EFFECT OF REPLACING CALF STARTER FEED WITH LUCERNE LEAF-MEAL ON DIET INTAKE, RUMEN DEGRADATION AND GROWTH OF HOLSTEIN HEIFER CALVES

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture has not previously been submitted by me for a degree at this or any other university, that it is my own work in design and in execution, and that all material contained herein has been duly acknowledged.

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Mangena JL Date

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ABSTRACT

A series of experiments were carried out to investigate the effect of replacing calf starter feed with lucerne leaf-meal (LLM) on diet intake, rumen degradation and growth of Holstein heifer calves. The treatments were calf starter pellets (PEL), a mixture of 65% calf starter concentrate pellets and 35% LLM ($P_{65}L_{35}$), and a mixture of 50% calf starter concentrate pellets and 50% LLM ($P_{50}L_{50}$). The first part of the study determined nutrient composition of lucerne leaf-meal (LLM), calf starter pellets and lucerne leaf-meal substituted diets. The experimental design was a completely randomised design (CRD). Lucerne leaf-meal had high protein (25% DM) and gross energy (GE) (16.2 MJ/kg DM) levels; low starch (0.2% DM) and fibre fractions. All diets had similar ($P>0.05$) crude protein (CP) and GE values. The concentrate diet was higher ($P<0.05$) in starch, ether extracts and fibre bound CP (neutral detergent insoluble crude protein).

The second part of the study estimated the supply of energy and protein fractions and carbohydrate composition from LLM and the diets. A CRD was used. Lucerne leaf-meal had high energy density and protein supply with low unavailable fibre and protein contents. All the treatments had TDN above 80%. Non-fibre carbohydrate levels differed ($P<0.05$) across treatments. The energy fractions were similar ($P>0.05$) across all dietary treatments. However, Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ had higher ($P<0.05$) soluble and non-fibre carbohydrates than PEL diet.

The third part of the study determined *in vitro* degradation of LLM and the three dietary treatments using the ANKOM Daisy^{II} incubator system. Lucerne leaf-meal had high *in vitro* dry matter (IVDMD), organic matter (IVOMD), crude protein (IVCPD) and neutral detergent fibre (IVNDFD) degradation. All diets had similar ($P>0.05$) IVDMD and IVOMD at 0, 4, 10 and 48 hours of incubation. Higher ($P<0.05$) IVNDFD, IVCPD and effective degradation (ED) were observed in Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ than in Diet PEL. No differences ($P>0.05$) in IVNDFD and IVCPD were observed at 24 and 48 hours of incubation. The rate of degradation ('*c*') was similar ($P>0.05$) across all the diets. The data demonstrated that LLM diets had higher ($P<0.05$) degradation values than Diet PEL.

The fourth part of the study determined the effects of replacing calf starter pellets with lucerne leaf-meal on diet intake, feed conversion ratio and growth of pre-weaned (21 to 42 days old calves) and transition (43 to 56 days old calves) Holstein heifer calves. The experimental design was a completely randomised design, with a total of 24 calves housed in individual pens. This study was divided into two experimental phases, namely, pre-weaning (Experiment 1) and transition (Experiment 2) phases. In each experiment, different calves were used. Body weights were taken weekly. The balance of ruminal nitrogen (% RNB) was predicted using Large Ruminant Nutrition System (LRNS) model. In Experiment 1 calves had free access to clean water and fed 4 litres/calf/day of unpasteurised milk. During the pre-weaning phase, differences ($P<0.05$) were observed in solid feed dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and starch intakes with Diet $P_{50}L_{50}$ having higher ($P<0.05$) intakes than Diets PEL and $P_{65}L_{35}$. Similarly, higher ($P<0.05$) % RNB and daily weight gains (ADG) were observed with calves on Diet $P_{50}L_{50}$. However, calves had similar ($P>0.05$) initial and final weights and feed conversion ratio (FCR).

Calves in Experiment 2 were fed 2 litres/calf/day of unpasteurised milk. Calves were weaned at the age of 56 days. During the transition phase, calves on Diet $HP_{50}L_{50}$ had higher ($P<0.05$) CP intake and % RNB than those on HPEL and $HP_{65}L_{35}$ diets. However, higher ($P<0.05$) solid feed starch intake was observed with calves on Diet HPEL. All dietary treatments had similar ($P>0.05$) DM intake, initial and final live weights, ADG and FCR.

The fifth part of the study predicted diet concentrations of Holstein heifer calves under specific conditions using the level 1 solution of Large Ruminant Nutrition System (LRNS) model. During the pre-weaning phase, $P_{65}L_{35}$ and $P_{50}L_{50}$ diets indicated higher ($P<0.05$) energy density values than Diet PEL. However, all diets had similar ($P>0.05$) metabolisable energy levels. No differences ($P>0.05$) in net energy for maintenance (NEm) and gain (NEg) during the transition phase were observed. However, Diet HPEL had higher ($P<0.05$) apparent TDN and ME levels compared to other dietary treatments. Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ had higher ($P<0.05$) protein, energy density and degradation values than Diet PEL. It is concluded LLM inclusions in the calf diet improved Holstein heifer calves' performance.

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LIST OF ABBREVIATIONS

'a '	Soluble fraction in the equation PD = *a*+ *b* (1 - e^{-ct}).
ADF	Acid detergent fibre.
ADG	Average daily gain.
ADICP	Acid detergent insoluble crude protein.
ADIN	Acid detergent insoluble nitrogen.
ADL	Acid detergent lignin.
ANOVA	Analysis of variance.
AOAC	Association of Analytical Chemists.
AS	Cellulose (washed off in ADL solution).
ATMS	Agricultural Modelling and Training System.
'b'	Insoluble slowly degraded fraction in the equation PD = *a*+ *b* (1 - e^{-ct}).
'c'	Fractional degradation rate in the equation PD = *a*+ *b* (1 - e^{-ct}).
Ca	Calcium.
CA	Sugar.
$CaCl_2.2H_2O$	Calcium chloride.
CB_1	Starch.
CB_2	Available fibre.
CC	Unavailable fibre.
CHO	Carbohydrates.
CNCPS	Cornell Net Carbohydrate and Protein System.
CO_2	Carbon dioxide gas.
$CoCl_2.6H_2O$	Copper chloride.

CP	Crude protein.
CRD	Completely randomised design.
DE	Digestible energy.
DM	Dry matter.
DMI	Dry matter intake.
dTDN	Digestible total digestible nutrient.
ED	Effective degradation.
EE	Ether extract.
FAO	Food and Agricultural Organisation.
FCR	Feed conversion ratio.
$FeCl_3.6H_2O$	Iron chlorite.
g	Grams
GE	Gross energy.
GLM	General linear model
HCL	Hydrochloric acid.
h	Hours
IADICP	Indigestible acid detergent crude protein.
INRA	Institut National de la Recherche Agronomique. French National Institute for Agricultural Research.
IVCPD	*In vitro* crude protein degradability.
IVDMD	*In vitro* dry matter degradability.
IVNDFD	*In vitro* neutral detergent fibre degradability.
K	Potassium.

kg	Kilogram.
KOH	Potassium hydroxide.
Kp	Fractional passage rate.
L	Litres
Lig	Lignin.
LIGNIN	Lignin of the feedstuff's neutral detergent fibre.
LLM	Lucerne leaf-meal
LRNS	Large Ruminant Nutrition System
LSD	Least significant difference.
m	Metres
ME	Metabolisable energy.
Mg	Magnesium.
$MgCl_2.4H_2O$	Magnesium chloride.
$MgSO_4.7H_2O$	Magnesium sulphide.
ml	Millilitres.
N	Nitrogen.
Na_2CO_3	Sodium carbonate
$Na_2S.9H_2O$	Sodium sulfide
NaCl	Sodium chloride.
NDF	Neutral detergent fibre
NDFn	Neutral detergent fibre corrected for nitrogen.
NDICP	Neutral detergent insoluble crude protein.
NDIN	Neutral detergent insoluble nitrogen.
NEg	Net energy for gain.

NEm	Net energy for maintenance.
NFC	Non-fibre carbohydrates.
NH_3	Ammonia.
NLIN	Non-linear.
NPE	Non-polar extracts (dissolved in the NDF wash).
NPN	Non-protein nitrogen.
NRC	National Research Council.
ºC	Degree centigrade.
OM	Organic matter.
OMD	Organic matter digestibility.
P	Phosphorus.
PA	Non-protein nitrogen.
PB_1	Rapidly degraded protein.
PB_2	Intermediately degraded protein.
PC	Bound protein.
PD	Potential degradation of dry matter at the incubation time '*t*'.
PEL	Calf starter pellets.
pH	The whole number referring to the number of hydrogen ions present. Negative logarithm of the hydrogen ion concentration.
PROC NLIN	Non-linear procedures.
RH	Relative humidity.
RNB	Ruminal nitrogen balance.
SAS	Statistical Analysis System.
SEM	Standard error of the means.

SOLP	Soluble protein.
TDN	Total digestible nutrient.
TDN_{1x}	Total digestible nutrients at maintenance level of intake;
VFA	Volatile fatty acids.
HS	Hemicellulose (washed off in the ADF solution).

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Forages constitute the basic diets of ruminants. Prime forages such as *Medicago sativa* (lucerne) have been used in the production of many animal species (Gawel and Grzelak, 2012). Lucerne is a high quality leguminous forage that is high in protein, energy, minerals and vitamins, and it is widely adaptable (Hartnell *et al.,* 2005). The forage, generally, contains high concentrations of valuable nutrients that are required by animals (Myer and Cheeke, 1975). It is, also, a good source of B-carotene (a precursor of vitamin A) and other nutrients including thiamine, vitamin B_6, vitamin B_9, vitamin C, vitamin E, vitamin K, calcium, potassium, iron and zinc (NRC, 2001). In addition, lucerne has antioxidant activities (Xie *et al.,* 2008). Lucerne is a drought-tolerant crop, summer growing perennial legume which provides high quality forage for livestock feeding (Gault *et al.,* 1995). Lucerne has been utilized effectively in lactating cow diets as a protein feed to reduce the cost of concentrate supplements. However, the feeding values and levels of replacement in calf diets are not defined.

1.2 Problem statement

The dairy industry depends on the successful rearing of calves and heifers for herd replacements (Göncü *et al.,* 2010). In early life rumen development is critical for growth in ruminant animals (Doescher, 2010). The rumen of neonates is not yet developed and ingestion of solid feed is vital for transition of a calf from a pre-ruminant animal to a functioning ruminant animal (Jones and Heinrichs, 2007). New-born calves also lack rumen microbial population and hence their ability to utilize solid feed is limited. Thus, for a calf starter to be efficiently utilized by calves it should have a minimum amount of fibre and optimum protein contents (Chester-Jones and Broadwater, 2009). Most systems do not expose calves to forage until after weaning, which might limit early development. Concentrates are costly and not easily available to most emerging smallholder farmers and hence alternative sources high in protein and energy should be considered. Lucerne which is highly adaptable and grows in a wide range of production systems including dry-land pasture on smallholder dairy farms may be suitable as a feed in neonatal and weaned dairy cattle when

processed as a leaf-meal. It is, therefore, important to determine lucerne leaf-meal replacement levels for optimal productivity of Holstein heifer calves.

1.3 Motivation of the study

Information on the nutrient value, preparation and utilization of lucerne leaf-meal as an alternative feed for dairy calves will be generated. This information will be useful to dairy farmers, particularly for emerging smallholder commercial dairy farmers in rural areas of South Africa, in sustaining growth of pre- and post-weaned Holstein heifer calves. Smallholder dairy farming faces immense challenges of nutritional management of replacement heifers and most are not viable. Prime forage that provides nutritional similarity to good quality concentrate are vital for development of these entities.

1.4 Aim and objectives

The aim of this study was to determine the effects of replacing calf starter pellets with lucerne leaf-meal on intake and growth of neonatal and transition Holstein heifer calves.

The objectives of this study were as follows:

i. determine nutrient composition of lucerne leaf-meal, calf starter pellets and mixtures of calf starter pellets and lucerne leaf-meal.
ii. estimate concentrations of rapid and slowly degradable fractions of protein and carbohydrates in lucerne leaf-meal, calf starter pellets and mixtures of calf starter pellets and lucerne leaf-meal.
iii. determine *in vitro* degradation of dry matter, organic matter, protein and neutral detergent fibre fractions of lucerne leaf-meal, calf starter pellets and mixture of lucerne leaf-meal and calf starter pellets.
iv. determine the effects of replacing calf starter concentrates with LLM on feed intake, feed conversion ratio and growth of pre-weaned and transition Holstein heifer calves.
v. predict ruminal nitrogen balance and energy density using Large Ruminant Nutrition System (LRNS).

1.5 Hypotheses

i. Lucerne leaf-meal, calf starter pellets and diet composites of the forage and concentrate have different nutrient composition.
ii. Rapid and slowly degradable fractions of protein and carbohydrates in LLM and a commercial dairy calf concentrate have different concentrations.
iii. Lucerne leaf-meal, commercial calf concentrate and composites of the forage and concentrate have different levels of dry matter, protein and fibre fractions.
iv. Replacement of calf starter pellets with lucerne leaf-meal has effect on feed intake, feed conversion ratio and growth of pre-weaned and transition Holstein heifer calves.
v. Lucerne leaf-meal, commercial dairy calf concentrates and diet composites of the forage and the concentrate have different ruminal nitrogen balance and energy density when Large Ruminant Nutrition System (LRNS) is used.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Dairy farming is an important income generating activity which provides regular cash income and high returns to land and family labour (Staal and Mullins, 1996). Nutritional management of the neonatal dairy calves is crucial in successful rearing of replacement heifers. At birth the calf is a monogastric animal with a rudimentary rumen (Doescher, 2010) and without microbial flora, hence calves cannot digest and utilize fibrous forages (Chester-Jones and Broadwater, 2009). The protein requirement of calves is high as muscle growth is in an exponential phase and rations need to maintain a good balance between protein and energy (Moss, 1993) for efficient nutrient metabolism. At birth calves consume milk and small amounts of solid feed concentrates supplemented with minerals and vitamins, especially fat soluble vitamins, as the calf grows to promote rumen development (NRC, 2001). Such essential nutrients are also high in forages such as lucerne (*Medicago sativa*), which has been utilized for two centuries as animal and human feed. Lucerne utilized effectively in lactating dairy cow diets as forage and protein source to reduce the cost of concentrate supplements. Among leguminous forages lucerne is peculiar because of its high nutrients and anti-oxidant levels (Xie *et al.,* 2008). Lucerne is processed into various products such as pellets, mash and LLM for use in monogastric and human diets. However, information on lucerne leaf-meal supplementation for optimal feed intake and digestion in pre- and post-weaned dairy calves is limited. It is, therefore, important to determine lucerne leaf-meal replacement levels of calf starter diets for optimal intake, rumen metabolism and growth of Holstein heifer calves.

2.2 Nutrient requirements of dairy calves

The nutrient requirements of dairy animals depend on rate of growth, body size, reproduction status, level of milk production, previous nutritional plane, acclimatization and environmental conditions (Sahlu *et al.,* 2004). Nutrient requirements are based on maintenance of normal body functions over time and they are adjusted with changes in physiological states (growth, pregnancy, lactation and work) (Sahlu *et al.,* 2004). Even animals of uniform breed, age and sex differ from one another in nutritional needs, and the needs of individuals change within a meal, from meal to meal, and across days (Provenza, 1996; Provenza *et al.,* 2003).

Nutrient limitations prevent ruminant animals from attaining their genetic potential. Thus, an optimum growth rate and feed utilization efficiency according to inherent genetic potentiality of a particular category of animals can be achieved only through accurate evaluation of their nutrient requirements (Mandal *et al.,* 2005).

Nutrient requirements can also provide satisfactory guidelines for formulation of rations for rearing the animals and also provide guidelines in developing supplementary feeding strategies. The minimum energy and protein requirements of dairy calves from birth to weaning as suggested by Van Amburgh and Drackley, (2005) are presented in Table 2.01. The table summarizes the current knowledge about the requirements for growth of the calf based on the body composition data derived since the NRC (2001) was published. The protein requirement is higher than the NRC (2001). The NRC (2001) indicated that absorbed protein was used with an efficiency of 0.80, whereas findings of Van Amburgh and Drakely (2005) suggest the efficiency is 0.70, and requirements are 10 to 12% higher than the predictions of NRC (2001).

Table 2.01 Energy and protein requirements of dairy calves from birth to weaning

Rate of gain (kg/d)	Dry matter intake (kg/d)	Metabolisable Energy (Mcal)	Crude protein (g/d)	Crude protein (% DM)
0.20	0.54	2.4	94	18.0
0.41	0.64	2.9	150	23.4
0.60	0.77	3.5	207	26.6
0.80	0.91	4.1	253	27.5
1.00	1.09	4.8	307	28.7

Adapted from Van Amburgh and Drackley (2005)

2.3 Feeding of dairy calves and rumen development

Feeding young dairy calves is critical to raising replacements. During the first 2 months neonatal ruminants are unique in that at birth they are physically and

functionally two different types of animals with respect to their gastro-intestinal system (Heinrichs and Zanton, 2007). The stomach development of the calf with age is presented in Table 2.02. At birth, calves have small, sterile and underdeveloped rumen. However, by one day of age, a large concentration of bacteria can be found which is mostly aerobic bacteria. Normally After about 2 months of age, they begin to function more like a full-fledged ruminant. During these first few weeks of age abomasum is functional whereas rumen, reticulum, and omasum are relatively small in size and are quite inactive. Due to developing abomasal and intestinal enzymatic state neonatal ruminants are regarded as monogastric animals (Longenbach and Heinrichs, 1998), and they survive on milk-based diets, which are digested and assimilated quite efficiently (Van Soest, 1994; Davis and Drackley, 1998). For this reason, young dairy calves have special requirements for protein, energy and vitamins in their diets as indicated in Table 2.03.

New-born calves cannot utilize vegetable protein before their rumen is functional because they have limited digestive enzymes. Therefore, following colostrum feeding, whole milk, or concentrates should be used. By the time calves are weaned, they can utilize most fibrous feedstuffs very efficiently. Young calves cannot digest starch or some sugars such as sucrose, because certain digestive enzymes are not present. Calves are limited by the type of fat they can utilize but can digest saturated fats. They are less able to digest unsaturated fats such as corn oil and soybean oil. Major sources of energy for new-born calves should be derived primarily from lactose (milk sugars) and milk fat. It is important that calves have adequate energy because the metabolic rate is greatest during the first two weeks of life. Within two weeks, the calves develop the ability to digest starch. Shortly thereafter, they develop the ability to digest complex carbohydrates. The rate of rumen development dictates how rapidly young calves can digest complex starches and carbohydrates (Coverdale *et al.,* 2004). Therefore, during pre-weaning an important objective is to make sure the rumen develops properly.

Vitamins that are critical in early growth include the water soluble B vitamins such as thiamine, riboflavin, niacin, choline, biotin etc.; which are found in colostrum, whole milk, or good milk replacers. Rumen microorganisms are able to produce these when

the calves' rumen begins to function. Calves require the fat soluble vitamins A, D and E; they are in short supply at birth but are found in colostrum (NRC, 2001). Dairy calves require the same minerals for growth as do other animals. Milk, fermented colostrum, and milk replacers generally supply adequate amounts of minerals necessary during the first few weeks of life. Therefore, inclusion of forage to the calf diet facilitates rumen development, mainly due to the production of volatile fatty acids (VFA) (Coverdale *et al.,* 2004).

Table 2.02 Development of the ruminant stomach at various ages

Compartments (% of total)	Birth	28 days	56 days
Reticulo-rumen	35	52	60
Omasum	13	12	13
Abomasum	49	36	27

Adapted from Church (1988)

Based on the information presented in Table 2.03, the primary aim is to achieve the starter intake of at least 0.75 kg/day for three consecutive days so that the calves can be weaned successfully. In addition, the average daily gain (ADG) of 0.6 kg should be achieved in four weeks to gain 15 kg from entry weight so that weaning can take place at 28 days (NRC, 2001).

Table 2.03 Nutrient specifications for a calf starter feed and weaner feed

Nutrient	calf starter feed	Calf weaner feed
Metabolisable energy (Mcal/d)	3.2-3.4	3.3
Crude protein (%)	18-21	16
Crude fat (%)	Min. 3; max 4.5	Min. 3; max 4.5
Neutral detergent fibre (%)	Less than 20	18
Acid detergent fibre (%)		8
Calcium (%)	0.70	0.60
Phosphorus (%)	0.45	0.40

Adapted from NRC (2001)

2.4 The use of the antibiotics and probiotics in the calf feed

The administration of the antibiotics in the calf feed to promote growth was replaced by the use of the probiotics such as monensin, capsicum, megasphera, garlic etc. Numerous benefits were observed with the use of probiotics in the calf diets such as introduction of the beneficial microorganisms into the gut which act to maintain optimal conditions within the gastrointestinal tract and inhibit the growth of pathogenic/undesirable bacteria (Windschitl *et al.,* 1991). Based on the existing commercial information about probiotics is that feeding probiotics increases feed conversion efficiency and live weight gain (Sissons, 1989) and prevent diarrhoea. However, lucerne leaves contain protein-xanthophyll which has lot of bioactive compound such as saponins, flavonoids, pro-vitamins, etc. (Ben-Aziz *et al.,* 2006; Grela *et al.,* 2013). Flavonoids have been reported that they acts as antioxidants, precursor of the toxic substances and defend against infections (Smith and Banks, 1986; Carroll *et al.,* 1998; Hertog and Katan, 1998). This indicates that the inclusion of lucerne in the calf diets might minimise the amount/levels of the probiotics use in the calf feeds.

2.5 Forage feeding for dairy cattle

Forages are defined as edible parts of plants, other than separated grain, generally above ground, that can provide feed for grazing animals, or can be harvested for feeding. They are considered to be the staple diets for ruminant animals, and high quality forage is essential to productivity and profitability of dairy farms. A wide variety of fresh and conserved forages are available for dairy feeding. Therefore, they are valuable for ruminants as they provides excellent, generally dilute sources of nutrients, supply valuable fibre for rumen digestion and being home grown and, in many cases, more sustainable and economic than other feeds. In addition, provision of well-managed forages can be more cost-effective sources of nutrients for dairy feeding than concentrates in many circumstances.

Legumes and grasses are a major source of forage for dairy animals. Livestock consume mostly the vegetative portion of the plant, mainly leaves and stems. Forage crops are fibrous in nature and ruminant livestock, such as cattle and sheep, require this fibre in their diet for proper digestion. They also obtain nutrients such as protein,

minerals, and vitamins from forage crops. As forage plants mature, they become more fibrous and have lower concentrations of essential nutrients. Forage crops can be grazed directly by animals in pastures, or conserved for winter feeding as hay or silage (Barnes *et al.,* 1995).

2.5.1. Composition of grasses versus legumes

The nutrient composition of grasses and legumes is variable depending on many factors such as species, maturity, fertilization and soil fertility, growing environment and harvesting conditions. Forage quality can be defined as the extent to which forage has the potential to produce a desired animal response. Forage quality, therefore, also influences intake and digestibility (Ball *et al.,* 2001). Maturity stage at harvest is the most important factor determining forage quality of a given species. Maturity at harvest also influences forage consumption by animals. As plants mature, protein, energy, calcium, phosphorus, and digestible DM levels decreases, while fibre level also increases (Table 2.04). Chemically, this dietary fibre can be determined as crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). As fibre, NDF, and especially ADF, increases, the lignin content of the plant also increases. Lignin is indigestible and makes other nutrients less available. Legumes were found to have high nutritive value due to their chemical composition (Figure 2.01 and Table 2.04) (Collins, 1988). Grasses contain higher concentrations of NDF and ADF compared to legumes. Black *et al.* (1980) reported that an increase in NDF concentration reduces feed intake of the forage.

Compared to grasses, legumes have relatively higher concentration of crude protein. The majority of CP in fresh legumes or grasses is true protein with approximately 10 -15% as non-protein nitrogen (NPN; primarily peptides, free amino acids and nitrates). The amount of NPN increases, as a percent of the CP, when grasses are heavily fertilized with nitrogen or when either legumes or grasses are fermented (30-65% of CP) (Reid, 1994; NRC, 2001). McDowell and Valle (2000); Jukenvicius and Sabiene (2007) reported that legumes tend to accumulate more total macro- and micro-minerals and ash. Of the major minerals in forages, legumes contain two to three times the calcium found in grasses, while potassium and phosphorus concentration is only slightly higher or similar to grasses (Cherney[1] and Cherney[2],

2011). Grasses have higher fibre concentration than legumes at an optimum stage of growth for harvest. Collins (1988) found an optimum NDF concentration of 50-55% in grass forage, while alfalfa is optimum at around 40% NDF.

Several grasses, such orchardgrass, rye grass, napier grass, *Erasgrostis* species, *Panicum maximum* and legumes, such as lablab, clover, lucerne, etc. have been used for dairy feeding. Lucerne is an old age outstanding forage and research that enhances its utilization as value added product is envisaged.

Table 2.04 Typical test value of lucerne and grass harvested at various stages of plant maturity (all values on DM basis)

Type of forage/Stage	CP (%)	ADF (%)	NDF (%)	TDN (%)
Lucerne				
Pre-bloom	> 19	< 30	< 35	> 62
Early bloom	17-19	30-35	35-39	57 – 62
Mid bloom	13-16	36-41	41-47	51 – 56
Late bloom	< 13	> 41	> 48	< 51
Grass				
Prehead	17	< 29	< 55	> 54
Early head	12-17	30-35	56-61	47 - 54
Head	8-12	36-44	60-65	44 - 46
Post-head	< 8	> 45	> 65	< 44

Adapted from Van Saun (undated)

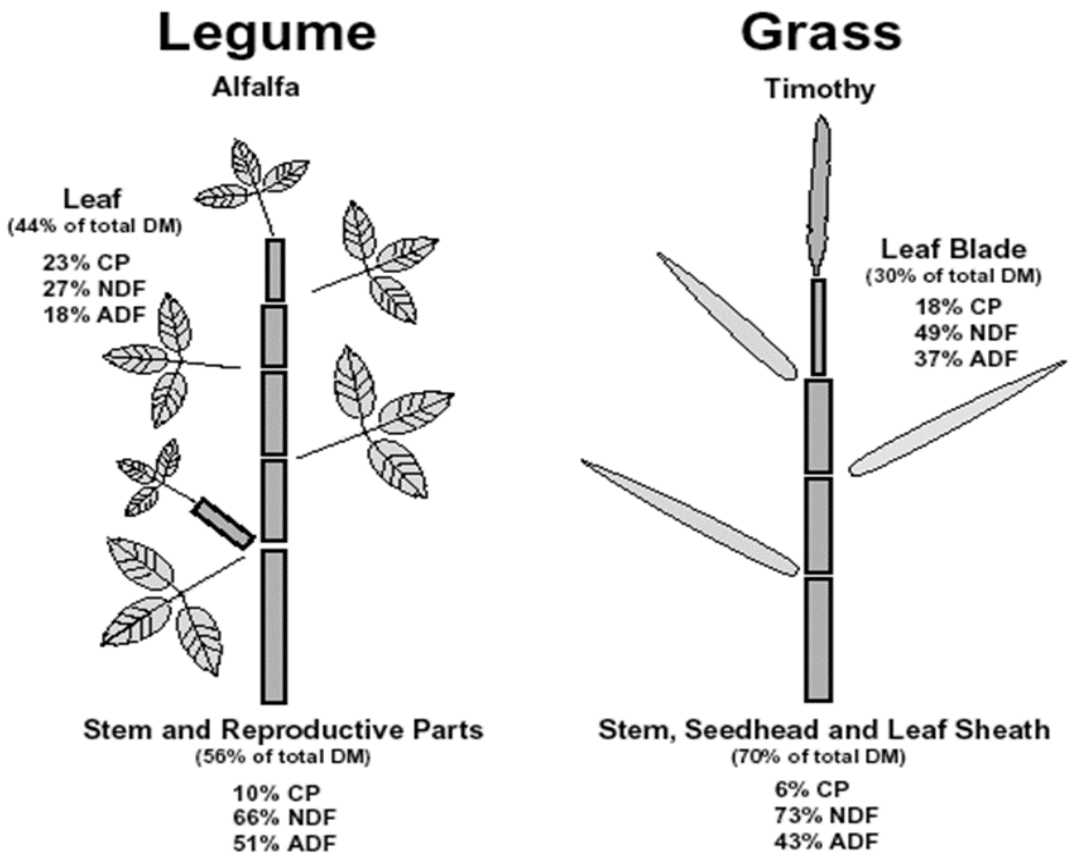

Figure 2.01 Chemical composition of leaf and stem tissue from alfalfa/lucerne and timothy (Adapted from Collins, 1988)

2.6 Lucerne

Lucerne, *Medicago sativa*, also called alfalfa, is a perennial flowering plant in the pea family Fabaceae cultivated as an important forage crop in many countries around the world (www.wikipedia.org). It is probably the oldest cultivated forage crop in the world and has been called the "Queen of forages". Although it originated in the Mediterranean climate of the Near East and Central Asia it is grown in almost all parts of the world, including Africa (Keftasa and Tuvesson, 1993) and is most often harvested as hay, but can also be made into silage, grazed, or fed as green-chop. Lucerne is native to warmer temperate climates (www.wikipedia.org). World production of lucerne was around 436 million tons in 2006 (FAO, 2006). Lucerne is a reliable, high yielding perennial crop with excellent forage quality and it is well adapted to both irrigated and non-irrigated land in most climates. It is widely grown throughout the world as forage for cattle. It is used less frequently as pasture.

Its primary use is as feed for high-producing dairy cows, because of its high protein content and highly digestible fibre, and secondarily for beef cattle, horses, sheep, and goats. The cost of growing and harvesting lucerne varies greatly between districts. It is used also in the poultry industry, whereby lucerne leaf concentrates are used in the layer and broiler diets as a source of pigment for pigmenting eggs and meat, because of their high content in carotenoids for colouring egg yolk and body lipids (Guenthner *et al.,* 1973). Moreover, it is also been used by humans because they eat lucerne leaves in salads and sandwiches and also play an important role in the aquaculture (Rechulicz *et al.,* 2014). Irrespective of its impressive profile, lucerne can also cause bloating in livestock, so there is an extra care that must be taken with livestock grazing on lucerne (Cook *et al.,* 2005; Frame, 2005).

Currently, there are numerous cultivars/varieties of lucerne, selected and breed for specific abilities, such as winter hardiness, drought resistance, tolerance to heavy grazing, high feeding value (i.e. digestibility and fibre content) or tolerance to pests and diseases (Brummer and Bouton*,* 1991; Julier *et al.,* 2000; Frame, 2005). The current role of lucerne is mostly as a supplement dry feed on dairy farms. Lucerne became of interest to the dairy industry due to its ability to produce more dry matter than conventional pasture and the fact that it also has the ability to grow without nitrogen inputs (Campion, 2011).

2.6.1 Lucerne leaf-meal processing

There several method whereby lucerne is processed for animal feeding during harsh conditions and this include hay and silage (Suttie, 2000). However, making hay or silage with lucerne decreases protein content by 2.1 to 3.2% for hay and by 1.6% for silage when compared to green lucerne (INRA, 2007). Although hay and silage making are considered to be the good conversation methods for animal feeding during harsh conditions in the small-scale farms, they have hindrances that can reduce animal production. For example, making silage with fresh lucerne may cause nutrient losses due to its high water content while with hay leaf losses becomes a major problem and cause protein loss (Mauriès, 2003). In addition, lucerne can be dehydrated which was reported to be the best way as it dries and stabilizes lucerne while preserving its high protein content, vitamins and overall nutritive value

(Renaud, 2002). Moreover, Coop de France (2010) reported that dehydrated lucerne is a good source of xanthophylls and beta-carotenes for poultry farmers. Irrespective of the impressive benefits of the dehydrated lucerne, it has many disadvantages including pre-wilting, chopping in the field, transportation to the plant and drum-drying to reduce moisture to 10% at a temperature between 250°C and 800°C (Coop de France, 2010) and reported to reduce feed efficiency and body weight gain in growing pigs (Kass *et al.,* 1980). Another industrial method includes pelletising of lucerne leaves whereby total or partial Millard reactions takes place and this lead to more protein bound to the fibre. Desialis (2013) reported that during the application of this method certain protein content of the pellets are often standardized to 17 or 18%. Therefore, all these methods are laborious, time-consuming, experience and require equipment; and these methods reduce the quality of this forage.

Considering the fact that lucerne leaf-meal prepared by drying the leaves under the shade for a short period (3-4 days), is the best solution to animal feeding and reduces the costs to the farmer. This processing method results in high protein, energy density; and low fibre content and protein-bound fibre.

2.6.2 The nutritive value of lucerne

Lucerne is a high quality leguminous forage and a good source of protein in livestock diets (Hartnell *et al.,* 2005). Lucerne is the most important forage legume for dairy cows (Figure 2.02). Energy and protein levels in lucerne are generally high, particularly in the early growth stage (Broderick and Satter, 1998; Campion, 2011). It is rich in a variety of nutrients that are required for general good health of livestock (Myer and Cheeke, 1975). Lucerne is a good source of B-carotene, a precursor of vitamin A, vitamins K and E and also contains significant amounts of the water soluble vitamins riboflavin, pantothenic acid, nicotinic acid and other nutrients (Aganga and Tshwenyane, 2003). It is also an outstandingly good source of vitamin C (1.78 mg/g) (Frame, 2005). Nutrient composition of different plant components of lucerne are presented in Table 2.05. The values are very variable, possibly because of different soils, climates, stage of growth, etc. for the different studies. In addition to this impressive nutritional profile, lucerne has anti-oxidant activities (Xie *et al.,* 2008).

Lucerne at the pre-bloom stage is highly palatable and often maximizes intake and production of dairy cows. It is low in fibre and high in protein compared to other forages, which makes it an excellent complement for grains and other forages in dairy rations (Martin and Mertens, 2005). During the early flowering stage, the leaves contain a greater concentration of digestible nutrients, proteins, fats, fibre, total non-structural carbohydrates and other micronutrients than the stems. Stems have more sugars, fibre, potassium and chlorine, therefore it is clear that the lucerne leaves contain more nutrients than the stems (Scholtz, 2008). Weiss *et al.* (undated) found that high-quality lucerne contain 35 to 40% NDF (25 to 30% ADF). Lucerne also contains anti-nutritional factors such as saponins and tannins that have both negative and positive impact on animal production.

Table 2.05 Nutrient composition of lucerne leaves and stem (units are expressed in % DM except MJ/kg DM for ME)

Nutrient	Leaves	Stems	Author
CP	-	13.4	Collins (1988)
CP	27.3	-	Titgemeyer *et al.* (1991)
CP	22-35	10-20	Putnam (2000)
CP	189.9	-	Niwińska *et al.* (2005)
CP	173.5	103	Tyrolová and Výborná (2008)
ME	8.0	-	AFRC (1993)
Ca	15	-	Frame *et al.* (1998)
Mg	1.1-6.4	-	Spedding and Diekmahns (l972)
Mg	2.0-3.5	-	Frame *et al.* (1998)
K	10.6-39.2	-	Spedding and Diekmahns (l972)
K	8.0-22.0	-	Frame *et al.* (1998)
NDF	32.8	-	Titgemeyer *et al.* (1991)
NDF	18-28	35-70	Putnam (2000)
NDF	50.1	-	Niwińska *et al.* (2005)
ADF	-	38.6	Collins (1988)
ADF	12-20	30-55	Putnam (2000)
ADF	40.8	-	Niwińska *et al.* (2005)
ADL	46	115	Titgemeyer *et al.* (1992)

Figure 2.02 Lucerne plant

Anti-nutritional factors in lucerne

Tannins in lucerne

Tannins are high-molecular-weight and phenol-rich polymers that exist in many feeds, including legumes (Chang *et al.,* 1994). Tannins are secondary plant metabolites that can bind proteins making them inaccessible by enzymes or microbes in the animal, thereby preventing bacterial proteolysis (Tabacco *et al.,* 2006). Condensed tannins are flavonoid polymers linked through acid-labile carbon-carbon-bonds (Figures 2.03); Hydrolysable tannins are polymers composed of gallic acid and ellagic acid, esterified to a core molecule. Hydrolysable tannins are usually present in low amounts in plants while condensed tannins reduce forage quality, the hydrolysable tannins cause poisoning in animals if sufficient quantities are consumed. Condensed tannins are regarded to be non-toxic because they are not absorbed, but they are associated with lesions of the gut mucosa, which could decrease absorption of other nutrients, especially essential amino acids (Reed, 1995). High concentrations of condensed tannins in forage legumes have been reported to reduce intake, digestibility of protein and carbohydrates, and animal performance (Barry, 1985; Reed *et al.,* 1990). McSweeney *et al.* (1999) found no condensed tannin detected in lucerne.

Beneficial effects of tannins

Rumen escape, urea recycling and microbial efficiency are mechanisms by which tannins in forage legumes may increase the efficiency of protein utilization by ruminants. Tannins may complex protein at the pH of the rumen and protect protein form microbial enzymes. Woodward (1988) reported that tannins lower the rate of protein degradation and deamination in the rumen and therefore lower ruminal ammonia (NH_3). Tannins may increase the glycoprotein content and excretion of saliva, which could lead to more nitrogen (N) recycled to the rumen (Robbins *et al.,* 1987). At low to moderate concentrations, tannins may prevent bloat and increase protein utilization (Waghorn *et al.,* 1987; McNabb *et al.,* 1993).

Effect of saponins on animal production

Lucerne saponins are important anti-nutritional factors, which may hinder the nutritive value of this forage species. Saponins are a mixture of diverse glycosides deriving from various sapogenins (aglycone moieties) (Pecetti *et al.,* 2006) which on acid hydrolysis yield pentoses, hexoses, uronic acids and aglycones (Oleszek *et al.,* 1992). Chemically, saponins contain one or more sugar chains on a triterpene or steroid aglycone backbone also called a sapogenin present in many families of plants (Patra and Saxena, 2009). Triterpenoid saponins have been detected in many legumes including lucerne, etc. (Francis *et al.,* 2002). They are categorized according to the number of sugar chains in their structure as mono, di-, or tridesmosidic. Howarth (1988) reported saponins concentration of 2.62% in lucerne hay fed to calves. Saponins also selectively affect specific rumen bacteria and fungi, which may alter the rumen metabolism beneficially or adversely (Patra and Saxena, 2009).

2.7 Effect of lucerne leaf-meal on productivity of dairy cattle

Lucerne is the most important forage legume for dairy cows. However, it is low in fibre digestibility and high protein (Martin and Mertens, 2005). The fibre content of the forage is best measured by NDF and ADF. Neutral detergent fibre is used to measure the amount of cellulose, hemicellulose, and lignin in the plant cell wall and is less digestible when compared to non-structural carbohydrates (NRC, 2001). The presence of these structural components helps to determine energy intake and

rumen fill. The NRC (2001) recommendation for the minimal amount of NDF is 25% of the dietary dry matter for young calves. The NRC (2001) recommends at least 17% ADF be present in the total diet for large dairy ruminants. Heifers fed diets as low as 19% NDF have done very well and have not acquired metabolic or lameness problems under routine management (Zanton and Heinrichs, 2008).

Stobo *et al.* (1966), Anderson *et al.* (1982) and Hill *et al.* (2009) reported that calf starters containing forages increased feed intake, improved weight gain, average daily gain (ADG) and improved rumen development of calves at an early age. However, roughage intake of calves is greater when the roughage and concentrate are incorporated together into a single ration than when they are offered separately (Bartley, 1973). Kariuki *et al.* (1999[a,b]) found that lucerne hay supplementation improved nutrient intake, rumen degradation, feed conversion efficiency and weight gain of dairy heifers. Thus, the authors indicated that lucerne-containing diets with dry matter (DM) and crude protein (CP) content levels of 845 g/kg DM and 190 g/kg DM, respectively, were adequate for optimal feed intake, feed conversion efficiency and growth performance of Sahiwal and Holstein-Friesian heifers. Göncü *et al.* (2010) observed that choice-fed calves on a diet containing 97% calf starter and 3% lucerne hay improved average daily gain but yielded poor feed conversion ratios. Harris and Sheare (2003), Jones and Heinrichs (2007) and Heinrichs and Lesmeister (2000) reported that hay inclusion in the diet is not recommended during pre-weaning period (3-6 weeks) of dairy calves because it is low in energy values. Coverdale *et al.* (2004) reported an improved performance of calves fed up to 15% of the diet as forage. Average daily body weight gain and hip width change decline as amount of roughage in the diet increased (Hill *et al.,* 2010).

2.8 Effect of lucerne on the rumen development

Rumen development in calves is important for the ability to digest solid feeds and to reduce their nutritional dependence on milk. Diets have the greatest influence on rumen development (Brownlee, 1956; Harrison *et al.,* 1960; Göncü *et al.,* 2010) as illustrated on Figure 2.03 and 2.04. Therefore, roughage inclusion in the calf diets plays an important role in maintaining a normal, healthy rumen environment and growth (Lengemann and Allen, 1959; Davenport, 1987; Quigley, 2011) (illustrated on

Figure 2.04). Kertz (2005) indicated that the use of roughages at any level is problematic to calves due to the negative impact on rumen development, intake and daily gain. At birth, neonates does not have enzymes to digest fibre, and they do not have a large enough and established microbial population to ferment fibre. Therefore, feeding bulky feeds like hays, straws, silages, and hay takes up rumen space and displaces space for grains and proteins that can be digested. In so doing, feeding of roughage to calves soon after weaning is detrimental to the calf. A 3-month-old calf does not have the rumen capacity or digestive capability via rumen microbes to utilize diets with free-choice hay or large amounts of fibrous feeds (Heinrichs and Zanton, 2007). Jones and Heinrichs (2007) reported that feeding a calf solid feeds, for example, concentrates and roughage, can have dramatic and positive effects on the process of rumen development. Kertz (2005) found an increase in gut fill with roughage inclusion from levels of 4 up to 16% but a considerably greater increase in gut fill above 16%. Coverdale *et al.* (2004) and Hill *et al.* (2010) reported that early weaned calves fed forages consumed more feed and had improved weight gains. The increase in feed intake has been shown to lead to an increase in volatile fatty acids (VFA) concentrations, which in turn stimulates rumen development (Coverdale *et al.,* 2004). Thus, rumen microbes are formed and rumen papillae develop when VFA are being produced in the rumen (Lane and Jesse, 1997). The production of the volatile fatty acids, mainly propionate and butyrate, in the rumen will cause a decrease in ruminal pH (Doescher, 2010).

There is some evidence that forages are able to promote rumen development. There is limited information on the effects of supplementing lucerne leaf-meal on productivity of both pre-and post-weaned dairy calves. Therefore, forages may be used to replace some of the expensive concentrates in the calf starter diets (Coverdale *et al.,* 2004).

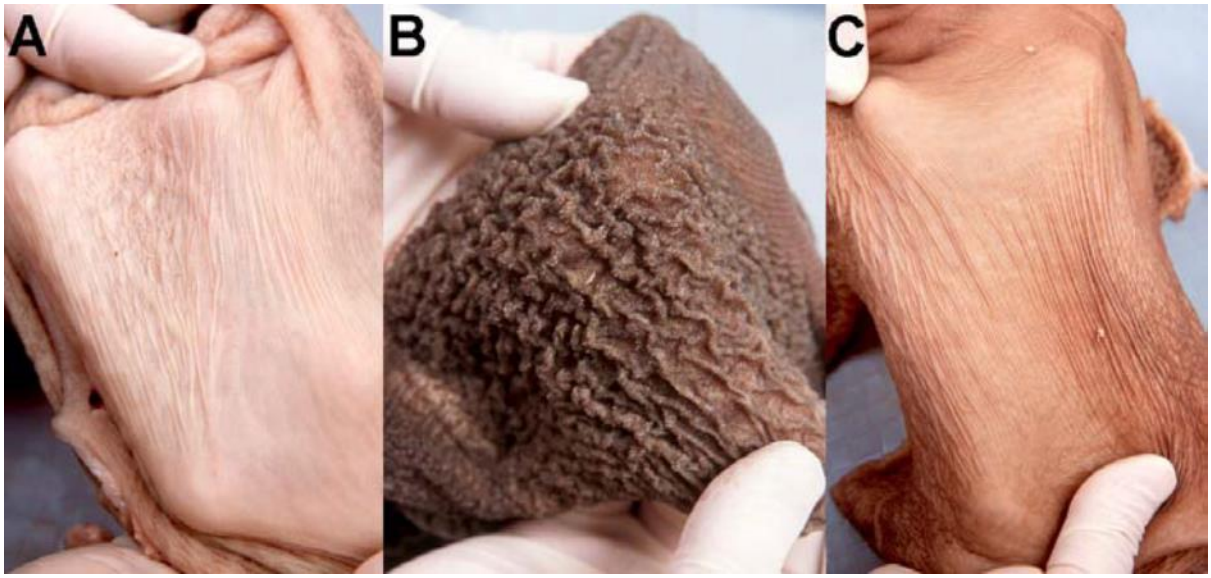

Figure 2.03 Rumen development of the 6 weeks old calf fed A: milk only; B: milk and grain and C: grain and hay. (Adapted from Heinrichs, 2005)

A: Milk, hay and grain B: Milk and hay

Figure 2.04 Effect of diet on rumen development of the calf aged 12 weeks. (Adapted from Heinrichs, 2005)

2.9 Methods of evaluating feeding value

Feeding or nutritive value is defined as a function of the feed intake and the efficiency of extraction of nutrients from the feed during digestion (digestibility) (Madsen *et al.,* 1997). According to Getachew *et al.* (2004), the feeding or nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion. Feed evaluation is a description of feeds in terms that allow for prediction of the animal performance offered the feeds (Seven and Cerci, 2006). Feed evaluation methods tend to

reproduce what is happening at the gastro-intestinal tract when the feed is eaten by the animal. The methods to evaluate the feed value tend to simulate or measure the effect of the digestion on that foodstuff. The most common definition of a feed states that it is a product that contains nutrients and possibly other components that are not nutrients (Ribeiro and Moreira, 2000). The aim of feed evaluation is to provide guidance about the best feeding methods. There are mainly different types of methods for the evaluation of feeding value, which include the chemical, biological and enzymatic.

2.9.1 Chemical methods

Chemical methods give information on the main chemical composition present in the feeds. However, this information has been used to predict the nutritive value of the feedstuff. Some of the parameters have the impact on digestion, which justifies their utilization to predict the nutritive value. Among those are nitrogen (crude protein) and the cell wall constituents. The CP content is a good predictor of organic matter digestibility (OMD) since it diminishes as the plant grows and also varies inversely with the indigestible cell wall fraction. Van Soest fractionation (Van Soest and Wine, 1967) makes it possible to measure the total cell wall content (neutral detergent fibre, NDF), ligno-cellulose content (acid detergent lignin, ADL) of forages. In particular acid detergent fibre (ADF) determination allows the evaluation of practically all the cellulose and lignin. To predict the digestibility of forages, total cell wall content or NDF is a less accurate predictor of OMD than ADF. Lignin content is the variable most closely linked to *in vivo* digestibility. It is necessary to establish separate relations, at least for grasses and legumes. For the same lignin content, legumes have less indigestible cell wall material and higher digestibility than grasses (Demarquilly and Andrieu, 1987).

2.9.2 *In vitro* and *in sacco* techniques

Using *in situ* technique has been strongly criticized by public opinion for the need of fistulated animals which raise ethical and moral issues about animal welfare (Stern *et al.,* 1997). Moreover, high associated costs and limited analytical capacity of *in situ* technique have led to the development of alternative *in vitro* techniques, which carried out some important advantages: i) they do not involve the direct use of

animals; ii) they are less laborious and more suitable for a large-scale evaluation of ruminant feeds. The *in vitro* techniques can be classified as: i) methods which measure the digestibility of feeds (Tilley and Terry, 1963; Goering and Van Soest, 1970; Czerkawski and Breckenridge, 1977); ii) methods which measure gas production from feed fermentation (Menke *et al.,* 1979). Amongst other *in vitro* techniques, Daisy^{II} incubator (Ankom Technology®, Macedon, NY, USA) was identified to be more efficient and less animal dependent techniques. Compared to Tilley and Terry (1963) and Goering and Van Soest (1970) methods, the Daisy^{II} one leads to an improvement of labour efficiency, as it allows to analyse simultaneously up to 100 feed samples.

2.10 Modelling

Diverse nutrition modelling systems have been used to formulate diets suitable for specific animals and to balance nutrients based on their requirements (Tedeschi *et al.,* 2005). Modelling systems for ruminants include Large Ruminant Nutrition System (LRNS), Cornell Net Carbohydrate and Protein System (CNCPS), Agricultural Modelling and Training System (ATMS), National Research Council (NRC) (Tedeschi *et al.,* 2005*;* Parsons *et al.,* 2012). However, these systems are interlinked and widely used by feed producers. There is a need to formulate proper ration to ensure satisfactory animal performance (e.g. meat, milk, egg production), to adequately plan for long-term financial stability, and competitiveness in the agriculture market (Parsons *et al.,* 2012). Feed resource planning is important for ruminant livestock production and this involves, knowing how much and what type of feeds are likely to be available; having information on their nutritive characteristics; understanding the animal's nutrient requirements for particular purposes; and using this information to design feeding strategies (Doyle *et al.,* 2008).

2.11 Sustainability of dairy businesses

Nutritional management of dairy calves is critical to improve the rumen development and reduce their dependence on milk and solid feed concentrates. The preceding review highlights the critical need for evolution in neonatal and weaned dairy calf nutrition in the face of intense global competition in the milk industry. Large-scale operations that operate at feed: milk ration will continue to face enormous pressure

against markets fluctuate, climate change, and prices of inputs escalates. The rapid loss in number of commercial dairy producers is evidence of failure to adapt to changing environments especially the management of feed costs which are usually more than 60% of total costs. Maize prices have soared increasing the cost of energy concentrates and with importation of various ingredient commodities such as soybean from Brazil, wheat bran, hominy chop and cotton seed from Swaziland management of early calf nutrition becomes more critical. Concentrates are mostly industrially distilled, although on-farm production is also practised, and variability in ingredient quality also increases the costs of producing products of consistent quality. It is in view of these economic, and market forces that LLM, be assessed as potential concentrate commodity to maintain viability of the milk industry at both large scale and on smallholder systems where feeding management of the neonates is substandard and characterised by high mortality.

There are limited studies in ruminant neonates and this study undertakes to lay the foundation on utilization of LLM in neonates and also weaned/transition heifers that have no prior exposure to grazing.

2.12 Conclusion

There is some evidence that forage inclusions in the calf diets can improve productivity. However, data on lucerne leaf-meal supplementation for optimal feed intake, digestion and growth of pre- and post-weaned dairy calves is limited and inconclusive. This study will, therefore, determine lucerne leaf-meal replacement levels for optimal intake, rumen metabolism and growth of Holstein heifer calves.

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CHAPTER THREE

CARBOHYDRATE AND PROTEIN FRACTIONS, RUMEN DEGRADATION AND SUPPLY OF METABOLISABLE ENERGY AND PROTEIN FROM LUCERNE LEAF-MEAL AND CONCENTRATE DIETS FOR HOLSTEIN HEIFER CALVES

3.1 Introduction

Lucerne is a valuable feedstuff for all livestock types, with a balanced profile of amino acids, vitamins, minerals and organic acids. Lucerne leaf-meal (LLM) is prepared by harvesting lucerne leaves and drying under shed to preserve nutrients and colour (chlorophylls). This forage mash contains 200-300 CP/kg DM and 18.09-20 MJ GE/kg DM (Collins, 1988; Broderick and Satter, 1998; Leming and Lindberg, 2001; Campion, 2011). Utilization of high quality forage minimizes the amount of concentrate supplementation because the forage supplies the bulk of required nutrients for maintenance and production (Oba and Allen, 2005; Zebeli *et al.,* 2010). Industrial or cottage preparations of LLM are incorporated into diets of deprived communities, mainly, the elderly and children (Rechulicz *et al.,* 2014). Some preparations of LLM have been used in feeding neonatal animals and in chickens which make LLM an attractive feed for neonatal dairy cattle that are usually raised with little exposure to grazing (Guenthner *et al.,* 1973; Coop de France, 2010). There is opportunity to harness the nutrients and compliment nutrients from concentrates and hence optimize costs. The dairy industries rely, mainly, on concentrates for nourishing neonates, and adding forage rich in protein and degradable fibre would improve growth at reduced production costs (Moran, 2005).

The aim of this study was to evaluate nutritional value of lucerne leaf-meal as complementary premium forage, feed fractions of protein and carbohydrates, and predict metabolisable energy and protein supply for growth.

The objectives of this study, therefore, were to:

i. determine nutrient composition of lucerne leaf-meal, calf starter pellets and mixtures of calf starter pellets and lucerne leaf-meal.

ii. estimate concentrations of rapid and slowly degradable fractions of protein and carbohydrates in lucerne leaf-meal, calf starter pellets and mixtures of calf starter pellets and lucerne leaf-meal.

iii. determine *in vitro* degradation of dry matter, organic matter, protein and neutral detergent fibre fractions of lucerne leaf-meal, calf starter pellets and mixture of lucerne leaf-meal and calf starter pellets.

3.2 Materials and methods

3.2.1 Study site

The study on nutrient composition and *in vitro* degradation of lucerne leaf-meal, calf starter pellets and LLM substituted diets was conducted at Irene, Animal Production Institute of the Agricultural Research Council of South Africa in 2013 and 2014. Irene is located at longitude of 28º 13 S, latitude of 25º 55 E, altitude of 1524 m (Nkosi *et al.,* 2009). The ambient temperatures in winter (May to July) range between 5 and 20 ºC, and in summer (November to January) they range between 18 and 29 ºC (Muya *et al.,* 2011).

3.2.2 Experimental design, treatments and procedures

Forage harvesting and processing

Lucerne forage was hand-harvested using secateurs at the pre-bloom stage, cut 5 cm above the ground and air-dried under the shade over four days to minimise nutrient losses. After drying, petioles were separated from stems and small twigs were crumbled by hand. The dried and crumbled forage was then thoroughly mixed with the concentrate pellets according to the different ratios. The prepared meal was used for feeding the calves. Ground samples were stored individually in airtight containers until nutrient analysis.

The experimental design was a completely randomised design with three dietary treatments, each having four replicates. The three dietary treatments were calf concentrate pellets (PEL), 65% calf concentrate pellets mixed with 35% LLM ($P_{65}L_{35}$), 50% calf concentrate pellets mixed with 50% LLM ($P_{50}L_{50}$). The lucerne leaf-meal (LLM), calf starter concentrate pellets and LLM substituted diets were analysed for nutrient composition. The *in vitro* degradation measurements were made on the lucerne leaf-meal, calf concentrate pellets and LLM substituted diets using ANKOM Daisy^{II} incubator system (Ankom Technology Corp., Fairport, NY). The protein and fibre fractions and total digestible nutrient (TDN) estimations were done using Weiss *et al.* (1992) and NRC (2001) equations.

Dairy Calf Concentrate

Commercial calf starter feed used in this study was obtained from Animal Feed Company in Gauteng Province. The feed is used by ARC for dairy calves aged three days till six months and the feed was in the form of pellets. The nutrient composition of a commercial calf starter feed was labelled on the bag as 18% for CP, 88% for DM, 10-15% for fibre, 25-70% for fat, 8% for Ca and 3.5% for P.

3.2.3 Chemical analysis

Chemical contents analysed were dry matter, organic matter, crude protein, gross energy, ether extract, ash, neutral detergent fibre, acid detergent fibre, acid detergent lignin, neutral detergent insoluble crude protein, acid detergent insoluble crude protein, starch, calcium and phosphorus.

Dry matter of ingredients and diets was determined according to AOAC (2000) (Procedure 930.15). Ash contents and organic matter were determined according to AOAC (2000) (Procedure 942.05) by placing a sample in muffle furnace at 550 ºC for eight hours. Ether extract (EE) was determined according to the method described by AOAC (2006) (Procedure 2003.05). Crude protein (CP) was determined by measuring nitrogen content using the Kjeldahl procedure (AOAC, 2000) (Procedure 968.06). Gross energy (GE) values of the feed samples were determined by combustion in an adiabatic bomb calorimeter (PARR model 2081). Starch was determined by a modification of the method of Holm *et al*. (1986) as cited by Hall (2000). Calcium (Ca) was determined according to Giron (1973) using a Perkin elmer atomic spectrophotometer. Phosphorus (P) was assayed according to AOAC (2000) (Procedure 965.17). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest *et al.* (1991) and acid detergent lignin (ADL) was determined according to Goering and Van Soest (1970). Acid detergent insoluble crude protein (ADICP) and neutral detergent insoluble crude protein (NDICP) (Licitra *et al.,* 1996) were determined by measuring the CP content of the ADF and NDF residue by Kjeldhal analysis and contents were expressed as a percentage of total nitrogen (Van Soest *et al.,* 1991). Soluble protein content was determined (Licitra *et al.,* 1996). All samples were analysed in triplicates.

Calculations: Feed fractions

Estimation of Non-fibre carbohydrates (NFC) (NRC, 2001) and Van Soest *et al.* (1991)

Non-fibre carbohydrates of the i^{th} feedstuff were calculated as:

NFC = [100- (%NDF + %CP + %EE +%ASH)]

Hemicelluloses and bound proteins (HS) (washed off in ADF solution)
%HS = [(NDF dry weight-ADF dry weight)/ (sample @ 105)] * 100

Cellulose (AS) (washed off in ADL)
%AS = [(ADF weight – ADL dry weight)/ (sample @ 105)] * 100

Non-polar extracts (NPE) such as fats, oil, waxes and soluble cell contents (carbs, lipids, starch and soluble proteins) dissolved in NDF wash.
%NPE = [(NDF dry weight – bag weight)/ (sample @ 105)] * 100

Neutral detergent insoluble N (NDIN) (expressed as N*6.25).
Acid detergent insoluble N (ADIN) (expressed as N*6.25).

3.2.4 Estimation of apparent total digestible nutrient and metabolic energy supply

Total digestible nutrient (TDN_{1x}) at maintenance was calculated as described by Weiss *et al.* (1992) and NRC (2001):

$$TDN_{1x}\ \% = 0.98 \times (100 - CP - Ash - EE + IADICP) + (KDcp \times CP) + 2.70 \times (EE - 1) + 0.75 \times (NDFn - Lig) \times (1 - (Lig / NDFn)^{2/3}) - 7$$

Where:

ADICP: ADF indigestible crude protein: (ADFIP / 100) x CP;

EE: ether extract

IADICP: indigestible ADICP; and is computed as:

Forages: (0.7 x ADICP);

Concentrates: (0.4 x ADFIP);

KDcp: calculated factor for the degradability of the CP, and is calculated as:
Forages: exp (-0.0012 x ADFIP);
Concentrates: 1 – (0.004 x ADFIP);

Lig: lignin (% of DM) and is computed as (Lignin / 100) x NDF;

NDFn: NDF corrected for nitrogen: NDF – NDFCP + IADICP;

NDICP: NDF indigestible crude protein: (NDFIP / 100) x CP.

Digestible TDN (dTDN) was calculated separately for forage and concentrate as described by Tedeschi (2001) and Fox *et al.* (2004). Digestible, metabolisable and net energy for growing dairy cattle were estimated based on dTDN.

Forages: $dTDN = 0.53 + 0.99\ TDN_{1x} – 0.009 \times 0.00005 \times TDN_{1x} \times NDF + 8.96 \times DMIFactor – 0.1 \times TDN_{1x} \times DMIFactor – 0.13 \times NDF \times DMIFactor + 0.00005 \times TDN_{1x} \times NDF \times DMIFactor$

Concentrates: $1.01 \times TDN_{1x} – 1.77 \times DMIFactor – 0.99$

The following equations were used to convert dTDN to DE, ME and NE:

DE, Mcal/kg DM = (dTDN / 100) x 4.409

ME, Mcal/kg DM = 0.82 x DE (growing dairy)

NEm, Mcal/kg DM = $1.37 \times ME – 0.138 \times ME^2 + 0.0105 \times ME^3 – 1.12$

NEg, Mcal/kg DM = $1.42 \times ME – 0.174 \times ME^2 + 0.0122 \times ME^3 – 1.65$

Partitioning of carbohydrate fractions

Estimation of structural and non-structural carbohydrates for supporting rumen fermentation was done based on feed chemical analyses and equations of Fox *et al.* (2003).

Carbohydrate composition of the i^{th} feedstuff

Equation 1: $CHO_i\ (\%DM) = 100 – CP_i – FAT_i – ASH_i$

CHO_i (%DM) is percentage of carbohydrate (total carbohydrates) of the i^{th} feedstuff

Equation 2: CC_i (%DM) = NDF_i x 0.01 x $LIGNIN_i$ x 2.4

CCi (%DM) is percentage of DM in the i^{th} feedstuff that is unavailable fibre

Equation 3: CB_{2i} (%DM) = NDF_i – ($NDIP_i$ x 0.01 x CP_i) – CC_i

CB_{2i} (%DM) is percentage of DM of the i^{th} feedstuff that is available fibre

Equation 4: CB_{1i} (%DM) = $STARCH_i$ x NFC_i / 100

CB_{1i} (%DM) is percentage of DM of the i^{th} feedstuff that is starch

Equation 5: CA_i (%DM) = NFC_i – CB_{1i}

CA_i (%DM) is percentage of DM of the feedstuff that is sugar

Where:

ASH_i (%DM) is percentage of ash of the i^{th} feedstuff

CP_i (%DM) is percentage of crude protein of the i^{th} feedstuff

FAT_i (%DM) is percentage of fat of the i^{th} feedstuff

$LIGNIN_i$ (%NDF) is percentage of lignin of the i^{th} feedstuff's NDF

$NDFIP_i$ (%CP) is the percentage of neutral detergent insoluble protein in the crude protein of the i^{th} feedstuff

NDF_i is the percentage of the i^{th} feedstuff that is neutral detergent fibre.

$STARCH_i$ (%NFC) is the percentage of starch in the non-fibre carbohydrates of the i^{th} feedstuff

Partitioning of Protein fractions (Fox *et al.,* 2004)

The following equations were used to calculate the five protein fractions contained in the i^{th} feedstuff from the % crude protein, % protein solubility, % NDFIP, and % ADFIP:

Equation 1: PA_i (%DM) = NPN_i x 0.0001 x $SOLP_i$ x CP

PA_i (%DM) is % of CP in the i^{th} feedstuff that is non-protein nitrogen (fraction A)

Equation 2: PB_{1i} (%DM) = $SOLP_i$ x CP_i x 0.01 – PA_i

PB_{1i} (%DM) is % of CP in the i^{th} feedstuff that is rapidly degraded protein (fraction B_1)

Equation 3: PC_i (%DM) = $ADFIP_i$ x CP_i x 0.01
PC_i (%DM) is % of crude protein in the i^{th} feedstuff that is bound protein (fraction B_2)

Equation 4: PB_{2i} (%DM) = CP_i – PA_i – PB_{1i} – PB_{1i} – PC
PB_{2i} (%DM) is % of the CP in the i^{th} feedstuff that is intermediately degraded protein (fraction C)

Where:

$ADFIP_i$ (%CP) is % of the i^{th} feedstuff that is ADICP.

CP_i (%DM) is % of crude protein of the i^{th} feedstuff.

NPN_i (%soluble protein) is % of soluble protein in CP of the i^{th} feedstuff that is non-protein nitrogen times 6.25.

$SOLP_i$ (%CP) is % of crude protein in the i^{th} feedstuff that is soluble protein.

3.2.5 *In vitro* degradation of LLM and diets

Degradation of the three dietary treatments was tested using ANKOM Daisy^{II} incubator system (Ankom Technology Corp., Fairport, NY, 2005).

Preparation and incubation of samples

A 0.5 g sample of the dietary treatment (PEL, $P_{65}L_{35}$ or $P_{50}L_{50}$) was weighed into ash free and N free nylon bags and then heat sealed. *In vitro* micro mineral solution was prepared by addition of approximately 13.2 g calcium chloride ($CaCl_2.2H_2O$), 10.0 g Magnesium chloride ($MnCl_2.4H_2O$), 1.0 g cobalt chloride ($CoCl_2.6H_2O$) and 8.0 g Iron chlorite ($FeCl_3.6H_2O$) into a 100 ml volumetric flask. *In vitro* macro mineral solution of 2000 ml was also prepared by addition of 12.4 g of KH_2PO_4 anhydrous, 1.18 g of magnesium sulphide ($MgSO4.7H_2O$), 4.44 g of sodium chloride (NaCl), 0.2 g of $CaCl_2.2H_2O$ and 1.0 g of urea in 2 L of deionized water. A magnetic stirrer bar was used to dissolve the mixtures slowly. Buffer solution was also prepared by addition of 15 g Na_2CO_3 and 1 g $Na_2S.9H_2O$ into 100 ml of distilled water (Holden *et al.,* 1999).

The final buffer solution of 4000 ml was prepared by 5 g tryptose, 0.5 ml micro mineral solution, 1000 ml buffer solution, 1000 ml macro mineral solution and 5 ml of rezasurin. Reducing solution was prepared in a beaker labelled A by adding 1.25 g of cysteine HCl and 20 g potassium hydroxide (KOH) pellets into 100 ml distilled water and magnetic stirrer bar was used to dissolve the chemicals slowly. Reducing solution was also made using a beaker marked B by mixing 1.25 g of sodium sulphide non-ahydrate with 100ml of distilled water. After preparing the solutions, beaker A and B was mixed together to make 1000 ml reducing solution where 500 ml of the mixed reducing solution was added into each digestion vessel.

All the solutions were prepared before each digestion was run by warming final buffer in a water bath to 39 °C and adding 20 ml of buffer solution to 1 L of micro mineral solution (Holden *et al.,* 1999).

Rumen fluid/liquor was collected from calves aged 50 days old directly using oesophageal tube method under mild vacuum suction (Tufarelli *et al.,* 2010) and mixed in a pre-warmed CO_2-filled thermos flask. The fluid was obtained in the morning before the calves were offered the morning feed. The rumen fluid pH was 6.9. The ruminal fluid was transferred to a domestic blender and homogenised while being gassed with CO_2, strained through double layers of cheese cloth directly into pre-warmed thermos flask (Hayes *et al.*, 2003). The ruminal fluid was purged with CO_2 before and during addition to samples to ensure an anaerobic environment. Approximately 1600 ml of buffer solution and 400 ml of the rumen fluid were added, each jar were flushed with CO_2. The sealed jars were placed into the pre-warmed Daisy^{II} incubator. The incubator maintained a constant temperature of 39°C throughout the incubation and continuously rotated.

The dietary treatment samples were incubated in triplicates for 0, 4, 10, 18, 24, 48 and 72 hours. Zero-hour bags were washed with water. Lucerne leaf-meal samples were incubated up to 48 hours. At termination bags were washed under running water. Samples were placed into a 100°C oven for 48 hours, weighed for dry matter degradability (IVDMD) estimation and then ashed in a muffle furnace at 550°C for 8 hours for estimating *in vitro* organic matter degradability (IVOMD). The NDF

(IVNDFD) and CP (IVCPD) disappearance were estimated at 24 and 48 hours because these are slowly degradable fractions.

Incubation of samples using a Daisy^{II} incubator

In vitro digestion kinetics

Non-linear procedures (PROC NLIN) in SAS (2009) were used to estimate *in vitro* degradation kinetics in the rumen. Data were fitted into exponential model without lag time (Ørskov and McDonald, 1979) to determine the rate constants and potential degradation according to the exponential model:

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

Where:

PD is the potential degradation of DM at incubation time '*t*'

a is the soluble fraction (%; fraction washed out at t = 0; this value resulted from the incubation of 0 h bags

b is the insoluble slowly degraded fraction

c is the fractional degradation rate (h^{-1}) and *t* is the incubation time (h).

Effective degradability (ED; %) was calculated from the afore mentioned parameters (*a*, *b* and *c*) assuming a fractional passage rate (*kp*) of 5%/h:

$$ED = a + b\,(c / (c + kp))$$

3.2.6 Statistical analysis

All data on chemical composition, nutrient fractions, rumen fermentation and nutrient balances were tested for normality and equal variance in Minitab Statistical Software, version 17 (Minitab, 2010). Analysis of variance (ANOVA) procedures in Minitab (Minitab 17.0) were used to test differences in ingredients (LLM and pellets) and differences in quality of simulated diets. Treatment means were compared using Fischers' LSD test and significant differences declared at $P<0.05$.

Fitted NLIN degradation parameters and ED were analysed as completely randomised design (CRD) with diet as a factor using ANOVA procedures in SAS (2009). The model used for analysis was:

$Yi = \mu + \tau_i + \varepsilon_i$

Where: Y_i is an observation of the dependent variable,

μ_i is the population mean for the variable,

τ_i is the random effect of the treatment, and

ε_i is the random error associated with the observation *i*.

3.3 Results

3.3.1 Nutrient composition of lucerne leaf-meal and diets

The results of the nutrient composition of lucerne leaf-meal are presented in Table 3.01. Lucerne leaf-meal contained 25% CP with very low fractions of bound nitrogen, less than 1%. Neutral detergent fibre was low and so was the lignin (less than 1%) with relatively high levels of NFC. Ash content was high (9.5%). However, phosphorus content was low (0.3%).

The results of the nutrient composition of the diets are presented in Table 3.02. The diets had similar ($P>0.05$) organic matter, crude protein, acid detergent insoluble crude protein, gross energy, ash, neutral detergent fibre, acid detergent fibre, hemicellulose, non-fibre carbohydrates and non-polar extracts contents. However, Diet PEL had higher ($P<0.05$) neutral detergent insoluble crude protein and acid

detergent lignin contents than Diets $P_{65}L_{35}$ and $P_{50}L_{50}$. Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ had similar ($P>0.05$) neutral detergent insoluble crude protein and acid detergent lignin contents. Diet PEL had higher ($P<0.05$) ether extracts, phosphorus and starch contents than Diets $P_{65}L_{35}$ and $P_{50}L_{50}$. Diet $P_{65}L_{35}$ had more ($P<0.05$) ether extracts, phosphorus and starch contents than Diet $P_{50}L_{50}$. Diet $P_{65}L_{35}$ had higher ($P<0.05$) cellulose and calcium contents than PEL and $P_{50}L_{50}$ diets. Similarly, Diet $P_{50}L_{50}$ had higher ($P<0.05$) cellulose and calcium contents than PEL diet.

Table 3.01 The nutrient composition of lucerne leaf-meal (units are in % dry matter except MJ/kg DM for gross energy)

Variable	Amount
Dry matter	94.0
Organic matter	90.5
Crude protein	25.0
Gross energy	16.2
Neutral detergent insoluble crude protein	0.6
Acid detergent insoluble crude protein	1.3
Ether extracts	1.6
Ash	9.5
Neutral detergent fibre	22.5
Hemicellulose	7.7
Cellulose	14.0
Acid detergent fibre	14.8
Acid detergent lignin	0.8
Non-fibre carbohydrates	40.8
Non-polar extracts	83.9
Calcium	1.4
Phosphorus	0.3
Starch	0.2

Table 3.02 The nutrient composition of diets (units are in % dry matter except MJ/kg DM for gross energy)

Variable	Treatment			SEM
	PEL	$P_{65}L_{35}$	$P_{50}L_{50}$	
Dry matter	92.3	92.3	92.6	0.142
Organic matter	91.3	91.4	91.3	0.248
Crude protein	20.4	22.6	22.8	0.626
NDICP	3.3[a]	2.0[b]	2.2[b]	0.127
ADICP	0.8	1.0	1.1	0.091
Gross energy	15.8	15.7	15.8	0.111
Ether extracts	4.7[a]	2.2[b]	2.0[c]	0.070
Ash	8.7	8.6	8.7	0.249
Neutral detergent fibre	34.1	33.4	33.3	2.520
Acid detergent fibre	11.8	13.8	13.0	1.456
Hemicellulose	22.3	19.7	20.3	1.637
Cellulose	9.9[c]	18.0[a]	13.4[b]	0.887
Acid detergent lignin	1.8[a]	0.4[b]	0.2[b]	1.036
Non-fibre carbohydrates	39.7	40.8	40.2	2.674
Non-polar extracts	74.3	74.9	74.7	2.520
Calcium	1.0[c]	1.7[a]	1.5[b]	0.006
Phosphorus	0.6[a]	0.5[b]	0.4[c]	0.012
Starch	13.4[a]	10.1[b]	8.5[c]	0.029

PEL: calf concentrate pellets

$P_{65}L_{35}$: 65% calf concentrate pellets mixed with 35% LLM

$P_{50}L_{50}$: 50% calf concentrate pellets mixed with 50% LLM

NDICP: neutral detergent insoluble crude protein;

ADICP: acid detergent insoluble crude protein;

SEM: Standard error of the means.

[abc]: Means in the same row not sharing a common superscript are significantly different ($P<0.05$).

3.3.2 Estimated supply of energy and protein fractions from lucerne leaf-meal and diets

The results of estimated supply of energy and protein fractions of the lucerne leaf-meal are presented in Table 3.03. Lucerne leaf-meal had high levels of carbohydrates (CHO), metabolisable energy (ME) and TDN_{1x} (maintenance level of intake); and low starch and fibre fractions.

Table 3.03 Estimated supply of energy and protein fractions of lucerne leaf-meal

Variable	Value
Carbohydrate (%)	62.7
Non-fibre carbohydrates (%)	40.8
Starch (%)	0.2
Available fibre (%)	19.8
Unavailable fibre (%)	1.8
Non-protein nitrogen (fraction A) (%)	0.05
Intermediately degraded protein (fraction B_1+ B_2) (%)	24.0
Bound/unavailable protein (fraction C) (%)	0.3
TDN_{1x} (%)	77.4
dTDN (%)	75.5
Digestible energy, DE (Mcal/kg DM)	3.3
Metabolisable energy, ME (Mcal/kg DM)	2.7
Net energy for maintenance, NE_m (Mcal/kg DM)	1.8
net energy for gain, NE_g (Mcal/kg DM)	1.2

TDN_{1x}: total digestible nutrients at maintenance level of intake;
dTDN: digestible total digestible nutrients.

The results of estimated supply of energy fractions of the diets are presented in Table 3.04. The diets had similar (P>0.05) carbohydrates, available fibre, TDN1x, digestible total digestible nutrients (dTDN), digestible energy (DE), metabolisable energy (ME), net energy for maintenance (NEm) and net energy for gain (NEg). However, differences (P<0.05) were observed in non-fibre carbohydrates, soluble carbohydrates, starch and unavailable fibre contents across all the dietary treatments. Diet PEL had higher (P<0.05) starch (fraction B_1) and unavailable fibre

(fraction C) than Diets $P_{65}L_{35}$ and $P_{50}L_{50}$. Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ had similar (P>0.05) starch and unavailable fibre contents. Diet $P_{65}L_{35}$ had more (P<0.05) non-fibre carbohydrate contents compared to $P_{50}L_{50}$ and PEL diets. However, Diets $P_{50}L_{50}$ and PEL had similar (P>0.05) non-fibre carbohydrate contents. Furthermore, Diet $P_{50}L_{50}$ had higher (P<0.05) soluble carbohydrate (fraction A) contents than Diets PEL and $P_{65}L_{35}$. Similarly, Diet $P_{65}L_{35}$ had higher (P<0.05) soluble carbohydrate contents than Diet PEL.

Table 3.04 Estimated supply of energy fractions and carbohydrates composition of the diets

Variable	Treatment			SEM
	PEL	$P_{65}L_{35}$	$P_{50}L_{50}$	
Carbohydrate (%)	64.7	64.0	63.7	0.523
Non-fibre carbohydrates (%)	39.7^{b}	40.8^{a}	40.2^{b}	0.122
Soluble CHO (fraction A) (%)	24.0^{c}	28.6^{b}	30.6^{a}	0.261
Available fibre (fraction B_2) (%)	26.0	23.8	22.9	1.172
Starch (fraction B_1) (%)	14.5^{a}	10.6^{b}	9.0^{b}	0.835
Unavailable fibre (fraction C) (%)	4.4^{a}	3.5^{b}	3.1^{b}	0.274
TDN_{1x} (%)	82.9	81.0	80.1	1.062
dTDN (%)	81.0	79.1	78.2	3.503
DE (Mcal/kg DM)	3.6	3.5	3.5	0.252
ME (Mcal/kg DM)	2.9	2.9	2.8	0.100
NE_m (Mcal/kg DM)	2.0	1.9	1.9	0.041
NE_g (Mcal/kg DM)	1.3	1.3	1.2	0.170

PEL: calf concentrate pellets

$P_{65}L_{35}$: 65% calf concentrate pellets mixed with 35% LLM

$P_{50}L_{50}$: 50% calf concentrate pellets mixed with 50% LLM

CHO: carbohydrates

TDN_{1x}: total digestible nutrients at maintenance level of intake;

dTDN: digestible total digestible nutrients;

DE: digestible energy; ME: metabolisable energy;

NEm: net energy for maintenance;

NEg: net energy for gain;

SEM: Standard error of the means.

[abc]: Means in the same row not sharing a common superscript are significantly different (P<0.05).

3.3.3 *In vitro* degradation of the lucerne leaf-meal and diets

The results of *in vitro* degradation of lucerne leaf-meal are presented in Table 3.05. Lucerne leaf-meal had high degradation values of dry matter, organic matter, neutral detergent fibre and crude protein. Dry and organic matter of LLM were rapidly degraded within four hours of incubation, with a high '*a*' component (42.5%) and high rate of degradation (6.4%/hr.). The DM component was completely degraded by 24 hours with a '*b*' of 96% and an ED of 97%. The NDF (97%) and CP (92%) contents were highly degraded within 48 hours of incubation.

The results of in vitro degradation of the diets are presented in Table 3.06 and Figure 3.01. No differences (P>0.05) were observed in *in vitro* dry matter (IVDMD), organic matter (IVOMD) and neutral detergent fibre (IVNDFD) degradation across all the diets at 0, 4, 10 and 48 hours of incubation. Furthermore, *in vitro* organic matter (IVOMD) and crude protein (IVCPD) degradabilities were similar (P>0.05) across all the dietary treatments at 24 hours of incubation period. Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ had similar (P>0.05) IVDMD and IVOMD within 18 and 72 hours of incubation. However, $P_{65}L_{35}$ and $P_{50}L_{50}$ diets had higher (P<0.05) IVDMD and IVOMD within 18 and 72 hours of incubation than Diet PEL. *In vitro* DM and NDF degradabilities differed (P<0.05) at 24 hours of incubation with $P_{50}L_{50}$ and $P_{65}L_{35}$ diets being similar (P>0.05) but higher (P<0.05) than Diet PEL. Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ had similar (P>0.05) IVCPD within 48 hours of incubation and both values were higher (P<0.05) than those of PEL. All the diets had similar (P>0.05) soluble fraction (*a*), insoluble fraction (*b*) and fractional degradation rate (*c*). Diets $P_{65}L_{35}$ and $P_{50}L_{50}$ had similar (P>0.05) effective degradability (ED). However, $P_{65}L_{35}$ and $P_{50}L_{50}$ diets had higher (P<0.05) effective degradability (ED) values compared to those of PEL diet.

Table 3.05 *In vitro* degradation, rate of degradation, potential and effective degradability of lucerne leaf-meal

Incubation time (h)	Degradability (%)			
	Dry matter	Organic matter	Neutral detergent fibre	Crude protein
0	55.3	57.8	-	-
4	61.1	65.0	-	-
10	73.1	79.3	-	-
18	79.4	85.6	-	-
24	91.5	96.0	91.5	86.0
48	97.3	99.2	97.3	91.5
Degradation kinetics of dry matter				
a (%)	42.52			
b (%)	96.03			
c (h^{-1})	0.064			
ED (%)	96.5			

PD = $a + b\ (1 - e^{-ct})$ is the exponential equation (Ørskov and McDonald, 1979)

Where:

a: soluble fraction or material small enough to immediately come out of the bag (%);

b: insoluble fraction but potentially degradable material (%);

c: fractional degradation rate (h^{-1});

t: incubation time (h);

ED: effective degradability (%).

Table 3.06 Diet *in vitro* degradation at different times of incubation in nylon bags and constants in the exponential equation: PD = *a*+ *b* (1 - e^{-ct}) (Ørskov and McDonald, 1979)

Variable	Incubation time (h)	Treatment			SEM
		PEL	$P_{65}L_{35}$	$P_{50}L_{50}$	
DM (OM)	0	53.6 (53.9)	56.6 (58.4)	55.6 (57.6)	1.975 (2.424)
(%)	4	63.0 (65.2)	62.3 (64.4)	62.1 (64.6)	2.225 (2.597)
	10	69.3 (72.0)	71.6 (75.2)	73.6 (77.8)	2.552 (3.099)
	18	76.3^{b} (79.8^{b})	77.8^{a} (82^{a})	78.5^{a} (82.9^{a})	0.805 (1.058)
	24	84.1^{b} (90.9)	88.3^{a} (95.8)	88.9^{a} (94.3)	0.555 (1.759)
	48	85.4 (92.6)	89.3 (97.2)	92.2 (97.7)	4.060 (4.320)
	72	90.9^{b} (96.6^{b})	92.8^{a} (99^{a})	93.1^{a} (98.7^{a})	0.564 (0.764)
NDF (%)	24	83.5^{b}	88.3^{a}	88.9^{a}	1.334
	48	83.6	89.8	92.5	8.270
CP (%)	24	71.7	82.9	82.3	7.621
	48	77.0^{b}	88.3^{a}	85.6^{a}	1.319
Degradation kinetics of dry matter					
a (%)		32.5	37.9	39.7	3.992
b (%)		86.3	93.4	94.4	3.816
c (h^{-1})		0.08	0.06	0.06	0.018
ED (%)		83.6^{b}	88.0^{a}	92.0^{a}	3.298

PEL: calf concentrate pellets

$P_{65}L_{35}$: 65% calf concentrate pellets mixed with 35% LLM

$P_{50}L_{50}$: 50% calf concentrate pellets mixed with 50% LLM

DM: dry matter;

OM: organic matter;

NDF: neutral detergent fibre;

CP: crude protein.

a: soluble fraction or material small enough to immediately come out of the bag (%);

b: insoluble fraction but potentially degradable material (%);

c: fractional degradation rate (h^{-1});

t: incubation time (h);

ED: effective degradability (%);

SEM: Standard error of the means.

[abc]: Means in the same row not sharing a common superscript are significantly different (P<0.05).

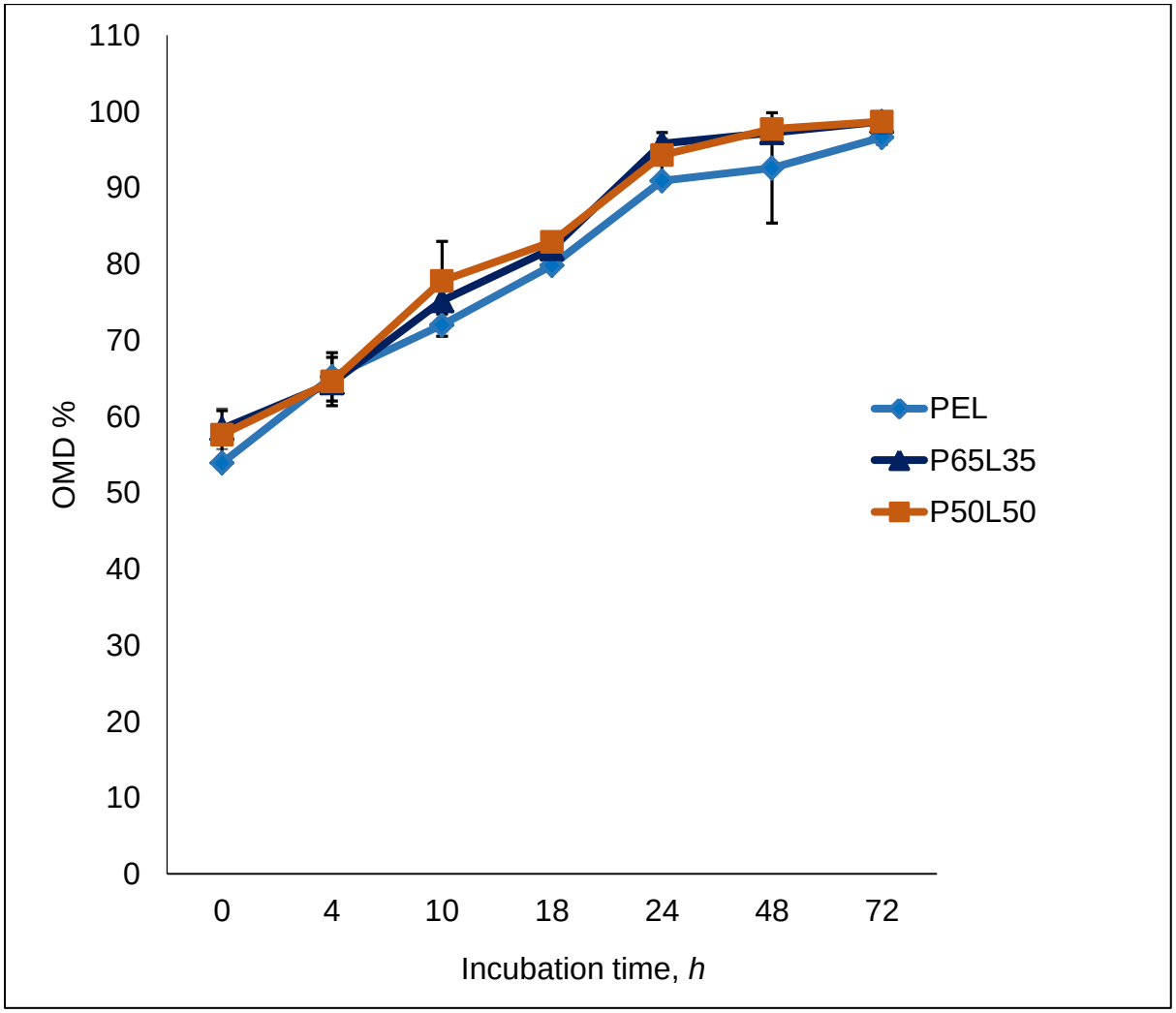

Figure 3.01: Average *in vitro* organic matter degradability (IVOMD, %) for each dietary treatment

3.4 Discussion

Lucerne leaf-meal was rich in crude protein and soluble carbohydrate fractions with high energy density as was also noted by Kuan *et al.* (1983), Broderick and Satter (1998) and Leming and Lindberg (2001). This is typical of leguminous forages harvested before maturity. Popovic *et al.* (2001) and Arias *et al.* (2003) reported

higher values for lucerne harvested pre-bloom but the differences may reflect variations in production environments, processing methods and cultivars (Lindberg *et al.,* 1995; Salem, 2005). Considering that calves are monogastric at birth and require feeds high in protein and low in fibre, LLM would be a suitable forage for neonates and monogastric animals (Leming and Lindberg, 2001; Fuller, 2004). In early growth of calves, muscle accretion is high.

All the dietary treatments had CP above 20%. However, Hill *et al.* (2006) reported that 22% CP is needed for young calves to achieve growth rates of at least 0.6 kg/day. Diets low in nutrients result in low growth rates and delayed attainment of breeding weight, consequently affecting age at first calving. This has economic consequences as heifers that calve late tend to be less productive (BhattI *et al.,* 2007). The Ca: P ratio of Diet $P_{50}L_{50}$ is 3.8:1 which is much higher than the ratio in the calf starter pellets (1.7:1), therefore, Diet $P_{50}L_{50}$ would be expected to perform better.

There was a high amount of unavailable carbohydrate in the concentrate, possibly reflecting damage of carbohydrates due to heat processing during feed manufacturing or poor quality of ingredients. The LLM was low in unavailable carbohydrates and the forage meal was green, indicating less breakdown of chlorophyll pigments and preservation of micronutrients. Proteins and carbohydrates are required for rumen microbial protein synthesis and production of volatile fatty acids that provide metabolisable energy to calves (Bannink *et al.,* 2006; Jones and Heinrichs, 2007). Concentrate diet (PEL) had higher ADICP and this fraction is completely unavailable for digestion and does not contribute to absorbable amino acids (NRC, 1985). Repetto *et al.* (2000) reported that application of thermal treatment results in increased ADICP and NDICP contents of the forage or concentrate and this results in an increased amount of the undegradable protein in the feed, making the nutrients unavailable to the animal. Thus, the increase in ADICP gives evidence of the occurrence of partial or total Maillard reactions, which increase with the intensity of the thermal treatment (Van Soest, 1982). In this reaction, proteins are permanently bound with carbohydrates/fibre and become lignified and indigestible or poorly digested by rumen microbes (de Ondarza, 2003).

The lucerne leaf-meal had lower level of NDF and ADF which is within the recommended range for the calf starter feed and this forage may be used as main feed for neonates including monogastric animals such as poultry and pigs (Leming and Lindberg, 2001), although energy may have to be supplemented. A higher starch level was observed in calf starter pellets (PEL) and this observation is also reported by Sniffen *et al.* (1992). Based on the estimations made in this study for the carbohydrate fractions, Diet PEL had higher content of sugars (CA) and is fermented rapidly by ruminal microorganisms (Sniffen *et al.,* 1992). Similarly, higher bound/unavailable fibre was also observed in Diet PEL and this could be associated with the lower lignin because fraction 'C' is assumed to be structural N associated with lignin or lignin artifacts (Marichal *et al.,* 2010). Higher LLM ash concentration was observed in this study and this is also reported by Frame (2005). The crude ash concentration reported in this study for LLM was similar to the value reported by Leming and Lindberg (2001).

The ME density of LLM substituted diets were similar to the results reported by Chester-Jones and Broadwater (2009) with a mean of 2.7 Mcal/kg DM. Similarly, higher CP degradation observed with LLM may be due to less bound proteins to the carbohydrates (Table 3.03). This is in agreement with the studies of Broderick and Albrecht (1997). Kirilov (2001) reported that the decrease in degradability and intake of lucerne is related to its maturity stage; and its quality depends on both time of harvesting and method of preservation. The higher IVDMD in the LLM substituted diets may probably be due to more cell contents in the diets, which are almost completely digestible and are not affected by lignin concentration in cell walls (Van Soest and Moore, 1965). McAllister *et al.* (1994) and McSweeney *et al.* (2001) also observed that higher NDF and lignification can reduce attachment of ruminal microbes to feed particles, as well as inhibit microbial growth and enzyme activity or intestinal activity. Giger-Reverdin (1995) and Wilson (1997) stated that cell wall thickness, surface area available to rumen microorganisms, tightness and packing of cells influence cell wall degradability by ruminants.

This study affirms that nutrient rich lucerne leaf-meal, which is also low in concentrations of secondary metabolites, is appropriate as a feed for young dairy cattle as all carbohydrate and protein components of the forage are easily degraded in the rumen.

3.5 Conclusion

Based on the present study, lucerne leaf-meal had high crude protein (25%) and low fibre fractions, this may be a good indication that inclusion of LLM in the calf diets would be beneficial to dairy heifer calves.

The diet containing 50% calf starter concentrate and 50% lucerne leaf-meal had higher crude protein and energy contents, indicating that it has a potential as an alternative calf starter feed for dairy calves. However, it also contained low amounts of starch, which can impede rumen papillae development in dairy calves. In addition, the diet was highly degradable, which would improve intake and nutrient utilisation in dairy calves

In a globally competitive production environment where dairy feed costs constitute over 70% of the production costs, it would be prudent to manage/optimise the use of commercial feeds by widening the feed resource base of neonates. In systems were calves are enclosed LLM is recommended in augmenting concentrates fed during early calf growth, for energy and protein coupling and increased rumen efficiency.

3.6 References

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CHAPTER FOUR

EFFECTS OF REPLACING CONCENTRATE FEED WITH LUCERNE LEAF-MEAL ON INTAKE AND GROWTH OF NEONATAL AND TRANSITION HOLSTEIN HEIFER CALVES

4.1 Introduction

The proper management of replacement heifers is an essential component in the dairy industry. Overall, management of dairy heifers must be handled in a manner that yields the best quality heifer, with a high potential to be productive, and at minimal cost to the farm and the environment. The sooner heifers are bred, the sooner they can enter the milking herd and become productive (Bach *et al.,* 2007). Nutritional management of the neonatal calf is the first and most important step in successful heifer rearing. If proper nutritional management is not met, then a delay in rumen development could occur and weaning will also be delayed. At birth neonates are considered to be monogastric animals due to non-functional rumen and they require amongst others, fat soluble vitamins, such as A, D and E (NRC, 2001). Hence, they cannot utilize fibrous feedstuffs due to lack of symbiotic microbial populations. Commercial calf concentrates are costly and not easily available to most emerging smallholder dairy farmers (Heinrichs and Jones, 2003).

Nutrient rich LLM would be optimal as concentrate replacement. However, low digestibility values of fibre during the neonatal stage are recognised. Kuan *et al.* (1983) reported that increasing proportion of LLM in the diet resulted a linear decrease in the digestibility of dry matter, crude protein and crude fat. However, other authors did not find any decrease in diet digestibility with LLM supplementation (Lindberg *et al.,* 1995). Thus, there is need to assess the value of LLM in neonatal feeding.

The objectives of this study were to:

i. determine the effects of replacing calf starter concentrates with LLM on feed intake, feed conversion ratio and growth of pre-weaned and transition Holstein heifer calves.
ii. predict ruminal nitrogen balance and energy density using Large Ruminant Nutrition System (LRNS).

4.2 Materials and methods

4.2.1 Study site

This study was conducted at Irene, Animal Production Institute of the Agricultural Research Council of South Africa in 2012 (Refer to Section 3.2.1).

4.2.2 Collection, drying and storage of forage material

Forage lucerne was grown in the summer of 2012 in large plots under irrigation at the Animal Production Institute of the Agricultural Research Council (Refer to Section 3.2.2).

Lucerne plant at the pre-bloom stage

4.2.3 Animals, experimental design and diets

A total of 48 Holstein heifer calves were used for this study. Calves were randomly housed in individual pens (10 m^2) with rubber mats and without bedding. Each calf was identified with a numbered ear-tag. All calves were dosed (anthelmintic, Ivomec, Bio Onderstepoort Production) against internal worms before the start of the experiment. From one to 20 days of age, before the commencement of the experiment, calves were fed 4 litres of unpasteurised milk and *ad libitum* calf starter pellets. Milk contained 3.8% fat, 3.5% protein and 4.8% lactose.

The study comprised of two experimental phases, pre-weaning phase or Experiment 1 (calves aged 21 to 42 days) and transition phase or Experiment 2 (calves aged 43 to 56 days).

Experiment 1 (Pre-weaning phase)

This study commenced with 24 Holstein heifer calves aged 21 days and was terminated when the calves were 42 days old. The calves were randomly allocated to three dietary treatments as specified in Table 4.01, having eight replicates (animals) per treatment, in a completely randomised design (CRD). Each calf was offered 4 litres of unpasteurised milk per day, and *ad libitum* feed. Calves were allowed free access to clean water from a bucket drinker in each pen throughout the experimental period.

Table 4.01 Dietary treatments for Experiment 1

Diet code	Diet description
PEL	Holstein heifer calves fed 4 litres of milk/calf/day and *ad libitum* commercial calf starter pellets
$P_{65}L_{35}$	Holstein heifer calves fed 4 litres of milk/calf/day and *ad libitum* diet containing 65% commercial calf starter pellets and 35% lucerne leaf-meal
$P_{50}L_{50}$	Holstein heifer calves fed 4 litres of milk/calf/day and *ad libitum* diet containing 50% commercial calf starter pellets and 50% lucerne leaf-meal

Experiment 2 (Transition phase)

A total of 24 Holstein heifer calves aged 43 days were used in this study. It was terminated when the calves were 56 days old. Before the start of the experiment (1-42 days of age) calves were raised on a diet of 2 litres of unpasteurised milk and *ad libitum* calf starter pellets. These calves were different from those used in Experiment 1. The calves were randomly allocated to three dietary treatments as specified in Table 4.02, having eight replicates per treatment, in a completely randomised design. Thus, there were 24 pens. Clean water was provided *ad libitum* throughout the experimental period. All the calves were weaned at the age of 56 days.

Table 4.02 Dietary treatments for Experiment 2

Diet code	Diet description
HPEL	Holstein heifer calves fed 2 litres of milk/calf/day and *ad libitum* commercial calf starter pellets
$HP_{65}L_{35}$	Holstein heifer calves fed 2 litres of milk/calf/day and *ad libitum* diet containing 65% commercial calf starter pellets and 35% lucerne leaf-meal
$HP_{50}L_{50}$	Holstein heifer calves fed 2 litres of milk/calf/day and *ad libitum* diet containing 50% commercial calf starter pellets and 50% lucerne leaf-meal

4.2.4 Feed, milk and live weight measurements

The amount of feed and milk offered to each animal was weighed and recorded daily and feed refusals were weighed every morning to determine the dry matter amount consumed by difference. The daily feed and milk intake was determined during the collection period by the difference in amount of feed/milk offered and feed/milk refusals or leftovers. Sub-samples of the feed offered and refusals were dried at 60 ºC for 72 hours to constant weight for dry matter determination. The calves were weekly weighed on Tuesdays at 08.00 hours until the termination of each experiment. The difference between the initial and final live-weight was used to compute live-weight change (gain/loss) for calves in each dietary treatment (McDonald *et al.,* 2002). No calves died during the experiment.

4.2.5 Chemical analysis

Chemical analysis of the feeds were as described in Chapter 3, Section 3.2.3.

Measurements and calculations of feed fractions

Non-fibre carbohydrates, hemicellulose, cellulose, non-polar extracts, neutral detergent insoluble crude protein and acid detergent insoluble crude protein were as described in Chapter 3, Section 3.2.3.

4.2.6 Simulation of LLM and diets

Large Ruminant Nutrition System (LRNS) model of the University of California-Davis (LRNS version 1.0.31, 2014) was used to predict energy nutrient density supply and balances of the three diets of LLM and commercial concentrates. The LRNS is based on the Cornell Net Carbohydrate and Protein System (CNCPS) Version 5.0.40 (Fox *et al.,* 2004). Level 1 solutions of the LRNS model were used to predict % ruminal nitrogen balance (RNB) and diet concentrations, and the results are presented in Tables 4.03, 4.04 and 4.05. The LRNS predictions were based on tropical climatic conditions of 22 ºC, 30% relative humidity (RH) and wind-speed of zero for calves restrained in a 10 m^2 cubicle with no exposure to storms and sunlight.

4.2.7 Statistical analysis

All the data on nutrient analyses, intake, and growth were subjected to analysis of variance using GLM procedures in Minitab Statistical Software, Version 17 (Minitab, 2010). Treatment means were compared using a Fishers' least significant difference (LSD) and significant differences were declared at $P<0.05$. The model used for analysis was:

$Y_i = \mu + \tau_i + \varepsilon_i$

Where: Y_i is an observation of the dependent variable,

μ_i is the population mean for the variable,

τ_i is the random effect of the treatment, and

ε_i is the random error associated with the observation i.

4.3 Results

4.3.1 Nutrient composition of the dietary treatments

Results of the nutrient composition of the dietary treatments were as described in Chapter 3, Section 3.2.7.1 and presented in Tables 3.01 and 3.02.

4.3.2 Effect of diet on intake, rumen metabolism and growth of pre-weaned dairy heifer calves

Table 4.03 shows results on intake, % rumen nitrogen balance, feed conversion ratio and growth rate in pre-weaned calves. Calves on Diet $P_{50}L_{50}$ had higher (P<0.05) daily solid feed dry matter, organic matter, crude protein and neutral detergent fibre intakes than those on Diets PEL and $P_{65}L_{35}$. However, calves on Diets PEL and $P_{65}L_{35}$ consumed similar (P>0.05) amounts of solid feed dry matter, crude protein and neutral detergent fibre contents. Diet PEL had higher (P<0.05) starch intakes than Diets $P_{65}L_{35}$ and $P_{50}L_{50}$. Calves on PEL and $P_{50}L_{50}$ diets consumed similar (P>0.05) starch amounts. Diet $P_{50}L_{50}$ had higher (P<0.05) dry matter and crude protein intakes and % ruminal nitrogen balance (RNB) than PEL and $P_{65}L_{35}$ diets. Similarly, calves on Diet $P_{65}L_{35}$ had higher (P<0.05) % RNB than those on Diet PEL. Calves on $P_{65}L_{35}$ and PEL diets consumed similar (P>0.05) amounts of dry matter and crude protein contents. There were no differences (P>0.05) observed in the initial and final weights and feed conversion ratio (FCR) of the calves across all the dietary treatments. Calves on Diet $P_{50}L_{50}$ attained higher (P<0.05) average daily gains (ADG) than those on Diets PEL and $P_{65}L_{35}$. However, calves on $P_{50}L_{50}$ and $P_{65}L_{35}$ diets had similar (P>0.05) ADG but differed (P<0.05) from ADG of those on PEL diet.

4.3.3 Effect of diet on intake, rumen metabolism and growth of transition dairy heifer calves

The results of the effect of replacing calf starter pellets with lucerne leaf-meal on diet intake, % ruminal nitrogen balance, feed conversion ratio and growth of transition Holstein heifer calves are presented in Table 4.04. Daily dry matter intakes of the diets were similar (P>0.05) across all the dietary treatments. Similarly, calves consumed the same (P>0.05) amounts of solid feed organic matter and neutral detergent fibre contents. However, calves fed a diet having 50% of the calf starter concentrate and 50% lucerne leaf-meal ($HP_{50}L_{50}$) had higher (P<0.05) crude protein intakes than those on HPEL and $HP_{65}L_{35}$ diets. Calves on a $HP_{50}L_{50}$ diet had similar (P>0.05) amount of CP intakes with those on Diet $HP_{65}L_{35.}$ Calves fed HPEL had higher (P<0.05) starch intakes than those on Diets $HP_{65}L_{35}$ and $HP_{50}L_{50}$. Calves on Diets $HP_{65}L_{35}$ and $HP_{50}L_{50}$ had similar (P<0.05) amounts of starch intakes. However,

calves on Diet $HP_{50}L_{50}$ had higher (P<0.05) % RNB compared to those on other diets. Calves attained similar (P>0.05) final weight, ADG and FCR across the dietary treatments.

4.3.4 Diet concentrations

Pre-weaning phase

The results of the predicted diet energy density during the pre-weaning phase are presented in Table 4.05. The diets had similar (P>0.05) metabolisable energy (ME) levels. However, Diet $P_{50}L_{50}$ had higher (P<0.05) apparent total digestible nutrients (TDN) than Diets PEL and $P_{65}L_{35}$. Diet $P_{65}L_{35}$ had more (P<0.05) apparent total digestible nutrients than Diet PEL. Similarly, the $P_{50}L_{50}$ diet had higher (P<0.05) net energy for gain (NEg) and maintenance (NEm) than PEL and $P_{65}L_{35}$ diets. Diets PEL and $P_{65}L_{35}$ had similar (P>0.05) NEg and NEm.

Transition phase

The results of the predicted of diet energy density during the transition phase are presented in Table 4.05. During the transition phase, diets had similar (P>0.05) NEg and NEm. However, Diet HPEL had higher (P<0.05) apparent TDN and ME than other dietary treatments. Diets $HP_{65}L_{35}$ and $HP_{50}L_{50}$ had similar (P>0.05) apparent TDN and ME.

Table 4.03 Effect of replacing calf starter pellets with lucerne leaf-meal on diet intake, % ruminal nitrogen balance, feed conversion ratio and growth of pre-weaned Holstein heifer calves

Variable	Treatment			SEM
	PEL	$P_{65}L_{35}$	$P_{50}L_{50}$	
Intake of solid feed (g/calf/day)				
Dry matter	506[b]	538[b]	634[a]	2.95
Organic matter	460[b]	489[b]	577[a]	268.80
Crude protein	96[b]	113[ab]	133[a]	59.61
Neutral detergent fibre	162[b]	167[b]	196[a]	92.82
Starch	66[a]	54[b]	57[ab]	32.97
Intake of milk (g/calf/day)				
Milk (litres/day)	4.0	4.0	4.0	0.00
Dry matter	400	400	400	0.00
Crude protein	140	140	140	0.00
Intake of solid feed and milk (g/calf/day)				
Dry matter	905[b]	938[b]	1033[a]	295.4
Crude protein	236[b]	253[b]	273[a]	59.61
Predicted % RNB (% of required)	61.3[c]	110.3[b]	126.7[a]	1.00
Initial weight (kg/calf)	35.2	41.1	37.1	8.46
Final weight (kg/calf)	44.9	52.8	53.6	9.79
Average daily gain (kg/calf/day)	0.46[b]	0.56[ab]	0.79[a]	0.16
Feed conversion ratio	2.2	1.9	1.1	0.73

PEL: calf concentrate pellets

$P_{65}L_{35}$: 65% calf concentrate pellets mixed with 35% LLM

$P_{50}L_{50}$: 50% calf concentrate pellets mixed with 50% LLM

RNB: ruminal nitrogen balance;

SEM: Standard error of the means.

[abc]: Means in the same row with different superscript are significantly different ($P<0.05$).

Table 4.04 Effect of replacing calf starter pellets with lucerne leaf-meal on diet intake, % ruminal nitrogen balance, feed conversion ratio and growth of transition Holstein heifer calves.

Variable	Treatment			SEM
	HPEL	$HP_{65}L_{35}$	$HP_{50}L_{50}$	
Intake of solid feed (g/calf/day)				
Dry matter	1094	1114	1143	283.3
Organic matter	996	1017	1041	257.8
Crude protein	208^{b}	235^{a}	240^{a}	58.19
Neutral detergent fibre	350	347	355	88.51
Starch	142^{a}	112^{b}	103^{b}	29.96
Intake of milk (g/calf/day)				
Milk (litres/day)	2.0	2.0	2.0	0.00
Dry matter	200	200	200	0.00
Crude protein	70	70	70	0.00
Intake of solid feed and milk (g/calf/day)				
Dry matter	1294	1318	1343	283.30
Crude protein	278^{b}	305^{a}	310^{a}	58.19
Predicted % RNB (% of required)	59.7^{c}	119.7^{b}	142.0^{a}	1.890
Initial weight (kg/calf)	44.9	53.2	55.5	8.952
Final weight (kg/calf)	56.1	64.6	68.9	8.700
Average daily gain (kg/calf/day)	0.86	0.88	1.03	0.230
Feed conversion ratio	1.57	1.73	1.46	0.337

PEL: calf concentrate pellets

$P_{65}L_{35}$: 65% calf concentrate pellets mixed with 35% LLM

$P_{50}L_{50}$: 50% calf concentrate pellets mixed with 50% LLM

RNB: ruminal nitrogen balance;

SEM: Standard error of the means.

^{abc}: Means in the same row with different superscript are significantly different ($P<0.05$).

Table 4.05 Predicted energy density of pre-weaning and transition diets using Large Ruminant Nutrition System (LRNS)

Variable	Treatment			SEM
	PEL (HPEL)	$P_{65}L_{35}$ ($HP_{65}L_{35}$)	$P_{50}L_{50}$ ($HP_{50}L_{50}$)	
Pre-weaning phase				
Apparent TDN (%DM)	79.0^{c}	80.0^{b}	82.7^{a}	0.333
ME (Mcal/kg DM)	2.9	2.9	3.0	0.530
NEm (Mcal/kg DM)	1.9^{b}	1.9^{b}	2.0^{a}	0.003
NEg (Mcal/kg DM)	1.3^{b}	1.3^{b}	1.4^{a}	0.003
Transition phase				
Apparent TDN (%DM)	78.0^{a}	77.0^{b}	76.9^{b}	0.060
ME (Mcal/kg DM)	2.8^{a}	2.8^{b}	2.8^{b}	0.006
NEm (Mcal/kg DM)	1.9	1.9	1.9	0.004
NEg (Mcal/kg DM)	1.24	1.23	1.23	0.005

PEL: calf concentrate pellets

$P_{65}L_{35}$: 65% calf concentrate pellets mixed with 35% LLM

$P_{50}L_{50}$: 50% calf concentrate pellets mixed with 50% LLM

TDN: Total digestible nutrients;

ME: metabolisable energy;

NEm: net energy for maintenance;

NEg: net energy for gain;

SEM: Standard error of the means.

^{abc}: Means in the same row with different superscripts are significantly different ($P<0.05$).

: The codes in brackets are for the Transition Phase.

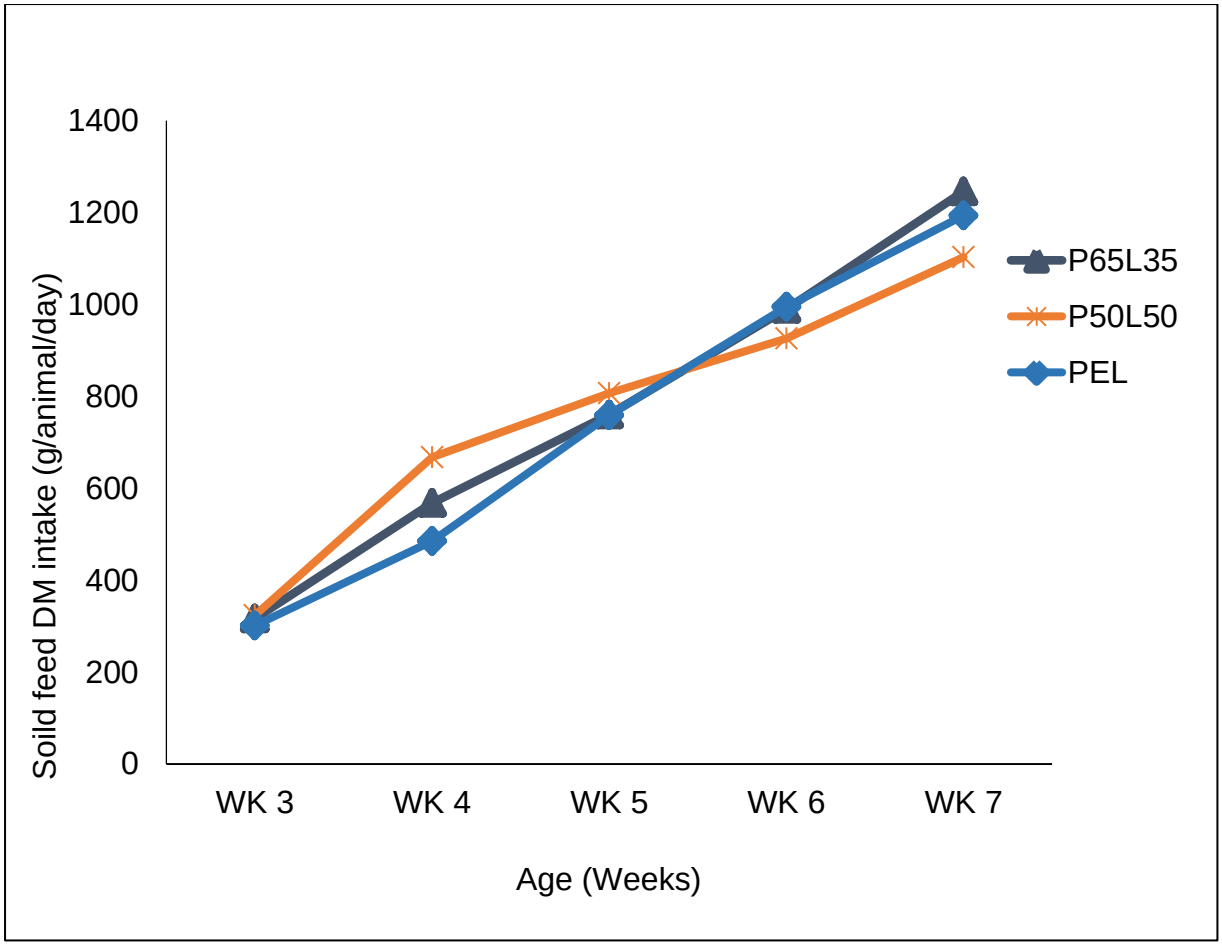

Figure 4.01 Change in dry matter intake (g/animal/day) for the dietary treatments

4.4 Discussion

Lucerne leaf-meal diets had high CP concentrations, which were influenced by LLM inclusion. The minimum required CP for dairy calves is 18% to support rumen bacterial growth (NRC, 2001; Drackley *et al.,* 2002). Bach (2014) stated that a starter feed containing above 20% CP influences growth of the calves after weaning. The NDF contents across all dietary treatments were higher than the recommended NDF content for dairy calf starter of 15 to 25% (Davis and Drackley, 1998), depending on the digestibility of the feed. The LLM fibre was highly degradable and, therefore, would not impact on nutrient availability. The diets were also low in ADL which, according to Goering and Van Soest (1970) and Buxton *et al*. (1996), would not negatively affect digestibility. Acid detergent fibre bound nitrogen (ADIN) was higher in the commercial starter feed for calves. Thus, inclusion of LLM reduced the proportion of indigestible nitrogen. Acid detergent fibre bound nitrogen represents

the portion of protein in a feedstuff that is unavailable for use by the animal because it is completely indigestible (Sniffen *et al.,* 1992). Additionally, Van Soest (1994) stated that as the concentration of ADIN increased, total N digestibility decreased. This negative association has been observed in several studies (Yu and Thomas, 1976; Thomas *et al.*, 1982; Weiss *et al.*, 1986; Van Soest, 1994). The energy density in diets were within the range of 2.9-3.2 Mcal/kg DM recommended by NRC (1989) for dairy calves.

Average daily starter intake during the pre-weaning phase were lower than the 680 g per calf recommended by NRC (2001) at approximately 41-42 days of age. However, higher intakes of DM, CP, NDF and ADF were observed in calves fed Diet $P_{50}L_{50}$ during pre-weaning and transition phases and this may be ascribed to the differences in ruminal development and thus the calf's metabolic and structural capacity to accommodate and digest solid feed (Khan *et al.,* 2007). In addition, these higher average daily CP and NDF intakes during pre-weaning phase in calves fed Diet $P_{50}L_{50}$ were the function of higher starter feed consumption. These findings are supported by higher ADG and better FCR observed in calves on Diet $P_{50}L_{50}$. During the pre-weaning and transition phase, the DM and CP intakes were within the recommended range for the dairy calves as reported by Van Amburgh and Drackley (2005); however, lower CP intake was observed with calves fed a 50% LLM inclusion diet during the transition phase. Total DM intake and FCR for the solid feed during the transition phase did not differ across treatments. It has been reported that feeding a fibre source to young dairy calves was necessary because it improved rumen health than if no forage was provided to calves (Thomas and Hinks, 1982). The high starch intake by calves on Diet PEL might be due to high NFC (39%), falling within the recommended range of 37-42%. Carbohydrates are degraded to VFA, and butyrate stimulates rumen papillae development (Coverdale *et al.,* 2004). Lucerne leaf-meal had low starch content, typical of lucerne and, therefore, should be supplemented with an energy feed. The lower starter intakes for the PEL diet was also found by Göncü *et al.* (2010) for the calf starter fed from day 4 to 56 with ADG and average starter intake of 0.51 and 0.62 kg/day, respectively.

Calves on lucerne leaf-meal diets had higher dry matter intakes. These results are evidence that fibre from LLM was highly digestible and less filling with low rumen retention time in the calves' rumen (Hall, 2003). Higher DM intakes of lucerne results in higher protein intakes hence saving of protein concentrates (Kirilov, 2001). Furthermore, the results of the present study indicate that LLM at the pre-bloom stage was highly digestible and provided more energy in the ration and, therefore, more energy was available for ruminal protein synthesis. The higher the quality, the faster the rate of digestion and the higher the potential intake. Chester-Jones and Broadwater (2006) reported the highest average daily calf starter intakes of 0.09, 0.51, 1.12 and 2.18 kg for periods 1-14, 15-28, 29-42 and 43-56 days, respectively. These results are higher than those of the present study at 21-42 and 43-56 days with intake values of 0.91-1.03 and 1.29-1.34 kg/calf/day, respectively. Moran (2005) reported that weaning can only take place by the age of six weeks if the starter consumption is 0.75 kg per animal per day. In contrast, these findings are higher than the findings of the present study during pre-weaning phase with intake ranging from 0.51 to 0.64 kg/calf/day. However, the differences may be due to breed differences (Moran, 2005). Heinrichs and Jones (2003) reported that the amount of starter eaten by calves must be used as the primary indicator of weaning time. Thus, they suggested that calves that eat 0.68 to 0.91 kg of calf starter per day for three consecutive days are ready to be weaned.

The higher ADG observed for LLM substituted diets than calf starter concentrate pellets (PEL) during the pre-weaning phase could be attributed to a better development of the rumen. This higher ADG observed during pre-weaning phase may be an indication that calves would reach puberty by 12 months and bred to calve by 22 months. Kertz *et al.* (1979) stated that high intake before weaning helps to ensure intake and sustain a desirable growth rate after weaning which improves the age at first calving.

Lucerne leaf-meal inclusion in the calf diet improved average daily weight gain. During the pre-weaning phase (21-42 days), an average daily gain of 0.68 kg/animal/day was observed for calves on diets with LLM inclusions. These findings are higher than those of Chester-Jones and Broadwater (2007) who observed that

lucerne leaf-meal inclusion of up to 25% improved average daily gain of calves up to 0.49 kg per calf over 60 days.

The PEL diet had low RNB. This is a serious hindrance for early growth as calves require more protein for muscle accretion and immune-development. The slower growth for calves on this diet confirmed these observations. During the transition phase, higher RNB was observed in calves on Diet $HP_{50}L_{50}$ and this indicates opportunities to improve the efficiency of N use by rumen microbes. However, lower value of RNB observed with Diet HPEL, indicates lower rumen fermentation, lower microbial yield and insufficient rumen degradable N relative to carbohydrate supply (Maglione and Russell, 1997). Ruminal nitrogen limitation can decrease microbial flow from the rumen (Kang-Meznarich and Broderick, 1980; NRC, 1985; Russell *et al.,* 1992), depress fibre fermentation (Russell *et al.,* 1992) and reduce DMI (NRC, 1987; Van Soest, 1994).

The lucerne leaf-meal diets seemed to provide well balanced supply of energy and protein. The diet with higher levels of LLM would be more ideal for transition or neonatal dairy calves.

4.5 Conclusion

Inclusion of 50% lucerne leaf-meal in the calf diet increased total digestible nutrients and crude protein intake by calves during pre-weaning and transition phases. However, inclusion of 50% LLM did not affect dry matter intake, average daily gain and feed conversion ratio during the transition phase. Inclusion of lucerne leaf-meal increased % RNB and ADG. Calf starter concentrate feed had low % RNB, which can impede rumen fibre fermentation and efficiency of N usage by rumen microbes.

The results of the present study indicate that supplementation of lucerne forage at the neonatal stage provided sufficient amount of energy and protein required for early growth. Thus, LLM can be included at higher proportions, especially in calves with matured rumen. There is a need for further studies to investigate rumen microbial dynamics of a rapidly degradable LLM, production of volatile fatty acids

and determine the effects of LLM on rumen papillae development and the outflows of microbial protein to the lower gut of neonates fed higher proportions of LLM.

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CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Lucerne leaf-meal (LLM) had high crude protein content, degradation and energy density values and lower fibre fractions. This has a potential to be utilised as the sole feed to the calves, although it hat short-fall of phosphorus level. Lucerne leaf-meal diets had higher CP content, calcium, fibre degradation and energy density values than the calf starter pellets. Higher *in vitro* degradation; protein and energy supply were observed with LLM diets compared to calf starter feed. The reason for low degradation and supply of nutrients may be due to more bound fibre fractions. Substitution of calf commercial starter feed with lucerne leaf-meal would reduce the total feed costs. However, the effects of the anti-nutritional factors in LLM substituted diets should also be taken into consideration.

Generally, LLM substituted diets improved diet intake, % ruminal nitrogen balance and growth of Holstein heifer calves. These findings are also supported by the predicted results using Large Ruminant Nutrition System (LRNS) during the pre-weaning phase. However, an improved performance was observed with LLM inclusion level of 50%. This may indicates that substitution of calf starter concentrate feed by 50% LLM would improve the performance of dairy heifer calves.

5.2 Recommendations

Lucerne leaf-meal provided nutrients for higher growth comparable to the concentrate feed. Feeding of premium forage to neonates would increase competitiveness of dairy businesses. Reliance on industrial feeds increases the cost of production during the growth period. Further research is envisaged on the effects of total substitution of concentrate and assessing the effects of organic compounds and antioxidants on nutrient metabolism in calves.