Effects of varying inclusion levels of canola meal for grass-fed cattle

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Certificate of Authorship

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Ethics Approval

All experiments had animal ethics approval from Charles Sturt University Animal Care and Ethics Committee (CSU ACEC) and New South Wales Department of Primary Industries Animal Ethics Committee (NSW DPI AEC) and were compliant with the Animal Research Act 1985 (as amended) and the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC 2013).

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Glossary of Terms and Abbreviations

a	Soluble (a) fraction
AA	Amino acid(s)
ABS	Australian Bureau of Statistics
Ac:Pr	Ratio of acetic acid to propionic acid proportion
ACEC	Animal Care and Ethics Committee
AEC	Animal Ethics Committee
ADF	Acid detergent fibre
ADFD	Acid detergent fibre digestibility
$ADFD_{OM}$	Acid detergent fibre digestibility on an organic matter basis
ADG	Average daily gain(s)
AFIA	Australian Fodder Industry Association
AFRC	Agricultural and Food Research Council
AIC	Akaike information criterion
aNDF	Neutral detergent fibre with heat stable amylase
aNDFD	Neutral detergent fibre digestibility with heat stable amylase
$aNDF_{OM}$	Neutral detergent fibre on an organic matter basis with heat stable amylase
ATP	Adenosine triphosphate
b	Not soluble, but slowly degradable (b) fraction
BCS	Body condition score
BCVFA	Branch chain volatile fatty acid(s)
BIC	Bayesian information criterion
c	Fractional rate of degradation of degradable fraction b (/h)
CH_4	Methane
CI	Confidence interval
CLA	Conjugated linoleic acid
CO_2	Carbon dioxide
CP	Crude protein
CPD	Crude protein digestibility
CSU	Charles Sturt University
DM	Dry matter
DMI	Dry matter intake
DMD	Dry matter digestibility
DOMD	Digestible organic matter in the dry matter
DMTP	Digestible microbial true protein
DNA	Deoxyribonucleic acid
DPI	Department of Primary Industries

DUP	Digestible undegradable protein
ED	Effective degradability at outflow rate (r)
EE	Ether extract
EMA	Eye muscle area
FA	Fatty acid(s)
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FQS	Feed Quality Service
GC	Gas chromatography
HCl	Hydrochloric acid
hd	Head (of cattle)
HSCW	Hot standard carcase weight
iNDF	Indigestible fraction of neutral detergent fibre
k_g	Efficiency of use (of energy or protein) for growth/gain
k_l	Efficiency of use (of energy or protein) for lactation
k_m	Efficiency of use (of energy or protein) for maintenance
LoF	Level of feeding
LW	Liveweight
M/D	MJ of metabolisable energy per kg of feed on a dry matter basis
MDA	Malondialdehyde
ME	Metabolisable energy
ME_m	Metabolisable energy requirement at maintenance
MLA	Meat and Livestock Australia
MP	Metabolisable protein
MQ4	Meat quality score
MSA	Meat Standards Australia
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre digestibility
NE	Net energy
NE_m	Net energy for maintenance
NH_3-N	Ammonia nitrogen
NHMRC	National Health and Medical Research Council
NIRS	Near infrared spectroscopy
NPN	Non-protein nitrogen
NRC	National Research Council
NSW	New South Wales
OM	Organic matter
OMD	Organic matter digestibility
PCAS	Pasture-fed Cattle Assurance System

PD	Potential degradability after 72 h incubation
PDNDF	Potentially digestible fraction of neutral detergent fibre
PMR	Partial mixed ration(s)
Pr: Ac + 2 X Bu	Ratio of propionic acid to acetic acid + 2 x butyric acid
PSA	Particle size analysis
PUFA	Polyunsaturated fatty acid(s)
r	Rumen outflow rate; 0.02/h, 0.05/h and/or 0.08/h
R^2	Coefficient of determination
RDP	Rumen degradable protein
REML	Restricted maximum likelihood
RIPA	Radioimmunoprecipitation assay
RNA	Ribonucleic acid
RUP	Rumen undegradable protein
se	Standard error
SCA	Standing Committee on Agriculture
SEM	Standard error of the mean
TBARS	Thiobarbituric acid reactive substance
TMR	Total mixed ration(s)
VFA	Volatile fatty acid(s)
VFI	Voluntary feed intake
WSC	Water soluble carbohydrate(s)

Abstract

Managing variability in pasture quality and quantity and maintaining a consistent supply of cattle that meet specific Meat Standards Australia (MSA) and Pasture-fed Cattle Assurance System (PCAS) requirements throughout the year is a challenge for beef producers supplying to certified grass-fed markets in Australia. Energy and/or protein supplementation is often practiced to overcome livestock nutritional deficits. In recent years canola meal has become readily available throughout south-eastern Australia due to an increase in canola cropping and processing. The overall objective of the research reported in this thesis was to determine the maximum inclusion level of canola meal as a PCAS approved supplement to roughages typical of that normally available during the summer-autumn period in southern New South Wales for grass-fed beef cattle. A combination of pen feeding, meat quality and consumer sensory evaluations, *in vivo* digestibility, and degradation studies were undertaken to determine the effects of varying inclusion levels of canola meal on animal production, ruminal parameters and nutrient digestibility.

Dry matter intake (DMI) and average daily gain (ADG) were improved when weaner calves fed a basal diet of canola hay were supplemented with 34% canola meal compared with 20% canola meal. Both DMI and ADG decreased when the weaner calves were offered 68% canola meal. Thus, an upper limit to the inclusion level of canola meal for weaner cattle fed roughage diets was established between 47% and 68%. Supplementing canola meal up to 43% in a low-quality roughage ration increased ruminal ammonia (NH_3-N) concentrations, while total volatile fatty acid (VFA) concentrations and molar proportions were within normal range for microbial synthesis. Ruminal pH did not decrease below 6.3, indicating canola meal was safe to include at 43% in a basal, low-quality ration for cattle. Meat quality traits and consumer sensory evaluations were not adversely affected when finishing Angus and Angus cross steers were supplemented with either canola meal or a grain-based pellet and offered *ad libitum* lucerne hay. The overall consumer satisfaction of the meat samples equated to “good everyday quality” and there was no difference between dietary treatments.

Supplementing cattle fed low-quality roughage with up to 47% canola meal had no detrimental effects on apparent nutrient digestibility. In addition, supplementing donor (of ruminal fluid) steers fed a low-quality ration up to 43% canola meal had no negative effects on either the degradation of lucerne hay standard or the degradation of canola meal dietary treatments when using either the *in sacco* or *in vitro* ANKOM Daisy™ incubator methods. The relationship between *in sacco* and *in vitro* Daisy™ was moderately correlated (R^2 = 0.59) indicating the *in sacco* method as the preferred method (of the two) as it better reflected *in vivo* apparent digestibility.

Canola meal can be used as an approved PCAS supplement for grass-fed cattle grazing low-quality roughages at levels up to 43% of DMI without any adverse effects on ADG, DMI, feed efficiency, apparent nutrient digestibility, or ruminal parameters. However, there is an upper limit of between 46% and 68% of DMI when supplementing canola meal to cattle fed low-quality rations.

Chapter 1: Introduction

1.1 Overview of southern Australian beef industry

Australian cattle and calf production (including live export) is one of the largest contributors to the Australian agriculture economy with a gross value worth over $15.1 billion in 2019-2020 (ABARES Agriculture Commodities, 2020). In recent years, the national cattle herd has continued to decline, from 29.3 million head in 2013 to 24.7 million head in June 2019 (MLA 2018; 2020b). New South Wales (NSW) accounted for 22% of Australian beef and veal production in 2019. Slaughter numbers for NSW from July 2019 to June 2020 were 1 745 000 and of this, 790 400 cattle were from feedlots (MLA 2020a) and the remainder were pasture-fed cattle.

There is an increasing consumer demand for grass-fed beef, both domestically and internationally (MLA 2018). Grass-fed beef certification programs, such as Pasture-fed Cattle Assurance System (PCAS), have been developed within Australia. Underpinning PCAS are standards which govern the on-farm requirements such as Meat Standards Australia (MSA) compliant, individual identification and lifetime traceability, no confinement for the purpose of intensive feeding, lifetime pasture-fed (no cereal grain or grain products) and minimum eating quality standards (on-farm) which influence meat quality (PCAS 2016). Producers who meet and comply within these market specifications can receive premium prices for their product.

1.2 Pasture variability in southern New South Wales

Southern NSW has a Mediterranean-type climate with most of the rainfall occurring in the winter and spring months. The generally favourable climate promotes the growth of highly digestible, high protein pastures for grazing during late autumn, winter and spring. Livestock then typically graze on mature annual pastures or crop residues during summer and early to mid-autumn (Hennessy *et al.* 1981; Dove *et al.* 2008). Nutritional inadequacies, even with adequate supply of dry forage available for grazing, can be a constraint for livestock. Normally, as forages mature their digestibility decreases which decreases feed intake (Leng 1990). The quality of these forages is often below the nutrient requirements for growing and finishing livestock (Lee *et al.* 1985; Leng 1990). Therefore, managing variability in pasture quality and quantity throughout the year is a challenge for beef producers supplying to certified grass-fed markets in Australia.

Energy and/or protein supplementation is often practiced to meet these requirements and production demands (Smith and Warren 1986a; Moore *et al.* 2009; McLennan *et al.* 2017a). Traditionally, beef producers supplement livestock with grain or grain-based pellets to enhance the energy (and protein) supply when grazing dry quality pastures; however, grain or grain based pellets are not approved for use under PCAS (PCAS 2016). Protein supplements that are approved for use under the PCAS guidelines include canola meal, cottonseed meal and soybean meal (PCAS 2016). Protein meals, such as cotton seed meal, have previously been shown to increase growth rates of cattle fed dry, low-quality pastures or forages; however, the protein supplements were only at relatively low inclusion levels (Hennessy 1980; Hennessy *et al.* 1981; Smith and Warren 1986a; McCollum and Horn 1990; Fordyce *et al.* 1996; Dixon *et al.* 2017). Urea, a non-protein N (NPN) source, is another approved PCAS supplement (PCAS 2016) and has been typically used in northern Australia for livestock fed low-quality roughages (Hennessy 1980; Lee *et al.* 1987). Although urea is an approved PCAS supplement and has been shown to improve DMI and ADG when steers were grazing low-quality roughages (McLennan *et al.* 1991; Huntington and Archibeque 1999; Zinn *et al.* 2003; Xu *et al.* 2019), there are risks associated with urea toxicity (Chalupa 1968; Patra 2015).

In recent years canola meal has become readily available throughout southeastern Australia, due to an expansion in canola cropping and an increase in its processing. Australia produced approximately 2.4 million tonnes of canola in 2018-2019 (ABS 2020) and 19% of this was produced in NSW. Canola is now the largest oilseed crop and the third largest broad-acre crop (behind wheat and barley) in Australia (ABS 2020). Canola is grown predominantly as a source of oil; however, the remaining meal component (the residue after the oil has been extracted from the seed) is a good source of energy and protein for livestock (Mailer *et al.* 2008). In Australia, canola meal is most commonly used as a protein source for pigs, poultry (Mailer 2004) and dairy cattle (White *et al.* 2004; Auldist *et al.* 2014b; McDonnell and Staines 2017; Wright *et al.* 2017; Auldist *et al.* 2019).

Canola meal has been used as the protein source in both total mixed rations (TMR) (Van De Kerckhove *et al.* 2011; He *et al.* 2013; Nair *et al.* 2016; Good 2018) and partial mixed rations (PMR) (Seoane *et al.* 1992; Seoane *et al.* 1993; Petit *et al.* 1994) for both dairy and beef cattle (Williams *et al.* 2008; He *et al.* 2013; Nair *et al.* 2015). In PMR, the inclusion levels of canola meal have been less than 1.2 kg/hd/d (Seoane *et al.* 1992; 1993; Petit *et al.* 1994). In beef cattle in particular, the effects of canola meal supplementation on dry matter (DM) intake (DMI), average daily gain (ADG),

apparent nutrient digestibility and ruminal parameters can vary depending on the other ration components and feeding regimen (Bergman 1990; France and Dijkstra 2005). The optimum inclusion levels of canola meal for beef cattle production and the effects on apparent nutrient digestibility are not well defined. The Canadian Canola Council feeding guide has not recommended either a maximum or optimal inclusion level of canola meal in feedlot rations or for grazing cattle (Newkirk 2009). Blackwood and Clayton (2007) suggested feeding canola meal to weaner calves and dry cows and cows at 0.3 – 0.7 kg/hd/d with roughage available. Therefore, the effect of varying inclusion levels of canola meal as a protein supplement for pasture-based beef production systems warrants further investigation.

The rate and extent of microbial fermentation of dietary ingredients that ruminants are fed can have significant effects on the rumen environment and the overall health of the animal (Bergman 1990). Assessing the potential effects on rumen degradation as well as effects on animal production are critical when evaluating any supplement, including canola meal. The degradation kinetics of low-quality rations containing varying inclusion levels of canola meal using the *in vivo*, *in sacco* or the *in vitro* Daisy™ method has not been investigated. Although, ruminal parameters have been investigated when steers were fed TMR containing 15% and 30% canola meal (Nair *et al.* 2015), ruminal parameters have not been investigated when steers are fed a low-quality PMR containing more than 40% canola meal. Assessment of meat quality traits and eating quality are also important factors when determining the overall benefit of any feed supplement for livestock. Carcase assessments were not different when finishing steers were fed canola meal compared with a grain-based pellet (Lynch 2017); however, meat quality and consumer sensory evaluations to determine if there is a difference between dietary treatments warrants further investigation.

1.3 Research objectives and aims

Managing variability in pasture quality and quantity and maintaining a consistent supply of cattle that meet specific MSA and PCAS standards throughout the year is a challenge for beef producers supplying to certified grass-fed markets in Australia. Therefore, the overall objective of the research reported in this thesis was to determine the maximum inclusion level of canola meal as a PCAS approved supplement to roughages typical of that normally available during the summer-autumn period in southern NSW for grass-fed beef cattle. A combination of pen feeding, and animal house studies were undertaken (in a controlled and replicated environment) to

investigate the effect of varying inclusion levels of canola meal as an approved PCAS supplement for certified grass-fed beef cattle.

The specific aims of the research reported in this thesis were:

1. To determine whether meat quality and consumer sensory traits were negatively affected when steers were supplemented with either canola meal or a grain-based pellet;
2. To investigate whether production responses were higher when weaner calves were fed varying inclusion levels of canola meal and moderate quality forage;
3. To determine whether ruminal parameters (ruminal ammonia (NH_3-N) concentrations, and volatile fatty acid (VFA) concentrations and molar proportions) were higher when steers were fed low-quality ration containing increasing inclusion levels of canola meal;
4. To determine whether the *in sacco* and *in vitro* Daisy™ degradation kinetic parameters of a standard feed (lucerne hay) were not different when the donor steers were fed rations containing varying inclusion levels of canola meal;
5. To determine whether the *in vivo* apparent digestibility, *in sacco* and *in vitro* Daisy™ potential degradation (PD) were higher when steers were fed rations containing varying inclusion levels of canola meal; and,
6. To assess and determine whether the relationships between *in vivo* DM digestibility (DMD), *in sacco* DM PD and *in vitro* DM Daisy™ PD were similar when steers were fed rations containing varying inclusion levels of canola meal.

Chapter 2: Literature Review

2.1 Introduction

Ruminant production from a nutrition perspective is a function of the nutritive value of the feed consumed, the availability of microbial nutrients and the voluntary feed intake (VFI). Gaining an understanding of the energy and protein requirements of the host animal and its associated rumen microbes is necessary to identify the limitations to animal production on forage-based diets. The addition of energy and/or protein supplements is often practiced to meet energy requirements and production demands, especially during seasonal feed deficit periods or when the quality of the available pasture is low. Therefore, the scope of this literature review included investigation of digestibility methodologies for assessing the nutritive value of diets, assessment of the nutrient requirements for ruminants, investigation of the nutritive value of canola meal as a feed ingredient for ruminants and the effect of feeding it on animal production responses, nutrient digestibility, and meat and eating quality.

2.2 Assessing the nutritive value of diets

Nutritive value is used to quantify the chemical composition (nutrients) of a feed type which is available to the animal. The nutrient value is influenced by several factors such as the concentrations of nutrients, the availability of these nutrients and the efficiency with which available nutrients can be absorbed by the animal (see review Chapman, 2014). Other factors that can influence the nutrient value of the plant include genetics/ variety of the plants fed to livestock (Abberton *et al.* 2008), environmental conditions (Kemp and Dowling 1991), and the management of the forage (Moore *et al.* 2020).

Factors that contribute to forage nutritive value include crude protein (CP), soluble sugars, structural carbohydrates (hemicellulose and cellulose), lignin, pectin, lipids, tannins and minerals (Schut *et al*. 2009; Chapman *et al*. 2014). Typically, wet chemistry is used to assess the nutritive value/ chemical composition of feeds, whereby the feed composition is analysed to determine DM, organic matter (OM), N (CP = N x 6.25), fibre (neutral detergent fibre (NDF) and acid detergent fibre (ADF)), lipids, soluble sugars and starches (AFIA 2014). Near infrared reflectance spectrometry (NIRS) is another technique that can be used to determine the chemical composition

of feed; however, the suitability of the NIRS is dependent on referenced data to enable the instrument to be calibrated (Landau *et al*. 2006).

The quality of the ration consumed by the animal is determined by the supply of nutrients and the energy obtained by the animal. This can be determined by the digestibility (%) of a feed type and the metabolisable energy (ME, MJ/kg DM) available for the animal to use (CSIRO *et al.* 2007). Further description of measuring and assessing digestibility and the rate of degradation is presented below.

2.2.1 Digestibility

The ability of a feedstuff to sustain animal growth and production depends on its digestibility. In ruminants, digestibility of a feedstuff is influenced by its chemical (carbohydrate, protein and fat contents) and physical (particle size) characteristics that affect the ability of the microbial enzymes to colonise and digest feed particles (McDonald *et al.* 2011). There are various methodologies available to determine digestibility and rumen degradation and the subsequent energy and protein value of feeds (López 2005). Digestibility can be measured either in the animal (*in vivo* and *in sacco/in situ*), or by laboratory methods (*in vitro* methods). Near-infrared reflectance spectroscopy is increasingly being used as a rapid, relatively cheap and accurate method of evaluating some chemical characteristics and predicting the digestibility of forages, concentrates and grains (Park *et al.* 1998; Jafari *et al.* 2003; Catunda *et al.* 2021). This non-invasive technique is based on developing calibrations from laboratory analysis of a large number and wide range of forges, concentrates and grains (Coleman and Wyatt 1982). The largest limitation of this methodology is the prediction is only as good as the data that is used for the calibration, and like any method, variation can still occur (McDonald *et al.* 2011).

In vivo apparent nutrient digestibility methodology

The reference method to evaluate the nutritional values of feed/ feedstuff is *in vivo* apparent nutrient digestibility (Van Soest 1994; McDonald *et al.* 2011). This method is commonly known as the "total collection" method, where the feed(s) under examination is normally given to the animals daily in known amounts (feed intake determined) and the faecal output is subsequently measured, typically using metabolism crates or harnesses in ruminants (Van Straalen and Tamminga 1990; McDonald *et al.* 2011). Feed offered, any orts (feed refusals) and faeces are collected, subsampled daily and chemically analysed to calculate apparent nutrient digestibility (Rymer 2000). The *in vivo* method is not; however, a true measure of digestibility as

the faeces will also contain nutrients of endogenous and/or microbial origin (McDonald *et al.* 2011).

The *in vivo* method is the most accepted and reliable for the evaluation of feed digestibility; however, it is laborious, time consuming, expensive and not suitable for routine and/or large scale feed evaluation (Rymer 2000; CSIRO 2007). Furthermore, apparent nutrient digestibility only provides information on total tract digestibility and not the degradation kinetics of the feed(s) in the rumen (Ørskov *et al.* 1980; Pellikaan *et al.* 2013). In addition, other sources of variation occur with this technique including animals selected (age, breed and sex), level of feeding (LoF), use of supplements, and the preparation of feeds (Rymer 2000).

An alternative *in vivo* technique is using internal indicators such as lignin (Sein and Todd 1988) or chromium (Binnerts *et al.* 1968). These methods are suitable if animals are either grazing, housed in groups or where the use of metabolism pens is impractical. Digestibility can be determined as a ratio between the known concentrations of indicator in the feed and faeces, assuming that the concentrations of indicator in the feed can be measured and is completely indigestible (McDonald *et al.* 2011).

In sacco methodology

A method of estimating the degradation of a feedstuff(s) in the rumen is using *in sacco* (*in situ* or dacron bag or nylon bag technique) incubation method which requires the use of rumen-fistulated animals. Ground feed samples are weighed in small bags typically made of permeable synthetic material (dacron or nylon) with a standard pore size (400 – 1600 μm^2), which are then incubated in the rumen for various lengths of time (typically 72, 48, 24, 18, 12, 9, 6, 3 and 0 h for forages) yielding a degradation curve from which the rate of degradation can be estimated (Ørskov and McDonald 1979; López *et al.* 2005; McDonald *et al.* 2011). The *in sacco* method closely relates to the rumen environment in which degradation of different feedstuffs can occur; however, it can vary depending on the passage rate used to estimate the extent of rumen degradation of the feedstuff (SCA 1990; NRC 2001; CSIRO 2007). Although it can be laborious, time consuming and has ethical challenges, this method has been widely used and accepted due to the similarities to the environment of the rumen in which degradation of different feedstuffs can occur (Ørskov *et al.* 1980; Chaudhry and Mohamed 2011).

Interpreting and comparing *in sacco* results to other studies must be done with care (Ørskov *et al.* 1980; Chaudhry and Mohamed 2011) as there are many factors that can influence the rate of degradation and degradation kinetics such as animals (Nandra *et al.* 2000), ration fed to the animals (Ganev *et al.* 1979), sample grind size (Damiran *et al.* 2008), bag type (Valente *et al.* 2011), bag pore size (Lindberg and Knutsson 1981) and size of sample incubated (Nocek 1985). Therefore, the inclusion of a standard such as good quality hay is recommended when examining the degradability of various feedstuffs (Ørskov 2000). Lucerne hay has been widely examined in both *in sacco* (Julier *et al.* 2001; Danicke 2002) and *in vitro* (Wilman and Adesogan 2000; Valentine *et al.* 2019) methodologies and can be used as a comparison.

The porosity of the bag used should allow for an influx of ruminal fluid and associated microbes, efflux of digested feed particles, and the retention of feed particles not yet digested (Mehrez and Ørskov 1977). When the bag pore size is too small (< 35 um), it can limit the influx of microbes, especially the relatively larger protozoa species (Mehrez *et al.* 1977); if the pore size is too large (> 60 μm) new feed particles can enter back into the bag (via the ruminal fluid) and undigested feed particles escape from the bag (Nocek 1985) influencing the accuracy of the degradation loss of a feedstuff (Ørskov and McDonald 1979).

The grind size of feedstuff incubated can be between < 1 to 6 mm (Michalet-Doreau and Ould-Bah 1992; Dewhurst *et al.* 1995); however, generally 1 mm screen has been preferred to simplify and standardise chemical analysis in the laboratory. The ratio between sample size and bag surface area can alter the influx of ruminal fluid, and the efflux of digested feed particles (Nocek 1985). Some studies have also utilised Ankom filter bags as opposed to dacron bags for *in sacco* studies (Valente *et al.* 2011; Tagliapietra *et al.* 2012) that allows for a standardisation of bag size, pore size, sample grind size for the *in sacco* technique.

The effectiveness of the washing technique used after incubation is critical to suspend microbial activity and microbial attachment to the bag as it can influence the accuracy and comparison of results (Nozière and Michalet-Doreau 1996). There is no standard washing technique recommended and the impact of difference in washing techniques may vary between different feedstuffs as well as the length of the incubation period (Chaudhry and Webster 2001; Chaudhry and Mohamed 2011).

In vitro methodologies

The *in vitro* digestion method was first developed as an alternative to the *in vivo* method, as the latter can be laborious and costly, has ethical considerations, is time consuming and not practical for large quantities of samples to be examined quickly. There are several different *in vitro* techniques that can be used to predict the degradation of various feedstuffs. These techniques commonly use ruminal fluid/ liquor from a donor animal(s) and a buffered artificial salvia which combined mimic the rumen environment. *In vitro* assays utilising flasks, such as the two stage method of Tilley and Terry (1963), and using pepsin cellulase (McLeod and Minson 1978) or NDF (Goering and Van Soest 1970), are widely used to assess the digestibility of the feedstuff(s) (Tilley and Terry 1963).

The Ankom filter bag method (referred to as the *in vitro* Daisy™ incubation) utilising an Ankom Daisy™ Incubator^{II} (Ankom Technology, Mecedon, New York, USA) is a simpler procedure than traditional *in vitro* digestibility flask methods. The *in vitro* Daisy™ incubator allows for routine determination of digestibility of large numbers of samples in batches. The assay is relatively quick, easy to perform and resolves some analytical errors such as manual filtration steps (Weiss 1994). *In vitro* Daisy™ digestibility values have been found to be comparable with traditional *in vitro* methods for varied incubation time for forages (Wilman and Adesogan 2000; Valentine *et al.* 2019), grains (Holden 1999; Brons and Plaizier 2005), and protein meals (Mabjeesh *et al.* 2000). Like other *in vitro* techniques, *in vitro* Daisy™ incubation has sources of variation that can affect the digestibility or degradability of the feedstuff being incubated. These include inoculum source (Mabjeesh *et al.* 2000), sample size (Valente *et al.* 2011), sample preparation (Damiran *et al.* 2008), bag type and surface area (Vogel *et al.* 1999; Adesogan 2005).

The rumen environment, which influences the rate and extent of microbial fermentation, varies depending on the ration fed to the animal. Both the dietary ingredients and the total ration fed to the animal can impact on the *in vitro* degradation kinetics (Holden 1999). The ruminal liquor used for *in vitro* methodologies including *in vitro* Daisy™ incubation can represent the largest source of uncontrolled variation (Marten and Barnes 1979; Holden 1999; Wilman and Adesogan 2000), with contributing factors to this variability including the time of feeding (Van Soest 1994; France and Dijkstra 2005; López 2005), and the LoF (Adesogan, 2005; Valentine *et al.* 2019).

There are no standardised protocols for *in vitro* Daisy™ incubation, including the ration fed to the (rumen-fistulated) donor animals from which the ruminal fluid/liquor is collected. Differences in available feeds and the cost associated with various feedstuffs in different countries (Trujillo *et al.* 2010; Valentine *et al.* 2019) has contributed to this lack of a standard dietary protocol. Differences also exist in the characteristics of ruminal fluid collected from different animals fed the same ration (Weiss 1994) and this may also account for differences in results between laboratories.

The buffered artificial saliva solution used for *in vitro* Daisy™ incubation (McDougall 1948) varies to that used for some other *in vitro* methodologies. Modifications may include the addition of urea and ammonium sulphate (Kaiser *et al.* 2007) to ensure an adequate supply of N (Schmid *et al.* 1969; Weiss 1994; Tassone *et al.* 2020) such that NH_3-N concentrations are not below the lower threshold for optimum microbial activity (Satter and Slyter 1974; Slyter *et al.* 1979). Low ruminal NH_3-N concentrations can reduce the rate of digestion, especially that of fibre (Lee *et al.* 1985).

The pH of the ruminal fluid used for *in vitro* Daisy™ incubation should be within the optimal range of 6.0 to 6.9 for microbial growth (Kamra 2005; Sung *et al.* 2006; Raffrenato *et al.* 2018). When ruminal fluid pH is < 6.0, fibre degradation is adversely affected due to the direct (adverse) effects on cellulolytic bacteria (Dijkstra *et al.* 2012).

A sample size 0.5 ± 0.02 g DM for the F57 filter bag (pore size 57 μm) has been previously used in some *in vitro* Daisy™ digestibility studies (Holden 1999; Mabjeesh *et al.* 2000; Trujillo *et al.* 2010; Bender *et al.* 2016); however, others have used 0.25 g DM of sample (Wilman and Adesogan 2000; Adesogan 2005; Cattani *et al.* 2009). Similar with the *in sacco* techniques, the pore size of the filter bags is a compromise between allowing an influx of microorganisms, and efflux of digested material and preventing loss of undigested material (Kitessa *et al.* 1999; Valente *et al.* 2015). A lower sample size could facilitate the release of soluble and fine particles, while a larger sample size can exert a barrier effect, blocking the bag's pores and limiting the passage rate of ruminal fluid in and out of the bag (Cattani *et al.* 2009). For incubations of 72 h or longer, a smaller sample size may limit the amount of undigested material remaining that is subsequently available for analysis, especially when conducting the analysis of NDF and ADF sequentially. Ensuring enough residue for subsequent chemical analysis has led to the recommendation of a sample size of 0.75 ± 0.02 g DM

as opposed to 0.5 ± 0.02 g DM for F57 bags for *in vitro* Daisy™ incubation (Raffrenato *et al.* 2018).

Degradation kinetics

The extent and rate of degradation within the rumen is important when evaluating a feedstuff, especially for protein degradation (Rymer 2000). Furthermore, the fractional rate of passage is critical for the determination of microbial protein efficiency in ruminants (Pellikaan *et al.* 2013). The fractional rate of passage of feedstuffs can vary depending on diet composition (Alcaide *et al.* 2000), diet quality (Krämer *et al.* 2013) and LoF (van Gastelen *et al.* 2021). Typically, the passage rate is: 0.02/h for animals fed at a low LoF, approximately 1 x maintenance; 0.05/h for calves, low yielding dairy cows, beef cattle and sheep on a higher LoF but < 2 x maintenance; and 0.08/h for high yielding dairy cows, fed at > 2 x maintenance (AFRC 1993; CSIRO 2007).

Throughout the literature many equations and models that can predict potential degradability (Assuncao *et al.* 2022; Mertens 2015; Ohlsson *et al.* 2007; Ørskov and McDonald 1979; Ørskov *et al.* 1980, Raffrenato and Van Amburgh 2010; Raffrenato *et al.* 2018). The percentage loss and degradation kinetics of each constituent (DM, CP, aNFD or ADF) are used to determine degradability coefficients using the non-linear regression (exponential) equation: potential degradability (PD) = a + b (1 - e^{-ct}), as described by Ørskov and McDonald (1979) and Ørskov *et al.* (1980), and as shown in Figure 2.1. To account for any microbial attachment to the bag, the degradability of each component is corrected using a blank (Ørskov and McDonald 1979; Ørskov *et al.* 1980). One of the main issues when quantifying and describing digestion kinetics are any residues remaining at any incubation time (t), as the residues can be either undigested and indigestible (or both) dietary components (Raffrenato and Van Amburgh 2010). Therefore, the duration of incubation of a sample influences PD. Ørskov *et al.* (1980) described that to reach or almost reach PD, the appropriate incubation time for concentates is 12-36 h, for good quality forages is 48-60 h and for poor quality roughages it is 48-72 h. For *in vitro* Daisy™ assay longer inbuation times of up to 240 h have been used (Valentine *et al* 2019); however, issues arise such as refreshing ruminal fluid and more complex models are required to interpret the data (Tassine *et al* 2020). Forage NDF consists of an indigestible fraction (iNDF) and potentially digestible fraction (PDNDF). Therefore, to determine the NDF digestibility of a forage, it is critical to accurately predict iNDF (Bender *et al.* 2016).The NDF component has been assumed to be digested at a rate of zero and therefore does not

have a soluble fraction (i.e., a = 0; Chaudhry 2000; Spanghero *et al.* 2007; Raffrenato *et al.* 2009). The degradation of NDF/ ADF can be adjusted for a lag phase when required (Ohlsson *et al.* 2007; Raffrenato and Van Amburgh 2010; Raffrenato *et al.* 2018). Therefore, in this thesis potential degradation refers to PD after 72 h incubation.

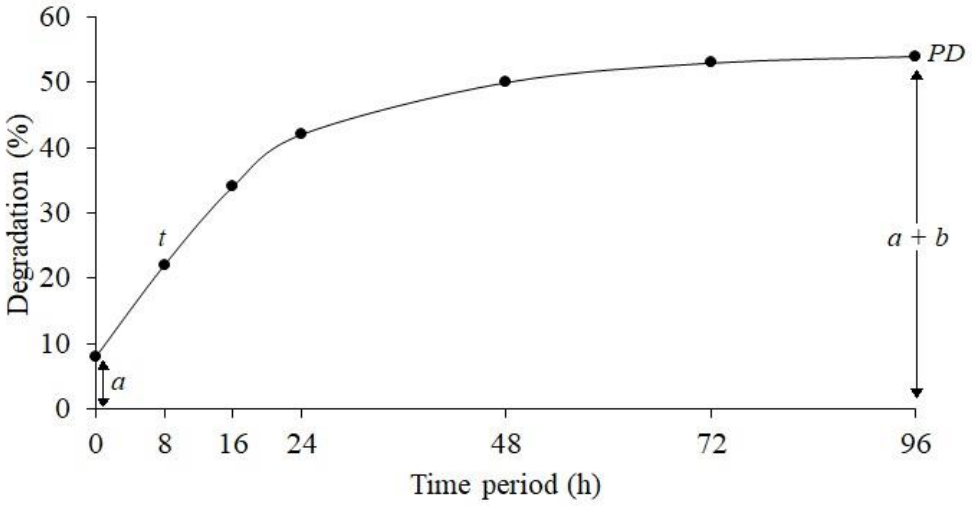


Figure 2.1 Schematic representation of the degradation of a typical roughage diet expressed by the formula PD = a + b (1 - e^{-ct}). Where; PD = potential degradability is the fraction of material degraded at 96 h, a = immediately degradable (water soluble) fraction at 0 h, b = not soluble, but slowly degradation fraction (96 h - 0 h), c = fractional rate of degradation of fraction b (per h (at time t)), t = incubation time (0, 8, 16, 24, 48, 72 and 96 h) and e = base for natural logarithm (adapted from Ørskov and McDonald 1979; Ørskov *et al.* 1980).

2.3 Nutrient requirements

2.3.1 Energy

The primary energy value of feeds is the amount of ME and is expressed as M/D (MJ/kg DM) (AFRC 1993; CSIRO 2007). Metabolisable energy represents energy that is available to use by the animal and the efficiency of its use (k value) varies depending on whether it is used for maintenance (k_m), gain (k_g) or lactation (k_l) (CSIRO 2007; McDonald *et al.* 2011). Energy requirements will be discussed in more detail below. Energy partitioning is diagrammatically represented in Figure 2.2, where ME is the gross energy less the energy in faeces, urine, and methane (CH_4). Metabolisable energy represents the amount of digestible energy within a feed that can be used for maintenance of body tissues, growth and production (milk, meat and fibre) (CSIRO

2007; Ørskov 2012). Net energy (NE) is energy that is gained by the animal from its diet and can be used as maintenance (NE_m), stored for liveweight gain (NE_g) and/or secreted as milk (NE_l) (Figure 2.2, CSIRO 2007).

Direct determination of ME is expensive, time consuming and therefore ME is routinely predicted from digestibility using published equations (CSIRO 2007). The preferred digestibility estimate from which to calculate ME is digestible organic matter in the dry matter (DOMD). Other equations exist to predict either DOMD or M/D from DMD or OM digestibility (OMD) (SCA 1990). Determining DOMD *in vivo* is preferred, but more often DOMD is determined using a wet chemistry method like those previously referred to or by NIRS (CSIRO 2007).

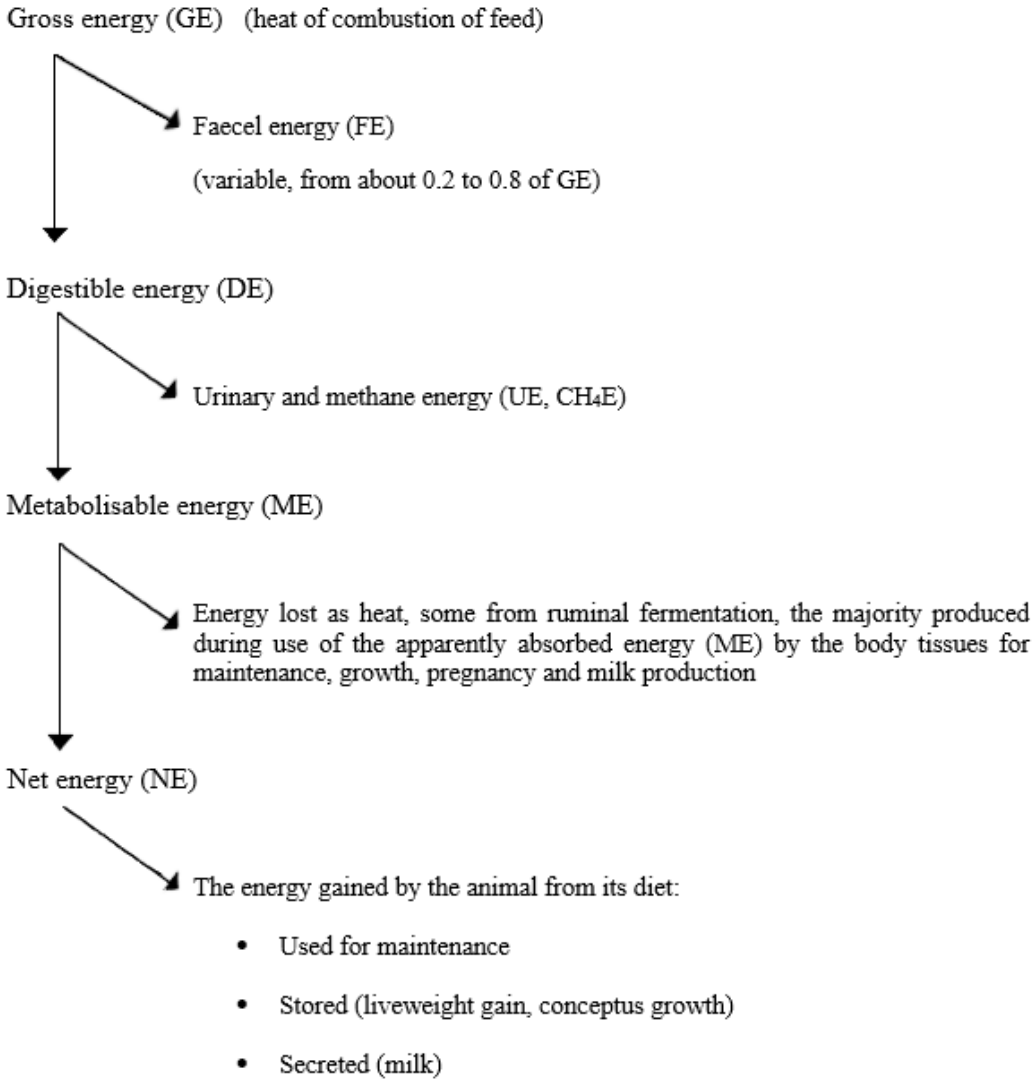


Figure 2.2 Energy partition of feed in the ruminant (source; CSIRO. 2007).

Volatile fatty acids

In ruminants, energy is derived primarily from the digestion of dietary cellulose, hemicellulose, pectin, starch and soluble sugars. Digestion is predominantly in the form of microbial fermentation in the rumen, releasing energy and producing VFA, which account for at least 50% of total digested energy (Sutton 1985). Diets rich in rumen degradable protein (RDP) and deaminated amino acids (AA) also significantly contribute to VFA yield (Noziere *et al.* 2010). Depending on the LoF, significant amounts of starch may escape ruminal degradation (Overton *et al.* 1999). Most post-ruminal starch digestion (on average 70%) occurs in the small intestine; however, starch digestibility in the intestine is variable (Noziere *et al.* 2010).

The major pathways of carbohydrate metabolism in the rumen that produce VFA are shown in Figure 2.3. The major source of energy for ruminants are VFA, mainly acetic, propionic and butyric acids (Ørskov *et al.* 1967) and these are also the predominant VFA produced (van Houtert 1993; France and Dijkstra 2005). Iso-butyric, iso-valeric, valeric, hexanoic and heptanoic acids are produced to a lesser extent (Aluwong *et al.* 2010; Noziere *et al.* 2010). All VFA can generate energy in the form of adenosine triphosphate (ATP). Carbon dioxide (CO_2) and methane (CH_4) are produced as waste products (van Houtert 1993) and represent a loss of energy.

Total VFA concentrations in the rumen vary between 70 and 130 mmol/L (France and Dijkstra 2005). Acetic, propionic and butyric acids are normally produced in a ratio varying from 75:15:10 to 40:40:20 (Bergman 1990). Acetic and butyric acids directly contribute to the energy supply of the animal, which can affect the overall efficiency of feed utilisation (Cronje *et al.* 1991) and propionic acid is the major contributor for gluconeogenesis in the body (Bergman 1990; Dijkstra 1994; France and Dijkstra 2005). As only a small amount of net glucose is available for absorption within the gastrointestinal tract in ruminants, propionic acid is, therefore, the most important VFA for ruminant production (Dijkstra 1994). Absorption of VFA through the reticulo-rumen wall accounts for 65% to 85% of the ruminal production, with the remainder absorbed in the omasum and abomasum (Noziere *et al.* 2010).

Valeric acid is formed from ruminal catabolism of the AA, arginine, proline and lysine (El-Shazly 1952). There is limited knowledge; however, on the other minor VFA including the branch chained VFA (BCVFA), iso-valeric and iso- butyric acids, and the longer chained VFA, heptanoic and hexanoic acids. Branched-chain VFA are formed from microbial deamination and decarboxylation of the branched-chain AA,

valine, isoleucine and leucine (Allison *et al.* 1962). Therefore, higher BCVFA concentrations may reflect increased protein degradation in the rumen (Alison and Bryant, 1983).

The total VFA concentration and molar proportions of individual VFA are mainly determined by the composition of the microbial population, which will vary depending on intake level, time of feeding, and dietary composition, particularly the nature of carbohydrates and their degradation rates (Bergman 1990; France and Dijkstra 2005). The composition of the ration can have significant effects on the rumen environment which then impacts on the rate and extent of microbial fermentation. Typically, the molar proportion of acetic acid is greatest for forage-based diets, whereas the feeding of starch-based diets increases the molar proportion of propionic acid (Brossard *et al.* 2004). Concentrate diets, and particularly those containing high levels of water soluble carbohydrates (WSC), may also increase the size of the rumen protozoal population, which induces an increase in butyric acid rather than propionic acid production (Brossard *et al.* 2004). The particle size of the diet can also impact the molar proportions of individual VFA. Longer particle size of forage/roughage can result in increased ratio of acetic acid to propionic acids as well as an increase in ruminal pH (Alamouti *et al.* 2014).

The feeding of low-quality (low protein, low digestibility) forages also affect VFA concentrations and proportions and typically yield low propionate: acetate ratio (Lee *et al.* 1987). As a consequence of lower propionic acid production, glucose may be the major limiting factor which can affect the utilisation of absorbed nutrients by ruminants. However, protein supplements can increase glucose availability by increasing DMI or increasing the availability of AA (of either microbial and/or dietary origin) for intestinal absorption. Total VFA concentrations were higher when a urea supplement was fed to steers offed low-quality forage diets (Lee *et al.* 1987).

The concentrations and molar proportions of VFA can also be affected by time after feeding. After feeding, there is normally a rise in VFA (and ruminal NH_3-N) concentrations and a fall in ruminal pH (Van Soest 1994). After feeding total VFA concentrations are normally between 70 and 130 mmol/L, but can be as low as 30 mmol/L or be in excess of 200 mmol/L (France and Dijkstra 2005). Ruminal pH has been found to be positively correlated with total VFA concentrations (Seymour *et al.* 2005; Packer *et al.* 2011).

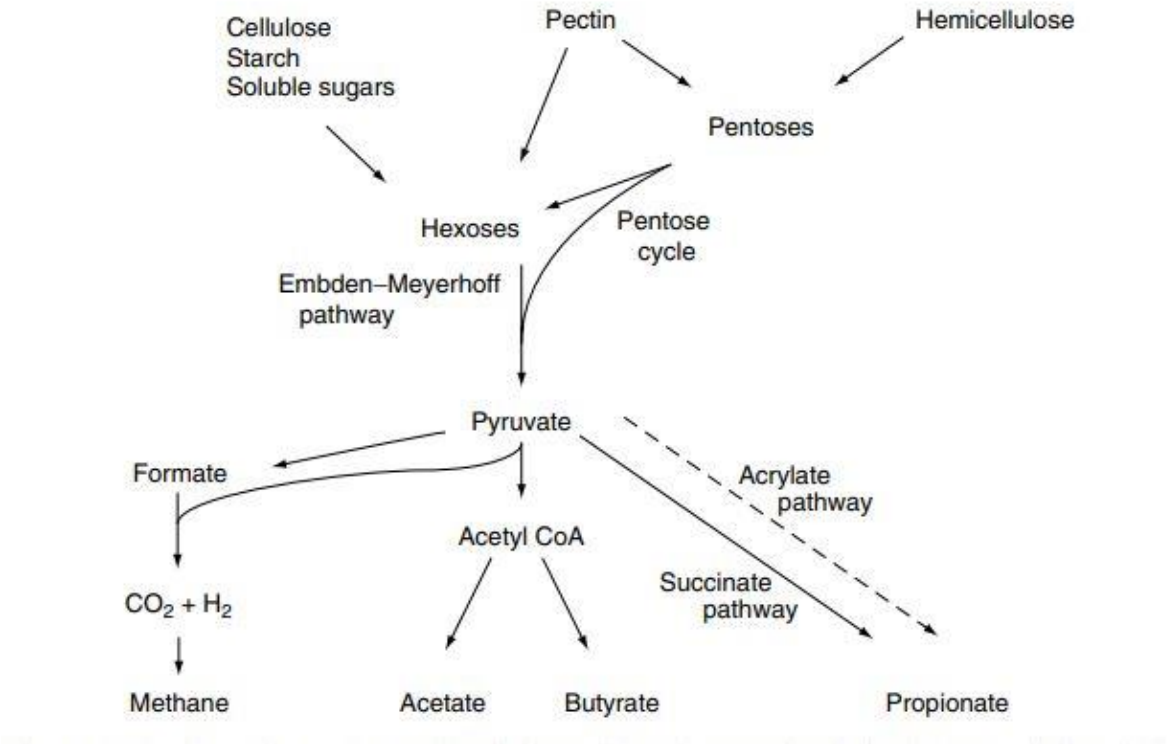


Figure 2.3 Schematic representation of the major pathways of carbohydrate metabolism in the rumen (source; France and Dijkstra 2005, p. 158).

The production of CH_4 in the rumen is influenced by the molar proportions of VFA (as reviewed by Moss *et al.* 2000; Williams *et al.* 2019). The amount of rumen fermented OM or the amount of digestible OM is also correlated to the amount of CH_4 produced. Roughage-based diets yield higher concentrations of acetic and butyric acids, which are associated with increased CH_4 production. In contrast, diets high in starch/ grain favour propionate acid production and decreased CH_4 production. Methane production increases as forage matures or when forages are roughly chopped rather than finely ground or pelleted (as reviewed by Moss *et al.* 2000). A rapid passage rate improves propionic acid production and the associated use of hydrogen (H) thereby decreasing CH_4 production. Increasing the LoF increases passage rate and consequently decreases CH_4 losses as a percentage of daily energy intake (Williams *et al.* 2019), thereby increasing the efficiency of use of energy.

Maintenance energy requirements

When livestock are given the maintenance LoF, the basal energy requirements are exactly met so the net gain or loss of energy from tissue of the animal is zero (Figure 2.4) (Corbett *et al.* 1990; CSIRO 2007; Ørskov 2012). The net energy (NE)

requirement for maintenance (NE_m) varies depending on the efficiency of use (k_m) of ME (CSIRO 2007; McDonald *et al.* 2011). When MEI increases above maintenance requirements, the animal begins to retain energy and this energy can be used for gain (k_g) or lactation (k_l).

Varying feeding levels and feed type can influence the maintenance costs, which are not necessarily related to metabolic size of the animal. In ruminants, energy for maintenance is largely absorbed in the form of VFA (Bergman 1990). In mature ruminants, the majority of energy used for maintenance is consumed by visceral tissues (digestive tract, liver, kidneys, heart and lungs) (Blaxter and Wainman 1964). In mature ruminants, at maintenance LoF approximately 50% of the energy used for maintenance is consumed by the liver and portal drained viscera and this value is likely to increase at higher LoF (Ortigues and Durand 1995). The liver and portal drained viscera contribute approximately 70% to total visceral tissues (Hegarty et al. 1999; Dougherty et al. 2022) that make up approximately 10% of empty body protein (Oddy et al. 2021).

Energy requirements for growth

The efficiency of utilisation of ME for growth is dependent on the composition of gain since the efficiencies of use of energy for protein and fat deposition differ (CSIRO 2007). Consequently, the ME requirement for any level of gain, varies between animals based on sex, growth rate, maturity type, stage of growth and LW. Early maturing animals deposit more fat at a lower LW and generally at a younger age compared to later maturing animals (McDonald *et al*. 2011).

In grazing ruminants, a considerable amount of energy is also consumed by muscle tissue, and this will vary depending on the amount of work required for grazing forages/diets and digestion of the forages/diets (Caton and Dhuyvetter 1997). The efficiency of use of ME for growth (k_g) is considered to be much lower when animals are fed roughage or forages in comparison to concentrate diets (Leng 1990). Furthermore, as forages mature, the digestibility of the forage typically declines, which can also affect the efficiency of use for ME for growth (Van Soest 1994).

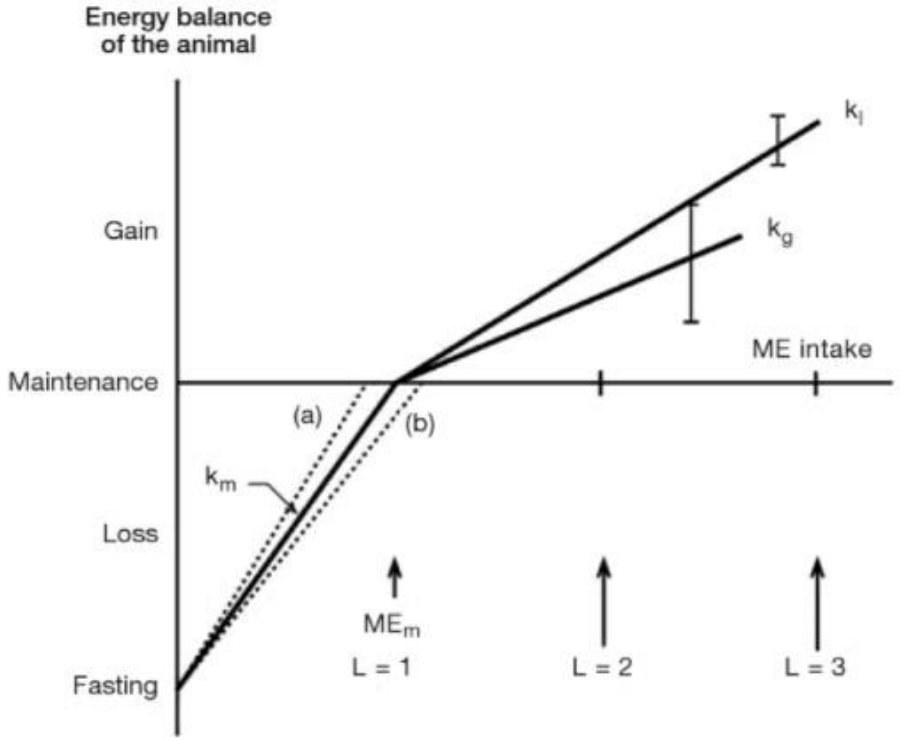


Figure 2.4 Change in energy balance of a ruminant with, on the same scale, change in the metabolisable energy (ME) intake. MEm is the ME intake that results in zero energy balance (maintenance); level of feeding (L) is unity. The solid lines showing the net efficiencies of use of ME (i.e., MJ net energy gain per MJ of ME) for maintenance (k_m), milk production (k_l), and growth and fattening (kg) are for mixed diets providing 10 MJ of ME per kg DM (M/D = 10). With higher or lower M/D, the net efficiencies (NE/ME) are respectively higher or lower within the range indicated by the dotted lines (a) and (b) for k_m and the vertical bars for k_l and k_g (source; CSIRO 2007).

The efficiency of use of ME for protein deposition is lower than for fat deposition and can also be more variable (CSIRO 2007). As the empty body weight of the animal increases, the relative proportions of energy, protein and fat increase. When the animal grows, the gain mainly consists of water, protein and ash required for bone and muscle growth; furthermore, as the animal ages the gain contains a higher proportion of fat (McDonald *et al.* 2011).

2.3.2 Protein

Metabolisable protein (MP) is the total digestible true protein (in the form of AA) available to the ruminant for metabolism after digestion and absorption in the small intestine (CSIRO 2007). Protein available to the ruminant is either supplied by microbial and/or dietary sources. Metabolisable protein can be divided into two

components: 1) digestible microbial true protein (DMTP); and 2) digestible undegradable protein (DUP). The DMTP is produced by the activities of the rumen microbes which synthesise protein from fermentable energy sources provided by the feed sources together with NH_3-N and AA from the breakdown of feed proteins in the rumen and recycling of NH_3-N via saliva (Alderman and Cottrill 1996). Microbial protein often accounts for two-thirds to three-quarters of the AA absorbed by ruminants (Dewhurst *et al.* 2000). The DUP is the fraction of the feed protein that has not been degraded during its passage through the rumen; however, it is digested and absorbed in the small intestines.

The protein value of feeds for ruminants is related to the degree of protein degradation in the rumen. Ruminal NH_3-N (and to a lesser extent AA) from the breakdown of dietary RDP and NPN sources (including urea) is utilised for the synthesis of microbial protein, to form MP and AA for absorption in the small intestine (Allen 2019). Dietary rumen undegradable protein (RUP) evades fermentation in the rumen to supply microbial protein and absorbable AA to the small intestine. To increase ruminant production, RDP is crucial for optimum microbial efficiency, while adequate RUP is required to optimise post-ruminal AA absorption (CSIRO 2007).

Rumen degradable protein

Rumen degradable protein can be classified as either true protein or NPN. True protein is composed of AA and is degraded to firstly peptides and AA and then eventually to NH_3-N. Non-protein N is composed of N present in deoxyribonucleic acid (DNA), ribonucleic acid (RNA), NH_3-N, free AA and small peptides (Alderman and Cottrill 1996; Bach *et al.* 2005). The end-products of rumen fermentation of dietary protein include NH_3-N, RUP and microbial protein (Figure 2.5). Some of the NH_3-N is used for microbial protein synthesis in the rumen, some is recycled back into the intestines, and the remainder is absorbed across the rumen wall into the portal blood vessels, then carried to the liver where it is converted to urea (McIntyre 1970; Reynolds and Kristensen 2008). Some of the urea may be recycled back to the rumen via saliva produced during rumination or through the ruminal epithelium (Reynolds and Kristensen 2008), with the remainder excreted in the urine (McIntyre 1970). Excess ruminal NH_3-N can result in poor N utilisation (Lapierre and Lobley 2001) and has an associated energy cost (Tyrrell *et al.* 1970; Reed *et al.* 2017; Jennings *et al.* 2018). There is variation in the amount of total N excreted at any given N intake, which may

be due to varying effects of experimental variation, animal variation, dietary or other environmental factors (Reynolds and Kristensen 2008).

The proportion of RDP ultimately captured in microbial protein depends on the CP content of the feed, the proportion of RDP in the CP, the availability of sufficient rumen degradable carbohydrates and the efficiency of microbial protein production (Tamminga 2006). The minimum CP requirements for adequate ruminal fermentation and for preventing the reduction of either DMI or apparent nutrient digestibility was proposed by CSIRO (2007) to be 70 g CP/kg DM, and from 60 - 80 g CP/kg DM by Van Soest (1994). Therefore, CP above 8% of the total diet DM typically results in improved apparent nutrient digestibility (Van Soest 1994) and normally meets microbial N requirements. The minimum threshold ruminal NH_3-N concentration to support microbial growth is 50-80 mg NH_3- N/L (Satter and Slyter 1974; Slyter et al., 1979). If this threshold is not met (due to inadequate RDP), microbial activity is suppressed, thereby reducing carbohydrate digestion (Leng 1990; McCollum and Horn 1990; Van De Kerckhove *et al.* 2011) and microbial yield, and ultimately decreases VFI and reduced animal production (Lee *et al.* 1987; Poppi and McLennan 1995; Mathis *et al.* 2000). Feeds high in RDP include young leafy fresh grass as well as grass silage (Tamminga 2006). Supplementation of RDP to cattle consuming low-quality forage generally results in improved forage intake and digestion (Olson *et al.* 1999; Mathis *et al.* 2000).

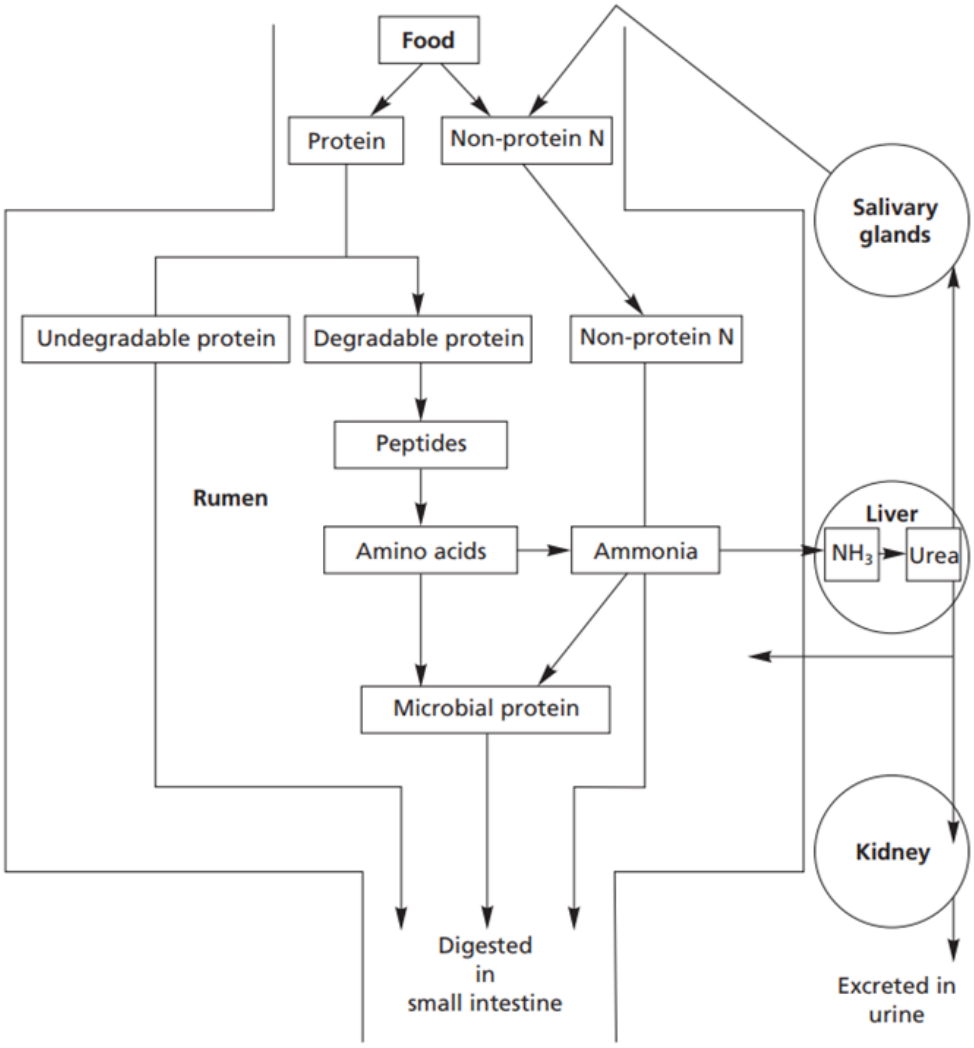


Figure 2.5 Digestion and metabolism of nitrogenous compounds in the ruminant (source; McDonald *et al*. 2011).

Microbial protein

Microbial protein contributes to a significant proportion (0.42 to 0.93) of the total protein available for absorption in the small intestine (Shingfield and Offer 1999). Microbial protein supply to the animal is affected by the composition, growth and turnover (Hespell and Bryant 1979) as well as lysis of bacteria in the rumen, the predation on bacteria by protozoa, and the digestibility of microbial protein in the intestines (Thomas 1973; Russell *et al.* 1992). The composition of the microbial population will vary depending on the rumen environment and substrate supply, which is influenced by both diet and feed intake. Generally, as DMI increases, microbial

protein synthesis also increases (Djouvinov and Todorov 1994); however, there is an upper limit of microbial protein synthesis (Leng 1990).

Microbial protein synthesis is maximised when the availability of fermentable energy and degradable N in the rumen are synchronised (Dewhurst *et al.* 2000). Ammonia is the primary N source for bacterial growth in the rumen (Nolan and Leng 1972) with some 82% of bacteria isolated from the rumen using NH_3-N as their principal source of N (Bryant 1973). Ruminal NH_3-N concentrations vary depending on feed intake, the CP content of the diet, the rate of degradation of feed protein, and the synchrony between the degradation of protein and carbohydrates (Tamminga 2006). Ruminal NH_3-N concentrations are affected by the time after feeding, typically peaking at 1-2 h after feeding (De Boever *et al.* 2005).

The efficiency of microbial protein synthesis (as opposed to yield) is lower for diets containing high levels of concentrates compared to forage-based diets (Cantalapiedra-Hijar *et al.* 2009). When soluble sugars and starches are fed, microbial growth is high for up to 2 h post-feeding but has decreased substantially by 4 h post-feeding. Degradation of soluble sugars and starch provide higher amounts of ATP than degradation of structural carbohydrates (cellulose and hemicellulose). However, structural carbohydrates are degraded much slower than soluble sugars and starch, allowing for a longer period of ATP production, thus supporting microbial growth for a longer period (Atkinson *et al.* 2010).

Rumen pH also impacts the efficiency of microbial protein synthesis. Feeding high starch diets leads to low ruminal pH, which is deleterious to many rumen microbes (Firkins 1996). Low rumen pH decreases digestibility of structural carbohydrates (Trevaskis *et al.* 2001). Thus, despite the potentially high intakes of starch-based diets, the efficiency of microbial protein synthesis may be reduced due to energy spilling to maintain cellular pH when rumen pH is low (Dewhurst *et al.* 2000).

Rumen undegradable protein

Feeding diets that are high in RUP can lead to a deficiency of fermentable energy (or end-products of fermentation) for rumen microbes and, consequently, reduced microbial protein synthesis (Cecava and Parker 1993; Christensen *et al.* 1993). As RUP avoids microbial fermentation in the rumen, it serves no benefits for the growth of rumen microbes, however, RUP does supply a direct source of AA to the animal to use for production (McDonald *et al.* 2011). Meeting the nutritional requirements of the rumen microbes as well as the host animal is essential for effective beef production.

As previously discussed, requirements for both energy and metabolisable protein are higher for growth and production than for maintenance.

2.4 Nutritional limits to animal production from low-quality pastures

In Australia, approximately 373 million ha of land is used for agricultural production and can be subdivided into temperate, tropical and arid grasslands (Michalk *et al.* 2017). Of this, approximately, 341 million ha is used for grazing, of which 36 million ha contains improved pastures (ABS 2017). In the Riverina region and Australian Capital Territory, 5.56 million ha is used for agricultural production, 1.12 million ha is mainly used for grazing on improved pastures, and 2.41 million ha is used for grazing on unimproved/ native pastures (ABS 2017).

Improved pastures in southern Australia are commonly based on subterranean clover (*Trifolium subterraneum* L.) and annual grasses (Hackney *et al.* 2019). In areas with higher and more reliable annual rainfall, perennial grasses such as phalaris (*Phalaris tuberosa* L.) and ryegrass (*Lolium perenne* L.) are more common (Michalk *et al.* 2017). These pastures, however, typically only provide small amounts of green feed during summer and early autumn months (Morley *et al.* 1978). The seasonal growth rates (Figure 2.6a) and the yield variability (CV%, Figure 2.6b) of pastures in southern NSW are depicted in Figure 2.6.

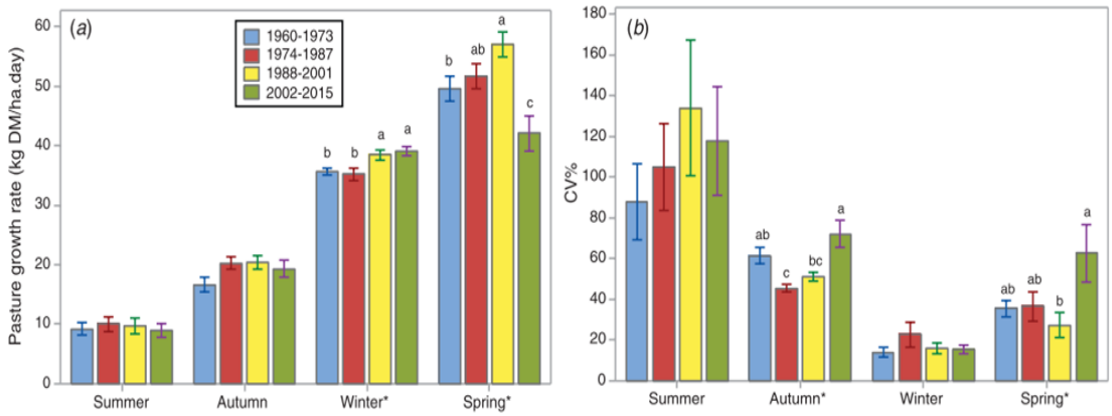


Figure 2.6 Seasonal (a) pasture growth rate (kg DM/ha.d) and (b) pasture yield variability (CV%) from 1960 to 2015 across six sites in southern NSW (Wagga Wagga without C4 grasses, Wagga Wagga with C4 grasses, Dookie, Hamilton, Ellinbank and Elliot (source; Perera *et al.* 2020).

Grazing livestock performance is determined by the quantity and quality of pastures available. Therefore, variation in growth rates of grazing beef cattle is strongly influenced by the season (temperature) and rainfall because it determines the type and quality of available pasture (Hill et al., 2009) as well as the amount of energy utilised by the animal to graze the pasture (Corbett et al., 1990). Rainfall is the major source of pasture growth variation in southern NSW (Chapman et al., 2014).

Determining nutrient intake of animals grazing on pastures is difficult, especially as the diet is continually changing. Changes in the quality of the diet ingested by grazing animals can be due to multiple factors including soil nutrient status (Bell *et al.* 2012), rainfall (Kemp and Dowling 1991), pasture composition (Hill *et al.* 2009), stage of plant growth (Perera *et al.* 2020), diet selection (Ciavarella *et al.* 2000) and other nutrients (Moore *et al.* 2009).

Nutritional imbalances in grazing animals are often associated with pasture, roughage or forage-based diets. Low-quality roughages typically contain high levels of structural carbohydrates and low levels of non-structural carbohydrates which can negatively impact digestibility (Köster *et al.* 2002). Low-quality forages are low in CP which may limit microbial growth due to low ruminal NH_3-N concentrations (Figueiras *et al.* 2010) and low supply of minerals, especially S, which may limit microbial growth (Saini *et al.* 2005; Jha *et al.* 2011). In addition, pasture species, particularly legume species, may contain plant secondary metabolites such as proanothocyanidins/ condensed tannins (Barry and McNabb 1999), which can limit the performance of grazing ruminants (Min *et al.* 2003; Naumann *et al.* 2017). The efficiency of feed utilisation is the major determinant of animal production responses. Preston and Leng (1987) and Leng (1990) suggested that with adequate and appropriate supplementation, ruminants grazing low-quality roughages can achieve high feed conversion efficiencies (FCE) and medium to high levels of production.

2.4.1 Forage quality

Generally, plants in the vegetative stages of growth have a relatively high digestibility and high CP content. As plants mature, a decrease in digestibility is generally observed due to an increase in the stem to leaf ratio and the associated increase in the proportion of the structural fibre and indigestible compounds, such as lignin (Van Straalen and Tarnminga 1990; Jung and Allen 1995).

Low-quality forage can be defined as those forages that are less than 55% digestible, are deficient in protein (< 80 g CP/kg DM) and low in soluble sugars and starches

(usually < 100 g/kg DM) (Lee *et al.* 1985; Leng 1990). As the stage of maturity increases, the proportion of cell wall components of the forage (cellulose, hemicellulose and lignin) increases, whereas the proportion of cell contents decreases (Bosch *et al.* 1992). Generally, when the quantity of roughages/ forages is not limited, provision of dietary supplements high in protein are beneficial to cattle grazing low-quality pastures. Responses to protein supplementation occurs when the basal roughage/ forage contains less than 6–8% CP (Lee *et al.* 1987; review by DelCurto *et al.* 2000). Production responses by ruminants will depend on the roughage/ forage availability, digestibility, other limiting nutrients and the energy and protein requirements of the animal.

2.4.2 Dietary supplementation

Energy and protein utilisation can be improved when supplementing low-quality roughages with protein meals (Lee *et al.* 1987; McCollum and Horn 1990). Supplemental protein can improve DMI and ADG by correcting N deficiencies, increasing post ruminal supply of AA and/or the correction of AA deficiency or imbalances at the tissue level that can stimulate DMI and increase ME utilisation (McCollum and Horn 1990). The improved growth rates of ruminants when supplemented with protein and fed low-quality forage is related to glucose availability (Leng 1990). Glucose availability is increased either by increasing feed intake (thereby increasing total VFA, especially propionic acid production) or by increasing the availability of glucogenic-AA for absorption in the intestines (Lee *et al.* 1987).

Energy and/or protein supplementation of grazing ruminants to meet maintenance requirements and production demands is often practiced (Smith and Warren 1986a; Moore *et al.* 2009; McLennan *et al.* 2017a). Traditionally, producers have supplemented grain or grain-based pellets (predominantly energy supplements) to ruminants grazing low-quality pastures, despite the first limiting factor of low-quality pastures being protein. Grain or grain based pellets are; however, not approved for use under PCAS (PCAS 2016). Supplements that are approved for use under the PCAS guidelines include canola meal, cottonseed meal, flax seed meal and soybean meal. These oilseeds meals are primarily classified as protein supplements and thus are ideal to meet maintenance requirement of animals grazing low-quality pastures. These oilseed meals also contain variable amounts of RUP and energy, both of which contribute to production responses in the animal.

Dietary supplementation of cottonseed meal (McCollum and Galyean 1985; Zinn *et al.* 1997), soybean meal (Albro *et al.* 1993; Mathis *et al.* 1999) and canola meal (He *et al.* 2013; Damiran *et al.* 2016; Good *et al.* 2017) have previously been associated with increased production in beef cattle (Hennessy *et al.* 1983; Poppi and McLennan 1995; Olson *et al.* 1999; Ipharraguerre and Clark 2005). Of these (true) protein supplements, cottonseed meal supplementation has traditionally been the most commonly used in Australia (Hennessy 1980) and has been shown to improve DMI and ADG of steers fed low-quality roughages (Hennessy *et al.* 1981; Hennessy *et al.* 1983; Lee *et al.* 1985; Smith and Warren 1986a, 1986b; McLennan *et al.* 2017a; McLennan *et al.* 2017b). Limited information exists on the effects of canola meal supplementation on digestibility, growth performance, and subsequent beef eating quality for cattle grazing on low-quality roughages and warrants further investigation.

2.5 Nutritive value of canola meal

Canola belongs to the Brassicas family (*Brassica napus*) (Mejicanos *et al*. 2016). In 1985, rapeseed and canola were officially recognised as different species, with the name "canola" derived from "Canadian oil, low acid" (Canola Council of Canada 2021). To be classified as "canola", the oil must contain < 2% erucic acid and the meal must contain < 30 µmol of four individual glucosinolates *(*3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate*)* per gram of oil-free matter of the seed (Bell 1993; Mailer 2004; Obied *et al.* 2013; Canola Council of Canada 2021).

Extraction of oil from the seed results in the production of meals or cakes. Traditionally, rapeseed meal contained high concentrations of erucic acid and glucosinolates, with the latter the limiting factor when using the meal in livestock diets (Mailer *et al.* 2008; Alashi *et al.* 2014). Glucosinolates are S containing glycosides that can reduce animal performance and impair thyroid function in developing foetuses and growing animals (Mailer *et al.* 2008; Mejicanos *et al.* 2016). Previous reviews have concluded inclusion of high levels of rapeseed meal in the diets for ruminants has negative effects on palatability due to the high concentrations of glucosinolates (Bell 1993; Mawson *et al.* 1993b; Spörndly and Åsberg 2006; Tripathi and Mishra 2007). Rapeseed also contains sinapine, a cholerine ester of sinapic acid, which is required for biosynthesis of lignin and flavonoids in plants (Bell 1993). Sinapine is bitter tasting, which results in the feed being less palatable and therefore, can limit DMI (Mailer *et al.* 2008; Mejicanos *et al.* 2016). Selective breeding of canola with lower

concentrations of both erucic acid and glucosinolates, has allowed canola meal to be more widely utilised as a livestock feed than rapeseed meal.

Variations in the oil extraction process changes the chemical composition and therefore the nutritive value of the resultant canola meal (Mejicanos *et al.* 2016; Seberry *et al.* 2017), as highlighted in Table 2.1. There are three main oil extraction methods, viz. cold pressed where the oil is extracted without heat, expeller-extracted, where the oil is physically extracted and heat is added, and solvent-extracted where the oil is extracted with the combined physical 'expeller' extraction and hexane (Mailer 2004; Seberry *et al.* 2017). Solvent-extraction is the most common method used (Mejicanos *et al.* 2016; Heim and Krebs 2018b), is the most efficient for extraction of the oil, and produces a meal with less than 5% oil residue (Mailer *et al.* 2008). Solvent-extracted meal is placed in a desolventiser-toaster, where the solvent (hexane) is removed using steam, as the solvent vaporises with the heat (95 - 115^{o}C). The final meal normally contains 10% moisture (Mejicanos *et al.* 2016). The cold-press and expeller oil extraction methods are less efficient resulting in meals containing 8-15% residual oil (Woyengo *et al.* 2010).

In addition to the oil extraction method used, the nutrient composition of canola seed and, therefore, canola meal can be affected by several factors such as variety, environmental factors, and storage (Bell 1993). The composition of the canola seed can be affected by excess moisture, frost damage, seed size, colour and the hull of the seed (Mailer *et al.* 2008). The nutrient composition of the canola seed can affect the energy, protein and fibre content of the residual meal (Bell 1993; Newkirk 2011). Heat and pressure used during canola meal processing can also affect the degradability of its protein and fibre components (Heim and Krebs 2018a).

Table 2.1 Nutritional value of Australian canola meals produced by expeller pressed, solvent extraction and cold pressed oil extraction techniques (adapted from Heim and Krebs 2018b).

Component[1] (g/kg DM)	Cold pressed	Expeller pressed	Solvent extraction
DM (fresh)	931	927	900
OM	930	941	930
CP	380	384	395
NDF	253	288	255
ADF	224	243	186
IVDMD	712	760	715
EE	120	102	33.3
ME (MJ/kg DM)	11.6	11.3	12.3

[1]DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; IVDMD = *in vitro* dry matter digestibility; EE = ether extract; ME = metabolisable energy.

2.5.1 Protein

The CP content of canola meal also varies depending on environmental conditions throughout the growing season (Mejicanos et al., 2016), varietal differences (Theodoridou and Yu 2013; Chen *et al.* 2015), and yield (Bell, 1993) of the canola crop. The CP content of Australian canola meals was found to vary from 36.7% to40.1% with total CP digestibility ranging from 85.1% to 90.8% (Heim and Krebs 2018b). These results are similar to those reported for Canadian-produced canola meals, as reviewed by Bell (1993); although Newkirk (2009) reported CP levels as low as 36% for some Canadian produced canola meals. Varying values for estimation of CP and other nutritive values for canola meal can depend on the model and equations employed (McDonald *et al.* 2011) and may account for some of the differences in reported values.

Based on the Cornell Net Carbohydrate and Protein System (Russell *et al.* 1992; Sniffen *et al.* 1992), Australian produced canola meals consist of rapidly degradable CP (A fraction, 12.9-26.6%), potentially degradable CP (B fraction, 62.4-80.1%) and undegradable CP (C fraction, 0.03-0.4%) (Heim et al., 2018c). Thus, canola meal is a source of both RDP, for efficient microbial growth in the rumen (Brito and Broderick 2007), and RUP; although, it is not considered to have a high RUP content (Bell 1993). The AA profile of the RUP includes high levels of histidine, methionine and cystine (Newkirk 2009; Seberry *et al.* 2017). Estimated RUP is lower for cold-press than expeller- and solvent-extracted meals (Heim and Krebs 2018b). The heat used during

the extraction process has a significant effect on both CP content and rumen degradability of that protein (Nia and Ingalls 1992). Expeller heat may increase RUP by establishing cross-linkages among and within peptide chains, and to carbohydrates (Deacon *et al.* 1988). Increased dry heat temperature can reduce both CP and the rapidly degradable protein fraction (Heim and Krebs 2018a), as well as acid detergent insoluble CP (Heim and Krebs 2018c).

2.5.2 Energy

Canola meal is often used in livestock feed as a source of protein; however, it can also be used as a source of energy. The M/D of canola meal is typically 11.3-12.3 MJ/kg DM but varies due to the amount of residual oil in the meal. As shown in Table 2.1, for Australian canola meals solvent-extracted meals have been found to have a higher M/D than cold-press and expeller extracted meals, respectively (Heim and Krebs 2018b). The average lipid content of Australian produced solvent-extracted meal is similar to the 3.5% reported for Canadian produced solvent-extracted canola meals (Mawson *et al.* 1993a; Newkirk 2009).

Crude canola oil contains phospholipid material (commonly known as "gum") which is removed during oil processing and the oil refinery process. The phospholipids are removed with a mild organic acid such as citric acid, yielding acidulated soapstocks (Mailer 2004). These acidulated soapstocks may be added back into the meal (Newkirk 2009), typically at a rate of 1-2% (Heim and Krebs 2018b), increasing the M/D value and decrease the dustiness of the meal (Spragg and Mailer 2007).

Any residual oil in canola meal is high in polyunsaturated fatty acids (PUFA) (He *et al.* 2013); however, the fatty acid (FA) profile varies between expeller- and solvent-extracted meals (Newkirk *et al.* 2003a) and this may impact on carcase and meat quality characteristics. The PUFA and conjugated linoleic acid (CLA) concentrations of beef were higher when cold press canola meal was included in PMR for feedlot cattle (Mir *et al.* 2003; Dugan *et al.* 2011; He *et al.* 2013), although He *et al.* (2013) found inclusion of solvent-extracted canola meal did not alter carcase quality. The potential human health benefits of the increased levels of desirable FA (i.e., increasing omega-3 and CLA) in beef in response to canola meal supplementation warrants further investigation.

2.5.3 Fibre

Canola meal contains polysaccharides, lignin and polyphenols. The polysaccharides can be classified into total starch polysaccharides (17.7 - 21.2 %, neutral detergent insoluble and neutral detergent soluble), cellulose (4.6 - 6.0 %), non-cellulose polysaccharide (2.5-3.8% and 10.4-11.4%, non-cellulose polysaccharide soluble and insoluble, respectively), lignin and other polyphenols (5-10.5%) plus proteins and minerals associated with the fibre fraction (Bell 1993; Mejicanos *et al.* 2016).

The hulls of the canola seed account for approximately 16.8% to 21.2% of the total seed mass and the hulls consist mainly of non-starch polysaccharides and lignin (Mejicanos *et al.* 2016). Canola meal contains relatively more hulls and therefore has a higher fibre content compared to other oilseed meals such as soybean meal (Bell 1993; Newkirk 2011).

The average NDF and ADF contents of Australian solvent-extracted canola meal were found to be 25.5% and 18.6%, respectively (Heim and Krebs 2018b), with an average lignin content of 5.1% (Evans *et al.* 2016). The NDF content varies between canola varieties, with solvent-extracted canola meal derived from early or mid-maturity varieties reported to contain 32.6% and 35.6% NDF, respectively. Varietal differences have also been found to impact the ADF content of solvent-extracted canola meal, with early and mid-maturity solvent-extracted canola meal (produced in NSW) reported to contain 18.3% and 18.9% ADF, respectively (Mailer *et al.* 2008). In comparison, Canadian solvent-extracted canola meal contained 28.8% NDF, 18.4% ADF and 5.8% lignin (Evans *et al.* 2016), which is similar to Australian solvent-extracted canola meal.

2.6 Canola meal as a feed ingredient for ruminants

Canola meal is used as a feed ingredient in many ruminant livestock industries including dairy (Ha and Kennelly 1984; Russo *et al.* 2017; Auldist *et al.* 2019), feedlot cattle (He *et al.* 2013; Nair *et al.* 2015), cattle grazing systems (Seoane *et al.* 1992; Veira *et al.* 1995) and sheep (Stanford *et al.* 1995; Masters *et al.* 2002). Canola meal has been used as a source of additional protein in TMR (Van De Kerckhove *et al.* 2011; He *et al.* 2013; Nair *et al.* 2016; Good 2018) and PMR (Seoane *et al.* 1992; Seoane *et al.* 1993; Petit *et al.* 1994) for both dairy (Russo *et al.* 2017; Auldist *et al.* 2019) and beef cattle (Williams *et al.* 2008; He *et al.* 2013; Nair *et al.* 2015).

Canola meal has been included in TMR at levels up to 30% of total diet DM (He *et al.* 2013; Nair *et al.* 2015); however, in PMR its intake has been typically less than 1.2

kg/hd/d (Seoane *et al*. 1992; 1993; Petit *et al*. 1994). The effect of feeding canola meal on ruminant production, DMI, apparent nutrient digestibility and ruminal parameters is discussed in more detail in the following sections.

2.6.1 Sheep

In Australia, canola meal has been used as a protein supplement for lambs fed either low-quality pastures or roughages. The DMI, ADG and feed conversion ratio (FCR) of lambs fed a ration consisting of lucerne and oaten hays (30:70) increased when canola meal was supplemented at the rate of 84 g/hd/d; however, there were no significant effect on hot standard carcase weight (HSCW) (Ponnampalam *et al.* 2005). The DMI, ADG, FCR, HSCW and meat quality traits did not differ when comparing canola meal with lupins as a protein source in TMR (Wiese *et al.* 2003). Replacing soybean meal with canola meal in TMR had no effects on ADG, FCR (Stanford *et al.* 1995), carcase characteristics or meat quality (Sekali *et al.* 2020). Canola meal has thus been found to be a suitable alternative protein source for sheep; however, there is no literature on the effects of or appropriate level of canola meal supplementation of sheep grazing low-quality roughages and warrants further investigation.

2.6.2 Dairy cattle

In Australia, canola meal has become a widely acceptable protein source when formulating TMR and PMR for dairy cattle due to its availability, high protein quality and palatability (Russo *et al.* 2017; Wright *et al.* 2017). The use of canola meal as a source of dietary protein in dairy systems has been extensively researched with many meta-analyses and reviews undertaken (e.g., Santos *et al.* 1998; Huhtanen *et al.* 2011; Martineau *et al.* 2013; Evans *et al.* 2016). From their review of 122 experiments, Huhtanen *et al.* (2011) concluded canola meal could be a successful substitute for soybean meal on an isonitrogenous basis in dairy rations. Furthermore, they concluded DMI and milk yield were higher and there was a more balanced supply of AA to the small intestines when soybean meal was replaced with canola meal (Huhtanen *et al*. 2011). Martineau *et al.* (2013) concluded that in comparison to several other protein sources, canola meal fulfilled both RDP and RUP requirements resulting in an increase in milk production.

Dry matter intake and milk yield increased when canola meal was included in PMR (Auldist *et al*. 2014a; 2019; Russo *et al.* 2017). Increased ruminal NH_3-N concentrations, a lower molar proportion of propionic acid, a higher molar proportion

of butyric acid and an increase in the ratio of acetic + butyric/propionic were observed when 16% canola meal was included in a PMR for dairy cows grazing perennial ryegrass (Auldist *et al.* 2014a). Conversely, ruminal NH_3-N concentrations, total VFA concentrations and the molar proportions of individual acids (acetic, propionic and valeric acids) did not differ when 22% canola meal was included in a PMR based on ryegrass (Russo *et al.* 2017). Similarly, total VFA concentrations were unaffected when lactating dairy cows were fed rations containing either 11% or 17% canola meal (Broderick *et al.* 2015). Dry matter intake, ADG and FCE were not altered when soybean meal was replaced with up to 20.7% canola meal in a pelleted starter rations formulated for Holstein calves (Burakowska *et al.* 2021).

Milk yield was improved when canola meal was included in a TMR based on lucerne and corn silage, and this was likely due to the RUP and AA profile of canola meal (Brito and Broderick 2007). Replacing soybean meal with canola meal increased milk fat and protein yield and N utilisation in lactating cows fed TMR based on corn and lucerne silage (Broderick *et al.* 2015). Energy corrected milk yield and milk protein were improved for cows in early lactation but not those in late lactation when some wheat in a PMR was replaced with canola meal (Auldist *et al.* 2019).

Supplementing high levels of CP in dairy rations (> 17.5% CP) may have adverse effects, especially in regards to the efficiency of N use and excess N (Russo *et al.* 2017). Excess N is converted to urea in the liver and is wasted as an energy cost to the animal (Reynolds and Kristensen 2008) which also may have adverse environmental impacts (Russo *et al.* 2017) as a consequence of nutrient overloading of soils.

2.6.3 Beef feedlot cattle

Canola meal has been used as a protein source throughout the beef feedlot industry, both domestically and internationally. In Australia, cottonseed meal remains the most used true protein source although other oilseed meals as well as lupins are also used as true protein sources in feedlot rations (Cowley *et al.* 2019; Greenwood 2021). The effects of any protein supplement on DMI, growth performance, and FCE will vary depending on other ration components (Koenig and Beauchemin 2011), feeding regimen (Islam *et al.* 2021) and the genetics of cattle (Robinson and McQueen 1994).

Dry matter intake and ADG have been investigated in several studies where canola meal was included in feedlot TMR (Van De Kerckhove *et al.* 2011; He *et al.* 2013; Nair *et al.* 2016; Good 2018). Dry matter intake was not affected when canola meal was offered at either 0, 10 or 20% of total diet (Nair *et al.* 2016) or when included at

7.3% in a TMR offered *ad libitum* to feedlot cattle (Van De Kerckhove *et al.* 2011). Dry matter intake and ADG were also not affected when canola meal was offered at 15% or 30% of a TMR for the entire (growing plus finishing) feeding period; however, DMI and FCR were significantly higher when canola meal was included in the TMR fed during the finishing period (He *et al.* 2013).

Varying the inclusion level of canola meal in TMR has had variable effects on nutrient digestibility. Dry matter digestibility and OMD were higher when canola was included at 7.2% (Koenig *et al.* 2004), 10% or 20% (Nair *et al.* 2016) in TMR. In contrast, DMD was unaffected by the inclusion of 7.3% in a TMR (Van De Kerckhove *et al.* 2011). Crude protein digestibility (CPD) was not affected when canola meal was added at either 10% or 20% in TMR (Nair *et al.* 2016). In comparison, CPD increased (79.2% vs 68.6%) when canola meal was added to the TMR compared with the basal TMR (Zinn 1993). Neither NDF digestibility (NDFD) nor ADF digestibility (ADFD) were affected when barley grain was replaced with canola meal at either 10% or 20% inclusion level in a TMR for finishing steers (Nair *et al.* 2016) or when canola meal was included at 7.3% compared with the control TMR based on barley straw and grass hay (Van De Kerckhove *et al.* 2011).

Increasing the level of inclusion of protein in feedlot rations has been found to have variable effects on rumen parameters, including ruminal NH_3-N concentrations and VFA concentrations and molar proportions. Ruminal NH_3-N concentrations were higher when increasing levels (12 %, 20%, 30% or 39%) of soybean meal were offered to cattle fed *ad libitum* wheat straw (Beaty *et al.* 1994). When heifers fed a basal ration of 75% ground barley straw and 25% ground bromegrass hay were supplemented with barley grain and canola meal, ruminal NH_3-N concentration increased (Van De Kerckhove *et al.* 2011). Ruminal NH_3-N concentrations were higher when 20% but not 10% canola meal was included in a TMR compared to the control (0%) (Nair *et al.* 2016). Total VFA concentrations and the ratio of propionic acid to acetic acid (Pr:Ac) did not differ when heifers were offered 0%, 10% or 20% canola meal in TMR.

2.6.4 Grazing cattle

Literature and feeding recommendations for the supplementary feeding of canola meal to cattle grazing low-quality pastures or confinement fed low-quality roughages in Australia is limited. Recommendations from the NSW Department of Primary Industries (DPI) are that for 150 kg weaner calves, protein supplements should be fed

at rates of 0.3–0.7 kg/hd/d (Blackwood and Clayton 2007), whereas a report from the Australia Oilseeds Federation suggested canola meal can be fed to cattle at inclusion rates of 2-3 kg DM/hd/d (Mailer 2004). International studies have utilised canola meal in grazing situations at low LoF (1.2 kg/hd/d; Petit *et al.* 1994). The Canadian Canola Council's, "Canola Meal Feeding Guide" does not include a maximum recommended inclusion level (percentage of ration) for beef cattle (Newkirk 2009).

Studies have investigated the effect of including canola meal in PMR (Seoane *et al.* 1992; Seoane *et al.* 1993; Petit *et al.* 1994; Veira *et al.* 1995; Koenig *et al.* 2004). Average DMI, ADG and FCR were higher when cattle were offered canola meal in PMR based on quackgrass hay (Seoane *et al.* 1992) or timothy silage (Petit *et al.* 1994). There is need for further research to determine the effect of feeding varying inclusion levels of canola meal when animals graze on or are fed low-quality forages or roughages.

There is a lack of data on the production responses of supplementary feeding canola meal to cattle grazing on low-quality pastures in southern Australia. With any feedstuff, there is need for such research before beef producers would consider this a useful protein supplement. Therefore, the effects on DMI and ADG of supplementing varying inclusion levels of canola meal when cattle are offered a low-quality roughage in a controlled pen feeding study is warranted. In addition, there is also need for investigation of the effects of feeding canola meal on meat (beef) quality as this may impact consumer preferences.

2.7 Beef market specifications and eating quality

Cattle and calf production (including live export) is one of the largest contributors to the Australian agriculture economy (ABARES Agriculture Commodities, 2020). In NSW, from July 2019 to June 2020, approximately 54% of cattle were pasture-fed (MLA 2020a). Australian beef is considered a high value product and has the perception of being "clean" and "green" and there is an increasing consumer demand both domestically and internationally for grass-fed beef (MLA 2018). Ensuring producers meet grass-fed market specification is critical for both domestic and export markets.

Carcase characteristics, meat quality traits and consumer sensory evaluations (Robbins *et al.* 2003) are also important factors to consider when determining the overall benefit of any feed supplement for livestock, particularly when assessing the acceptance of any potential PCAS approved supplement for grass-fed beef.

2.7.1 Meat quality traits

Managing variability in pasture quality and quantity and maintaining a consistent supply of cattle throughout the year can be a challenge for beef producers supplying to certified grass-fed markets. There are many factors that can affect meat quality and thus the price received. Dark cutting is the major contributing factor of poor meat quality and thus is the major cause of carcase downgrades and as a consequence is an economic problem worldwide (Jose *et al.* 2015; Knee *et al.* 2004; Knee *et al.* 2007; McGilchrist *et al.* 2014).

Dark cutting or dark, firm and dry beef is defined as beef with an ultimate pH which is greater than 5.71 or if the MSA meat colour is greater than 3 at the quartering site (10th/11th or 12th/13th ribs) of the *M. longissimus thoracis* (McGilchrist *et al.* 2014). High ultimate pH is caused when muscle glycogen concentrations are low at slaughter and consequently there is a reduced production of lactic acid within the muscle during post mortem glycolysis (Gardner *et al.* 2014). Meat Standards Australia graded beef should have an ultimate pH of ~5.5 and 1-1.5% muscle glycogen. Carcases with an ultimate pH greater than 5.7 are typically dark in colour, have a shorter shelf life, are bland in flavour and have variable tenderness (McGilchrist *et al.* 2012; McGilchrist *et al.* 2014). These characteristics have been identified as issues with grass-fed beef in Australia (Slack-Smith *et al.* 2009) and has been estimated to cost beef producers approximately $7.09 per carcase graded by MSA (Jose *et al.* 2015; McGilchrist *et al.* 2012).

Nutritional variation affects muscle glycogenolysis which in turn affects the incidences of dark cutting in carcases (McGilchrist *et al.* 2012). Energy and/or protein supplementation has the potential to increase muscle glycogen concentrations thereby reducing the incidence of dark cutting meat prior to slaughter (Knee *et al.* 2007). Therefore, further investigation of the effects of supplementing canola meal when available pasture quality is low is warranted.

Tenderness is the ease by which consumers can cut, bite and masticate meat and is one of the most important palatability attributes for consumer preference (Holman *et al.* 2020). Tenderness of beef is influenced by both intrinsic and extrinsic factors. Intrinsic factors include muscle fibre structure, type and dimension, and the distribution of connective tissue and fatty deposits within the muscles fibres, and extrinsic factors include cut or muscle selection, preparation method (degree of doneness), production,

processing and preservation effects (Holman *et al.* 2020). To determine these effects on beef tenderness, either laboratory objective measurements and/or subjective consumer sensory evaluations can be employed. Tenderness can be objectively measured by shear force (Huffman *et al.* 1996; Miller *et al.* 2001) and muscle fibre can be measured by particle size analysis. The acceptable levels for shear force and particle size are 36.8 N and 142 µm, respectively (Holman *et al.* 2020).

Another important aspect of meat quality is the degree of lipid oxidation, which is measured by thiobarbituric acid reactive substance (TBARS) and provides an estimate of potential shelf-life and eating quality characteristics of beef (Campo *et al.* 2006; Zhang *et al*. 2019). Typically, as TBARS values increase, the shelf life of the product decreases (Zhang *et al.* 2019). The proposed threshold value for the acceptability of oxidised beef is approximately 2 mg of malondialdehyde (MDA)/kg (Campo *et al.* 2006). When grass-fed beef was aged for up to 12 weeks, TBARS values were 1.00 ± 0.03 mg MDA/kg (Holman *et al.* 2019) compared with 2.60 and 3.11 mg MDA/kg when beef was aged up to 20 weeks (Hughes *et al.* 2015). Dietary factors may also potentially affect TBARS value and warrants further investigation.

Consumer sensory evaluations are important when considering the overall acceptability of a product. Sensory evaluations are critical to obtain coverall feedback and acceptance of a product by consumers. Individual consumers evaluate the meat sample for tenderness, juiciness, flavour and the overall liking which are weighted by 0.3, 0.1, 0.3 and 0.3, respectively and then used to calculate a palatability or meat quality score (MQ4) out of 100. Meat samples with scores closer to 100 are considered to have higher eating quality compared with those with scores closer to 0 that are considered to have a poor eating quality.

Assessing both consumer evaluations and objective measurements when evaluating a product is ideal. It has been found that consumers could not detect any discernible change in tenderness of beef unless the shear force value was greater than 4.1 kg (or 40.21 N) (Huffman *et al*. 1996).

2.7.2 Dietary effects on meat and eating quality

Diet can influence meat and eating quality traits in both sheep (Hopkins *et al.* 2014) and cattle (Berge *et al*. 1993). Diet can influence meat and eating quality traits such as the rate of oxidation (Gobert *et al.* 2010), colour (Lanari *et al.* 2002), tenderness (Bakker *et al.* 2021), flavour (Aldai *et al.* 2010) and the overall liking of a product (Mezgebo *et al.* 2017). Ageing the product can also influence meat and eating quality

traits (Jiang *et al.* 2010; Holman *et al.* 2019), although meat quality traits including TBARS and particle size were not negatively affected when beef *longissimus lumborum* muscle (striploin) was aged for up to 12 weeks (Holman *et al.* 2020).

Evaluating dietary effects on meat and eating quality have mainly focused on grain versus pasture-based feeding systems (French *et al.* 2001; Pethick *et al.* 2005). Finishing cattle on high quality pastures can alter the biochemistry, especially the aroma and flavour of beef (Daley *et al.* 2010; He *et al.* 2013; Hopkins *et al.* 2014). Diet can alter the FA profile and oxidative status of the meat (Hopkins *et al.* 2014; Holman *et al.* 2022), with pasture-fed livestock found to have higher omega-3 PUFA compared with livestock fed grain. The PUFA content of meat has been found to be higher when feeding oilseeds or oilseed meals (De Mello *et al.* 2018; Segers *et al.* 2011), as was the case when cattle were fed flaxseed meal (Kronberg *et al.* 2011) and when lambs were fed pelleted rations containing canola and flaxseed oil (Nguyen *et al.* 2017). Polyunsaturated fatty acids were higher when canola meal was included in the PMR for beef feedlot cattle (Dugan *et al.* 2011; He *et al.* 2013; Mir *et al.* 2003).

Very few studies have been undertaken to investigate the effects of feeding canola meal on carcase characteristics and (beef) eating quality. Carcase yield, HSCW, and eye muscle area (EMA) were not altered when steers were fed a ration containing either canola meal or soybean meal (Good *et al.* 2017; Good 2018) or when canola meal replaced barley grain (He *et al.* 2013) in a TMR. In these studies, meat quality parameters and consumer sensory evaluations were not investigated. There is a lack of information on how the level of protein supplementation affects carcase and meat characteristics and eating quality.

2.8 Conclusion

There is an increasing consumer demand for grass-fed beef, both domestically and internationally. To meet grass-fed beef certification programs the cattle must be lifetime pasture-fed. Maintaining cattle production levels becomes challenging when the quality of the pasture available is low and no cereal grain or grain products supplements can be used, although, oilseeds meals are allowable supplements under grass-fed beef certification programs.

Relatively large quantities of canola meal are available within Australian but only limited research has been undertaken with regards to its usefulness as a supplement for cattle consuming low-quality roughage diets. Assessing the suitability of canola meal

including feeding it at varying levels involves determining the overall effect on livestock production, including DMI, ADG, nutrient digestibility, rumen parameters, carcase characteristics, meat quality traits and consumer sensory evaluations.

Chapter 3: General Methods and Materials

This chapter is a description of methods and materials that were common to more than one experiment. This includes details on the experimental location, animal housing, sample collection and preparation, common analytical methodologies, calculations, and statistical analysis.

3.1 Animal ethics approvals

All animal-based experiments had approval from Charles Sturt University (CSU) Animal Care and Ethics Committee (ACEC) and the NSW DPI Animal Ethics Committee (AEC) and were compliant with the Animal Research Act 1985 (as amended) and the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC 2013).

3.2 Location

All experiments were conducted at Wagga Wagga, NSW. The pen feeding studies (Chapters 4 and 5) were conducted at the CSU Ruminant Research Complex (35°06'S, 147°34'E). The animal house studies for the determination of apparent nutrient digestibility (Chapter 6), ruminal parameters (Chapter 7), and the rate of degradation and digestion kinetics (Chapters 7 and 8) were conducted at the NSW DPI Animal Nutrition Unit (35°05'S, 147°33'E).

3.3 Animal housing and animal data collection

3.3.1 Ruminant Research Complex

The animals were housed in 12 pens (50 m x 8 m) with a cement bunker for feeding. The first six pens had full shade over the feeding bunker (5 m x 8 m) compared with minimal shade (1 m x 8 m) over the last six pens. Animals were randomly allocated to pen, and "shade" was included as a random term in statistical models. The flooring of the feeding pens was dirt, with a cement apron (3 m x 8m) around feeding bunker. All animals had continuous access to water toughs (0.5 m x 1.2 m) and shade cloth (8 m x 8 m) was provided at the back of each pen. Adjacent to the feeding pens were cattle yards which were used for drenching, vaccination, and fortnightly weighing.

3.3.2 Animal Nutrition Unit

Four ruminal fistulated Red Poll steers, (n = 2, 7 years old, LW = 938 ± 7.86 kg; and n = 2, 5 years old, LW = 636 ± 8.76 kg) were housed together at the Animal Nutrition Unit. Each steer had permanent, surgically placed Ankom™ 10 cm, pliable rumen cannula with rubber stopper and U-bolt (Ankom Technology, Macedon, NY).

The steers were individually penned for 2 h during feeding and were fed twice daily at 0800 and 1600. The steers were moved into a crush during allocated times of ruminal fluid collection and the addition and removal of dacron bags. The steers always had access to clean water and shade.

3.3.3 Liveweight and body condition score

The fasted (Chapters 5, 6, 7, and 8) and unfasted (Chapter 4) liveweight (LW) of animals were measured using an electronic scale (Tru-Test XR5000, Australia). The scales were calibrated with a known weight prior to each weighing. Animals were weighed in the morning, at approximately the same time, at allocated intervals. For fasted LW, the animals were still offered water, as the experiments were conducted in the warmer months of the year; however, feed residuals were removed approximately 12–14 h before weighing.

Body condition score (BCS) is an assessment of the amount of adipose tissue and muscle on the animal at the short loin and the backbone (Wright and Russel 1984). The BCS of the animals was assessed while they were being weighed in a crush. A scoring system of 1 to 5 (Appendix A) was used, where 1 represented an animal in poor condition and 5 represented an animal in fat condition. Assessments were made by one operator to minimise variation.

3.4 Sample collection

3.4.1 Feed samples

The canola meal used throughout the studies reported in this thesis was solvent extracted. Representative offered samples (approximately 500 g) of the experimental rations and any feed refusals (daily: Chapters 5, 6, 7 and 8; weekly: Chapter 5) were taken and stored frozen at -18˚C until subsequent analysis.

3.5 Rumen degradability studies

Rumen degradability of a lucerne standard (Chapter 7) and the canola meal dietary treatments (Chapter 8) were determined using two methods, viz. *in sacco* nylon bag method and *in vitro* Daisy™ incubation. Both of these methods were used to examine degradability loss over time and rumen degradability kinetics, as described previously (Ørskov and McDonald 1979; Ørskov *et al.* 1980).

3.5.1 Bag preparation

Ankom™ F57 filter bags (25 μm porosity, Ankom^{TM} Technology, Macedon, NY) were used for both the *in sacco* and *in vitro* Daisy™ incubations. To prepare the filter bags they were pre-rinsed in acetone for 3-5 min to remove surfactant that inhibits microbial digestion, and air-dried overnight (Ankom Technology 2021). The filter bags were then numbered using an indelible marker and dried at 80°C for 2 h before being placed in a desiccator to cool. Either 1.0 ± 0.2 g (*in sacco* incubation) or 0.5 ± 0.2 g (*in vitro* Daisy™ incubations) of the canola meal dietary treatments or lucerne hay was weighed into a filter bag and the filter bag was sealed immediately using an electronic heat sealer (Ankom^{TM} Heat sealer, 120V 50/60HZ).

Prior to commencement of the *in sacco* incubations, it was established that there was significant loss of the soluble (a) fraction of the lucerne standard if the sample was placed directly into dacron bags (mesh size: 50 ± 10 μm; bag size 140 x 90 mm, Ankom^{TM} Technology, Macedon, NY). Thus, instead of being placed directly into the dacron bags, the samples were weighed into Ankom™ F57 filter bags (25 μm porosity, Ankom^{TM} Technology, Macedon, NY) which were then placed inside the dacron bags, together with a marble (to prevent the bag floating to the top of the rumen) and then the bag was tied off using fine cord, with approximately 20 cm of extra cord to suspend the bag down into the rumen.

3.5.2 *In sacco* incubation

Duplicate dacron bags (each containing three filter bags) were prepared for the lucerne standard (Chapter 7) or the canola meal dietary treatments (0%, 13%, 27% or 43% canola meal inclusion level, Chapter 8) for each incubation period (3, 6, 9, 12, 18, 24, 48, and 72 h) (Figure 3.1).

Incubation

Dacron bags were randomly pre-allocated to "time" to ensure randomisation throughout the experimental periods. Dacron bags were inserted into the rumen via the cannulae at 72, 48, 24, 18, 12, 9, 6, 3 h and all bags were removed at 0 h.

a) b)

Figure 3.1 a) One dacron bag with three Ankom bags which contained either canola meal dietary treatments (0%, 13%, 27% or 43%) or the lucerne standard as the sample diet and b) four dacron bags to be incubated at each time point (two x dacron bags contain three Ankom bags of the pre-allocated sample diet [the same as the steer was fed] and two x dacron bags contain three Ankom bags of the lucerne standard).

Time (h:min) was recorded for all samples being inserted and removed from the rumen, to establish an accurate degradability timeline. At the end of 72 h incubation period, the dacron bags were removed from each steer via the cannulae and placed in a tub of cold water and thoroughly rinsed, replacing the water several times until the water ran clear and no residue was apparent on the outside of the bags. The incubated dacron bags, together with time zero dacron bag (containing non-incubated filter bags, to determine the soluble (a) fraction) were placed in a mesh bags (30 cm x 20 cm) and then washed in a washing machine for at least 10 min or until the water ran clear, then squeezed to remove excess water before removing from the mesh bag and then arranging them flat on a foil tray, without overlapping and dried in an oven at 80°C for 24 h. Once dried, the dacron bags were placed in a desiccator to cool, after which the filter bags were removed (from the dacron bags), weighed and then stored in airtight plastic bags in a dark dry room until analysis of DM, CP, aNDF and ADF contents.

3.5.3 *In vitro* Daisy™ incubation

The lucerne hay standard (Chapter 7) and the canola meal dietary treatments (Chapter 8) were incubated in a mixture of ruminal liquor and a buffered artificial saliva according to previously published methods (Adesogan 2005) with reference to the Daisy™ II technical manual (Daisy™ Technical Manual 2021). Samples were incubated without the second acidified pepsin stage to mimic the *in sacco* incubation (Chaudhry and Mohamed 2011).

Daisy™ incubator preparation

On the day prior to the incubations (Day 10 of the experimental period), 1.2 L of distilled water was measured into each digestion jar, which was fitted with a plastic agitator baffle and placed into the Ankom Daisy™ II Incubator (Figure 3.2) set at 39 ± 0.5°C to continuously rotate. All other glassware and porcelain ware to be used for the sample preparation of ruminal fluid were placed into an incubator set at 39 ± 0.5°C to warm overnight. On Day 11, prior to the collection of the ruminal liquor, a buffered artificial saliva solution was prepared (McDougall 1948) with the modification of adding urea and ammonium sulphate (Kaiser *et al.* 2007) to ensure an adequate supply of N (Schmid *et al.* 1969). The artificial salvia solution was prepared by adding the quantities of chemicals listed in Table 3.1 into a total of 6.4 L of warm, distilled water. A total of 1.6 L was used per jar.

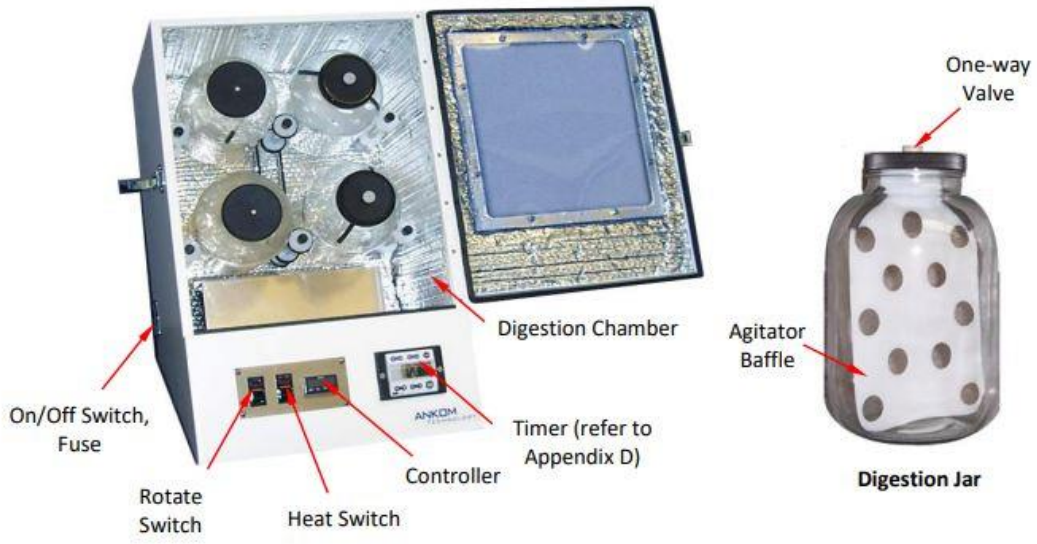


Figure 3.2 *In vitro* Ankom Daisy™ incubator machine and digestion jars (source; Ankom Technology Manual 2021).

Ruminal liquor

Approximately, 2 L of rumen contents (including fluid and particulate matter) was manually collected (via the rumen cannula and from the dorsal ventral rumen sac) from each steer prior to feeding (at 0800 h on Day 11) and immediately placed into an incubator set at 39 ± 0.5°C to keep warm until processing. For each collection (i.e., for each steer per treatment), approximately half of the collected amount was filtered through coarse and then fine muslin, which was supported in a warm porcelain funnel, into a warmed vacuum flask. The remainder was placed into a blender and blended on high/pulse to extract microbes present on the feed particles. The blended material was filtered through the two types of muslin and then combined with the previously filtered ruminal fluid. The pH was then measured, and subsamples taken and frozen for subsequent analysis.

Table 3.1 Chemical constituents required to produce 6.4 L of the buffered artificial saliva solution.

Chemical	**Weight (g)**
Sodium bicarbonate ($NaHCO_3$)	62.70
Sodium dihydrogen phosphate dodecahydrate (Na_2HPO_4)	29.50
Sodium chloride (NaCl)	3.000
Potassium chloride (KCl)	3.650
Magnesium chloride ($MgCl_2$)	0.380
Urea ($CO(NH_2)_2$)	0.800
Ammonium sulphate ($(NH_4)_2SO_4$)	0.800
Calcium chloride ($CaCl_2$)	0.256

The pre-allocated digestion jar (in duplicate - one jar from each incubator) was removed from the Daisy™ incubator and 400 mL of the filtered ruminal liquor was added to the 1.6 L of artificial saliva solution to give a total jar volume of 2 L. The pre-allocated 72 h filter bags were placed into each jar and then each jar was gassed with CO_2 for approximately 1 min to ensure an anaerobic environment, prior to being placed into each incubator. This process was repeated for the collection from each steer over all periods.

Bag incubation

Pre-allocated filter bags (either the lucerne standard or the canola meal dietary treatment and a blank) were placed into the allocated digestion jar in the following incubation time order, 72, 48, 24, 18, 12, 9, 6 and 3 h, to ensure all samples were taken out at 0 h. Time (h:min) was recorded upon addition of filter bags to the digestion jars and at the end of the study (time 0 h) to establish an accurate degradability timeline. At the end of 72 h incubation period, the solution in each jar was drained and the jar was refilled with water and recapped and gently inverted, replacing the water multiple times until the water ran clear and no residue was apparent on the outside of the filter bags. Filter bags for time point zero were washed in a similar way to remove the soluble (a) fraction. Upon completion of washing, the bags were gently squeezed before arranging them flat in a foil tray, without overlapping for oven drying at 80°C for 24 h. The bags were removed from the oven, allowed to cool in a desiccator before weighing and then stored in a dark room for subsequent analysis.

3.5.4 Estimation of degradation parameters

In this thesis potential degradation refers to PD after 72 h incubation to compare with DMD *in vivo* apparent nutrient digestibility. The percent loss (degradability) of DM, CP, aNDF and ADF were calculated according to the following equation:

Degradability (%) = ([amount incubated (g) – amount remaining following incubation at time (t)]/ amount incubated) x 100

The percentage loss of each constituent (DM, CP, aNFD or ADF) was then used to determine standard degradability coefficients using the non-linear regression (exponential) equation described by Ørskov and McDonald (1979) and Ørskov *et al.* (1980) as follows:

$$PD = a + b\ (1 - e^{-ct})$$

where:

PD = potential degradability is the fraction of material degraded at 72 h

a = immediately degradable (water soluble) fraction at 0 h

b = not soluble, but slowly degradable fraction (72 h - 0 h)

c = fractional rate of degradation of fraction b (per h (at time t))

t = incubation time (0, 3, 6, 9, 12, 18, 24, 48, 72 h)

e = base for natural logarithm

The degradability of each component was corrected for a blank where required (Ørskov and McDonald 1979; Ørskov *et al.* 1980). The aNDF/ADF component was assumed to be not immediately soluble (therefore, a = 0) (Chaudhry 2000; Spanghero *et al.* 2007) and was thus adjusted for a lag phase from 0 to 12 h (Ohlsson *et al.* 2007; Raffrenato and Van Amburgh 2010; Raffrenato *et al.* 2018) where required.

The fractional rate of degradation of fraction b (per h (at time t)) was calculated using the following equation:

$$c = [(LN((a+b)-t/b))*-1]/t$$

Where, a, b and t were fitted from the equation above, t was calculated from incubation at time t and the LN function (in excel) returned the natural logarithm of a number.

Effective degradability (ED) or rumen degradability was calculated using the following equation also described previously by Ørskov and McDonald (1979) and Ørskov *et al.* (1980):

$$ED = a + (b \times c)/(c + r)$$

Where a, b and c were fitted from the equations above and r related to rumen outflow rates as described below.

The retention time in the rumen is negatively correlated with the LoF, as outflow rate per hour (r) is faster with a higher LoF. Effective rumen degradabilities were calculated using the following rumen outflow rates (AFRC 1993):

0.02/h = animals fed at a low LoF, approximately 1 x maintenance

0.05/h = calves, low yielding dairy cows, beef cattle and sheep on higher LoF but < 2 x maintenance

0.08/h = high yielding dairy cows, > than 2 x maintenance

Rumen undegradable protein, assuming a rumen outflow rate (r) of 0.05/h, was calculated using the following equation (NRC 2001):

$$RUP = b [r / (c + r)] + (100 - PD)$$

3.6 Analytical procedures

All analytical procedures were undertaken at the Feed Quality Service (FQS) laboratory of NSW DPI at Wagga Wagga. All analyses were based on AFIA (2014) procedures.

3.6.1 Dry matter and ash

Duplicate samples of approximately 70 g (known weight) of each of the dietary ingredients used for each experiment (each period), feed refusals and faeces were dried at 80ºC in a forced-air oven (Labec Oven; Laboratory Equipment Pty Ltd) for 24 h to determine the DM content. The samples were then ground, firstly through a 5 mm sieve followed by a 1 mm sieve, except for canola meal which was ground only through a 1 mm sieve. Once dried (and ground) the samples were then placed in plastic storage containers at room temperature pending chemical analysis.

The analytical DM content of ground samples was determined by drying a known weight (approximately 1 g) of sample into a (dried) porcelain crucible before being dried in an air-forced oven at 80°C for 24 h. For the determination of ash content, approximately 1 g of ground sample was weighed into a (dried) porcelain crucible and then ignited in the muffle furnace at 200°C for 2 h, then 550°C for 6 h until all carbon was removed. After cooling in a desiccator, the crucible and residual were weighed and recorded. The OM content was calculated by subtracting the ash weight from the DM weight and dividing by the DM weight.

The determination of the ash content for filter bags, after the sample had been analysed for ADF content (g), the filter bag was folded into a (dried) porcelain crucible and then ignited in the muffle furnace at 200°C for 2 h, then 550°C for 6 h until all carbon was removed. After cooling in a desiccator, the weight of ash was recorded.

3.6.2 Nitrogen

The N content of offered feed, feed refusals, faeces and the residual from the filter bags (approximately 0.2 g, 1 mm ground sample) was determined using the Dumas combustion method using a Leco CNS 2000® analyser (Leco, St Joseph, MI, USA). Before N analysis of the incubated filter bags, the filter bags were dried for 30 minutes at 105°C to remove any moisture and allow for an accurate DM weight. After drying, cooling in a desiccator and weighing the filter bags, the filter bags were cut open (close

to the seal) and a known weight (approximately 0.2 g) sub sample of the residual was weighed into ceramic boats for the determination of N content. The CP content of all samples was calculated by multiplying the N content by 6.25.

3.6.3 Neutral detergent fibre

The NDF content of the offered feed, feed refusals and faeces were determined by weighing approximately 0.5 g (known weight) of the ground samples into an Ankom 57 filter bag. For the *in sacco* and *in vitro* Daisy™ studies, the incubated filter bags were used (and sequentially used for ADF analysis). After ensuring each sample was spread evenly throughout the filter bag (by manually shaking the sealed bag), the bags were evenly placed bags into the Ankom Fiber Analyser (Ankom® 200/220 Fiber Analyser, Ankom Technology, Macedon NY, USA) vessel along with 2 L of neutral detergent solution, 20 g of sodium sulphite and 5.05 mL of heat stable alpha-amylase. The bags were heated and agitated for 75 min and then rinsed twice with 2 L of boiling water and 5.05 mL of alpha-amylase for 5 min each, followed by a final rise with boiling water for a further 5 min. The filter bags were removed, excess liquid removed and then dried in an oven at 105°C for 2 h. A reagent blank sample and two laboratory known standards were prepared precisely the same way and placed in with each batch. The NDF content was reported as either aNDF, to indicate it was assayed with heat stable amylase and inclusive of residual ash, or $aNDF_{OM}$, to indicate it was assayed with heat stable amylase and exclusive of residual ash.

3.6.4 Acid detergent fibre

The ADF content of all samples was analysed sequentially after the determination of aNDF content. After ensuring the sample (post NDF analysis) was spread evenly throughout the filter bag, the filter bags were placed in the ANKOM Fibre Analyser (Ankom® 200/220 fibre analyser, ANKOM technology, Macedon NY, USA) vessel along with 2 L acid detergent solution. The filter bags were heated and agitated for 60 min and then rinsed three times with boiling water for 5 min. The filter bags were removed, excess liquid removed and then dried in an oven at 105°C for 2 h. A reagent blank sample and two laboratory known standards were prepared the same way and placed in with each batch. The ADF was reported as either ADF, which was inclusive of residual ash, or as ADF_{OM}, which was exclusive of residual ash.

3.6.5 Ether extract

Total lipid concentration (represented as ether extract [EE]) was determined by solvent extraction using the Randall method (Randall 1974) with hexane, which has been validated for use with animal feed (Thiex *et al.* 2003) using a FOSS solvent extraction system (Soxtec 2050 Avanti Extraction Unit, FOSS, Hoganas, Sweden) according to the FOSS Application Note (FOSS 2005). Approximately 2.5 ± 0.1 g of dried canola meal and/or 5.0 ± 0.1 g of ground forage was placed into each thimble, thimbles were placed on the extractor with corresponding tins. Approximately, 80 mL of hexane was added, once complete the tins were removed and dried at 105°C for 2 h. Total lipid concentration (%) was calculated as the proportional difference between the weight of tin before and after solvent extraction and recorded as a percentage of wet sample weight.

3.6.6 Digestibility using the pepsin cellulase method

Laboratory DMD, OMD and DOMD were calculated using the pepsin cellulase method previously described by AFIA (2014). Approximately 0.25 ± 0.0001 g of dried sample (in duplicate) were weighed into 50 mL test tubes before 15 mL of acidified pepsin solution was added to each sample, and then the tubes were placed in a water bath (incubator) at 40°C for 24 h and agitated continuously. After 24 h, 4 mL of diluted α-amylase solution was added to each sample and the sample heated to 80°C for 45 min, after which 0.8 mL sodium carbonate solution was added. Then 10 mL of buffered cellulase and the sample was then incubated for a further 24 h at 40°C. The remaining residual was filtered under vacuum into weighed and cooled sintered glass crucibles before the disappearance of DM or OM was determined. This value was adjusted to predict *in vivo* digestibility using a linear regression based on samples of known *in vivo* digestibility.

3.7 Calculations

3.7.1 Prediction of M/D requirements for maintenance

The following equation was used to predict the ME (M/D) required for maintenance (CSIRO 2007).

$$ME_m\ (MJ/d) = K(0.26W^{0.75}\exp(-0.03A)/k_m + 0.09MEI$$

where:

K = Constant for breed (1.2)

W = LW (kg)

A = Age (years)

k_m = Efficiency of use of energy for maintenance (0.02 x M/D + 0.5)

0.09 MEI = adjustment for LoF (0.09 x estimated ME required, MJ/d

The ratio of ME intake (MEI) to MEm is the LoF above maintenance; MEI is the amount of ME offered (DMI multiplied by the estimated M/D of the feed) divided by the MEm, which is the maintenance requirement of the animal (LoF).

3.7.2 Metabolisable energy

The M/D of feed was calculated using either *in vivo* DOMD (Chapter 6) or DOMD from the pepsin cellulase assay (Chapter 4, 5 and 7) according to the following equations (CSIRO 2007; AFIA 2014):

For roughages other than silage (control ration);

M/D (MJ/kg DM) = 0.203 x DOMD (%) – 3.001

For rations containing any level of canola meal (the grains and concentrates equation);

M/D (MJ/kg DM) = 0.138 x DOMD (%) + 0.272 x ether extract (EE %) + 0.858

This equation was used to account for the EE of the canola meal in the ration.

3.8 Statistical analysis

3.8.1 Repeated measure analysis

Data collected from the same animal over time were analysed using Repeated Measures Analysis in the SAS statistical program (SAS Institute Inc.). The mixed model procedure was chosen over other methods, as it accounts for covariation both within and between animals over time (Littell *et al.* 2000). The most appropriate covariance structure (UN, CS, AR(1), ANTE(1), TOEP or SIMPLE) was determined for each analysis with reference to the Bayesian Information Criterion (BIC) and

Akaikes Information Criterion (AIC) (Littell *et al.* 2000). The analyses of fixed and random effects are outlined in each experimental chapter.

3.8.2 Mixed model analysis of variance

Data collected from animals in different treatment groups at a single point in time were analysed using the Mixed Model procedure in SAS.

Chapter 4: Canola Meal as a Potential Grass-Fed Beef Supplement: Meat Quality Traits and Sensory Evaluations

4.1 Introduction

Accredited grass-fed producers can obtain premiums for supplying compliant cattle into certified grass-fed beef schemes such as PCAS. Managing variability in pasture quality and quantity is a challenge for producers supplying to certified, grass-fed beef markets in Australia. In southeastern Australia, during the summer-autumn period the quality of pastures is often low, having a digestibility of less than 55 % and a CP content less than 80 g/kg DM (Leng 1990). These low-quality pastures are often below the required nutrient content to meet the requirements for growing and finishing livestock. The supplementation of energy and/or protein to meet these requirements is a widely adopted strategy (Moore *et al.* 2009; McLennan *et al.* 2017a).

Traditionally, producers often supplement livestock grazing low-quality pastures with grain or grain-based pellets to increase energy and/or protein supply, although, these types of supplements are not approved for use under some grass-fed certification schemes, such as PCAS (PCAS 2016). Plant-based protein supplements are acceptable alternatives and some that are approved for use in the certified grass-fed beef system include canola meal, cottonseed meal and soybean meal (PCAS 2016). Canola meal is high in CP and is also considered a good source of energy (Heim and Krebs 2018b), which makes it a viable option to feed to cattle. In addition, canola meal is readily available throughout southeastern Australia due to the expansion in canola cropping and processing in recent years. Cottonseed meal is more commonly available and used as a supplement for livestock in Northern Australia (McLennan *et al.* 2017a).

Dry matter intake and growth rates were not adversely affected when canola meal was included in various formulations of TMR (Van De Kerckhove *et al.* 2011; He *et al.* 2013; Nair *et al.* 2016; Good 2018) and PMR (Seoane *et al.* 1992; Seoane *et al.* 1993; Petit *et al.* 1994). Similarly, final LW and ADG were not altered when steers were fed either a grain-based pellet or canola meal; however, carcase weight was lower when steers were offered canola meal compared with the grain-based pellets (Lynch and Campbell 2017).

In addition to production responses and assessing carcase characteristics, meat quality traits and eating quality are important factors when determining the overall benefit of any feed supplement for livestock. There are limited studies on the effect of canola meal on carcase characteristics, in particular meat and eating quality. Results from two

studies conducted in Canada indicated that carcase yield, HSCW and EMA did not differ when steers were offered canola meal compared with soybean meal (Good 2018) or when canola meal replaced barley grain (He *et al.* 2013) in TMR. All carcase characteristics were within acceptable MSA limits (except one carcase from each treatment groups for fat depth and pHu) when steers were offered canola meal compared with the grain-based pellets (Lynch and Campbell 2017).

Given the limited data on the effect of canola meal supplementation in PMR on carcase characteristics and meat quality when finishing steers are offered low-moderate quality roughage, further investigation of meat quality traits and eating quality was warranted. Therefore, the aim of the current study was to determine whether meat quality and consumer sensory attributes were adversely affected when steers were supplemented with canola meal compared with a grain-based pellet typically fed in pasture-fed production systems in southeastern Australia.

4.2 Methods and materials

The study was conducted at the CSU Ruminant Research Complex. This study was approved by the CSU ACEC (Approval No: A17033) and was compliant with the Animal Research Act 1985 (as amended), as previously described in Chapter 3.1. The location, animal housing and animal data collection, sample collection, analytical procedures, calculations, and statistical analysis are presented in Chapter 3, Sections 3.2, 3.3, 3.4, 3.6, 3.7 and 3.8 unless stated otherwise.

The animal feeding experiment was part of an Honours research project (Lynch 2017). Animal production measures (including, LW, ADG and BCS) and MSA carcase grading data (including carcase weight) were collected and presented in the Honours dissertation and a summary of this data is included for the current study. The meat samples from this earlier study were collected, stored, and analysed as part of the current research.

Brief details of the experimental animals and housing, experimental design, dietary treatments, feed samples, slaughter and carcase sampling, are presented in section 4.2.1, 4.2.2, 4.2.3, 4.2.4 and 4.2.5. In addition, key animal production and carcase results are included in Section 4.2.1 to help with interpretation of meat quality and consumer sensory results.

4.2.1 Experimental animals and housing

Thirty-four Angus and six Angus-cross Hereford steers (n = 40, LW = 470.9 ± 5.0 kg) were purchased from Wagga Wagga sale yards, 10 d prior to the commencement of the feeding study. The steers were approximately 18 months old, all with a similar BCS (3.1 ± 0.07) and were purchased from three properties. Steers were vaccinated against clostridial diseases and leptospirosis (Ultravac® 7 in 1, Zoetis, Rhodes, Sydney) and treated with an anthelmintic drench (Maximus® Boehringer Ingelheim, North Ryde, Sydney) prior to being allocated to the feeding pens. The non-fasted LW and BCS of the steers were recorded fortnightly, prior to morning feeding, using electronic scales (Tru Test XR5000, Australia) and difference in recorded LW (over time) was used to calculate ADG.

As reported by Lynch (2017), the mean LW increased ($P < 0.001$) over time when steers were fed lucerne hay *ad libitum* together with either canola meal (final LW = 563 ± 7.60 kg) or grain-based pellets (final LW = 546.8 ± 7.60 kg). The overall ADG from day 0-60 did not vary ($P = 0.233$) between the canola meal (ADG = 1.34 ± 0.07 kg) or grain-based pellets (ADG = 1.46 ± 0.07 kg) treatment groups. Final LW did not differ ($P = 0.140$) between dietary treatments, however, carcase weight was lower ($P = 0.016$) when steers were supplemented with canola meal (HSCW = 276.7 ± 1.91 kg) compared with the grain-based pellets (HSCW = 283.6 ± 1.91 kg).

4.2.2 Experimental design

Steers were randomly allocated to one of eight pens (five steers per pen) using a randomised block design based on genotype (n = 2), property source (n = 3) and LW. The steers were placed into pre-allocated pens for 7 d adaptation period, followed by 60 d feeding prior to being sent to slaughter. A controlled pen feeding study was conducted in lieu of an extensive grazing study to allow for additional measures and replications of treatments. All animals had access to shade and clean water (pen area: 50 m x 8 m).

4.2.3 Dietary treatments

The steers were offered one of two supplements (daily), either canola meal (solvent-extracted, Riverina Oils and BioEnergy, Wagga Wagga, NSW) or a grain-based pellet (wheat dried distiller's grain and solubles from the Manildra Group, Nowra, NSW). The basal roughage was lucerne hay and was offered *ad libitum*. The canola meal and the grain-based pellets dietary treatments were initially offered at 2.0 or 2.5 kg/hd/d

(0.4 or 0.5% LW/d), respectively, to provide an equivalent MEI for each treatment group. Following weighing on Day 28 and conducting further proximate analysis the M/D content of the canola meal was found to be lower than originally estimated. Therefore, the amount of canola meal offered was increased from 2.0 to 2.5 kg/hd/d (0.4 - 0.5% LW/d).

4.2.4 Feed samples

Feed samples were collected fortnightly and stored frozen at -18°C. A representative sub-sample (approximately 150 g) of each feed was dried at 80˚C for 24 h for the determination of DM and subsequent analysis of nutritive value. Proximate analyses (% DM) were determined using NIRS with a Bruker multi-purpose analyser (MPA, Bruker Optik GmbH, Ettlingen, Germany) and OPUS software (version 5.1) with calibrations developed by the NSW DPI FQS, as described previously by Packer *et al.* (2011). The nutritive value of the dietary ingredients are presented in Table 4.1.

Table 4.1 Nutritive value (g/kg DM) of the dietary ingredients.

Component (g/kg DM)[1]	Grain-based pellets	Canola meal[2]	Lucerne hay
DM	931.0	906.0	893.0
OM	910.0	920.0	930.0
CP	201.0	377.0	119.0
aNDF	310.0	320.0	560.0
ADF	110.0	210.0	370.0
DMD	720.0	730.0	570.0
DOMD	710.0	730.0	550.0
EE	45.0	39.0	-
M/D (MJ/kg DM)	11.9	11.9	8.2

[1]DM = dry matter; OM = organic matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash, DMD = dry matter digestibility; DOMD = digestible organic matter in the dry matter; EE = ether extract; M/D = MJ metabolisable energy/kg DM = 0.203 x DOMD - 3.001 (for roughage) or 0.138 x DOMD + 0.272 x EE + 0.858 (for canola meal, AFIA, 2014).
[2]Canola meal was solvent extracted.

4.2.5 Slaughter

Steers were removed from their pens and weighed the morning (10:00 h) of Day 60 before being transported (1600 h) 11 km to a licensed, commercial abattoir. The steers

had *ad libitum* access to water prior to slaughter. The steers were slaughtered the following morning (10:30 h) in a single run and then dressed in accordance with the Australian standard practice. No electrical stimulation was used post-slaughter. Ear tag identification was matched with corresponding abattoir body number to maintain traceability throughout the chillers and boning room. Before entering the chillers, carcases were split in half down the vertebral column and HSCW recorded (AUSMEAT 2005). The carcases were chilled at 2–3°C for approximately 16 h before being split horizontally between the 11th and 12th ribs. The left side of the carcase was graded by a trained Meat Standards Australia (MSA) inspector (Polkinghorne *et al.* 2008). Meat Standards Australia data included carcase weight, meat colour, fat colour, fat depth, marbling, ultimate pH, hump height, MSA index, EMA and carcase ossification.

4.2.6 Carcase sampling

Although the following samples were collected, further sectioned, and sorted during the Honours project, all analyses were completed during the PhD thesis. The *longissimus lumborum* muscle (striploin) from the left side of each carcase was boned out. The untrimmed striploin primals were placed in vacuum bags, tagged, and chilled at 2–3 °C for 24 h before further sectioning.

As reported by Lynch (2017), carcase characteristics including fat and meat colour, rib fat, hump height, pH, MSA index, eye muscle area, MSA marble and ossification were not affected ($P > 0.05$) when steers were supplemented either grain-based pellets or canola meal when offered lucerne hay *ad libitum*.

4.2.7 Meat sample preparation

Seventy-two hours post-mortem, the striploin was sub-sectioned into further samples for the analysis of meat quality traits and consumer sensory analysis, aged and then stored. Briefly, the striploin was denuded of all fat, sinew, and epimysium. Starting from the anterior end of the striploin, the first 2.5 cm slice (A0) was aged for 3 weeks and retained at -18°C for future analysis. The remaining striploin was divided into three equal portions (A1; anterior position 1, A2; anterior position 2 and P3; posterior position 3). Each portion was sliced into 4 x 2.5 cm slices across the muscle to expose perpendicular muscle fibres (Figure 4.1). These portions (A1, A2 and P3) were randomly assigned to one of three ageing durations (3, 5 and 7 weeks).

The first three slices (Figure 4.1, white section) from each position (A1, A2 and P3) were further prepared for consumer sensory evaluations (Watson *et al.* 2008). Briefly, each slice was denuded of all fat and sinew and then cut into five sample slices (dimensions: 6.0 × 6.0 × 2.5 cm). Sample slices were individually wrapped in plastic sheets before being placed into labelled cryovac bags. Samples were aged (3, 5 or 7 weeks) at 2°C before being frozen at -18°C until consumer sensory analysis.

The last slice (Figure 4.1, grey section) from each position was aged for either 3, 5 or 7 weeks at 2°C. After the allocated ageing period, the slice (from each position) was cut into two (5 × 5 × 2.5 cm) portions. The first portion was frozen at -18°C for subsequent analysis of cooking loss, and shear force. From the second a core sample (diameter: 2.5 cm) was placed into EZ-DripLoss tubes for drip loss analysis immediately after preparation. The remainder of this portion was subdivided into two (thickness: 1 cm) sub-slices and stored frozen at -18^{o}C until subsequent analysis for TBARS (one sub-slice) and particle size analysis (PSA) and ultimate pH analysis (second sub-slice).

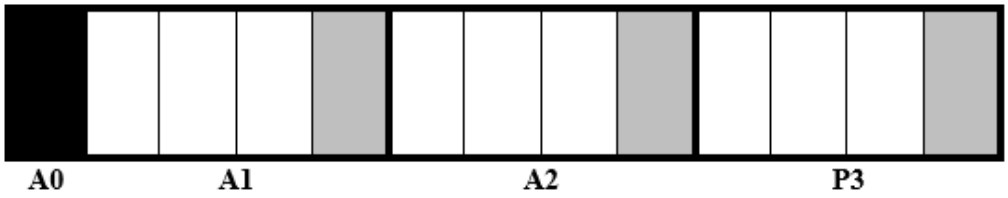


Figure 4.1 The beef *longissimus lumborum* muscle division. A0; anterior position 0, A1; anterior position 1, A2; anterior position 2, P3; posterior position 3. Black section denotes slice for future analyses, white sections denote slices for consumer sensory analysis and grey sections denote slices for meat quality analysis.

4.2.8 Meat quality parameters

Ultimate pH analysis

The thawed sample (approximately 1 g) was homogenised with a phosphate buffer, incubated in a 20°C water bath, allowed to equilibrate, and the pH (smartCHEMC-CP, TPS Pty Ltd., Queensland, Australia) measured in duplicate (De Brito *et al.* 2016). The pH meter was fitted with a polypropylene spear type gel electrode (IJ-44, Ionode^{TM},

Queensland, Australia), and calibrated using pH 4.0 and pH 6.8 standards (at 20°C, no.121382 and no. 121380, TPS Pty Ltd., Queensland, Australia).

Drip loss

Drip loss was determined using fresh sample cores removed using a specialised circular knife. The samples were weighed before being placed into individual EZ-DripLoss tubes (Danish Meat Research Institute, Taastrup, DEN). The samples were refrigerated (3–4°C) for 48 h before each core was reweighed and the drip loss was calculated as the percentage of weight change over this period.

Cooking loss and shear force

The individual vacuum-packaged and frozen shear force samples were cooked for 35 min in a 71°C water bath (90 L with a 2000 W heating element, Ratek Instruments Ltd., Melbourne, AUS). After cooking, samples were removed and submerged under cold water for 30 min to stop the cooking process (Hopkins *et al.* 2010). Packaging was removed and the cooked sample blotted dry with paper towel. Cooking loss was calculated as the percentage of weight change pre- and post-cooking.

All cooked samples were refrigerated overnight (4-5°C). Six cuboidal strips (cross section area: 1 cm^2) were removed parallel to muscle fibre direction and analysed (Holman *et al.* 2015) using a texture analyser (Model LRX, Lloyd Instruments, Hampshire, United Kingdom) fitted with a Warner Bratzler blade, set to a crosshead speed of 200 mm/min. The cutting line was positioned perpendicular to the fibre direction and care was taken to avoid connective and fatty tissue. The average peak shear force (SF) was reported in Newtons (N).

Particle size analysis

Approximately 1 g of meat sample (in duplicate) were homogenised at 16 000 rpm (Series X 10/25, Ystral, GER) with 20 mL of ice-cold buffer (0.1 M KCI, K_2HPO_4 and 0.001 M EDTA, balanced to pH 7.0) (Karumendu *et al.* 2009). The homogenised samples were then added drip-wise to a laser diffraction particle size analyser (Model LS-13/320, Beckman-Coulter, Miami, USA) and the results were averaged and expressed in µm.

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substance content determination was also conducted as per Hopkins *et al.* (2014). Briefly, 100 mg meat samples were placed into labelled micro-tubes with 500 µL ice cold radioimmunoprecipitation assay (RIPA) buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals, Michigan, USA). Each sample was homogenised using micro-tube pestles and held on ice before 5 min centrifugation at 5,600 x g (MiniSpin Plus, Eppendorf Pty. Ltd., Hamburg, Germany). The supernatant was immediately transferred into another labelled micro-tube and held frozen at -80 °C. These were analysed for their TBARS content as per the OXI-tek TBARS assay kit (no. ALX-850-287-KI01, Enzo Life Sciences Inc., New York, USA) technical bulletin (Zeptometrix 2006) and using a benchtop spectrophotometer set to 532 nm (AMR-100 Microplate Reader, Hangzhou Allsheng Instruments Ltd., PRC). All results were expressed as mg MDA per kg of fresh meat.

4.2.9 Sensory design and cooking protocol

Sensory evaluations were conducted as per established MSA protocols described by Watson *et al.* (2008). Specifically, grill samples were thawed (4°C for 24 h) before being cooked using a Silex clamshell grill (Silex Grills Australia Pty Ltd, Marrickville, NSW, Australia) that was set to 225°C. Samples were cooked for 5 min, with the grill closed, to achieve "medium" degree of doneness (70°C) using an electric thermometer to test for degree of doneness and temperature was recorded. Ten sample steaks were prepared for each cooking round. Before serving, steaks were rested for 3 min, and then each steak was cut into two equal-sized rectangular pieces and served to two separate pre-selected consumers.

A total of 1200 untrained consumers participated over seven nights and 20 tasting sessions (tasting sessions included three groups of 20 people). Untrained consumers who preferred meat cooked to a medium degree of doneness and who consumed meat at least once a week were selected. Each muscle sample was tasted by 10 different consumers. Briefly, each consumer tested a starter sample (link sample), followed by six experimental samples (pre-allocated within a 6 × 6 Latin square design). Consumers were asked to score each sample based on tenderness, juiciness, flavour and overall liking using a 10 cm line scale (where; 0 being not very tender and 10 being very tender). Individual consumer sensory scores for tenderness, juiciness, flavour and the overall liking were weighted by 0.3, 0.1, 0.3 and 0.3, respectively, and

used to calculate a palatability or MQ4 out of 100 (scores closer to 100 are considered to have higher eating quality compared with those scores closer to 0 that are considered to have a poor eating quality). Before calculating the mean sensory scores for each sample, the 10 individual scores for each sample were ranked (highest to lowest) and the two highest and two lowest scores were clipped to reduce the variance of the mean sensory scores (Polkinghorne *et al.* 1999; Watson *et al.* 2008). In addition, consumers then ranked the product on quality (lowest quality to highest quality) of the sample using categorical terms of "unsatisfactory quality", "good everyday quality", "better than everyday quality" or "premium quality".

4.2.10 Statistical analysis

Prior to analysis, data were assessed for assumptions of normality by histograms and plotting residuals as previously described in Chapter 3.8. Meat quality traits and clipped consumer sensory parameters were also analysed using the Mixed model procedure in SAS. Samples that were retained for drip loss (5 weeks aged), PSA and TBARS (7 weeks aged) were not included in the data set for analysis due to preparation error. The analysis examined the fixed effects of "dietary treatment" and "ageing period" as well as the interaction between the fixed effects, with "pen", "muscle position" and "batch" examined as random effects. The final model included the fixed effect of "dietary treatment" and "ageing period" and random effects of "pen" and "position". Least square means and differences of least square means were reported. An alpha of 0.05 was used for all statistical tests.

4.3 Results

4.3.1 Meat quality traits

Meat quality traits (Table 4.2) were not different ($P > 0.05$) when steers were fed either the grain-based pellets or canola meal. Particle size analysis, ultimate pH and TBARS were not affected ($P > 0.05$) by ageing period or by dietary treatment. Shear force and cooking loss values were lower ($P < 0.05$) as ageing period increased; however, drip loss percentage was higher ($P > 0.05$) when ageing period increased. There were no interaction ($P > 0.05$) effects between dietary treatment and ageing period for any meat quality traits.

4.3.2 Consumer sensory analysis

Consumer sensory analysis was not affected ($P > 0.05$) by dietary treatment, ageing period or the interaction between dietary treatment and ageing period (Table 4.3). Although all consumer sensory traits for tenderness, juiciness, flavour, overall liking and MQ4 score were numerically higher as ageing period increased, none of the differences were statistically significant. The overall satisfaction of samples equated to "good everyday quality" from steers that were supplemented with either canola meal or grain-based pellets, which scored as 3.35 and 3.34 out of 5, respectively.

4.4 Discussion

4.4.1 Meat quality traits

Meat quality traits did not differ between the dietary treatments. Diet can influence meat and eating quality traits such as the rate of oxidation (Gobert *et al.* 2010), colour (Lanari *et al.* 2002), tenderness (Bakker *et al.* 2021), flavour (Aldai *et al.* 2010) and the overall liking of a product (Mezgebo *et al.* 2017). Tenderness is one of the most important palatability attributes for consumer preference and can be objectively measured by shear force (Huffman *et al.* 1996; Miller *et al.* 2001). Acceptable levels for shear force and particle size analysis are 36.8 N and 142 µm, respectively (Holman *et al.* 2020), which are higher than reported in the current study. Shear force values decreased with longer ageing periods, which was expected (Holman *et al.* 2019); however, the interaction between length of ageing and dietary treatment was not significant, further supporting that canola meal had no adverse effects. Ageing the product can also influence meat and eating quality traits (Jiang *et al.* 2010; Holman *et al.* 2019). The effect of ageing the beef, when steers were fed higher levels of canola meal, for long periods of time may be worthwhile for further research to ensure there are no negative effects on self-life.

Table 4.2 Meat quality traits of *longissimus lumborum* (striploin) muscle of steers supplemented with either grain-based pellets or canola meal and offered lucerne hay *ad libitum*.

Meat quality traits[1]	Dietary treatment		Weeks aged			*P*-values		
	Grain-based pellets	Canola meal	3	5	7	Dietary treatment (D)	Weeks aged (W)	D x W
Shear force, N	30.4 (0.79)	30.1 (0.79)	32.8 (0.97)[b]	29.0 (0.97)[a]	28.9 (0.97)[a]	0.786	0.007	0.786
Cooking loss, %	20.1 (0.41)	20.1 (0.41)	20.9 (0.50)[b]	20.6 (0.50)[b]	19.2 (0.50)[a]	0.705	0.036	0.965
Particle size, µm	129.4 (8.90)	126.2 (8.90)	128.6 (8.79)	127.0 (9.02)	n.a	0.804	0.904	0.302
pHu, U	5.57 (0.02)	5.60 (0.02)	5.59 (0.02)	5.60 (0.02)	5.57 (0.02)	0.302	0.348	0.609
Drip loss, %	0.33 (0.02)	0.32 (0.02)	0.29 (0.02)	n.a.	0.36 (0.02)	0.428	0.015	0.310
TBARS, mg MDA/kg	0.75 (<0.01)	0.75 (<0.01)	0.75 (<0.01)	0.75 (<0.01)	n.a.	0.953	0.986	0.206

Values are least squares means ± the standard error of least squares means. Means within rows with different superscripts differ significantly ($p < 0.05$).
[1]N = newtons; pHu = ultimate pH; TBARS = thiobarbituric reactive substance; MDA = malondialdehyde. n.a. = data not available.

Table 4.3 Meat Standards Australia (MSA) consumer eating quality traits of *longissimus lumborum* (striploin) muscle of steers supplemented with either grain-based pellets or canola meal and offered lucerne hay *ad libitum*.

Meat eating quality traits	Dietary treatment		Weeks aged			*P*-values		
	Grain-based pellets	Canola meal	3	5	7	Dietary treatment (D)	Weeks aged (W)	D x W
Tenderness	56.6 (2.50)	57.2 (2.50)	55.4 (2.73)	56.4 (2.70)	58.9 (2.76)	0.788	0.388	0.806
Juiciness	59.2 (3.02)	59.3 (3.02)	58.4 (3.19)	59.1 (3.15)	60.1 (3.23)	0.968	0.817	0.891
Flavour	58.8 (2.09)	60.9 (2.09)	58.8 (2.25)	59.5 (2.22)	61.2 (2.29)	0.208	0.510	0.898
Overall liking	59.6 (2.35)	60.9 (2.35)	59.3 (2.52)	59.8 (2.48)	61.7 (2.56)	0.478	0.508	0.922
MQ4 score[1]	58.7 (2.27)	59.8 (2.27)	58.1 (2.43)	58.9 (2.40)	60.7 (2.47)	0.508	0.467	0.892
Overall satisfaction	3.35 (0.09)	3.34 (0.09)	3.29 (0.09)	3.33 (0.09)	3.40 (0.10)	0.870	0.444	0.767

Values are least squares means ± the standard error of least squares means. All parameters used clipped data as described in the materials and methods. Meat eating quality traits were scored out of 0-100 expect the overall satisfaction that was scored out of 0-5.

[1]MQ4 = composite overall quality score calculated from clipped tenderness, juiciness, flavour and overall liking.

The higher drip loss with increasing ageing was also expected (Savage *et al.* 1990) and may be associated with structural decomposition and higher sarcoplasmic protein in the intra- myofibrillar space (Pearce *et al.* 2011). The degree of lipid oxidation in meat (measured by TBARS) provides an estimate of potential shelf-life and eating quality characteristics of beef (Campo *et al.* 2006; Zhang *et al.* 2019). In the current study, TBARS values were not affected by the dietary treatment; and the interaction between diet and ageing period was also not significant. The TBARS values observed in the current study (approximately 0.75 mg MDA/kg) were lower than those observed when grass-fed beef was aged for up to 12 weeks (1.00 ± 0.03 mg MDA/kg) (Holman *et al.* 2019) and much lower than the threshold value proposed for the acceptability of oxidised beef (approximately 2 mg MDA/ kg) (Campo *et al.* 2006). Therefore, supplementing canola meal up to 2.5 kg/hd/d to cattle fed *ad libitum* lucerne hay does not have any adverse effects on self-life.

4.4.2 Consumer sensory analysis

Sensory evaluations are critical to obtain consumer's overall feedback and acceptance of a product. In the present study, instrumental values and consumer evaluations were not different between dietary treatments. These findings validate the laboratory objective measures, affirming that consumers could likewise not detect differences between the dietary treatments. The absolute eating quality values from the consumer sensory analysis were identified as "good everyday quality" (Watson *et al.* 2008). Although the samples were not considered to have a high eating quality value, the absence of a significant dietary effect on these measures is considered a positive result for the use of canola meal as a supplement for grass-fed beef. Producers could use canola meal as a supplement with dry forage typical of feed available during summer and autumn in Australia without negatively impacting eating quality.

In addition to consumer sensory data not being affected by dietary treatment, these parameters were also not affected by ageing period. This result was not expected given some objective measures were affected by ageing period. Consumers were unable to detect any effect of ageing period on tenderness; however, shear force values were significantly lower as ageing period increased. In general, consumers cannot detect any discernible change in tenderness unless the shear force value was greater than 4.1 kg (40.21 N) (Huffman *et al.* 1996). The relatively large variation observed in consumer sensory data compared with objective measures could also have precluded a statistically significant result from being detected in the present study.

4.5 Conclusion

Meat quality traits and eating quality were not affected when steers were supplemented with canola meal compared with the grain-based pellets (0.5% LW/d). While, some meat quality traits were altered as a result of the ageing period, there was no interaction with dietary treatment. Therefore, canola meal could be used as an approved PCAS supplement for a forage-based diet without negative effects on meat quality. Future research should investigate the DMI, ADG, FCR of weaners offered varying inclusion levels of canola meal on dry forage typical of feed available during summer and autumn in southern Australia.

Chapter 5: Response to Varying Inclusion Levels of Canola Meal when Weaner Calves are Fed a Low-Quality Roughage

5.1 Introduction

Domestic and export markets have been developed for certified grass-fed beef where producers must meet particular market specifications to receive certified grass-fed premiums for their product (Cummins 1996). For animals to be certified grass-fed, they must not consume any grain throughout their lifetime. This creates a challenge for producers especially during those periods of low pasture quality and/or quantity. During these times of the year, growing animals need to be provided a diet that is high in both protein and energy to gain weight. Therefore, any low-quality pastures on offer need to be supplemented with a non-grain, high protein, and high energy feed resource to meet grass-fed certification requirements.

Average daily gain did not differ when steers were fed a moderate-quality basal diet (lucerne hay) and supplemented with either a grain-based pellet or canola meal, although, canola meal was only included at 2.5 kg/hd/d (Lynch and Campbell 2017). Dry matter intake and growth responses were improved when canola meal was included in a variety of PMR (Seoane *et al.* 1992; 1993; Petit *et al.* 1994). There are no specific recommendations on the feeding levels of canola meal for ruminants fed low-quality roughage diets. General recommendations from the NSW DPI are that, where required, protein supplements should be fed at rates of 0.2-0.5% LW/d (0.3 – 0.7 kg/hd/d for 150 kg) for weaner calves (Blackwood and Clayton 2007), whereas a report from Australia Oilseeds Federation suggested canola meal can be fed to cattle (typically consuming 20-25 kg DM/hd/d) at inclusion rates of 2-3 kg DM/hd/d (Mailer 2004).

To gain a better understanding of the suitability of canola meal as a supplement in grass-fed beef systems, there is need to investigate the DMI and growth responses when animals, particularly growing animals such as weaner calves, are fed a low-quality roughage diet (to represent dry, mature pastures under practical application) and supplemented with varying levels of canola meal. Therefore, the objective was to determine whether DMI, ADG and FCR increased when weaner calves were provided with *ad libitum* access to a relatively low-quality hay (canola hay) and offered increasing inclusion levels of canola meal.

5.2 Methods and materials

The study was conducted at the Ruminant Research Complex, located on the CSU campus at Wagga Wagga, as previously described in Chapter 3.2. The feeding trial was approved by CSU ACEC (Approval number: A18090) as previously described in Chapter 3.1. The location, animal housing and animal data collection, sample collection, analytical procedures, calculations and statistical analysis, are presented in Chapter 3, Sections 3.2, 3.3, 3.4, 3.6, 3.7 and 3.8 unless stated otherwise.

5.2.1 Experimental animals and housing

Eighty-four Angus weaner calves (LW = 161 ± 1.6 kg) were selected from 113 weaner calves that were 4-5 months old. The weaner calves were sourced from the CSU commercial herd and were selected based on similar LW and BCS. Prior to the feedlot induction period, all weaner calves were vaccinated (Ultravac® 7in1, Zoetis, Victoria, Australia) and administered an anthelmintic drench (Ivomec® Pour-on (Ivermectin), Boehringer Ingelheim, Canada). All animals had access to shade and clean water, as previously described in Chapter 3.3.1; however, there was an additional shaded area (5 x 8 m) over the feed trough in the first six pens. The weaners were weighed fortnightly at 0600 h (12 h fasting; however, animals still had access to water due to extreme heat). Their BCS was also assessed at the time of weighing.

5.2.2 Experimental design

The weaner calves were allocated to one of four dietary treatment groups (n = 21 per treatment group) using a stratified randomised design based on sex (n = 46 male and 38 female) and LW. The weaner calves were then randomly allocated to one of 12 pens, with three pens per treatment and thus seven animals per pen. The feeding trial consisted of a 10 d dietary adaptation period and a 56 d feeding period.

5.2.3 Dietary treatments

During the adaption period, the weaner calves were offered 1 kg/hd/d of canola meal and *ad libitum* canola hay. The dietary treatments were formulated to achieve an intake of canola meal equivalent to either 0.5%, 1.0%, 1.5% or 2.0% of the average pen LW. This equated to four inclusion levels of canola meal viz. 20%, 37%, 46% and 68% of estimated DMI. A control treatment (0% canola meal) was not included in the feeding trial because the quality of the canola hay was not sufficient to support the

requirements of growing animals. The lowest inclusion level of canola meal that would meet the requirements of growing animals (CSIRO 2007) when fed a basal diet of canola hay (M/D of approximately 7.97 MJ/kg DM) was 20% dietary treatment group. The canola hay was chosen as the basal diet as it was readily available and of similar quality to that of non-irrigated forages available during the summer-autumn period in southern NSW. The nutritive value of the dietary ingredients and the dietary treatments are presented in Table 5.1 and 5.2, respectively.

Each pen of animals was provided *ad libitum* access to canola hay and offered their predetermined amount of canola meal daily at 0730. The amount of canola meal fed was adjusted every 2 weeks based on the average LW of the animals in each pen. Canola hay intake and supplement refusals were measured and recorded weekly.

5.2.4 Sample preparation

Representative samples (approximately 500 g) of the offered canola meal and canola hay, and the refusals from each pen were taken weekly, as previously described in Chapter 3.4.1. These samples were frozen at -18˚C until subsequent analysis of their nutrient content.

5.2.5 Analytical procedures

All chemical analytical procedures to determine the nutrient content of the feeds and feed refusals were undertaken at the Feed Laboratory of NSW DPI at Wagga Wagga, as described in Chapter 3.6.

Table 5.1 Nutritive value (g/kg DM) of the dietary ingredients.

Component (g/ kg DM)[1]	Dietary ingredients[2]	
	Canola hay	Canola meal[3]
DM	902.1	929.0
OM	933.3	930.0
CP	80.3	425.8
aNDF	543.3	260.0
ADF	400.0	175.0
DMD	556.7	760.0
DOMD	540.0	760.0
EE	12.8	18.5
M/D (MJ/kg DM)	7.97	11.85

[1] DM = dry matter; OM = organic matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash, DOMD = digestible organic matter in the dry matter; EE = ether extract; M/D = MJ metabolisable energy/kg DM = 0.203 x DOMD - 3.001 (for roughage) or 0.138 x DOMD + 0.272 x EE + 0.858 (for canola meal, AFIA, 2014).
[2]Ration ingredients were collected each fortnightly and analysed in duplicate and the average results are presented.
[3]Canola meal was solvent extracted.

Table 5.2 The estimated nutritive value (g/kg DM) of the experimental dietary treatments consumed by weaner calves.

	Inclusion level of canola meal (% estimated DMI)[2]			
Nutritive value[1] (g/kg DM)	20	34	46	68
OM	93.3	93.2	93.2	93.1
CP	148.6	197.5	240.0	314.1
aNDF	487.3	447.1	412.1	351.5
ADF	355.5	323.6	295.8	247.6
DMD	598.9	629.1	655.4	701.1
DOMD	583.5	614.7	641.9	689.0
EE	13.9	14.7	15.4	16.7
M/D (MJ/kg DM)	8.73	9.28	9.76	10.59

[1]OM = organic matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash; DMD = dry matter digestibility; DOMD = digestible organic matter in the dry matter; EE = ether extract; M/D = MJ metabolisable energy/kg DM = 0.203 x DOMD - 3.001 (for roughage) or 0.138 x DOMD + 0.272 x EE + 0.858 (for canola meal, AFIA, 2014).
[2]Canola meal was solvent extracted.

5.2.6 Statistical analyses

Prior to analysis, data were assessed for assumptions of normality by histograms and plotting residuals. Change in LW and BCS over time was analysed by repeated-measures analysis using the Mixed Model procedure in the SAS statistical program (SAS Institute Inc. 1997). The REML estimation used "pen" as the experimental unit and "animal" within "pen" as the sampling unit (Littell *et al.* 2000; Clayton *et al.* 2008). The most appropriate covariance structure used for each analysis was determined by the Schwarz's BIC (Wang and Goonewardene 2004). The analysis examined the fixed effects "dietary treatment" and "day" plus the interaction between the fixed effects and "pen" and "sex" as random terms. Analysis of LW included day -10 (pre-treatment) as a co-variate in the model.

The DMI, ADG, FCR, MEm and MEm x LoF were analysed using the Mixed Model procedure in SAS. The analysis included the fixed effect of "dietary treatment" with "pen" and "sex" and "shade" and the interactions as a random effect. The final model included the fixed effect of "dietary treatment" with "pen" and "sex" as a random effect. Least square means and differences of least square means were reported. An alpha of 0.05 was used for all statistical tests.

The relationships between actual MEI (MJ kg/DM) and predicted MEI (MJ kg/DM) and actual ADG (kg/hd/d) were assessed using the PROC REG procedure in SAS. An alpha of 0.05 was used for all statistical tests.

5.3 Results

5.3.1 Diet composition and dry matter intake

The average daily intakes of the canola hay and the canola meal, and total DMI are presented in Table 5.3. The dietary treatments were formulated to achieve an intake of canola meal equivalent to either 0.5%, 1.0%, 1.5% or 2.0% of the average pen LW (starting LW 161 ± 1.6 kg). This equated to four inclusion levels of canola meal viz. 20%, 37%, 46% and 68% of estimated DMI. Dry matter intake increased as the inclusion level of canola meal supplementation increased up to 46%; however, a decline in DMI was observed at the 68% canola meal inclusion. There were no refusals of canola meal when it was fed at 20%, 34% and 46% inclusion levels; however, there was residual canola meal remaining in the highest inclusion level (2.0 % LW) canola meal dietary treatment group.

The amount of canola hay consumed was higher ($P < 0.05$) when the weaners were supplemented with either 20% or 34% canola meal. The amount of canola hay consumed did not differ when the weaners were supplemented with either 34% or 46% canola meal and was significantly lower when supplemented with 68% canola meal. The total DMI was higher ($P < 0.05$) when 34% and 46% canola meal was fed compared with the 20% and 68% canola meal dietary treatments. The estimated MEI and MEI:MEm significantly increased ($P < 0.05$) as the level of canola meal increased up to 46%; however, the MEI and the MEI:MEm did not differ between the 46% and 68% canola meal inclusion levels (Table 5.3). The FCR was not altered ($P > 0.05$) by dietary treatment.

The polynomial relationship of estimated total DMI ($R^2 = 0.967$, $P = 0.183$) was highly correlated; but was not significantly ($P > 0.05$) related to the inclusion level of canola meal in the ration (Figure 5.1). The actual MEI ($R^2 = 0.387$, $P = 0.378$) had a polynomial relationship to ADG, whereas the relationship between predicted MEI and ADG was linear ($R^2 = 1$, $P < 0.001$) as the inclusion level of canola meal increased when weaner calves were fed canola hay (Figure 5.2).

5.3.2 Liveweight

Liveweight and BCS were significantly ($P <0.05$) affected by dietary treatment, experimental day and the interaction between dietary treatment and experimental day. Liveweight and BCS were higher ($P = 0.001$) at Day 42 and Day 56 when weaner calves were fed 34% and 46% canola meal compared with 20% and 68% canola meal (Figures 5.3 and 5.4, respectively). As shown in Table 5.3, ADG was higher ($P < 0.001$) when the weaner calves were fed 34% and 46% canola meal compared with 20% and 68% canola meal.

Table 5.3 Total dry matter intake (DMI), average daily gain (ADG) and estimated metabolisable energy intake (MEI) when weaner calves consumed varying inclusion levels of canola meal and canola hay.

Parameter[1]	Inclusion level of canola meal (% estimated DMI)				*P*-value
	20	34	46	68	
Estimated dry matter intake (DMI, kg DM/hd/d)					
Canola hay	3.41 (0.19)^{c}	3.34 (0.19)^{bc}	2.91 (0.20)^{b}	1.49 (0.20)^{a}	<0.001
Canola meal	0.84 (0.05)^{a}	1.79 (0.05)^{b}	2.68 (0.05)^{c}	3.57 (0.05)^{d}	<0.001
Total DMI[2]	4.23 (0.21)^{a}	5.12 (0.21)^{bc}	5.60 (0.21)^{c}	5.04 (0.21)^{b}	0.003
Estimated metabolisable energy intake (MEI, MJ kg/hd/d)					
MEI	36.91 (1.72)^{a}	47.68 (1.72)^{b}	54.73 (1.72)^{c}	53.95 (1.72)^{c}	<0.001
MEI:MEm	1.23 (0.04)^{a}	1.52 (0.04)^{b}	1.74 (0.04)^{c}	1.76 (0.04)^{c}	<0.001
ADG (kg/hd/d)	0.51 (0.09)^{a}	0.82 (0.09)^{b}	0.90 (0.09)^{b}	0.60 (0.09)^{a}	<0.001
FCR (kg feed/kg ADG kg/hd/d)	9.03 (1.18)	6.88 (1.18)	6.80 (1.19)	9.91 (1.19)	0.154

Values are least-squares means ± standard errors of least-squares means. Means within rows with different superscripts differ significantly ($p < 0.05$).
[1]MEI:MEm; amount of metabolisable energy offered (DM intake x estimated M/D of the feed MJ/d) divided by the maintenance requirement of the animal (the level of feeding), FCR, feed conversion ratio.

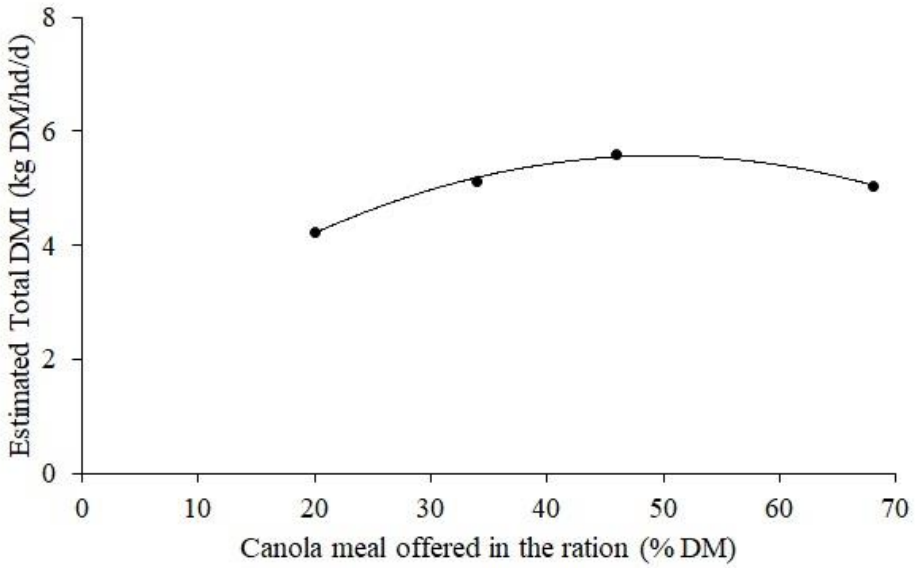


Figure 5.1 Estimated total dry matter intake (DMI = -0.002 x inclusion level of canola meal^2 + 0.153 x inclusion level of canola meal + 1.765 (R^2 = 0.967, *P* = 0.183) when steers were fed varying inclusion levels of canola meal and offered canola hay.

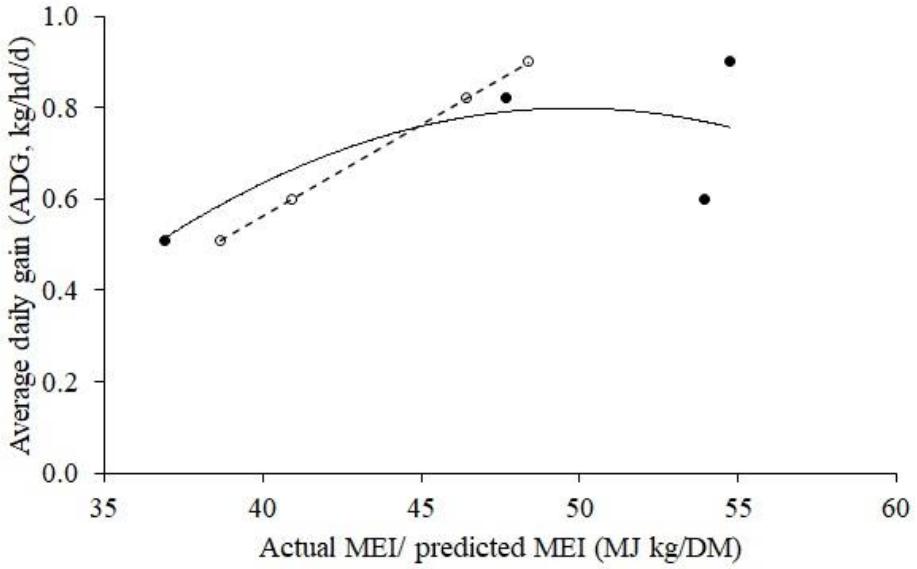


Figure 5.2 Correlation between average daily gain (ADG) and actual Metabolisable Energy Intake (MEI MJ/kg/DM, (●)) and predicted MEI (MJ/kg/DM, (○)) from CSIRO (2007) feeding standards when steers were fed varying inclusion levels of canola meal and offered *ad libitum* canola hay. Actual MEI = -0.002 x ADG^2 + 0.170 x ADG - 3.440 (R^2 = 0.387, *P* = 0.378). Predicted MEI = 0.040 x ADG - 1.044 (R^2 = 1, *P* < 0.001).

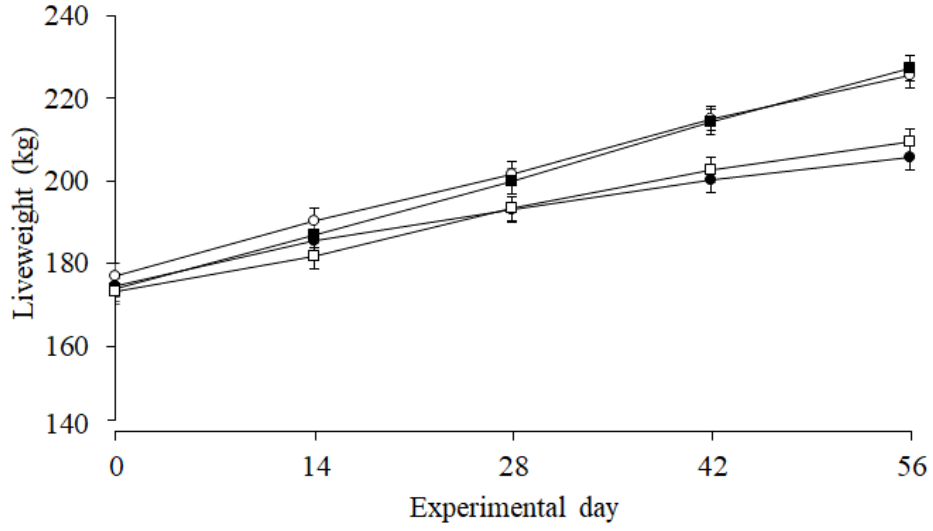


Figure 5.3 Liveweight changes over the experimental period when weaner calves were fed either; 20% (●), 34% (○), 46% (■) or 68% (□) canola meal and *ad libitum* canola hay. Error bars are the standard error of the least square means.

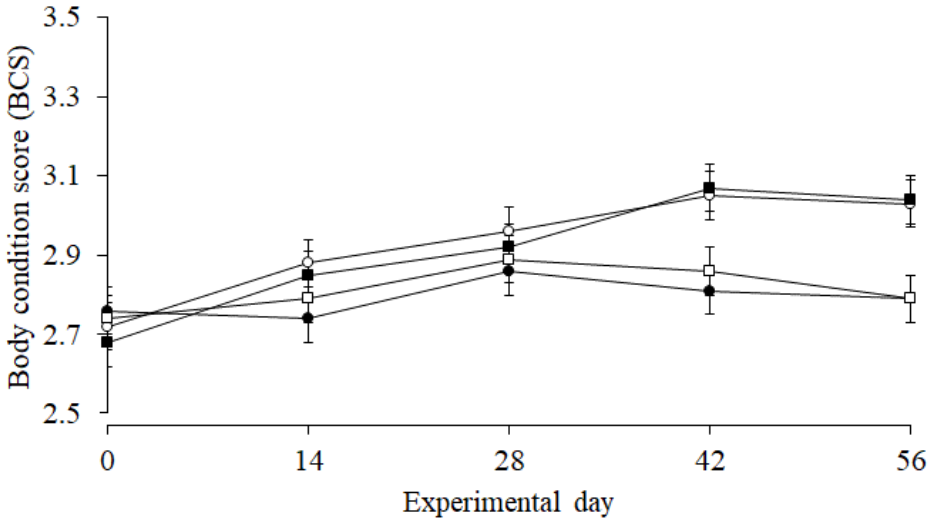


Figure 5.4 Body condition score (BSC) changes over the experimental period when weaner calves were fed either; 20% (●), 34% (○), 46% (■) or 68% (□) canola meal and *ad libitum* canola hay. Error bars are the standard error of the least square means.

5.4 Discussion

All of the weaner calves gained weight over the feeding period; however, increasing the level of canola meal supplementation from 20% to 34% or 46% (0.5% , 1.0% and 1.5% LW/d, respectively) resulted in a significantly higher DMI and ADG. Both DMI and ADG declined when 68% canola meal was offered compared with the 34% and 46% inclusion level. The following discussion is focused on how these responses might be linked to changes in energy and protein utilisation by the weaner calves.

5.4.1 Growth response

The ADG increased in response to feeding 34% and 46% canola meal compared with the 20% canola meal treatment group was expected as the M/D and CP of the diet also increased. The CP and M/D of the 20% inclusion level ration was 148.6 g/kg DM and 8.73 MJ/kg DM, respectively, indicating this inclusion level was not limiting and acceptable for growth for weaner calves (SCA 1990). The ADG (and DMI) was higher when feedlot calves were fed 16% CP compared with diets containing 12.5% CP (Fluharty and Loerch 1996). In addition, including canola meal up to 34% of the diet did not affect ruminal fermentation, nutrient digestion, and microbial growth using *in vitro* methodology (Paula *et al.* 2018). The increase in ADG in response to the higher CP from feeding canola meal at these higher levels (34% and 46%) indicates no

apparent differences in rumen function; however, further research when supplementing canola meal above 34% is warranted.

An increase in LW gain, has been reported when ruminants are fed cereal straw or hay and supplemented with protein, which provides additional N for rumen microorganisms (Dixon *et al.* 2017). Cottonseed meal has been shown to improve ADG of steers fed low-quality roughages (Hennessy *et al.* 1981; 1983; Lee *et al.* 1985; Smith and Warren 1986a, 1986b; McLennan *et al.* 2017a; 2017b). Average daily gains were higher when cottonseed meal was provided at varying levels (200, 400, 600, 800 and 1000 g/hd/d) to steers grazing on oaten stubble and dry annual pastures (Smith and Warren 1986a). Dry matter intake, ADG, and FCR were higher when cattle were offered canola meal in PMR based on quackgrass hay (Seoane *et al.* 1992), timothy silage (Petit *et al.* 1994) or barley grain (Seoane *et al.* 1993). Supplementing canola meal also resulted in improved DMI, LW gain and FCR of lambs fed either low-quality pastures or roughages (Ponnampalam *et al.* 2005). The inclusion levels of canola meal or cottonseed meal were not as high in any of these studies compared with the current study. The reason(s) for the lack of difference in ADG (and total DMI) between the 34% and 46% supplementation levels is unclear. Investigation of apparent nutrient digestibility and degradation kinetics in a controlled feeding experiment may provide some insight into the lack of difference in ADG and total DMI. Therefore, understanding how supplementation changes the digestibility of a diet, in conjunction with production responses is critical when evaluating the usefulness of canola meal, as an approved PCAS supplement, when cattle are consuming low-quality roughages.

The weaner calves fed 68% canola meal were offered an estimated 270.3 g N/kg DM which is above recommended N intake for weaners (SCA 1990; CSIRO 2007). The polynomial relationship between total DMI and increasing inclusion levels of canola meal also supports the decline in ADG (Figure 5.2). The lower ADG may have been due to the excess or wasted N in the diet resulting in a decrease in DMI. When relatively large quantities of RDP, such as canola meal (Maxin *et al.* 2013) are fed, it results in high ruminal NH_3-N concentrations that exceeds the ability of rumen microbes to utilise it (Slyter *et al.* 1979). The excess NH_3-N is absorbed across the rumen wall and ultimately converted to urea in the liver, and then excreted via the urine, which has an associated energy cost (Tyrrell *et al.* 1970; Reed *et al.* 2017; Jennings *et al.* 2018), resulting in a potential decline in ADG. Furthermore, regardless of whether the excess CP from canola meal is degraded in the rumen or used as a source of RUP, any excess NH_3 will get converted to and excreted as urea.

Investigation of ruminal NH_3-N concentrations in animals fed high levels of canola meal would provide critical information to better understand why there was a decrease in ADG of the animals.

5.4.2 Dry matter intake

Generally, if the basal diet has a CP between 6 and 8%, as was the case in this study, protein supplementation seems to improve DMI responses of cattle (Mathis *et al.* 2000). Total DMI increased in response to feeding 34% and 46% canola meal compared with the 20% canola meal diet, indicating that increasing the amount of canola meal fed did not result in a direct substitution effect. Conversely, canola hay intake was higher when 20% canola meal was fed compared with all the other canola meal inclusion levels, indicating that there was a partial substitution effect (Horn and McCollum, 1987). Substitutional effects were observed when cattle (McLennan *et al.* 2017a) and lambs (Dixon and Egan 2000) were offered cottonseed meal and a low-quality roughage. Total DMI increased linearly when young steers were fed hay and cottonseed meal (24 to 36 g/kg LW/ d) and a substitutional effect was observed as the inclusion level of cottonseed meal increased (0 to 18 g/kg LW d) and the hay decreased (McLennan *et al.* 2017a). Although, in the current study there was a polynomial relationship (R^2 = 0.992) of total DMI (Figure 5.1) when increasing inclusion levels of canola meal. In addition, the actual MEI to ADG relationship also declined between 46% and 68% of canola meal. Conversely, the predicted MEI to ADG using CSIRO (2007) feeding standards indicated that the response to increasing the inclusion level of canola meal should be linear; however, this was not the case indicating an upper inclusion level of canola meal had been reached when weaner calves were fed canola hay (Figure 5.2).

There was not a dietary treatment that contained canola hay only as this would not have provided adequate ME or CP requirements for growing weaners (CSIRO 2007). Therefore, the 20% canola meal dietary treatment was used as the positive control group. Further studies investigating varying inclusion levels of canola meal (including a negative control group) in a low-quality/ roughage paddock situation is warranted.

5.4.3 Energy and protein utilisation

Energy and protein utilisation can be improved when supplementing low-quality basal diets with protein meals (Lee *et al.* 1987; McCollum and Horn 1990). Supplemental protein can improve DMI and LW by correcting N deficiencies in the rumen, increase true protein flow (from dietary undegraded protein) to the small intestines and/or the

correction of AA deficiency or imbalances at the tissue level, all of which can stimulate DMI and ME utilisation (McCollum and Horn 1990). The M/D of the diets increased with increasing inclusion level of canola meal; however, there was no difference in MEI between 46 and 68% inclusion level. Similarly, the MEI:MEm was higher when 46% and 68% canola meal was offered compared with 34%, which was higher than for 20% canola meal inclusion level. This supports the polynomial relationship between the actual MEI and ADG (Figure 5.2), where there was a decrease in MEI and ADG between 46% and 68% canola meal, indicating there is a maximum inclusion level of canola meal.

The improved growth rates in response to protein supplementation of ruminants fed low-quality forage is related to increased glucose availability. This increase in glucose availability may be the result of either an increased feed intake increasing the production of propionic acid (as a consequence of increased microbial activity), and/or by increasing the availability of AAs for absorption in the small intestines (Lee *et al.* 1987). Further studies investigating the forage and canola meal intakes and the digestibility of the diets between the 34 and 46% inclusion level in a controlled environment may provide further information on energy utilisation.

The rate and extent of protein degradation (in the rumen) can be influenced by the diet the animal is offered (Ørskov and McDonald 1979; López 2005). Canola meal is mostly classified as a source of RDP (Heim and Krebs 2018b) and thus when fed it increases ruminal NH_3-N concentrations, thereby increasing microbial growth in the rumen (Satter and Slyter 1974; Brito and Broderick 2007) if NH_3-N was limiting. Once the N needs of the rumen microbes are met, the addition of more RDP will not yield additional microbial CP and the additional N (converted to urea in the liver) is wasted at an energy cost to the animal. Determining the rate and extent of degradation of diets containing varying inclusion levels of canola meal may assist in explaining why there were no differences in total DMI and ADG when weaner cattle were fed either 34% or 46% canola meal dietary treatments.

5.5 Conclusions

Dry matter intake and ADG were improved when 34% canola meal was offered compared with 20% canola meal; however, there was no further improvement at 46% canola meal dietary treatment. The decrease in DMI and ADG at the 68% inclusion level indicates this is not a viable inclusion level for weaner calves fed a low-quality

basal diet, indicating an upper inclusion level of canola meal has been reached when weaner calves were offed canola hay. Further investigation into apparent nutrient digestibility, degradation kinetic parameters and ruminal parameters would determine if there were differences between 34% and 46% canola meal. This would provide information on the ideal supplementation level of canola meal for cattle grazing low-quality forages and was the focus of the studies reported in Chapters 6 and 7.

Chapter 6: Increasing the Inclusion Level of Canola Meal in the Ration up to 47% has no Detrimental Effects on Apparent Nutrient Digestibility

6.1 Introduction

The ADG and DMI of weaner calves offered a basal diet of canola hay were improved when 34% canola meal was offered compared with 20% canola meal; however, there was no further improvement when 46% canola meal was offered and both DMI and ADG declined when 68% canola meal was offered. Therefore, investigation to determine if there was a difference in energy utilisation and apparent nutrient digestibility between 34 and 46% canola meal ration was warranted.

In ruminants, digestibility of a feedstuff is influenced by its chemical (carbohydrate, protein and fat contents) and physical (particle size) characterises that affect the ability of the microbial enzymes to colonise and digest feed particles (Rymer 2000; McDonald *et al.* 2011). *In vivo* apparent nutrient digestibility is the reference method to evaluate the nutritional values of feed/ feedstuff (Van Soest 1994; McDonald *et al.* 2011). This method provides information on total tract digestibility; however, *in vivo* apparent digestibility is not a measure of true digestibility as the faeces also contain nutrients of endogenous and/or microbial origin (McDonald *et al.* 2011). The *in vivo* method is the most accepted and reliable for the evaluation of feed digestibility although; it is laborious, time consuming, expensive and not suitable for routine and/or large scale feed evaluation (Rymer 2000; CSIRO 2007). In addition, this method has multiple sources of variation including animals selected (age, breed and sex), LoF offered, the use of supplements, and the preparation of feeds (Rymer 2000).

The ability of a feedstuff/s to sustain livestock growth and production depends on its digestibility (López 2005). Understanding how supplementation changes the digestibility of a diet, together with production responses is critical when evaluating the usefulness of a supplement such as canola meal when cattle are consuming low-quality roughages. Varying the inclusion level of canola meal in TMR for beef cattle has been shown to have variable effects on nutrient digestibility. Dry matter digestibility was higher when canola was included at 7.2% (Koenig *et al.* 2004), 10% or 20% (Nair *et al.* 2016) in TMR. Crude protein digestibility was not affected when canola meal was added at either 10% or 20% in TMR (Nair *et al.* 2016). In contrast, CPD was higher (79.2% vs 68.6%) when canola meal was added to the TMR compared

with the basal TMR (Zinn 1993). Neither NDFD nor ADFD were affected when barley grain was replaced with canola meal at either 10% or 20% inclusion level in a TMR for finishing steers (Nair *et al.* 2016) or when canola meal was included at 7.3% compared with the control TMR based on barley straw and grass hay (Van De Kerckhove *et al.* 2011). There are limited studies that have investigated varying inclusion levels of canola meal in grazing cattle.

The effects of supplementing high levels of canola meal (> 20%) on apparent nutrient digestibility of a low-quality basal diet has not been investigated. Therefore, the aim of this study was to determine whether apparent nutrient digestibility increased with increasing inclusion levels of canola meal when steers were fed a basal diet of low-quality roughage, typical of feed available during summer and autumn in Australia.

6.2 Methods and materials

The study was conducted at the NSW DPI Animal Nutrition Unit as previously described in Chapter 3.3.2. The feeding trial was approved by CSU ACEC and NSW DPI AEC (Approval number: A19059) as previously described in Chapter 3.1. The location, animal housing and animal data collection, sample collection, analytical procedures, calculations and statistical analysis are presented in Chapter 3, Sections 3.2, 3.3, 3.4, 3.6, 3.7 and 3.8 unless stated otherwise.

6.2.1 Experimental animals

Ten Angus steers (LW = 344 ± 6.42 kg) were sourced from the CSU commercial herd located on the campus farm at Wagga Wagga. The steers were approximately 10-12 months of age. When selecting the steers, care was taken to select those of similar BCS and within the same weight range. The steers were treated with an anthelmintic drench (Maximus® Boehringer Ingelheim, North Ryde, Sydney) prior to commencement of the study.

6.2.2 Experimental design

A partial crossover design was used and consisted of three experimental periods of 18 d. Each experimental period consisted of 11 d dietary adaptation period and a 7 d total collection period, during which time the steers were housed in individual metabolism pens. Each dietary treatment was offered to two steers per experimental period (Figure 6.1). Each steer received a different (pre-allocated) dietary treatment in each experimental period. Thus, there was a total of six replicates per dietary treatment.

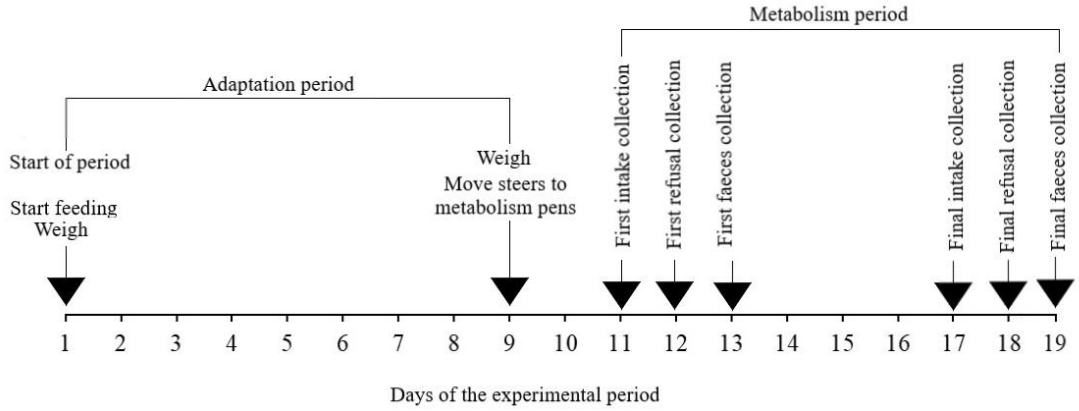


Figure 6.1 Schematic representation of a period during the experiment where major data collection was recorded.

6.2.3 Housing

Initially, the steers were group housed in an outdoor pen at the CSU Ruminant Research Complex for one week prior to being transported to the NSW DPI Animal Nutrition Unit. During this backgrounding period, the steers were offered 1 kg/hd/d of canola meal and *ad libitum* access to barley hay. At all times the steers had access to clean water. Following this period in the outdoor pens, the steers were weighed and randomly allocated on a stratified LW basis to individual pens inside the animal house facility.

Once housed indoors, the steers were maintained at varying times in either individual pens (2.4 m x 1.2 m) or metabolism pens (2.4 m x 1.2 m) fitted with trays for faeces collection. Upon completion of each total collection period (for experimental periods 1, 2 and 3) the animals were returned to their (same) individual pen. The steers were allowed to exercise in a group pen outside for 1 – 1.5 h/week during the adaptation phase of each period.

6.2.4 Experimental diets

The proportions of canola meal in the experimental diets were adapted from the previous feedlot study (Chapter 5), where canola meal was included at 20%, 34%, 46% and 68% of estimated DMI and the steers were offered *ad libitum* canola hay. As previously described in Chapter 5, DMI and ADG decreased when 68% canola meal was offered and thus, was not included in this study. Therefore, the steers were offered

diets based on one of five inclusion levels of canola meal; 0%, 15%, 26%, 36% or 47% of dietary DM. The roughage was a mix of barley hay and wheaten stubble to provide a M/D of approximately 8.35 MJ/kg DM. This was to simulate a typical quality forage available during the summer – autumn period in southern NSW. The nutritive value (% of DM) of the dietary ingredients is presented in Table 6.1.

The rations (Table 6.2) were offered at 1.2 times maintenance (CSIRO 2007), with half of the daily allocation being fed at 0800 and the remaining half fed at 1600. The amount of feed offered was 1.2 x ME_m to ensure each steer received at least maintenance LoF (Chapter 3.7.1). The amount of feed offered was adjusted based on each steer's LW when they were weighed at the beginning of each experimental period (Day 1) and before they were moved to the metabolism pens (Day 9 of each experimental period), as depicted in Figure 6.1. When changing diets between experimental periods, care was taken to ensure that for individual animals the level of canola meal in their (new) ration increased by a maximum of no more than two inclusion levels.

Table 6.1 Nutritive value (g/kg DM) of the dietary ingredients.

Component (g/kg DM)[1]	**Dietary ingredients[2]**		
	Canola meal[3]	**Barley hay**	**Wheaten stubble**
DM	922.3	912.7	917.0
OM	932.7	933.8	925.3
CP	414.1	99.2	45.0
aNDF	296.9	413.2	741.4
$aNDF_{OM}$	277.9	400.3	711.9
ADF	276.0	216.7	502.1
ADF_{OM}	271.2	198.8	443.6
EE	33.0	16.8	7.2

[1] DM = dry matter; OM = organic matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; $aNDF_{OM}$ = neutral detergent fibre assayed with heat stable amylase and exclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash, ADF_{OM} = acid detergent fibre exclusive of residual ash; EE = ether extract.
[2]Dietary ingredients were collected over each metabolism period and analysed in duplicate and the average results are presented.
[3]Canola meal was solvent extracted.

6.2.5 Sample collection

During the collection period of each experimental period, representative samples (approximately 200 g) of the dietary ingredients and any daily feed refusals were taken

for subsequent analysis of nutrient content, as previously described in Section 3.4.1. Each day during the metabolism period and prior to feeding, the amount of faeces voided was collected and the weight recorded. Representative sub samples (10%) of the faeces (of each animal) were taken daily and stored at -18°C for subsequent chemical analysis.

Table 6.2 The ration composition and nutrient composition of the dietary treatments based on the intake of steers fed increasing inclusion levels of canola meal.

	Inclusion level of canola meal (% of DM)[2]				
	0	**15**	**26**	**36**	**47**
Ration composition (% DM basis)					
Barley hay	50.73	43.17	37.46	32.24	26.90
Wheaten stubble	49.27	41.92	36.37	31.30	26.07
Canola meal	0.00	14.91	26.17	36.47	47.03
Nutrient composition (g/kg DM basis)[1]					
OM	933.4	931.5	931.6	931.9	933.1
aNDF	553.2	522.6	493.1	462.2	427.9
aNDFom	530.8	496.5	467.9	439.9	404.5
ADF	337.9	336.7	329.6	319.2	309.3
ADFom	301.5	304.0	300.4	293.3	288.4
CP	75.5	126.6	165.4	202.9	241.2
[3]M/D (MJ/kg DM)	9.25	9.47	9.66	9.75	10.03

[1] OM = organic matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; aNDFom = neutral detergent fibre assayed with heat stable amylase and exclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash, ADFom = acid detergent fibre exclusive of residual ash; M/D = MJ metabolisable energy/kg DM = 0.203 x DOMD - 3.001 (for roughage) or 0.138 x DOMD + 0.272 x EE + 0.858 (for canola meal, AFIA, 2014).
[1]Intake data presented in this table is from each metabolism period and the average over all periods is reported.

6.2.6 Sample preparation

Duplicate subsamples of approximately 70 g (known weight) of offered feed samples collected during the digestibility study for each experimental period were dried at 80ºC in a forced-air oven (Labec Oven; Laboratory Equipment Pty Ltd) for 24 h to determine the DM content (AFIA 2014). Feed ingredients were then ground, first through a 5 mm sieve followed by a 1 mm sieve. The ground samples were then placed in plastic storage containers and stored at room temperature pending chemical analysis, as previously described in Chapter 3.4.1.

The feed refusals (if any) collected from each steer during the digestibility study for each experimental period were similarly dried at 80ºC in a forced-air oven to determine their DM content. The dried feed refusals for each steer were then bulked together to create a composite sample. The composite samples were then ground through a 5 mm sieve followed by a 1 mm sieve and the ground samples retained for subsequent chemical analysis.

Faecal samples

The frozen subsampled faecal samples per animal, per period were thawed overnight at room temperature, subsampled (approximately 200 g, once thawed) in duplicate and then dried in a fan-forced oven at 80°C for 48 h (AFIA 2014) to determine their DM contents. The dried samples for each animal per period were then bulked together to create a composite sample. The composite samples were ground, first through a 5 mm sieve followed by a 1 mm sieve. Duplicate samples of the composite faecal sample (per animal per period) were then stored in plastic storage containers at room temperature pending chemical analyses.

6.2.7 Analytical procedures

All analytical procedures were undertaken at the FQS Laboratory of NSW DPI at Wagga Wagga as described in Chapter 3.6. Any differences of analytical methods are described below.

For the determination of aNDF and ADF content on an OM basis ($aNDF_{OM}$ and ADF_{OM}, respectively), the dried Ankom bag was folded into porcelain crucibles (previously dried and weighed) and then ignited in the muffle furnace at 200°C for 2 h, and then at 550°C for 6 h until all carbon was removed in order to determine the ash content, from which the OM content was calculated (AOAC 2004-5; M942.05). Assessing the aNDF and ADF content on both a DM and OM basis enabled fibre digestibility values to be reported as either aNDF digestibility (aNDFD) assayed with heat stable amylase inclusive of residual ash, or $aNDFD_{OM}$ assayed with heat stable amylase exclusive of residual ash ($aNDFD_{OM}$) and ADFD inclusive of residual ash or $ADFD_{OM}$ exclusive of residual ash.

6.2.8 Calculations

The apparent digestibility of a feed or individual nutrient is most accurately defined as the proportion, which is not excreted in faeces, and therefore assumed to be absorbed

by the animal. The amount of each constituent (DM, OM, CP, aNDF, $aNDF_{OM}$ ADF, and ADF_{OM}) was determined for the feed offered, feed refusals and faeces. Nutrient intakes (on a DM basis) were calculated by subtracting the amount of each constituent in the feed refusals from that in the offered feed.

The apparent digestibility of the nutrients (DMD, OMD, DOMD, CPD, aNDFD, $aNDFD_{OM}$ ADFD, and $ADFD_{OM}$) were then determined, using the following formulas (AFIA 2014):

DMD (%) = [(DMI – faecal DM output)/ DMI] x 100

OMD (%) = [(OM intake – faecal OM output)/ OM intake] x 100

DOMD (%) = [(OM intake – faecal OM output)/ DM intake] x 100

CPD (%) = [(CP intake – faecal CP output)/CP intake] x 100

aNDFD (%) = [(aNDF intake – faecal aNDF output)/aNDF intake] x 100

$aNDFD_{OM}$ (%) = [($aNDF_{OM}$ intake – faecal $aNDF_{OM}$ output)/$aNDF_{OM}$ intake] x 100

ADFD (%) = [(ADF intake – faecal ADF output)/ADF intake] x 100

$ADFD_{OM}$ (%) = [(ADF_{OM} intake – faecal ADF_{OM} output)/ADF_{OM} intake] x 100

The ME value (M/D; MJ/kg DM) of feed was calculated using the *in vivo* DOMD (as above) and as previously described in Chapter 3.7.2.

6.2.9 Statistical analysis

Prior to analysis, all data were assessed for assumptions of normality by histograms and plotting residuals. Two outliers were observed when plotting residuals that represented the same animal in Period 1 and Period 2 (offered 36% and 47% of canola meal, respectively) that had a large amount of dropped feed (> 1 kg DM/d). Data from this animal (for Period 1 and Period 2) were removed prior to analysis. All other feed refusals were < 0.5 kg DM/d for all other dietary treatments. Therefore, all dietary treatments had six measures of DMI and apparent nutrient digestibility except for dietary treatments which contained 36% and 47% of canola meal where only five measures of DMI and apparent nutrient digestibility were observed.

The analysis of estimated MEI, MEI:ME_m and apparent nutrient digestibility parameters were analysed using the Mixed Model procedure in the SAS statistical program (SAS Institute Inc. 1997). The most appropriate covariance structure used for each analysis was determined by the Schwarz's BIC (Wang and Goonewardene 2004). The analysis included the fixed effect of "dietary treatment" with "animal" and "period" and the interaction between "animal" and "period" as random effects. The final model included the fixed effect of "dietary treatment" with "animal" and the interaction of "animal" and "period" as random effects. For DMD, OMD and DOMD a linear equation was used to fit the best model; however, for CPD and fibre degradation parameters a polynomial equation was used to fit the best model. Least square means and differences of least square means were reported. An alpha of 0.05 was used for all statistical tests. Dry matter intake was not analysed statistically, as the DM offered to each group was deliberately altered to provide an equivalent intake on an MEI:ME_m basis.

The relationships between inclusion level of canola meal and DMD, OMD, DOMD and CPD were assessed using the PROC REG procedure in SAS. An alpha of 0.05 was used for all statistical tests.

6.3 Results

6.3.1 Feed intake and diet composition

The ration composition and nutrient composition of the dietary treatments (based on average actual intake data) are presented in Table 6.3. As the proportion of canola meal increased, the proportion of roughage in the diets decreased.

The average DMI and MEI of the experimental diets during the metabolism periods (excludes the adaptation period) are presented in Table 6.3. Average MEI (MJ/d) did not differ between dietary treatments; however, the MEI:ME_m was significantly lower ($P < 0.05$) when steers were offered the control diet (1.04:1) compared with all other dietary treatments (> 1.09:1). The steers offered the control diet had the most refusals (> 10 g/d) which mainly consisted of wheaten stubble.

Table 6.3 Average (± SEM) dry matter intakes (DMI) and metabolisable energy intakes (MEI) when steers were fed increasing inclusion levels of canola meal.

Feed intake[2]	**Inclusion level of canola meal (% of DM)[1]**					***P* - value**
	0	**15**	**26**	**36**	**47**	
Intake (kg/hd/d DM)						
DMI (kg/d)[1]	5.73	5.63	5.31	5.02	4.74	-
	(0.15)	(0.09)	(0.18)	(0.16)	(0.14)	
Relative DMI	16.84	16.68	16.11	15.31	14.55	-
(g/kg/LW/d)	(0.25)	(0.14)	(0.23)	(0.25)	(0.18)	
Estimated energy intake (MJ kg/hd/d)						
MEI	48.29	49.90	48.92	47.85	46.74	0.572
	(1.31)	(1.31)	(1.31)	(1.43)	(±1.43)	
MEI:ME_m[2]	1.04	1.09	1.10	1.09	1.09	0.044
	(0.01)[a]	(0.01)[b]	(0.01)[b]	(0.02)[b]	(0.02)[b]	

[1]MEI:ME_m; amount of metabolisable energy offered (DM intake x estimated M/D of the feed MJ/d) divided by the maintenance requirement of the animal (the level of feeding).
[2]Dry matter intake during the digestibility study (excludes adaption period).

6.3.2 Apparent nutrient digestibility

Increasing the inclusion level of canola meal had varied effects on apparent nutrient digestibility (Table 6.4). There was a positive linear relationship ($R^2 = 0.91$, $P = 0.011$) between DMD and the inclusion level of canola meal in the ration (Figure 6.2), although DMD did not vary between control and 15% canola meal. The inclusion of 36% canola meal increased DMD compared with the control ($P < 0.05$) but did not differ from either 15% or 26% canola meal ($P > 0.05$). The DMD was the highest when steers were offered 47% canola meal in the ration.

Organic matter digestibility and DOMD ($R^2 = 0.92$, $P = 0.009$ and $R^2 = 0.91$, $P = 0.012$, respectively) was positively correlated with the level of canola meal in the ration (Figure 6.2). As shown in Table 6.4, OMD and DOMD did not vary ($P < 0.05$) between 0%, 15% and 26% inclusion levels of canola meal. Organic matter digestibility and DOMD was higher ($P < 0.05$) when the ration contained 47% canola meal compared with all other inclusion levels; however, not different ($P > 0.05$) to the ration containing 36% canola meal

Crude protein digestibility (CPD) significantly increased ($P < 0.001$) as the inclusion level of canola meal in the ration increased. The polynomial relationship of CPD ($R^2 = 0.996$, $P = 0.007$) was significantly related to the ration containing increasing levels of canola meal (Figure 6.3). The aNDFD, $aNDFD_{OM,}$ ADFD and $ADFD_{OM}$ were not significantly affected by dietary treatment (Table 6.4).

6.4 Discussion

Low-quality forages and roughages are those with a digestibility of less than 55% and are deficient in true protein (less than 80 g CP/kg DM) (Leng 1990). Therefore, the control ration (75.5 g/kg DM of CP, with a DMD of 562.8 g/kg) fed to the steers would be classified as low-quality and likely had insufficient RDP to meet the N requirements for optimal microbial activity in the rumen (Leng and Nolan 1984; Lee *et al.* 1985). Investigation of ruminal NH_3-N concentrations, VFA concentrations and proportions and ruminal pH of steers fed these varying inclusion levels of canola meal was one of the objectives of the study reported in Chapter 7.

Increasing the inclusion level of canola meal had varied effects on apparent nutrient digestibility. Dry matter digestibility, OMD and DOMD had a positive linear relationship ($R^2 > 0.900$) with increasing inclusion levels of canola meal; although, there was not always a significant difference within these parameters with each incremental increase in the inclusion level of canola meal. The increase in DMD, OMD and DOMD could have also been due to the differences in the proportions of the dietary ingredients (roughages and canola meal) in the ration offered to the steers. The DMD of the individual ingredients within the ration could have also accounted for the differences. Increasing the inclusion level of canola meal increased the CP content of the diet (Table 6.3) and consequently would have also increased ruminal NH_3-N concentrations, as solvent-extracted canola meal contains both RDP and RUP (Heim and Krebs 2018c). The increase in DMD in response to increased dietary CP was expected as NH_3-N is normally the limiting nutrient for ruminal microbial fermentation when ruminants are fed low-quality roughages (Lee *et al.* 1987; DelCurto *et al.* 1990). Estimates of the minimum ruminal NH_3-N concentration required for maximum rates of DM digestion (and microbial synthesis) have varied from 50-80 mg NH_3-N/L (Satter and Slyter 1974; Morrison *et al.* 1988) to 150-200 mg NH_3-N/L (Krebs and Leng 1984; Boniface *et al.* 1986). The effect of feeding increasing levels of canola meal on ruminal NH_3-N concentrations (and other rumen parameters) was investigated in the research reported in Chapter 7.

There are no comparative studies investigating the effects of feeding high levels (> 40% DM) of canola meal supplementation on DMD; however, the effects of high levels of supplementation of other protein sources have been investigated. Dry matter digestibility increased linearly ($P < 0.05$) in response to increasing levels (12 %, 20%, 30% or 39% DM) of soybean meal offered to cattle with *ad libitum* wheat straw (Beaty *et al.* 1994). Similarly, DMD and OMD was higher when increasing amounts (0 g, 200 g, 400 g or 600 g) of Mulberry leaf pellets (high in CP) were fed to cattle and offered rice straw (Huyen *et al.* 2012). Therefore, the linear increase in DMD by increasing levels of canola meal is generally consistent with other reports using other protein sources; however, the increase in DMD could also be due to DMD of the dietary ingredients.

Studies that had included canola meal in PMR and feedlot rations have reported varying effects on DMD, likely due to differences in diet composition and quality. Dry matter digestibility was not affected when 4.8% canola meal was offered to steers offered *ad libitum* quack grass compared with the basal diet (Seoane *et al.* 1992). In addition, DMD was not affected when canola meal was supplemented at 0.6 kg/d with *ad libitum* silage compared with silage only (Veira *et al.* 1995). These inclusion levels of canola meal were much lower than used in the current study and may account for the differences in results. Furthermore, either ruminal NH_3-N concentrations and/or the DMD of the dietary ingredients in the ration may have accounted for the differences in DMD in the current study.

Dry matter digestibility decreased when 10% of canola meal (derived from *B. napus* but not that derived from *B. juncea*) was included in finishing feedlot TMR; however, no difference when 20% canola meal derived from either *B. napus* or *B. juncea* was included in a TMR (Nair *et al.* 2016). Similarly, DMD was not affected when steers were offered 0% or 7.3% canola meal in TMR (Van De Kerckhove *et al.* 2011). Feedlot TMR are normally balanced for energy and protein which would explain why, at lower inclusion levels, there were no effects on DMD as ruminal NH_3-N concentrations would not be limiting fibre digestion as was the likely case in the control diet used in the current study.

Table 6.4 Least square means (± SEM) of apparent nutrient digestibility when steers were fed increasing inclusion levels of canola meal.

	Inclusion level of canola meal (% of DM)					*P*-values
Parameters (g/kg DM)[1]	**0**	**15**	**26**	**36**	**47**	**Diet**
DMD	562.8 (±1.46)^{a}	593.0 (±1.43)^{ab}	601.8 (±1.55)^{b}	606.6 (±1.70)^{bc}	647.5 (±1.55)^{d}	0.002
OMD	629.3 (±1.33)^{a}	658.2 (±1.31)^{ab}	664.2 (±1.42)^{ab}	669.3 (±1.55)^{bc}	699.3 (±1.41)^{c}	0.006
DOMD	588.2 (±1.24)^{a}	614.5 (±1.23)^{ab}	616.0 (±1.32) ^{ab}	625.0 (±1.45)^{bc}	653.3 (±1.32)^{c}	0.006
CPD	459.7 (±1.23)^{a}	625.2 (±1.22)^{b}	701.4 (±1.31)^{c}	737.8 (±1.44)^{d}	782.3 (±1.31)^{e}	<0.001
aNDFD	456.9 (±2.15)	488.4 (±2.04)	477.6 (±2.26)	455.0 (±2.42)	482.2 (±2.29)	0.663
$aNDFD_{OM}$	518.2 (±2.09)	538.7 (±1.97)	521.1 (±2.19)	509.2 (±2.35)	521.1 (±2.22)	0.862
ADFD	321.0 (±2.15)	379.0 (±2.04)	365.4 (±2.43)	375.9 (±2.43)	394.7 (±2.29)	0.111
$ADFD_{OM}$	417.7 (±1.58)	462.7 (±1.50)	445.7 (±1.67)	455.5 (±1.79)	468.0 (±1.69)	0.128

Different superscripts within rows for each parameter indicates that there is a significant difference ($P < 0.05$) between diets containing varying inclusion levels of canola meal.
[1]DMD = dry matter digestibility; OMD = organic matter digestibility; DOMD = digestible organic matter in the dry matter, CPD = crude protein digestibility; aNDFD = neutral detergent fibre digestibility assayed with heat stable amylase and inclusive of residual ash; $aNDFD_{OM}$ = neutral detergent fibre digestibility assayed with heat stable amylase and exclusive of residual ash; ADFD = acid detergent fibre digestibility inclusive of residual ash, $ADFD_{OM}$ = acid detergent fibre digestibility exclusive of residual ash.

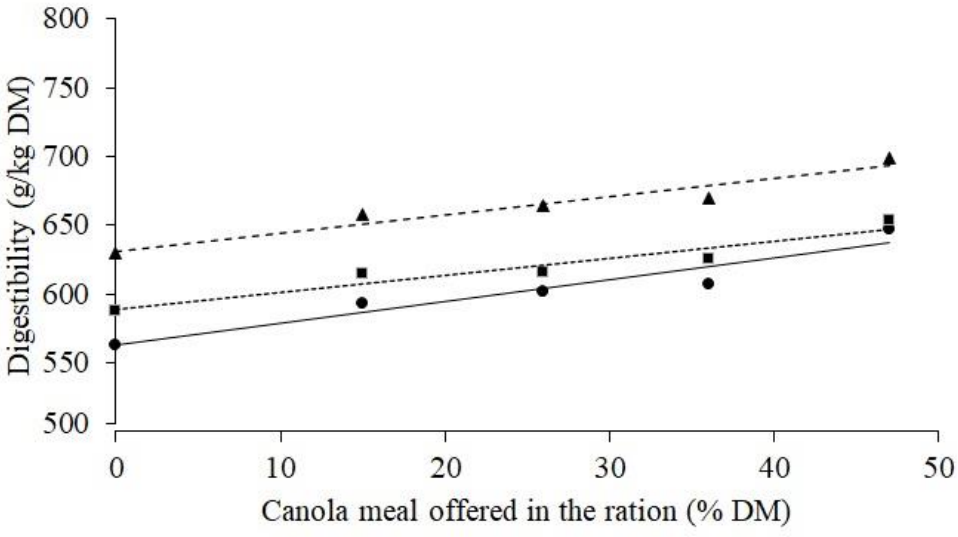


Figure 6.2 Change in dry matter digestibility (●, DMD = 0.15 x inclusion level of canola meal + 56.30 (R^2 = 0.91, *P* = 0.011)), organic matter digestibility (■, OMD = 0.13 x inclusion level of canola meal + 63.07 (R^2 = 0.92, *P* = 0.009)) and digestible organic matter in the dry matter (▲, DOMD = 0.12 x inclusion level of canola meal + 58.91 (R^2 = 0.91, *P* = 0.012)) when steers were fed increasing inclusion levels of canola meal.

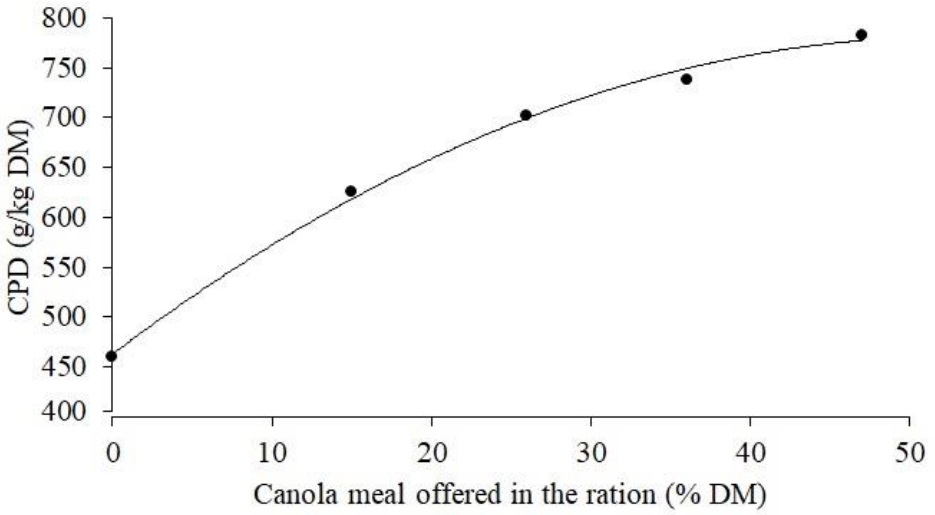


Figure 6.3 Crude protein digestibility (CPD = -0.01 x inclusion level of canola meal^2 + 1.16 x inclusion level of canola meal + 46.30 (R^2 = 0.996, P = 0.007)) when steers were fed increasing inclusion levels of canola meal.

Responses in OMD to protein supplementation have been variable. Organic matter digestibility was significantly higher for high-protein diets compared to low-protein diets (Van Horn *et al*. (1979), which is similar to the current study where there was a positive linear relationship between OMD and inclusion level of canola meal. Similarly, OMD was higher when increasing amounts (0 g, 200 g, 400 g or 600 g) of Mulberry leaf pellet were fed to cattle offered rice straw Huyen *et al.* (2012). In contrast, OMD was not altered by supplemental protein in steers consuming low-quality forage (Cappellozza *et al.* 2021).

Differences in dietary energy can also impact on apparent nutrient digestibility (Li *et al.* 2014; Zhou *et al.* 2015); however, this was not a contributing factor in the current study as MEI did not vary between the treatment groups (Table 6.2). The control diet, however, had a lower MEI:ME_m ratio (lower LoF). Lower levels of feeding are known to result in higher apparent nutrient digestibility (CSIRO 2007), which might have accounted for the higher than expected DOMD in the current study.

Diets with elevated CP content usually have greater apparent CPD (Olmos Colmenero and Broderick 2006). The relationship between dietary CP concentration and apparent CPD has previously been described as curvilinear due to the constant metabolic and endogenous N output per kg DMI (Van Soest 1994). In the current study, CPD had a positive polynomial relationship ($R^2 = 0.996$) with the inclusion level of canola meal. As discussed previously, there are no comparative studies involving high levels of canola meal supplementation on apparent CPD; however, increasing amounts (0 g, 200 g, 400 g or 600 g) of Mulberry leaf pellet linearly increased CPD in cattle fed rice straw (Huyen *et al.* 2012).

High proportions of fibre fractions in the cell wall of the plant (typical of low-quality roughages) can negatively impact on digestibility (Van Soest 1965). The addition of protein supplements to low-quality diets can improve fibre digestibility as ruminal microbes can metabolise higher proportions of fibre in the diet (DelCurto *et al.* 1990), although results have been variable. Neither $aNDFD_{OM}$ nor $ADFD_{OM}$ were affected by increasing inclusion levels of canola meal in this study which was expected due to similar ADF_{OM} content in the rations (control 301.5 g/kg DM compared with 47% inclusion 288.4 g/kg DM); however, the $aNDF_{OM}$ content of the rations was 530.8 g/kg DM for the control and 404.5 g/kg DM for the 47% inclusion level. Neutral detergent fibre digestibility was not different in response to increasing supplementation of soybean meal to steers fed low-quality roughage forage (grass seed straw; Chewings

fescue) (Cappellozza *et al.* 2021). Similar lack of response in fibre digestion was also found in feedlot TMR within varying inclusion levels of canola meal (Koenig *et al.* 2004; Van De Kerckhove *et al.* 2011; Nair *et al.* 2016). In contrast, NDFD and ADFD increased linearly when steers were supplemented at 4.8% canola meal and fed *ad libitum* quack grass (*Agropyron repens*) Seoane *et al.* (1992). Similarly, aNDFD and ADFD increased linearly with increased inclusion levels of soybean meal (Beaty *et al.* 1994) and Mulberry leaf pellet (Huyen *et al.* 2012) in low-quality diets offered to cattle.

The polynomial relationship of CPD plateaued out at the maximum LoF; therefore, it could be concluded that supplementing canola meal higher than 47% when animals are fed low-quality roughages is not beneficial. However, possible economic benefits and the fate of excess dietary N on the environment (Hristov *et al.* 2011) when supplementing high levels of canola meal need to be considered.

6.5 Conclusion

Supplementing cattle fed low-quality roughage with up to 47% canola meal had no detrimental effects on apparent nutrient digestibility. The addition of canola meal improved nutrient digestibility of low-quality roughages and consequently has the potential to improve animal productivity. Therefore, the addition of canola meal at any of the inclusion levels used in this study has the potential for steers to effectively utilise roughages/forages typical of the summer autumn period in southern NSW and remain PCAS certified.

Chapter 7: Supplementing Ruminal Fluid Donor Steers up to 43% Canola Meal had no Negative Effects on Either Ruminal Parameters or the Degradation Characteristics of Lucerne Hay

7.1 Introduction

The ADG and DMI of weaner calves fed a basal diet of canola hay increased with level of canola meal supplementation up to 34% of dietary DMI, with no further improvement at the 46% inclusion level of canola meal. The ADG and DMI of weaners calves decreased when canola meal was supplemented at 68% of dietary DMI (Chapter 5). Further investigation to evaluate the possible decline in ADG and DMI with increasing inclusion levels of canola meal was reported in Chapter 6, where *in vivo* apparent DOMD and CPD increased when the ration contained increasing inclusion levels of canola meal; however, fibre digestibility (aNDFD and ADFD) was not affected by the level of inclusion of canola meal. Ruminal parameters; however, were not assessed in the studies reported in Chapters 5 and 6.

The rumen environment and the rate and extent of microbial fermentation are affected by the composition of the rations fed to ruminants. The total VFA concentration and molar proportions of individual VFA are mainly determined by the composition of the microbial population (France and Dijkstra 2005). The total concentration of VFA in the rumen is normally between 60 and 150 mmol/L (Bergman 1990); however, this varies depending on intake level, time of feeding and dietary composition, particularly the nature of carbohydrates and their degradation rate (Bergman 1990; France and Dijkstra 2005). Ruminal NH_3-N concentrations and total VFA concentrations can be increased with the addition of a protein supplement such as canola meal to dairy (Auldist *et al.* 2014b) and feedlot rations (Nair *et al.* 2016). There is; however, a lack of information on the impacts of varying inclusion levels of canola meal on these ruminal parameters when cattle are fed low-quality roughage diets.

The degradation of feeds in the rumen can be determined by a range of methodologies, which all have advantages and limitations. The *in sacco* technique was originally applied to study the degradation of forages in the rumen (Ørskov *et al.* 1980). Although it can be laborious and time consuming, this method has been widely used and accepted because it closely relates to the rumen environment in which degradation of different feedstuffs can occur (Ørskov *et al.* 1980; López 2005; Chaudhry and Mohamed 2011). The *in vitro* Daisy™ incubation is a simpler procedure than traditional *in vitro* digestibility flask methods (Brons and Plaizier 2005). *In vitro*

Daisy™ incubations allows for routine determination of the digestibility of large numbers of samples in batches, and it is relatively quick, easy to perform and resolves some analytical errors such as manual filtration steps (Weiss 1994). Ruminal liquor, with a low NH_3-N concentration can restrict microbial growth and protein synthesis (Slyter *et al.* 1979) as well as reduce the rate of digestion of fibre (Lee *et al*. 1985). Ruminal liquor maybe N limited when the donor animals are fed a low-quality ration, such as the control ration utilised in the study reported in Chapter 6. Therefore, supplemental N can be included for *in vitro* Daisy™ incubations to overcome any limitations in N supply from the ruminal liquor used.

Interpreting *in sacco* and *in vitro* Daisy™ results must be done with care (Ørskov *et al.* 1980; Chaudhry and Mohamed 2011) as there are many factors that can influence the rate of degradation and degradation kinetics such as animals (Nandra *et al.* 2000), ration fed to the animals (Ganev *et al.* 1979), sample grind size (Damiran *et al.* 2008), bag type (Valente *et al.* 2011), bag pore size (Lindberg and Knutsson 1981) and size of sample incubated (Nocek 1985). Therefore, to determine if the ration fed to animal affects degradation parameters, it is common to include a standard feedstuff. Lucerne hay has been widely examined in both *in sacco* (Julier *et al.* 2001; Danicke 2002) and *in vitro* (Wilman and Adesogan 2000; Valentine *et al.* 2019) methodologies.

The aims of the study presented in this Chapter were to determine:

1. whether ruminal NH_3-N concentrations and VFA concentrations and molar proportions increased, while ruminal pH remained unchanged in response to feeding a low-quality roughage ration containing increasing inclusion levels of canola meal;
2. whether *in sacco* digestion kinetic parameters and the degradation of lucerne hay increased in steers fed a low-quality roughage ration containing increasing inclusion levels of canola meal; and
3. whether the ruminal digestion kinetic parameters and the degradation of lucerne hay were improved with the addition of supplemental N to the *in vitro* Daisy™ incubation when the donor steers were fed a low-quality roughage ration containing increasing inclusion levels of canola meal.

7.2 Methods and materials

The *in sacco* and *in vitro* Daisy™ studies were conducted at the NSW DPI Animal Nutrition Unit, allowing dual use of the steers. The use of the steers was approved by both the CSU ACEC (Protocol number: A19220) and the NSW DPI AEC (Protocol number: ORA 19/22/009) as previously described in Chapter 3.1. Further details of the use and care of the fistulated steers are described in Chapter 3.2 and 3.3.2. The location, animal housing and animal data collection, sample collection, analytical procedures, calculations and statistical analysis, are presented in Chapter 3, Sections 3.4, 3.5, 3.6, 3.7 and 3.8 unless stated otherwise.

7.2.1 Experimental design

Details of the animal housing have been previously described in Chapter 3.3.2. A 4 x 4 Latin square design was used and consisted of four 15 d periods, each consisting of 10 d dietary adaptation, 24 h for ruminal fluid collection and 72 h *in sacco* and *in vitro* Daisy™ incubation period (Figure 7.1). Each steer received a different (pre-allocated) ration in each experimental period. Care was taken to make sure the inclusion level of canola meal did not increase more than two inclusion rates between periods.

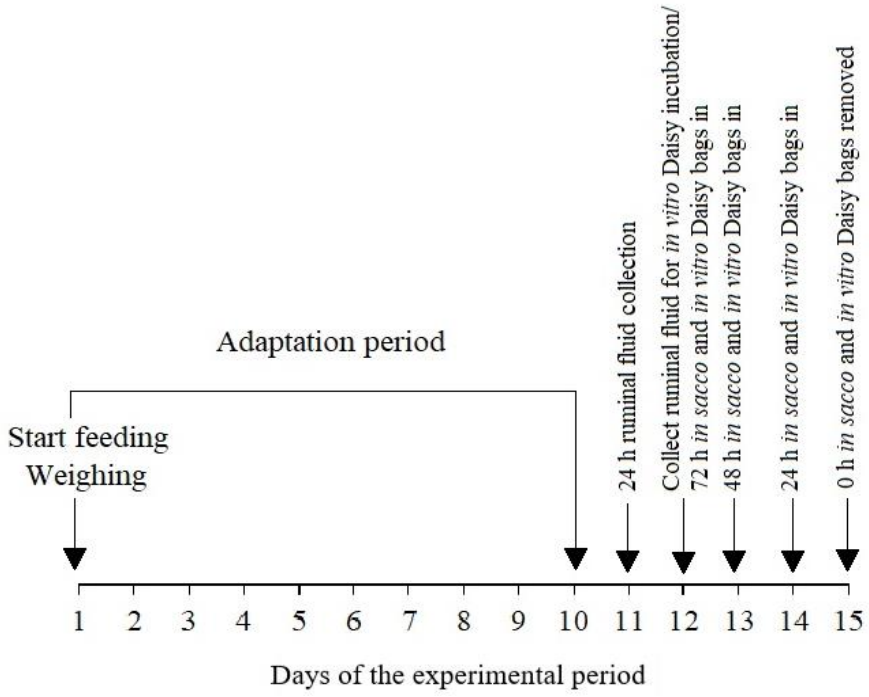


Figure 7.1 Schematic of a period during the *in sacco* and *in vitro* Daisy™ experiments where main data collection was obtained.

7.2.2 Dietary treatments

The steers were offered rations based on cereal roughages with one of four inclusion levels of canola meal, viz. 0% (control), 13%, 27% or 43% on a DM basis. The canola meal inclusion levels are slightly lower than previously reported in Chapter 6; however, the basal roughage composition was the same as previously described in Chapter 6. The nutritive value (g/kg DM) of the ration ingredients, and the composition (% DM) and nutritive value of the rations are presented in Tables 7.1 and 7.2, respectively. The ration ingredients were collected over each experimental period and analysed in duplicate, and the average results are presented. The ration was offered at 1.2 times maintenance (CSIRO 2007), with half of the daily allocation being fed at 0800 and the remaining half fed at 1600. The amount of feed offered was adjusted based on each steer's LW at the beginning of each experimental period (Day 1). The average DMI for each inclusion LoF (0% (control), 13%, 27% and 43% canola meal) was 9.48, 9.36, 9.42 and 9.48 kg/hd/d, respectively.

Table 7.1 Nutritive value (g/kg DM) of the dietary ingredients.

Component (g/kg DM)[1]	**Dietary ingredients[2]**		
	Canola meal[3]	**Barley hay**	**Wheaten stubble**
DM	919.9	914.5	919.2
OM	925.0	925.0	934.9
CP	414.4	83.8	28.8
aNDF	310.1	445.9	789.2
ADF	226.2	230.1	497.9
DMD	793.3	780.0	390.0
DOMD	783.4	730.0	380.0
EE	33.0	16.8	0.72
M/D (MJ/kg DM)[4]	12.57	11.82	4.71

[1]DM = dry matter; OM = organic matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash; DMD = dry matter digestibility; DOMD = digestible organic matter in the dry matter; EE= ether extract; M/D = MJ metabolisable energy/kg DM = 0.203 x DOMD - 3.001 (for roughage) or 0.138 x DOMD + 0.272 x EE + 0.858 (for canola meal, AFIA, 2014).
[2]Dietary ingredients were collected over each experimental period and analysed in duplicate and the average results are presented.
[3]Canola meal was solvent extracted.

7.2.3 Collection and assessment of ruminal fluid

To minimise sampling variation, only one person collected ruminal fluid throughout the experiments. To determine temporal changes in ruminal pH, NH_3-N concentrations and VFA concentrations and molar proportions, approximately 0.5 L of ruminal fluid was collected at 3 h intervals over a 24 h period (on Day 11 of each experimental period) from each steer by removing the plug of the cannulae and manually collecting the fibrous mat/fluid from the dorsal ventral rumen sac and placing into labelled individual, warmed plastic containers.

Table 7.2 The composition and nutritive value of the experimental rations and the lucerne standard.

	Inclusion level of canola meal (%)[1]				**Lucerne hay**
	0	**13**	**27**	**43**	
Diet composition (% DM)					
Barley hay	50.74	44.27	37.07	29.05	-
Wheaten stubble	49.26	43.00	36.00	28.25	-
Canola meal	-	12.72	26.93	42.70	-
Lucerne hay	-	-	-	-	100
Nutritive value[2] (g/kg DM)					
OM	929.9	929.0	928.5	927.8	900.0
CP	91.3	147.9	176.8	228.6	215.0
aNDF	614.3	563.2	530.1	482.1	412.5
ADF	361.5	338.7	324.2	302.8	230.0
DMD	587.9	614.0	643.2	675.5	678.5
DOMD	557.6	586.3	618.4	653.9	643.0
EE	12.07	14.73	17.71	21.01	15.80
M/D (MJ/kg DM)[3]	8.32	9.85	10.29	10.78	10.77

[1]OM = organic matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash; DMD = dry matter digestibility; DOMD = digestible organic matter in the dry matter; EE= ether extract; M/D = MJ metabolisable energy/kg DM = 0.203 x DOMD - 3.001 (for roughage) or 0.138 x DOMD + 0.272 x EE + 0.858 (for canola meal, AFIA, 2014).
[2]Based on intake from Day 11 to Day 15 of each experimental period.

Ruminal liquor was also collected for the assessment of ruminal characteristics and for the *in vitro* Daisy™ incubation of samples. Approximately 2 L of ruminal fluid was collected from each steer (as described above) and placed into warm plastic beakers, before being placed into an incubator set at 39 ± 0.5°C, before further preparation.

For both collections, the ruminal fluid was passed through coarse and then fine muslin cloth and the pH of the strained material was measured using an electronic pH meter (Activon model 210; Probe TPS serial number 1204 – 097217). The pH meter was calibrated prior to each sampling using buffer solutions of pH 4 and 7. To determine the VFA concentrations and molar proportions, a 10 mL sample of strained ruminal fluid was placed into a 70 mL plastic, yellow top container and immediately frozen at -18°C. To determine NH_3-N concentration, 10 mL of strained ruminal fluid was placed into a 70 mL plastic, yellow top container containing 0.5 mL of 0.1 M hydrochloric acid (HCl) to ensure pH was below 3.0. Samples were inverted thoroughly before being stored frozen at -18°C.

7.2.4 Estimation of degradation loss and kinetic parameters

The methodologies for *in sacco* degradability, and *in vitro* Daisy™ incubations including the bag preparation, incubation, estimation of degradation loss and kinetic parameters analysis and calculations are described in Chapter 3.5. In this thesis potential degradation refers to PD after 72 h incubation as previously described in Chapter 3, Section 3.5.4.

7.2.5 Preparation of samples

To prepare the lucerne standard, a 20 kg bale of lucerne hay was mixed thoroughly, before a subsample of approximately 4 kg was collected. The lucerne standard was processed by first grinding through a 5 mm sieve and then a 1 mm sieve.

7.2.6 Analytical procedures

All analytical procedures were undertaken at the NSW DPI's FQS laboratory as described in Chapter 3.6. For the determination of *in sacco* degradability of DM, all six filter bags, per period and per time point were used. One filter bag from each dacron bag (n=2 per period and per time point) was used for the analysis of either CP (n=1) or aNDF and ADF (n=1, analysed sequentially). For *in vitro* Daisy™ degradability

(of the bags incubated per ration, per period and per time point), two bags were used to determine DM loss, one was used to determine CP loss and one was used for the analysis of aNDF and ADF (analysed sequentially). Organic matter degradability was unable to be determined for either the *in sacco* or *in vitro* Daisy™ methodologies. Dry matter digestibility for the lucerne standard was determined using the pepsin cellulase methodology as previously described in Section 3.6.6.

Volatile fatty acids concentrations and molar proportions

After thawing at room temperature, the (non-acidified) ruminal fluid samples (approximately 10 mL) were centrifuged at 1500 x g for 10 min. A 100 µL aliquot of supernatant was transferred to a 1.5 mL plastic Eppendorf tube containing 900 µL of dilute acid (1% formic- 1% ortho-phosphoric acid mixture containing 184 ppm 4-methylvaleric internal standard). The Eppendorf tubes were then centrifuged for 5 min at 1500 x g and 800 µL of supernatant was transferred to a glass gas chromatography (GC) vial for analysis of VFA concentrations and proportions.

Volatile fatty acid concentrations were determined using an Agilent 6890 N GC with an autosampler and autoinjector. The method used a wide bore capillary column (SGE BP21 column; 12 m x 0.53 mm internal diameter (ID) and 0.5 µm film thickness, SGE International, Ringwood, VIC, Australia, P/N 054474) with retention gap kit (including a 2 x 0.53 mm ID guard column, P/N SGE RGK2).

For GC analysis, the carrier gas was helium with a total flow rate of 48.0 mL/min, a split ratio of 5:1 and a column flow of 7.84 mL/min. The inlet temperature was 155°C, inlet pressure was 19 kPa and injection volume was 1 µL. The oven temperature was set at 80°C for 2 min, increasing 6ºC/min to 122°C, 12°C/min to 144ºC, then increasing 40°C/min up to 220°C, which was then maintained to give a total run time of approximately 20 min. The flame ionisation detector temperature was set at 200°C, with the following gas flow rates: H at 40 mL/min, instrument air at 500 mL/min and N make-up gas at 30 mL/min (Packer *et al.* 2011).

Sample VFA peaks were identified by comparing their retention times with those of a standard mixture of genuine VFA (Supelco and Sigma Aldrich) and quantified using Agilent OpenLab (ChemStations C01.03) and Microsoft Excel using a 4-point standard curve with 4-methlyvaleric acid as the internal standard. All results were calculated as ppm and converted to mmol/L or molar % for subsequent analyses. The ratios of acetic acid to propionic acid (Ac:Pr) and the ratio of propionic acid to acetic acid plus two times butyric acid (Pr:Ac + 2 x Bu) were calculated from molar

proportions. These ratios were included in the experimental analysis as they help explain the energy partitioning of ATP between the individual VFA, e.g., 1 mole of butyric acid is formed from 1 mole of hexose while two moles of propionic or acetic acids are formed from 1 mole of hexose (Dijkstra *et al.* 2012).

Ammonia-N concentrations

After thawing at room temperature, the (acidified) ruminal fluid samples (10 mL) were centrifuged at 1500 x g for 10 min. A 1 mL aliquot of the supernatant was transferred in to a 10 mL round bottomed centrifuge tube containing 4 mL of 0.1 M HCL. The concentration of NH_3-N was determined by Flow Injection Analyser Spectroscopy. The analysis relies on the reaction of NH_3-N with salicylate in the presence of free chlorine to form an emerald green colour (Nelson 1983). The procedure was conducted according to the analysis of NH_4 in soil using sodium salicylate and nitro prusside (Prokopy 1992) as revised (Egan 2015) with the following modification. The carrier was 0.1 M HCl instead of 2 M KCl, as there was no requirement to displace bound NH_4^+, as is required for soil analysis, and available NH_3-N is captured as stable NH_4^+ prior to analysis. Known standards were used to create a standard curve and analysed before each batch.

The NH_3-N standards were made from ammonium chloride (NH_4Cl) dissolved in 0.1 Mol HCl. A stock standard solution of NH_4Cl (3818.059 mg NH_4-Cl/L, which is the equivalent of 1000 mg N/L) was prepared. Eight working standards (0.2, 0.5, 2.0, 4.0, 8.0, 20.0, 40.0 and 60.0 mg/L) were prepared from the stock standard solution.

A nine-point standard curve was fitted from the eight working standards and a blank solution containing 1M HCl using a polynomial regression equation. The peak area of unknown samples was used to calculate the concentration of NH_3-N in mg/L, with correction for the dilution factor. Any samples with a concentration above 40 mg/L NH_3-N were further diluted (1 in 25) and re-analysed.

The intra and inter-assay co-efficient of variation for the analysis was 0.60% and 1.31%, respectively, with a standard spike recovery of 100.2 ± 0.17%. The assay was also validated against the Kjeldahl NH_3-N method (Bremner 1965), recovery = 91.2 ± 2.02%.

7.2.7 Statistical analyses

Prior to analysis, data was assessed for assumptions of normality by histograms and plotting residuals. Ruminal NH_3-N data were moderately positively skewed; therefore, the data was transformed using the square root function (Tabacknick and Fidell 2001). For the analysis of ruminal parameters including pH, NH_3-N concentrations and VFA concentrations and molar proportions, the model included the fixed effects of "ration" and "sampling hour" as well as the interaction between the fixed effects, with "animal", "period" and "batch" examined as random effects. The final model included the fixed effect of "ration" and "sampling hour" and random effects of "animal" and "period".

The ruminal liquor characteristics were assessed for assumptions and residuals. The ruminal liquor characteristics analysis examined the fixed effects of "ration" with "animal by period" examined as random effects. Least square means and differences of least square means were reported.

The degradation loss and degradation kinetic parameters for the *in sacco* analysis examined the fixed effects of "ration" and "time" and the interaction between "ration" and "time" with "animal by period" examined as random effects. Least square means and differences of least square means were reported.

The degradation loss and degradation kinetic parameters for the *in vitro* Daisy™ incubation analysis examined the fixed effects of "ration/dietary treatment" and "time" and the interaction between "ration/dietary treatment" and "time" with "animal", "period", "machine" and "jar" examined as random effects. The final model included the fixed effects of "ration/dietary treatment" and "time" and the interaction between "ration/dietary treatment" and "time" with "animal by period by machine" examined as random effects. Least square means and differences of least square means were reported. An alpha of 0.05 was used for all statistical tests.

7.3 Results

7.3.1 Ruminal pH

Average ruminal pH was not affected ($P > 0.05$) by the ration fed to the steers. The interaction of the ration fed to the steers and time after initial feeding was also not significant (Table 7.3). Ruminal pH was lower at 3 h after feeding compared with pre-

feeding and remained low until 12 h after initial feeding (Figure 7.2). For all rations, ruminal pH did not fall below 6.2 over the 24 h monitoring period.

7.3.2 Ruminal ammonia concentrations

The average ruminal NH_3-N concentration increased ($P < 0.001$) with increasing levels of canola meal in the ration (Table 7.3). Average ruminal NH_3-N concentrations were higher ($P < 0.001$) 3 h after feeding compared with pre-feeding when steers were fed rations containing any level of canola meal but not the control ration (Figure 7.3).

7.3.3 Ruminal VFA concentration and molar proportions

Total VFA concentration was higher ($P < 0.05$) when the ration contained 43% canola meal compared with the control and 13% canola meal ration but not compared with the 27% canola meal ration (Table 7.3). Total VFA concentration was higher ($P < 0.05$) 3 h after initial feeding compared with pre-feeding and remained higher for all subsequent time points when steers were fed a ration containing either 27% or 43% canola meal. However, the control or 13 % canola meal ration where the total VFA concentration was not higher 6 h after feeding compared with pre-feeding (Figure 7.4a).

The molar proportion of acetic acid was lower ($P < 0.05$), and the molar proportion of propionic acid was higher ($P < 0.05$) when steers were fed the 43% canola meal ration compared with the 13% canola meal and control rations. The molar proportion of butyric acid was lower ($P < 0.05$) when steers were fed rations containing any inclusion level of canola meal (13%, 27% and 43%) compared with the control ration (Table 7.3).

The ratio of Pr:Ac + 2 x Bu was higher ($P < 0.05$) when steers were fed rations containing either 27% or 43% canola meal compared with the control or 13% canola meal rations (Table 7.3). The interaction between the ration and time after initial feeding was higher ($P < 0.05$) and the ratio of Pr:Ac + 2 x Bu was higher ($P < 0.05$) 21 h after feeding when steers were fed the ration containing 43% canola meal compared with all other rations (Figure 7.4b). Similar treatment effects were found for the Ac:Pr ratio (Figure 7.4c).

The molar proportion of valeric acid was higher ($P < 0.05$) when the ration contained 43% canola meal compared with all other rations. The molar proportions (Table 7.3)

and concentrations (Figure 7.5) of iso-butyric acid and iso-valeric acid were higher ($P < 0.05$) when the inclusion level of canola meal exceeded 13%. The molar proportions of hexanoic and heptanoic acids were lower ($P < 0.05$) when cattle were fed rations containing either 27% and 43% canola meal compared with control and 13% canola meal rations (Table 7.3).

Table 7.3 Ruminal pH, ammonia concentration and total volatile fatty acid (VFA) concentration and molar proportions when steers fed rations containing either 0%, 13%, 27% or 43% canola meal.

Ruminal fluid parameter	Inclusion level of canola meal (% of DM)[1]				*P*-values		
	0	13	27	43	Diet	Time	Diet x time
Ruminal pH	6.61 (0.11)	6.52 (0.11)	6.60 (0.11)	6.59 (0.11)	0.652	< 0.001	0.867
Ruminal ammonia concentration (mg NH_3–N/L)[1]	3.05 (0.35)^{a}	6.70 (0.35)^{b}	9.35 (0.35)^{c}	11.6 (0.35)^{d}	< 0.001	< 0.001	0.011
Total VFA concentration (mmol/L)	96.3 (3.99)^{a}	95.3 (3.99)^{a}	99.5 (3.99)^{ab}	102.9 (3.99)^{b}	0.037	< 0.0001	0.498
VFA proportion (Mol %)							
Acetic acid	69.1 (0.76)^{ab}	70.4 (0.76)^{b}	70.0 (0.76)^{ab}	67.4 (0.76)^{a}	0.017	< 0.001	< 0.001
Propionic acid	15.9 (0.76)^{a}	16.5 (0.76)^{ab}	17.7 (0.76)^{bc}	18.2 (0.76)^{c}	0.025	< 0.001	< 0.001
Butyric acid	12.5 (0.66)^{b}	10.3 (0.66)^{a}	9.9 (0.66)^{a}	10.0 (0.66)^{a}	0.021	< 0.001	< 0.001
Iso-butyric acid	0.43 (0.07)^{a}	0.57 (0.07)^{a}	0.82 (0.07)^{b}	1.11 (0.07)^{c}	< 0.001	< 0.001	< 0.001
Valeric acid	1.49 (0.18)^{a}	1.40 (0.18)^{a}	1.54 (0.18)^{a}	1.98 (0.18)^{b}	0.004	< 0.001	0.621
Iso-valeric acid	0.58 (0.11)^{a}	0.73 (0.11)^{a}	1.10 (0.11)^{b}	1.45 (0.11)^{c}	< 0.001	< 0.001	< 0.001
Hexanoic acid	0.30 (0.04)^{b}	0.26 (0.04)^{b}	0.16 (0.04)^{a}	0.13 (0.04)^{a}	< 0.001	< 0.001	< 0.001
Heptanoic acid	0.02 (0.01)^{b}	0.02 (0.01)^{b}	0.01 (0.01)^{a}	0.01 (0.01)^{a}	0.003	< 0.001	< 0.001
Pr:Ac + 2 x Bu[2]	0.17 (0.01)^{a}	0.18 (0.01)^{a}	0.20 (0.01)^{b}	0.21 (0.01)^{b}	< 0.001	< 0.001	< 0.001
Ac:Pr[3]	4.41 (0.18)^{b}	4.33 (0.18)^{b}	3.93 (0.18)^{a}	3.76 (0.18)^{a}	< 0.001	< 0.001	< 0.001

Values are least squares means ± the standard error of the least squares means. Different superscripts within rows for each parameter indicates that there is a significant difference ($P < 0.05$) between dietary treatments.

[1]Ammonia concentration data were transformed using a square root transformation prior to analysis.

[2]Pr:Ac+2xBu = ratio of propionic acid to (acetic acid + 2 x butyric acid)

[3]Ac:Pr = ratio of acetic acid to propionic acid

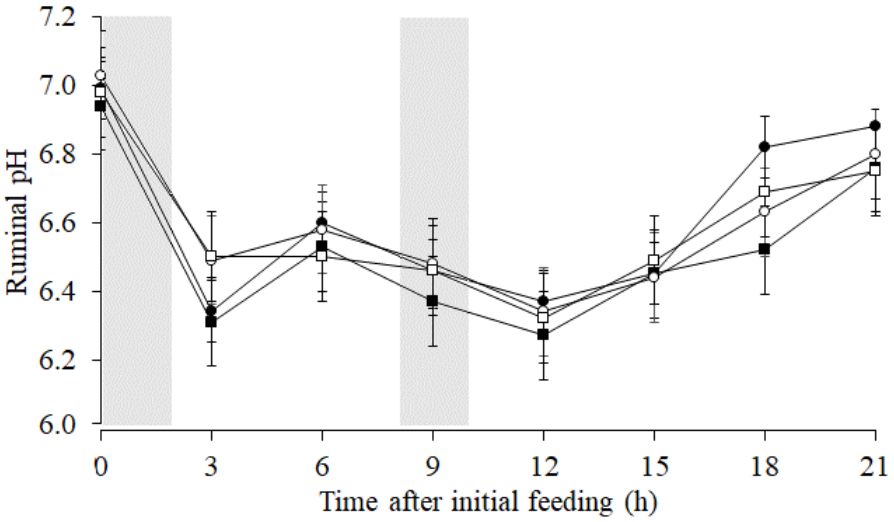


Figure 7.2 Diurnal variation in ruminal pH of steers fed rations containing either 0% (●), 13% (○), 27% (■) or 43% (□) canola meal. Shaded areas indicate the 2 h period during which steers were offered fed.

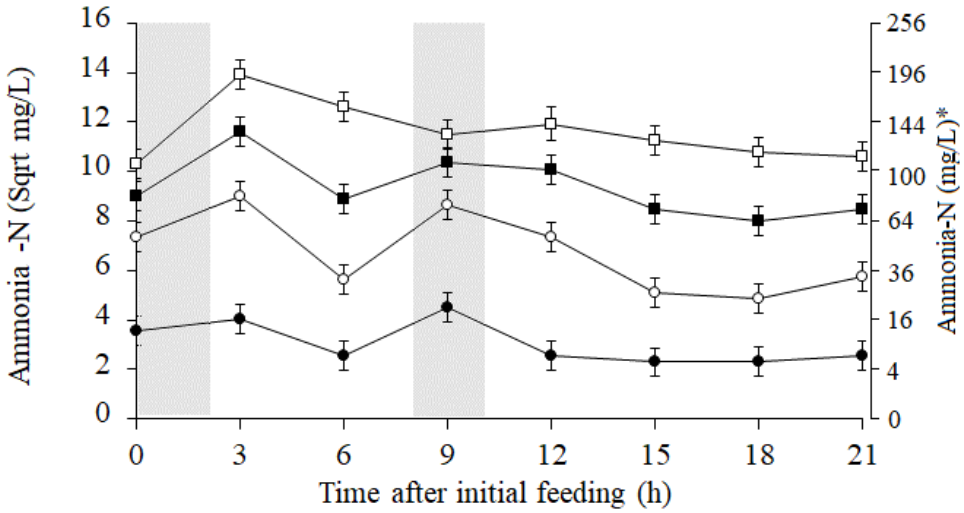


Figure 7.3 Diurnal variation in ruminal ammonia concentrations of steers fed rations containing either 0% (●), 13% (○), 27% (■) or 43% (□) canola meal. Shaded areas indicate the 2 h period during which steers were offered fed.*Ammonia-N presented in the untransformed scale for illustrative purposes only.

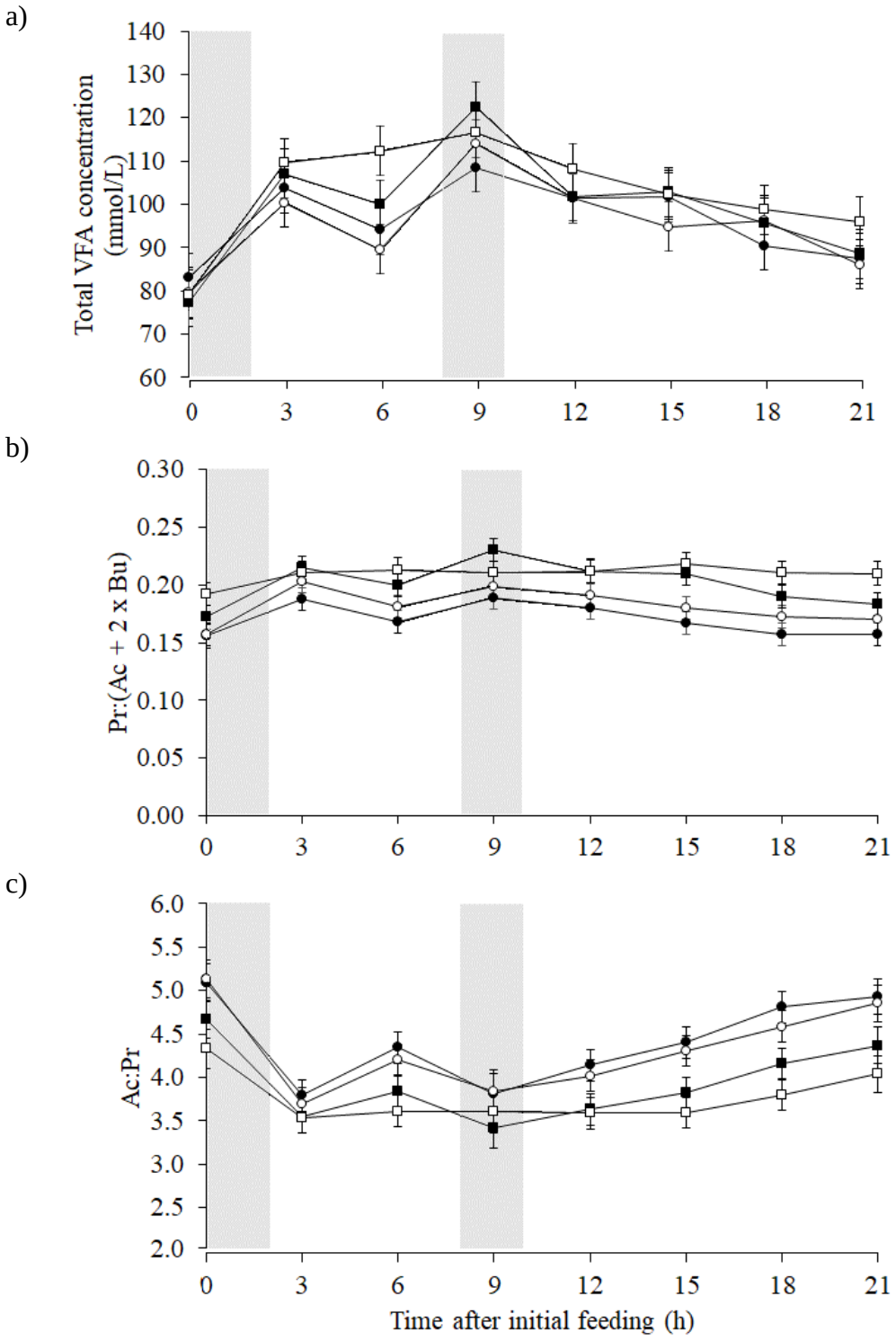
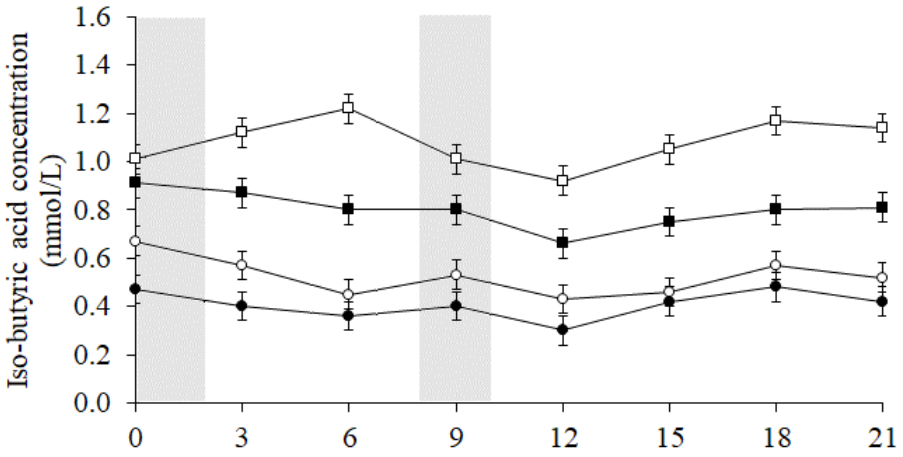


Figure 7.4 Diurnal variations in a) total volatile fatty acids (VFA) concentrations, b) propionic: (acetic + 2 * butyric) ratio; [Pr:(Ac + 2* Bu)] and c) acetic: propionic ratio (Ac:Pr) in steers fed rations containing either 0% (●), 13% (○), 27% (■) or 43% (□) canola meal. Shaded areas indicate the 2 h period during which steers were offered fed.

a)



b)

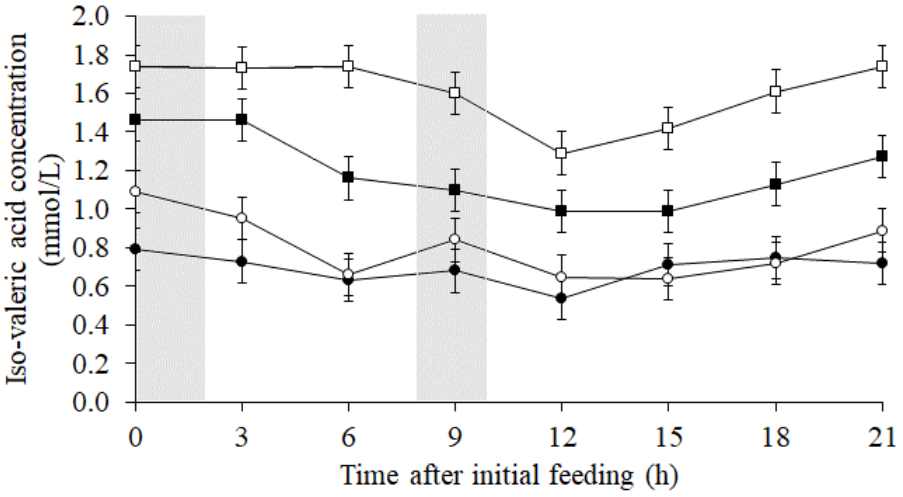


Figure 7.5 Diurnal variations in the concentrations of (a) iso-butyric, and (b) iso-valeric acid in steers fed rations containing either 0% (●), 13 % (○), 27% (■) or 43% (□) canola meal. Shaded areas indicate the 2 h period during which steers were offered fed.

The ruminal pH was significantly ($R^2 = 0.62$, $P < 0.001$) and negatively correlated to total VFA concentrations when the steers were fed rations containing varying inclusion levels of canola meal (Figure 7.6).

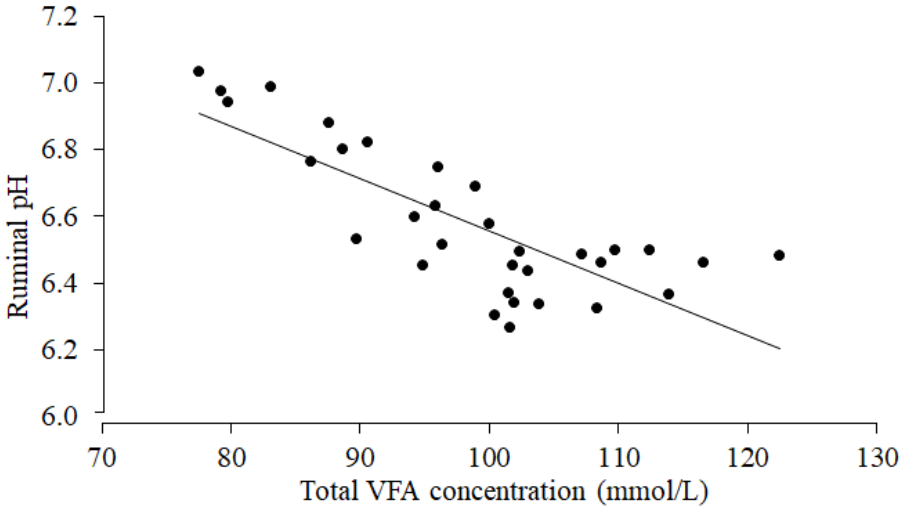


Figure 7.6 Correlation between total VFA concentrations (mmol/L) and ruminal pH over time when steers were fed all rations containing varying inclusion levels of canola meal. Ruminal pH = -0.02 x Total VFA concentration (mmol/L) + 8.12 (R^2 = 0.62, P < 0.001).

7.3.4 Characteristics of the ruminal liquor used for the *in vitro* Daisy™ incubations

The pH, NH_3-N concentration and total VFA concentration and molar proportion of the ruminal liquor used for the *in vitro* Daisy™ incubations are presented in Table 7.4. The pH of the ruminal liquor was not affected (P = 0.358) by the rations fed to the donor steers. Ammonia concentrations were significantly (P < 0.001) higher with increasing inclusion level of canola meal in the ration fed to the donor steers (Table 7.4). The estimated total NH_3-N concentration of the jars are presented in Table 7.4. Total VFA concentrations, and the molar proportions of propionic, hexanoic and heptanoic acids were not affected (P > 0.05) by the ration fed to the donor steers. The molar proportions of acetic and butyric acid decreased (P < 0.05), whereas those of valeric and the iso-acids increased (P < 0.05) with increasing inclusion levels of canola meal in the ration fed to the donor steers.

Table 7.4 Characteristics of the ruminal liquor (collected pre-feeding) used for *in vitro* Daisy™ incubation analysis when collected from steers fed rations containing either 0%, 13%, 27% or 43% canola meal.

Characteristic	**Inclusion level of canola meal (% of DM)[1]**				***P*-values**
	0	**13**	**27**	**43**	
Ruminal pH	7.01 (0.09)	6.90 (0.09)	7.04 (0.09)	6.96 (0.09)	0.358
Ruminal ammonia concentration (mg NH_3–N/L)	13.8 (5.11)^{a}	53.8 (5.11)^{b}	81.5 (5.11)^{c}	105.2 (5.11)^{d}	< 0.001
Estimated total N concentration per jar (mg /L)[1]	73.4 (2.05)	89.4 (2.02)	100.5 (2.36)	109.9 (1.68)	-
Total VFA concentration (mmol/L)	83.0 (7.63)	79.7 (7.63)	77.4 (7.63)	79.1 (7.63)	0.602
VFA proportions (Mol %)					
Acetic acid	73.4 (0.72)^{c}	73.7 (0.72)^{c}	71.9 (0.72)^{b}	70.3 (0.72)^{a}	0.001
Propionic acid	14.5 (0.82)	14.4 (0.82)	15.4 (0.82)	16.6 (0.82)	0.056
Butyric acid	9.93 (0.39)^{b}	8.91 (0.39)^{a}	8.91 (0.39)^{a}	8.38 (0.38)^{a}	0.007
Iso-Butyric acid	0.53 (0.12)^{a}	0.85 (0.12)^{ab}	1.13 (0.12)^{bc}	1.41 (0.12)^{c}	0.001
Valeric acid	0.56 (0.08)^{a}	0.66 (0.08)^{a}	0.74 (0.08)^{a}	1.05 (0.08)^{b}	0.005
Iso-Valeric acid	0.79 (0.17)^{a}	1.26 (0.17)^{ab}	1.69 (0.17)^{bc}	2.09 (0.17)^{c}	0.002
Hexanoic acid	0.27 (0.04)	0.27 (0.04)	0.18 (0.04)	0.16 (0.04)	0.090
Heptanoic acid	0.010 (0.003)	0.015 (0.003)	0.008 (0.003)	0.010 (0.003)	0.308
Pr:Ac + 2 x Bu[2]	0.16 (0.01)^{a}	0.16 (0.01)^{a}	0.17 (0.01)^{ab}	0.19 (0.01)^{b}	0.034
Ac:Pr[3]	5.09 (0.26)^{bc}	5.14 (0.26)^{c}	4.67 (0.26)^{ab}	4.33 (0.26)^{a}	0.007

Values are least square mean ± the standard error of the mean. Different superscripts within rows for each parameter indicates that there is a significant difference ($P < 0.05$) between dietary treatments.

[1] Estimated total N concentration per jar (mg/L) was calculated by the average concentration (mg/L) of NH_3-N from the ruminal fluid from each steer and the amount (mg/L) of NH_3-N added per jar from ammonium sulphate $(NH_4)_2SO_4$ and urea $CO(NH_2)_2$.

[2] Pr:Ac+2xBu = ratio of propionic acid to (acetic acid + 2 x butyric acid)

[3] Ac:Pr = ratio of acetic acid to propionic acid.

7.3.5 *In sacco* degradation

The DM PD after 72 h incubation and slowly degradable (b) fraction were higher (P = 0.008) when the steers were fed the 13% and 43% canola meal rations compared with the control and 27% canola meal ration (Table 7.5). The fractional rate of degradation (c) and the ED at all rumen outflow rates (0.02/h, 0.05/h and 0.08/h) were not affected ($P > 0.05$) by the ration the steers were fed. The *in sacco* DM degradability of the lucerne standard was significantly higher ($P < 0.001$) 48 h after initial incubation when the steers were fed the 13% canola meal ration compared with all other rations (Figure 7.8a).

The CP, aNDF and ADF degradability losses over time for the lucerne standard were not affected (P = 0.203, P = 0.264 and P = 0.236, respectively) by the ration the steers were fed; however, CP degradability was significantly higher at 72 h after initial incubation when the steers were fed the 43% canola meal ration compared with all other rations (Figure 7.8b). The CP, aNDF and ADF degradation kinetics of the lucerne standard was not affected ($P > 0.05$) by the ration fed to the steers.

Table 7.5 *In sacco* ruminal degradation kinetic parameters, potential degradation after 72 h incubation (PD) and effective degradability (ED), considering outflow rates of 0.02, 0.05 and 0.08/h) of the lucerne standard when steers were fed rations containing either 0%, 13%, 27% or 43% canola meal.

Degradation (%)[1]	**Dietary inclusion level of canola meal (% of DM)**				***P*-values**
	0	**13**	**27**	**43**	
DM					
a	13.61 (0.75)				
b	47.44 (1.31)[a]	51.72 (1.31)[b]	46.45 (1.31)[a]	51.31 (1.31)[b]	0.008
c	0.054 (0.01)	0.054 (0.01)	0.053 (0.01)	0.051 (0.01)	0.737
PD	61.05 (1.31)[a]	65.32 (1.31)[b]	60.05 (1.31)[a]	64.97 (1.31)[b]	0.008
$ED_{(0.02/h)}$	48.66 (1.37)	51.49 (1.33)	48.60 (1.47)	50.45 (1.41)	0.289
$ED_{(0.05/h)}$	38.54 (1.16)	40.55 (1.14)	38.35 (1.25)	39.41 (1.19)	0.478
$ED_{(0.08/h)}$	32.99 (0.99)	34.57 (0.98)	32.79 (1.06)	33.55 (1.01)	0.546
CP					
a	12.75 (1.91)				
b	49.15 (7.93)	47.50 (7.93)	46.43 (7.93)	58.75 (7.93)	0.686
c	0.049 (0.01)	0.054 (0.01)	0.045 (0.01)	0.040 (0.01)	0.758
PD	61.90 (7.93)	60.25 (7.93)	59.18 (7.93)	71.50 (7.93)	0.686
$ED_{(0.02/h)}$	45.95 (6.52)	46.48 (6.52)	43.98 (6.52)	47.13 (6.52)	0.987
$ED_{(0.05/h)}$	35.48 (5.17)	36.58 (5.17)	33.83 (5.17)	34.84 (5.17)	0.985
$ED_{(0.08/h)}$	30.12 (4.25)	31.27 (4.25)	28.69 (4.25)	29.17 (4.25)	0.974
RUP	64.52 (5.17)	63.42 (5.17)	66.17 (5.17)	65.16 (5.17)	0.985
aNDF					
c	0.017 (0.01)	0.021 (0.01)	0.030 (0.01)	0.016 (0.01)	0.742
PD	49.97 (4.56)	53.79 (4.47)	44.22 (4.56)	51.12 (4.66)	0.472
$ED_{(0.02/h)}$	23.60 (4.47)	29.68 (4.24)	24.81 (4.47)	26.98 (4.73)	0.666
$ED_{(0.05/h)}$	12.74 (3.95)	17.52 (3.75)	15.54 (3.95)	15.40 (4.19)	0.772
$ED_{(0.08/h)}$	8.58 (3.46)	12.28 (3.28)	11.44 (3.46)	10.52 (3.66)	0.810
ADF					
c	0.035 (0.02)	0.019 (0.01)	0.041 (0.01)	0.015 (0.01)	0.525
PD	34.99 (4.68)	41.10 (4.68)	30.07 (4.68)	37.60 (4.68)	0.434
$ED_{(0.02/h)}$	17.96 (7.74)	16.31 (6.32)	21.19 (6.32)	14.45 (6.32)	0.890
$ED_{(0.05/h)}$	12.11 (5.96)	9.49 (4.87)	14.98 (4.87)	7.86 (4.87)	0.755
$ED_{(0.08/h)}$	9.33 (4.75)	6.74 (3.88)	11.63 (3.88)	5.41 (3.88)	0.696

Values are least square means ± the standard error of the means. Different superscripts within rows for each parameter indicate there is a significant difference ($P < 0.05$) between dietary treatments.

[1] DM = Dry matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash. RUP = rumen undegraded protein (RUP = b [r / (c + r)] + (100 - PD) with rumen outflow rate of 0.05/h.

a = soluble fraction; b = not soluble but slowly degradable fraction; c = fractional rate of degradation of degradable fraction b (/h) from the fitted exponential equation $PD = a + b (1- e^{-ct})$; PD = potential degradable after 72 h incubation (a + b); ED = effective degradability (a + b [c / (c + r)] considering a rumen outflow rate (r) of 0.02, 0.05 and 0.08/h.

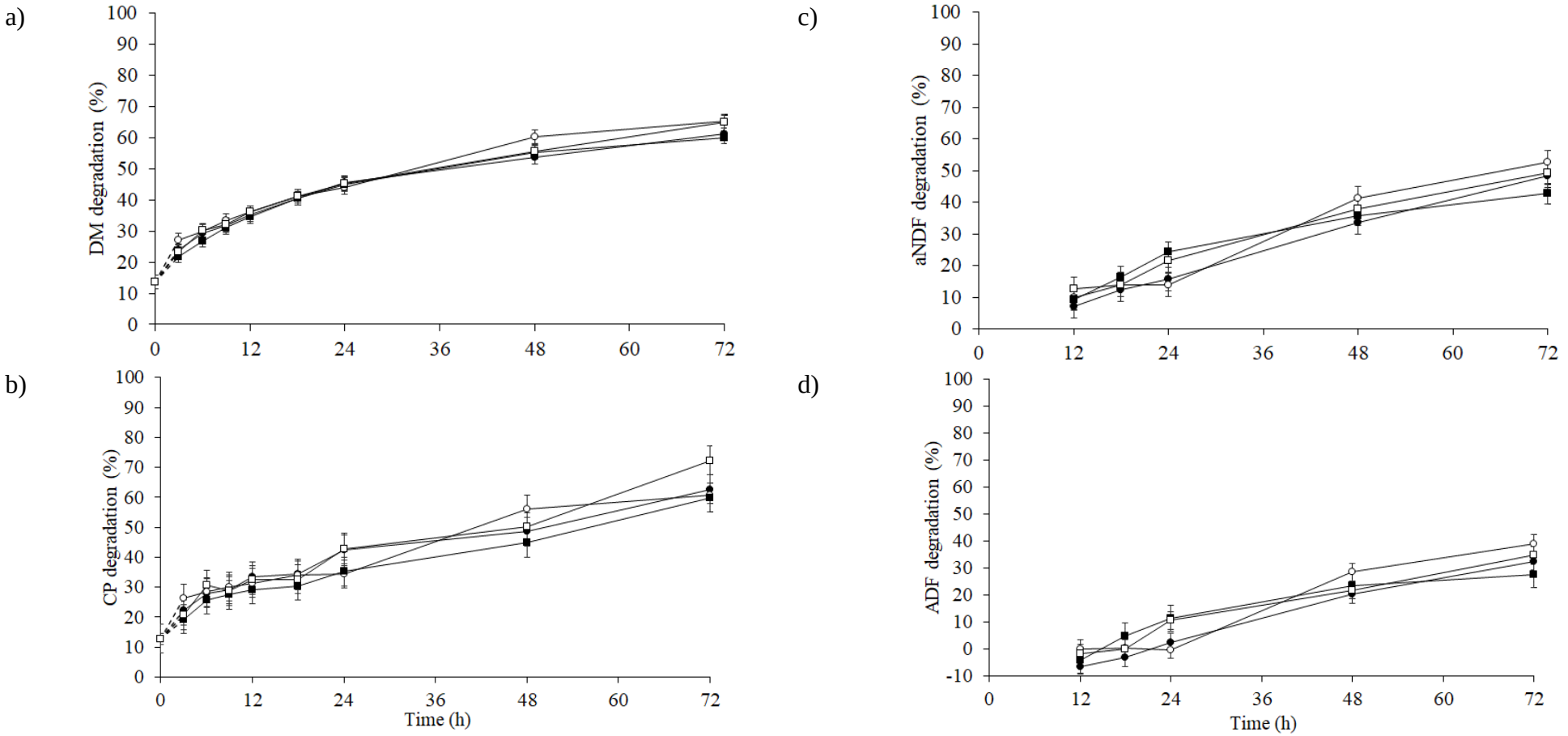


Figure 7.8 *In sacco* a) dry matter (DM), b) crude protein (CP), c) neutral detergent fibre (aNDF) and d) acid detergent fibre (ADF) degradation losses of the lucerne standard over a 72 h incubation period when steers were fed rations containing either 0% (●), 13% (○), 27% (■) or 43% (□) canola meal.

7.3.6 *In vitro* Daisy™ degradation

The DM and CP degradation kinetic parameters were not affected ($P > 0.05$) by the ration fed to the donor steers (Table 7.6). The aNDF and ADF PD after 72 h incubation were also not affected ($P = 0.277$ and $P = 0.109$, respectively) by the ration fed to the donor steers. The aNDF ED at all outflow rates (0.02/h, 0.05/h and 0.08/h) and the fractional rate of degradation (c) were higher ($P < 0.05$) with any inclusion level of canola meal in the ration compared with the control ration. The ADF ED at outflow rates of 0.02/h and 0.05/h were higher ($P < 0.05$) with any inclusion level of canola meal in the ration compared with the control ration.

The DM degradability loss over time for the lucerne standard was not affected ($P = 0.840$) by the ration the donor steer was fed (Figure 7.9a). Overall, the CP, aNDF and ADF degradation losses over time were higher ($P = 0.004$, $P < 0.001$ and $P < 0.001$, respectively) when the ruminal liquor came from steers fed rations containing canola meal. The CP degradation loss at 18, 24, 48 and 72 h after initial incubation was lower ($P = 0.004$) when the ruminal liquor was collected from steers fed a ration containing 43% canola meal compared with all other rations (Figure 7.9b). The aNDF (Figure 7.9c) and ADF (Figure 7.9d) degradation losses were significantly lower at 48 h after incubation when the ruminal liquor came from steers fed the control ration compared with all other rations.

Table 7.6 *In vitro* Daisy™ ruminal digestion kinetics, potential degradation after 72 h incubation (PD) and effective degradability (ED, considering outflow rates of 0.02, 0.05 and 0.08/h) of the lucerne standard when the ruminal liquor was collected from steers fed a ration containing either 0%, 13%, 27% or 43% DM canola meal.

Degradation (%)[1]	**Dietary inclusion level of canola meal (% of DM)[2]**				***P*-values**
	0	**13**	**27**	**43**	
DM					
a	16.89 (1.88)				
b	39.88 (1.58)	41.20 (1.58)	41.78 (1.58)	41.95 (1.58)	0.789
c	0.055 (0.01)	0.048 (0.01)	0.048 (0.01)	0.045 (0.01)	0.617
PD	56.77 (1.58)	58.08 (1.58)	58.67 (1.58)	58.83 (1.58)	0.789
$ED_{(0.02/h)}$	45.74 (2.28)	45.74 (2.28)	44.75 (2.28)	45.65 (2.28)	0.987
$ED_{(0.05/h)}$	37.42 (1.84)	36.88 (1.84)	36.71 (1.84)	36.61 (1.84)	0.990
$ED_{(0.08/h)}$	32.86 (1.51)	32.20 (1.51)	32.31 (1.51)	31.93 (1.51)	0.977
CP					
a	16.73 (3.78)				
b	46.53 (3.76)	49.43 (3.76)	50.43 (3.76)	43.56 (3.76)	0.582
c	0.024 (0.01)	0.018 (0.01)	0.021 (0.01)	0.016 (0.01)	0.385
PD	64.87 (3.76)	67.78 (3.76)	68.77 (3.76)	61.90 (3.76)	0.582
$ED_{(0.02/h)}$	42.67 (3.89)	40.73 (3.89)	45.05 (4.22)	36.39 (3.89)	0.353
$ED_{(0.05/h)}$	32.81 (2.71)	30.82 (2.71)	33.72 (2.23)	28.30 (2.71)	0.383
$ED_{(0.08/h)}$	28.66 (2.04)	26.99 (2.04)	29.14 (2.20)	25.24 (2.04)	0.393
RUP	67.19 (2.71)	69.18 (2.71)	66.28 (2.23)	71.70 (2.71)	0.383
aNDF					
c	0.034 (0.02)^{a}	0.119 (0.02)^{b}	0.123 (0.02)^{b}	0.074 (0.02)^{b}	0.012
PD	37.67 (2.13)	41.61 (1.93)	41.88 (2.00)	43.25 (2.08)	0.277
$ED_{(0.02/h)}$	25.49 (2.51)^{a}	35.47 (2.22)^{b}	35.91 (2.26)^{b}	34.07 (2.30)^{b}	0.036
$ED_{(0.05/h)}$	17.76 (2.63)^{a}	29.19 (2.28)^{b}	29.70 (2.28)^{b}	26.05 (2.28)^{b}	0.026
$ED_{(0.08/h)}$	13.65 (2.55)^{a}	24.74 (2.21)^{b}	25.22 (2.21)^{b}	20.98 (2.21)^{ab}	0.025
ADF					
c	0.030 (0.02)	0.098 (0.02)	0.106 (0.02)	0.057 (0.02)	0.116
PD	32.70 (1.96)	36.16 (1.70)	38.23 (1.70)	39.55 (1.70)	0.109
$ED_{(0.02/h)}$	17.57 (2.89)^{a}	28.77 (2.50)^{b}	30.77 (2.50)^{b}	28.61 (2.50)^{b}	0.028
$ED_{(0.05/h)}$	11.09 (3.15)^{a}	22.57 (2.73)^{b}	24.36 (2.73)^{b}	20.49 (2.73)^{b}	0.046
$ED_{(0.08/h)}$	8.16 (3.03)	18.75 (2.63)	20.34 (2.63)	16.03 (2.63)	0.058

Values are least square means ± the standard error of the means. Different superscripts within rows for each parameter indicate there is a significant difference ($P < 0.05$) between dietary treatments.

[1] DM = Dry matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash. RUP = rumen undegraded protein (RUP = b [r / (c + r)] + (100 - PD) with rumen outflow rate of 0.05/h.

a = soluble fraction; b = not soluble but slowly degradable fraction; c = fractional rate of degradation of degradable fraction b (/h) from the fitted exponential equation PD = a + b (1- e^{-ct}); PD = potential degradable after 72 h incubation (a + b); ED = effective degradability (a + b [c / (c + r)] considering a rumen outflow rate (r) of 0.02, 0.05 and 0.08/h.

[2]Sample diet containing lucerne hay when steers were offered varying inclusion levels of canola meal.

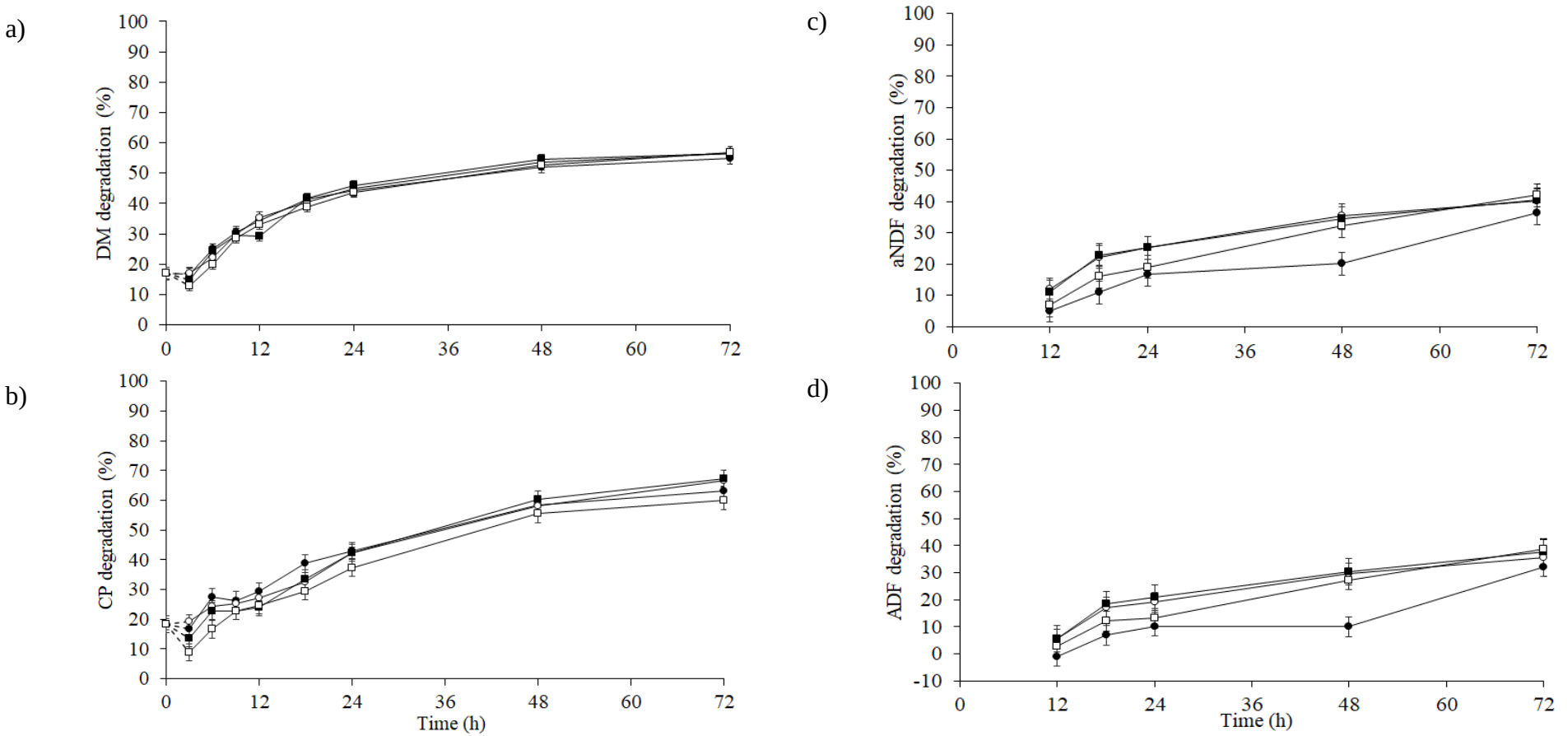


Figure 7.9 The *in vitro* Daisy™ predicted a) dry matter (DM), b) crude protein (CP), c) neutral detergent fibre (aNDF) and d) acid detergent fibre (ADF) degradation losses of the lucerne standard over a 72 h incubation period when the ruminal liquor was collected from steers fed a ration containing either 0% (●), 13% (○), 27% (■) or 43% (□) canola meal.

7.4 Discussion

The degradation loss and degradation kinetic parameters, including PD after 72 h incubation of the lucerne standard varied but not consistently for the *in sacco* and *in vitro* Daisy™ methodologies with increasing levels of canola meal in the ration fed to the ruminal fluid donor steers. Whether the observed effects for the *in sacco* incubation were due to the change in ruminal parameters over time and/or differences in the ration fed to the steers will be discussed. The effect of the supplemental N in the artificial saliva solution for *in vitro* Daisy™ incubation will also be discussed.

7.4.1 *In sacco* incubation

Ruminal fluid parameters

Ruminal pH was not different between rations and was within the optimal range of 6.0 to 6.9 for microbial growth (Kamra 2005) and was not below 6.3 at any time after initial feeding. Therefore, including canola meal in the ration should not affect fibre degradation of the lucerne standard (Dijkstra *et al.* 2012). This will be discussed later in relation to *in sacco* degradation.

The total VFA concentrations were higher with increasing inclusion levels of canola meal; however, they were within the normal expected range of 70 and 130 mmol/L (France and Dijkstra 2005). The increase in total VFA concentrations was expected as total VFA concentrations are affected by dietary composition, time of feeding and intake level (Bergman 1990; France and Dijkstra 2005). The M/D and CP content of the ration were higher as the inclusion level of canola meal increased (Table 7.2). Increasing dietary M/D has been associated with higher total VFA concentrations (Putnam *et al.* 1966; Furchtenicht and Broderick 1987; Cui *et al.* 2019; Ahmad *et al.* 2020). The total VFA concentration was higher when the ration contained 43% canola meal compared with the control and 13% canola meal rations but not compared with the 27% canola meal rations. Moreover, it is difficult to directly compare these results with others as no studies were identified that used such high levels of canola meal in a ration. Total VFA concentration was unaffected when lactating dairy cows were fed rations containing either 11% or 17% canola meal (Broderick *et al.* 2015), which somewhat aligns with the effects of feeding 13% and 27% canola meal in the current study.

Total VFA concentration increased and ruminal pH decreased, after initial feeding compared with pre-feeding which is typical of a forage based diet (Van Soest 1994). Ruminal pH has been found to be negatively correlated with the ruminal concentrations of total VFA (Seymour *et al.* 2005), which was also found in this study. The correlation between total VFA concentration and ruminal pH (R^2 = 0.62) when steers were fed all rations were less than reported for steers (R^2 = 0.74) grazing pastures and treated with or without a monensin capsule (Packer *et al.* 2011). Therefore, the addition of canola meal into a low-quality ration should not negatively impact degradation. This will be discussed later in relation to *in sacco* degradation

The proportion of acetic acid is relatively higher than propionic acid in forage-based diets compared with high starch, concentrate diets (Brossard *et al.* 2004) which was similar when canola meal was included at 27% or less in the ration. The molar proportion of acetic acid (control ration 69.1 ± 0.76 % vs 43% ration 67.4 ± 0.76 %) was lower and propionic acid (control ration 15.9 ± 0.76 % vs 43% ration 18.2 ± 0.76 %) was higher when the ration contained 43% canola meal compared with all other rations, which suggests this ration was more closely aligned with a concentrate diet. Thus, including 43% of canola meal in a low-quality ration resulted in an improvement in the overall efficiency of feed utilisation and may have increased gluconeogenesis as propionic acid is the major contributor for gluconeogenesis in the body (Bergman 1990; Dijkstra 1994; France and Dijkstra 2005), although further research is required to confirm this.

The lower Ac:Pr ratio when the ration contained either 27% or 43% canola meal was expected as the fibre content of the ration decreased (Table 7.2) and a decline in fibre is associated with a decrease of the Ac:Pr ratio (Ørskov and McDonald 1979). The lower Ac:Pr ratio may also be due to an increased level of PUFA (residual oil in the canola meal) in these rations (Palmquist and Conrad 1978); therefore, further investigation is needed to confirm this. The lack of effect on the Ac:Pr ratio when either 0% or 13% canola meal was included in the ration is in agreement with Nair *et al.* (2016) who found this ratio did not differ when heifers were fed either 0%, 10% or 20% canola meal in a TMR. Similarly, the Ac:Pr ratio was not affected when canola meal was included in a PMR based on ryegrass fed to dairy cattle (Russo *et al.* 2017).

Valeric acid is formed from ruminal catabolism of the AA, arginine, proline and lysine (El-Shazly 1952) and canola meal normally contains these AA (Newkirk *et al.* 2003b); although, lysine is normally the limiting AA. The increase in molar proportion of

valeric acid (Table 7.4) when the ration contained 43% canola meal was expected given the increase in dietary supply of these AA.

Branched-chain VFA are formed from microbial deamination and decarboxylation of the branched-chain AA, valine, isoleucine and leucine (Allison *et al.* 1962). The increase in the molar proportions and concentrations of the BCVFA iso-butyric acid and iso-valeric acid with increasing inclusion levels of canola meal in the ration may indicate an increase in ruminal protein degradation (Alison and Bryant, 1983). Further investigation to determine the effects on energy metabolism and efficiency of high concentrations of iso-acids when feeding varying inclusion levels of canola meal to steers is required.

Ruminal NH_3-N concentrations were higher as the inclusion level of canola meal in the ration increased, that was expected given the high CP content of the canola meal, that could also reflect the improved rate of degradation (Heim and Krebs 2018d). Similarly, ruminal NH_3-N concentrations were higher when increasing levels (12 %, 20%, 30% or 39% of total dietary DM) of soybean meal were offered to cattle with *ad libitum* wheat straw (Beaty *et al.* (1994). The minimum threshold ruminal NH_3-N concentration to support microbial growth is 50-80 mg NH_3-N/L (Satter and Slyter 1974; Slyter *et al.* 1979), and if this threshold is not met, the rate of fibre digestion can decrease (Lee *et al*. 1985). This minimum threshold was only met when the ration contained either 27% or 43% canola meal. Similarly, ruminal NH_3-N concentrations were only higher when 10% canola meal was included in a TMR for steers (Nair *et al.* 2016). The possible influences of ruminal NH_3-N concentrations in relation to degradability of the lucerne standard is highlighted in the following discussion.

Degradation kinetics

Including a standard feed for *in sacco* studies enables variations in rumen microbial activity to be taken into account (Michalet-Doreau and Ould-Bah 1992) and to determine if the ration fed affects degradation kinetics. The DM but not the CP, aNDF or ADF degradation kinetic parameters of the lucerne standard varied when steers were fed different rations. In contrast, the DM (and CP) degradation of hay samples over 72 h was not affected when steers were fed varying levels of soybean meal and *ad libitum* brome hay (Carey *et al.* 1993). The DM PD and the slowly degradable (b) fraction were higher when the steers were fed rations containing either 13% or 43% canola meal compared with the control or 27% canola meal rations. The increase in ruminal

NH_3-N concentration does not fully explain the lack of response in DM PD when 27% canola meal was included in the ration and warrants further research.

The DM PD for the lucerne standard ranged from 60.1% to 65.3% (Table 7.5) which was consistent with the laboratory predicted pepsin cellulase (as previously described in Section 3.6.6) estimate of 64.3% (Table 7.2). The DM PD in the current study was also comparable to that of 15 cultivars of lucerne hay (Julier *et al.* 2001), but lower than a lucerne standard when steers were fed roughage and wheat (McDonnell *et al.* 2017) or when sheep were fed roughages (Nandra *et al.* 1993). Therefore, the *in sacco* degradability of the lucerne hay used as a standard can be variable depending on the nutritive value of the standard used, the animal and the composition of the ration fed to the animal.

The soluble fraction (a) of the lucerne standard was lower than previous reports McDonnell *et al.* (2017) that could be due to the combined effects of genetics, plant maturity and structure, environmental differences (Julier *et al.* 2001), having filter bags contained inside of dacron bags or the variation in the technique used to wash the bags following their incubation in the rumen (López 2005). Effective washing after incubation is critical to stop microbial activity and remove any microbes attached to the bag (Nozière and Michalet-Doreau 1996). Excessive washing has been shown to increase losses of material (Cherney *et al.* 1990; Vanzant *et al.* 1998) and thus is a source of variation.

The ration fed to the steers did not alter the CP and fibre degradation (after 72 h) of the lucerne standard. The increase in DM PD could have been due to the PD of the non-structural carbohydrates (Huntington *et al.* 1998) or the DM fraction of the lucerne standard did not plateau at 72 h. Further research into the effects of feeding canola meal of the composition of rumen microbial populations (Jung and Varel 1988; Ding *et al.* 2014), especially cellulolytic bacteria (Van Gylswyk 1970; Russell *et al.* 2009) and protozoa (Dijkstra and Tamminga 1995; Newbold *et al.* 2015) in the rumen, may offer insight into why the fibre degradation was not affected by the ration.

7.4.2 *In vitro* Daisy™ incubation

Ruminal liquor characteristics

The ruminal liquor used for *in vitro* methodologies can represent the largest source of uncontrolled variation (Marten and Barnes 1979; Holden 1999; Wilman and Adesogan 2000), including the time of feeding (Van Soest 1994; France and Dijkstra 2005; López

2005), and the LoF (Adesogan, 2005; Valentine *et al.* 2019). Therefore, ruminal liquor was collected pre-feeding to minimise variation (Mabjeesh *et al.* 2000; Trujillo *et al.* 2010).

The pH of the ruminal liquors collected was not below 6.9 and thus was within normal range for *in vitro* Daisy™ incubation (Sung *et al.* 2006; Raffrenato *et al.* 2018). Total VFA concentration and pH of the ruminal liquor was not affected by the ration fed to the donor steers, with both being within the normal range (France and Dijkstra 2005; Kamra 2005). The molar proportion of acetic acid was lower when ruminal liquor was collected (before initial feeding) from steers fed 43% canola meal compared with all other rations; however, propionic acid was not different which was unexpected given these were the same rations fed to the steers for the *in sacco* study. The mean molar proportions of propionic acid reported for the *in sacco* study were; however, based on the average of samples collected at 3 h intervals over 24 h, and this was most likely the cause of the differences. The ruminal liquor used for *in vitro* Daisy™ incubation consisted of both ruminal fluid and blended (but strained) particulate matter whereas strained ruminal fluid was assessed for the *in sacco* study. Volatile fatty acids are the end-products of fermentation and are found in ruminal fluid, thus it is unlikely that processing (blending) would account for the difference.

The NH_3-N concentration of the ruminal liquor increased as the inclusion level of canola meal in the ration fed to the donor steers increased as was the case in the *in sacco* study. The minimum threshold NH_3-N concentration for optimal microbial growth (Satter and Slyter 1974; Slyter *et al.* 1979) was only met when the rations fed to the donor steers contained either 27% or 43% canola meal; however, this is not a true reflection of the N available to the rumen microbes. Supplemental N (urea and NH_3-N sulphate) was included in the artificial saliva solution, which was added to the ruminal liquor to ensure N was not limiting (Kaiser *et al.* 2007). An estimate of the additional NH_3-N added into each jar indicated all incubations had sufficient N to support microbial growth (Table 7.4). Consequently, the DM, CP, and aNDF PD of the lucerne standard were not affected by the initial NH_3-N concentration of the ruminal liquor. Further analysis of ruminal NH_3-N concentrations during the incubation period would provide further information on the degradation kinetics and losses of the lucerne standard.

Degradation kinetics

The *in vitro* Daisy™ DM degradation kinetic parameters for the lucerne standard were not affected by the source of the ruminal liquor (i.e., ration fed to the donor steers) which was similar when ruminal liquor was collected from donor sheep fed lucerne grass hay (Trujillo *et al.* 2010). Conversely, the PD of the lucerne standard varied but not consistently with increasing levels of canola meal in the ration fed to the donor steers using the *in sacco* methodology. Furthermore, the *in vitro* Daisy™ PD of DM (56.77 - 58.83%) for the lucerne standard was lower compared with the laboratory predicted pepsin cellulase digestibility (67.85%) (Table 7.2) highlighting the variability between *in vitro* methodologies. Determining digestibility of a feedstuff using the *in vitro* pepsin cellulase method is commonly used for routine analysis; however, the *in vitro* Daisy™ method described allows for the determination of rumen degradation kinetics.

The PD of DM of the lucerne standard was higher for the *in sacco* (61.05 - 64.97%) method compared with the *in vitro* Daisy™ (56.77 - 58.83%) method indicating the *in vitro* Daisy™ method was potentially underestimating the degradability of the lucerne standard. The DM PD of a range of grasses and concentrates were higher for *in sacco* compared with *in vitro* test tubes when bags were incubated in sheep and/or when ruminal fluid was collected from the same sheep fed a ration consisting of 65% grass hay and 35% concentrate (Chaudhry and Mohamed 2011). The lower DM PD values of the lucerne standard for the *in vitro* Daisy™ incubation maybe due to the method of collection of the ruminal liquor, including the unavoidable exposure to oxygen and temperature fluctuations (Chaudhry and Mohamed 2011). Furthermore, the ruminal liquor used for *in vitro* Daisy™ incubation might not have contained some microbial communities that were either present on longer feed particles or the rumen wall of the steers (Kong *et al.* 2010). The potential difference in the microbial populations associated with feed particles was unlikely to have been the cause. As the lucerne standard was incubated in filter bags that should have restricted the movement of feed particles and associated microorganisms into the filter bags, in both the *in sacco* and *in vitro* Daisy™ methods. Conversely, there could be an overestimate of the PD after 72 h of the lucerne standard due to the greater movement feed particles and associated microorganism into the dacron and filter bags using the *in sacco* methodology.

The degradation kinetics of the lucerne standard was not altered by the source of ruminal liquor, despite which method was employed, which was similar to a previous study when lucerne hay was incubated in *in vitro* flasks (Marinucci *et al.* 1992). In

contrast, *in vitro* DM digestibility for a range of hays and concentrates was found to be higher when donor cows were fed lucerne haylage and corn silage based TMR compared with grass hay (Holden 1999). Ruminal inoculum is one of the largest sources of variation for measuring degradation kinetics and will be discussed in Chapter 8.

The CP degradation kinetic parameters for the lucerne standard were not affected by the source of the ruminal liquor, as was observed with the *in sacco* method. The PD of aNDF and ADF was also not affected by the source of ruminal liquor when the steers were fed varying inclusion levels of canola meal. The aNDF PD of the lucerne standard was similar to that reported by Tagliapietra *et al.* (2012) for an *in vitro* Daisy™ incubation using ruminal liquor collected from cows fed *ad libitum* meadow hay Brown *et al.* (1991) reported; however, that *in vitro* fibre degradation varied depending on the source of inoculum; although they did not use the *in vitro* Daisy™ method and no supplemental N was added to the inoculum.

Fibre digestibility can improve with the addition of protein supplements to low-quality roughages due to increased microbial activity (DelCurto *et al.* 1990). Often with fibre degradation, there is a lag phase before the onset of fermentation (Chaudhry 2000; Raffrenato *et al.* 2009; Raffrenato and Van Amburgh 2010). The ED of aNDF at all outflow rates and the fractional rate of degradation (c) were higher when ruminal liquor was collected from steers fed any inclusion level of canola meal. The fractional rate of degradation (c) when the lucerne standard was incubated in ruminal liquor collected from steers fed the 43% canola meal ration was similar to when ruminal liquor was collected from sheep (Trujillo *et al.* 2010). In contrast, the ED and the fractional rate of degradation (c) of aNDF for the *in vitro* Daisy™ was lower compared with the *in sacco* method which was unexpected as the pH of the ruminal liquor was above 6.9 and fibre degradation was not affected (Sung *et al.* 2006; Dijkstra *et al.* 2012). The pH of the buffered ruminal liquor was not monitored throughout the *in vitro* Daisy™ incubation. Therefore, it is unknown whether the pH declined over the incubation period and thus warrants further research especially for longer incubation times. In addition, this could be due to the *in sacco* method reported in the current study loses more particulate matter during the 72 h incubation compared to the *in vitro* Daisy™ incubation.

7.5 Conclusion

Supplementing donor steers up to 43% canola meal in a low-quality ration had no negative effects on either ruminal parameters or the degradation of lucerne hay when using the *in sacco* or *in vitro* Daisy™ method. Ruminal NH_3-N concentration and total VFA concentration and molar proportions increased as the inclusion level of canola meal increased in the ration; therefore, including 27% or 43% canola meal in the ration has the potential to improve energy utilisation for the steers. Increasing the inclusion level of canola meal did not have a consistent effect on the degradation kinetics of the lucerne standard for the *in sacco* method. In addition, adding additional N to the artificial saliva buffer solution used in the *in vitro* Daisy™ method did not improve the PD kinetics of the lucerne standard. The high quality of the lucerne standard may have resulted in the lack of difference in the degradation kinetics between methods. Further investigation into the degradability of the canola meal rations when the steers are fed the same rations containing varying inclusion levels of canola meal using both the *in sacco* and *in vitro* Daisy™ method is warranted and was investigated in the studies reported in Chapter 8.

Chapter 8: *In sacco* and *In vitro* Daisy™ Ruminal Degradation Kinetics of Dietary Treatments Containing Increasing Levels of Canola Meal

8.1 Introduction

Apparent DMD and CPD of the ration increased as the inclusion level of canola meal increased in the ration; however, fibre digestibility (aNDFD and ADFD) was not affected by the ration fed to the steers, as previously reported in Chapter 6. Ruminal NH_3-N concentrations and total VFA concentrations also increased as the inclusion levels of canola meal in the ration increased (Chapter 7). In addition, the Ac:Pr ratio decreased when the rations contained 27% and 43% canola meal indicating higher inclusion levels of canola meal in the ration have the potential to improve energy utilisation of steers consuming low-quality roughages.

Apparent nutrient digestibility provides information on how a ration is digested in both the rumen and hindgut. *In sacco* and *in vitro* Daisy™ methodologies provide information on the extent and rate of degradation in the rumen. Directly comparing *in sacco* and *in vitro* values to the literature should be done with caution as there are multiple sources of variation (López 2005) and ideally a feed component should be validated against apparent nutrient digestibility (Mould 2003; Damiran *et al.* 2008). Only a few studies (Chaudhry and Mohamed 2011; Krizsan *et al.* 2013) have directly compared *in sacco* and *in vitro* Daisy™ DM and CP degradability of a ration (referred to hereafter as dietary treatments) using fistulated animals fed the same ration (i.e., the incubated material is the same composition as the ration fed to the animals) and no studies have involved increasing inclusion levels of canola meal in a low-quality roughage ration.

Therefore, the aims of the study presented in the current Chapter were to determine:

1. whether the ruminal kinetic parameters and the degradation loss of the canola meal dietary treatments were increased when steers were fed the same ration containing increasing inclusion levels of canola meal using the *in sacco* technique;
2. whether the ruminal kinetic parameters and the degradation loss of the canola meal dietary treatments were increased with the addition of supplementary N into the *in vitro* Daisy™ method when the ruminal liquor was collected from

steers which were fed the same ration containing increasing inclusion levels of canola meal; and,

3. the relationship between *in vitro* Daisy™ PD, *in sacco* PD and *in vivo* DMD when steers were fed rations containing varying inclusion levels of canola meal.

8.2 Methods and materials

The *in sacco* and *in vitro* Daisy™ incubations of the canola meal dietary treatments were undertaken at the same time as the lucerne standard study reported in Chapter 7, allowing dual use of the animals for the *in sacco* study as well as donors of ruminal fluid for the *in vitro* Daisy™ study and direct comparison of ruminal liquor. The study was conducted at the NSW DPI Animal Nutrition Unit and the use and care of the steers for this study were as described in Chapter 3.1 and Chapter 7.2.

The same general methods and materials are described in Chapter 3.1, 3.2, 3.3.2, 3.3.3, 3.4, 3.5 and 3.6, 3.7 and 3.8. Specific details including experimental animals and housing, experimental design, dietary treatments, *in sacco* and *in vitro* Daisy™ degradability and analytic procedures were the same as described in Chapter 7.2. Any differences are described below.

8.2.1 Preparation of samples

All feed ingredients to be included in preparation of the canola meal dietary treatments were collected during the *in vivo* apparent nutrient digestibility study (Chapter 6). Briefly, during each experimental period, a sample of each ration component was collected daily. On completion of the *in vivo* apparent nutrient digestibility study, approximately 10 g of each ration ingredient was taken from each day (across all periods) and bulked to provide a representative sample for each ingredient.

To prepare the canola meal dietary treatments the barley hay and wheat stubble were first ground through a 5 mm sieve and then these ground roughages as well as the canola meal were individually ground through a 1 mm sieve. A total of approximately 100 g (as-fed weight) of each canola meal dietary treatment was made up by weighing out the appropriate amount of each (ground) ingredient so that it represented the same proportion as in the ration fed to the steers.

Bag preparation, *in sacco* and *in vitro* Daisy™ incubation has previously discussed in Section 3.5.1. The same canola meal dietary treatment was incubated in the rumen of

steers offered the same dietary treatment (*in sacco)* and in ruminal fluid collected from steers offered the same dietary treatment inside the filter bags (*in vitro* Daisy™). The canola meal dietary treatments inside the filter bags and dacron bags were the same composition offered to the fistulated steers in Chapter 7 (nutritive value (g/kg DM) of the ration ingredients and composition (% DM) and nutritive value of the rations are presented in Tables 7.1 and 7.2.). The dietary treatments were cereal roughages with one of four inclusion levels of canola meal, viz. 0% (control), 13%, 27% or 43% on a DM basis. Either 1.0 ± 0.2 g (*in sacco* incubation) or 0.5 ± 0.2 g (*in vitro* Daisy™ incubations) of the canola meal dietary treatments were weighed into a prepared filter bag and the filter bag was sealed immediately. *In sacco* filter bags were then placed inside the dacron bags, together with a marble (to prevent the bag floating to the top of the rumen) and then the bag was tied off using fine cord, with approximately 20 cm of extra cord to suspend the bag down into the rumen. In the current thesis potential degradation refers to PD after 72 h incubation as previously described in Section 3.5.4.

8.2.2 Analytical procedures

All analytical procedures were undertaken at the FQS of NSW DPI at Wagga Wagga as described in Chapter 3.6. For *in sacco* degradability of DM loss, all six filter bags per dietary treatment, per period and per time point were used. One filter bag from each dacron bag (n=2, per dietary treatment, per period and per time point) was used for the analysis of either CP or the analysis of aNDF and ADF (analysed sequentially). For *in vitro* Daisy™ degradability of the bags incubated per rations, per period and per time point, two filter bags were used to determine DM loss, one filter bag was used to determine CP loss and one filter bag was used for the analysis of aNDF and ADF (analysed sequentially).

8.2.3 Statistical analysis

Prior to analysis, data were assessed for assumptions of normality by histograms and plotting residuals. The degradation loss and degradation kinetic parameters for the *in sacco* analysis examined the fixed effects of “ration” and “time” and the interaction between “ration” and “time” with “animal by period” examined as random effects. Least square means and differences of least square means were reported.

The degradation loss and degradation kinetic parameters for the *in vitro* Daisy™ incubation analysis examined the fixed effects of “ration/dietary treatment” and “time” and the interaction between “ration” and “time” with “animal”, “period”, “machine”

and “jar” examined as random effects. The final model included the fixed effects of “ration” and “time” and the interaction between “ration” and “time” with “animal” by “period” by “machine” examined as random effects. Least square means and differences of least square means were reported. An alpha of 0.05 was used for all statistical tests.

The relationships between *in sacco* PD and *in vitro* Daisy™ PD and *in vivo* apparent DMD or pepsin cellulase DMD were assessed using the PROC REG procedure in SAS. An alpha of 0.05 was used for all statistical tests. The *in vivo* apparent nutrient DMD for the control (0%), 15%, 26% and 47% canola meal in the ration as previously reported in Chapter 6.

8.3 Results

8.3.1 *In sacco* degradation

In sacco ruminal degradation kinetics, potential degradation after 72 h incubation (PD) and effective degradability (ED, considering outflow rates of 0.02, 0.05 and 0.08/h) of the canola meal dietary treatments are presented in Table 8.1. The DM degradability loss over time increased ($P < 0.001$) as the inclusion level of canola meal in the dietary treatments increased (Figure 8.1a). The DM degradation loss at 72 h was higher ($P < 0.05$) when the canola meal dietary treatment contained 43% canola meal compared with 13% or 27% canola meal, which was higher ($P < 0.05$) than the control dietary treatment. The DM soluble (a) fraction was highest ($P = 0.003$) for the dietary treatment containing 13% canola meal compared with all other dietary treatments. The DM slowly degradable (b) fractions, PD and ED at three different rumen outflow rates (0.02/h, 0.05/h and 0.08/h) were higher ($P < 0.05$) when the dietary treatment contained 43% canola meal compared with all other dietary treatments.

The CP degradability loss over time was higher ($P = 0.010$) when the canola meal dietary treatments contained increasing levels of canola meal (Figure 8.1b). The CP degradation loss at 72 h was higher ($P < 0.05$) when the canola meal dietary treatment contained 43% canola meal compared with 13% or 27% canola meal, which was higher ($P < 0.05$) than the control dietary treatment. The CP soluble (a), ED at three different rumen outflow rates (0.02/h, 0.05/h and 0.08/h) and RUP did not differ ($P > 0.05$) between canola meal dietary treatments. The CP PD was higher ($P = 0.033$) when the dietary treatment contained 43% canola meal compared with the control. The CP soluble (a) fraction, the fractional rate of degradation (c) and ED at any outflow rates did not vary ($P > 0.05$) between the canola meal dietary treatments.

The aNDF degradation loss over time was not significantly affected ($P = 0.102$) by dietary treatment (Figure 8.1c). In contrast, the ADF degradation loss over time was significantly ($P < 0.001$) affected by dietary treatment (Figure 8.1d). The ADF degradation loss was significantly higher ($P < 0.05$) at 48 h when the dietary treatment contained 43% canola meal compared with the control. The aNDF and ADF degradation kinetic parameters did not differ ($P > 0.05$) between the canola meal dietary treatments; however, the ADF ED at 0.05/h and 0.08/h was significantly higher when the dietary treatment contained 43% and 27% canola meal. The aNDF and ADF fractional rate of degradation (c) did not vary ($P > 0.05$) between the canola meal dietary treatments.

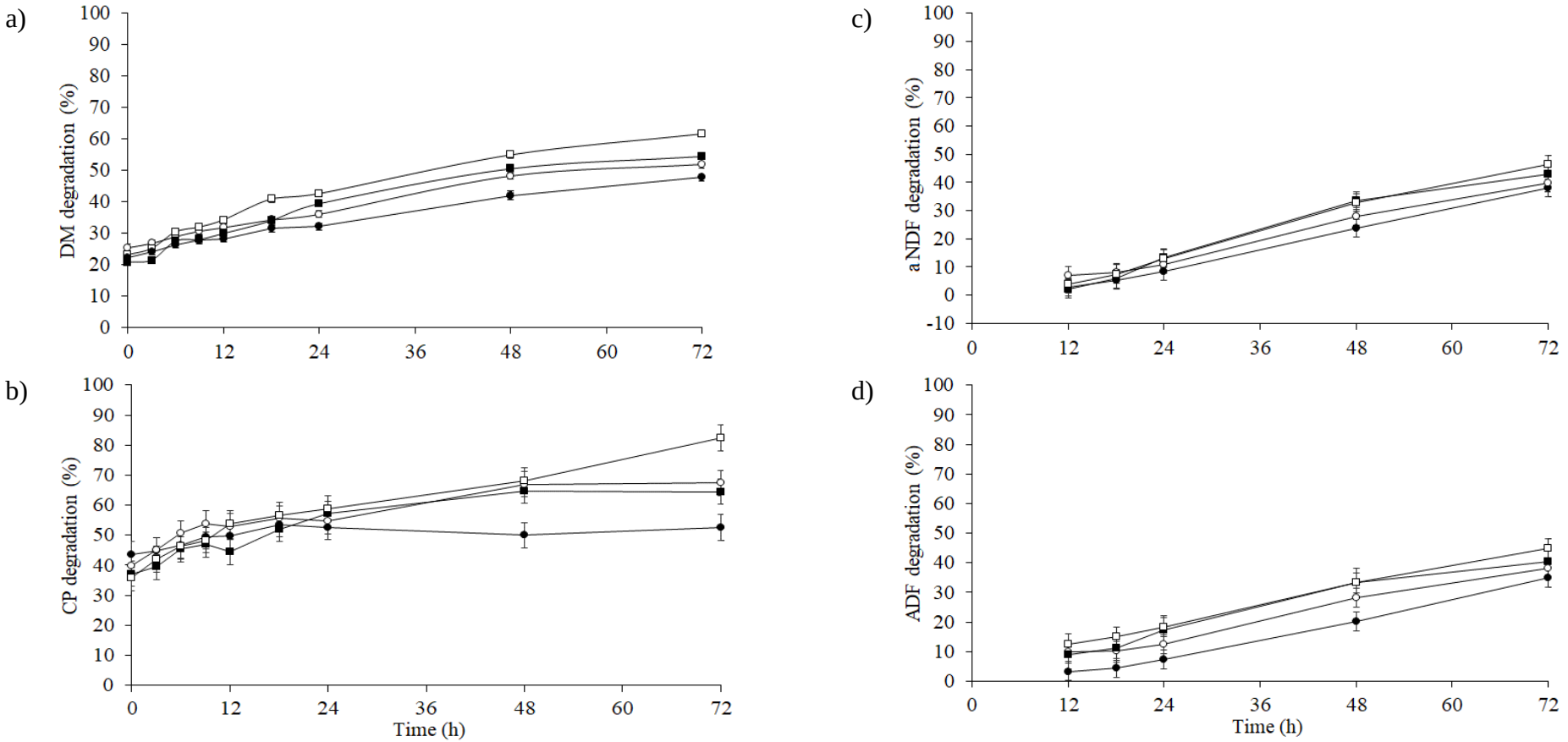


Figure 8.1 The *in sacco* predicted a) dry matter (DM), b) crude protein (CP), c) neutral detergent fibre (aNDF) and d) acid detergent fibre (ADF) degradability of the canola meal dietary treatments over a 72 h incubation period when the steers were fed the equivalent ration (same as incubated dietary treatment) containing either 0% (●), 13% (○), 27% (■) or 43% (□) canola meal.

Table 8.1 *In sacco* ruminal degradation kinetics, potential degradation after 72 h incubation (PD) and effective degradability (ED, considering outflow rates of 0.02, 0.05 and 0.08/h) of the canola meal dietary treatments when steers were fed the equivalent ration containing either 0%, 13%, 27% or 43% canola meal.

Degradation	**Dietary inclusion level of canola meal (% of DM)[2]**				***P*-value**
(%)[1]	**0**	**13**	**27**	**43**	
DM					
a	20.92 (0.93)^{ab}	24.45 (0.93)^{c}	19.55 (0.93)^{a}	21.57 (0.93)^{b}	0.003
b	26.00 (1.42)^{a}	29.25 (1.42)^{a}	34.15 (1.42)^{b}	39.16 (1.46)^{c}	< 0.001
c	0.034 (0.01)	0.029 (0.01)	0.032 (0.01)	0.038 (0.01)	0.407
PD	46.92 (1.42)^{a}	53.69 (1.41)^{b}	53.70 (1.41)^{b}	60.73 (1.46)^{c}	< 0.001
$ED_{(0.02/h)}$	36.88 (1.88)^{a}	39.72 (1.82)^{ab}	41.35 (1.88)^{b}	46.43 (1.98)^{c}	< 0.001
$ED_{(0.05/h)}$	31.46 (1.42)^{a}	34.66 (1.38)^{ab}	33.42 (1.42)^{a}	37.86 (1.50)^{b}	0.002
$ED_{(0.08/h)}$	28.85 (1.13)^{a}	32.17 (1.10)^{bc}	29.75 (1.13)^{ab}	33.77 (1.20)^{c}	0.001
CP					
a	40.53 (4.17)	37.16 (4.17)	34.13 (4.17)	32.37 (4.17)	0.550
b	9.05 (5.88)^{a}	27.77 (5.88)^{b}	27.33 (5.88)^{ab}	46.56 (5.88)^{c}	0.008
c	0.012 (0.03)	0.102 (0.03)	0.050 (0.03)	0.037 (0.03)	0.208
PD	49.58 (5.88)^{a}	64.92 (5.88)^{ab}	61.45 (5.88)^{ab}	78.93 (5.88)^{b}	0.033
$ED_{(0.02/h)}$	42.78 (7.93)	59.48 (5.61)	53.80 (5.61)	61.02 (5.61)	0.313
$ED_{(0.05/h)}$	42.03 (6.61)	54.77 (4.67)	48.07 (4.68)	51.43 (4.68)	0.470
$ED_{(0.08/h)}$	41.67 (5.62)	51.86 (3.97)	44.97 (3.97)	46.81 (3.97)	0.844
RUP	57.97 (6.61)	45.23 (4.67)	51.93 (4.68)	48.57 (4.68)	0.470
aNDF					
c	0.028 (0.01)	0.022 (0.01)	0.021 (0.01)	0.042 (0.01)	0.329
PD	38.30 (3.46)	35.55 (3.46)	31.68 (3.66)	35.06 (3.66)	0.477
$ED_{(0.02/h)}$	26.38 (4.45)	25.17 (5.14)	21.64 (5.14)	28.42 (4.45)	0.796
$ED_{(0.05/h)}$	16.27 (3.59)	15.24 (4.10)	13.31 (4.14)	19.50 (3.59)	0.716
$ED_{(0.08/h)}$	11.78 (2.92)	10.97 (3.37)	9.68 (3.37)	14.97 (2.92)	0.700
ADF					
c	0.031 (0.01)	0.045 (0.01)	0.063 (0.01)	0.073 (0.01)	0.088
PD	35.14 (4.31)	38.33 (4.25)	40.58 (4.31)	44.85 (4.37)	0.435
$ED_{(0.02/h)}$	24.79 (2.80)	26.78 (2.43)	33.02 (2.80)	35.43 (2.43)	0.052
$ED_{(0.05/h)}$	15.62 (2.65)^{a}	18.30 (2.30)^{a}	24.01 (2.65)^{ab}	26.67 (2.30)^{b}	0.040
$ED_{(0.08/h)}$	11.42 (2.41)^{a}	13.95 (2.09)^{a}	19.02 (2.41)^{ab}	21.41 (2.09)^{b}	0.042

Values are least squares means ± standard error of the least squares means. Values within a row with different superscripts are significantly different ($P < 0.05$).

[1] DM = Dry matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash. RUP = rumen undegraded protein (RUP = b [r / (c + r)] + (100 - PD) with rumen outflow rate of 0.05/h.

a = soluble fraction; b = not soluble but slowly degradable fraction; c = fractional rate of degradation of degradable fraction b (/h) from the fitted exponential equation PD = a + b (1- e^{-ct}); PD = potential degradable after 72 h incubation (a + b); ED = effective degradability (a + b [c / (c + r)] considering a rumen outflow rate (r) of 0.02, 0.05 and 0.08/h.

[2]The dietary treatment incubated *in sacco* was the same as the ration fed to the steers.

8.3.2 *In vitro* Daisy™ degradation

The DM degradation was higher ($P < 0.05$) at 12, 18, 24 and 48 h incubation when the dietary treatments contained 27% and 43% canola meal compared with the control and 13% canola meal (Figure 8.2a). As shown in Table 8.2, the DM PD after 72 h incubation, the soluble (a) fraction and the slowly degradable (b) fraction were not affected ($P > 0.05$) by the dietary treatment. The DM ED at any outflow rates (0.02/h, 0.05/h and 0.08/h) and the fractional rate of degradation (c) were higher ($P < 0.05$) when 27% and 43% canola meal was included in the dietary treatments compared with 13% canola meal but did not vary ($P > 0.05$) compared with the control.

The CP degradation losses were higher ($P < 0.001$) at 18, 24, 48 and 72 h incubation when the dietary treatments contained 27% and 43% canola meal compared with the control and 13% canola meal (Figure 8.2b). The CP slowly degradable (b) fraction was significantly different ($P = 0.044$) when the dietary treatments contained varying inclusion levels of canola meal. All other CP ruminal degradation kinetics were not significantly ($P > 0.05$) different when the dietary treatment contained varying inclusion levels of canola meal when the ruminal liquor came from steers fed varying inclusion levels of canola meal (Table 8.2).

The aNDF degradation was higher ($P < 0.001$) at 24 h incubation when the dietary treatment contained any inclusion level of canola meal, compared with the control (Figure 8.2c). The ADF degradation was higher ($P < 0.001$) at 24 and 48 h incubation when the dietary treatment contained any inclusion level of canola meal, compared with the control (Figure 8.2d). The aNDF degradation kinetics were not significantly different ($P > 0.05$); however, all ADF degradation kinetics were higher ($P < 0.05$) with any inclusion level of canola meal compared with the control dietary treatment (Table 8.2).

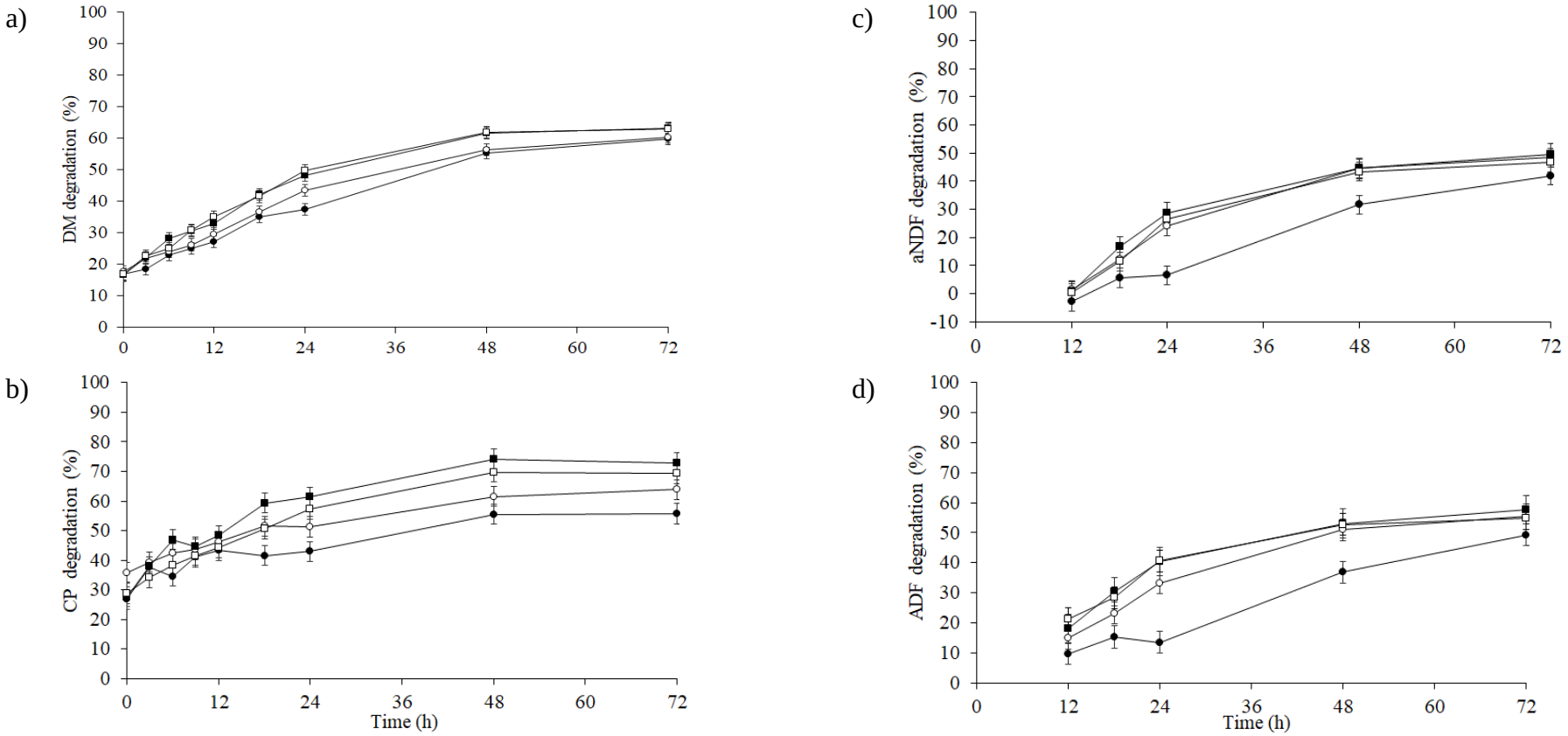


Figure 8.2 The *in vitro* Daisy™ predicted a) dry matter (DM), b) crude protein (CP), c) neutral detergent fibre (aNDF) and d) acid detergent fibre (ADF) degradability of the canola meal dietary treatments over a 72 h incubation period when the steers were fed the equivalent ration (same as incubated dietary treatment) containing either 0% (●), 13% (○), 27% (■) or 43% (□) canola meal.

Table 8.2 The i*n vitro* Daisy™ ruminal degradation kinetics, potential degradation after 72 h incubation (PD) and effective degradability (ED, considering outflow rates of 0.02, 0.05 and 0.08/h) of the canola meal dietary treatments when the ruminal liquor was collected from steers fed the equivalent ration containing either 0%, 13%, 27% or 43% canola meal.

Degradation (%)[1]	**Dietary inclusion level of canola meal (% of DM)**[2]				***P*-values**
	0	**13**	**27**	**43**	
DM					
a	18.18 (2.34)	22.77 (2.46)	20.13 (2.34)	21.17 (2.34)	0.589
b	43.60 (1.62)	39.22 (1.62)	45.06 (1.62)	44.11(1.62)	0.076
c	0.030 (0.01)^{b}	0.014 (0.01)^{a}	0.035 (0.01)^{b}	0.034 (0.01)^{b}	0.003
PD	61.78 (1.62)	62.30 (1.62)	65.19 (1.62)	65.29 (1.62)	0.256
$ED_{(0.02/h)}$	45.71 (2.45)^{ab}	39.40 (2.29)^{a}	48.42 (2.29)^{b}	48.67 (2.29)^{b}	0.028
$ED_{(0.05/h)}$	35.27 (2.13)^{ab}	31.57 (2.00)^{a}	38.48 (2.00)^{b}	38.80 (2.00)^{b}	0.032
$ED_{(0.08/h)}$	30.46 (1.79)^{ab}	28.59 (1.69)^{a}	33.66 (1.69)^{b}	34.09 (1.69)^{b}	0.039
CP					
a	29.00 (4.28)	40.41 (4.28)	33.13 (4.28)	34.63 (4.28)	0.335
b	29.00 (4.57)^{a}	28.31 (4.57)^{a}	46.19 (4.57)^{b}	40.91 (4.57)^{ab}	0.044
c	0.081 (0.01)	0.039 (0.01)	0.057 (0.01)	0.044 (0.01)	0.183
PD	58.96 (4.57)	66.21 (4.57)	76.13 (4.57)	73.38 (4.57)	0.087
$ED_{(0.02/h)}$	52.35 (4.87)	50.66 (4.87)	66.33 (4.87)	62.30 (4.87)	0.119
$ED_{(0.05/h)}$	46.66 (4.56)	44.14 (4.56)	56.57 (4.56)	52.92 (4.56)	0.208
$ED_{(0.08/h)}$	43.25 (4.05)	41.04 (4.05)	51.28 (4.05)	48.21 (4.05)	0.244
RUP	53.34 (4.56)	55.86 (4.56)	43.43 (4.56)	47.08 (4.56)	0.208
aNDF					
c	0.027 (0.01)	0.043 (0.01)	0.062 (0.01)	0.040 (0.01)	0.171
PD	42.11 (2.45)	48.62 (2.36)	49.76 (2.45)	46.76 (2.54)	0.118
$ED_{(0.02/h)}$	23.94 (3.95)	32.93 (3.43)	37.56 (3.45)	31.06 (3.48)	0.129
$ED_{(0.05/h)}$	14.88 (3.60)	22.41 (3.12)	27.51 (3.12)	21.01 (3.12)	0.131
$ED_{(0.08/h)}$	10.79 (3.14)	16.99 (2.72)	21.71 (2.72)	15.87 (2.72)	0.134
ADF					
c	0.044 (0.01)^{a}	0.073 (0.01)^{ab}	0.111 (0.01)^{b}	0.098 (0.01)^{b}	0.006
PD	49.22 (1.67)^{a}	55.40 (1.67)^{b}	57.73 (1.67)^{b}	54.80 (1.67)^{b}	0.024
$ED_{(0.02/h)}$	32.07 (2.24)^{a}	43.14 (2.24)^{b}	48.47 (2.24)^{b}	45.27 (2.24)^{b}	0.002
$ED_{(0.05/h)}$	21.57 (2.42)^{a}	32.46 (2.42)^{b}	39.16 (2.42)^{b}	35.96 (2.42)^{b}	0.002
$ED_{(0.08/h)}$	16.37 (2.31)^{a}	26.05 (2.31)^{b}	32.90 (2.31)^{b}	29.86 (2.32)^{b}	0.002

Values are least squares means ± standard error of the least squares means. Values within a row with different superscripts are significantly different ($P < 0.05$).

[1] DM = Dry matter; CP = crude protein; aNDF = neutral detergent fibre assayed with heat stable amylase and inclusive of residual ash; ADF = acid detergent fibre inclusive of residual ash. RUP = rumen undegraded protein (RUP = b [r / (c + r)] + (100 - PD) with rumen outflow rate of 0.05/h.

a = soluble fraction; b = not soluble but slowly degradable fraction; c = fractional rate of degradation of degradable fraction b (/h) from the fitted exponential equation PD = a + b (1- e^{-ct}); PD = potential degradable after 72 h incubation (a + b); ED = effective degradability (a + b [c / (c + r)] considering a rumen outflow rate (r) of 0.02, 0.05 and 0.08/h.

[2]The dietary treatment incubated *in vitro* Daisy™ was the same as the ration fed to the steers.

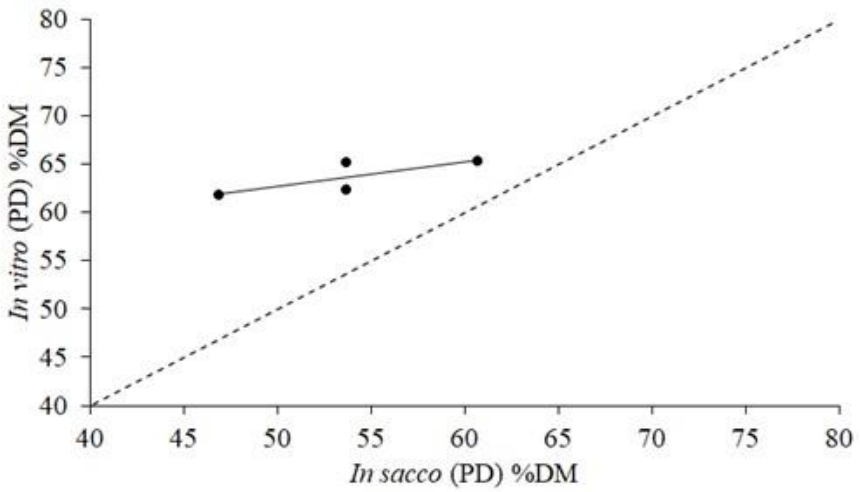


Figure 8.3 Relationship between *in sacco* potential degradability (PD) and *in vitro* Daisy™ potential degradability (after 72 h) for four dietary treatments contained varying inclusion levels of canola meal (●) when steers were fed a ration containing varying inclusion levels of canola meal. Canola meal: *In vitro* Daisy™ PD = 0.25 x *in sacco* PD + 49.99 (R^2 = 0.59, *P* = 0.201).

8.3.3 Relationship between *in sacco* and *in vitro* Daisy™

The relationship of the *in vitro* Daisy™ PD of the canola meal dietary treatments (R^2 = 0.59, *P* = 0.201) was not significantly related to *in sacco* PD (Figure 8.3). The *in sacco* PD (R^2 = 0.98, *P* = 0.012), but not *in vitro* Daisy™ PD (R^2 = 0.67, *P* = 0.183) of the four canola meal dietary treatments was significantly positively related to *in vivo* DMD (Figure 8.4). Both *in sacco* (R^2 = 0.90, *P* = 0.052) and *in vitro* Daisy™ PD (R^2 = 0.86, *P* = 0.073) were not significantly correlated with pepsin cellulase DMD (Figure 8.5).

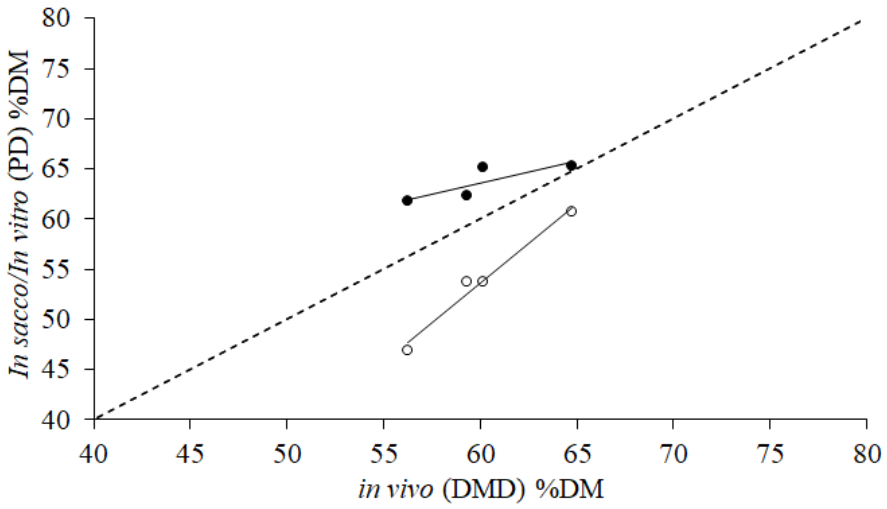


Figure 8.4 Correlation between *in vivo* dry matter digestibility (DMD, Table 6.4) and *in sacco* (○) or *in vitro* Daisy™ (●) potential degradability (PD, after 72 h) when steers were fed a ration containing varying inclusion levels of canola meal. *In sacco* PD = 1.59 x *in vivo* DMD - 41.82 (R^2 = 0.98, *P* = 0.012); *in vitro* Daisy™ PD = 0.43 x *in vivo* DMD + 37.58 (R^2 = 0.67, *P* = 0.183).

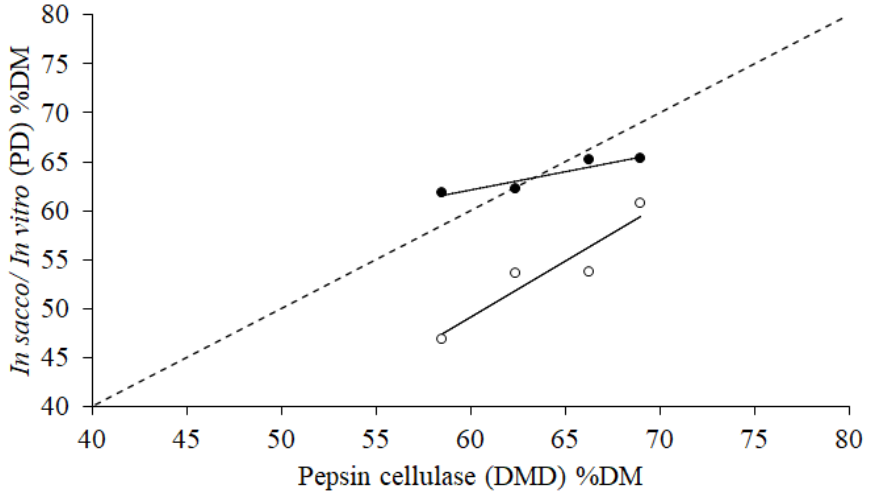


Figure 8.5 Correlation between predicted pepsin cellulase dry matter digestibility (DMD) and *in sacco* (○) or *in vitro* Daisy™ (●) potential degradability (PD, after 72 h) when steers were fed a ration containing varying inclusion levels of canola meal. *In sacco* PD = 1.42 x pepsin cellulase DMD - 35.53 (R^2 = 0.90, *P* = 0.052); *In vitro* Daisy™ PD = 0.46 x pepsin cellulase DMD + 34.84 (R^2 = 0.86, *P* = 0.073).

8.4 Discussion

The degradation kinetics for the canola meal dietary treatments varied depending on which method was used and which components (i.e., DM, CP, aNDF and ADF) were being assessed. The focus of this discussion is whether the observed *in sacco* degradability improved as the level of canola meal increased in the dietary treatments when the steers were fed a ration that was the same as the incubated dietary treatments. In addition, the effects of supplemental N into the artificial saliva on the *in vitro* Daisy™ digestion kinetics and the relationships between *in vivo* apparent DMD, *in sacco* DM PD (after 72 incubation) and *in vitro* Daisy™ DM PD (after 72 incubation) are also discussed.

8.4.1 Dry matter degradation

The *in sacco* DM PD increased as the inclusion level of canola meal in the dietary treatment increased, which was expected as *in vivo* apparent DMD increased with increasing inclusion levels of canola meal in the ration, as previously discussed in Chapter 6. The *in sacco* DM PD of the canola meal dietary treatments were lower than the *in vivo* apparent DMD values; although, highly correlated (R^2 = 0.98, Figure 8.4) as the inclusion level of canola meal increased in the ration. The *in vivo* apparent DMD values were higher compared with *in sacco* which was expected as this methodology is a measure of the total (gastrointestinal) tract digestibility, which encompasses hindgut fermentation (Rymer 2000); whereas, the *in sacco* degradation kinetic parameters only indicate the degradation of the feedstuff in the rumen (Ørskov and McDonald 1979).

The *in sacco* DM PD and the slowly degradable (b) fraction were higher when the dietary treatments contained 43% canola meal compared with the control which was expected as protein supplementation of low-quality (low N) rations increases DM degradability (Beaty *et al.* 1994; Olmos Colmenero and Broderick 2006; Cappellozza *et al.* 2021). The *in sacco* DM ED at all outflow rates were higher when the dietary treatment contained 43% canola meal compared with all other dietary treatments which was expected as vegetable protein meals, such as canola meal, have a more rapid degradation compared with concentrates and roughages (Ganev *et al.* 1979). Furthermore, the DMD of the dietary ingredients and increased ruminal NH_3 concentration when the steers were fed the 43% canola meal ration and when the 43% dietary treatments were incubated may be the reason for the higher PD and slowly

degradable (b) fraction compared with the control. The fractional rate of degradation (c) was not affected by dietary treatment which was unexpected given all other DM degradation kinetics were improved with the addition of canola meal in the dietary treatment.

The *in vitro* Daisy™ DM PD and the slowly degradable (b) fraction did not vary between dietary treatments. The lack of difference was expected as the supplemental N into the artificial saliva buffer increased the overall ruminal NH_3-N concentration of the incubation medium for adequate microbial synthesis (Satter and Slyter 1974) even for when ruminal liquor was collected from the steers fed the control ration. The relationship between *in sacco* and *in vitro* Daisy™, and *in vivo* DMD and *in vitro* Daisy™ were moderately correlated (R^2 = 0.59, Figure 8.3 and R^2 = 0.67, Figure 8.4, respectively). The *in vitro* Daisy™ DM PD values were higher than the *in sacco* PD and *in vivo* apparent DMD indicating *in vitro* Daisy™ overestimated the DM PD of the canola meal rations. Conversely, the relationship between *in sacco* and *in vitro* Daisy™ for a range of forages, concentrates and protein meals were higher (R^2 > 0.90; Tagliapietra *et al.* 2012) than the current study (R^2 = 0.59). The major difference between the two methodologies is that the *in sacco* method is considered a continuous system because the rumen experiences constant apparent turnover of microorganisms (Van Soest 1994; Valentine 2019) which does not occur in *in vitro* Daisy™ incubations, as the ruminal fluid and thus substrate supply for the microbes is not replaced. Therefore, care must be taken when comparing both between methodologies as well as with other studies.

The relationship between the predicted pepsin cellulase DMD and *in sacco* DM PD (R^2 = 0.90) method was highly correlated when compared with the *in vitro* Daisy™ DM PD (R^2 = 0.86, Figure 8.5). This suggests the *in vitro* Daisy™ values for the canola meal dietary treatments may have been overestimated, which could be due to the additional N added. Although, it would be expected that the *in sacco* values would be higher due to the addition of feed particles, refreshment of substrate supply for microbes (through feed intake) and larger microbial populations including a larger feed particle associated microbial population (Leng 2014). Despite this, the supplemental N may be the reason why the *in vitro* Daisy™ results were not different and higher compared with the *in sacco DM PD* and the *in vivo* apparent DMD.

Increasing inclusion level of canola meal in the dietary treatments the DM soluble (a) fraction did not differ for the *in vitro* Daisy™ method but varied, although not consistently, for the *in sacco* methodology. The washing of the bags used in these

methodologies is a subjective procedure and the probability of DM loss may vary between different methodologies, different feeds and at different times (Chaudhry and Webster 2001; Chaudhry and Mohamed 2011). The *in sacco* bags were first rinsed in a bucket of cold water before being placed in a washing machine, whereas the *in vitro* Daisy™ filter bags were washed in the incubation jars with continuous running cold water until the water ran clear. This difference in washing methods may also have contributed to the difference in the degradability values between methodologies.

8.4.2 Crude protein degradation

The *in sacco* CP PD increased in response as the inclusion level of canola meal increased. This aligned with the increase in *in vivo* apparent CPD as the level of canola meal in the ration fed to the steers increased as previously reported in Chapter 6. Similarly, CPD also increased when steers were fed increasing levels of soybean meal in addition to *ad libitum* wheat straw (Beaty *et al.* 1994).

The *in sacco* degradation kinetic parameters of a ration containing varying inclusion levels of canola has not previously been reported; however, the degradation of canola meal itself using the *in sacco* technique has. The CP PD and ED with an outflow rate of 0.05/h was 88.7% and 65.8% respectively, when 100% canola meal was incubated in the rumen for 48 h of steers fed a concentrate mix and lucerne-brome hay (Ha and Kennelly 1984). Similar results to those of the current study were obtained when sheep were fed lucerne hay and canola meal (Eghbali *et al.* 2011). The PD and ED at an outflow rate of 0.05/h, when the ration contained 43% canola meal, were 78.9% and 51.4%, respectively, indicating the canola meal used in the current study was highly degraded in the rumen.

The *in sacco* CP PD increased with increasing the inclusion level of canola meal; however, the PD for the control dietary treatment was lower (49.58 ± 5.88 %) using the *in sacco* method compared with the *in vitro* Daisy™ method (58.96 ± 4.57 %). The CP PD values for the 43% canola meal dietary treatment values were similar between assays. Although, as previously discussed in Chapter 7, the addition of N for the *in vitro* Daisy™ would have ensured the ruminal NH_3-N concentrations for the ruminal liquor collected from steers fed the control and the 13% canola meal ration was not limiting for microbial synthesis (Satter and Slyter 1974; Slyter *et al.* 1979). The addition of the control ration used as a standard for both methodologies would confirm

if the improved CP PD was due to the increasing inclusion levels of canola meal or the combination of ration ingredients.

The *in sacco* CP slowly degradable (b) fraction increased with increasing inclusion levels of canola meal which was expected due to the increasing CP content of the dietary treatment. Conversely, the *in vitro* Daisy™ CP slowly degradable (b) fraction varied with increasing the inclusion levels of canola meal in the dietary treatment; however, the reason for this is not readily apparent.

The CP ED were similar for both methods; however, the *in vitro* Daisy™ ED at an outflow rate of 0.02/h for the control dietary treatment was numerically higher than the *in sacco* values. This could be due to the addition N in the artificial rumen buffer solution in the *in vitro* Daisy™ method compared with the *in sacco* method. In general, when both the diet fed to the donor animal and the sample had low CP concentrations (less than 90 g/kg), such as the control in this study, variation among donor animals was greater than when diets fed to donor animals and samples contained higher concentrations of CP (Weiss 1994).

8.4.3 Fibre degradation

Forage iNDF consists of indigestible fraction and potentially digestible fraction (PDNDF) and thus, to determine the NDF digestibility of a forage, it is critical to accurately predict iNDF (Bender *et al.* 2016). Therefore, the NDF components (hemicellulose, cellulose and lignin) has been assumed to be zero (a = 0) as NDF is not immediately degradable in the rumen and therefore, not soluble (Chaudhry 2000; Spanghero *et al.* 2007; Raffrenato *et al.* 2009). In addition, there is often a lag phase before the onset of fermentation of fibre degradation (Chaudhry 2000; Yu *et al.* 2004; Raffrenato *et al.* 2009; Raffrenato and Van Amburgh 2010) which was also evident in this study where the lag phase was 12 h after initial incubation for both methods.

Fibre digestibility typically improves with the addition of protein supplementation to low-quality roughages as a result of increased microbial activity (Leng 1990; DelCurto *et al.* 2000). In addition, the NDF degradability of forages were positively correlated with dietary CP content (Hoffman *et al.* 1993). Conversely, the *in sacco* aNDF PD was not affected by increasing the inclusion level of canola meal in the ration, which is similar to *in vivo* apparent aNDFD results reported in Chapter 6. The lack of difference in PD of aNDF and aNDFD was expected as the inclusion level of canola meal in the dietary treatment and/or the ration fed to the steers increased, the CP content also increased, whereas the fibre content decreased. The NDF (and ADF) PD of guinea

grass (*Panicum maximum*) was not affected when sheep were fed protein meals and *ad libitum* guinea grass; however, loss of NDF (and ADF) overtime was increased using the *in sacco* method (Jetana *et al.* 1998) which was similar to the current study. Furthermore, the *in vitro* Daisy™ NDF PD did not differ between dietary treatments which was expected given the lack of difference between apparent *in vivo* aNDFD and *in sacco* aNDF PD.

The *in sacco* ADF PD was not affected by dietary treatments, which were similar to the apparent *in vivo* ADFD (Chapter 6). Although, the *in vitro* Daisy™ ADF PD was higher with any inclusion level of canola meal compared with the control dietary treatment, which was unexpected as the artificial buffer solution contained addition N, which is within the suggested range to support microbial synthesis (Satter and Slyter 1974) (Table 7.4). The *in sacco* ADF ED at high outflow rates (0.05/h and 0.08/h) were significantly higher when the dietary treatment contained 27% or 43% canola meal; however, the reason(s) for this increase is unclear. Therefore, use of a low-quality roughage (such as used for the control standard) as a second standard would confirm whether NDF and ADF degradation kinetics are improved with increasing the level of canola meal in a ration or the ration components.

The *in sacco* aNDF digestion kinetic numerical values were lower compared with the *in vitro* Daisy™ numerical values, which could have been due sample size (Valente *et al.* 2011; Valentine *et al.* 2019), the composition of the buffer solution with the addition of N (Trujillo *et al.* 2010), and ruminal liquor (Marten and Barnes 1979). The ratio of sample amount, pore and grind size must be considered, to ensure adequate loss of soluble fraction and fine particulates without over and/or underestimating the slowly degradable fraction of the feedstuff as larger pore sizes can cause barrier effect by blocking the bag's pores and limiting the passage of ruminal fluid in and out of the bag (Cattani *et al.* 2009). The sample size for the *in vitro* analysis was 0.5 ± 0.02 g, which is recommended (Mabjeesh *et al.* 2000; Damiran *et al.* 2008). Conversely, 0.75 g of sample was suggested after incubation and doing sequential NDF/ ADF to ensure there is enough residual remaining for ADF analysis (Raffrenato *et al.* 2018). Therefore, if sequential NDF and ADF of samples is required, the sample size must be considered prior to incubation.

8.5 Conclusion

Supplementing donor steers up to 43% canola meal in a low-quality ration had no negative effects on the degradation of the canola meal dietary treatments when using the *in sacco* or *in vitro* Daisy™ methods. The *in sacco* DM and CP PD improved as the inclusion level of canola meal in the dietary treatment increased; however, fibre PD was not affected by the dietary treatments. Conversely, the *in vitro* Daisy™ DM, CP and NDF PD of the dietary treatments were not affected because of the additional N in the artificial saliva buffer solution; however, ADF PD was higher when the dietary treatments contained any inclusion level of canola meal. Furthermore, the DM PD for the *in vitro* Daisy™ appeared to overestimate the DM PD for the *in sacco* method when the dietary treatments contained increasing inclusion levels of canola meal. In addition, the *in sacco* PD had a higher correlation with the *in vivo* DMD compared with the *in vitro* Daisy™ indicating the *in sacco* methodology closely aligns with *in vivo* DMD as the preferred method when evaluating a feedstuff and/or ration. Further research to investigate the degradation kinetics of a low-quality roughage, such as the control ration, when steers are fed varying inclusion levels is warranted.

Chapter 9: General Discussion

A combination of pen-feeding and animal house studies were undertaken to investigate the maximum inclusion level of canola meal as a PCAS approved supplement to roughages typical of that normally available for grass-fed beef cattle during the summer-autumn period in southern NSW. The results of these studies indicated that canola meal can be supplemented at up to 43% of total DMI without any adverse effects on ADG, DMI, FCR, apparent nutrient digestibilities or ruminal parameters. Furthermore, an upper limit between 46% and 68% inclusion level of canola meal was established for cattle fed a low-quality ration. The following discussion highlights the production responses, practical suggestions, ruminal parameters and meat and eating quality responses and the effects on digestibility and degradation kinetics of increasing the inclusion levels of canola meal to cattle offered low quality diets.

9.1 Production responses and practical suggestions

9.1.1 Production responses

Dry matter intake and ADG were higher when steers were fed 34% and 46% canola meal compared with 20% canola meal; however, there was a decline in DMI and ADG when canola meal was fed at 68% of total DMI. The polynomial relationship between actual MEI and ADG indicated there was an upper inclusion level of canola meal between 46% and 68% of total DMI (Figure 5.4). Measuring ruminal NH_3-N concentrations could have provided additional information to help explain the decrease in ADG and DMI when the steers were fed 68% canola meal. Feeding high levels of RDP (as contained in canola meal) was found to elevate plasma urea concentrations and consequently decrease conceptions rates in cattle (Canfield *et al.* 1990; Butler 2000; Doran *et al.* 2022). Therefore, the long-term effects of supplementing high levels of canola meal to cattle, in particular breeding cattle warrants further investigation.

Feeding high levels of rapeseed meal has been associated with decreased DMI and ADG due to high concentrations of glucosinolates (Lardy and Kerley 1994), although, canola meal contains low levels of these compounds (Mailer 2004; Obied *et al.* 2013). The glucosinolates concentrations of canola meal used in the pen-feeding study was

not analysed. The estimated glucosinolates concentrations when the weaners were fed ration containing 68% canola meal would likely range from 5 to 18 µmol g^{-1} (Ayton 2014). Dietary glucosinolates concentration of 11 µmol g^{-1} or less are considered safe to feed to cattle (as review by Tripathi *et al*. 2007). Therefore, the glucosinolates concentrations associated with feeding 68% canola meal may have been the reason for the decrease in DMI and ADG of the weaner cattle.

The increased ADG response to feeding 34% and 46% canola meal compared with the 20% canola meal was expected as the M/D and CP content of these rations were higher. Protein meals can improve DMI, ME utilisation, MEI and ADG by correcting N deficiencies, increasing post-ruminal supply of protein and AA, and/or correction of AA deficiency or imbalances at the tissue level (McCollum and Horn 1990). In addition, increases in ADG have been well documented for ruminants fed a low-quality roughage, cereal straw or hay diet and supplemented with protein sources such as cottonseed meal that provide additional N for rumen microorganisms (Hennessy *et al.* 1981; Hennessy *et al.* 1983; Lee *et al.* 1985; Smith and Warren 1986a, 1986b; Dixon *et al.* 2017; McLennan *et al.* 2017a; McLennan *et al.* 2017b).

The optimal inclusion level of canola meal in weaner cattle rations was between 34% and 46%, as ADG did not varying between these two levels of supplementation. Thus, supplementing canola meal at between 1.0% LW/d and 1.5% of LW/d to weaners during low-quality pasture availability allows them to gain weight and comply with PCAS accreditation. A farm case study modelling the economics of supplementing up to 34% canola meal and selling cattle that are PCAS approved would be useful; however, it was outside the scope of the research reported in this thesis.

A roughage only diet was not included in the feedlot study (Chapter 5) due to the low-quality of the roughage used; feeding weaner cattle this as a sole diet would likely have resulted in weight loss. Production responses of weaners and/or cattle grazing low-quality roughage or stubble in a paddock situation in response to varying supplementation levels of canola meal warrants further investigation. The effects of frequency of feeding and delivery mechanism of canola meal in paddocks and the subsequence effects of animal responses and meat and eating quality is also required before practical recommendations can be given.

9.1.2 Ruminal parameters

No comparative studies have evaluated ruminal parameters when high levels (greater than 40% of total DMI) of canola meal have been used to supplement low-quality roughage rations (Chapter 7). Ruminal pH was not affected by increasing the inclusion level of canola meal in the ration and thus would not induce ruminal acidosis (Packer *et al.* 2011).

Total VFA concentrations were higher when the ration contained 43% canola meal compared with all other rations, but remained within the normal expected range of 70 to 130 mmol/L (France and Dijkstra 2005). Increased dietary M/D (and CP) has been associated with higher total VFA concentrations (Putnam *et al.* 1966; Furchtenicht and Broderick 1987; Cui *et al.* 2019; Ahmad *et al.* 2020). The molar proportion of acetic acid was lower and that of propionic acid was higher when the ration contained 43% canola meal. Propionic acid is the major contributor for gluconeogenesis in the body (Bergman 1990; Dijkstra 1994; France and Dijkstra 2005), thus including 43% of canola meal in the ration not only resulted in an improvement in the overall efficiency of feed utilisation but may have increased gluconeogenesis.

The increase in ruminal NH_3-N concentrations with increasing the inclusion level of canola meal in the ration was expected given the high CP (and RDP) content of the canola meal. Similarly, ruminal NH_3-N concentrations were higher when increasing levels of soybean meal were offered to cattle with *ad libitum* wheat straw (Beaty *et al.* 1994).

9.1.3 Meat and eating quality

Carcase quality and consumer sensory traits were not altered when finishing cattle were supplemented with either canola meal or a grain-based pellet (Chapter 4). The overall eating quality value, assessed by the consumer sensory analysis ranked the products as “good everyday quality” (Polkinghorne *et al.* 2008; Watson *et al.* 2008). These results support research from Canada, where grading data were not altered when steers were offered canola meal compared with soybean meal (Good 2018) or when

canola meal replaced barley grain (He *et al*. 2013) in a TMR. Some meat quality traits were altered as a result of ageing period which was expected (Holman *et al.* 2019); however, there was no interaction with dietary treatment. Therefore, canola meal could be used as an approved PCAS supplement for cattle without impacting on meat and eating quality; however, the acceptance of canola meal by certified grass-fed beef producers would depend on an economic evaluation.

The canola meal used in the feeding trials was solvent extracted and the residual oil is high in PUFA (He *et al.* 2013). Polyunsaturated fatty acid and CLA concentrations were higher when canola meal was included in PMR for beef feedlot cattle (Mir *et al.* 2003; Dugan *et al.* 2011; He *et al.* 2013), which may have beneficial human health benefits (such as increasing omega-3 in meat). Therefore, further investigation of the FA profiles of beef when steers are fed rations containing varying inclusion levels of canola meal would be useful given increasing consumer demand for products with human health benefits (Holman *et al.* 2019).

9.2 Digestibility and degradability techniques

9.2.1 Ruminal fluid

Currently, when undertaking *in vitro* digestibility studies there is no "standard" feedstuff/s that the ruminal fluid donor animal(s) should be fed. Ruminal liquor used for *in sacco* and *in vitro* methodologies can represent the largest source of uncontrolled variation (Marten and Barnes 1979; Holden 1999; Wilman and Adesogan 2000). The degradation kinetics of the lucerne standard for both the *in sacco* or the *in vitro* Daisy™ studies were not consistently affected by the ration fed to the steers.

The *in sacco* DM PD of the lucerne standard was lower when 27% compared with 13% and 43% canola meal was included in the rations fed to the donor steers; however, the increase in NH_3-N concentration did not fully explain the differences between rations. The provision of additional N to the artificial saliva buffer solution used in the *in vitro* Daisy™ method did not alter the DM PD of the lucerne standard. The lucerne standard used was high quality (relatively high CP content) which could have accounted for the lack of difference in the degradation kinetics between methods. Including a range of standard feedstuffs for *in sacco* and *in vitro* Daisy™ studies would

enable variations in rumen microbial activity to be taken into account (Michalet-Doreau and Ould-Bah 1992).

The inclusion of a low-quality roughage standard, such as the control ration (used in Chapter 6), may have allowed the determination of whether the observed differences in apparent nutrient digestibility (Chapter 6) were due to either an increase in the digestibility of the low-quality basal diet and/or inclusion of the canola meal *per se*. Monitoring of pH, and NH_3-N and VFA concentrations during the *in vitro* Daisy™ incubation would provide information on microbial substrate supply and microbial activity which may help explain the observed differences when comparing *in sacco* and *in vitro* degradation kinetics.

9.2.2 Digestibility and degradability of canola meal

Increasing the inclusion level of canola meal up to 47% in the ration had no negative effects on apparent nutrient digestibility (DMD, OMD and DOMD; Chapter 6). The *in sacco* DM PD after 72 h incubation was improved as the inclusion level of canola meal in the dietary treatment increased which was expected as protein supplementation of low-quality (low N) rations increases DM degradability (Beaty *et al.* 1994; Olmos Colmenero and Broderick 2006; Cappellozza *et al.* 2021). The increase in DMD could have also been due to the differences in the proportions and the DMD of the dietary ingredients (roughages and canola meal) in the ration offered to the steers. Furthermore, the addition of each dietary ingredient during incubation (*in sacco* and *in vitro* Daisy™) would have provided additional information regarding the degradation kinetics of the canola meal dietary treatments. Conversely, *in vitro* Daisy DM PD after 72 h of incubation the dietary treatments was not affected (Chapter 8). This was expected as the supplemental N in the incubation medium liquor would have overcome any potential limitations of N supply for the microorganisms (Satter and Slyter 1974), even when ruminal liquor was collected from the steers fed the control (low-quality roughage) ration. Crude protein digestibility and degradability kinetics and losses increased with increasing inclusion levels of canola meal in the ration, which was expected as the amount of CP also increased in each of the rations. The minimum threshold ruminal NH_3-N concentrations to support microbial synthesis was

only met when 27% and 43% canola meal was included into the ration fed to the fistulated steers (Chapters 7 and 8). In addition, the increase in CPD and CP PD after 72 h incubation for *in sacco* assay could have also been due to the CP content in the proportions of the dietary ingredients (roughages and canola meal) in the ration offered to the steers.

In vivo fibre digestibility and *in sacco* fibre PD were not affected by the dietary treatments. Conversely, the *in vitro* Daisy™ NDF PD of the canola meal dietary treatments were not affected by the additional N in the incubation medium. The *in vitro* Daisy™ PD of ADF was higher when the dietary treatments contained any inclusion level of canola meal. This was unexpected as the incubation medium contained additional N. Although, it could be that aNDF and ADF PD had not reach a plateau by 72 h of incubation. Extending the incubation time beyond 72 h may have provided additional information about the maximum aNDF and ADF PD of the lucerne standard and the dietary treatments. Longer incubation times as previously described by Raffrenato et al (2018) and Mertens (2015) focusing on iNDF. Furthermore, the degradation kinetics after 72 incubation were similar to the *in vivo* apparent nutrient DMD results, supporting that the 72 h incubation period was appropriate.

9.2.3 Technique relationships

The DM PD for the *in vitro* Daisy™ incubation was higher ($R^2 = 0.59$) compared with the DM PD for the *in sacco* method, when the (incubated) dietary treatments contained increasing inclusion levels of canola meal. The DM PD for the *in vitro* Daisy™ appeared to overestimate the DM PD for the *in sacco* method when the dietary treatments contained increasing inclusion levels of canola meal. The difference could have been due to the sample size (*in sacco* 1.0 g ± 0.02 g and *in vitro* Daisy™ 0.5 ± 0.02 g, respectively), pore ratio, grind size, placing filter bags inside dacron bags, washing technique and/or the addition of N into the *in vitro* Daisy™ artificial buffer solution. Furthermore, the number of filter bags incubated for the canola meal dietary treatments were less than those for the lucerne standard and thus there were a lower number of replicates used in the analyses, which could have resulted in a larger error. Therefore, when choosing the appropriate method to estimate digestibility or degradability of feedstuff/s the overall objective of the study must be considered first.

The addition of urea and ammonium sulphate (Kaiser *et al.* 2007) to the incubation medium for the *in vitro* Daisy studies was to ensure an adequate supply of N (Schmid *et al.* 1969). The differences between the *in sacco* and *in vitro* assay could have been due to the supplemental N, especially since threshold ruminal NH_3-N concentrations to support microbial synthesis were only meet when 27% and 43% canola meal were included in the ration fed to the (ruminal fluid donor) steers (Chapter 7 and 8). A limitation of this study was not being able to determine the effect of supplemental N and the rate of degradability between *in sacco* and *in vitro* Daisy. Further investigation into the effect of supplemental N into the incubation medium for a range of feedstuff/s is warranted.

9.3 Conclusion

An upper limit of between 46% and 68% inclusion level of canola meal was established for weaner cattle fed a low-quality ration. Average daily gain, DMI, FCR and apparent nutrient digestibility were improved when weaners and steers fed a low-quality ration and supplemented with 47% canola meal. Meat and eating quality were not adversely affected when supplementing canola meal. Supplementing cattle fed low-quality roughage with canola meal up to 47% had no detrimental effects on apparent nutrient digestibilities. Similarly, supplementing donor (of ruminal fluid) steers fed a low-quality ration up to 43% canola meal had no negative effects on the degradation of either lucerne hay standard or canola meal dietary treatments when using the *in sacco* or *in vitro* ANKOM Daisy™ incubator methods. The *in sacco* was found to be the preferred method when evaluating rumen parameters and associated digestion kinetic parameters compared with the *in vitro* Daisy™. Canola meal can be supplemented at up to 1.5% of LW as an approved PCAS supplement for grass-fed cattle grazing low-quality roughages without any adverse effects; however, further economic consideration and practical application of supplementing canola meal in a farm setting is warranted.

Chapter 10: References

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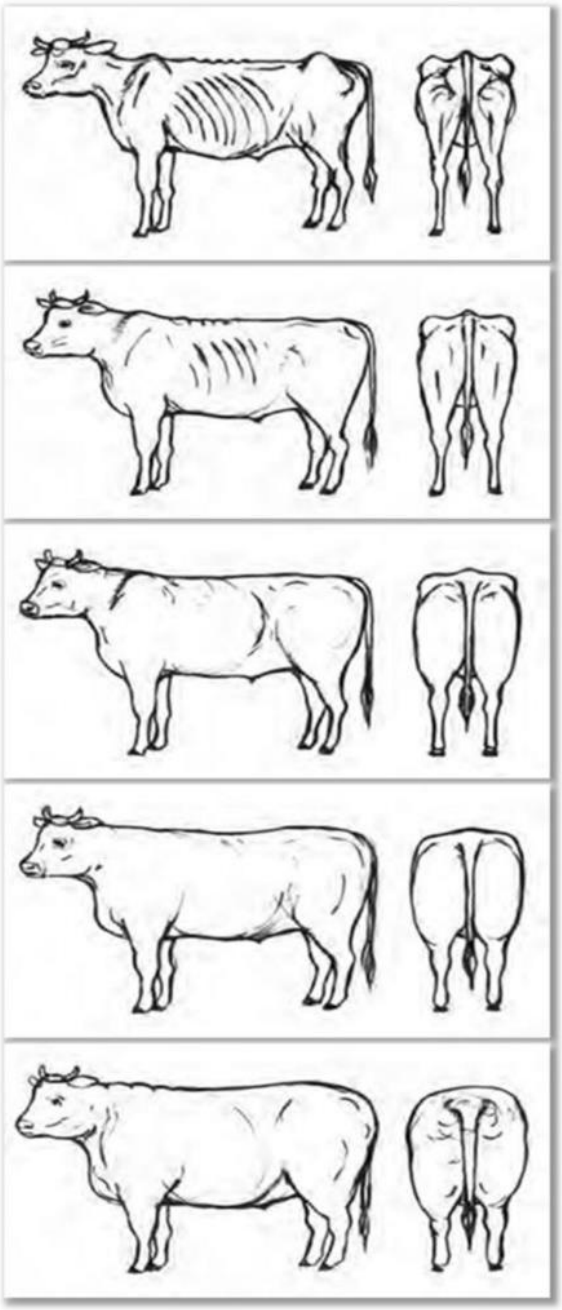
Appendix A - Cattle Body Condition Scoring



Condition score 1
Backbone prominent.
Hips and shoulder bones prominent.
Ribs clearly visible.
Tail-head area recessed.
Skeletal body outline.

Condition score 2
Backbone visible.
Hips and shoulder bones visible.
Ribs visible faintly.
Tail-head area slightly recessed.
Body outline bony.

Condition score 3
Hip bones visible faintly.
Ribs generally not visible.
Tail-head area not recessed.
Body outline almost smooth.

Condition score 4
Hip bones not visible.
Ribs well covered.
Tail-head area slightly lumpy.
Body outline rounded.

Condition score 5
Hip bones showing fat deposit.
Ribs very well covered.
Tail-head area very lumpy.
Body outline bulging due to fat.

Figure A.1 Schematic of beef cattle body condition scoring (1 out of 5; source Queensland Department of Agriculture and Fisheries, 2019).