to the lambs had to be adjusted due to the variation in the daily DM content of the pasture samples throughout the experimental periods.

The treatments throughout the text will be referred to as PKEM 0, 15, 30 and 45%; whereas in reality, the lambs were offered and consumed diets which differed slightly for the above planned in each period, as shown presented in Table 5.2.

In both periods at maintenance the percentage of PKEM eaten (DM basis) by the lambs in the diet was similar to the amount offered (DM basis); however when the diet was offered *ad libitum* animals chose to eat more PKEM than pasture, which increased the percentage of PKEM eaten in the total diet. The increase in the PKEM eaten was greater in period 2, when the quality of the pasture was lower than that in period 1 (Table 5.1).

Table 5.2. Percentage of palm kernel expeller plus molasses (PKEM) offered initially, PKEM actually offered (based on pasture and PKEM dry matter (DM)) and actual PKEM intake on the four diets at maintenance and *ad libitum* in period 1 and 2.

	Period 1							
	Maintenance				*Ad Libitum*			
PKEM initially offered	0%	15%	30%	45%	0%	15%	30%	45%
PKEM actually offered[1] (DM basis)	0%	16.8%	32.8%	48.3%	0%	16.8%	32.8%	48.3%
Actual PKEM intake[1](DM basis)	0%	16.9%	33.0%	48.4%	0%	17.2%	34.4%	50.6%
	Period 2							
	Maintenance				*Ad Libitum*			
PKEM initially offered	0%	15%	30%	45%	0%	15%	30%	45%
PKEM actually offered[1] (DM basis)	0%	12.0%	24.9%	38.8%	0%	12.0%	24.9%	38.8%
Actual PKEM intake[1] (DM basis)	0%	12.4%	25.4%	39.2%	0%	16.4%	28.1%	46.6%

[1]Calculated based on actual DM content of pasture and PKEM

Daily total DM intake (g or g/kg $LW^{0.75}$) in periods 1 and 2 are presented in Table 5.3. In period 1, lambs offered diets *ad libitum* presented an average intake 1.8 times above maintenance, while in period 2 the average intake for the *ad libitum* treatments was 1.5 times above maintenance. In both periods, for lambs fed at maintenance, no significant differences between diets were found for total DM intake and DM intake adjusted for metabolic liveweight. However, in period 1, for lambs fed *ad libitum*, pasture DM intake adjusted for $LW^{0.75}$ was significantly higher ($P<0.05$) for the pasture-only diet than for all three diets containing PKEM (Table 5.3).

In this study, PKEM supplementation resulted in a decrease in pasture DM intake. Rates of substitution were generally slightly greater at *ad libitum* than at maintenance feeding level. Substituion rate (SR) increased as the amount of PKEM was increased in the diet, except at the *ad libitum* feeding level in period 2, and SR ranged from 0.27 to 0.97 in period 1; and from 0.55 to 1.10, in period 2.

During period 1, average feed refusals were 1% (0.3-3%) and 5% (1-14%) of the feed offered for maintenance and *ad libitum*, respectively, while in period 2, they were 2% (1-13%) and 28% (11-33%) for maintenance and *ad libitum*, respectively, as a consequence of the lower quality of pasture offered in period 2.

The chemical composition of the diet treatments consumed by the lambs in each diet treatment is shown in Table 5.4. The concentrations of each nutrient in the diet were calculated by multiplying the proportion of each feed eaten in the diet by the respective content of each nutrient in each feed. Treatment diets consumed either at maintenance or at *ad libitum*, showed that increasing the intake of PKEM resulted in an increase in the NDF, EE and GE consumed by the lambs. However, the intake of starch and sugars, and ash were reduced in both periods. In period 1, CP content decreased, whereas in period 2 CP increased with the addition of PKEM.

Table 5.3. Dry matter (DM) intake of PKEM (g/day), pasture DM (g/day), total DM intake (g/day and g/kg $LW^{0.75}$/day), substitution rate and Liveweight (LW) gain (g/day) of lambs fed diets containing different amounts of PKEM at maintenance and *ad libitum* in period 1 and 2. Each value is the average of three lambs.

	Percentage of PKEM offered in the diet											
	0%	15%	30%	45%	SE[1]	P value	0%	15%	30%	45%	SE[1]	P value
	Period 1 - Maintenance						Period 1 – *Ad Libitum*					
PKEM DM intake (g/day)	-	133^{c}	235^{b}	347^{a}	12.6	0.01	-	215^{c}	430^{b}	608^{a}	17.7	0.01
Pasture DM intake (g/day)	602^{a}	566^{a}	439^{b}	358^{c}	15.0	0.01	1154^{a}	1035^{b}	819^{c}	594^{d}	25.3	0.01
Total DM intake (g/day)	602	699	674	705	34.3	ns	1154	1250	1249	1202	22.9	ns
Total DM intake (g/kg $LW^{0.75}$.day)[2]	47.7	50.1	49.2	51.5	0.09	ns	78.8^{b}	86.5^{a}	84.6^{a}	85.7^{a}	1.38	0.02
Substitution rate	-	0.27	0.77	0.74	0.127	ns	-	0.55	0.78	0.92	0.131	ns
LW gain (g/day)	163	160	87	90	70.3	ns	147	360	157	317	59.9	ns
	Period 2 – Maintenance						Period 2 – *Ad Libitum*					
PKEM DM intake (g/day)	-	113^{c}	224^{b}	330^{a}	6.2	0.01	-	215^{c}	389^{b}	599^{a}	40.1	0.01
Pasture DM intake (g/day)	831^{a}	800^{a}	658^{b}	510^{c}	25.9	0.01	1333a	1096^{b}	987^{b}	702^{c}	59.7	0.01
Total DM intake (g/day)	831	913	882	840	30.5	ns	1333	1311	1376	1301	56.7	ns
Total DM intake (g/g $LW^{0.75}$.day)[2]	58.8	60.6	60.0	57.2	1.34	ns	82.4	83.3	83.4	82.4	4.17	ns
Substitution rate	-	0.27	0.77	0.97	0.305	ns	-	1.10	0.89	1.05	0.111	ns
LW gain (g/day)	50	442	296	171	90.1	ns	213	288	167	204	93.5	ns

[1]SE = standard error.
[2] Total dry matter intake adjusted for metabolic liveweight ($LW^{0.75}$).
[a, b, c, d] Means with common superscripts within columns do not differ significantly (Pr < 0.05).
ns = non significant (P> 0.05).

Table 5.4. Chemical composition of diets consumed by lambs fed either at maintenance or *ad libitum* with different amounts of palm kernel expeller plus molasses (PKEM) in period 1 and 2 (g/100g)*.

Nutrient (g/100g DM)	Percentage of PKEM consumed in the diet							
	0%	15%	30%	45%	0%	15%	30%	45%
	Period 1 - Maintenance				Period 1 - *Ad Libitum*			
Crude protein	20.0	19.3	18.6	18.0	20.0	19.3	18.6	17.9
Neutral detergent fibre	43.6	47.0	51.1	54.4	43.6	47.5	51.4	55.1
Ether extract	3.8	4.7	5.5	6.3	3.8	4.7	5.6	6.5
Starch and sugars[1]	22.4	18.9	15.6	12.4	22.4	18.8	15.2	11.9
Ash	10.2	9.7	9.2	8.8	10.2	9.7	9.2	8.7
Gross energy (MJ/kg DM)	18.2	18.4	18.6	18.8	18.2	18.4	18.6	18.9
	Period 2 - Maintenance				Period 2 - *Ad Libitum*			
Crude protein	12.9	13.3	13.8	14.4	12.9	13.5	14.0	14.7
Neutral detergent fibre	49.2	51.0	52.9	55.0	49.2	51.6	53.4	56.1
Ether extract	3.0	3.8	4.6	5.5	3.0	4.1	4.8	6.0
Starch and sugars[1]	26.4	23.5	20.4	17.0	26.4	22.4	19.6	15.1
Ash	8.5	8.4	8.3	8.1	8.5	8.4	8.2	8.1
Gross energy (MJ/kg DM)	18.0	18.2	18.3	18.5	18.0	18.2	18.4	18.6

* Calculations based on the intake of each feed and its chemical composition.
[1] Calculated by difference (100 – crude protein – neutral detergent fibre – ether extract – ash).

5.3.3. Apparent in vivo digestibility

Digestibility results for both periods are shown in Table 5.5 and 5.6, and Figures 5.2-5.6. In each period, the data obtained from both levels of feeding (maintenance and *ad libitum*) were combined for the determination of the regression equations due to the limited number of data points for each level of feeding and to the variability between data points. Although, regression equations were estimated using the intake level.

Overall, pasture quality differed significantly between periods with the apparent digestibilities of DM, CP, NDF and GE being significantly ($P<0.05$) greater in periods 1 than period 2, however, the estimated PKEM digestibility based on the regression equations showed similar values for period 1 and period 2 (Table 5.5).

The addition of PKEM into the diet resulted in linear decreases in the apparent digestibility of DM and CP of the diet, which were similar in both periods. The

reduction in the apparent CP digestibility was significantly ($P<0.05$) greater in period 1 than period 2 (Figure 5.3). In period 2, there was only a small alteration of the NDF and GE digestibilities when PKEM was increased in the diet, while in period 1, the apparent NDF and GE digestibilities decreased linearly with addition of PKEM ($P<0.001$).

The DE during periods 1 and 2 showed linear ($P<0.05$) but opposite changes with the addition of increasing amounts of PKE into the diet. Whilst in period 1 there was a 0.4 unit reduction in the DE with the addition of 45% of PKE in the diet, in period 2 there was 0.4 unit increase of DE in the diet, either at maintenance or *ad libitum* (Table 5.6). Additionally, significant differences between period 1 and 2 in the pasture DE were found, with the DE of pasture used in period 1 being around 1.7 units higher ($P<0.05$) than the DE of the pasture used in period 2. These differences between the energy content of both pasture samples could also be observed through the relationship between DE intake vs. GE intake (Figure 5.7).

Table 5.5. Calculated[a] apparent *in vivo* digestibility (%) and concentration of digestible energy (MJ/ kg DM) of diets containing different amounts of palm kernel expeller plus molasses (PKEM) and estimated apparent *in vivo* digestibility digestible energy of PKEM at maintenance and *ad libitum*, during periods 1[b] and 2[c]. (Note: The values for 100% PKEM, shown in italics, present a wide extrapolation from measured data).

	Percentage of PKEM offered in the diet									
	0%	15%	30%	45%	100%	0%	15%	30%	45%	100%
	Period 1 – Maintenance					Period 1 – *Ad Libitum*				
DM digestibility	76.1	74.3	72.5	70.7	64.1	74.8	73.0	71.2	69.4	62.8
CP digestibility	71.8	68.8	65.8	62.8	51.8	71.0	68.0	65.0	62.0	51.0
NDF digestibility	81.3	79.2	77.1	75.0	67.3	79.5	77.4	75.3	73.2	65.5
GE digestibility	74.0	72.6	71.2	69.8	64.7	72.2	70.8	69.4	68.0	62.9
Digestible energy	13.5	13.4	13.3	13.1	12.7	13.2	13.1	12.9	12.8	12.4
	Period 2 – Maintenance					Period 2 – *Ad Libitum*				
DM digestibility	69.3	68.2	67.2	66.1	62.3	67.2	66.2	65.1	64.1	60.2
CP digestibility	60.9	59.7	58.5	57.3	52.9	56.3	55.1	53.9	52.7	48.3
NDF digestibility	68.1	68.0	67.9	67.8	67.5	66.3	66.3	66.2	66.1	65.7
GE digestibility	66.5	66.2	65.9	65.6	64.4	64.0	63.7	63.4	63.1	61.9
Digestible energy	11.9	12.0	12.1	12.2	12.6	11.5	11.6	11.7	11.9	12.2

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; GE: gross energy.

[a]Calculations based on the regressions equations of Table 5.5.

[b] DM intake used in the calculations for maintenance and *ad libitum* were obtained from data of Table 5.3 (0.67 and 1.21 kg, respectively).

[c] DM intake used in the calculations for maintenance and *ad libitum* were, obtained from data of Table 5.3 (0.87 and 1.33 kg, respectively).

Table 5.6. Linear regression equations for apparent digestibility and concentration of digestible energy of diets containing different amounts of palm kernel expeller plus molasses (PKEM), and fed to lambs at maintenance and *ad libitum*, during periods 1 and 2.

Period	Regression equation	Linear	Quadratic	R^2
	DM Digestibility (%)			
1	Y = 77.7 (± 1.59***) - 2.4 (± 1.51)Intake - 0.12 (± 0.022***)%PKEM	***	***	0.63
2	Y = 73.1 (± 1.95***) - 4.4 (± 1.71*)Intake - 0.07 (± 0.025*)%PKEM	***	***	0.41
	CP Digestibility (%)			
1	Y = 72.9 (± 2.41***) - 1.6 (± 2.4)Intake - 0.20 (± 0.036***)%PKEM	***	***	0.58
2	Y = 69.7 (± 3.15***) - 10.1 (± 2.75**)Intake - 0.08 (± 0.041)%PKEM	***	***	0.51
	NDF Digestibility (%)			
1	Y = 83.6 (± 1.75***) – 3.4 (± 1.76)Intake - 0.14 (± 0.026***)%PKEM	***	***	0.68
2	Y = 71.4 (± 2.28***) – 3.8 (± 1.99)Intake - 0.006 (± 0.029)%PKEM	Ns	ns	0.14
	GE Digestibility (%)			
	Y = 76.3 (± 1.70***) – 3.4 (± 1.72)Intake – 0.093 (± 0.0256**)%PKEM	***	***	0.49
	Y = 71.2 (± 2.03***) – 5.4 (± 1.77**)Intake – 0.021 (± 0.0265)%PKEM	**	*	0.33
	Digestible Energy (MJ kg/ DM)			
1	Y = 13.9 (± 0.30***) - 0.6 (± 0.30)Intake - 0.008 (± 0.0044)%PKEM	*	*	0.27
2	Y = 12.6 (± 0.41***) - 0.8 (± 0.35*)Intake + 0.007 (± 0.0052)%PKEM	*	ns	0.30

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; GE: gross energy. Units for intake are kg.
ns: no significant (P> 0.05).
* P< 0.05, ** P< 0.01, ***P< 0.001

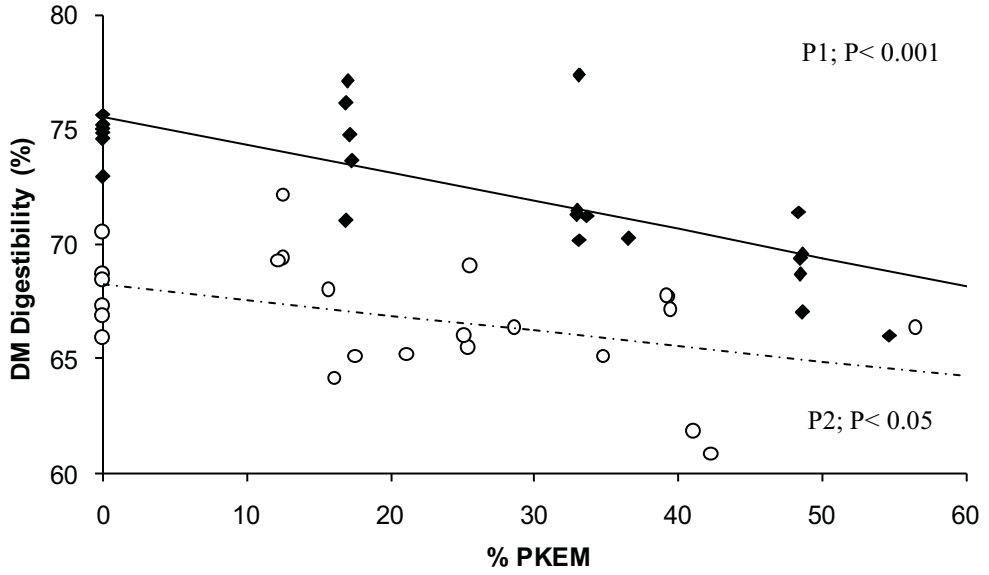


Figure 5.2. Apparent *in vivo* dry matter (DM) digestibility in diets containing different amounts of pasture and PKEM, including lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (− · −).

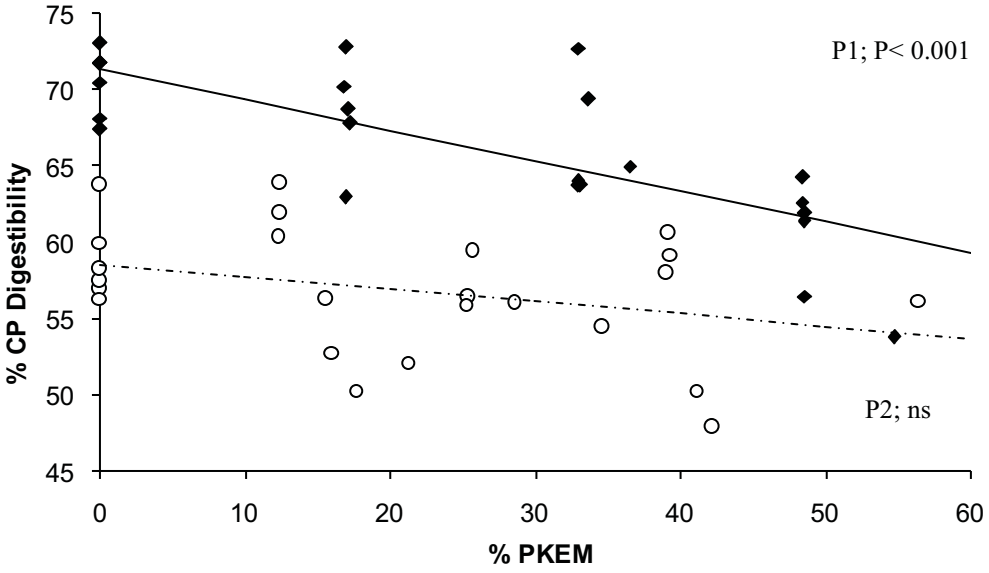


Figure 5.3. Apparent *in vivo* crude protein (CP) digestibility in diets containing different amounts of pasture and PKEM, including lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (− · −).

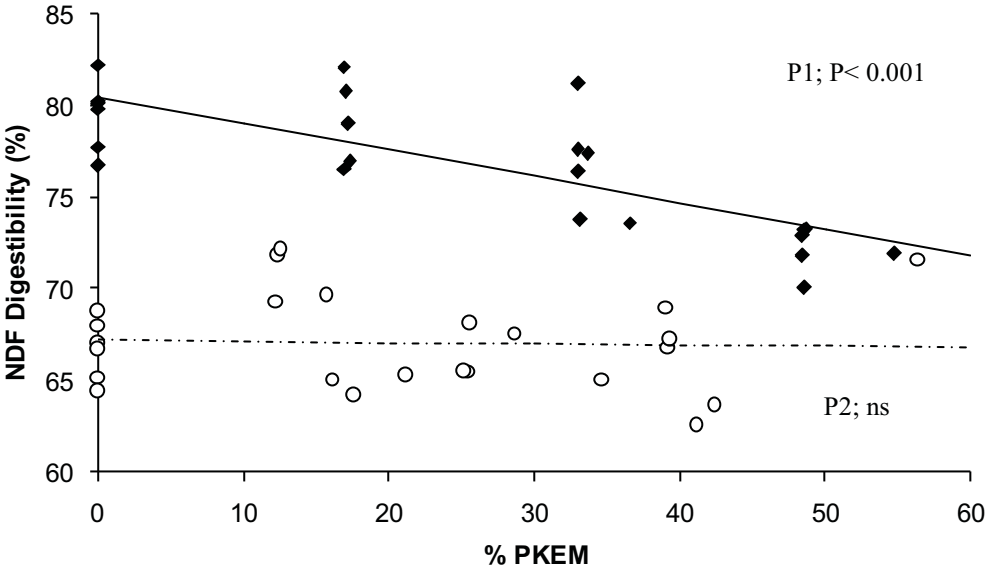


Figure 5.4. Apparent *in vivo* neutral detergent fibre (NDF) digestibility in diets containing different amounts of pasture and PKEM, including lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (– · –).

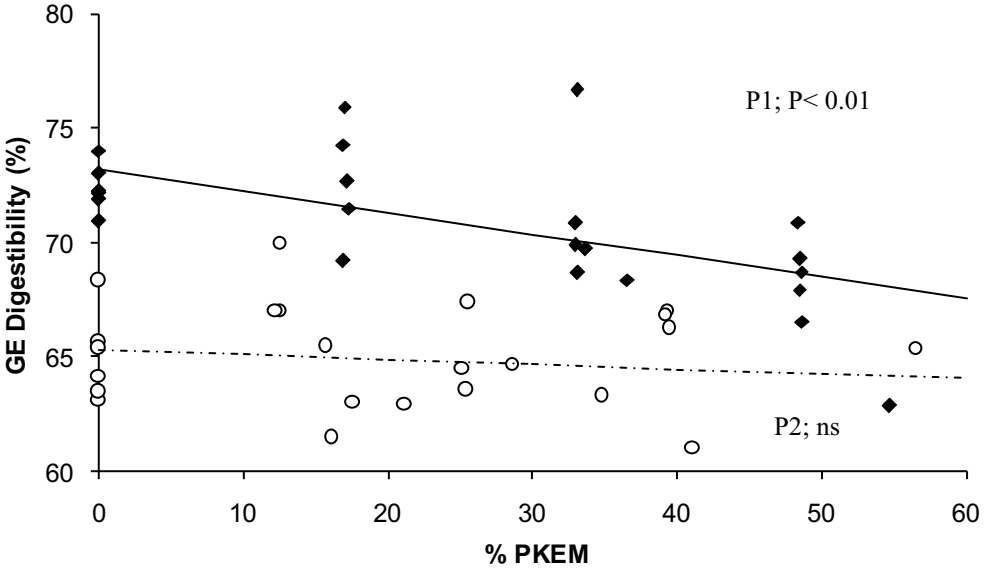


Figure 5.5. Apparent *in vivo* gross energy (GE) digestibility in diets containing different amounts of pasture and PKEM, including lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (– · –).

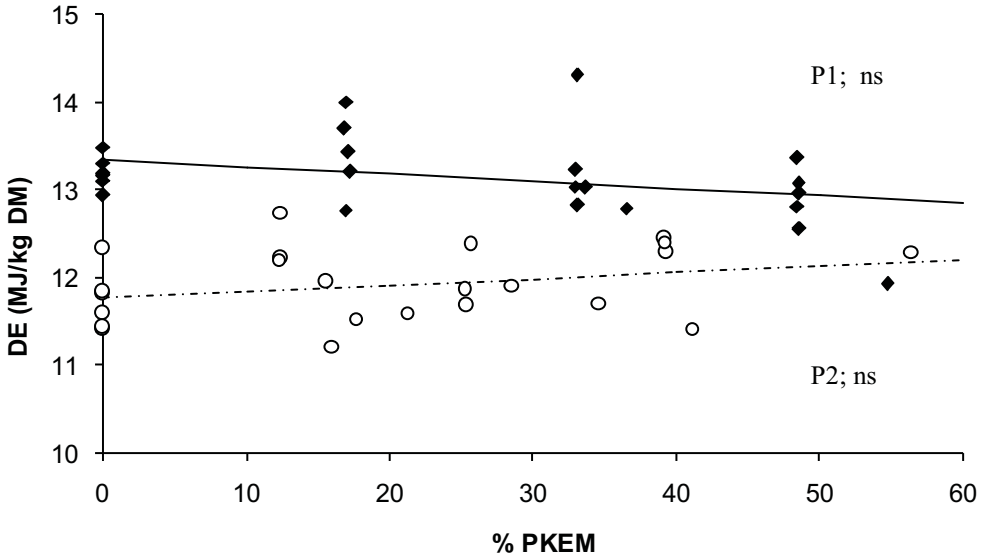


Figure 5.6. Concentration of digestible energy (DE) in diets containing different amounts of pasture and PKEM, including lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (− · −).

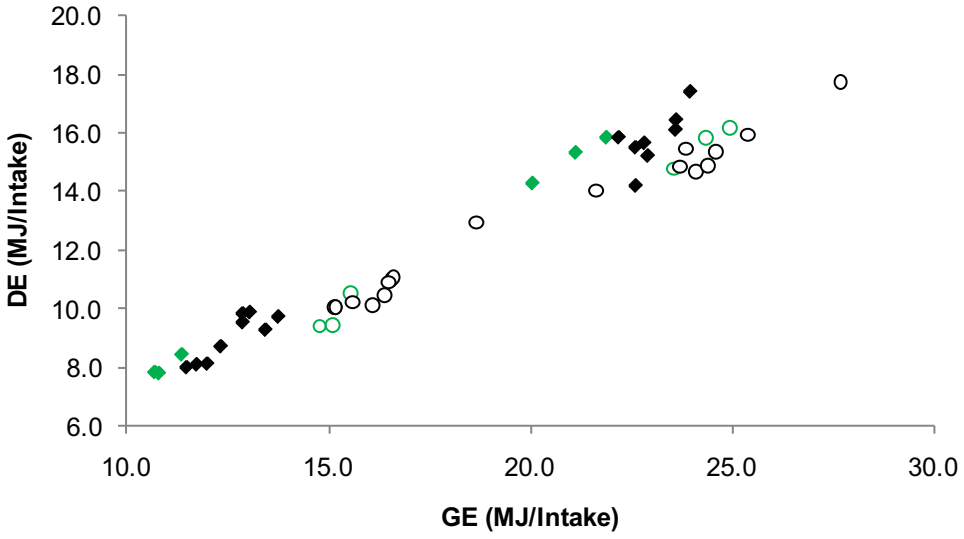


Figure 5.7. Concentration of gross energy (GE) and digestible energy (DE) in diets containing different amounts of pasture and PKEM, including lambs fed at maintenance or *ad libitum*, during period 1♦) and 2 (○) . Points with green colour in the chart symbolize pasture-only samples.

5.3.4. Nitrogen balance

Nitrogen balance results are shown in Table 5.7 and 5.8, and Figures 5.8 - 5.11. Intake of N is a consequence of the DM intake of the diet and its N concentration and, there was a linear decrease ($P<0.001$) in the N intake as the amount of PKEM increased in the diet in period 1. In contrast, there was a linear increase ($P<0.001$) in the amount of N intake with the addition of PKEM in period 2 (Figure 5.8). Furthermore, there were significant differences in the N intake when pasture-only ($P<0.001$) was fed during period 1 and 2 (Table 5.8).

In both periods, faecal N was higher when increasing amounts of PKEM were fed (Table 5.7; Figure 5.9), with linear responses ($P<0.001$). Faecal N output varied little between periods with slightly higher values for period 2 than period 1, but no significant differences were found between periods. In contrast, there was a gradual linear decrease in urinary N excretion in both periods as PKEM intake increased, with differences between slopes ($P<0.001$), but the effect of increasing amounts of PKEM was significant only in period 1 ($P<0.01$) (Table 5.7, Figure 5.10). Moreover, there were differences ($P<0.01$) in the urinary N excretion of pasture-only treatments between period 1 (1.03 and 1.27 g/kg $LW^{0.75}$ at maintenance and *ad libitum*, respectively) and period 2 (0.64 and 0.79 g/kg $LW^{0.75}$ at maintenance and *ad libitum*, respectively).

The results of N retention showed that progressive increases of PKEM in the diet (from 0 to 45%) led to a lower retention of N in period 1 (from 0.15 to 0.01 and from 0.56 to 0.42 g/kg $LW^{0.75}$ at maintenance and *ad libitum*, respectively).However in period 2 there was a gradual increase in the retention of N (from 0.13 to 0.31 and 0.24 to 0.42 g/kg $LW^{0.75}$ at maintenance and *ad libitum*, respectively), with significant differences between the slope of the two periods ($P<0.01$) (Figure 5.11).

The relationship between nitrogen intake and output of faeces and urine, and retention of nitrogen are presented in Table 5.9 and Figures 5.12- 5.14. These results are unlike any other data presented before in this study as the data is presented without looking into specific treatment or mention of PKEM. In both periods there was a linear increase in the faeces and urine excretion as the intake increased. Additionally, independent of the treatment, faeces excretion was greater in period 2 than period 1 (Figure 5.12), while the opposite pattern was found for urine excretion, which was greater in period 1 than 2 (Figure 5.13).

Table 5.7. Calculated[a] intake, losses (faecal and urinary) and retention of nitrogen (N) of diets containing different amounts of palm kernel expeller plus molasses (PKEM) at maintenance and *ad libitum*, during periods 1[b] and 2[c].

	Percentage of PKEM offered in the diet							
	0%	15%	30%	45%	0%	15%	30%	45%
	Period 1 – Maintenance				Period 1 – *Ad Libitum*			
N intake (g/kg $LW^{0.75}$)	1.61	1.55	1.49	1.43	2.64	2.58	2.52	2.46
Faeces N (% of N intake)	27.1	31.0	35.3	40.0	30.8	33.3	35.9	38.6
Urine N (% of N intake)	63.9	62.5	60.9	59.3	48.1	46.8	45.6	44.3
Retention N (% of N intake)	9.0	6.5	3.7	0.7	21.1	19.9	18.5	17.2
	Period 2 – Maintenance				Period 2 – *Ad Libitum*			
N intake (g/kg $LW^{0.75}$)	1.20	1.29	1.38	1.47	1.75	1.84	1.93	2.02
Faeces N (% of N intake)	35.8	38.0	39.8	41.5	40.9	42.2	43.3	44.4
Urine N (% of N intake)	53.7	47.7	42.4	37.7	45.1	41.2	37.8	34.6
Retention N (% of N intake)	10.5	14.4	17.8	20.8	14.0	16.6	18.9	21.0

[a]Calculations based on the regressions equations of Table 5.7.
[b] DM intake used in the calculations for maintenance and *ad libitum* were obtained from data of Table 5.3. (49.6 and 83.9 g/kg $LW^{0.75}$, respectively).
[c] DM intake used in the calculations for maintenance and *ad libitum* were, obtained from data of Table 5.3 (59.2 and 82.9 g/kg $LW^{0.75}$, respectively).

Table 5.8. Linear regression equations for intake, faecal and urinary losses, and retention (intake less urinary and faecal losses) of nitrogen (N) based on the % of palm kernel expeller plus molasses (PKEM) in the diet, with data for lambs fed at maintenance and *ad libitum* included, in periods 1 and 2.

Period	Regression equations	Linear	Quadratic	R^2
	Intake N (g/kg $LW^{0.75}$)			
1	Y = 0.12(± 0.051*) + 0.030(± 0.0004***)Intake - 0.004(± 0.0007***)%PKEM	***	***	0.99
2	Y = - 0.16(± 0.080) + 0.023(± 0.0014***)Intake + 0.006(± 0.0009***)%PKEM	***	***	0.93
	Faecal N (g/kg $LW^{0.75}$)			
1	Y = - 0.11(± 0.051*) + 0.011(± 0.0009***)Intake + 0.003(± 0.0007**)%PKEM	***	***	0.90
2	Y = - 0.28(± 0.079**) + 0.012(± 0.0008***)Intake + 0.004(± 0.0009**)%PKEM	***	***	0.93
	Urinary N (g/kg $LW^{0.75}$)			
1	Y = 0.68 (± 0.08***) + 0.007(± 0.0012***)Intake - 0.004(± 0.0011**)%PKEM	***	***	0.64
2	Y = 0.29 (± 0.12*) + 0.006 (± 0.0015**)Intake - 0.002 (± 0.0014)%PKEM	**	**	0.45
	Retention N (g/kg $LW^{0.75}$)			
1	Y = -0.43 (± 0.08**) + 0.012(± 0.0011***)Intake - 0.003(± 0.0011*)%PKEM	***	***	0.87
2	Y = -0.17 (± 0.13) + 0.005(± 0.0019*)Intake + 0.004 (± 0.0015*)%PKEM	*	*	0.35

Units for intake are g/kg $LW^{0.75}$.
* P< 0.05, ** P< 0.01, ***P< 0.001.

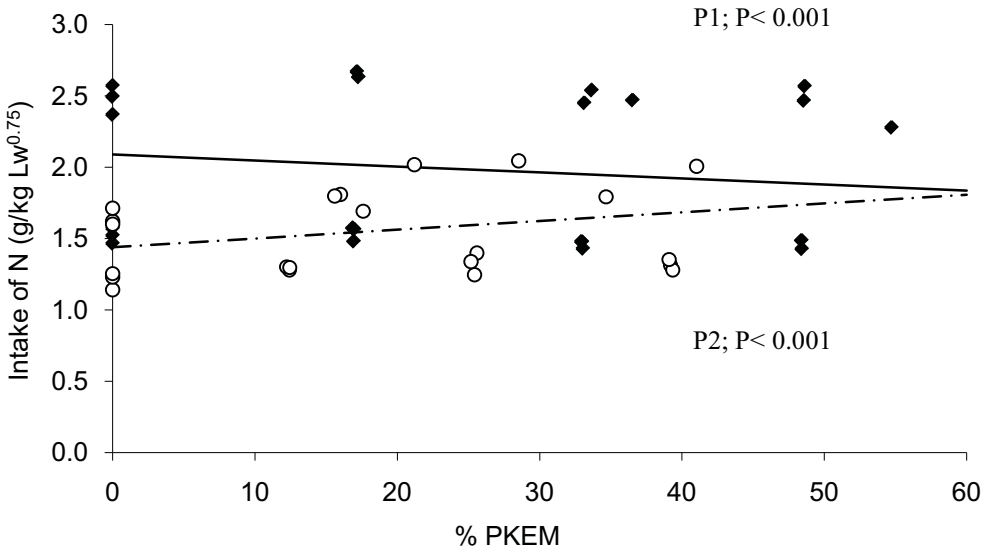


Figure 5.8. Nitrogen (N) intake of lambs fed diets containing different amounts of pasture and PKEM for lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (– · –).

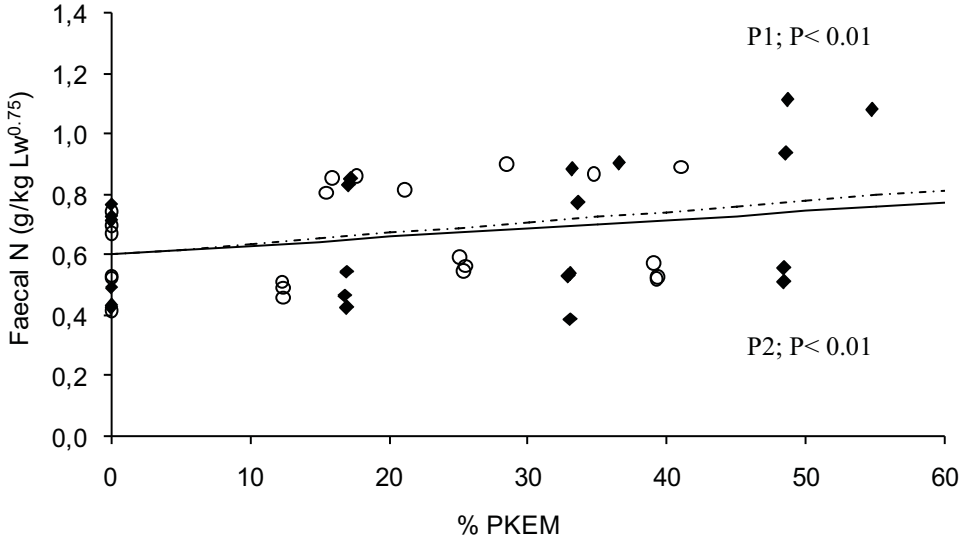


Figure 5.9. Faecal nitrogen (N) of lambs fed diets containing different amounts of pasture and PKEM for lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (– · –).

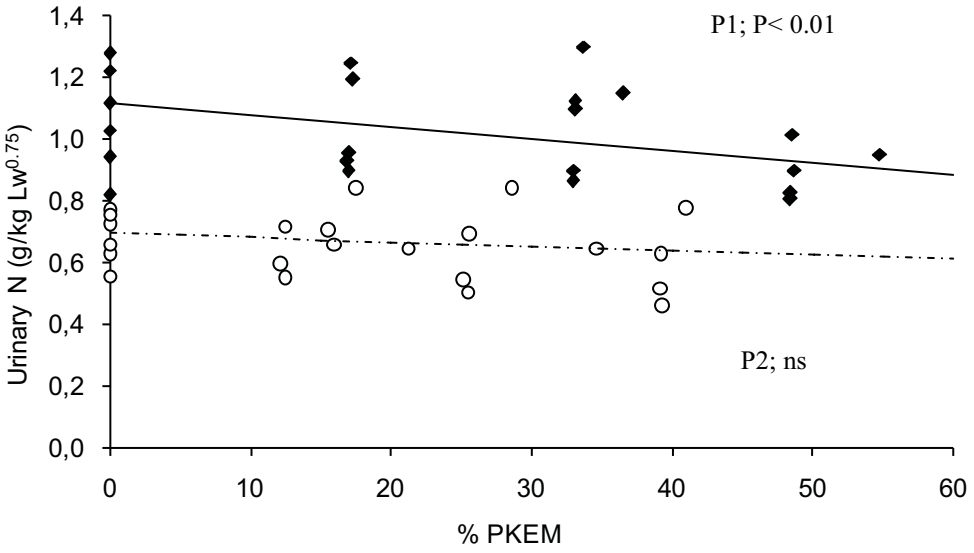


Figure 5.10. Urinary nitrogen (N) of lambs fed diets containing different amounts of pasture and PKEM for lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (– · –).

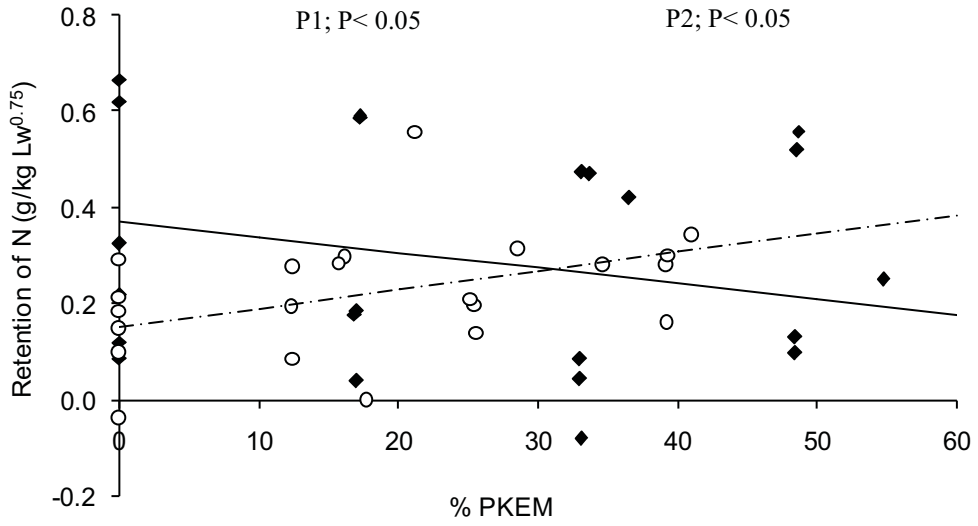


Figure 5.11. Retention of nitrogen (N) of lambs fed diets containing different amounts of pasture and PKEM for lambs fed at maintenance or *ad libitum*, during period 1 (♦) and 2 (○), and corresponding linear regression lines for period 1 (—) and 2 (– · –).

Table 5.9. Linear regression equations for faecal and urinary losses, and retention of nitrogen (N) based on the amount of nitrogen consumed in the diet, with data for lambs fed at maintenance and *ad libitum* included, in periods 1[a] and 2[b].

Period	Regression equations	Maintenance	*Ad Libitum*	Linear	Quadratic	R^2
	Faecal N (g/kg $LW^{0.75}$)					
1	Y = - 0.08(± 0.067) + 0.377(± 0.0327***)NInt.	0.49	0.88	***	***	0.83
2	Y = - 0.05(± 0.090) + 0.458(± 0.0584***)NInt.	0.88	0.99	***	***	0.82
	Urinary N (g/kg $LW^{0.75}$)					
1	Y = 0.60 (± 0.058***) + 0.209(± 0.0282***)NInt.	0.75	1.22	***	***	0.73
2	Y = 0.32 (± 0.043***) + 0.218 (± 0.0275***)NInt.	0.61	0.73	***	***	0.76
	Retention N (g/kg $LW^{0.75}$)					
1	Y = - 0.53 (± 0.062***) + 0.414 (± 0.0301***)NInt.	0.10	0.53	***	***	0.95
2	Y = -0.27 (± 0.083***) + 0.324 (± 0.0538***)NInt.	0.16	0.34	***	***	0.54

NInt: nitrogen intake (g/kg $LW^{0.75}$).
[a] Nitrogen intake used in the calculations for maintenance and *ad libitum* were obtained from data of Table 5.7 (1.52 and 2.55 g/kg $LW^{0.75}$, respectively).
[b] Nitrogen intake used in the calculations for maintenance and *ad libitum* were, obtained from data of Table 5.7 (1.34 and 1.88 g/kg $LW^{0.75}$, respectively).
***P< 0.001.

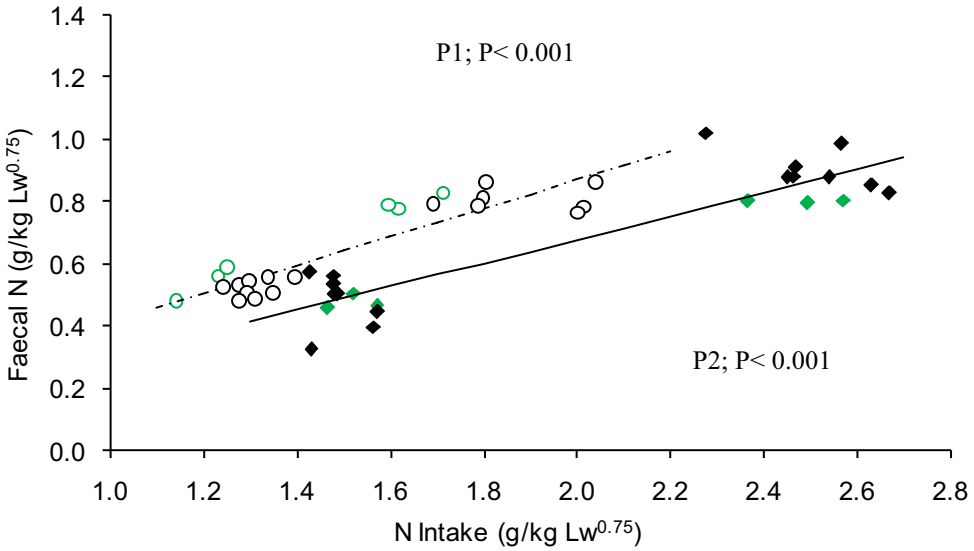


Figure 5.12. Nitrogen (N) intake and faecal N of lambs fed diets at maintenance or *ad libitum* with different combinations of pasture and PKEM during period 1 (♦) and 2 (○) and estimated faecal N excretion using linear regression in period 1 (—) and 2 (– · –). Points with green colour in the chart symbolize pasture-only samples.

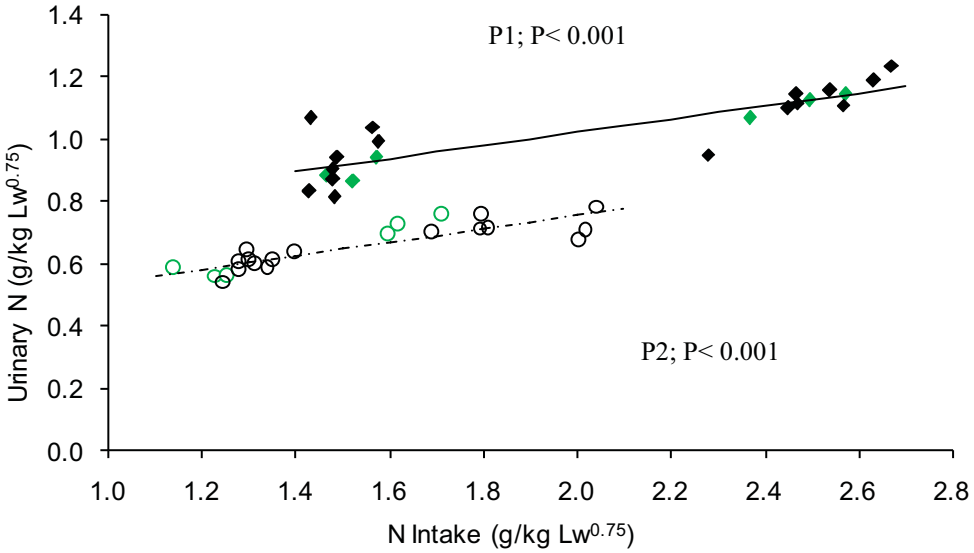


Figure 5.13. Nitrogen (N) intake and urinary N of lambs fed diets at maintenance or *ad libitum* with different combinations of pasture and PKEM during period 1 (♦) and 2 (○) and estimated urinary N excretion using linear regression in period 1 (—) and 2 (– · –). Points with green colour in the chart symbolize pasture-only samples.

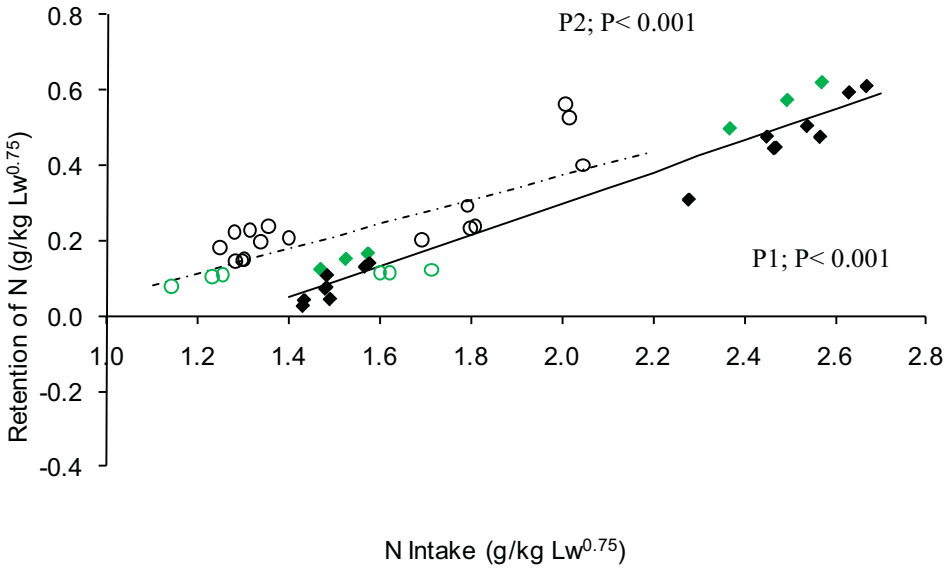


Figure 5.14. Nitrogen (N) intake and retention of lambs fed diets at maintenance or *ad libitum* with different combinations of pasture and PKEM during period 1 (♦) and 2 (○) and estimated retention of N using linear regression in period 1 (—) and 2 (– · –). Points with green colour in the chart symbolize pasture-only samples.

5.3.5. Rumen volatile fatty acids and ammonia

The mean results of rumen VFA concentration and molar percentages obtained during 12 hours of sampling after feeding lambs different amounts of pasture and PKEM are shown in Table 5.10. Because only one pooled sample was analysed from each time of collection (4, 8 and 12 hours after feeding) the data for each time sample were combined.

In period 1, increasing the amounts of PKEM in the diet reduced VFA concentration in the rumen by around 30%, both at maintenance and at *ad libitum*. Additionally, the results showed that diets containing increased amounts of PKEM tended to reduce the proportion of acetate and increase the proportion of butyrate in the rumen, except at the maintenance feeding level in period 2 (Table 5.10).

In contrast, in period 2 the addition of PKEM to the diet led to an increase in the VFA concentration, either at maintenance or *ad libitum*, with PKEM 15% showing the greatest VFA concentration compared to all dietary treatment (Table 5.10). At maintenance the molar percentages of acetate, propionate and butyrate were similar for all four diets; however, at *ad libitum*, the results were generally similar to those in

period 1, except PKEM 45%, which showed a greater butyrate concentration than pasture-only.

Results of rumen NH_3 concentrations obtained during period 1 and 2 are presented in the Figure 5.15. In period 1, PKEM 45% presented the lowest NH_3 concentration out of all the diets both at maintenance (27%) and at *ad libitum* (45%). The reduction in the NH_3 concentration was also observed for treatment diets containing 15 and 30% of PKEM and fed at *ad libitum*, however no other significant differences were found at maintenance.

During period 2, all diets presented similar NH_3 concentrations, with no significant differences between them, either at maintenance or *ad libitum*.

Table 5.10. Average concentration of rumen volatile fatty acids and molar percentages of actetate, propionate and butyrate for the four diets containing different amounts of palm kernel expeller and molasses (PKEM) and fed to lambs at maintenance or at *ad libitum* during periods 1 and 2. Molar ratios are given for acetate:propionate (A:P). Least square means (LSMeans) ± SE (standard error) from three samples collected across 4, 8 and 12 hours after feeding lambs.

	Total	Percentage of			A:P ratio
	mMol/L	Acetate	Propionate	Butyrate	
			Period 1		
	Maintenance				
Pasture	79.2	73.7[ab]	16.1	7.0[b]	4.6
PKEM15%	67.9	75.3[a]	14.5	6.8[b]	5.2
PKEM30%	64.6	75.1[a]	13.0	7.7[b]	5.8
PKEM45%	52.8	70.2[b]	15.5	10.0[a]	4.6
	Ad Libitum				
Pasture	92.8	73.5[ab]	16.0	7.1[b]	4.7
PKEM15%	80.6	75.1[a]	14.5	7.3[b]	5.3
PKEM30%	71.0	72.9[ab]	14.5	8.9[a]	5.1
PKEM45%	65.3	70.9[b]	15.3	10.1[a]	4.7
			Period 2		
	Maintenance				
Pasture	88.9	74.5	15.3	8.2	4.9
PKEM15%	98.9	74.6	15.4	7.4	4.8
PKEM30%	90.0	75.1	15.1	7.3	5.0
PKEM45%	93.2	75.5	14.2	7.4	5.3
	Ad Libitum				
Pasture	74.1	74.9[ab]	14.9	7.5[b]	5.0
PKEM15%	112.2	76.7[a]	14.1	7.2[b]	5.5
PKEM30%	102.6	75.0[ab]	15.3	7.2[b]	4.9
PKEM45%	90.2	71.6[b]	16.0	9.5[a]	4.5
SEM	14.08	1.77	1.26	0.79	0.50
Period	0.01	0.04	ns	ns	ns
Diet	ns	ns	ns	ns	ns
Feed	ns	0.01	ns	0.01	ns
Period x Feed	0.04	ns	ns	0.02	ns

[a, b, c] LS means with common superscripts within columns do not differ significantly ($P < 0.05$).
ns = non significant ($P > 0.05$).

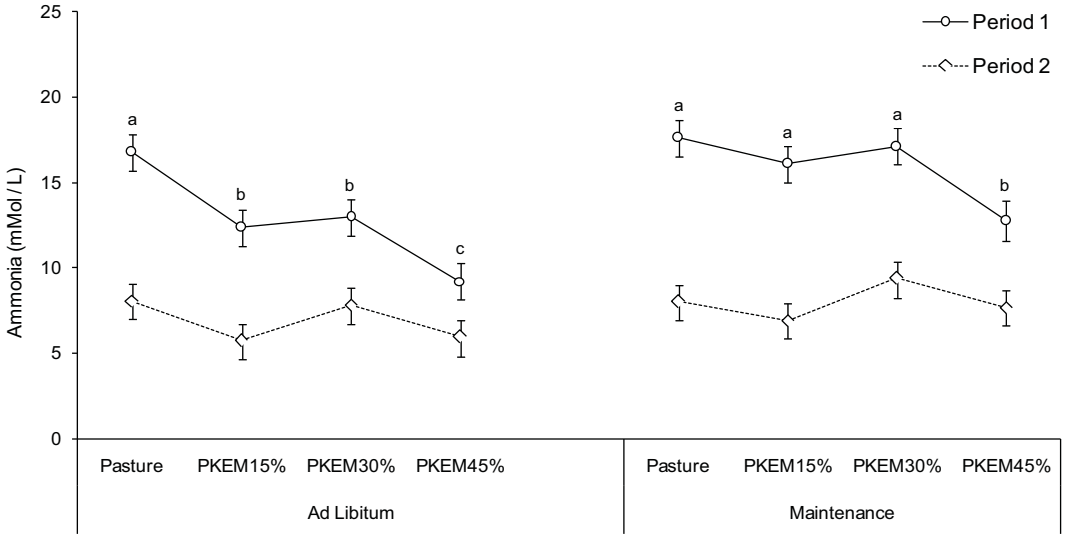


Figure 5.15. Rumen ammonia concentration (mMol/L) for lambs fed at maintenance or at *ad libitum*) on four diets containing different amounts of palm kernel expeller and molasses (PKEM) in period 1 and 2. Least square means (LSMeans) from three samples collected across 4, 8 and 12 hours after feeding lambs. LSMeans within periods with common superscripts do not differ significantly ($P < 0.05$).

5.4. Discussion

5.4.1. The quality of pasture offered

Whilst the prime objective of this experiment was to measure the effects of PKE supplementation on the apparent digestibilities and nitrogen balance of ruminants fed fresh pasture, differences in the chemical composition of the pasture harvested between the first and second periods were encountered. As a consequence, the estimations of apparent digestibility and nitrogen balance differed significantly between the two periods. Therefore, the interaction between the quality of the grass and the supplementation of PKE will also be discussed.

The chemical composition of the pasture offered in the first and second periods were distinctly different, particularly in the protein and fibre fractions. Higher CP and lower NDF concentrations were found in the pasture harvested in period 1 than the pasture harvested in period 2. Additionally, the present data also showed that apparent digestibilities of DM, CP, NDF and GE in pasture of period 1 were higher than those for the pasture in period 2, which is consistent with the corresponding differences in the chemical composition results of pastures in the two periods.

During spring time, pasture grows rapidly (Valentine and Kemp, 2007, Matthews et al., 1999), and this is associated with botanical changes caused by flowering, and also with characteristic changes in chemical composition (Waghorn and Barry, 1987). As the plant matures, NDF and lignin contents increase, while crude protein content decreases (Cherney et al., 1993; Chaves et al., 2002; Chaves et al., 2006), and the nutritional quality of pasture decreases. Changes in plant composition due to maturation are also related with the increment in the proportion of less digestible components of the plant (eg. stem) and the reduction of the digestibility of the main nutritional components of the plant, such as protein and structural carbohydrates (Waghorn and Barry, 1987), which is in agreement with the results found here.

Despite the differences between the two periods in terms of composition and quality, the results for the apparent DM digestibility in the present study agree with the values reported by Rattray and Joyce (1969), when different proportions of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens* L.) were tested, and by Minson (1990) for ryegrass (*Lolium perenne*) during different stages of growth.

5.4.2. Feed intakes and substitution

The effect of the diet on voluntary intake could be measured when the treatments were offered at *ad libitum* amounts. Total DM intake of pasture treatments in both periods (1154 g DM /day, period 1 and 1333 g DM /day, period 2) were in the upper range of values reviewed by Minson (1990) and Dulphy and Demarquilly (1994) for temperate grasses (60 to 90 g/kg $LW^{0.75}$).

However, pasture DM intake was unexpectedly higher in period 2, when the pasture quality was lower. It has been recognized that one of the main factors controlling intake is the fill capacity of the digestive tract (Forbes, 2007), which the NDF content of forages is inversely related to (Waldo, 1986; Mertens, 1994). However, in this study NDF was not a good predictor of pasture intake, as the pasture with higher NDF content showed higher intakes than the pasture with lower NDF fraction. This result may be explained by the fact that NDF content obtained from pasture with higher NDF (period 2) is not the same NDF ingested by the lambs in their diet due to the selection factor, as they showed greater refusals than lambs consuming pasture with low NDF in period 1.

The DM content of the forage is another important factor affecting intake and could be the reason for the differences in DM intake obtained here, as pasture DM content in period 1 (15% of DM) was lower than in period 2 (21%). John and Ulyatt (1987) demonstrated that forage DM content was positively correlated with voluntary DM intake at all stages of forage maturity. The volume of water trapped inside the plant cells contribute to the bulk of the food. Therefore, in period 1 when PKEM was added to the diet there was an increase in the DM content of the diet, which probably reduced the physical bulk effect of pasture and consequently increased the total DM intake to a greater extent than expected, when compared with the pasture-only diet. In other words, the beneficial effects on intake of the decrease in water content between periods 1 and 2 may have been greater than the adverse effects of the increase in NDF.

The differences in the substitution rates found between the two feeding levels, maintenance and *ad libitum*, is probably a consequence of the pasture amount offered to the lambs. In both periods, at maintenance lambs were in a restricted allowance, while at *ad libitum* lambs were offered an allowance of at least 1.6 times the maintenance level. In a review article Bargo et al. (2003) reported that as the pasture allowance increases SR also increases. Similarly, in sheep Dove (2002) reviewed some studies and reported similar results between pasture allowance and substitution rate.

An increased SR was also described in the experiments of Faverdin et al. (1991) and Wales et al. (1999). However, other studies have reported either no effect of supplement amount (Opatpatanakit et al., 1992), or an inconsistent effect of the amount of supplement on SR (Peyraud and Delaby, 2001), which was also observed in period 2 at *ad libitum*. Bargo et al. (2003) in their review reported that usually when grazing and confined cows are fed energy supplements, pasture DM intake decreases, but the level of response is affected by pasture, animal and supplement, in particular feeding level. The difference in the forage quality could be one of the reasons for the differences in the results. As forage digestibility increases substitution rates also increase (Dixon and Stockdale, 1999; Kellaway and Harrington, 2004), which is the opposite found in this study as lambs in period 2 presented higher substitution rates than in period 1.

5.4.3. Digestibilities of PKEM

The estimated PKEM digestibility obtained in this study was based on the regression equations (in Tables 5.5 and 5.6) and is an extrapolation of the measured data.

However, the similarity of values from periods 1 and 2 (eg. 64 and 63%; digestibility of DM in period 1; and 62 and 60%, in period 2) in the PKEM digestibility results (Table 5.5) gives confidence in the data, especially since this consistency was achieved despite wide differences in the digestibility of the basal pasture fed in the two periods.

The small differences between periods and feeding levels of the estimated apparent digestibilities of DM, CP, NDF and GE are probably due to the interactions caused by the differences in pasture quality. The estimated DM, NDF digestibilities and DE of PKEM found in this study in both periods were inferior to the values published by Carvalho et al. (2005) in a study where the digestibility was measured in rams fed a diet comprising dehydrated alfalfa and increasing levels of PKE. The chemical composition of their samples presented higher values for EE, CP and ash, but the NDF and DE values were similar to the present values. However, their study also showed a linear increase in the DM and NDF digestibilities, and also in the DE when increasing amounts of PKEM were fed with dehydrates alfafa, which is similar to the results found in period 2 of this study, when the quality of pasture was lower than that of PKEM.

Nevertheless, the estimated results of DM, CP, NDF and GE were similar to the average values published by O'Mara et al. (1999) for PKE, when the *in vivo* digestibility of several samples of palm kernel were fed to sheep in a diet in a diet composed of molasses and hay. However, DE values obtained in the current study are at the lower end of the values reported by O'Mara et al. (1999) for PKE. Nevertheless, the mean GE content obtained by these authors was higher (20.6 MJ /kg DM versus 19.4 MJ /kg DM reported in the current experiments), and despite a lower average EE content (78 g/ kg DM versus 93 g/kg DM in the current experiments). Moss and Givens (1994) also published the *in vivo* digestibility of 11 samples of PKE determined in sheep, and they found considerably higher values for CP, NDF digestibility and DE than the current study. However, their average contents of CP and GE were higher than the values reported in this experiment, which explains the higher digestibility and DE recorded by them, but similar values for NDF content were recorded in both studies (666 g/ kg DM versus 685 g/ kg DM in the current experiments), which does not explain the differences in the NDF digestibility.

The decrease in the DM digestibility with the addition of PKEM to the pasture basal diet in both periods seems to be associated with the lower CP digestibility of PKEM found here, as the NDF digestibility of PKEM was only lower than the digestibility of the pasture fed to the lambs in period 2. The lower digestibility of the CP

in PKEM (48 to 53%) found here was also observed in another study (O'Mara et al., 1999) and in the Chapter 3 of this thesis, which showed that effective degradability of PKE CP was around 60%. The low digestibility of PKE is probably due to the heat damage caused to the protein fraction during the expeller process of PKE (O'Mara et al., 1999). Van Soest (1994) reported that heat can produce insoluble protein, with reduced rates of proteolytic hydrolysis. The high percentage of acid detergent insoluble nitrogen (ADIN) in the CP fraction of PKE (18% of the CP content; AFRC, 1993) is an indication of the unavailable N caused by the heat process, as N digestibility is negatively associated with ADIN (Van Soest, 1994). Additionally, the increase in the amount of faeces N observed in the current study, due to the supplementation with PKEM, could be due to an increment in the indigestible N.

Despite its low DM digestibility, PKEM increased the DE content of the diet composed of low quality pasture (period 1), but decreased the DE of the diet composed of good quality pasture (period 2) (Table 5.5). According to de Ruiter et al. (2007) the energy of PKE comes mainly from its digestible fibre and its oil content, which is consistent with the present results; where the apparent digestibility of NDF in PKEM was higher than the apparent digestibilities of DM and CP; and the study of Moss and Givens (1994), where the oil fraction was totally digestible. Additionally, molasses with PKE led to an increase in the energy content of the diet, as molasses has a high concentration of sugars (70-80%) (Drysden, 2008).

5.4.4. Nirogen metabolism

Independent of the diet, up to 90% of N intake consumed by the lambs was excreted in faeces and urine in both periods of this study, which is in agreement with the range of values reported by Haynes and Williams (1993) for grazing animals. Differences in the faecal N of pasture used in periods 1 and 2 (27 vs. 36% of N intake) are probably due to the differences N intake and CP digestibility, as low N intake and lower CP digestibility should result in greater amount of faeces N, which is the case of the pasture used in period 2. The increase in the amount of faeces N with the supplementation of PKEM, even in period 1 when the N intake is reduced with the supplementation of PKEM, is probably a result of the low digestibility of PKE CP caused by the heat process, as discussed before.

Differences in urine excretion between the two pasture samples used here is probably due to the higher N content of pasture used in period 1, and to the relatively limited abilities of these lambs to anabolise N into protein (N retention). Normally, as the amount of N in the diet increases, the proportion of N excreted in the form of urine also increases (Castillo et al., 2000; Kebreab et al., 2002). Good quality pasture has high CP content, and high degradation as showed in the Chapter 3 of this study and by Van Vuuren (1990), which leads to a high NH_3 concentration in the rumen (Figure 5.12). The excess of NH_3 in the rumen is excreted as urea in the urine (Huntington and Archibeque, 2001), which increases N excreted in the urine.

Therefore the reduction in the urinary N output when PKEM was supplemented to lambs in period 1 was expected, because N intake was reduced due to the lower CP content of PKEM. The PKEM has lower degradability than high quality pasture (Chapter 3). This result could be beneficial in terms of environment impact, as the animal's urine is the main source of nitrate leaching in grazed pasture systems (Cameron et al., 2007).

However, the fact that in period 2, the urinary N output was decreased when PKEM in the diet was increased is unexpected, because the addition of PKEM to the diet increased the lamb's N intake (Table 5.7). However, it is important to note that PKEM presented low CP digestibility in both periods. Additionally, the NH_3 results showed no differences in period 2 between pasture only or mixed with PKEM diets, which demonstrates that even with PKEM showing higher CP content than pasture in period 2, N was not totally available for the microorganisms in the rumen, and therefore no excess of NH_3 was produced in the rumen and excreted as urea in the urine. According to the *in sacco* results of this thesis (Chapter 3) when the outflow rate is 3%/h, around 20% of the CP content of PKE is not digested in the rumen, and this value can reach more than 30% when the outflow rate increases to 8%/h.

The decreased N retention found in period 1 were supplemented with PKEM is probably due to the reduction in the CP intake; however all of the diets supplemented with PKEM presented positive N balance. In contrast, when low quality pasture (period 2) was supplemented with PKEM there was an increase in the N retention, due presumably to the increase in dietary N intake. Other reports where low-quality forage diets were supplemented with different sources of N has reported similar results (Bohnert et al., 2002; Currier et al., 2004; Salisbury et al., 2004). Currier et al. (2004) found an increase in the N retention in wethers consuming low quality forage (4.3% of

CP) and supplemented with different sources of N, urea or biuret, when compared with wethers receiving just forage. Salisbury et al. (2004) also reported an increment in N retention of wethers consuming low quality forage (mix of blue grama and love grass hay) were supplemented with protein concentrates, one high and the other low in rumen degradable protein. Therefore, the result of the current study suggest that PKEM can be used as a source of energy when ruminants are consuming low quality pasture (<14% CP).

5.4.5. VFA concentrations in the rumen

The lack of statistically significant differences in rumen VFA concentrations between the diets is probably due to the small number of samples analysed, as only one bulked sample was analysed for each time point. Nevertheless the numerical reduction in the rumen VFA concentrations with high quality pasture due to the supplementation of PKEM is probably a consequence of the lower digestibility of energy from PKEM, and the fact that addition of PKE reduced the lamb's DE intake. In contrast, in period 2, addition of PKEM to the low quality pasture increased numerically the VFA concentration in the rumen, which could be related to better rumen fermentation, as PKEM presented higher DE and CP content than the pasture fed to the lambs in period 2, and the addition of PKEM increased the lamb's intake of DE.

The change in the ratios of VFA concentrations in the rumen caused by the addition of PKEM to the lamb's diet, in particular the increased ratio of butyrate:acetate, was also observed *in vitro* incubations when different amounts of PKE were incubated with ryegrass and white clover, or ryegrass only (Chapter 4). As explained in the Chapter 4, PKE may cause a shift in the microbial population to organisms that produce high proportions of butyrate. Additionally, the reduction of acetate concentration in the rumen due to the high proportions of PKE in the diet (> 30% of the diet) was also observed *in vitro* (Chapter 4) and is probably due to the detrimental effect of lipids on rumen fermentation, as those diets had more 5% of fat in their composition. According to several authors (Palmquist, 1984; Jenkins, 1993; Doureau and Chilliard, 1997) lipid supplementation often reduces the digestion of ruminal fibre digestion, and thereby affects the proportions of VFA produced in the rumen, particularly the ration of acetate:propionate. The negative effects of fat supplementation have been associated with inhibition of the activity of ruminal microorganisms concerned with cellulose

digestion and methane production (Palmquist, 1984). However, in this study negative effects of PKEM on the digestion of NDF were only observed in period 1.

5.5. Conclusions

Overall, PKEM supplementation decreased pasture DM intake but increased total DM intake when it was fed *ad libitum* with good quality pasture; however there was no effect of PKEM supplementation on intake when it was fed with lower quality pasture offered *ad libitum* (period 2). Increased amounts of PKEM supplemented to lambs fed fresh pasture based diets led to a reduction in DM and CP apparent digestibility of the diet, in both periods, with pasture of low or high quality, due to the low DM and CP digestibility of PKEM. However, NDF apparent digestibility of the diet was only reduced by PKEM supplementation when pasture quality was higher (period 1), as NDF digestibility of PKEM and mature pasture, with low quality, were similar in period 2. The addition of PKEM to the mature pasture increased DE of the diet; in contrast, PKEM supplementation decreased the DE of a diet based on high quality pasture. Faeces nitrogen were similar for both supplemented pastures, however, urine N was only reduced when PKEM was supplemented to high quality pastures. Intake and retention of N was increased as PKEM was added into a diet of mature pasture, but decreased when PKEM was added to a good quality pasture.

VFA concentration was only increased with the supplementation of PKEM to more mature pasture, as a result of an increase in the energy and protein of the diet. Based on the results of this study, PKEM supplementation can be used as an alternative to improve the diet quality of more mature pasture, even with PKEM having a lower DM and CP apparent digestibility, which is usually done during summer droughts by NZ farmers. However, if farmers intent to supplement their animals when the quality and quantity of pasture is good, which is not common situation in NZ, they need to be aware of the great differences in digestibility between PKEM and high quality pasture, and in this situation PKEM supplementation can decrease DE energy of the diet, retention of N and VFA production, and consequently affect animal performance. But at the same time decreased urine excretion, which can be beneficial to the environment.

CHAPTER 6:
The effect of palm kernel expeller as a supplement for grazing dairy cows at the end of lactation

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Abstract

In recent years dairy farmers in New Zealand have fed increasingly quantities of palm kernel expeller, but with little supporting evidence for grazing cows. The objective of this research was to evaluate the effect of different amounts of PKE on the milk production of grazing dairy cows. A herd of 60 spring calved cows were used during a 28 day experimental period in the autumn of 2007. Cows were offered one of four treatments, which were either a restricted pasture allowance (20 kg dry matter/cow/day) with either 0 (RG0P), 3 (RGLP) or 6 kg (RGHP) of PKE fresh weight/cow/day or an *ad libitum* pasture allowance (40 kg dry matter/cow/day) with no PKE (HG0P). Total daily DM intake and milksolids (MS) production was highest for the cows on the HG0P treatment (12.5 kg DM/day and 1.06 kg MS/cow/day, respectively, $P<0.001$). At the restricted pasture allowance, cows under the RG0P, RGLP and RGHP treatments ate 0, 2.7 and 3.6 kg of PKE and produced 0.76, 0.86 and 0.94 kg MS/cow daily, respectively, with the treatment HG0P producing significantly more milk than the RGLP and RGHP treatments. On the restricted allowance, offering supplements significantly increased total dry matter intake compared to the treatment offered only-pasture. RGLP had a lower substitution rates (0.30 kg/kg) than RGHP (0.54 kg/kg), with significant differences between those treatments. Milk fat concentration was significantly higher in all treatments supplemented with PKE, but differences in protein concentration were not significant. The supplemented groups also had higher concentrations of short- and medium-chain fatty acids in milk, and lower concentrations of long chain fatty acids.

Liveweight gain was significantly ($P<0.05$) lower for RG0P cows than the other treatments, but no differences in the BCS were detected between treatments. Calculated marginal returns from feeding 1 kg of PKE were 42 and 53 g of milksolids when cows were offered lower and higher amounts of PKE, respectively. It is concluded that PKE can be used as a source of additional feed for grazing cows at the end of lactation, and that it could therefore be used to enable the dry off date to be delayed.

Keywords: palm kernel, dairy cows, pasture, supplement, production, milksolids, substitution rate.

6.1. Introduction

Pastoral dairy systems are based on the use of grass as the cheapest and main source of feed for milk production. However, in those systems, milk production can be limited by seasonal variations of pasture growth and composition. In the case of spring calving systems, which are common in NZ and South East Australia, the herd's requirements are synchronized with pasture growth and pasture surplus is conserved. In addition, a tactical drying-off date is necessary to coincide with decreased pasture growth which generally leads to a short lactation length. In this situation, supplementary feed can have an important role when pasture growth is insufficient and/or in situations where an increase in feed demand is created. For example, systems that have high stocking rates, high genetic merit cows or even extended lactations.

However, supplements are more expensive than pasture, and to be economic, their total cost should not surpass their value to the farm activity, directly (extra milk per animal or hectare) or indirectly (body condition score, reproductive performance, maintain post-grazing residual in order to maximize pasture production) (Clark and Woodward, 2007). In the past decade the increase in the cost of land and farm expenses (specially pasture and supplements) in New Zealand (Clark et al., 2007) has led farmers to increase the output per hectare, which results in an increase in the use of cheap alternative feeds or by-products such as palm kernel expeller, copra meal, and tapioca to supplement their herd.

Palm kernel expeller is a solid by-product from the palm oil industry remaining after the palm kernel oil has been extracted. Its use as a supplementary feed on dairy farms in New Zealand has increased steadily in the past few years. The imports of PKE

into NZ have increased from virtually nothing in 2000/2001, to almost 900,000 tonnes in 2007/08 (MAF, 2008). In some cases it is used mixed with other supplements, such as maize silage or grains, while in others it has been used as the sole supplement to pasture, in order to fill short term or emergency pasture deficits.

Despite its importance as a feedstuff for ruminants in New Zealand, very little information exists about the nutritional value of PKE and its effects on milk production when it is incorporated into the diet of pasture grazing ruminants. Research from overseas has shown that milk production of dairy cows was not affected when 0 or 15% of palm kernel solvent extracted was included in a total mixed ration (Carvalho et al., 2006), or when cows grazed a low allowance of pasture plus corn silage and a concentrate composed of different proportions of grain and PKE (Davison et al., 1994). However, the inclusion of palm kernel solvent extracted in the total mixed ration diet tended to increase the protein and lactose contents of milk (Carvalho et al., 2006), but in the study with cows grazing pasture plus supplementation (corn silage and concentrate), only the fat content of milk was increased (Davison et al., 1994).

In New Zealand, apart from limited information provided by the feed importers, the only data comes from one on-farm comparative study by a private company, which estimated a response of 72 g milksolids/kg of dry matter of PKE (51g fat and 21 g protein) (Pyke, 2006). However, it is unclear if there were any improvements in the efficiency of feed utilization, since the stocking rate was the same for the control herd and the herd fed PKE. There is a need to ascertain if those responses are due to additive or substitutive effects on pasture intake and if the higher milksolids production is due to an increase of either fat and/or protein concentration in the milk composition.

Therefore, the aim of this research was to evaluate the production (milk yield and composition) and marginal response when different amounts of PKE were offered to dairy cows grazing pasture in late lactation. We hypothesized that milk production of dairy cows grazing restricted pasture and supplemented with 6 kg of PKE would be similar to that of cows grazing high pasture allowance (or cows grazing ad libitum), and that cows on the restricted pasture allowance with no PKE would have produce the least amount of milk. Cows supplemented with PKE would also produce milk with a higher concentration of milk fat.

6.2. Material and methods

An experiment was conducted at Massey University's No. 4 Dairy Farm over a period of 28 days during autumn 2007. A group of 60 Friesian cows was selected from the main herd, 59 were multiparous cows and one was primiparous. At the beginning of the experiment, the cows were 272 ± 17 days in milk (mean ± s.d.), producing 10.4 ± 2.3 kg milk/cow/day and had a liveweight of 468.5 ± 31.4 kg.

After a covariate period of five days, when all the cows grazed as one group, the animals were allocated to one of four treatments, each of 15 cows, balanced for their pre-treatment live weight, milk yield, days in milk, age and production worth. Animals were adapted to the experimental treatments for 11 days, with the last 19 days used for measurements. Treatments offered to the animals were composed of an *ad libitum* pasture allowance without PKE or a restricted pasture allowance with or without supplementation with PKE at different levels:

- Pasture only – restricted allowance (20 kg DM/cow/d; RG0P)
- Restricted pasture + low PKE supplementation (3 kg fresh weight /cow/d; RGLP)
- Restricted pasture + high PKE supplementation (6 kg fresh weight /cow/d; RGHP)
- Pasture only – *ad libitum* allowance (40 kg DM/cow/d; HG0P).

The HG0P treatment was included to simulate an unrestricted feed allowance to the cows and also as a comparison with the restricted pasture plus high PKE supplementation. Additionally, RG0P was used for the calculations of substitution rate when PKE was offered.

6.2.1. Feedstuffs and cow management

Each treatment was offered a new break of fresh pasture every 24 h, with all four treatments grazing in the same paddock, with a total of 6 paddocks being grazed during the experiment. Electric fences were used to control daily pasture allowances, and water was available at all times through the use of portable water troughs. Each day, the treatments were positioned in a different sequence across the paddock to minimise

effects due to variation in pasture composition over the paddock. Cows were milked twice a day at 06:30 and 15:00 h. PKE was fed to the treatments after each milking in two equal amounts, in portable feed troughs (one trough per five cows) on a concrete pad. Cows were allowed to eat PKE for about 40 minutes in each period before returning to the paddock. Water was available at all times.

6.2.2. Measurements

Individual milk yields were determined automatically every day, and milk samples were collected twice a week from each cow using proportional in-line samplers in the afternoon and following morning milking. These samples were analysed for fat and protein concentration by Livestock Improvement Corporation (Hamilton, NZ) using a FT 6000 Fourier Transform infrared analyzer (Foss Electric, Hillerød, Denmark). Additionally, a bulk sample was taken once a week for each treatment, stored frozen, for later milk urea analyses. These samples were skimmed to remove fat, and analysed for milk urea concentration (MU: mMol/L) (Pacheco, 2008), and milk urea nitrogen (MUN) was calculated by the formula MUN (mg/dL) = MU (mMol/L) x 2.8 (Gooden et al., 2001). Also, a bulk milk sample (am and pm milking) from the 15 cows in each treatment was collected and stored frozen for later determination of fatty acids profile in the milk. Milk fatty acid methyl esters were quantified by gas chromatography using a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector fitted with a SupelcoTM-2560 Capillary Column 100m x 0.25mm x 0.2um film thickness. The carrier gas was helium. The oven temperature was programmed to hold at 140^{o}C for 5 minutes then to increase to 240^{o}C at the rate of 4^{o}/minute, hold for 38min. Injector temperature was 250^{o}C, Detector temperature 255^{o}C. Each peak was identified using methyl ester standards. Standards were purchased from Sigma-Aldrich Corporation (Nutrition Laboratory - IFNHH, Massey University).

The amount of PKE left in the troughs by the treatment was measured daily while the amount of PKE spilt on the concrete was measured when conditions allowed. Several samples of the PKE offered were taken, bulked for the whole trial, ground (1mm) and analysed by wet chemistry for ash (942.05), CP (968.06) and lipid (991.36) (AOAC, 2005). The neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) were determined as described by Robertson and Van Soest (1981).

The amount of pasture eaten by each group of cows was estimated every day using a sward sampling technique similar to that described by Stockdale and King (1983). Intake was based on the difference between the pre- and post-grazing pasture mass. A rising plate meter was used daily to estimate pre- and post-grazing pasture masses of each treatment break grazed per 24 hours. Between 50 and 80 rising plate meter height measurements were taken in every treatment break grazed by each group of cows. Pre-grazing measurements were also used to calculate the area required each day to offer the nominated pasture allowances of 20 or 40 kg DM/cow.

In addition, five pre- and five post-grazing calibration quadrats (0.245 m^2) were cut to ground level with hand shears from each treatment in each paddock, washed and dried to allow for conversion of meter reading to herbage mass. The relationship between pasture meter readings (MR) and herbage mass (Y) estimated from the pooled data was:

Y (kg DM/ha) = 572.4 + 123.37 x MR (cm) (R^2 = 0.68; CV=49%).

A pre-grazing sample of pasture offered was taken at 5 cm above ground level from each of the paddocks during the measurement period to indicate the quality of feed offered. Also, a second pasture sample was harvested from each treatment and paddocks grazed to simulate the herbage consumed by the cows at the different pasture allowances offered. For this "consumed herbage", samples were hand plucked at 4, 6, 6 and 8 cm for RG0P, RGLP, RGHP and HG0P, respectively.

Both pasture samples were oven dried at 60°C for 48h and ground to pass a 1-mm sieve, and analysed using a near-infrared reflectance spectrometer (FeedTech, AgResearch Grasslands, Palmerston North) to estimate concentrations of CP, NDF, ADF, lipid, ash, soluble sugars and starch, organic matter digestibility and metabolisable energy.

Liveweight gain (BW) and body condition score (BCS) of each cow was measured at the beginning and at the end of the experiment. For each treatment the LW gain was calculated as the difference between the animal's liveweight at the beginning and at the end of the experimental period.

6.2.3. Statistical analysis

Data were analysed using the PROC Mixed procedure (SAS, 2003) to compare treatments for milk yield, fat corrected milk yield, milk composition, MUN, BW and

BCS. The model included the fixed effects of treatment, time (day) as the repeated measurement, the random effect of cow nested within treatment and the residual error. Cow nested within treatment was subject to a ante-dependence (ANTE) covariance structure.

Pasture and supplement measurements were also analysed using the PROC MIXED procedure (SAS, 2003), and the treatments were considered fixed effects and the paddock was the random effect.

All means presented in this study were least squares means tested with the PDIFF option in SAS (2003) and differences were considered to be significant at $P<0.05$.

6.3. Results

6.3.1. Composition and feeds

The pasture from the six paddocks grazed throughout the experiment presented similar chemical composition with mean values for crude protein, neutral detergent fibre and metabolisable energy of 18.2, 47.7 g/100g DM and 11.6 MJ/kg DM, respectively (Table 6.1). Compared with pasture, PKE presented less than half of ash content (less 5.9 g/100g DM) and a slightly lower concentration of protein (less 3.2 g/100g DM), but much higher concentrations of fibre and fat (more 22 and 5.9 g/100g DM, respectively).

Table 6.1. Chemical composition of the pasture and palm kernel expeller (PKE) offered to cows throughout the experiment period.

Chemical component	Pasture[1, 2]	PKE[3]
Crude protein (g/100g DM)	18.2 ± 3.23	15.7
Neutral detergent fibre (g/100g DM)	47.7 ± 1.53	69.6
Acid detergent fibre (g/100gDM)	27.3 ± 1.61	38.7
Fat (g/100g DM)	2.9 ± 0.21	8.8
Soluble sugars and starch (g/100g DM)	13.7 ± 0.79	-
Starch (g/100g DM)	-	0.3
Ash (g/100g DM)	10.2 ± 0.36	4.3
Organic matter digestibility (g/100g DM)	76.6 ± 3.31	-
Metabolisable energy (MJ/kg DM)	11.6 ± 0.45	-

[1]Means ± standard deviation.
[2] determined by NIRS
[3] determined by wet chemistry
Items with a slash (-) were not measured.

Mean daily pasture allowance was similar for all restricted allowance treatments (18.2-18.8 kg DM/cow/day), and half that offered to cows fed *ad libitum* pasture (36.9 kg DM/cow/day) (Table 6.2). The average pre grazing pasture mass over the experimental period was 3100 kg DM/ha with no significant differences between treatments.

6.3.2. Pasture intakes and supplementation

Pasture DM intake for cows in the unsupplemented treatments was 8.9 kg DM/cow/day for the restricted pasture allowance and 12.5 kg DM/cow/day on the *ad libitum* pasture allowance. As expected, pasture DM intake was significantly higher for the high pasture allowance. However, for the restricted pasture treatments, the small differences in DM intake of pasture were also significant, with the lowest value for the treatment offered the highest amount of PKE. Intakes of PKE were 3.4 and 2.4 kg for RGHP and RGLP, respectively; with significant difference between the two treatments Of the 6 kg of PKE (fresh weight) offered to the RGHP treatment almost 40% was refused or wasted by the cows, while the corresponding value for the cows offered 3 kg PKE (RGLP) was 17%.

Estimated substitution rate was calculated by the difference between pasture DM intake of the treatment under restricted pasture allowance and pasture DM intake of the supplemented treatments, divided by the supplement intake. The results showed that the SR for RGLP was significantly lower than for RGHP (0.30 kg/kg vs. 0.54 kg/kg).

Overall, offering PKE significantly increased total DM intake compared to the diet offered pasture-only at a restricted allowance. Total DM intake increased from 8.9 kg DM/cow/day in the diet fed pasture-only at restricted allowance to 10.8 and 10.6 kg DM/cow/day when PKE was fed at 3 and 6 kg (fresh weight), respectively. Nevertheless, supplemented groups had lower total DM intake than the group fed pasture-only at high allowance.

Table 6.2. Daily pasture allowance, pre and post grazing pasture height and mass, dry matter (DM) intake and substitution rate for cows fed either a restricted pasture allowance plus 0 (RGOP), 3 (RGLP), or 6 (RGHP) kilograms of fresh PKE/cow/day or *ad libitum* pasture allowance (HG0P).

	RG0P	RGLP	RGHP	HG0P	P
Pasture allowance (kg DM/cow/day)	18.2 ± 0.28^{b}	18.5 ± 0.23^{b}	18.8 ± 0.32^{b}	36.9 ± 0.36^{a}	0.001
Pre grazing pasture height (cm)	20.5 ± 1.95	21.2 ± 1.91	20.8 ± 1.93	20.6 ± 1.90	ns
Pre grazing pasture mass (t DM/ha)	3.1 ± 0.24	3.2 ± 0.23	3.1 ± 0.24	3.1 ± 0.23	ns
Post grazing pasture height (cm)	8.2 ± 0.57^{d}	9.3 ± 0.56^{c}	10.4 ± 0.57^{b}	11.6 ± 0.59^{a}	0.001
Post grazing pasture mass (t DM/ha)	1.54 ± 0.29^{d}	1.69 ± 0.039^{c}	1.82 ± 0.039^{b}	1.97 ± 0.052^{a}	0.001
Pasture DM intake (kg DM/ cow/day)	8.9 ± 0.50^{b}	8.3b ± 0.50^{c}	7.2 ± 0.51^{d}	12.5 ± 0.65^{a}	0.001
Supplement DM intake (kg DM /cow./day)	-	2.4 ± 0.02^{b}	3.4 ± 0.18^{a}	-	0.001
Total DM intake (kg DM /cow/day)	8.9 ± 0.50^{c}	10.8 ± 0.50^{b}	10.6 ± 0.55^{b}	12.5 ± 0.65^{a}	0.001
Substitution rate (kg DM/kg DM)	-	0.30 ± 0.077^{b}	0.54 ± 0.070^{a}	-	0.02

[a, b, c] LSMeans within each row with a different superscript are significantly different from each other ($P < 0.05$);
ns, not significant.

6.3.3. Diet quality, milk production and liveweight change

The chemical composition of the diets in the four treatments are shown in Table 6.3. These values were calculated from the composition of the pasture and PKE in the diet offered and the proportional daily intakes of pasture and PKE. There were

significant differences between the CP concentration in the diet of the different treatments, with greater values for HG0P (P<0.002). However no significant differences were found between the treatments offered restricted allowances of pasture. The fibre concentrations of the treatments supplemented with PKE were greater than the pasture-only treatments as a consequence of the higher NDF content in the PKE (Table 6.1). Additionally, the concentration of dietary fat was lower in the treatments offered pasture-only. These treatments contained 4 and 5 g/100g DM of fat for RGLP and RGHP, respectively, with this difference being significant (P<0.001) (Table 6.3).

Milk yield was 7.3 and 11.4 kg/cow/d in the restricted and *ad libitum* pasture allowance treatments with no supplement, respectively, with intermediate values for the supplemented treatments (Table 6.4). For the treatments offered restricted pasture allowances, significant differences in milk yield were found between the treatments without supplement and the supplemented treatments (P <0.001). Milk fat concentration was significantly lower (P<0.001) for the treatments without PKE supplementation than for the groups without supplementation. Milk lactose was significantly greater (P<0.001) in HG0P (4.9 g/kg) than the other three treatments. There were no significant difference in the milk protein concentration and MUN, with values around 4.1 g/kg and 19.2 mg/dL, respectively.

The analysis of one bulk milk sample for fatty acid content, for each treatment, showed that supplementing with PKE increased the concentration in fat of the short- and medium-chain FA, and reduced the concentration of long chain fatty acids; with small differences between high and low pasture allowance. Fat in PKE contained a much higher concentration of saturated FA than pasture (82% versus 23%), but a much lower concentration of unsaturated fatty acids (18 versus 74%). Therefore, the supplementation of PKE to cows on a restricted pasture allowance, at either 3 or 6 kgDM/cow/day, resulted in milk with more saturated fat and less unsaturated fat than cows fed pasture only.

Liveweight changes were significantly lower for RG0P (+1.1 kg/cow/day) cows than for the high allowance and supplemented cows (+ 1.6 to 1.8 kg/cow/day). No significant differences in BCS were detected between treatments.

Short term marginal responses, defined as the increase in MS yield for each increment in feed DM intake from supplements or pasture, were calculated from the data in Table 6.2 and 6.4. The marginal returns from feeding 1 kg of PKE were an extra 42 and 53 g of MS when cows were offered lower and higher amounts of PKE,

respectively, with fat contributing more to this increase in yield of MS than protein. In comparison, the marginal response from feeding an additional 1 kg of pasture was 50 g of MS.

Table 6.3. Chemical composition (g/100 gDM) of diets eaten by cows during the experiment. Data are calculated based on the composition of PKE eaten by the supplemented treatments, and the composition of pasture cut at 4, 6, 6 and 8 cm above the ground for the restricted pasture allowance (RG0P), restricted pasture allowance plus 3 (RGLP) and 6 (RGHP) kilograms of fresh PKE /cow/d and *ad libitum* pasture allowance (HG0P), respectively[1].

Chemical component	RG0P	RGLP	RGHP	HG0P	P
Crude protein	17.0 ± 1.19^{b}	18.2 ± 1.19^{b}	17.6 ± 1.19^{b}	21.2 ± 1.19^{a}	0.002
Neutral detergent fibre	48.1± 0.72^{b}	52.6± 0.89^{a}	55.2 ± 0.24^{a}	45.9 ± 0.86^{b}	0.001
Acid detergent fibre	27.9 ± 0.63^{c}	29.7 ± 0.63^{b}	31.2 ± 0.63^{a}	25.7 ± 0.63^{d}	0.001
Fat	2.8 ± 0.11^{d}	4.4 ± 0.11^{b}	5.0 ± 0.11^{a}	3.2 ± 0.11^{c}	0.001
Ash	10.0 ± 0.19^{b}	8.7 ± 0.08^{c}	8.3 ± 0.12^{d}	10.6 ± 0.15^{a}	0.001

$^{a, b, c}$ LSMeans within each row with a different superscript are significantly different from each other ($P < 0.05$);

[1]Pasture eaten was based on the difference between pre and post measurements at each paddock and treatment. PKE eaten was based on the difference between the amounts offered less the amount refused and wasted.

Table 6.4. Milk production and changes in live weight (LW) and body condition score (BCS) of cows fed either a restricted pasture allowance offered either 0 (RG0P), 3 (RGLP) or 6 (RGHP) kilograms of fresh PKE /cow/day or an *ad libitum* pasture allowance (HG0P) without any supplement.

	RG0P	RGLP	RGHP	HG0P	P
Milk yield (kg/cow/day)	7.3± 0.29[d]	8.3± 0.30[c]	9.1± 0.30[b]	11.4± 0.30[a]	0.001
3.5% FCM (kg/cow/day)	10.3± 0.39[d]	11.9± 0.40[c]	13.1± 0.40[b]	14.4± 0.40[a]	0.001
Milk solids yield (kg/cow/d)	0.76 ± 0.026[d]	0.86 ± 0.027[c]	0.94 ± 0.027[b]	1.06 ± 0.027[a]	0.001
Milk protein (g/kg)	4.1 ± 0.03	4.2 ± 0.03	4.2 ± 0.03	4.1 ± 0.03	ns
Milk fat (g/kg)	5.9 ± 0.08[b]	6.4 ± 0.10[a]	6.5 ± 0.12[a]	5.5 ± 0.08[b]	0.001
Milk lactose (g/kg)	4.6 ± 0.02[b]	4.6 ± 0.02[b]	4.6 ± 0.02[b]	4.9 ± 0.01[a]	0.001
Milk urea-N (mg/dL)	19.4 ± 1.98	18.8 ± 1.98	18.1 ± 1.98	20.6 ± 1.98	ns
BCS change (units of BCS)[2]	-0.1 ± 0.07	0.0 ± 0.07	0.0 ± 0.07	0.1 ± 0.07	ns
LW change (kg/day)	1.1 ± 0.09[b]	1.6 ± 0.12[a]	1.8 ± 0.15[a]	1.7 ± 0.09[a]	0.001

[a, b, c] LSMeans within each row with a different superscript are significantly different from each other (P <0.05);
ns, not significant.
[1]3.5% Fat Corrected Milk (FMC) = (0.4324 x kg milk) + (16.216 x kg fat).
[2]Differences in BCS between the begin and end of the trial.

Table 6.5. Fatty acid composition of pasture and palm kernel expeller (PKE) offered to cows during the experiment; and fatty acid composition of bulk milk fat samples taken from cows fed a restricted pasture allowance offered either 0 (RG0P), 3 (RGLP) or 6 (RGHP) kg of fresh PKE /cow/day or an *ad libitum* pasture allowance (HG0P).

	Feeds		Bulk milk			
Fatty acids (g/100g)	PKE	Pasture	RG0P	RGLP	RGHP	HG0P
C6:0	0.4	ND	2.8	2.7	2.5	3.0
C 8:0	4.2	0.3	1.1	1.0	0.9	1.2
C 10:0	3.5	ND	2.2	2.1	2.0	2.4
C11:0	ND[a]	ND	0.3	0.3	0.3	0.3
C 12:0	46.9	0.5	2.5	7.0	9.0	2.8
Total short-chain	*55.0*	*0.8*	*8.9*	*13.1*	*14.8*	*9.7*
C13:0	0.4	ND	ND	0.2	0.3	0.1
C 14:0	15.6	0.5	10.6	13.3	14.1	10.7
C 14:1	ND	ND	1.0	1.2	1.6	1.0
C15:1	ND	ND	ND	ND	ND	ND
C 16:0	8.3	18.0	28.6	27.6	28.3	27.8
C 16:1	ND	0.2	1.9	2.1	2.2	2.0
C17:0	ND	0.3	0.8	0.6	0.5	0.8
Total medium-chain	*24.3*	*19.0*	*42.8*	*45.0*	*47.1*	*42.5*
C 18:0	2.4	2.6	13.8	12.2	11.1	14.1
C18:1 -trans9	ND	ND	0.4	0.3	0.4	0.4
C18:1 -trans11	ND	ND	3.5	2.5	1.8	3.6
C 18:1-cis9	15.6	2.9	25.5	23.6	22.2	24.9
C18:1 -cis11	0.1	0.2	0.3	0.3	0.3	0.3
C 18:2-trans9,12	ND	ND	ND	ND	ND	0.0
C18:2 -cis9,12	2.4	10.8	1.0	0.7	0.6	0.9
C18:3 -cis6,9,12	ND	0.2	ND	ND	ND	ND
C 18:3 -cis9,12,15	ND	59.9	1.1	0.5	0.4	1.1
C 20:0	0.1	0.5	ND	ND	ND	ND
Total long-chain	*20.6*	*77.1*	*45.7*	*40.1*	*36.8*	*45.3*
Others	0.1	3.3	1.4	1.0	0.7	1.5
Total Unsaturated[a]	18.1	74.3	34.8	31.2	29.6	34.3
Total Saturated[b]	81.8	22.6	62.7	67.0	69.0	63.3

ND=non determined.
[a]Sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0.
[b]Sum of C14:1, C16:1, C18:1, C18:2 and 18:3.

6.4. Discussion

The nutritive composition of the pasture used during the experiment can be considered of medium quality (Burke, 2004), with some paddocks being grazed at a more mature stage. As pasture matures there is a progressive decrease in its nutritive value (Chaves et al., 2006), which in association with the late stage of lactation, could explain the low milk yields reported in this study.

The chemical analyses of PKE agree with those reported by O'Mara et al. (1999), except for NDF concentration, which was lower (70 g/100g DM) in the present study. However, the present NDF content agrees with the values reported by Carvalho et al. (2005). When the data in Table 6.1 is compared with published data, the relatively high fat (ether extract) concentration suggests that the PKE used in this trial was produced by mechanical pressing rather than by solvent extraction (O'Mara et al., 1999; de Ruiter et al., 2007).

Significantly lower post-grazing pasture height and mass for the treatment offered unsupplemented restricted pasture indicated that those cows were forced to graze harder (post grazing residuals below 1600 kg DM/ha) than the cows fed restricted pasture with supplements and the cows offered a high allowance of pasture (post grazing residuals above 1700 kg DM/ha). As expected, cows on restricted pasture allowance with no supplements were energy deficient (Penno et al., 2001, Holmes and Roche, 2007), which can be measured as the decrease in MS yield of the control treatment (RG0P) after changing from high pasture allowance, in the covariate period, to restricted pasture allowance. (Penno et al., 2006). The level of feeding offered to the cows before the experiment, in the covariate period, was probably higher than the RG0P but lower than the HG0P, as indicated by the changes in the kg MS/cow daily between the two periods for these groups (-0.20 kg MS/cow/day vs. +0.10 kg MS /cow/day).

The increase in pasture DM intake with increasing pasture allowance, (0.19 kg DM/kg DM of extra herbage offered) is in agreement with the studies of Wales et al. (1998, 1999), which found values ranging from 0.13 to 0.28 kg DM/kg DM when cows were offered similar pasture allowances. These results are also in agreement with Bargo et al. (2003), who reviewed several studies on the effect of pasture allowance on pasture DM intake, and suggested that overall pasture DM intake increase 0.19 kg/kg of increment in the pasture allowance.

Increasing the level of supplementation with PKE resulted in a decrease of pasture DM intake, known as substitution, but increased total DM intake. This same result was observed in many other studies where cows were fed different levels of supplements (Reis and Combs, 2000; Sayers et al., 2003; McEvoy et al., 2008). According to Bargo et al. (2002a), the reduction in the grazing time is the main cause of substitution when concentrates are supplemented at low pasture allowances.

The increase in the SR as the intake of PKE increased, observed in this study, is in agreement with by Kellaway and Harrington (2004) and previous experiments (Faverdin et al., 1991; Wales et al., 1999). However, other studies have shown either no effect of supplement amount (Opatpatanakit et al., 1992), or an inconsistent effect of the amount of supplement on SR (Peyraud and Delaby, 2001).

Supplementation of PKE in cows offered restricted pasture allowance increased total daily metabolizable energy intake and the amount of milksolids produced daily. Nevertheless, fat-corrected milk and milksolids yield of cows fed the restricted pasture allowance plus PKE, even with the largest amount of PKE offered, were not as high as those of cows on the *ad libitum* pasture allowance alone. The main reason for those results is the lower intake of PKE by the cows at high supplementation. The greater refusals by cows on the RGHP treatment suggest that 6 kg of PKE could be above the maximum voluntary intake for this type of feed, when it is offered only for short periods of time, such as for 40 minutes twice daily in this study. Sporndly and Asberg (2006) reported lower intake rates for PKE than ground barley (29 vs. 128 g/minute) when heifers with limited experience in concentrate feeding were fed 1kg/twice day of either feed for a short period of time (10 minutes). The low intake of PKE by cattle could be associated with either the dry nature of PKE or its palatability (McDonald et al., 2002). According to Phillips (1993), dusty meals (e.g. PKE) are not eaten rapidly, and more saliva is necessary to produce a bolus suitable for swallowing. During this study it was also observed that PKE can have great wastage when fed alone and in great quantities, which could be also an important aspect on the intake of these supplements by dairy cows.

The differences in milk production between the treatments under PKE supplementation, even with similar intake, may be due to the higher energy intake of the RGHP treatment compared to the RGLP treatment, as energy is the most limiting nutrient for milk production with pasture based systems (Kolver and Muller, 1998). Calculations of the diets eaten by the cows in showed that intakes of fat and fibre

increased, as the amount of PKE offered in the diet increased from 22 to 32%. Increasing the amount of fat in the diet increased its energy density, as fat contains more energy for lactation than protein or carbohydrates (Tyrrell, 2005), which is probably the reason for difference in milk production between the supplemented treatments. However it is important to notice that the excess fat in the diet (above 5% DM based) can cause a negative effect in the DM intake and fibre digestibility in the rumen (Schroeder et al., 2004), and the fat content of PKE is not enough to increase its digestible energy to the same levels of a good quality temperate pasture (see chapter 5).

The extra milk obtained from 1 kg of PKE supplemented is in the range of responses reported by Holmes and Roche (2007) for short-term experiments, 30-60 g MS/kg DM. However, the inclusion of PKE in the diet resulted in lower marginal response in MS production than extra pasture, 48 g MS/kg DM of extra PKE vs. 83 g MS/ kg DM extra pasture offered, a difference that may be due to the higher ME/kg DM in grazed forage (see Chapter 5). Additionally, the marginal response calculated for PKE in this study does not take into account the extra liveweight gained or the extra pasture obtained from the substitution. Therefore, if all those factors plus the fact that PKE supplementation could be used to delay the drying off date and thus to extend the lactation period, larger long-term responses could be obtained on dairy farms (Holmes and Roche, 2007).

The highly saturated composition of the FA found in the fat content of PKE is in agreement with the values reported by Cornelius (1977) for palm kernel oil, where the short-chain FA C12:0 (lauric acid) is its main component. The increased milk fat percentage found in this experiment where PKE was supplemented to cows is in agreement with the review of Shroeder et al. (2004), who reported an average 5% unit increase in the milk fat percentage with the supplementation of saturated fat. Milk fat percentage and composition is dependent on the balance between an increase in the exogenous FA uptake and transfer to the mammary gland, and a decrease in the de novo synthesis (Salado et al., 2004; Shroeder et al., 2004). The milk FA results of cows supplemented with PKE showed a higher percentage of short- and medium-chain milk FA, C4:0 to C14:0, which are derived from de novo synthesis of acetate and β-hydroxybutyrate in the mammary gland (Bauman and Griinari, 2003; Bargo et al., 2006), therefore, the supplementation of PKE suggests a positive balance and an increase in the milk fat percentage. The increased proportion of saturated FA in milk of cows supplemented with PKE can be of some concern, as the concentration of saturated fatty

acids in ruminant products, such as milk, is considered to be negative for human health. The consumption of diets with elevated concentration of saturated fats (C12:0, C14:0 and C16:0) has been connected with an increase in the cholesterol levels (Grundy and Denke, 1990; Katan et al., 1994).

Cows in all treatments were gaining liveweight as would be expected for the treatment supplemented with PKE and offered a high allowance of pasture, because of their advanced stage of lactation (272 ± 17.0 days in milk at the start of the experiment); when energy requirements for milk production are lower than energy intake (Vazquez-añon et al. 1997). During late lactation higher amounts of feeding results in energy partitioning towards body reserves and less into milk production (Garnsworthy, 1997), which will result in greater gains of live weight. However it is difficult to explain the higher weight gains found in this experiment for the group under restricted allowance of pasture and no supplementation (1.1 kg/day), as all treatments showed negative energy balance if we take into account the liveweight gain obtained here, which is not true.

Generally, liveweight change is not a precise prediction of mobilization or storage of energy, as they can be affected by gut fill and weight of the uterus and fetal-placental unit (Enevoldsen and Kristensen, 1997; Schroeder and Staufenbiel, 2006). In this study, the BCS change show a more realist result, with zero or positive change for supplemented cows and cows fed high pasture allowance, respectively, and a negative result for low pasture allowance without supplements. These results are in agreement with the DM intake obtained by the different treatments, and which reflects the amount of energy consumed by the cows on these treatments.

6.5. Conclusions

The results of this study showed that PKE can be fed successfully as an alternative supplement in late lactation. However, the response to PKE supplementation was less than that to feeding extra pasture of moderate quality, probably as consequence of the lower intake of PKE when offered at high amounts (6 kg DM/cow/day). But, if the cows had not been supplemented with PKE they would probably have had to be dried off, to increase the pasture cover of the farm. When supplements are used to extend lactation, they deliberately replace grazed pasture in order to save pasture, while at the same time maintaining feed levels (Holmes and Roche, 2007). In addition the present experiment was relatively short, so that its short-term responses to PKE

supplementation did not include the possible effect of extra liveweight gain or of pasture saved.

CHAPTER 7:
General discussion, final considerations and future work

7.1. General discussion and final considerations

The present thesis investigated the nutritive value of palm kernel expeller (PKE), and its use as a supplement for grazing dairy cows in late lactation. A series of studies were undertaken to describe the nutritive characteristics of PKE and, generate information about its chemical composition, rates of degradation, products of digestion and digestibility. Additionally, the effect of PKE on the milk production, and its composition, of grazing dairy cows in late lactation was also determined. This has provided the first scientific database in New Zealand on the nutritive and feeding values of PKE when used to supplement the diets of dairy cows and other ruminants, grazing on pasture.

7.1.1. Composition of PKE

Knowledge about the composition of feedstuffs is essential in order to supply, in quantity and quality, the amounts required by the different categories of farm animals. The nutritive characteristics of PKE have been described in New Zealand only by its chemical composition, provided mainly by feed companies, despite its widespread use by farmers in the last decade as a supplement for ruminants. However, this feed is a by-product from the palm oil industry, and therefore, its composition and quality can be expected to show wide variations, as reported by de Ruiter et al. (2007). Surprisingly, the chemical composition of the samples (Table 7.1) analysed throughout this thesis (samples 5 and 6) and of samples collected from around New Zealand (samples 1 to 4) showed more consistent values in the PKE chemical composition than expected, even though they were randomly collected from different sources and over different years. However, it is important to note that the number of samples analysed were small (only six samples) and the samples were always analysed by wet chemistry using the same laboratory and the same methods, which normally reduces the variability within results (Gizzy and Givens, 2004)

The results of chemical analyses showed that PKE is a unique feed with fibre, fat and protein being the main components (Table 7.1). Additionally PKE has low starch and sugar content (0.03 g/100g DM), which reduces the chances of acidosis. The average gross energy content of PKE was moderate (18.6 MJ GE/kg DM), and similar to the values obtained for both pasture samples (around 18 MJ GE/kg DM) used in the digestibility study (Chapter 5) and between the values reported by Waghorn et al. (2007) for medium and high quality pasture (17.9 and 18.9 MJ GE/kg DM).

Table 7.1. Chemical composition of palm kernel expeller obtained from different sources in New Zealand.

	DM	CP	NDF	ADF	Fat	Ash	Starch	GE
	g/ 100g DM							MJ/kg DM
Sample 1	93.3	16.7	70.7	38.4	8.8	4.3	-	18.9
Sample 2	92.0	15.8	69.7	37.5	9.4	4.4	-	18.6
Sample 3	92.1	16.6	70.0	40.0	9.4	4.5	-	18.8
Sample 4	91.4	16.3	70.1	44.3	8.5	4.5	-	18.2
Sample 5 (Chapter 3 and 4)	94.8	16.0	70.3	39.3	9.0	4.1	0.03	-
Sample 6 (Chapter 6)	94.6	15.7	69.6	38.7	8.8	4.3	-	-
Average	92.2	16.2	70.1	39.7	9.0	4.4	0.03	18.6
SD	0.79	0.42	0.39	2.40	0.37	0.17	0.02	0.33

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; GE, gross energy.
SD = standard deviation.

7.1.2. Nutritive value, as assessed by in sacco and in vitro methods

In addition to the data for chemical composition obtained during the period of this thesis, the nutritive characteristics of PKE were also described using *in sacco* (Chapter 3) and *in vitro* (Chapter 4) techniques, which allow feeds to be evaluated quickly and cheaply. Digestion kinetic results showed that PKE was digested slowly in the rumen with a large proportion (around 60%) of its components (DM, CP and NDF) being degraded at rates ranging from 9.8 to 13.4%/h (Chapter 3) (Table 7.2). Compared to pasture, PKE presented low crude protein degradation rates (34 vs. 10%/h). This fact, when combined with the low estimated CP digestibility of PKE (48-53% for PKEM in Chapter 5 compared to 71-72% for good quality pasture and 56-61% for poor quality pasture), could be the reason for the brief period of net ammonia (NH_3) surplus

followed by deficiency of NH_3 for microbial growth found for PKE in the *in vitro* incubations (Chapter 4), and also for the low VFA yields found for PKE in the *in vitro* incubations (Chapter 4). According to O'Mara (1999) the heat damage caused by the expeller process could be the reason for the drop in the CP digestibility in their study, which can reduce the availability of nitrogen (N). Low CP digestibility of PKEM (48-53%) was also observed in both periods of the *in vivo* experiment (Chapter 5). The amount of acid detergent insoluble nitrogen (ADIN) in the protein fraction of the feed could be an indication of the unavailable nitrogen and its determination may help to clarify how much CP of PKE is actually available for the ruminant. Currently this is not measured. According to the AFRC (1993) approximately 18% of PKE CP content is present as ADIN fraction, however, some variation is expected as this value is a consequence of the process used during the oil extraction.

Table 7.2. Mean values for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) degradation characteristics of palm kernel expeller (PKE) defined by the soluble fraction (A), slowly degradable fraction (B), fractional disappearance rate (k), lag phase, undegradable residue (U), potential degradability (PD).

	DM	CP	NDF	ADF
A (%)	24.8	37.8	27.8	27.0
B (%)	61.4	57.5	58.8	51.5
k (%/h)	13.4	9.8	10.7	11.1
lag (h)	5.0	7.7	3.2	3.4
U (%)[1]	13.7	4.7	13.4	21.6
PD (%)[2]	86.2	95.1	86.4	78.3

[1] U=100-A-B
[2] $PD = A + B\ (1-e^{-k\ (t-lag)})$

When compared with the pasture used in this thesis, the addition of PKE to high quality pasture containing a high CP content (24%), resulted in the *in sacco* CP degradation rates of the mix to be reduced (Chapter 3) and a dilution in the CP content, which led to a decrease in the *in vitro* net NH_3 production of pasture (Chapter 4) and in the *in vivo* rumen NH_3 concentration of lambs (Chapter 5, period 1). This indicates that supplementation of PKE could result in a better CP utilization of high quality temperate pastures, which are known to have high rates and extents of degradation in the rumen

(observed in Chapter 3), and high N excretion in the urine (observed in Chapter 5). The reduction in the urine nitrogen excretion observed here when PKE plus molasses (PKEM) was supplemented to lambs fed a good quality pasture (≥ 20% CP), indicates that PKE could be used to improve CP utilization. This may have positive environmental benefits due to the decrease in the urinary nitrogen excretion, but it is also important to notice that N retention was reduced, which in terms of animal productivity this could have a negative impact. In contrast, when PKE was fed with low quality pasture (≤15% and high fibre content), N retention was increased by feeding increasing amounts of PKE into the diet of lambs (Chapter 5). These two facts demonstrate that there are likely to be interactions between pasture quality offered as a base diet and the effects of the PKE offered as a supplement, in terms of the ruminant's utilization of dietary N.

The degradation rates of the fibre fractions were similar for both PKE and pasture (between 10 and 14%) which showed that, for PKE, digested materials and undigested residues will be cleared from the rumen at the same rates as those from high quality pasture, indicating that rates of passage through the rumen and excess rumen-fill will not have important effects on intake of PKE. Nevertheless, the farm study (Chapter 6) showed that the amount of PKE may have been limited by its dry and gritty physical characteristics, as the cows grazing the restricted pasture allowance and supplemented with PKE were not able to consume all of the 6 kg of PKE offered in two short feeding periods per day, despite their restricted pasture allowance. During the adaptation period of the *in vivo* study with lambs (Chapter 5), the addition of molasses to PKE (to produce PKEM) helped to improve its palatability to the lambs that had never been in contact with this supplement before.

7.1.3. In vivo studies with lambs

The prediction of digestibility and estimated energy values for PKEM were determined in an *in vivo* study, in which lambs were fed different amounts of PKEM plus fresh harvested pasture of either high (high CP and digestibility; period 1) or low quality (low CP and digestibility; period 2). The estimated apparent digestibility of PKE can be estimated if we consider a digestibility value of 100% for molasses (Oliveira et al., 2003), and a 10% fraction in the PKEM pellet (Table 7.3). The results obtained here are in the range of values found by O'Mara et al. (1999) and showed that PKE is a

moderate quality feed with an average DM digestibility of 68.2 g/100g DM, with its fibre fraction being more digestible than its protein fraction (72.8 vs. 55.6 g/100g DM). This result suggested that for PKE, fibre and fat (using an assumed high digestibility for fat; Moss and Givens, 1994) are the main sources of dietary energy to the ruminant. The addition of PKEM to good quality pasture caused a reduction in the digestibility of DM and CP, but this negative effect of PKE was not found for fibre and GE digestibility, when the quality of pasture was lower (Chapter 5). Similarly, the addition of PKEM reduced the DE content of a diet containing high quality pasture, but increased the DE content of a diet containing low quality pasture.

These results showed that the lower digestibility values of PKEM can have a strong impact on the nutritive value of diets based on high quality pasture, causing a reduction in the overall quality of the diet and consequently in animal productivity, as observed by the reduction in the VFA concentration when PKE was added to a high quality pasture (Chapter 5, period 1). Surprisingly, the *in vitro* incubations showed an increase in the rate and amount of VFA produced when PKE was added to high quality pasture, and these results should be studied further.

7.3. Estimated digestibility (%) and energy (MJ/kg DM) content of PKE (the values for 100% PKE are based on the measured data of Chapter 5, and calculated based on digestibility and energy of molasses[1]).

	DMD	CPD	NDFD	ME
Period 1 – Maintenance (670 g/day)	70.1	56.4	73.7	10.2
Period 2 – Maintenance (670 g/day)	68.1	57.7	73.9	10.1
Period 1 – *Ad Libitum* (1214 g/day)	68.7	55.6	71.7	10.0
Period 2 – *Ad Libitum* (1214 g/day)	65.8	52.6	71.9	9.8
Mean	68.2	55.6	72.8	10.0
SD	1.8	2.2	1.2	0.2

[1]100% DM and CP digestibility and 12 MJ ME/kg DM.
DMD, dry matter digestibility; CP, crude protein digestibility; NDF, neutral detergent fibre digestibility;DE, digestible energy; ME, metabolic energy.
SD = standard deviation.

In New Zealand, metabolisable energy (ME; MJ ME/kg DM) is the unit adopted to express the energy supplied by a feed to ruminants, however only one report has included a value for the ME content of PKE (13.1 MJ/kg DM) (Moss and Givens, 1994), and this was a study in which some of the energy losses were determined using

equations. In this thesis the ME content of PKE can be estimated from the DE value obtained for PKEM, which was estimated by regression back to a diet with zero pasture included. For most forages and cereal grains the conversion of DE into ME is estimated using a conversion factor of 0.82, which is smaller than the rate presented by Moss and Givens (1994) of 0.86. However, in both cases, the assumption is that methane output is a constant proportion of the net rumen fermentation, and no consideration is made to the fact that methane production is a consequence of rumen VFA ratios (Van Soest, 1994). Therefore, it is important to take into consideration that those conversion factors may vary considerably depending on feed intake, age of the animal and feed source. However, if the conversion factor 0.82 is used to calculate the energy content of PKEM the results would show moderate ME values for PKEM, ranging from 10.2 to 10.4 and 10.0 to 10.3 MJ/kg in period 1 and 2, respectively (Table 7.3). However, it is important to remember that the DE of PKEM estimated here had the addition of 10% of molasses in the pellet (PKEM), therefore, ME values of PKE, without the addition of molasses, would be expected to be ranging from 10.0 to 10.2 and 9.8 to 10.1 MJ/kg DM in period 1 and 2, respectively, if we consider a estimated value of 12 MJ ME/kg DM for molasses (de Ruiter et al., 2007). Considering the conversion factor 0.82 used above to determine the ME values of the two pasture samples used in the *in vivo* experiment (Chapter 5), the results would be between 11.0 and 9.6 MJ of ME/kg DM for the high (high protein and high digestibility) and low (low protein and low digestibility) quality pastures, respectively. These values are similar to the range of values reported by Waghorn and Barry (1987) for perennial ryegrass at different stages of maturity, fed to lambs at maintenance level, and lends support to the validity of the estimated values for ME of PKE reported above.

It is important to highlight the fact that the apparent digestibility and energy values found in this study were determined using lambs as the experimental unit (Chapter 5) and not dairy cows, due to practical and economic reasons. But, most of the PKE fed in New Zealand is used as a supplement for dairy cows, and therefore, the aim of this study was that the dataset built here could also be used as a reference for dairy cows. But, it is important to acknowledge that, compared with cattle, sheep normally have poorer abilities to digest fibre fractions, but better abilities to digest protein (Mertens and Ely, 1982; Cottyn et al., 1989; Cottyn et al., 1989; Woods et al., 1999).

7.1.4. On farm study with dairy cows in late lactation

The on-farm study with cows (Chapter 6) showed that the supplementation of PKE to a diet of restricted pasture allowance resulted in moderate immediate (short-term) responses in milk production (30 to 60 g extra MS/kg DM eaten as PKE) (Holmes and Roche, 2007). Larger responses would have been expected if the study had been carried out over a longer period of time, and in a whole-farm system, because the extra liveweight gained during the short-term feeding period may have contributed to some extra milk production, and because later consumption of some of the pasture not eaten in the short term because of substitution, may have also contributed to some extra milk as well (Holmes and Roche, 2007). Larger long-term responses would also be expected if the feeding of PKE had been used to delay the decision to dry-off the cows, and so to extend their lactations (Clark, 1993; Pinares and Holmes, 1996). However it is important to highlight that the effects of PKE on milk production were obtained when a pasture deficit was offered to the cows (restricted allowance) and therefore ME intake was a limiting factor. But, if there is no pasture deficit, and high allowances of high quality pasture can be offered, then ME is not a limiting factor, and the supplementation of PKE in this conditions will reduce the diet quality (lower digestibility) and probably animal performance (lower rumen VFA production), as observed in Chapter 5 – period 1. With a high allowance of lower quality pasture, PKE may increase the quality of the diet and the level of animal performance, as observed in Chapter 5 – period 2.

The composition of milk was modified by the supplementation of PKE, with an increase in its fat concentration (mainly due to an increase in the short- and medium-chain fatty acids). This is probably a consequence of a higher dietary fat intake, as PKE has around 9.0 g/100g DM, associate with an increase in the concentration of butyrate caused by the supplementation of PKE, which was observed both *in vitro* (Chapter 4) and *in vivo* (Chapter 5). This is the first study that has looked at the fatty acid content of milk produced by cows fed PKE, and the results showed that PKE supplementation increased the concentration of saturated fatted acids, which may have a negative impact for human health (Grundy and Denke, 1990; Katan et al., 1994), but more studies are needed to understand the effect of PKE on the composition of milk and its components, and on possible effects on the milk's value for processing into dairy products.

7.1.6 Overall summary and Recommendations

The present thesis has demonstrated that PKE is a feed of moderate quality for ruminants, with some unique characteristics. The estimated ME of PKE found in this study (between 9.8 – 10.2 MJ/kg DM) comes mainly from the fat and fibre fractions, however, the high fibre content is probably not fully functional as "effective fibre", because it is finely ground in the preparation of the PKE (Kolver, 2006). The main fractions of PKE are digested slowly in the rumen, which could be a beneficial characteristic when it is supplemented to a feed containing rapidly degradeable protein, such as high quality pasture. The apparent digestibilities of DM, NDF and GE showed moderate values (ranging from 60 to 65%), with CP digestibility being lower (around 50%), as a result of the expeller extraction and drying processes. When PKE was fed with high quality pasture (high CP and digestibility) the digestibility of the combined diet was lower than pasture for the main fractions, except for the NDF digestibility when PKE was mixed with low quality pasture (low CP and digestibility) The addition of increased amounts of PKEM to the diets of lambs fed pasture increased N retention and VFA concentration in diets based on mature pasture, but in contrast, reduced N retention and VFA concentration of diets based on high quality pasture. Additionally, the supplementation of PKE to lambs fed high quality pasture decreased urinary excretion.

The results of this thesis have also demonstrated that PKE supplemented to grazing dairy cows in late lactation can extend the lactation, and at the same time save some pasture, but will never substitute good quality pasture. Additionally, intake of this supplement can be limited when fed alone and milk fat concentration will be increased, which may have an impact in the value of the extra milk produced.

All these results clearly showed that when PKE is used in situations where the pasture quality is not good (low CP and digestibility; eg. as a supplement in a summer drought) farmers can expect PKE to improve the quality of the combined diet (compared with the pasture only). Whereas, when used with good quality pasture (high CP and digestibility; eg. spring), PKE supplementation will reduce the quality of the combined diet. However it is important to remember that animal performance will depend on pasture allowance that can be offered to the animals, as mention in earlier.

Recommendations

- Based on the chemical composition obtained here it seems that PKE imported into New Zealand is of consistent quality (eg. very little difference in composition between the six samples we analysed from around the country), however it is important for farmers to monitor from time to time the main fractions (eg. fibre, fat and protein) to ensure they are in the range of values expected for PKE (NDF around 70%, and fat between 8-10%). If the values change, especially for fat (< 7-8%) then it is likely that the PKE imported into New Zealand is being produced by solvent extraction, not mechanically extraction, and the results found in this PhD may not apply.

- Fibre and fat are the fractions that are the most digestible components in PKE and are therefore responsible for most of the energy value of this feed. However, farmers need to pay attention that estimations of the ME value of PKE obtained here in a feeding trial with animals showed results ranging from 9.8 to 10.3 MJ ME/ kg DM, and not 11 MJ ME/kg DM as reported in New Zealand.

- The fibre (NDF and ADF) fraction of PKE are degraded moderately fast in the rumen (around 11%/h), however its fibre is not effective due to the small particle size of this feed.

- Crude protein of PKE is degraded slower than pasture CP in the rumen (7-10% for PKE vs. 34-48% for pasture). This characteristic associated with lower CP digestibility of PKE make this feed suitable to be fed in conjunction with feeds that have high CP degradation in the rumen (eg. high quality pasture). However, PKE when fed alone is not able to sustain the minimum ammonia concentration in the rumen required for normal microbial growth (3.5 mMol/L).

- The lower digestibility of PKE compared to good quality pasture can have an impact on the nutritive value of diets composed of good quality pasture, causing a reduction in the overall energy content of the diet (from 13.5 to 12.7 MJ DE/kg DM, at maintenance and 13.2 to 12.4 MJ DE/kg DM, at *ad libitum*) and VFA concentration in the rumen (from 79 to 53 mMol/L, at maintenance and 93 to 65 mMol/L, at *ad libitum*) . In contrast, PKE supplemented with more mature pasture can increase the energy of the diet (from 11.9 to 12.6 MJ DE/kg DM, at maintenance and 11.5 to 12.2 MJ DE/kg DM, at *ad libitum*) and improve VFA concentration in the rumen (from 89 to 93 mMol/L, at maintenance and 74 to112 mMol/L, at *ad libitum*), which can positively affect animal productivity.

- The excretion of nitrogen through the urine of cows fed good quality pasture can be reduced by an average of 7.6 % with the supplementation of PKE.

- PKE intake is limited by its gritty and dusty characteristic when it is fed alone, and therefore we suggest that no more than 3-4 kg of PKE/day should be offered alone or mixed with other supplements such as molasses or corn silage.- Marginal returns between 40 and 55 g of milksolids/ kg DM PKE can be expected when fed during late lactation without taking into account the extra liveweight gained or the extra pasture saved. This shows that PKE supplementation could be an alternative supplement to extend lactation and/or save some grass for the winter.

- The supplementation of PKE to dairy cows can have an impact on the milk composition. Milk fat percentage is normally increased (around 5-10% unit) due to the great fat content of PKE, which is composed mainly by saturated fatty acids.

7.2. Future work

The present work was based on six samples of PKE collected from only four sources over a period of only 36 months. Therefore, a larger number of samples from a wider range of sources, locations and times and places should be analysed to build a more robust and widely applicable database of PKE chemical composition, and confirm or deny the existence of a variation for this feed. These samples could also be subjected to analysis by near infrared reflectance spectroscopy (NIRS) in order to develop a reliable calibration curve that would make it possible to predict the chemical composition of PKE to be predicted quickly and cheaply.

Some of the samples should also be subjected to *in sacco* incubations, in order to assess their degradations and nutritive characteristics. Additionally, it has been reported in the literature that the prediction of PKE digestibility through enzymatic methods is very poor, and further research in this area should be carried out to facilitate the determination of the digestibility of PKE without the use of animals in expensive *in vivo* digestibility trials.

The information presented here showed that PKE can be used as a supplement for grazing dairy cows in late lactation, but the responses were moderate but in the range of values found by other short-term experiments. Normally, carry over effects are not measured in short-term experiments, it would be interesting to evaluate the use of

PKE to supplement cows during a whole year, in which the availability of extra pasture that was saved during the supplementation period and the effects of the improved body condition would be taken into account in the final results. Also, the combination of other supplements (eg. maize silage, or barley grain) with PKE should be investigated in terms of diet balance and animal performance as farmers often feed more than one supplement to their cows, and associative effects may occur. Another interesting aspect to investigate is to evaluate the performance of grazing animals when PKE is supplemented during spring time, when the quality of pasture is good, as the results obtained here showed that lambs fed increasing amounts of PKE on a good quality pasture diet can reduce animal performance. But, it is important to note that results of grazing animals under supplementation will depend on the pasture allowance offered during the experiment, as explained above.

The effects on the environment due to the supplementation of PKE to grazing animals should also be examined in future research, as the *in vivo* results obtained here has shown that PKE supplementation can reduce nitrogen excretion of sheep fed high quality pasture with high protein content. Additionally, the determination of methane emissions of animals fed pasture and PKE could give useful information both for the determination of a more precise value of PKE metabolic energy and for environmental purposes, as the addition of fats and oil seeds (eg. palm kernel oil) in the diet of ruminants is one of the alternatives to reduce the emissions of methane (Beauchemin et al., 2008).

Last but not least, it would be interesting to look at the mineral balance of grazing animals supplemented with PKE, as PKE has a low ratio of calcium to phosphorus and a low sodium concentration, and therefore increasing amounts of this supplement could cause a deficiency of these two minerals in the diet. In addition, PKE has also a high copper content, which can cause chronic toxicity, primarily in sheep (Akpan et al., 2005) when fed in large quantities. However, in regions of New Zealand where the soil is deficient in copper (eg. the Northland soils; Sherrell and Rawnsley, 1982), supplementation of PKE to the diet of pasture may help to overcome this deficiency, and research about this topic may clarify availability of this mineral to the ruminant.

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APPENDICES

Appendix 3.1. Soybean meal used as a standard to measure variations between incubations. Least square means of soybean meal (SBM) dry matter loss at different times and rumen parameters (pH, ammonia (NH_3) and volatile fatty acid (VFA) concentrations) observed during the four *in sacco* incubations.

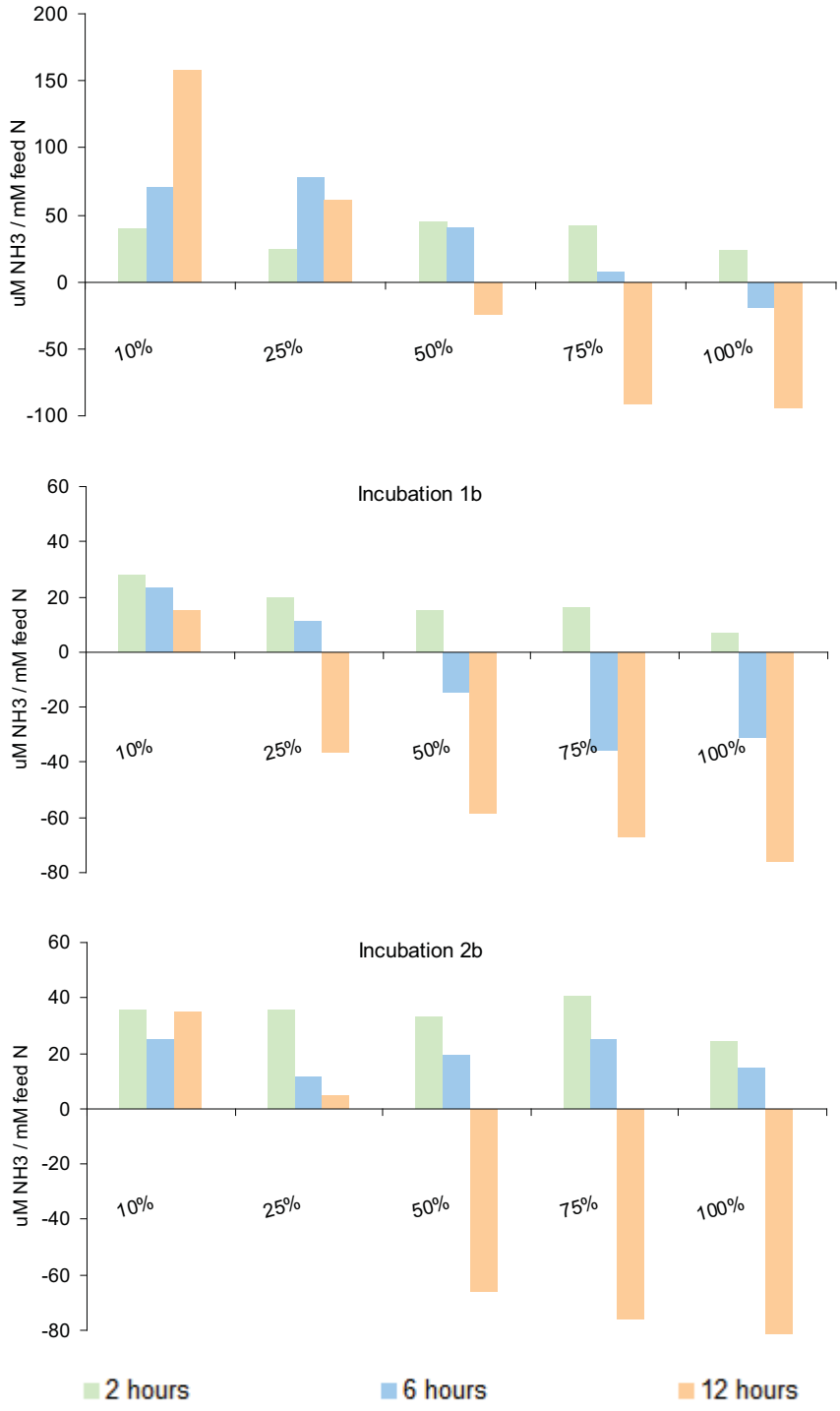
Experiment	Experiment 1			Experiment 2
Incubation	Incubations			
	1	2	3	4
Time of incubation (h)	% DM Loss			% DM Loss
0	19.6	19.6	19.6	19.6
2	-	36.0	37.0	36.4
4	42.1	-	-	-
6	47.0	49.6	53.7	50.3
12	77.6	75.3	83.0	80.9
24	93.3	96.0	-	95.0
Average	55.9	55.3	48.3	56.5
Incubation effect (Pr)	ns	ns	Ns	ns
Cow (incubation) (Pr)	ns	<0.05	<0.05	ns
	DM kinetics			DM kinetics
A (%)	18.4	20.8	19.4	19.3
B (%)	92.1	94.3	81.2	81.9
K (%/h)	8	7	11	10
Predicted 2h	31.3	-	-	-
Predicted 4h	-	43.8	48.4	46.5
Predicted 24h	-	-	94.9	-
	Rumen parameters[1]			Rumen parameters
pH	7.0	6.9	6.9	7.3
NH_3 (mMol/L)	24.7	24.3	24.9	-
Total VFA (mmol/L)	98.2	116.5	113.6	-
Propionic (%)	12.4	13.9	15.9	-
Acetic (%)	74.8	72.6	71.0	-
Butyric (%)	7.2	6.2	7.1	-
Minor (%)	5.6	7.2	5.9	-

[1] Rumen parameters only measured in the three incubations of Experiment 1.

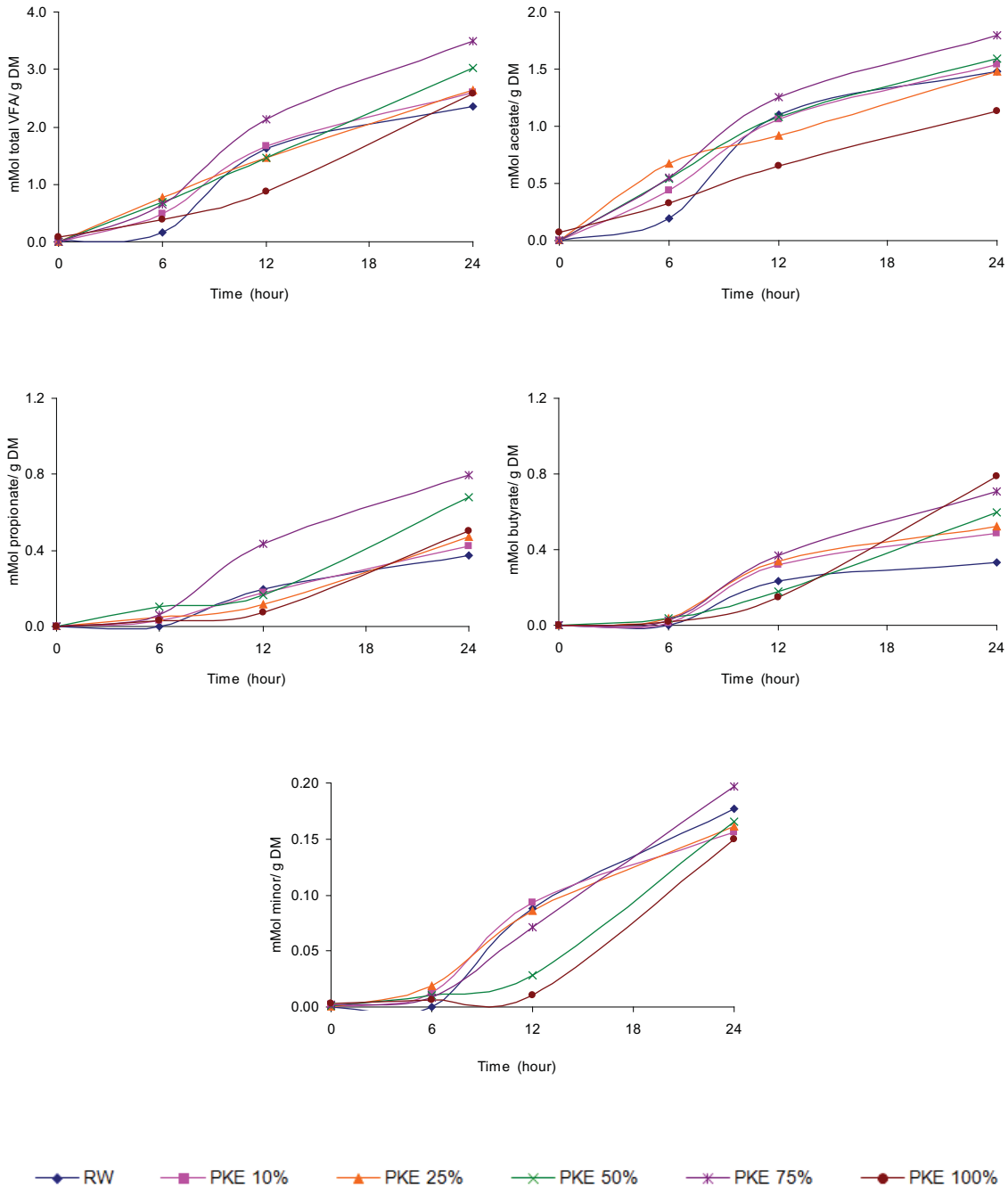
Appendix 4.1. *In vitro* pH and ammonia (NH_3) concentration (mMol/L) of freeze-dried and ground lucerne and soybean meal (SBM) incubated in four *in vitro* incubations. *In vitro* NH_3 and pH data of the standard feeds are the least square means of triplicate samples at each time and incubation.

		Incubations			
		Experiment 1		Experiment 2	
Incubations		1st	2nd	1st	2nd
Parameters					
	Time (h)	SBM		Lucerne	
pH[1]	2	7.0	7.1	6.9	6.9
	6 or 10	6.8	6.8	6.4	6.2
		ns		ns	
Ammonia (mMol/L)	2	5.9	6.4	6.2	8.7
	6 or 10	9.3	5.4	8.6	16.5
		ns		ns	

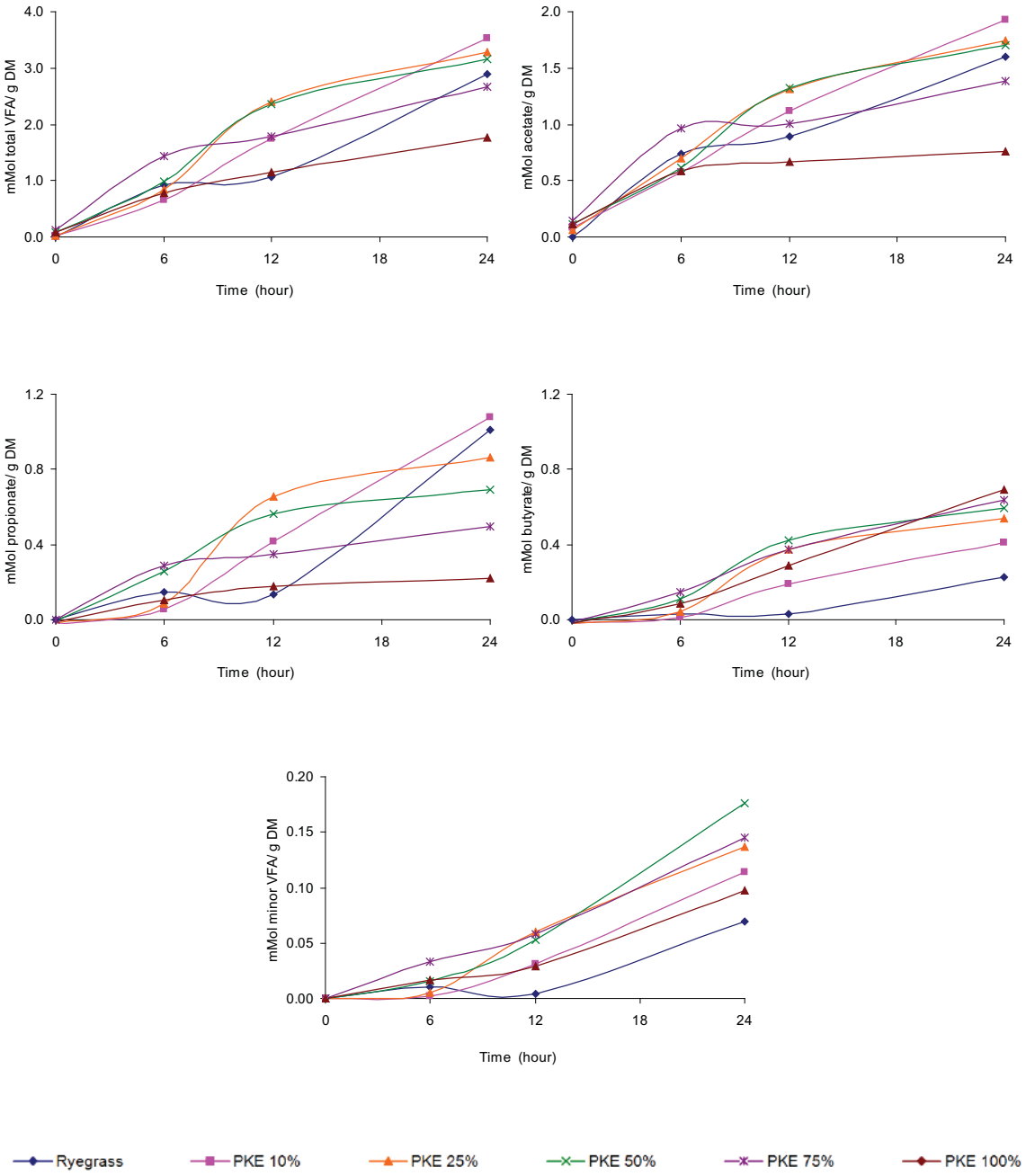
[1] pH at 6 hour for lucerne or 10 hours for soybean meal. ns, not significant.



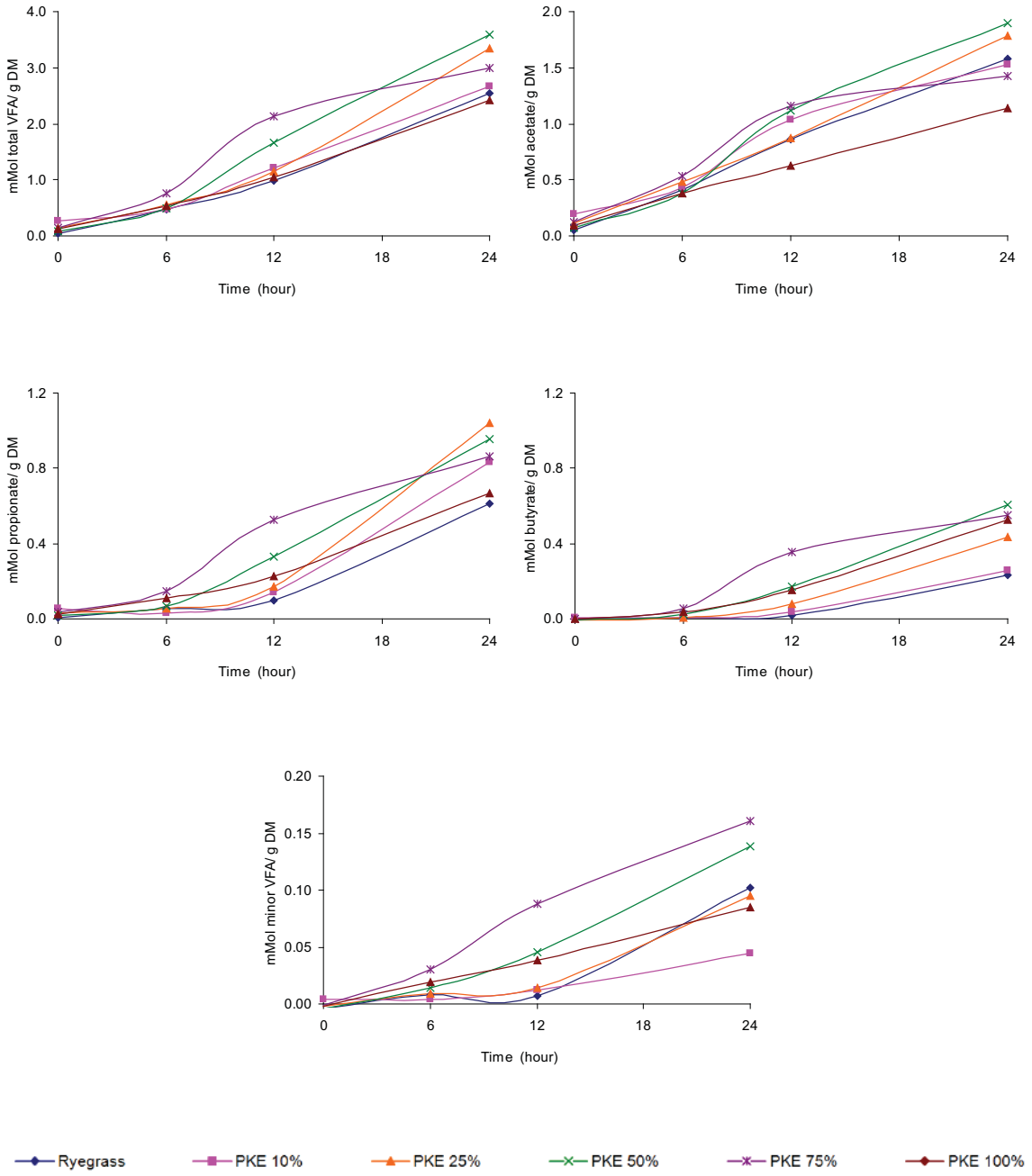
Appendix 4.2. Average *in vitro* net ammonia production of PKE mixed with either ryegrass and white clover (RW) (Experiment 1; a), or ryegrass-only (Ryegrass) (Experiment 2; b) after 2, 6, and 12 hours of incubation.



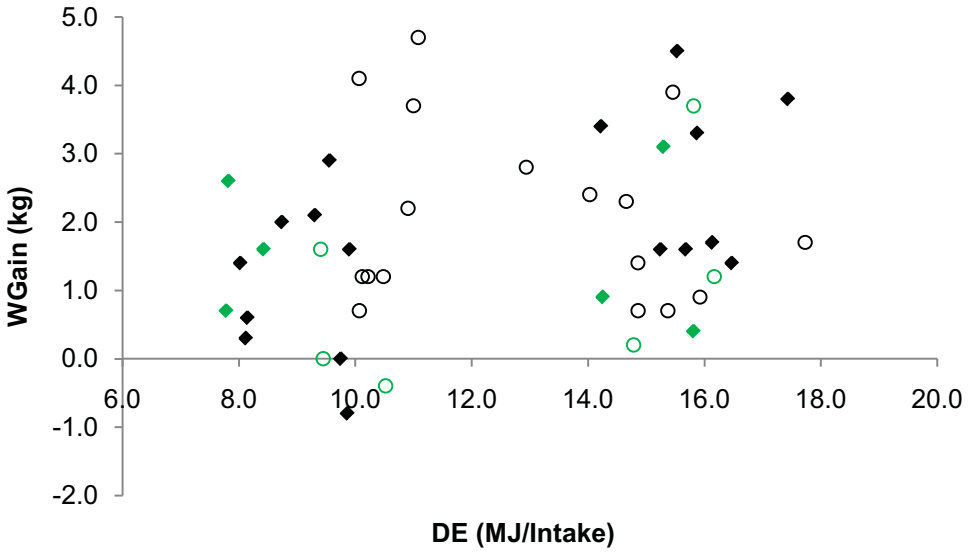
Appendix 4.3. Experiment 1: cows fed lucerne chaffage plus PKE: Concentrations of total VFA, acetate, propionate, *n*-butyrate, and minor VFA (iso-butyrate, *n*-valerate and iso-valerate, *n*-caprionic) of diets with different proportions of PKE and ryegrass and white clover (RW).



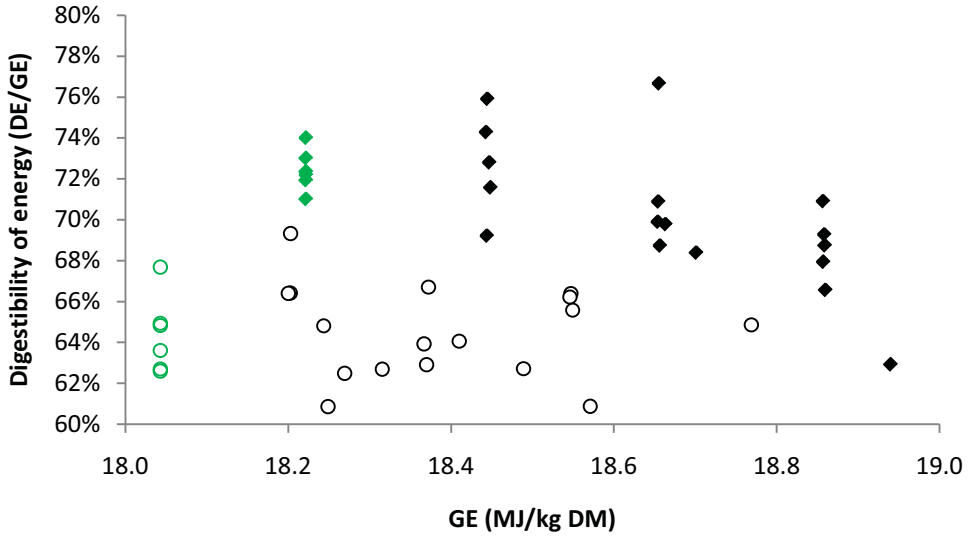
Appendix 4.4. Experiment 2: cows fed lucerne chaffage plus PKE: Concentrations of total VFA, acetate, propionate, *n*-butyrate, and minor VFA (iso-butyrate, *n*-valerate and iso-valerate, *n*-caprionic) of diets with different proportions of PKE and ryegrass-only (Ryegrass).



Appendix 4.5. Experiment 2: cows fed lucerne chaffage only: Concentrations of total VFA, acetate, propionate, *n*-butyrate, and minor VFA (iso-butyrate, *n*-valerate and iso-valerate, *n*-caprionic) of diets with different proportions of PKE and ryegrass-only (Ryegrass).



Appendix 5.1. Digestible energy (DE) of diets containing different amounts of pasture and PKEM and the weight gain (Wgain) of lambs fed those diets, including data for lambs fed either at maintenance or ad libitum, during period 1 (♦) and 2 (○). Points with green colour in the chart symbolize pasture-only samples.



Appendix 5.2. Gross energy (GE) and digestibility of energy (digestible energy divided by gross energy) in diets containing different amounts of pasture and PKEM, including data for lambs fed either at maintenance or ad libitum, during period 1 (♦) and 2 (○). Points with green colour in the chart symbolize pasture-only samples.