Effects of forage-based diet on milk production and body reserves of dairy cows on smallholder farms in South Africa

by

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ABSTRACT

Low nutrient intake affects metabolism and growth in pregnant heifers and limits milk production in lactating cows on communal area smallholder dairy farms of the subtropics. Two studies were conducted during the current research. The first study evaluated effects of nutrient supply in standardized dairy diets on the growth and body reserves of pregnant Jersey heifers raised on communal area smallholder farms in a semi-arid zone of South Africa. Twenty-two farms with a total of 42 heifers, aged 22 to 28 months which were seven months pregnant at the beginning of the study were selected for the study. These represented the total number of farms with dairy cows in the area that were supported through a structured Dairy Development Program (DDP) of South Africa. Each farm had at least two pregnant Jersey heifers during the summer season of 2016. Each heifer was supplied 2.5 kg of a far-off (60-30 d prepartum) dry cow concentrate and increased to 3.3 kg of the same concentrate at close-up period (29-0 d prepartum). Feeding of concentrate was based on a standardized feeding program as recommended by DDP. During this study, no feeding treatment was imposed on the heifers. *Eragrostis curvula* hay was supplied by DDP. Daily intake of 7.2 and 5.4 kg; respectively for heifers at 60-30 d prepartum and 29-0 d prepartum was determined based on residual hay. Heifer diet (HD1) and heifer diet HD2 were therefore simulated respectively for cows at 60-30 d preparpartum and 29-0 d prepartum, respectively. Diets were assessed for nutrient composition using chemical analyses and *in vitro* ruminal degradation. Post ruminal nutrient absorption and animal responses were predicted using the Large Ruminant Nutrition System (LRNS) version 1.0.33 (level 1). Actual measurements of body weight (BW), body condition score (BCS) were done and blood was collected and analysed for proteins monthly. Heifers' responses were validated against the model predicted values and comparative analysis of animal performance during pregnancy was done against the National Research Council (NRC, 2001) reference values. Relative to the minimum requirement for ruminants, both HD1 and HD2 diets had relative feed value (RFV) below 144. About 35% of HD1 dietary crude protein (CP) was within the slowly degrade neutral detergent fibre (NDF) fraction which is the neutral detergent fibre insoluble crude protein (NDFICP) while 32% was not available as the acid detergent insoluble crude protein (ADICP). Equally, HD2 diet had effectively 5.2% of CP as available protein and the fraction of the slowly degraded NDF constituted only 52.3% of the effective available protein. Energy density of HD1 and HD2 were 25% and 16% higher than expected at far-off and close-up period, respectively. The intake of metabolzable protein (MP) were 32 and 25% higher than predicted for the far-off and close-up period, respectively. Supply of MP was 37 % and was higher than NRC predictions of daily requirement in Jersey cow. This allowed BW gain of 29 kg and BCS of 0.33 which was within 25th percentile for pregnant heifers. Mean concentration of blood urea at both far-off and close-up periods deviated by 25% from NRC values. Creatinine (CR) concentration was 145 µmol /L at far-off and 155 µmol /L at close-up period.

The second study assessed the adequacy of two lactation diets fed to 42 primiparous Jersey cows, aged 24 to 30 months during early (1-30 d postpartum) and peak (31-60 d postpartum) periods on the lactation performance of the cows. Cows received 4.5 and 5 kg of dairy concentrate at 1-30 d postpartum and peak milk (31-60 d postpartum) respectively. *Eragrostis curvula* hay was supplied *ad libitum* and dry matter intake (DMI) was estimated at 7.2 kg of hay/cow/day from residual hay. No feeding treatment was imposed except for the standardised diets typical to the production environment. Two simulated lactation diets (LD1 and LD2) were prepared based on dry matter intake (DMI) of grass hay and lactation concentrate. Diets were assessed for

nutrient composition using wet chemistry and *in vitro* ruminal degradation. Nutrient supply of diets and absorption from the small intestines as well as cows' responses were predicted using the Large Ruminant Nutrition System (LRNS) version 1.0.33 (level 1). Body weight and BCS were monitored, blood was collected and analysed for proteins monthly. A record of milk yield was taken daily, and milk was analysed for fat, protein, lactose and urea nitrogen weekly. Cows had DMI of 11.2 kg which was 12% higher than the expected at 1-30 d postpartum period and 11.6 kg which was 21% higher than the expected in 31-60 d postpartum cows. Diets had low available protein as % of dietary protein (LD1=46%; LD2=45%) and the slowly degraded NDF fraction (NDFICP) constituted 64% of the available protein. Intake of energy was 20% and 17% lower than the predicted value for the cows, respectively, at 1-30 d postpartum and 31-60 d postpartum period. Cows had negative energy balance of -6.5 and -5.6 Mcal respectively at 1-30 d postpartum and 31-60 d postpartum cows. Protein intake of lactating cows was low, which resulted in negative protein balance of 59% and 42% of cow's daily requirement, respectively, at 1-30 d postpartum period and 31-60 d postpartum period. There was loss of BW and BCS, low milk yield, energy corrected milk (ECM: 9.50 kg/d) and feed efficiency (FE) of less than 1 (LD1= 0.85; LD2 =0.89) in cows at both periods. Composition of fat, protein and lactose in milk were negatively affected by the low level of dietary protein. Somatic cell count (SCC) in milk was 121 ± 13 x 10^3/ml and cows did not show signs of illness. Mean milk urea nitrogen (MUN) concentration was 12 ± 2.7 mg/dl reflecting the low protein status of the lactating cows. Cows had high creatinine concentration of 116 and 102 µmol /L at 1-30 d postpartum and 31-61 d postpartum period, respectively, which may indicate muscle breakdown due to heat stress relative to the hot production environment. Results showed that diets fed to dairy cows on communal area smallholder farms in Sekhukhune and Vhembe districts in Limpopo province had low feeding value and their low nutrient supply affected rumen fermentation, heifers' 'growth, body reserves and early lactation in Jersey dairy cows. In conclusion, diets supplied to dairy cows raised on smallholder farms are low in nutrients and do not support efficient growth in heifers and optimal milk production in early lactation. Development of a nutrition plan for improved dairy diets is required to maximise production and longevity in cows and enhance sustainability of dairy production on the smallholder farms in South Africa.

Key words: Standardized diets, dietary nutrients, diet degradation, pregnant Jersey heifers, communal smallholder dairy farm, early lactation, primiparous cow

TSHOBOKANYO

Go ja dijo tse di nang le dikotla tse di kwa tlase go ama metaboliseme le kgolo ya meroba e e dusang mme e ngotla tlhagiso ya mašwi ya dikgomo tse di tlhagisang mašwi mo dipolaseng tse dinnye tse di tlhakanetsweng mo mafelong a a mogote. Go dirilwe dithutopatlisiso di le pedi jaaka karolo ya patlisiso ya ga jaana. Thutopatlisiso ya ntlha e sekasekile ditlamorago tsa tlamelo ya dikotla mo dijong tsa teri tse di rulagantsweng mo kgolong le dirasefe tsa mmele tsa meroba ya Dijeresi e e dusang mo dipolaseng tse dinnye tse di tlhakanetsweng mo karolong e e batlileng e nna sekaka mo Aforika Borwa. Go tlhophilwe dipolase di le 22 tse di nang le meroba e le 42, e e bogolo jo bo magareng ga dikgwedi tse 22 le 28 mme e na le dikgwedi tse supa e ntse e dusa kwa tshimologong ya thutopatlisiso. Tsone di emetse palogotlhe ya dipolase tse di mo karolong eo tse di tshegediwang ke Lenaneo le le rulaganeng la Tlhabololo ya Teri (DDP). Polase nngwe le nngwe e ne e na le bonnye meroba ya Jeresi e le mebedi e e dusang ka paka ya selemo sa 2016. Moroba mongwe le mongwe o ne o fepiwa ka 2.5 kg ya dijo tse di omileng tsa dikgomo tsa fa go sa ntse go le kgakala (malatsi a le 60-30 pele ga go tsala) mme tsa okediwa go nna 3.3 kg fa malatsi a atamela (malatsi a le 29-0 pele ga go tsala). Dijo tseno di ne di di rulagantswe go ya ka lenaneo le le rulagantsweng la kotlo le le atlenegisitsweng ke DDP. Mo nakong ya thutopatlisiso eno, ga go na kalafi epe ya kotlo e e neng e patelediwa meroba. DDP e ne e tlamela ka furu ya *eragrostis curvula*. Go ja ga letsatsi le letsatsi ga meroba ga 7.2 le 5.4 kg ka nako ya malatsi a le 60-30 pele ga go tsala le malatsai a le 29-0 pele ga go tsala go ne go ikaegile ka furu e e setseng. Ka jalo go ne ga tlhagisiwa gape kotlo ya meroba ya 1 (HD1) le kotlo ya meroba ya 2 (HD2) mo dikgomong tse di mo malatsing a le 60-30 pele ga go tsala le malatsi a le 29-0 pele ga go tsala. Dikotlo tseno di ne tsa sekwasekwa go bona go nna gona ga dikotla mo go tsona go dirisiwa tshekatsheko ya dikhemikale mo mogodung. Go ne ga bonelwa pele monyelo ya dikotla morago ga go feta mo mpeng ya ntlha le tsibogo ya diphologolo go ya ka Thulaganyo ya Kotlo ya Diotli tse Dikgolo (LRNS) mofuta wa 1.0.33 (legato 1). Go dirilwe tekanyo ya boima jwa mmele (BW) le maduo a seemo sa mmele (BCS) mme go ne ga tsewa madi le go a sekaseka go bona diporoteini kgwedi le kgwedi. Tsibogo ya meroba e ne ya tlhomamisiwa ka dipalo tse di bonetsweng pele tsa sekao mme ga dirwa tshekatsheko e e tshwantshanyang ya tiragatso ya diphologolo ka nako ya go dusa go dirisiwa dipalo tsa Lekgotla la Bosetšhaba la Dipatlisiso (NRC, 2001). Malebana le ditlhokegopotlana tsa diotli, HD1 le HD2 di ne di na le boleng jo bo tshwantshanyegang jwa kotlo (RFV) jo bo kwa tlase ga 144. Poroteini e e tala (CP) ya dijo e e ka nnang 35% ya HD1 e ne e le mo karolwaneng ya tekanyetso ya faeba e e bolang ka iketlo (NDF) e leng poroteini e e tala ya faeba e e lekanyediwang (NDFICP), fa 32% di ne di seyo jaaka poroteini e tala e e sa monyelegeng ya esete (ADICP). Fela jalo, HD2 e na le 5.2% tsa CP e e dirang jaaka poroteini e e teng mme karolo ya NDF e e bolang ka iketlo e ntse fela 52.3% tsa poroteini e e dirang e e gona. Bogolo jwa maikatlapelo a HD1 le HD2 bo ne bo le kwa godimo ka 25% le 16% go na le jaaka go ne go solofetswe mo dipakeng tse di kgakala le tse di atamelang. Go jewa ga poroteini e e

silegang (MP) go ne go le kwa godimo ka 32% le 25% go na le jaaka go ne go solofetswe mo dipakeng tse di kgakala le tse di atamelang. Tlamelo ya MP e ne e le 37%, e leng e e kgolwane go na le diponelopele tsa NRC tsa ditlhokego tsa letsatsi le letsatsi tsa dikgomo tsa Jeresi. Seno se letlile gore go nne le koketsego ya BW ya 29 kg le BCS ya 0.33 e leng se se neng se le mo diperesenteng tsa bo25 tsa meroba e e dusang. Go nna teng ga *urea* ya madi mo dipakeng tse dikgakala le tse di atamelang go ne go farologane ka 25% go tswa mo dipalong tsa NRC. Go nna teng ga kereitini (CR) e ne e le 145 µmol/L mo pakeng e e kgakala le 155 µmol/L mo pakeng e e atamelang.

Thutopatlisiso ya bobedi e sekasekile ditlamorago tsa dijo tse pedi tsa tlhagiso ya maswi mo tiragatsong ya tlhagiso ya maswi ya dikgomo tsa Jeresi di le 42 tse e leng la ntlha di tsala tsa bogolo jwa dikgwedi tse di magareng ga 24 le 30 mo pakeng ya ntlha (malatsi a le 1-30 morago ga go tsala) le ya setlhoa (malatsi a le 31-60 morago ga go tsala). Dikgomo di amogetse 4,5 le 5 kg ya motswako wa teri mo dipakeng tsa maswi tsa ntlha (malatsi a le 1-30 morago ga go tsala) le tsa setlhowa (malatsi a le 31-60 morago ga go tsala). Go ne go tlamelwa ka furu ya *eragrostis curvula* go ya ka tlhokego mme go ja dijo tse di omileng (DMI) go ne go lekanyediwa go 7.2 kg ya furu/ka kgomo/ka letsatsi go tswa mo furung e e neng e setse. Go ne go sa patelediwe kalafi epe ya phepo, kwa ntle fela ga dijo tse di rulagantsweng tse di tshwanetseng tikologo ya tlhagiso. Go ne ga baakanngwa dijo tsa tlhagiso ya maswi tse di tlhagisitsweng gape (LD 1 le LD 2) di ikaegile ka go jewa ga tse di omileng (DMI) e leng furu ya tlhaga le metswako ya tlhagiso ya maswi. Go nna teng ga dikotla ga dijo tseno go ne ga lekanyediwa go dirisiwa khemisitiri e e bongola le go bola mo mpeng ga *in vitro.* Go ne ga bonelwa pele tlamelo ya dikotla ya dijo, monyelo go tswa mo maleng a mannye mme go ne ga bonelwa pele tsibogo ya dikgomo go dirisiwa Thulaganyo ya Kotlo ya Diotli tse Dikgolo (LRNS) mofuta wa 1.0.33 (legato 1). Go ne ga elwa tlhoko boima jwa mmele le BCS, go ne ga tsewa madi mme a sekasekwa go bona diporoteini kgwedi le kgwedi. Go ne ga rekotiwa tlhagiso ya maswi letsatsi le letsatsi mme maswi a sekasekwa go bona mafura, poroteini, laketose le *urea* naeterojini beke le beke. Dikgomo di ne di na le DMI ya 11.2 kg, e e neng e le kwa godingwaga ka 12% go na le jaaka go ne go solofetswe mo pakeng ya malatsi a le 1-30 morago ga go tsala, le DMI ya 11.6 kg, e e neng e le kwa godingwana ka 12% go na le jaaka go ne go solofetswe mo dikgomong tse di nang le malatsi a le 31-60 di tsetse. Dijo di ne di na le poroteini e e gona e e kwa tlase jaaka peresente ya poroteini ya dijo (LD1=46% le LD2=45%) mme karolwana ya NDF e e bodileng ka bonya (NDFICP) e nnile 64% tsa poroteini e e gona. Go jewa ga maikatlapelo go ne go le kwa tlasenyana ka 20% le 17% go na le dipalo tse dineng di bonetswe pele mo dikgomong mo dipakeng tsa malatsi a le 1-30 morago ga go tsala le malatsi a le 31-60 morago ga go tsala. Go rekotilwe balanse ya maikatlapelo a a tlhaelang a dikgomo ya -6.5 le -5.6 Mcal mo malatsing a le 1-30 morago ga go tsala le 31-60 morago ga go tsala. Go jewa ga poroteini ke dikgomo tse di tlhagisang maswi go ne go le kwa tlase, mme seo sa baka balanse e e tlhaelang ya poroteini ya 59% le 42% tsa ditlhokego tsa letsatsi le letsatsi tsa dikgomo mo pakeng ya malatsi a le 1-30 morago ga go tsala le malatsi a le 31-60 morago ga go tsala. Go rekotilwe tatlhegelo ya BW le

BCS, tlhagiso e e kwa tlase ya maŝwi, maŝwi a a baakantsweng maikatlapelo (ECM: 9.50 kg/ka letsatsi) le bokgoni jwa furu (FE) jo bo kwa tlase ga 1 (LD1=0.85; LD2=0.89) mo dikgomong mo dipakeng tseo tsotlhe. Go nna teng ga mafura, poroteini le laketouse mo maŝwing di amegile ka tsela e e sa siamang ka ntlha ya seelo se se kwa tlase sa poroteini e e kwa tlase. Tekanyetso ya disele tsa somatiki (SCC) mo maŝwing e ne e le 121±13x10³/ml mme dikgomo ga di a bontsha matshwao ape a bolwetsi. Motswako wa urea naeterojini ya maŝwi (MUN) e ne e le 12±2.7mg/dl, e leng se se bontshang seemo se se kwa tlase sa poroteini sa dikgomo tse di tlhagisang maŝwi. Dikgomo tseno di ne di na le motswako wa kereitine wa 116 le 102 μmol/L mo dipakeng tsa malatsi a le 1-30 morago ga go tsala le malatsi a le 31-61 morago ga go tsala, mme seo se ka supa go fokotsega ga mesifa ka ntlha ya kgatelelo ya mogote e e bakwang ke tikologo e e mogote e go tlhagisiwang mo go yona. Dipholo di bontshitse gore dijo tsa dikgomo tsa teri mo dipolaseng tse dinnye tse di tlhakanetsweng mo dikgaolong tsa Sekhukhune le Vhembe kwa Porofenseng ya Limpopo di na le boleng jo bo kwa tlase jwa kotlo le gore dijo tse di nang le dikotla tse dinnye di amile titielo ya dijo, kgolo ya meroba, dirasefe tsa mmele le tlhagiso ya maŝwi ka bonako mo dikgomong tsa teri tsa Jeresi. Kwa bokhutlong, dijo tsa dikgomo tsa teri tse di godisediwang mo dipolaseng tse dinnye di na le dikotla tse di kwa tlase mme ga di tshegetse kgolo e e mosola ya meroba le tlhagiso e e siameng ya maŝwi mo nakong ya ntlha ya tlhagiso ya maŝwi. Go tlhokega leano la dikotla go tokafatsa dijo tsa teri go tokafatsa tlhagiso le go tshela sebaka ga dikgomo le go tokafatsa go nnela leruri ga tlhagiso ya teri mo dipolaseng tse dinnye mo Aforika Borwa.

Mafoko a botlhokwa: Dijo tse di rulagantsweng, dikotla tsa dijo, go bola ga dijo, meroba e e dusang ya Dijeresi, polase e nnye ya teri e e tlhakanetsweng, tlhagiso ya maŝwi ka bonako, kgomo e e leng la ntlha e tsala

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
AL	Albumin
AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Council
ATP	Adenosine triphosphate
BCS	Body condition score
BHB	β-hydroxybutyrate
BUN	Blood urea nitrogen
BW	Body weight
Ca	Calcium
CHO	Carbohydrate
CP	Crude protein
CR	CreatininE
DAFF	Department of Agriculture, Fisheries and Forestries
DCAD	Dietary cation-anion differences
DM	Dry matter
DMI	Dry matter intake
DRDLR	Department of Rural Development and Land Reform
FAO	Food Agricultural Organisation
GDP	Gross Domestic Product
GE	Gross energy
GHG	Greenhouse gas
GL	Globulin
GP	gas production
IMI	Intramammary infection

IVDMD	*In vitro dry* matter digestibility
Mcal /kgDM	Mega calories per kilogram dry matter
ME	Metabolizable energy
MP	Metabolizable protein
MUN	Milk urea nitrogen
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
NFC	Non-fibre carbohydrates
NRC	National research council
N	Nitrogen
NSC	Non-structural carbohydrate
OM	Organic matter
P	Phosphorus
SC	Structural carbonhydrate
SCC	Somatic cell count
TP	Total protein
VFA	Volatile fatty acids

CHAPTER 1

INTRODUCTION

Milk production from smallholder dairy farms is mostly channelled through informal markets (Migose *et al*., 2018). Contribution of milk to food security is significant in South America, India, South Asia and East Africa, (McDermott *et al*., 2010; Dugdill *et al*., 2013; Balcão *et al*., 2017). Swai *et al*. (2005) reported milk yields of 1,800 kg/cow/year in Tanzania where indigenous East African Zebu and Ankole breeds are predominantly used for dairy production. In Sudan, Eastern Kenya, Brazil and India cow milk production ranged between 2,700 to 3,000 kg /cow/annum (Amani *et al*., 2007; Kimenchu *et al*. (2015). Although the levels of milk produced are low, the contributions to food and health security in these areas are notable. However, in other sub-Saharan countries such as South and South West Africa, communal area milk production systems are poorly developed.

In South Africa, the smallholder sector contributes less than 1% of formally marketed milk and the informal milk sector is also under developed (South Africa Year book, 2012/2013). Dairy smallholder farming is practised in the sub-tropical and mediterrranean regions (Tsitsikama, Eastern Cape), coastal areas of KwaZulu-Natal province and open savanna of the Free State province (Meissner *et al*., 2013). Semi-zero grazing is the main dairy production system in the aforementioned provinces and in the North West of South Africa (Mansana *et al*., 2014). Napier grass (*Pennisetum purpureum*), kikuyu grass (*Pennisetum* clandestinum), Lucerne (*Medicago sativa*), cactus and soghurm (*Sorghum bicolor)* are mostly utilized for nutritional support. Some smallholder dairy cows are raised on natural pasture (Damptey *et al*., 2014). However, cyclic losses in body mass continue to hamper smallholder dairy development as natural pasture and dryland pastures dimish in nutrients during the dry seasons.

In South Africa, there are no guidelines on nutritional management of smallholder dairy cows and hence most feeding is *ad-hoc* and application guidelines are from other tropical envrionments (Milk SA, 2014). Hence, these application guidlines and utilization of poor grazing might affect growth of the smallholder dairy system. The grasses are mostly increaser species including *Eragrostis curvula* and *Sporobolus fimbriatus,* with fibre content above 70% and hence poorly degradable (Cabezas-Garcia, 2017). Metabolic disorders and infertility are mostly linked to poor fibre (Mapekula, 2009)

1.1. Problem statement

Growth in the livestock industry occurs under smallholder farming systems, which have the largest herds of cattle worldwide (Jayne *et al*., 2003; Mc Dermott *et al*., 2010). However, production environments of smallholder farming are not conducive as water and other resources to manage negative effects of climate change are limited. Regardless of the challenges, the smallholder sector is critical as it hosts the largest cattle population and hence greater opportunity to positively affect nutrition quality and livelihood security.

Zero-grazing or semi-zero grazing is desirable as it minimises partitioning of nutrients towards maintenance, managing heat stress as animals are less exposed to effects of direct solar radiation, rain and wind. Besides, under this system, isolation of dairy cows from other animals for biosecurity minimises infections. In East Africa, where smallholder farming has been practised for over 40 years, the recommendations on daily consumption of dairy concentrate was as low as 2 kg/lactating cow which represents 0.5% BW/day in Holsteins and 0.8% in small framed animals such as the Jersey. Agboola, (2015) recognises that some of the concentrates are of poor quality and mostly procured from unregulated sources. In Kenya, dairy cows consummed up to 52 kg of roughages daily on smallholder farms (Kimenchu *et al*., 2015). Furthermore, the management dynamics around cows foraging at different communal areas make the management assessment of the nutritional status more difficult. It is essential to define levels of nutrient supply of the forage-based diets for dairy cows during gestation and lactation on communal area smallholder farms. Therefore, this research aimed at evaluating the effects of forage-based diet on milk production and body reserves of dairy cows on smallholder farms in Sekhukhune and Vhembe districts of Limpopo, South Africa.

1.4. Objectives of the study

1.4.1. Main objective

The study aim was to assess the effects of forage-based diet on milk production and body reserves of dairy cows raised on smallholder farms in Sekhukhune and Vhembe districts of Limpopo, South Africa.

1.4.2. Specific objectives

1. To assess deviations of standardized diets on nutrient support for the growth of Jersey heifers during pregnancy.

2. To determine the effects of low supply of dietary nutrients on body reserves and early lactation in primiparous Jersey cows.

1.5. Hypotheses

1. Deviations of standardized diets affect nutrient support for the growth of Jersey heifers during pregnancy**.**

2. Low supply of dietary nutrients affects body reserves and early lactation in primiparous Jersey cows.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

In South Africa, rearing of cattle for milk production is a common practice on smallholder farms. Most of the cattle in this farming system are indigenous breeds with few exotic pure breeds and sometimes the cross between the exotic and indigenous breeds. The South African indigenous dairy breeds that are on the smallholder farms and communal areas include Boran, Saga, Afrikaner and Nguni. The cross between the exotic and indigenous breeds are Drankensberger and Bonsmara (Myburgh *et al*., 2012). According to Milk South Africa, the most common breeds on South Africa smallholder dairy herds include crossbreed, Jersey and Holstein, with average of 5 cows per herd (Milk SA, 2017).

Milk production of indigenous breeds is very low due to inadequate intake of quality feeds (Ngongoni *et al*., 2006; Tavirimirwa *et al*, 2013; Damptey *et al*., 2014). There are variations in the quality of forages fed to dairy cows on smallholder farms. The impact of season on availability of good quality forages has been reported (Khan *et al*. 2009). Feeds supplied to dairy cows during winter are less palatable with low DMI and are poorly digested. This low-quality feed commonly results in poor metabolism and nutrient absorption by animals.

In South Africa, the contribution of smallholder dairy production improved household food security, income, and general living standard while its contribution to national GDP is insignificant (Altman *et al*. 2009). The popular dairy value chain initiated by the Department of Agriculture, Fisheries and Forestries and Department of Rural Development and Land Reform was an intervention launched to increase production, farmers' income and the overall contribution to the countrys local and national economy (NDP, 2011). The intention to improve household income through dairy production means considering different approaches such as using improved animal breeds for milk production and improved feeds and feeding management for optimal production (Tavirimirwa *et al*., 2013). The total diets supplied to dairy cattle on smallholder farms are indequate quantity and quality (Ndlovu *et al*., 2007; Manzana *et al*., 2014). Hence, the protein and energy demand of the animals are not satisfied. This became a challenge to growing a viable and profitable dairy business.

High prevalence of diseases and parasites infestation and low feed intake associated with poor forage quality are the major constraints to optimal production on communal smallholder farms (Tavirimirwa *et al*., 2013; Damptey *et al*., 2014). Zero-grazing or semi-zero grazing is commonly

practised in communal areas of smallholder dairy farms in South Africa (Manzana *et al*., 2014). This system is recommended as animals conserve more nutrients for maintenance and less exposed to harsh weather conditions such as direct sunlight, wind and rain.

2.2. Forage fraction of diets supplied to dairy cows on smallholder farms

Increased fodder production and consequent reduction of seasonality in forage availability, as commonly practiced by smallholders on zero-grazing and stall-feeding system in Eastern Africa, would improve productivity. For maximum utilization of forage by the dry and lactating cows, it is important to supplement fed forage with limiting nutrients such as energy and crude protein required for optimal rumen function, improved animal health and lactation performance (Lee *et al*., 2002). Similarly, Khan *et al*. (2009) indicated that a regular supply of adequate quantity and quality feed sources to dairy cows is key to achieve high production performance and enhanced animal health.

2.3. Forage structural and chemical composition

Forage cell wall digestibility greatly determines the production performance and efficiency of animals (Jung, 2012; Allen & Segarra, 2001). Forages consist of high cell wall materials surround the plant cell, providing the physical support needed for plant rigidity. The structural composition of forage plants varied with the species resulting in high variations in its digestibility (Jung, 2012). The major components of cell wall are cellulose, hemicellulose and lignin. The components can be separated using neutral and acid detergent fibre analysis respectively (van Soest, 1963).

Cellulose is the largest component of cell wall consisting mainly of direct glucose chains (Jung, 2012). Hemicelluloses are described as polysaccharides in plant cell walls including xylan, xyloglucans, mannans and glucomannans (Scheller & Ulvskov, 2010). Lignification impairs digestibility of hemicellulose and pectin of forages, but rumen microbiota can completely digest the plant intervening walls through access to the connecting cells (Engels & Jung, 2005). Moore and Jung (2001) described lignin as a polymer formed from monolignols, which include guaiacyl, syringyl and *p*-hydroxyl phenyl-type lignin that are found in forages while hydroxycinnamic acids, ferulic and *p*-coumaric are found only in grasses (Jung, 2012). The chemical link of lignin to carbohydrates and proteins in the plant cell wall forms macromolecules (Moore & Jung, 2001).

2.4. Benefits of feeding forages to dairy cows

Feeding forages to dairy cows is valuable for their importance in gut health, growth, reproduction and lactation (Stokes, 2012). The quality of forage is determined by intake and its nutritive value that in turn is determined by a combination of its chemical and physical characteristics (Allen &

Segarra, 2001). The physical structure of forages when offered to the animal influences the effective utilization and benefits to the animal. Long or chopped forage enhances better rumen functioning. This is due to the increased chew, secretion of saliva and the formation of a rumen mat for the smaller particles (Allen & Segarra, 2001). In contrast, pelleted hay increased voluntary intake and microbial protein production efficiency due to increased rate of digestion and passage rate of residual particles in the gut (Skerman & Riveros, 1990; Bernard *et al*., 2000). This results in increased daily weight gain and fat content in milk.

According to Stokes (2012), an approximately 40% of dairy cow DMI from forage is recommended. There are important determinant factors in meeting the nutritional demands of animals fed forage-based diets. These include forage palatability, the level of dry matter intake, rate of passage of particulate matter, amount of nutrients and the ratio that are available to the animals. The nutritional demands of dairy cows differ and depend on the age, breed, BW and BCS, lactation level and reproduction cycle.

2.5. Environmental impact of feeding low concentrate diets to dairy cows

According to Walsh (2013) cows in sub-Saharan Africa consumed 10 times more feed (mostly grasses) to produce a kilogram of protein compared to cows raised in resource-rich farms regions. The low conversion of the fed diets to protein could be due to poor feed fermentation efficiency resulting in loss of undigested nutrients in the faeces and production of greenhouse gases (GHG) including carbon dioxide (CO_2), nitrogen oxide and methane (CH_4) (IPCC, 2007). The report indicated that poor feeding of cows contributed to the global warming challenge. These challenges include up to 32% of air and water pollution as well as loss of about 2-12% of the GE intake in ruminants in the form of CH_4 (Patel *et al*., 2011). The high cost of feeds, health risk, and low production performance became a huge drawback to viability and sustainability of the smallholder dairy sector IPCC (2007).

Supplementing ruminant diets with concentrates rich in starch favours propionate production and increased concentration in the rumen. Feeding forage-based diets results in slower digestion rate and high methane production per unit of feed digested (Patel *et al*., 2011). Ellis *et al*. (2007) reported a positive correlation of NDF with methane production (kg per day) but a negative correlation when expressed in percentage per DM for dairy cow. The positive correlation between cow/day consumption of NDF (kg) and the corresponding methane production was explained by possible slower rate of digesta passage, optimal rumen fermentation and the subsequent increased ratio of acetate: propionate which was observed. Boadas *et al*. (2012) showed that the incidence of negative correlation might be due to high proportion of fibre fractions, increased rate of passage and the ensuing increased DMI.

2.6. Inefficiencies in carbohydrate digestion

Methane, CO_2 and nitrous oxide are produced from enteric fermentation on livestock and management of manure. Methane contributes about 14.5% of the total anthropogenic emissions, posing a huge threat to the environment from livestock production (IPCC, 2014). Nonetheless, the extent of methane emissions significantly differs between production systems and within units of production system. In recent years, the awareness of GHG emissions and increased environmental impact has prompted concerns and increased interest of several researchers in relevant topics on GHG.

Studies on methane emissions from livestock production involve proposals and development of different management and dietary mitigating strategies for the reduction of methane emissions (Kim *et al*., 2013; Patra *et al*., 2017). The use of dietary mitigation strategies for dairy cows through diet modification includes addition of unsaturated fats and ionophores to diets and processed forages. Mitigation options result in a shift of rumen fermentation inhibiting activity of methanogens and certain protozoa to favour production of propionate, thereby improving efficiency of feed utilisation and reduced methane synthesis (Kim *et al*., 2014; Fox *et al*., 2004). Feeding dairy and beef cows with high concentrate diets resulted in decreased methane production due to the negative relationship between starch, concentrate and methane (Hatew *et al*., 2015)

Ionophores like monensin are known to inhibit the growth of Gram-positive bacterial genera such as *Clostridium* and *Ruminococcus* (acetate and H_2 producer), and Gram-negative bacteria (formate and H_2 producer) resulting in decreased H_2 and CH_4 production (Tomkins *et al*., 2015; Kim *et al*., 2013). Higher dosage (250 versus 60 mg/day/cow) is required to effect a reduction in tropical cattle (Tomkins *et al*., 2015). The use of popular ionophore antibiotics has residual effects on animal products and resulted in the ban of the antibiotic for animal food production in many countries (Patel *et al.,* 2011). Bacterocins such as bovicin HC5 and nisin produced by *Streptococcus* spp. and *Lactobacillus lactis*, respectively, decrease methane production by modulating rumen fermentation and consequently increase propionate production (Lee *et al*., 2002; Sar *et al*., 2005). *In vivo* evaluation of the effectiveness of this option and the cost efficiency are recommended (Patra *et al*., 2017).

The addition of essential oils from plant extracts such as found in garlic, clove bud (eugenol), hop pepper (capsaicin), cinnamon (cinnamaldehyde) and coconut oil to ruminant diets have been shown to reduce production of methane (Calsamiglia *et al*., 2007; Bodas *et al*., 2012; Brask *et al*., 2013; Kim *et al*., 2014). Reduction in feed intake and decreased dry matter and fibre digestion limit the use of canola oil (Beauchemin & McGinn, 2006).

Chemical compounds such as sulphonate, halogenated aliphatic compounds inhibit the activity of archaea in the rumen reducing methane production by 6o% (Denman *et al*., 2007; Tomkins *et al*., 2009; Abecia *et al*., 2012). The short-lived efficacy of these compounds, depletion of ozone and the identification of chloroform as a carcinogenic are some of the limitations to the practical application of this mitigation option (Patra *et al*., 2017). The use of seaweed (*Asparagopsis taxiformis (*Machado *et al*., 2014) and nitro-compounds such as 3-nitrooxypropanol (3NOP) and sulphate (Anderson *et al*., 2010; Hristov *et al*., 2015) and vaccination by injecting animal with anti-methanogen antibodies (Wright *et al*., 2004) also reduced methane production. Although, the small quantity of antibodies available in the rumen and possible degradation of the antibodies by the ruminal proteolytic bacteria are limitations to this approach (Patra *et al*., 2017).

The anti-microbial activities of certain plant secondary metabolites such as tannin, saponins, flavonoids and organic sulphur compound have been identified to be potentially inhibitory to rumen methanogens and methane emissions (Patra & Sexana, 2010; Oskoueian *et al*., 2013; Patra & Yu. 2013; Saminathan *et al*., 2016). The review of Patra and Yu (2012) indicated that the extent of the effectiveness of these compounds significantly differred and was found to depend on several factors including sources, type, molecular weight, dose and the type of diet to which the compounds were added. Differences found in their modes of action could elucidate variations in the reported effects of these compounds in the mitigation strategy to reduce production of methane.

The IPCC warned against further warming with long-lasting changes to all components of climate system with possible harsh and permanent impacts on people and the ecosystems should GHG emissions continue (IPCC, 2014). Therefore, it is important that the use of different mitigation options should aim at limiting climate change, sustainable reductions of GHG emissions and managing the risks climate change at all levels of livestock production.

2.7. Effects of diet quality on nutrient intake, digestion and availability

2.7.1. Feed dry matter intake

High DMI has been observed in cows fed diets high in energy and highly digestible fibre (Emanuelson *et al*., 2006). Increased DMI relates to enhanced microbial degradation of the feed substrates and the subsequent quick digesta passage and increased intake. On the other hand, diets high in fibre (NDF) decrease digestibility and cause declined intake. It became imperative that diets for dairy cows be balanced with adequate effective NDF (eNDF) and potentially effective NDF (peNDF) content for maximum microbial activity in the rumen (Mertens, 1997). The peNDF is described as specific effectiveness of diet NDF fraction to stimulate chewing in relation to particle size and recommended peNDF of 22% of diet DM in dairy cow diets.

The level of feed intake is determined by stage of lactation (early, mid and late) with the highest intake observed during early lactation. Friggens *et al*. (1998) reported a decrease in DMI with increased lactation stage for cows fed high concentrate mixed diets. Demand at early lactation is usually high resulting in increased DMI to compensate for the negative energy balance (NEB) typical at this stage of lactation. Gradual make-up for energy balance could explain the observed decreased DMI as lactation progresses. Again, heat stress and the state of animal health are other factors that influence feed intake in dairy cows. West (2003) found increased air temperature, temperature to humidity index and excessive rectal temperature to be associated with decrease DMI, milk yield and reduction in efficiency of milk yield. However, the recommended ration re-formulation optimized DMI, increased diet nutrient density and prevention of excessive nutrient loss which compensated for changes in nutritional needs during heat stress.

2.7.2. Rumen microbiota population

There are millions of microorganisms including bacteria, fungi, protozoa and archaea residing in the rumen that are responsible for fermentation and degradation of fibrous plant feeds consumed by ruminants (Flint *et al*., 2008). Cellulotic bacteria break down cellulose and hemi-cellulose and amylolytic bacteria break down starch and activation of their activity in the rumen is dependent on the feed type. Low pH has negative effects on the activity of cellulotic bacteria resulting in low degradation of fibre fraction and underutilization of forage.

The low fibre (high concentrate) diets stimulate the production of quick-working 'floating' microbes (starch and sugar degrading microbes) resulting in improved passage rate of feeds in the digestive system and the ensuing increased feed intake. On the other hand, high fibre diets such as found in matured forages stimulate the growth of slow working, fibre-digesting microbes and subsequently slow digesta in the digestive system causing declined intake (Barbers *et al*., 2013). Bacteria involved in hydrolysis of feed substrates are extremely diverse in the rumen indicative of their vast metabolic flexibility. Polysaccharides such as cellulose are substrate of cellulolytic bacteria including *Ruminococcus albus* and *Fibrobacter succinogenes* (Atasoglu *et al*., 2001). According to Schwarz (2001) cellulolytic anaerobes degrade cellulose by means of binding to cellulose substrates with a multi-enzymatic complex called cellulosome.

Methanogenesis is the production of methane facilitated by anaerobic microbes, also known as methanogens, mainly from the archaea family called methanogens for the generation of adenosine triphosphate (ATP). The major methanogenesis during fermentation (anaerobic digestion), is the reduction of carbon dioxide (CO_2) by hydrogen (H) to form methane. Low fibre diets with high starch content are related to high proportions of propionate in the rumen total VFA production with reduced methane production per mole of substrates fermented (Wilkinson, 2012). The molar

proportion of the total VFA produced during ruminal fermentation affects the production of methane. Moss *et al*. (2000) showed that acetate and butyrate promote production of methane while the formation of propionate indicates its competition with methanogenesis as an alternative pathway for hydrogen use in the rumen. This points to the ability of starch-fermenting bacteria to compete against methanogens for hydrogen.

Production of methane is an indication of less fibrolytic bacterial activity, low nutrient availability and microbial attachment in the rumen. The consequential low DM digestibility with about 12% loss in digestible energy in form of methane links with inadequate microbial activity (Wanapat *et al*., 2015). A negative relationship between the concentration of propionate and methane production has been reported. Moss *et al*. (2000) showed that availability of highly fermentable carbohydrate favoured increased proportion of propionate in total VFA production and consequential reduction in methane production.

2.7.3. Volatile fatty acids production

Volatile fatty acids are absorbed into the ruminal and intestinal walls to serve as major source of energy to ruminant body tissues and mammary glands during lactation (Getachew *et al*., 2004). Acetate was confirmed by Nafikov and Beitz (2007) as the major end-products of fermentation. It is indicated that diet composition such as ratio of starch to cellulose influences the proportion of individual end-product of fermentation. Other studies on ruminants showed significant increases in total VFA production with higher concentrate to roughage ratios but not with molar proportion of individual VFAs or ratio of acetate to propionate (Suharti *et al*., 2011; Kumar *et al*., 2013; Dung *et al*., 2014). Conversely, McDonald *et al*. (2002) suggested high fermentable carbohydrates such as starch diets could have resulted in lower acetate production in favour of high propionate proportion. Hence, high production of VFA is an indication of effective microbial activity due to higher DMI, improved DM digestibility, increased rate and extent of degradability and the ensued positive energy balance available for optimal animal performance.

2.7.4. Energy balance and gluconeogenesis

Carbohydrates in diets are broken down to glucose. Gluconeogenesis is a metabolic pathway that results in the synthesis of glucose from non-carbohydrate precursors such as propionate, valerate, lactate, amino acids and glycerol. These precursors provide between 70 to 90 % of the required glucose in ruminants, young calves and piglets (Nafikov & Beitz, 2007). This metabolic pathway is quantitatively crucial in lactating ruminants since sufficient daily glucose is needed for the corresponding milk production. The release of optimal amounts of glucose from the liver to the blood stream to meet animal's demands at that point in time is crucial. This is to avoid energy mobilisation via breakdown of fat from adipose tissues (Lafontan *et al*., 2009). The breakdown

of adipose tissue fat is associated with circulating serum non-esterified fatty acids (NEFA) which are metabolised to energy following absorption in primiparous cows (Van Saun, 2000; Meikle *et al*., 2004). The higher incidences of periparturient disease with fatty acid infiltration of the liver to exceptionally high NEFA concentrations. In ruminants, glucose is always released into the blood stream from the liver. The rate at which gluconeogenesis and lipogenesis occur depends on the energy balance where there is increase with a positive energy balance (Nafikov & Beitz, 2007).

Conversely, feeding high fibre diets that are limiting in required energy and protein to dry and lactation cows will result in a negative energy balance (NEB) and negative impact on animal health, lactation and reproduction performance. In the conversion of pyruvate to propionate, increased flow of lactate to propionate through acrylate and succinate pathways has been associated with feeding animals with high starch diets (Nafikov & Beitz, 2007). The authors suggested that the observed improved VFA could be linked to the addition of propionate precursors. Li *et al*. (2015) showed an increased concentration of VFA and the molar propionate proportion in cattle diet supplemented with linseed oil and propionate precursors. This was due to the response of diverse rumen microbes to bio-hydrogenation during the metabolism of unsaturated fatty acids.

Li *et al*. (2015) showed an increased concentration of VFA and the molar propionate proportion in diet of cattle supplemented with linseed oil and propionate precursors due to the response of diverse rumen microbes to bio-hydrogenation during the metabolism of unsaturated fatty acids.

2.8. Protein nutrition of dairy cows

The use of biochemical profile tests involving analysis of blood metabolites to assess the nutritional and health status of animals has gained popularity (Lohakare *et al*., 2006; Ndlovu *et al.*, 2007; Damptey *et al*., 2014). These metabolites are reflections of energy status (glucose, cholesterol, NEFA and β-hydroxybutyrate (BHB)) protein status (total protein, albumin, globulin, urea-nitrogen and creatinine) and other nutrients such as calcium, phosphorus, magnesium and potassium. Changes in the circulating concentration of these metabolites at a particular time is an indicator of the animal's metabolic status (Agenas *et al.*, 2006).

Breed, physiological status, age, season, nutrition, metabolic efficiency of liver and kidney as well as extent of muscle tissue breakdown may influence the concentration of blood metabolites (Ndlovu *et al.*, 2007; Damptey *et al*., 2014). The level of dietary protein and carbohydrate intake, effective rumen degradable protein intake, diet amino acids and energy compositions may play a major role in determining the level of different blood metabolites in dairy cows. Serum protein is the total amount of blood protein present at a particular point in time. Protein fractions in the

blood include total protein, albumin, globulin in the serum with each protein and metabolites playing a significant role in the animal's well being.

Kaneko *et al*. (1997) described albumin as a reservoir of major amino acids and its metabolism provides precursors required for growth and certain physiological needs of the animal. Agenas *et al.* (2006) showed that the concentration of total blood protein and albumin is a reflection of the animal's protein status. However, it is indicated that the concentration of these protein fractions in turn depends on the level of metabolism of protein. Their low concentration indicates a negative balance of dietary protein. According to Mazzaferro *et al*. (2002) albumin is a protein produced in the liver that maintains the colloid osmotic pressure in the circulatory system thus keeping fluid in the vasculature and preventing fluid leakage to the tissue.

Albumin has been identified as the plasma primary transport protein responsible for transporting long chain fatty acids during lipolysis (Ordway *et al*., 1991; Contreras *et al*., 2011) There is a significant correlation of blood concentration of albumin with better animal nutritional status and BCS (Damptey *et al*., 2014). There have been inconsistent reports on the influence of dietary treatments and animal age on total serum protein, albumin and globulin. Alberghina *et al.* (2011) conducted a study using 20 cows of different ages and observed albumin as the highest protein concentration electrophoretogram of cows and constituted 39-58% of the total serum protein. The findings showed that the age of the animals did not influence the proportion of each protein fraction. In contrast, influence of animal age on the total serum protein and albumin has been reported in which total protein and albumin are lower for younger animal compared to the older ones (Otto *et al*., 2000; Lohakare *et al*., 2006; Maurya & Singh, 2015). The study of Lohakare *et al*. (2006) on the effect of feeding different protein levels to weaned crossbred calves showed no significant influence of dietary treatments on the corresponding total serum protein, albumen and globulin. Other studies showed increased total serum protein and albumin with higher protein intake (Ndlovu *et al.*, 2007; Damptey *et al*., 2014).

The circulating concentrations of globulin in the blood was described as a signal of the animal's immune state and ability to combat diseases and infections (Kapele *et al.*, 2008). The study by Damptey *et al*. (2014) showed a higher globulin concentration (52.9 g/L) above the normal range of 30-44.3 g/L in Sanga dairy cows and suggested possible susceptibility of the cows to infection. Whitaker *et al*. (1999) showed that high globulin concentration, low concentration of albumin increased BHB concentration were observed in the incidence of inflammatory disease indicating under-nutrition in cows. However, availability of grass improves the nutritional status of animals in summer

The blood urea nitrogen (BUN) concentration is linked with various factors including level of dietary protein in cow's diets, level of intake of rumen degradable protein, balance of ruminal nitrogen, presence of recycled urea and urea from catabolized tissue (Tan & Murphy, 2004: Fenton & Knepper, 2007). Higher BUN concentration was observed in ruminants (Hammond, 2006) and dairy calves (Lohakare *et al*., 2006) fed supplemental protein. The report of Bovolenta *et al*. (2013) showed that feeding of low dietary protein resulted in decreased BUN concentration. The results indicated that the presence of animal malnutrition, starvation or when metabolic activity in the liver is impaired, could result in low dietary protein. Conversely, Whitaker *et al.* (1999) observed low blood urea concentration with low dietary protein in dairy cow diets. This observation may have resulted from excessive rumen undegradable protein from forage causing nutrient imbalances (Kohn, 2007). Óðinsdóttir (2009) reported a significant effect of dietary composition on urea concentration in milk, where urea concentration increased as energy in prepartum diet decreased.

Creatinine is a waste product of normal muscle tissue catabolism subsequently filtered via the kidney and finally excreted in urine and can be measured in the blood (Dampty *et al*., 2014). The use of creatinine metabolic profile tests in assessing the nutritional status of dairy cows has been demonstrated in several studies (Otto *et al*., 2000; Grunwaldt *et al.*, 2005; Ndlovu *et al.*, 2007; Dampty *et al*., 2014). The blood concentration of creatinine was observed to be associated with the animal's live weight (tissue mass). Dampty *et al*. (2014) observed higher concentration of creatinine in cows with higher BW compared to those with lower BW.

Evaluation of total protein and the albumin to globulin ratio in the blood of dairy cows is key to determining the role of serum protein in growth, reproduction and lactation performance. The effects of diet composition on the corresponding total protein yield of albumin and globulin fractions when dairy cows are fed different diets with different forage to concentrate ratio is crucial to improved diets. Further understanding of the relationship between diet composition and blood biochemical profile of dairy cows will be a guide to meeting their nutritional demands to improve health and lactation performance.

2.9. Body condition and growth

Effects of diet quality on BW of dairy cows and the subsequent influence on reproduction and production performance have been reported (Grummer, 1995; Heinrichs *et al*., 2011). At different stages of production, the BW of dairy cows is expected to meet the requirements for maintenance, growth and lactation. It is important that a common ground be found at which the animal's BW is balanced for the purpose of maintenance, growth, lactation and reproduction for a successful and sustainable dairy production. The BW of a dairy cow indicates balanced diet with adequate

nutrient, dry matter intake, effective utilisation of feed due to efficient feed conversion, optimal microbial activity and the resultant energy balance. In addition, a response of dairy cows by gaining BW is an indication of positive energy balance in which animals store excess energy and protein after body maintenance and production (Varga & Ishler, 2010). Loss of BW indicates a NEB during which fats are constantly mobilised from body fat reserves (Sundrum *et al*., 2015).

A BCS of 3.5 is ideal at dry-off (Gillund *et al*., 2001) allowing for assimilation of body reserves. At this stage, cows should not be overweight or there will be decreased intake with associated metabolic disorders including ketosis and milk fever prepartum and postpartum (Radostits *et al*., 2007). Furthermore, BCS is a measure of fatness during positive energy balance or thinness for the period of negative balance marked with health problems and production loss. Body condition score of cows has been correlated to body fat and energy (NRC, 2001). Fatness or overconditioning in dairy cows usually occurs in late lactation when milk production is reduced without a corresponding decrease in dietary energy.

According to Chiwome *et al*. (2017), excessive body fat should be avoided at calving while a BCS of 3.5 is maintained for adequate body fat and protein reserves. Implications of underconditioning or thinness include decreased lactation performance due to negative energy and protein balance, low fat content of the milk and delayed oestrous (Bovolenta *et al*., 2013). Influence of feeding high dietary energy postpartum to improve performance was greater in underconditioned (thin) cows than in overconditioned cows (Grummer, 1995). Loss of BCS in the first 60 days post parturition is common in cows and should be maintained at 0.5-1.5 loss postpartum (Heinrichs *et al*., 2011).

The body reserves are useful under NEB where they act as a reservoir of energy and make it available at the animal's demand. Adequate feeding of dietary protein and energy should support the building of body reserves that is generally crucial in maintaining or increasing milk quality and yield (Sundrum *et al*., 2015). Ferguson and Otto (1989) stated that overconditioning or underonditioning of cows disposed them to high risk of metabolic disorder and diseases, poor foetus health, low milk yield and reproductive efficiency.

2.10. Milk production and composition

The composition of milk is generally influenced by nutrition and breed. In early lactation, NEB is typical during which reserved body fat is mobilised to the required energy for body tissue and milk fat production (NRC, 2001). Cows with a BCS of 3.0 to 3.5 at calving produce more milk than those calving either at a lower or higher score (Heinrichs, 2011). The relationship may be due to increased availability of energy from body stores up to a BCS of 3.5 and negative effects of BCS on dry matter intake after that point.

There is a high and positive correlation (0.74) between genetics and milk composition in which Jersey cows are known for the highest heritability of milk fat of 0.71 % compared to other breeds with a range of 0.51 to 0.57 % (NRC, 1989). This may probably make it difficult for genetic selection of milk yield only to modify milk composition. According to Promkot and Wanapat (2005), milk urea nitrogen (MUN) increased with increased dietary CP. However, Flis and Wattiaux (2005) showed that a small percentage change (1%) in dietary CP did not result in a change in MUN content.

Mastitis is an inflammation of the mammary glands caused by bacteria in dairy cows marked with high economic loss in the industry (Santos *et al*., 2003; Halasa *et al*., 2007). It causes reduction in milk yield and composition including fat and casein content and subsequent increased whey content and lower cheese yield (Cunha *et al*., 2008). The somatic cell count (SCC) is used in screening of intramammary infection (IMI) status for individual cow or at herd level (Schukken *et al*., 2003; Sharma *et al*., 2011; Reis *et al*., 2013). According to Bytyqi *et al.* (2010), high SCC in milk (>1 x 10^5 or 10 x 10^3 cells /ml) is an indication of disease condition in lactating cows. The SCC in milk from a healthy cow should be < 1 x 10^5 cells/ml. Mastitis in cows caused reduction in quality and milk yield and milk products. Reduction in milk casein, fat and lactose, extended period of milk coagulation, formation of weaker milk curds and shorter shelf life of milk products have been linked with mastitis in cows (Sharma *et al*., 2011).

On the other hand, in summer with very humid conditions marked with low availability of forage resulted in reduced dry matter intake and depressed milk fat content (Linn, 1988). A relationship between dietary fibre content, fibre particles and milk fat has been observed in dairy cows. Feeding cows with diets less than 25% NDF and 16% forage NDF depressed milk fat percentage (NRC, 2001). Furthermore, high concentrate: forage ratio feeding has been associated with low milk fat and acidosis when reduction in milk yield was observed (Benchaar *et al*., 2012).

2.11. Minerals and vitamins requirements of dairy cows

Maintaining mineral and vitamin balance in dairy cow diets not only produces a positive impact on the foetus growth but also increases milk yield and quality. The deficiency of any of these required minerals and vitamins in the diet may result in dysfunction of regulatory systems and increased production losses (NRC, 2001). Macro-minerals such as calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), sulphur (S) and sodium chloride (NaCl) are required in moderate amounts in dairy cattle diets and play a vital role in the proper functioning of the animal organs and systems (NRC, 2001). The roles of macro-minerals in the well being and the production performance of the animals depend on their concentration in the blood. Their sufficient concentration is beneficial to the physiological needs of the animals. The deficiency or excessive

amounts of any of these minerals may pose a threat to the health and production capacity of the animals. Blood concentration of these macro-minerals does not imply the dietary status of the animal when the homeostatic system is functioning properly (van Saun, 2000). The blood concentration of minerals such as P, K, Mg and S respond to changes in dietary intake while the concentrations of Na and Cl may reflect renal and digestive dysfunction or in cases of extreme dietary deficiency.

Calcium and P dairy diets are complementary and maintaining a good balance is key to optimising their utilisation and prevention of the negative effects of their deficiency or surplus. The goal of ensuring adequate P in the diet is to maximize health and production performance, minimize waste cost effective dairy management (Amaral-Philips, 2011). Excess release of P in the environment poses the greatest potential risk compared to other minerals resulting in the contamination and eutrophication of surface water (NRC, 2001). Therefore, the use of absorption coefficients is recommended to avoid an excessive intake of P from diets and microbes. The highest proportion of P requirement for lactation and maintenance while growth and reproduction require only a small proportion except for foetal growth at a later stage of development. The recommended P concentration is 0.3-0.4% of diet DM intake for dairy cows (NRC, 2001). The prevention of excess P excretion is crucial to avoid high production costs and environmental hazards (Amaral-Philips, 2011).

An adequate supply of Ca in the dairy diet results in increased Ca blood concentration. This is essential for the proper functioning of skeletal muscles and nerves (Oetzel, 2012). According to Goff (2008), compromising the normal functioning of muscles and nerves in dairy cows could result in reduced DMI linked with weak gastrointestinal motility, increased metabolic diseases and decreased milk yield. Evaluating blood Ca concentration in dairy cows has become a useful indicator for the positive balance required in a normal regulatory system (NRC, 2001). Hypocalcaemia is a clinical condition indicating blood Ca concentration below requirement in dairy cows. Chiwome *et al*., (2017) reported a low 1.87 mmol/L of blood Ca concentration compared to reference range of 2.0 -2.5 mmol/L in early lactating cows. This finding was related to rapid increase in calcium requirements for the production of colostrum and milk resulting in the observed milk fever condition.

Potassium plays a key role in influencing dietary cation-anion differences (DCAD) of a ration together with sodium and chlorine. A low DCAD (indicative of lower dietary potassium and sodium but high chlorine) has been suggested for periparturient cows in the prevention of milk fever and higher DCAD post calving for the optimization of milk production (Razzaghi *et al*., 2012). Potassium content of 1.0% of diet DM intake is suggested to be adequate. This is to prevent udder oedema resulting from feeding high concentrations of K through forages to close-up dry

cows. Udder oedema in periparturient cows resulting from impaired Ca and Mg metabolism has been linked to feeding cows with forages high in K (NRC, 2001).

The importance of micro-minerals such as iodine (I), selenium (Se), zinc (Z) as well as fat-soluble vitamins cannot be underestimated in their contribution to the optimal functioning of the immune system. Animals with compromised immune system condition resulting in subclinical diseases has been associated with deficiency of certain trace minerals and vitamins (van Saun, 2000). Good storage capacity of the liver for trace minerals and vitamins is key to overcoming moderate dietary deficiencies and maintaining the biochemical functions. Selenium is a trace element identified for its important role in dairy cows' health, reproduction and production performance and its deficiency can lead to infertility, placenta retention, mastitis and metritis. It may also lead to production losses (Sordillo, 2013). Vitamins A, D, E and K are commonly found in the pigmentation of plants, such as the carotene causing discolouration, wilting and drying of forages when these vitamins are deficient (NRC, 2001). The vitamin A deficiency in dairy cows is linked to fertility problems, placenta retention, abortions and incidences of infections.

2.12. Nutritional management of dairy cows

2.12.1. Pregnant heifer and cow

Pregnant heifers and mature cows are placed on rest (dry) for a maximum of 60 days period before the next calving (Smith & Becker, 1995). During this period, they are prepared for optimal milk production in the next lactation. The nutritional demands of pregnant dairy cows can depend on the number of parities, gestation period, breed and their health status (Hall *et al*., 2005). During dry-off period, pregnant heifers/dry cows are separated from the rest of lactating cows to maintain their body condition by feeding them low energy diet with protein, minerals and vitamins to avoid overweight. Dry cows are not expected to be overconditioned during dry-off period or to lose BCS. Overconditioned dry cows are pre-dispose to metabolic disorders resulting in health problems such as displaced abomasum, udder oedema and ketosis after calving (Heinrichs *et al*., 2011). The minimum forage dry matter intake of 1.8 - 2.0% of body weight of the cow per day should be offered to keep dry cows in a proper condition.

An understanding and implementation of the nutritional demands of pregnant cows at different stages of gestation is crucial in attaining optimal production and reproduction performance as well as prevention of health problems. The NRC (2001) recommended 40% NDF of which 80 % should be of forage source, 10-13 % and 15% of CP for mature dry cows and pregnant heifers, respectively; 2-3% and 3-4% of starch and sugar content for mature dry cows and pregnant heifers, respectively.

2.12.2. Lactating cows

Cows in first lactation are referred to as primiparous cows while multiparous cows are mature dairy cows that are in their second lactation or more. Negative energy balance occurs in early lactation in cows, during which nutrient supply lags behind requirements causing fat mobilisation from adipose tissues (Grummer, 1995). Feeding a concentrate diet with sufficient supply of energy to close-up cows is critical for supporting early lactation. According to Ingvartsen (2006) and Óðinsdóttir (2009) this preparation promotes the growth of rumen papillae with subsequent increase in the absorptive capacity of the rumen epithelium. The enhanced functioning of rumen epithelium results in reduced incidence of lipolysis by increasing the production of glucogenic precursors and optimum nutrients supply to transition cows.

2.13. Effects of nutrient deficits in transition dairy cows

Inadequate feeding of energy in close-up cows was a contributing factor to increased lipolysis (Drackley *et al*., 2006; Tieken *et al*., 2015). Milk fever has been described as the most crucial and common metabolic disorder in the liver of transition cows and nutrition was suggested as one of the factors that determines the pathogenesis of this disorder (Radostits *et al*., 2007). Increased incidence of several metabolic disorders and diseases such as mastitis, dystocia and displaced abomasum in transition cows developed from clinical hypocalcaemia or milk fever has been reported (Mulligan *et al*., 2006; Chiwome *et al*., 2017). Adequate particle size in cow diet is important to enhance chewing activity and the subsequent production of saliva as a natural buffer required for keeping a healthy rumen environment of normal ruminal pH (Varga & Ishler, 2010). Doepel *et al*. (2002) and Sundrum *et al*. (2015) reported incidences of metabolic disorders such as ketosis, acidosis, metritis, displaced abomasum and udder oedema during rapid change to high-energy ration postpartum. Hence, it is important to feed a low level of high-energy source to close-up cows and gradually increase postpartum to enhance microbial adaptation.

2.14. Modelling dairy cattle nutrition

Application of a mathematical nutrition model for ruminants to evaluate the required and supplied nutrients to cattle enhances the identification of the variation relative to the animal's performance, thereby minimising the high cost of harmful environmental impact due to excessive nutrient waste. These models include the Large Ruminant Nutrition System (LRNS); Agricultural Modelling and Training System (AMTS) and Co-operative Feed Dealer (CFD) Dairy version 5. Prediction of nutrient concetration and intake in composite diets for dairy cows in any functional state is crucial in assisting ration formulation based on requirements. This is to prevent nutrient waste and its environmental consequences thereby reducing production cost and maximizes profits. Nutrient requirements of a dair cow largely depend on her physiological state such as

increased nutrient demand as gestation advances and may experience poor health, production and reproductive performance in the face of nutrient defficits. Prediction of nutrient concetration and intake in composite diets for dairy cows in any functional state is crucial in assisting ration formulation based on requirements. This is to prevent nutrient waste and its environmental consequences thereby reducing production cost and maximizes profits.

Summary

Smallholder dairy production plays a vital role in providing income, improving livelihood and food security across various smallholder households in South Africa. Currently, communal smallholder dairy production in South Africa is not structured for dairy enterprise compared to other countries where smallholder farms contribute significantly to the global dairy market. The use of indigenous breeds in this sector coupled with their susceptibility to infections and limited potential for high milk production limits the prospect in dairy enterprise. Dairy production on smallholder farms has other limitations such as poor feed resources and sometimes harsh weather conditions. Nutrition plays an important role towards better growth and production performance and improved longevity in cows. However, standardised diets supplied to dairy cows on communal smallholder dairy farms are low in nutrients essential for optimal production performance. Dairy cows need adequate quality diets at gestation for foetal and mammary growth and during lactation for optimal milk synthesis. The quality of forage fraction such as fibre and lignin level, in dairy diets determines extent of diet degradation and nutrient availability for absorption in the lower gut of the animal. Evaluation of dairy diets in smallholder farms is essential to determining how the on-farm fed dairy diets deviated from the NRC (2001) recommendations for the optimal performance of pregnant and lactating cows. The application of wet chemistry and nutrition models are common tools in feed evaluation. Nutrition models are used for further evaluation of nutrient absorption in the small intestine of ruminants in response to nutrient availability.

CHAPTER 3
EFFECTS OF DEVIATIONS OF STANDARDIZED DIETS ON NUTRIENT SUPPORT FOR THE GROWTH OF JERSEY HEIFERS DURING PREGNANCY ON COMMUNAL SMALLHOLDER DAIRY FARMS

ABSTRACT

Intake of net energy is often a limiting factor for growth and pregnancy and is directly affected by dietary proportion of forage carbohydrates and proteins. The study evaluated nutrient supply and growth of pregnant Jersey heifers on smallholder farms in a semi-arid zone of South Africa. Twenty-two farms, all with Jersey cattle and within a communal area districts, were selected for the study. Each farm had at least two pregnant Jersey heifers during the summer season of 2016. A total of 42 heifers, aged 22 to 28 months were on the selected farms. The heifers were considered for measurements from 60 days prepartum. Farmers supplied 2.5 ± 0.25 kg of a far-off (60-30 d prepartum) dry cow concentrate (CP=16%; NDF=32%; ADF=16%). The amount was increased to 3.3 kg at 29-0 d prepartum. *Eragrostis curvula* hay (CP=12%; NDF-75%; ADF=47%) was supplied daily, Simulations of far-off and close-up diets were prepared (HD1 and HD2) based on observed intake of concentrate and hay. The quality of simulated diets was assessed based on chemical analysis. The Large Ruminant Nutrition System (LRNS) version 1.0.33 (level 1) was applied in the prediction of dietary nutrient supply, post ruminal nutrient absorption and animal responses. Actual measurements of body weight (BW), body condition score (BCS) were done monthly; blood was collected and analysed for proteins at the same time. The responses were validated against model predicted values and comparative analysis of animal performance during pregnancy. Heifers consumed all concentrate offered and intake of hay was 7.2 and 5.4 kg/cow/day, respectively, at far-off and close-up period. Both HD1 and HD2 had RFV below 144, which is the minimum requirement for ruminants. Grass hay had a high level of unavailable carbohydrates (CC = 18 % DM). Energy density of HD1 and HD2 were 25% and 16% higher than expected at far-off and close-up period. About 35% of HD1 CP was within the slowly degraded NDF fraction (NDFICP) while 32% was not available (ADICP). The NDFICP component was 52.3% of the effective available protein. Predicted intake of metabolizable protein (MP) were 32% and 25% higher than predicted for the far-off and close-up period respectively. Supply of MP (37%) was higher than NRC predictions of daily requirement in Jersey cow. This allowed BW gain of 29 kg and BCS of 0.33 which was within 25th percentile for pregnant heifers. Mean concentration of blood urea nitrogen (BUN) was 6 and 4.6 mmol/L at far-off and close-up period, respectively, and deviated by 25% from NRC values. Creatinine (CR) concentration was 145 µmol /L at far-off and 155 µmol /L at close-up period. High concentration of CR than the reference value observed might have resulted from heat stress in helfers due to muscle breakdown.

Diets supplied to pregnant Jersey heifers by smallholder farmers had low nutritive value, hence, could not support adequate growth in heifers.

Key words: Standardized diets, dietary nutrients, pregnant heifers, communal smallholder dairy farm

3.1. Introduction

High quality forage rations sustain heifers during puberty, however, nutrient demand increases during the last trimester of pregnancy to support foetal, uterine and mammary gland development (Grummer *et al*., 2004). Improved rumen function and nutrient uptake is critical to the building of body reserves and minimising metabolic disorders such as ketosis (Holcomb *et al*., 2001; Doepel *et al*., 2002) and degeneration of rumen epithelium (Oõinsdóttiir, 2009). Rabelo *et al*. (2003) reported high dry matter intake and blood glucose concentration in close-up heifers. High concentrate intake supports rumen microbial production by stimulating papillae growth, increasing epithelium absorptive capacity and increase production of glucogenic precursors (Ingvartsen, 2006; Oõinsdóttiir, 2009).

Grummer *et al*. (2004) reported -0.8 and -5.8 Mcal/d of energy loss in late gestation primiparous and multiparous cows. This was related insufficient feeding of protein to loss of protein reserves and poor milk production. Most communal area herds average DMI of 8 kg/d (Manzana *et al*., 2014) which is similar to grazing beef and unsupplemented dual purpose cows. Efficiency of milk production in these systems is therefore low and unsustainable.

Dairy cattle graze on communal area pastures or are stall fed (cut and carry system). Recommendations by Franzel *et al*. (2004) of 2 kg dairy meal concentrate, which are widely adopted in East and Southern Africa, support low levels of milk production but result in metabolic retrogression of the dairy cow. There are no clear feeding guidelines for communal area environments; hence the perpertual challenge of unsustainable dairy production continues to negatively impact livelihoods in resource limited areas. Milk production and longevity in dairy cows on smallholder farms could be impoved with carefull implementation of good nutritional regimes that support pregnancy and lactation. Information on analysis and descripton of adequacy of standardized dairy diets in the environment of production are lacking. Therefore, the objective of this study was to evaluate and assess deviations of standardized diets on nutrient support, and growth in pregnant Jersey heifers on communal area smallholder dairy farms. The study tested the hypothesis that deviations of standardised dairy diets affect nutrient support and growth of pregnant heifers raised on on communal smallholder farms

3.2. Materials and methods

Ethical approval for animal experimental protocol

The ethics committees of the University of South Africa (2015/CAES/007) and Agricultural Research Council (APIEC16/023) approved ethical clearance for animal experimental protocol. Standard guidelines established by ARC for care and use of animals were applied accordingly during rumen fluid collection for *in vitro* ruminal fermentation procedure.

3.2.1. Study area

The field study was conducted at two communal areas, Vhembe (Latitude 22^{o} 45' 17'' S, Longitude 30^{o} 12' 37'' E; Altitude 1206 m) and Sekhukhune (Latitude 24^{o} 50'41'' S, Longitude 29^{o} 50' 17'' E; Altitude 1206 m) both in Limpopo province, South Africa. The average annual rainfall was 560 mm for the districts with a mean minimum and maximum temperature of 8 and 31^{o}C respectively in the two districts (ARC-ISCW, Irene, South Africa).

3.2.2. Study design

Ten households with at least two pregnant Jersey heifers respectively from Sekhukhune and Vhembe districts were selected for the study. The ten households selected in each district represented the total population of smallholder farmers with dairy cattle in each area. Forty-two heifers that were six to seven months pregnant were included in the study. The milk producers applied a standardized feeding program based on a prescribed diet by Dairy Development Program (DDP) of South Africa and with one sole supplier of dietary ingredients. The diets are defined in Table 3.1 as HD1 for far off (60-30 days to calving) and HD2 for close up heifers (-30 days to calving). The NRC (2001) provided baseline data to determine the extent of deviation in nutrient support.

Table 3.1 - Ingredients of HD1 and HD2 diets

Diet ingredients (g/kg DM)	Feeding regime	
	[1]HD1	[2]HD2
Concentrate (Commercial heifer pellet)	270	330
Weeping love grass hay *(Eragrostis curvula)*	730	670

[1]HD1 = Heifer diet at 60 - 30 d pre-partum; [2]HD2 = Heifer diet at 31-0 d postpartum

During stage one (far-off period) each Jersey heifer was fed HD1 diet which consisted of grass (*Eragrostis curvula*) hay and 2.5 kg concentrate (heifer pellets). At stage two (close-up period) each pregnant heifer was fed grass hay and 3.3 kg of concentrate (heifer pellets). Grass hay was fed *ad-libitum* at both periods and the cows had free access to clean drinking water. Feed intake was adjusted for 10% weekly refusal. All the pregnant heifers at both experimental sites (Vhembe and Sekhukhune districts) were on similar feeding management during far-off and close-up periods. Diets were analysed for chemical composition and *in vitro* ruminal fermentation to assess their nutritive value.

3.2.3. Chemical analyses

Diet ingredients (concentrate and grass hay) and heifer diets (HD1 and HD2) were analysed for chemical composition. The estimated values were subsequently used to calculate the nutrient composition of both ingredients and diets and the results are presented in Table 3.3. Dry matter and moisture were determined according to AOAC, (1990) and AOAC (2000) respectively. The dried samples were incinerated in a muffle furnace set at 600ºC for 5 hours to determine the organic matter (OM) content (AOAC official method 942.05). Organic matter content was calculated as the loss in weight of dried samples after incineration and ash as the residual content. Total nitrogen was analysed using a standard macro Kjeldahl method (AOAC official method 984.13: 1990). Crude protein content (% DM) was subsequently calculated by multiplying the estimated percentage of total nitrogen by 6.25 (a factor to obtain protein content). Ether extract was determined by method 920.39 (AOAC, 1992). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the method described by van Soest *et al*. (1991) using ANKOM F57 bags in ANKOM$^{2000/220}$ Fibre Analyser (Ankom Technology®, Macedon, NY, USA). Heat stable α-amylase (ANKOM Alpha-amylase) and sodium sulphite were added to the NDF solution to reduce the nitrogenous contamination (Hintz *et al*., 1996; Mertens, 2002). For acid detergent lignin (ADL) residues were subsequently solubilised in sulphuric acid (72% by weight) for 1 h 15 minutes. Bags were washed after extraction and dried in a forced draught oven at 100ºC for 8 hours. Neutral detergent fibre and ADF residues were further analysed for CP for the determination of the neutral detergent indigestible crude protein

(NDICP) and acid detergent indigestible crude protein (ADICP). All fibre estimates were corrected for DM and expressed in % DM including residual ash. Non-fibre carbohydrates were calculated as NFC = [100- (percentage (NDF + CP + Fat + Ash))]. Calcium content was determined according to AOAC (1999) method 968.08 and phosphorus by method 965.17. Cellulose and hemicellulose were estimated using equations of van Soest *et al.* (1991). The dTDN was estimated according to the NRC (1989) and conversion factors were used as described by Fox *et al*. (2004) to convert dTDN to the evaluated nutrients in the equations below. The equations were based on the requirement of dry and lacting cows at level 1 (maitenance level)

- Cellulose = ADF - ADL
- Hemicellulose = NDF-ADL
- Neutral detergent fibre indigestible crude protein (NDFICP) = (grass = 2.3; concentrate = 5.4; HD1 = 4.2; HD2 = 4)
- Indigestible acid detergent fibre insoluble crude protein (IADFICP) = 0.4 x ADFICP
- Non-structural carbohydrates = 100 (NDF+CP+fats+ash)
- Available carbohydrate (B2) = NDF-(2.5+ADL)
- Indigestible carbohydrate (C) = 2.4 x ADL
- Unavailable carbohydrate (CC) = ADL x 2.4 x NDF x 0.01
- Neutral detergent fibre corrected for nitrogen (NDF_N)= NDF – (NDFICP+IADFICP)
- Total digestible nutrients on intake at maintenance level (TDN_{1x}) = 0.98 x (100- NDF_N – CP- fat – ash +IADFICP-0.0072 + 2.25 x (fat-1) +0.75 x (NDF_N - lgnin) x (0.7) - 7
- Digestible total digestible nutrients (dTDN) = 1.01 x NDF_N – 1.77 x 1 – 0.99
- Digestible energy (DE) = NDF_N/100 x 4.409
- Metabolizable energy (ME) (dry and lactating cows) = (DE x 1.1.01) – 0.45
- Net energy maintenance (NEm) = 1.37 x ME – 0.138 x 5.0798 + 0.0105 x 11.4498 – 1.12
- Net energy gain/growth (NEg) = 1.42 x ME – 0.174 x 5.0798 + 0.0122 x 11.4492 – 1.65
- Digestible dry matter (DDM) = 88.9 – (0.779 x ADF)
- Expected dry matter intake (%BW) (EDMI) = 120/NDF
- Relative feed value (RFV) = DDM x EDMI/1.29
- Acid detergent fibre indigestible crude protein (ADFICP) = NDFICP - 0.38

3.2.4. *In vitro* ruminal nutrient degradation of HD1 and HD2 diets

Samples of the simulated HD1 and HD2 were diets milled (1mm), weighed (0.5 ± 0.05 g) into pre-weighed labelled ANKOM bags nd heat sealed (ANKOM Technology®, Macedon, NY, USA). Rumen inoculum was collected from two ruminally cannulated lactating Holstein cows (Age: 36 months; BW: 585 ± 40 kg). Donor cows for rumen fluid were fed *Eragrostis curvula ad-libitum* supplemented with lactating cow pellets. Freshly collected rumen fluid was blended and strained into a pre-heated flask using a pre-warmed four layers of cheese cloth to remove fibrous particles from getting into the rumen fluid. Anaerobic conditions and a constant temperature of 3 9ºC were maintained throughout collection and preparation of rumen fluid.
In the present study, the method described by Goering and van Soest, (1970) was adapted for the preparation of buffer solution with a modification. The modification was the addition of tryptose (Sigma-Aldrich: Aviation Park Sarenta, Pomona Rd, Kempton Park, South Africa), as a protein source to Goering and van Soest buffer. Goering and van Soest buffer consisted of two separate buffer A and (Appendix 1)

In vitro ruminal dry matter degradation (IVDMD) of diets was determined according to the ANKOM Technology Method 3 using ANKOM Daisy[11] incubator (ANKOM Technology®, Macedon, NY, USA). The incubator consists of a four (4 L) jars placed on a rotating rack which intermittently rotated during incubation to optimise substrate-microorganism contact. Each diet sample bags in triplicates plus one empty bag (total of 25 sample bags) were placed in each jar. Incubation medium constituted buffer solution and rumen fluid respectively in ratio of 4:1. 1600 mL of prepared Goering and van Soest buffer solution was transferred into each of the jar containing sample bags and pre-heated to 39ºC. Jars with sample bags and buffer were subsequently taken out of the incubator after warming and inoculated each with 400 mL of freshly prepared rumen inoculum.

Incubation was done for 2, 4, 8, 12, 18, 24, 30 and 36 hours. At termination, bags were washed with running water and dried at 100ºC for 8 hours. Disappearance of nutrients from sample bags after each incubation periods was regarded as degraded substrates. Dry matter degradability was determined by the difference between the initial sample weight and the weight after incubation divided by the initial sample weight. The IVDMD was expressed as % DM.

For the determination of nitrogen degradation residues of *in vitro* DM degradability were pooled the N content of the residues was determined according to the described standard macro-Kjeldahl Method no 984.13 (AOAC, 1999) using UDK 159 Automatic Kjeldahl Analyser. Loss in the total N post-incubation was regarded as estimates of N degraded. It was calculated as difference in total nitrogen and nitrogen content in diet residues after incubation (expressed as % DM)

In vitro ruminal organic matter degradability (IVOMD) was determined by further incinerating individual diet fermentation residues (in filter bags) in pre-weighed and labelled crucibles in a muffle furnace at 550^{o}C for 5 hours. Notably, filter bags are completely combustible without influencing the ash result according to the manufacturer (ANKOM Technology®, Macedon, NY, USA). Crucibles with ash residues were cooled and weighed. Estimates of OM degraded was calculated considering the initial dried sample weight before and after incineration and the value was expressed as percentage of diet DM.

3.2.5. *In vitro* ruminal gas production and fermentation kinetics of HD1 and HD2 diets

The HD1 and HD2 diets were evaluated for gas production and fermentation kinetics (rate of gas production, effective and potential gas production) using ANKOM^{RF} gas production system (ANKOM Technology®, Macedon, NY, USA). The gas production system was fully automated with the use of mobile pressure sensors programmed to record gas pressure at pre-set intervals for the period (36 hours) of the experiment. About 1 g of milled HD1 and HD2 diet samples were placed in separate ANKOM^{RF} bottles with buffered rumen fluid. The bottles were subsequently sealed with pressure censored modules and placed in an incubator and incubated. Gas pressure from the headspace was automatically measured at a pre-set interval (15 minutes) until 36 h after inoculation. The measured gas pressure was converted to moles of gas produced which in turn converted to gas volume produced in millilitres (mL) using Boyles (ideal) gas law and Avogadro's law, respectively. The laws were presented as:

Boyles (Ideal) gas law:

$$n = p\ (V/RT)$$

Avogadro's law;

$$y = n \text{ x } 22.4 \text{ x} 1000 \text{ I ..}$$

Where; y = Volume of gas as produced (mL); *n* = gas produced in moles; *P* = pressure in Kpa; *V* = volume in the bottle headspace (L); *T* = temperature (Kelvin); *R* = the gas constant.

Gas production kinetics were determined by fitting the generated data into the non-linear equation of Ørskov and McDonald (1 979);

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

Where: *y* = cumulative gas volume at time t; *a* = gas production (mL) from the immediately soluble fraction; *b* = gas production (mL) from insoluble fraction; *c* = rate of gas production constant for the insoluble fraction; *t* = incubation time (h); *e* = exponential'

Potential gas production (GP_P) was calculated as the sum of *a* and *b* fractions from the equation of Ørskov and McDonald (1979).

Effective gas production was calculated using the equation below;

$$GP_E = a + \frac{bc}{k+c},$$

Where GP_E = effective gas production; a, b and c = constants from the non-linear model of Ørskov and McDonald (1979) for cumulative gas production; *k* = rate of gas production assumed at 3% per hour. (a = the initial gas production)

3.2.6. Evaluation of required and supplied nutrients of HD1 and HD2 diets

The two diets were estimated for required and supplied nutrients. Estimated nutrient concentrations of diets were inputted into the Large Ruminant Nutrition System (LRNS) version 1.0.33 at level 1 solution in Cornell Net Carbohydrate and Protein system (CNCPS) Model (Fox *et al*., 2004). Animal type and condition differing in production group, managed under different conditions, environment and feeding system (were considered under the system. The feed libraries of the system were edited by replacing the existing feeds with the ingredients and composition of the HD1 and HD2 diets.

Description, management/environment, production and ration inputs for individual animal were inputted into the model to generate the actual/observed and predicted/required nutrients at the two lactation stages. Information on the animals under different inputs for far-off and close-up pregnant heifers are presented in Table 3.2. The required metabolizable energy (ME), metabolizable protein (MP), calcium and phosphorus for foetus and mammary growth8 and maintenance were also predicted in the model.

Table 3.2 - Imputed parameters for pregnant heifers at far-off and close-up periods.

Inputted parameters	Bred heifers	
Descriptions	Far-off	Close-up
Animal type	Replacement heifer	Replacement heifer
Age (month)	22	24
Current body weight (kg) (SBW*)	349	365
Mature weight (kg)	450	450
Days pregnant	250	280
Production		
Body weight	349	376
Body condition score	2.15	2.46
Management/environment		
Temperature (^oC)	29	29
Relative humidity (%)	75	77
Minimum night temperature (^oC)	11	11
Ration (kg)		
Grass hay	6.7	6,7
Commercial heifer pellets	2.5	3.3
Total dry matter intake (TDMI)	9.20	8.7

*SBW= shrunk body weight

3.2.7. Evaluation of the performance of pregnant Jersey heifers

3.2.7.1. Body weight and condition scoring

All the linear measurements including body length, heart girth, wither girth, wither height on the experimental animals were taken every week using tailor measuring tape from 60 days prepartum to calving. The BW was calculated using heart girth according to the predicted equation of Francis *et al*. (2004). The equation showed a high correlation between body weight and heart girth (r^2 =0.96) and therefore can be used alone in developing a prediction equation reliable for body weight.

The prediction equation for body weight for cattles;

Body weight = 73.11 - 1.958GTH + 0.01899GTH2 + 0.0000216GTH3 (R^2 = 0.97).

Where GTH = heart girth.

The All the experimental animals were condition scored according to the method and scale of Klopcic *et al*. (2011). Condition score of 1 denotes very thin while 5 denotes very fat.

3.2.7.2. Blood sampling for the evaluation of protein metabolic profile

The protein profile test conducted in the present study aimed at evaluating the nutritional status of pregnant heifers at far-off and close-up periods. Blood sampling was carried-out once a month until calving. Once a month blood sampling was preferable to forthnight collection to minimise handling stress to the cows during this late gestatation period. Blood was collected in anti-coagulant free vacutainer tubes from the coccygeal vein before morning feeding. The tubes were immediately placed in an ice-bath to prevent possible metabolic reactions (Oõinsdóttiir, 2009). Blood samples were maintained motionless for 30 min for coagulation and further centrifuged (3000 rpm for 10 min) for serum separation. Serum samples were subsequently analysed for blood proteins including total protein (TP), albumin (AL), globulin (GL), creatinine (CR) and blood urea nitrogen (BUN). Serum protein concentration in heifers was analysed using biuret reagent according to the standard method of Doumas *et al*. (1981). The concentration of the TP in the serum was determined by the intensity of the colour formed and was directly proportional to the colour formed. The TP concentration was read in the analyser and was expressed as g/L.

The concentration of albumin in serum was determined by using bromocresol green dye as described by Doumas *et al*. (1971). The intensity of the colour produced is directly proportional to albumin concentration in the serum sample. Globulin concentration was calculated as the difference between the total protein and albumin in the serum. Globulin and albumin concentrations were expressed as g/L. The concentration of urea (mmol/L) in the serum sample was quantified according to the enzymatic method of Sampson *et al*. (1980). Creatinine level (μmol/L) in the serum was analysed according to the method of Ambrose *et al*. (1983) using high performance liquid chromatography

3.2.8. Statistical analyses

Data were analysed separately for far-off and close-up pregnant heifers using a completely randomised design (CRD). The *in vitro* DM, N and OM degradation and fermentation parameters of heifer diets were analysed using analysis of variance (ANOVA) procedures in SAS software package (version 9.3: SAS Institute, Inc., Cary NC, USA). Variation within animals and between units of all tested parameters were assessed. Fischer's test was used to separate means for different periods and time intervals in the *in vitro* fermentation. Tests were performed at 95% confidence limit ($P \leq 0.05$) and the results were presented as mean ± standard deviation. Paired t-test was used to compare the observed and expected nutrient composition and intake of diets in a general linear model (GLM) of SAS.

3.3. Results

3.3.1. Nutrient supply of HD1 and HD2 diets for pregnant heifers

Adding grass hay to the lactation concentrate (diets constituted grass hay and heifers concentrate) had a negative effect on RFV as grass hay had a high level of unavailable carbohydrates (CC = 18 % DM) which affected diet quality. Both HD1 and HD2 had RFV below 144, which is the minimum requirement for ruminants. In HD1, 35% of dietary protein was within the slowly degraded NDF fraction (NDFICP) while 32% was not available (ADICP). Equally, HD2 diet had effectively 5.2% of CP as available protein. The fraction of the slowly degraded NDF constituted only 52.3% of the effective available protein.

At 60-30 d prepartum period, the low i*n vitro* dry matter degradation of the HD1 (Fig 3.3.1) confirmed the low feed qualityof the diet. At 36 hours, DM and N degradation were 44% and 51% respectively. The N components were degraded better than the carbohydrate fractions; about 45% within 18 hours, however, increase in degradation was insignificant thereafter. Extending the incubation time beyond 18 hours did not improve degradation of fibrous components. Gas production and fermentation kinetics of HD1 diet are presented in Table 3.4. Potential gas production (*GPp*) and effective gas production (*GPe*) were 64 mL/g and 41 mL/g DM respectively. However, *GPp* and *GPe* were estimated at 72 and 56 mL/g respectively. The rate (c) was 5%/h within 18 hours and reduced to less than 2%/h thereafter.

Table 3.3 – Chemical and nutrient composition of concentrate, grass hay, HD1 and HD2 diets

Parameters	*Concentrate	**Grass hay	HD1 diet	HD2 diet
Dry matter	88.7	93.1	92.3	91.6
Organic matter	92.0	93.0	92.9	92.7
Calcium	0.88	0.37	0.67	0.70
Phosphorus	0.45	0.16	0.32	0.36
Crude protein	16	12	129.7	134.0
Fat	3.1	1.3	17.6	19.8
Ash	8	7	7.12	7.33
Structural proteins and carbohydrates				
Neutral detergent fibre	32	75	65.0	61.0
Acid detergent fibre	16.2	46.9	40.1	37.5
Acid detergent lignin	7.3	21.1	12.4	11.5
Cellulose	8.9	25.8	27.7	26.0
Hemicellulose	24.7	53.9	52.6	49.4
Lignin	5.10	37.7	19.6	18.3
ADFICP (% CP)	1.92	5.02	4.18	4
NDFICP (% CP)	2.3	5.4	4.56	4.38
IADICP (% DM)	0.77	2.0	1.67	1.6
NDF_n (% DM)]	28.8	67.5	58.7	54.9
Non- structural carbohydrates				
Non-fibre carbohydrate (% DM)	40.9	4.7	13.2	16.4
Available carbohydrate (B2) (% DM)	22.2	19.0	50.1	46.9
Indigestible carbohydrate (C) (% DM)	17.5	50.6	29.7	27.6
Unavailable carbohydrate (CC) (% DM)	5.6	38.0	19.3	16.8
TDN_{1x} (% DM)	61.0	31.5	46.9	49.8
dTDN (% DM)	58.9	26.1	45.6	48.5
Digestible energy (Mcal/kg)	2.3	1.0	2.0	2.1
Metabolizable energy (Mcal/kg) for lactation	1.9	0.5	1.6	1.7
Metabolizable energy (Mcal/kg) for growth	1.9	0.8	1.6	1.8
NEm (Mcal/kg)	0.8	0.5	0.92	1.04
NEg (Mcal/kg)	0.2	0.4	0.86	1.1
Digestible dry matter (% DM)	76.3	52.4	57.7	59.7
Expected dry matter intake (% BW)	3.8	1.6	2.6	2.7
Relative feed value	216.6	61.4	125.8	156.0

***Concentrate**- Commercial lactation diet; ****Grass hay** - *Eragrotis curvula*; **HD1** - Diet for 60-30 d prepartum heifers; **HD2 -** Diet for 29-0 d prepartum heifers; **ADFICP** – Acid detergent fibre indigestible crude protein; **NDFICP** – Neutral detergent fibre indigestible crude protein; **IADFICP** - Indigestible acid detergent fibre insoluble crude protein; **NDFn** – Neutral detergent fibre corrected for nitrogen; **TDN_{1x}** - Total digestible nutrients on intake at maintenance level; **dTDN** - Digestible total digestible nutrients; **NEm -** Net energy maintenance**; NEg -** Net energy gain**.**

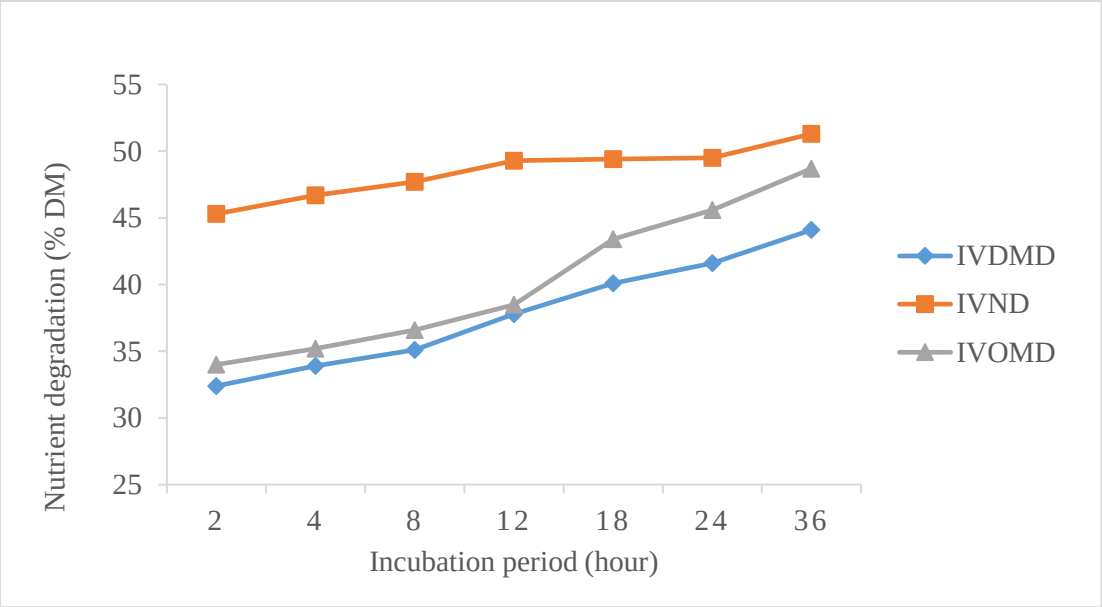

Figure 3.3-1 In vitro dry matter degradability, in vitro nitrogen degradability and in vitro organic matter degradability (% DM) of HD1 diet

Table 3.4 - *In vitro* gas production and fermentation kinetics of HD1 diet after 36 h of incubation

Parameters	*In vitro* gas production and fermentation kinetics of HD1 diet
Total gas production	58.2
[1]a	2.92
[2]b	61.0
[3]*GPp*	64
[4]c (% in 18 h)	5
[5]*GPe*	41

a = gas production from immediate diet degraded fraction; [2]b = gas produced from diet slowly degraded fraction; [3]*GPp*= potential gas production; [4]c = rate of gas production within 18 h; [5]*GPe* = effective gas production

Fig 3.3-1 shows *in vitro* degradation of HD2 diet, and fermentation kinetics are in Table 3.4. Dry matter and nitrogen degradation were 48 and 53% respectively with 90% of degradation occuring before 24 hours. Potential gas production and *GPe* recorded was 66 and 45.4mL/g DM respectively. The estimated *GPp* and *GPe* at 48 h was 76 and 60 mL/g respectively. The rate (c) within 18 hours was 6% /h, declining rapidly thereafter.

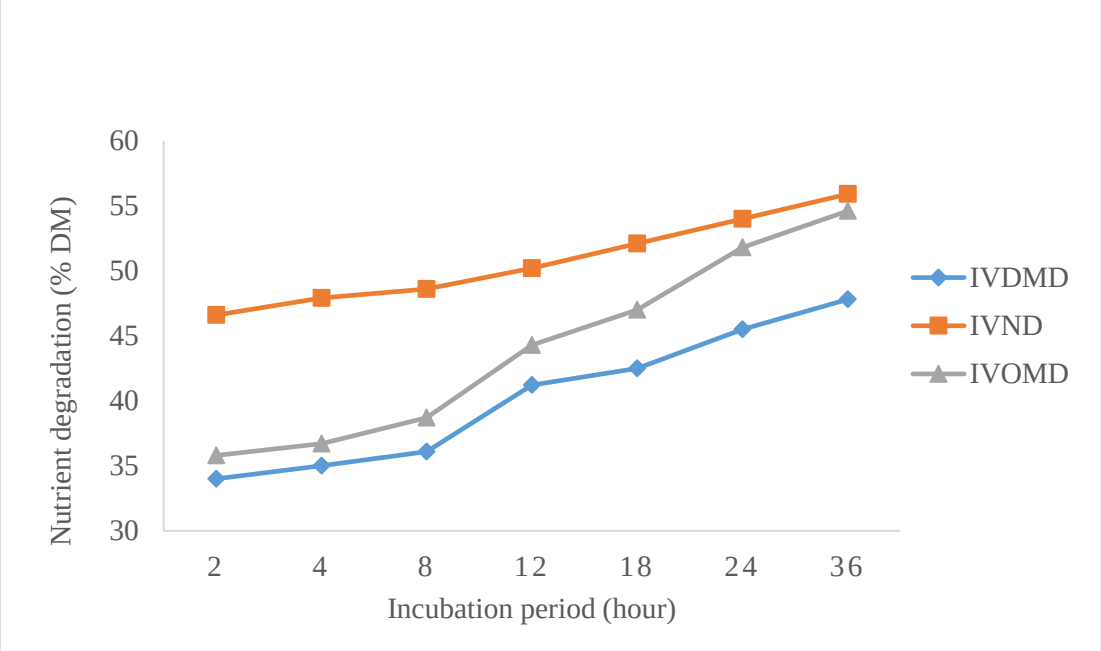

Figure 3.3-2: - In vitro dry matter degradability, in vitro nitrogen degradability and in vitro organic matter degradability (% DM) of HD2 diet

Table 3.6 - *In vitro* gas production and fermentation kinetics of HD2 diet after 36 h of incubation

Parameters	*In vitro* gas production and fermentation kinetics of HD2 diet
Total gas production	62.6
[1]a	3.7
[2]b	62.2
a+b	65.9
[4]c (% in 18 h)	6.1
[5]*GPe*	45.4 (61

[1]a = gas production from immediate diet degraded fraction; [2]b = gas produced from diet slowly degraded fraction; [3]*GPp*= potential gas production; [4]c = rate of gas production within 18 h; [5]*GPe* = effective gas production.

3.3.2. Supplied and predicted nutrients in HD1 and HD2 diets for pregnant heifers

Table 3.5 shows a comparison between observed nutrient concentration in HD1 diet and expected values. Observed concentration of CP in diet was not significantly different (*p=0.054*) from expected value. Significant difference (*p<0.05*) was found between observed concentrations of metabolizable protein (MP), rumen degradable protein (RDP), rumen undegradable protein (RUP), metabolizable energy (ME) and non-fibre carbohydrate (NFC) in diet than expected values. The observed values of these nutrients were lower (*p<0.05*) than expected (MP: 11.0 vs 5.5%; RDP: 8.3 vs 4.1%; RUP: 3.19 vs 1.37%; ME: 3.19 vs 1.73%; NFC: 33 vs 18%). However, observed concentration of dietary fibre such as NDF (65%), total forage in diet (78%), forage NDF (84%) were higher (p<*0.05*) compared to expected values.

Observed and expected daily nutrient intake of the HD1 diet are presented in Table 3.6. Observed mean daily CP (1196 g) was higher (*P<0.05*) than the expected value (929 g). Dry matter intake (9.2 kg/d) and DMI expressed as a percentage of BW (2.6) were higher (*p<0.05*) than expected values of 6.7 kg and 1.9 respectively. Mean daily intake of MP (503 g), RDP (377 g), RUP (126 g) and ME (1.6 Mcal) observed in the diet were significantly lower (*p<0.05*) than expected values. On the other hand, observed NDF (6.0 kg/d) intake and forage intake as percentage of BW (2.2) were higher (*p<0.05*) than expected values.

Table 3.5 - Comparison of observed and expected nutrient concentration in HD1 diet

Nutrient concentration (% diet DM) of [1]HD1 diet			
Parameters	Observed	Expected	*p*-value
Crude protein	13.0	13.8	0.054
Metabolizable protein	5,47	11.0	0.003
Rumen degradable protein	4.1	8.32	0.001
Rumen undegradable protein	1.4	2.8	0.01
Metabolizable energy (Mcal)	1.7	3.2	0.01
Neutral detergent fibre (NDF)	65	60	0.017
Forage NDF	78	57	0.002
Forage neutral detergent fibre	84	61.2	0.02
Non-fibre carbohydrate	18	33	0.001

[1]HD1= heifer diet for far-off (60-30 d prepartum) heifer

Table 3.6 Comparison of observed and expected daily nutrient intake of HD1 diet

Nutrient intake of [1]HD1 diet			
Parameters	Observed	Expected	*p*-value
Dry matter intake (kg /d)	9.2	6.7	0.04
Dry matter intake (% body weight)	2.6	1.9	0.03
Crude protein (g/d)	1196	929	0.001
Metabolizable protein (g/d)	503	743	0.001
Rumen degradable protein (g/d)	377	557	0.001
Rumen undegradable protein (g/d)	126	186	0.001
Metabolizable energy intake (Mcal/d)	16.0	21.4	0.01
Neutral detergent fibre (kg/d)	6.0	4.0	0.04
Forage (% of body weight)	2.20	1.60	0.02

[1]HD1= heifer diet for far-off pregnant heifers

Observed and expected concentration of nutrients including MP, ME, RDP, RUP and NFC in HD2 diet for close-up heifers are presented in Table 3.7. Lower ($p<0.05$) nutrient concentration was observed in diet than expected values while no difference was found in CP content (observed

=13.4% vs expected= 13.0%). On the other hand, observed concentration of NDF (61%), total fibre in ration (74%) and forage NDF (77.3%) were higher ($p<0.05$) than predicted values (NDF = 43; total fibre in ration = 52.5; forage NDF = 55.1).

Table 3.8 indicates observed and expected mean daily nutrient intake of the HD2 diets. Mean daily CP intake observed in diet was 1340 g and significantly higher ($p<0.05$) than expected value of 923 g/heifer. Observed DMI (8.7 kg/d) and DMI (% BW: 2.3) were significantly higher ($p<0.05$) compared to expected values (DMI = 7.1 kg/d; DMI (% BW) =1.9). Mean daily intake of the MP (555 g), RDP (416 g), RUP (139 g) and ME (18.2 Mcal) was observed in the diet and were significantly lower ($p<0.001$) than expected values (MP = 738 g; RDP = 554 g; RUP = 185 g; ME = 21.7 Mcal). The results showed higher significant ($p<0.05$) intake of NDF (6.10 kg/d) and forage as percentage of BW (2.0) were observed compared to expected values of NDF (3.10 kg/d) and forage (% BW) of 1.4.

Table 3.7 - Comparison of observed and predicted nutrient concentrations of HD2 diet

Nutrient concentration (% diet DM) of HD2 diet			
Parameters	Observed	Predicted	*P*
Crude protein	13.4	13.00	0.25
Metabolizable protein	5.60	10.4	0.01
Rumen degradable protein	4.16	7.80	0.001
Rumen undegradable protein	1.39	2.60	0.01
Metabolizable energy (Mcal)	1.82	3.06	0.001
Neutral detergent fibre	61.0	43.0.	0.0002
Total forage in diet	74.0	52.5	0.0002
Forage neutral detaergent fibre	77.3	55.1	0.013
Non-fibre carbohydrates	20.0	34.0	0.003

[1]HD2= heifer diet for close-up (29-0 d prepartum) pregnant heifers

Table 3.8 - Comparison of observed and expected nutrient intake of HD2 diet.

Nutrient intake of [1]HD2 diet			
Parameters	Observed	Predicted	*P*
Dry matter (kg/d [2]DM)	8.7	7.10	0.012
Dry matter intake (% body weight)	2.30	1.90	0.02
Crude protein (g/d)	1340	923	0.0001
Metabolizable protein (g/d)	555	738	0.001
Rumen degradable protein (g/d)	416	554	0.001
Rumen undegradable protein (g/d)	139	185	0.001
Metabolizable energy (Mcal/d)	18.2	21.7	0.003
Neutral detergent fibre (kg/d)	6.10	3.10	0.04
Forage (% of body weight)	2.0	1.4	0.01

[1]HD2= heifer diet for close-up (29-0 d prepartum) pregnant heifers

3.3.3. Energy and protein balance in pregnant heifers

Energy and protein balance during the period 60-30 d prepartum are shown in Table 3.11. Mean energy supply at 21.1 ± 2.1 Mcal/heifer/day was higher ($p<0.05$) than requirement (16.0 ± 1.2 Mcal). However, 25% of the cows achieved mean energy balance of 5.1 Mcal/d and most were within the range 0.8–2.1 Mcal/d. Energy supply was 32% relatively higher than requirement. However, based on calculation, the NRC (2001) recommend minimum requirement of 112% for pregnant cows. Mean daily protein supplied (743 ± 8 g/d) was higher ($p=0.001$) than requirement (503 ± 9 g/d resulting in a positive balance of 240g/d. Pregnant heifers had sufficient energy and protein to support fetal development but to varying levels.

Table 3.9- Mean daily energy and protein balance in 60-30 d prepartum heifers.

Parameters	Energy and protein balance in 60-30 d prepartum heifers				
	Required	Supplied	Balance	supplied as % of required	[*]% recommended
Energy (Mcal/d)	16.0 ±1.1	21.0 ±2.1	5.3	132	112 (NRC 2001)
Protein (g/d)	503 ± 9	743 ± 8	240	148	117 (NRC 2001)

[*]minimum requirement

Table 3.10 shows mean daily balance of supplied and expected energy of close-up heifers. The results indicated a significant difference between supplied and required energy and protein. Energy supplied was 19% higher relative to requirement at close-up period. The results showed that heifers showed positive energy balance of 3.5 Mcal; the NRC recommends 128% supply at this stage. Mean protein supplied was high compared to requirement. Heifers had 8 units of protein above recommended levels (133 vs 118).

Table 3.10 - Mean daily energy and protein balance 29-0 d prepartum heifers

Parameters	Energy and protein balance in 29-0 d prepartum heifers				
	Required	Supplied	Balance	Supplied (% of requirement)	*% recommended
Energy (Mcal/d)	18.2± 0.4	21.7± 0.1	3.5	119	128 (NRC, 2001)
Protein (g/d)	555 ± 9	738 ± 4	183	133	118 (NRC, 2001)

*minimum requirement

3.3.4. Performance of pregnant heifers

Body weight and BCS during 60-30 d prepartum are presented in Table 3.11. Mean BW and BCS were 349 ± 36 kg and 2.15 ± 0.12 respectively. Mean change (gain) in BW was 26.4 kg/heifer. Mean changes in BCS was 0.26 in which body fat, protein and energy reserves contributed 3.5 kg, 18 kg and 103 Mcal of energy respectively of the total gain. Figure 3.3-3 represents the protein metabolic profile of far-off pregnant heifers including concentration of serum total protein (TP), albumin (AL), globulin (GL) blood urea nitrogen (BUN) and creatinine (CR) and the reference values as adapted from Otto *et al*. (2000). The concentration of the TP obtained was 80 g/L and was within the reference range. The concentration of albumin (34 g/L) was below the reference value range for pregnant cows. On the other hand, mean concentration of globulin of 46 g/L was higher than the reference value. Albumin to globulin ratio (AL: GL) was 0.8- a value less than the reference value of 1.1 for pregnant cow. The concentration of BUN (6.3 mmol/L) and CR (150 μmol /L) obtained were higher than the reference value range.

Table 3.11 - **Mean body weight and condition score of far-off pregnant heifers**

Parameter	Far-off (60-30 d prepartum) pregnant heifers
Mean body weight (kg)	349 ± 36
Mean change in body weight (kg)	26.4 ± 11.34
Mean body condition score	2.15 ± 0.12
Mean change in body condition	0.26
Body fat reserves gain (kg)	3.5
Body protein reserves gain (kg)	18
Body energy reserves gain (Mcal)	103

Table 3.11 shows BW and BCS pregnant heifers at close-up period. Heifers had mean BW and BCS of 376 ± 43 kg and 2.46 ± 0.45 respectively. The unit increase in BW was 29.6 kg and 0.33 for BCS. About 160 Mcal was contributed by 4.6 kg fat, 28 kg protein to body gain. Figure 3.3-4 represents the serum concentration of serum TP, AL, GL, BUN and CR in close-up heifers. Mean concentration of TP obtained was 77 g/L and the value was within the reference range. Heifers had mean concentration of the albumin of 36 g/L and was within the reference range for pregnant heifers. The globulin concentration was 40 g/L and higher than the reference value range. The ratio of AL: GL at 0.9 was lower than the reference. Concentration of BUN and creatinine was 5.7 mmol/Land 136 μmol /L respectively; and were higher than reference value range.

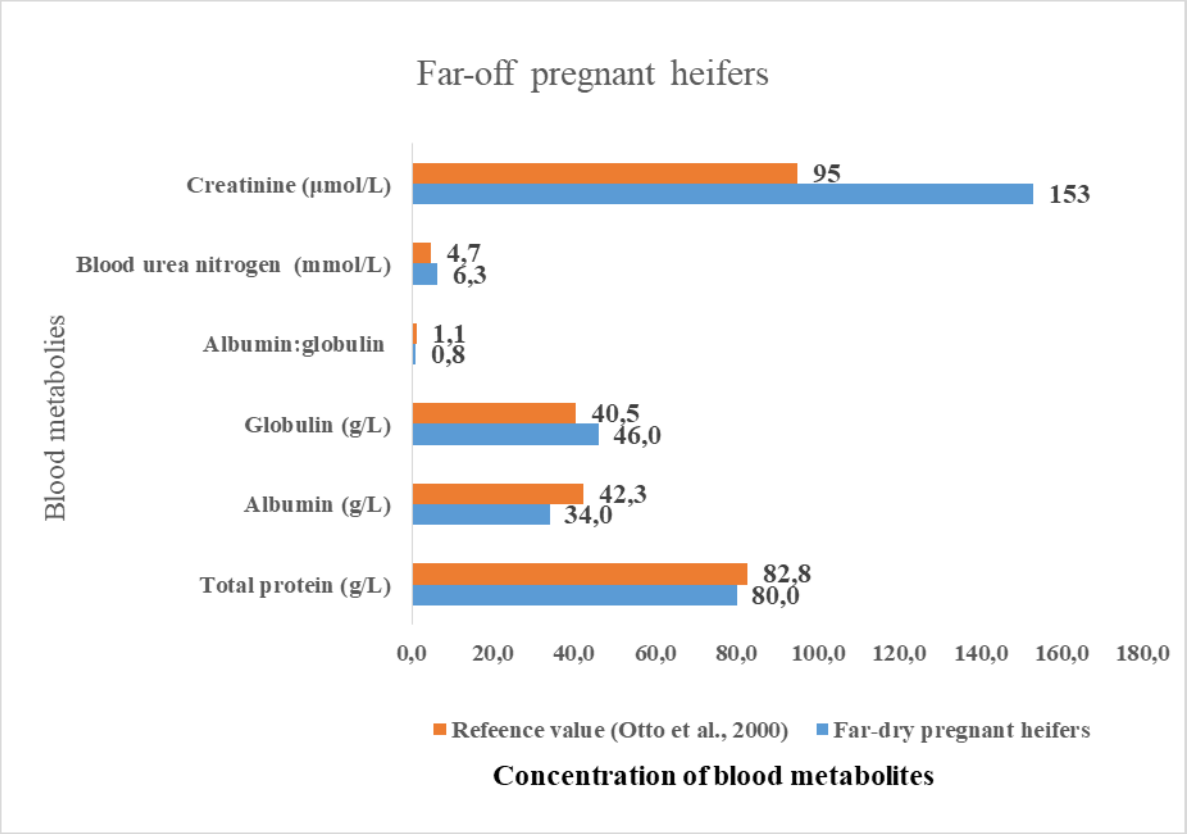

Figure 3.3-3 *Serum concentration of blood proteins at far-off period. Reference value range (Otto et al., 2000): TP = 77.9 - 87.7 g/L; AL = 40 - 44.5 g/L; GL = 36.9 - 43.8 g/L; AL: GL = 0.9 - 1.2; BUN = 4.3 - 5.0 mmol/L; CR = 56 - 134.2 µmol/L.*

Table 3.12 - Mean body weight and condition score of close-up pregnant heifers

Parameter*	Close-up (29-0 d prepartum) heifers
Mean body weight (kg)	376 ± 43
Mean change in body weight (kg)	29.6
Mean body condition score	2.46 ± 0.45
Mean change in body condition	+0.33
Body fat reserves mobilized (kg)	4.6
Body protein reserves mobilized (kg)	28
Body energy reserves mobilized (Mcal)	130

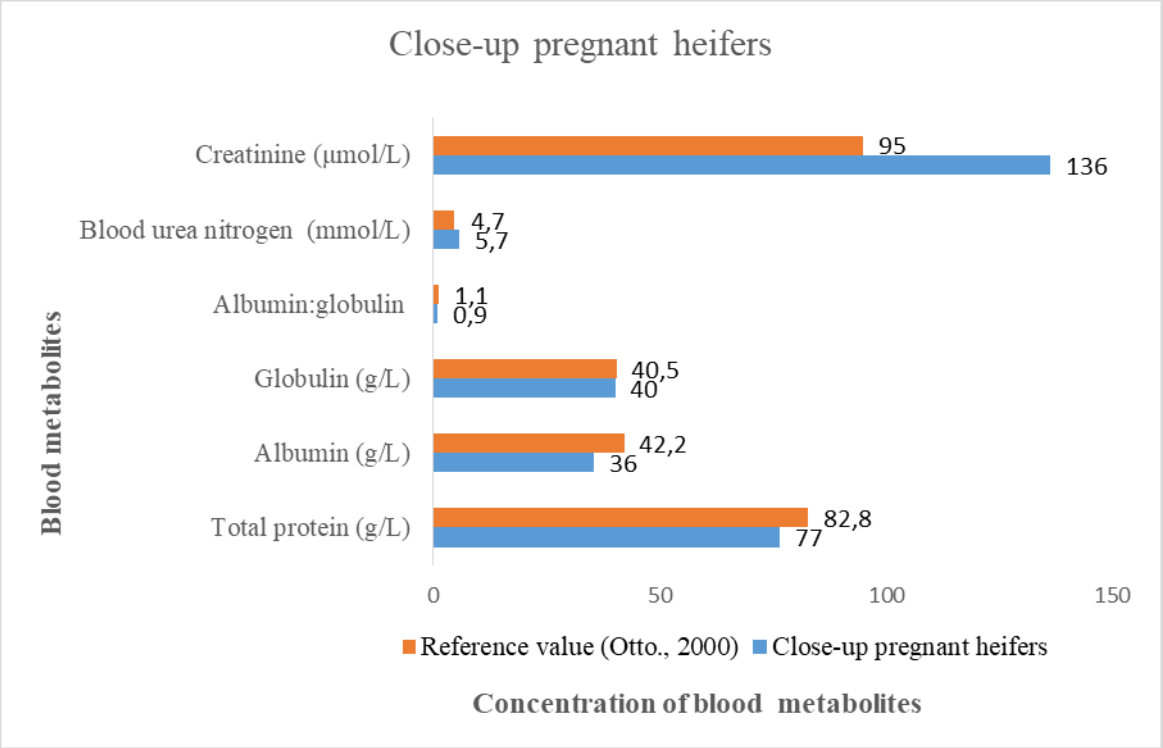

Figure 3.3-4: Serum concentration of blood proteins at close-up period. Reference value range (Otto et al., 2000): TP = 77.9 - 87.7 g/L; AL = 40 - 44.5 g/L; GL = 36.9 - 43.8 g/L; AL: GL = 0.9 - 1.2; BUN = 4.3 - 5.0 mmol/L; CR = 56 - 134.2 µmol/

3.4. Discussion

3.4.1. Nutrient supply of HD1 and HD2 diets for pregnant heifers

Low diet DMD was related to poor rumen degradation as grass hay content was high. The inclusion level of forage in diets was higher than the range of 20-50% DM suggested by Mertens, (2005) and NRC (2001) recommendations for pregnant cows. Tyasi *et al.* (2015) noted that ≥ 65% dietary forage affected dietray energy density. Hay based diets tend to degrade more slowly (Aung *et al.*, 2016; Patra, 2012) as nutrients are not accessible due to limited sloughing of epidermal layers and feed particles (Beauchemin, 2018). High proportions of slowly degradable carbohydrates (B2) and low non-structural carbohydrate fraction including starch and soluble sugars resulted in poor fermentation as noted by Njidda and Nasiru (2010); Suharti *et al.* (2011). There is a higher enregy cost in fermenting high fibre diets; most energy is lost as heat increment.

Low NSC intake which is associated with high fibre intake affects non-structural bacterial growth. Efficiency of microbial protein production has been linked to diet carbohydrate and nitrogen degradation and rumen environment favourable for microbial growth (Pathak, 2008; Hamid *et al.*, 2007). The total digestible nutrient (TDN) of less than 50% was lower than the range

recommended for pregnant cows (NRC, 2001). Consequently, RFV was low. In this study, it was noted that intake of metabolisable energy increased for pregnancy (6.3%) and gain (13%) as gestation progresses, reflecting the partitioning of energy for gain (foetus, heifers' body and mammary tissues).

One the hand, high fibre content enabled longer mastication increasing particle breakdown, thereby creating access for microbial attachment and subsequent enzymatic degradation of substrate (Beauchemin, 2018; van Soest, 1991). Rumen pH was within the optimal range of 6 to 6.7 that promotes the growth of various bacteria that degrade SC (*Ruminococcus flavefasciens*, *Bacterioride saccinogene*) and NSC (*Bacteriodes ruminocola*, *Bacteriodes amylophilus*, *Streptococcus bovis* (Russell & Wilson, 1996). Therefore, degradation of diets is expected to be enhanced, Indigestible and unavailable carbohydrate fractions were high and represented an energy loss, which could be methane (Moss *et al*., 2000; Cabezas-Garcia, 2017). Methane production can contribute up to 12% loss in gross energy of diet when diets are high in fibre (Partel *et al*., 2011). The role of non-structural carbohydrate bacteria in the production of N compounds needed for efficient growth of SC bacteria is crucial in ruminant nutrition. Nevertheless, the high inclusion level of grass hay fraction and its physico-chemical characteristic such as lignification may have limited fibre and overall diet digestibility.

It is possible that the growth of bacteria that degrade soluble carbohydrates such as *Streptococcus bovis* was depressed, affecting microbial outflow (Tedeschi *et al*., 2001). Energy supply to proteolytic and structural (cellulolytic) bacteria is reduced resulting in unbalanced coupling of protein and energy degradation. Supplementation of dairy diets with readily degradable carbohydrate improves N utilization and synchronization of carbohydrate and protein degradation (Zhao *et al*., 2015). Effects of NEB can be reduced by providing high-energy boosters such as propylene glycol during late pregnancy (Ayoub *et al*., 2015). In this case however, glucose cannot be used for supplementation in diets to improve nutrient degradation as smallholder farmers are resource poor.

The low ruminal supply of urea N for the synthesis of microbial protein reflected the low rumen degradation of dietary CP. Maccarana *et al*. (2016) reported increased ammonia N content with increased crude protein in diet. However, rumen N balance was in excess of 112%, which may not indicate high dietary CP but a reflection of low dietary energy and uncoupled protein to energy ratio hence, N utilization for bacterial protein synthesis was negatively affected. These pre-duodenal losses of nitrogen may constitute up to 30% of ingested nitrogen as noted by Beever and Siddon (1986). The NRC, (2001) recommends 15% dietary CP for first-calf pregnant heifer. Diets had low degradation of N (IVND) indicating inefficiency in rumen fermentation of dietary CP.

In this study, effective CP of less than 5.3% of diets was below the minimum recommendation of 7.5% (Jusoh *et al*., 2014). The microbial protein was not efficiently synthesized in the rumen of the pregnant heifers fed low quality (high fibre) diet as proteolytic bacteria such as *Prevotella ruminicola* and *Clostridium proteoclaticum* resident in the rumen were starved resulting in compromised bacteria growth. High dietary fibre induces increased population of ruminal cellulytic bacteria and methanogens resulting in decreased rumen papillae length (Steele *et al*., 2015). Hence, reduced nutrient absorption into ruminal mucosa and less utilization of diets to support adequate growth and increased body reserves in heifers. Feeding pregnant cows with high concentrate diets especially at prepartum transition period is recommended for rumen microfloral and ruminal mucosa adaptation to high energy density diet post calving (Zebeli *et al*., 2015). However, changes from high fibre diet to high concentrate diet needs to be assessed as stimulation of incidence of acidosis in early lactation has been linked with such changes. In the current study, the diets were low in highly fermentable NSC. This is typical of the communal environment of production requiring a need for an upgrade in an anticipation of improving productivity.

Excess and unbalanced supply of nutrients represents a major cost in production as excess N is converted to urea; about 0.2 Mcal/day was used in urea synthesis. Bauman *et al*. (1980) indicated that overconditioned cows have lower fertility and lower milk production. Although cows in this study gained weight, BCS was below the optimal of 2.5 to 3.5; most averaged 2.5. The fact that estimated energy protein supply was high, the environment, including heat, water stress and other physiological factors, affected degradation, absoprtion and utilization post-absorption.
Supplied and required nutrients for pregnant heirfer

Metabolizable protein (MP) describes the underlying concept in which the required protein content for ruminants at the gastro-intestinal level as well as its availability for animal use (Das *et al*., 2014). The total diet MP represents available protein and has been described as the aggregate of microbial supply (rumen degradable protein (RDP) and dietary supply (rumen undegradable protein (RUP)) and both are considered in accurate and precise prediction of available protein (Das *et al*., 2014) gestation stages were significantly Lower ($p<0.05$) daily concentration and intake of MP, metabolizable energy (ME), and non-fibre carbohydrate (NFC) with their corresponding intake observed in the current study than predicted values may be explained by the high grass hay to concentrate ratio in the heifer diets (73% in HD1 diet for far-off heifers and 67% for close-up heifers) and the negative impact on degradation

The ratio of RDP to RUP in diets were on the high side (>74%) for the observed and predicted values. This may be due to the extreme differences between dietary forage and concentrate as indicated in the diets composition These results were consistent with (Fox *et al*., 2003), who

showed in the Cornell Net Carbohydrate and Protein System (CNCPS) formulations that the contribution of MP from microbes (RDP) should be ≥ 50%. Predicting MP in these diets provides a detail explanation on available energy (ME) for rumen microbial growth (supplied from the end-product of fermentation and fat supplementation) and the net absorption of required amino acids from the small intestine. The low level of CP in the fed diets may explain the lower MP estimates in the observed situation. Besides, MP estimates of 11 % in diet and 743 g/d intake with CP protein of 13.8% (138g/kg DM) are predicted for far-off heifers while MP estimates of 10.4% in diet and 738 g/d with 12.9% (129 g/kg DM) CP in close-up heifers.

According to Das *et al*., (2014), low level of MP in diet results in insufficient microbial protein synthesis and subsequent reduction in available energy (ME) for the animal benefits. Hence, supply of MP in terms of total supply of RDP and RUP sources should be balanced with adequate energy source for better utilization of N by ruminal microbes. This is also to prevent N waste and its negative environmental impact (Kebreab *et al*., 2002). The consumption of HD1 and HD2 by the pregnant heifers at far-off and close-up respectively may possibly result in excretion of N. Predicted ME at both gestation periods were higher than the observed values indicative of a negative ME balance. This could also indicate a low availability of energy from microbial origin and/or dietary source resulting in less synchronization with the available MP for efficient utilization by the animals (Das *et al*., 2014).

3.4.2. Body weight and condition score

Pregnant heifers had increased BW and BCS at far-off and close-up periods. The gain in BW and BCS reflected increased foetal and placetomal weight because of foetal development (Reynolds *et al*., 1990). First-calf heifers are expected to have BW of 3-3.5 of BCS on a 5 point scale and maintained until calving (Gillund *et al*., 2001; Óðinsdóttir, 2009; Heinrichs, 2011). Increased nutrient requirements in the last trimester of gestation for foetal and mammary growth, and increased uterus size, placenta and foetal fluid explained increased metabolizable and the corresponding net energy partitioned for gain recorded in this study.

During far-off and close-up periods, diets supported foetal and mammary growth, maintenance and continued growth as first-calf heifers (NRC, 1989) hence, the increased BW and BCS observed in the heifers. Based on NRC (2000), it was estimated in this study that pregnant heifers had average daily gain (ADG) of 183g/day at far-off period and 85g/day at close-up period. The higher ADG recorded at 60-30 d prepartum (250 days of gestation) was consistent with Reynolds *et al*. (1990) who reported an exponential increase in all the gravid uterine tissues between 100 and 250 days of gestation.

Pregnant heifers had initial relative rate of ADG of 0.6% per day at 60-30 prepartum and 0.3% per day at 29-0 d prepartum period indicating a decreased relative rate of ADG as gestation advances. This result agreed with those reported by Ferrell *et al*. (1976) and Reynolds *et al*. (1990). The reserchers noted decreased percentage per day (relative rate) of foetal growth as gestation advances. Despite decreased per day of relative rate of ADG, absolute rate (kg/d) of ADG increased with increased gestation period due to increased foetal mass. Therefore, increased weight of heifers from 60-30 d prepartum and 29-0 d prepartum was 26.4 and 29.6 kg, respectively. Increased (gain) in BCS has been associated with increased foetal weight as days in gestatation increase and the nutritional status improves due to improved energy balance (Reynolds *et al*., 1990; Damptey *et al*., 2014).

3.4.3. Blood proteins

Diets had low CP content that resulted in low TP concentration. The results showed more impact of low concentration of TP in close-up (29-0 d prepartum) heifers. This may be due to increased nutrient demands for heifers in close-up period. The low dietary crude protein may explain the low circulating albumin levels observed. The concentration of albumin in pregnant heifers was below the reference range of 40 – 44.5 g/L, the corresponding low BW and BCS. Kubkomawa *et al*. (2015) and Agenas *et al*. (2006) indicated the serum concentration of albumin reflects the animal's protein status and its low level indicates inadequate feeding of CP.

High globulin concentration in heifers could also reflect an infection in the heifers' udder including mastitis. Besides, high globulin concentration in the blood may also indicate the level of immunity in animals or immune related diseases and consequential reaction to the presence of antigen; a foreign substance to the body as noted by Kapele *et al*. (2008). Heifers had decreased globulin concentration as gestation progressed. The comparative globulin concentration to normal value observed in the close-up (29-0 d prepartum) heifers is an indication of improved health status in this period. However, this does not equate to good nutritional status as heifers had low mean BCS of 2.45, which was below the 3-3.5 suggested by Gillund *et al*. (2001) and Heinrichs (2011). Pregnant heifers had lower albumin to globulin ratio indicating a low dietary CP. Amanlou *et al*. (2017) indicated that an increase in serum AL concentration and AL: GL ratio was due to feeding higher levels of dietary CP.

Blood urea nitrogen concentration in heifers at both far-off and close-up periods were high. Low dietary energy is indicative of poor nutritional status and high BUN concentration in cows (Chomonyo *et al*., 2002). In this study, diets had low dietary CP hence, low CP intake, resulting in low gain in body reserves. The reason for increased BUN concentration despite low-level CP diets is not very clear. However, inadequate utilization of N in diets resulting from uncoupling of

protein and carbohydrate degradation due to low dietary NSC of diets could explain N loss in form of urea. Feeding heifers with diets high in NSC such as starch could inprove N utilization and consequentially reduce urea in the blood. Damptey *et al*. (2014) showed that decreased concentration of urea in cows is associated with an improved nutritional status.

Concentration of creatinine in the blood has a direct link with muscle breakdown and the level of its excretion at any point in time is affected by muscle mass. The high creatinine concentration indicated its high excretion because of muscle breakdown (Cozzi *et al*., 2011). Metabolic responses to extreme exercise or hot weather conditions related to dehydration considering the environment of production (semi-arid zone) may explain possible breakdown of tissue protein. Hence the high concentration of creatinine observed in the heifers.

3.4.4. Conclusion

Diets supplied to pregnant Jersey heifers at far-off and close-up gestation stages on the smallholder farms had low nutritive value. The low RFV reflected a linear relationship between diet nutrient composition and degradation. The chemical composition of diets influenced their degradation. Although the metabolic protein profile of the pregnant heifers did not show any sign of illness (globulin concentration within the reference value range) at close-up period, poor performance in terms of low BW and BCS was observed. High dietary NDF in diets supplied to pregnant heifers had a negative affect on rumen fermentation and degradation of nutrients.

In this study, the LRNS program in CNCPS model was utilized to determine nutrient supply and requirements for growth and pregnancy of first-calf heifers raised on a zero grazing milk production system. The consistent higher DMI of the pregnant heifers observed compared to expected values might be due to increased nutrient required for pregnancy and continued growth as first-calf heifers. However, high DMI of the heifers did not result in high nutrient intake, as diets were low in structural carbohydrates such as starch. Feeding an energy dense diet to pregnant heifers could improve DMI, nutrient intake, body reserves and reduction of fat mobilization typical to the early lactation period.

Poor management ractices such as irregularities in the qualitative and quantitative supply of diet recipes, feeding time and quantity of diet supplied relative to the climatic condition of the production environment may also explain discrepancies in actual fed and predicted nutrients for the pregnant heifers. Higher DMI as a percentage of BW of observed \was above the recommendations of NRC (2001) for pregnant cows. High rumen fill and rate of passage could be expected in the heifers which resulted in low nutrient degradation and subsequently reduced the available nutrients for the benefits of the animals.

Heifers could not used diets for optimum benefits due to low available energy from microbial origin and/or dietary source as available nutrients are less synchronized for efficient utilization by the animals. The observed nutrient values for pregnant heifers could not supply adequate nitrogen and energy requirements for efficient microbial protein synthesis and amino acids required for foetal growth and mammary development. The high forage intake and forage intake as a percentage of BW than expected, resulted in low ME (due to low energy density in diets) and low gain in body reserves. The results of this study demonstrated that the growth of the heifers was affected by the high composition of structural carbohydrate in diets supplied to heifers at late gestation. However, the marginal increased body weight and condition score of the heifers from far-off to close-up period do not justify increased benefit from the diets. Therefore the hypothesis that the nutrient supply of standardised dairy diets on smallholder farm affects growth of heifers +during pregnancy proved to be true.

CHAPTER 4

EFFECTS OF LOW SUPPLY OF DIETARY NUTRIENTS ON BODY RESERVES AND EARLY LACTATION IN PRIMIPAROUS JERSEY COWS

ABSTRACT

Nutrient intake is the first limiting factor in milk production on communal area dairy farms of the subtropics. Effects of nutrient supply in standardized lactation diets on body reserves and early lactation was evaluated on primiparous Jersey cows raised on communal area smallholder farms. Forty-two primiparous cows, aged 24 to 30 months were used in the study. The study assessed the adequacy of two lactation diets fed to communal area primiparous Jersey cows during early (1-30 d postpartum) and peak (31-60 d postpartum) periods. Cows received 4.5 kg of dairy concentrate with 19.2% CP, 33% NDF and 15.5% ADF which was increased to 5 kg during peak lactation. *Eragrostis curvula* hay (3%, 77% and 48% of CP, NDF and ADF respectively) was supplied *ad libitum* and dry matter intake (DMI) estimated from residual hay. Two simulation diets were prepared based on DMI and assessed for composition and nutrient supply using both wet chemistry and model predictions. Body weight and BCS were monitored, blood was collected and analysed for proteins monthly. A record of milk yield was taken daily, and milk was analysed for fat, protein, lactose and urea nitrogen every week. Energy corrected milk was also calculated. Simulated diets were assessed for nutritive value using chemical analysis, *in vitro* nutrient degradation of diets. Dietary nutrient supply and intestinal absorption were predicted using the Large Ruminant Nutrition System (LRNS) version 1.0.33 (level 1) and cows' responses were validated against predicted values. Hay intake was 7.2 kg of hay/cow/day. Cows had DMI of 11.2 kg which was 12% higher than expected at 1-30 d postpartum period and 11.6 kg which was 21% higher than expected. Diets had low available protein as % of dietary protein (LD1=46%; LD2=45%) and the slowly degraded NDF fraction (NDFICP) constituted 64% of the available protein. Intake of energy was 20% and 17% lower than predicted for Jersey cows at 1-30 d postpartum and 31-60 d postpartum period, respectively. Cows had negative energy balance of -6.5 and -5.6 Mcal respectively at 1-30 d postpartum and 31-60 d postpartum cows. Protein intake of lactating cows was low, resulting in negative protein balance of 59% and 42% of daily requirement respectively, at 1-30 d postpartum period and 31-60 d postpartum period. There was loss of BW and BCS, low milk yield, energy corrected milk (ECM: 9.50 kg/d) and feed efficiency (FE) of less than 1 in cows at both periods. Composition of fat, protein and lactose in milk were affected by the low level of dietary protein. Somatic cell count (SCC) in milk was 121 ± 13 x 10^3/ml and cows did not show signs of illness. Mean MUN concentration was 12 ± 2.7 mg/dl reflecting the protein status and the ensuing performance of the lactating cows. Cows had high

creatinine concentration of 116 and 102 μmol /L at 1-30 d postpartum and 31-61 d postpartum period respectively, which may indicate muscle breakdown due to heat stress relative to the hot production environment. Diets could not supply adequate microbial N to the small intestine due to inefficiency in rumen fermentation of nutrients resulting in negative energy balance (NEB), loss in body reserves (BW and BCS) and poor lactation of cows at both lactation periods.

Key words: Dietary nutrients, body reserves, early lactation, primiparous cow

4.1. Introduction

The process of lactation commences after calving and the lactating cow may or may not continue suckling the calf in addition to milking. Lactating cows are usually grouped into stages; early lactation (0-100 d postpartum), mid lactation (101-200 d postpartum) and late lactation (201-300 d postpartum). The fresh lactation period can further be distinguished from early lactation as the first 4 weeks after calving (that is 1-30 d postpartum). Cows are recovering from calving during this transitional period and demand for increased intake of a nutrient-dense diet formulated for the maintenance of good BW and BCS, and lactation remains high (NRC, 2001). The amount and nutritive value of the diet fed to cows at each lactation stage influence their performance. This is due to variation in nutrient demands of dairy cows at different stages of lactation. Early lactation period is marked with progressive increase in milk production, onset of peak milk production with loss in BW (Grummer, 1995; Agenas *et al*., 2003) as energy supply lags behind demand. Hence, the state of NEB in cows at this stage of lactation (Hayirli *et al*., 2002). The effect of NEB depends on level of milk production, prepartum body reserves, diet composition and the resulting feed intake and energy demand for further growth such as in first-calf lactating cows (Grummer, 1995; Hayirili *et al*., 2002). The state of NEB in lactating cows results in high fat content and increased urea concentration in milk (Keady *et al*., 2000; Agenas *et al*., 2003; Oõinsdóttiir, 2009) and can be corrected with improved diet quality through increased energy balance (Busato *et al*., 2002). Ensuring adequate body reserves during late gestation and feeding of adequate energy diet that boosts dry matter intake in early lactation seems logical to reducing the level and the length of time of negative energy commonly identified with fresh period.

Rumen degradable protein enhances rumen microbial growth and efficient microbial protein synthesis for maintenance, growth and lactation of the host animal (Das *et al*., 2014; Tedeschi *et al*., 2015). Improvement in fibre digestion results from the deamination of amino acids and availability of the branched-chain VFA that stimulate the growth of fibre digesting bacteria (cellulolytic) and the corresponding enzyme activity (Eugene *et al*., 2004).

Impacts of feeding low concentrate diets to lactating cows on milk yield and composition has been well researched. Feeding dietary CP of 12% depresses nutrient digestibility and ruminal microbial protein synthesis crucial to optimal lactation performance (Aschemann *et al*., 2012). Improvement of amino acid profile increased dietary fermentable carbohydrate content and decreased feeding of excess dietary protein increase milk yield and protein content (Das *et al*. 2014). Inadequate dietary energy in dairy nutrition results in a NEB inducing mobilization of fatty acids from adipose tissue, amino acids from muscle tissues and a high level of circulating non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) in the animal's body (Oõinsdóttiir, 2009).

Lactating cows raised in communal areas of South Africa fed on high forage-based diets with little or no concentrate supplementation results in low productivity in this sector. Diets are limited in supplying required nutrients for lactation and little information is known about the supply of dietary nutrients to dairy cows and the effect on the lactation performance. Therefore, the objective of this study was to evaluate the effect of dietary nutrient supply on changes in body reserves and early lactation in primiparous Jersey cows. **The hypothesis tested was that** low supply of dietary nutrients affects body reserves and early lactation in primiparous Jersey cows.

4.2. Materials and methods

The ethics committees of the University of South Africa (2015/CAES/007) and Agricultural Research Council (APIEC16/023) approved the ethical clearance for the animal experimental procedures. The animals were managed according to the standard guidelines established by ARC for care and use of animals during rumen fluid collection for the *in vitro* ruminal fermentation procedure.

4.2.1. Study area

The study on lactating Jersey cows was conducted in two communal areas (Vhembe and Sekhukhune) in Limpopo province. Forty-two lactating Jersey cows (age: 24-30 months) were used in the evaluation of early lactation performanc. The same geographical location of the production site for pregnant heifers (chapter 3, section 3.2.1) was used.

4.2.2. Study design

Forty-two pregnant lactating cows selected from 10 households were used in the study during 1-60 days post-calving. A standard feeding regime for lactating cows in both Sekhukhune and Vhembe districts was applied by farmers. Diets were defined as LD1 and LD2 based on lactation periods, on grass hay and on concebtrate (commercial lactation diet) supplied to the cows (Table 4.1) Nutrient composition of diets was determined based on predictions and recommendation of NRC (2001).

Table 4.1 - Ingredients of LD1 and LD2 diets for lactating cows

Diet ingredients (g/kg DM)	Feeding regime	
	[1]LD1	[2]LD2
Concentrate (Commercial lactation diet)	400	430
Weeping love grass hay (*Eragrostis curvula*)	600	570

[1]LD1 = Lactation diet for 1-30 d postpartum cows; [2]LD1 = Lactation diet for 31-60 d postpartum cows

4.2.3. Chemical analyses

Concentrate, grass hay and diets (LD1 and LD2) were analyzed for chemical composition following the described procedures in Chapter 3 section 3.2.3 and the estimated values were used in calculating the nutrient composition. The chemical and nutriet composition are presented in Table 4.3.

4.2.4. *In vitro* ruminal nutrient degradability of lactation diets

Milled samples of formulated LD1 and LD2 for lactating cows were weighed (0.5 ± 0.05 g) into separate pre-weighed and labelled F57 ANKOM bags, re-weighed and heat sealed. Inoculum preparation and procedures for incubation, rinsing and drying for *in vitro* ruminal DM, N and OM degradation were as described in chapter 3 section 3.2.6.

4.2.5. *In vitro* ruminal gas production and fermentation kinetics of lactation diets

Lactation diets (LD1 and LD2) were evaluated for total gas production and fermentation kinetics in an ANKOM^{RF} gas production system (ANKOM Technology®, Macedon, NY, USA). Fermentation kinetics evaluated include; the rate of gas production, potential and effective gas production. The gas production and determination of fermentation kinetics of the LD1 and LD2 was done using the same procedures in chapter 3 section 3.2.7. Data generated for total gas production was fitted into the exponential Model of Ørskov and McDonald (1 979) to determine fermentation kinetics of diets.

4.2.6. Evaluation of required and supplied nutrients in diets for lactating cows

The nutrient supplied and requirements of lactating cows at 1-30 d postpartum and 31-60 d postpartum periods were estimated using the Large Ruminant Nutrition System (LRNS) program (version 1.0.33) in Cornell Net Carbohydrate and Protein System (CNCPS) Model (Fox *et al*., 2004). Data on nutrient composition of diets were imputted into the solution level 1 of the model. The feed libraries of the system were edited to accommodate ingredients and composition of the

newly simulated LD1 and LD2 diets. A cow has mature weight of 450 kg; mean BW = 360 kg; BCS = 2.4; early milk production = 9.3 kg/day; peak milk production = 10 kg; milk fat = 3.5% and milk protein = 3.2%. The expected (required) and observed (actual supply from diets) concentration (%/DM) and intake of metabolizable nutrients and the corresponding balances for lactation, maintenance and BW changes were also predicted.

4.2.7. Evaluation of the performance of lactating Jersey cow

4.2.7.1. Body weighing and condition scoring

The linear measurements of body length, heart girth, wither girth and wither height were measured as described in section 3.2.8.1. Animals were condition scored following the method of Klopcic *et al*. (2011) on 5 points scale where 1 signifies very thin and 5 for very fat cow.

4.2.7.2. Milk production record and sampling

Cows were milked twice a day at 08:00 and 15:00 hour. Milk samples were pooled for morning and evening production once a month and analyzed for protein, fat, lactose, milk urea nitrogen (MUN) and somatic count (SCC). Milk samples were analysed for composition using FOSS CombiFoss™ FT+ electronic instrumentation (Foss Alle 1 DK-3400 Hilleroed, Denmark). The instrument consists of MILKoScan™ FT+ and Fossomatic™ FC analyzer. Milk protein, fat and lactose were analysed using MILKoScan™ FT120.

Milk composition was determined using the measuring principle of Fourier which transforms infra-red spectrophotometer measuring milk composition through infrared light. Fossomatic™ FC was used for the automatic counting of somatic cells in milk samples. The milk sample was first mixed with a fluorescent dye which dyed DNA molecules in somatic cells. The sample was afterwards passed under a counting unit where it is exposed to blue light. Cells emitted red light making them feasible for the instrument to count. Milk components are expressed in percentage of the milk sample.

Feed efficiency (FE) was also determined by measuring the amount of milk produced (kg) per kg of dry matter consumed by cows. These parameters were calculated according to Heins *et al*., (2008) as follow;

ECM (kg) = 0.327 x milk yield (kg) + 12.96 x fat (kg) + 7.2 x protein (kg)

Where ECM= Energy corrected milk

FE = ECM/DMI

Where FE = feed efficiency; ECM= energy corrected milk; DMI = dry matter intake

4.2.7.3. Evaluation of blood protein

Sampling of blood from the lactating cows was done monthly (i.e twice from day 1 to 60 days postpartum). Blood samples were analyzed following the described procedures in Chapter 3, section 3.2.7.2) to determine concentration of total protein (TP), blood urea nitrogen (BUN), albumin (AL), globulin (GL) and creatinine (CR) in cows.

4.2.8. Statistical analyses

Data on the *in vitro* evaluation of lactation diets and cow performance 1-30 d postpartum and 31-60 d postpartum periods were analyzed in a completely randomized design. Data on the ruminal fermentation, BW and BCS, milk yield and composition, and blood parameters were subjected to analysis of variance (ANOVA) procedures in statistical analytical systems (SAS) Software package (version 9.3: SAS Institute, Inc., Cary NC, USA) to assess variation within animals and between units of all tested parameters. Means were separated using Fischer's test. Tests were performed at 95% confidence limit (declared significance at $p \leq 0.05$). The results were presented as means and standard deviation (mean ± STD). Observed and predicted diet nutrient concentrations and intake were compared using the paired t-test.

4.3. Results

4.3.1. Nutrient supply of LD1 and LD2 diets for lactating cows

Table 4.2 chemical and nutrient composition of concentrate, grass hay and total diets (LD1 and LD2). Diets had low RFV reflecting high level of unavailable carbohydrates (CC) of 18 % DM from the grass hay component in diet. Lactation diet (LD1) had low RFV of 125.8. However, the value improved in LD2 diet to 156 and was above the minimum recommended for ruminants of 144. Dry matter and nitrogen degradability were 49.4% and 55.9, respectively (Figure 4.3-1). However, 76 and 91% of the observed DM and N degradability, respectively, degraded within 18 h of incubation. Total gas production and fermentation kinetics of LD1 diet after 36 h of incubation were presented in Table 4.3. The gas production from the slowly fermented fraction (b) was 62.8 mL/g DM. Diet had *GPp* of 67 mL/g DM and *GPe* was 47 mL/g DM. At 48 h, the estimated *GPp* and *GPe* were 78 and 62 ml/g, respectively.

Table 4.2 – Chemical and nutrient composition of concentrate, grass hay, LD1 and LD2 diets

Parameters	*Concentrate	**Grass hay	LD1 diet	LD2 diet
Dry matter	95.8	93.1	91.3	93.4
Organic matter	92.0	92.8	92.7	92.5
Calcium	8.21	0.37	0.71	0.72
Phosphorus	5.5	0.16	0.38	0.30
Crude protein	19.2	3	13.6	14.2
Fat	2.6	1.2	2.01	2.6
Ash	8	7.2	7.3	7.5
Structural proteins and carbohydrates				
Neutral detergent fibre	33.0	77.0	57.6	54.7
Acid detergent fibre	15.5	48.9	34	33.4
Acid detergent lignin	7.00	21.3	9.6	7.3
Cellulose	8.50	27.6	24.4	26.1
Hemicellulose	26.0	55.7	48.01	47.4
Lignin	5.10	37.7	19.6	18.3
ADFICP (% CP)	1.06	5.02	3.44	3.21
NDFICP (% CP)	1.44	5.4	3.82	3.59
IADICP (% DM)	0.42	2.01	1.37	2.48
NDF_n (% DM)]	31.1	69.6	52.4	49.8
Non- structural carbohydrates				
Non-fibre carbohydrate (% DM)	39.2	11.6	19.5	21
Available carbohydrate (B2) (% DM)	23.5	20.5	45.5	44.9
Indigestible carbohydrate (C) (% DM)	16.9	51.1	23.1	17.5
Unavailable carbohydrate (CC) (% DM)	5.60	39.4	13.3	9.6
TDN_{1x} (% DM)	57.9	37.8	52.4	53.9
dTDN (% DM)	55.7	37.8	51.2	52.6
Digestible energy (Mcal/kg)	2.5	1.3	2.3	2.3
Metabolizable energy (Mcal/kg) for lactation	2.0	0.8	1.8	1.9
Metabolizable energy (Mcal/kg) for growth	2.0	1.0	1.8	1.9
NEm (Mcal/kg)	2.2	0.6	1.0	1.1
NEg (Mcal/kg)	0.5	0.3	1.0	1.1
Digestible dry matter (% DM)	76.8	50.8	62.4	62.9
Expected dry matter intake (% BW)	3.6	1.6	2.6	3.2
Relative feed value	216.6	61.4	125.8	156.0

***Concentrate**- Commercial lactation diet; ****Grass hay** - *Eragrotis curvula*; **LD1** - Diet for 1-30 d postpartum cows; **LD2** - Diet for 31-60 d postpartum; **ADFICP** – Acid detergent fibre indigestible crude protein; **NDFICP** – Neutral detergent fibre indigestible crude protein; **IADFICP** - Indigestible acid detergent fibre insoluble crude protein; **NDFn** – Neutral detergent fibre corrected for nitrogen; **TDN_{1x}** - Total digestible nutrients on intake at maintenance level; **dTDN** - Digestible total digestible nutrients; **NEm** - Net energy maintenance**; NEg** - Net energy gain**.**

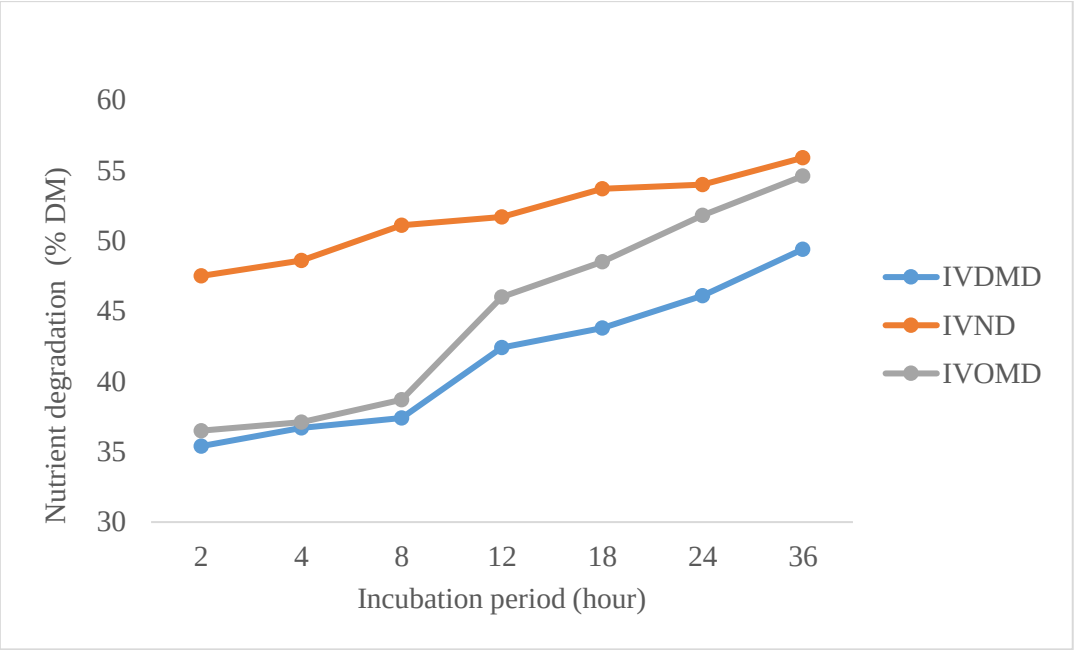

Figure 4.3-1 - In vitro dry matter degradability, in vitro nitrogen degradability and in vitro organic matter degradability (% DM) of LD1 diet

Table 4.3 -Total gas production and fermentation kinetics of LD1 diet at 36 h of incubation

Parameters	*In vitro* gas production and fermentation kinetics of LD1 diet
Total gas production	63.7
[1]a	4.04
[2]b	62.8
[3]*GPp*	66.9
[4]c (% in 18 h)	6.6
[5]*GPe*	47.3

[1]a = Gas produced from the immediate soluble fraction; [2]b = Gas produced from slowly degraded fraction; [3]*GPp* = Potential gas production; [4]c = Rate of gas production within 18 h; [5]*GPe* = Effective gas production.

The estimated values for the *IVDMD*, *IVND* and *IVOMD* of LD2 diet are depicted in Figure 4.3-2. Dry matter, nitrogen and organic matter degradability of diet was 55.2, 65.4 and 60.7%, respectively. Diets had over 94% of observed degradability at 48 h. Total gas production and fermentation kinetics of diet are presented in Table 4.5. Total gas production was 65.1 mL/g DM following 36 h of incubation. Potential gas production and *GPe* recorded was 68.9 and 49.6 mL/g DM respectively. The estimated *GPp* and *GPe* at 48 h was 80 and 65 mL/g respectively. The rate (c) within 18 hours was 7% /hours and declined as incubation progresses.

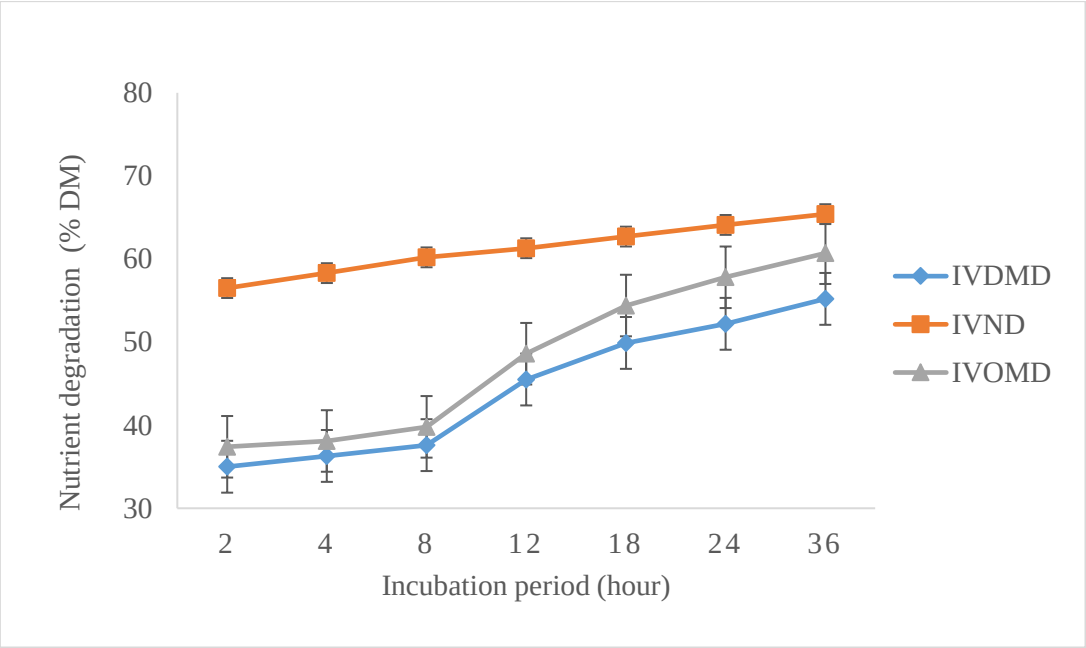

Figure 4.3-2 - In vitro dry matter degradability, in vitro nitrogen degradability and in vitro organic matter degradability (% DM) of LD2 diet.

Table 4.4 - Total gas production and fermentation kinetics of LD2 diet

Parameters	*In vitro* gas production and fermentation kinetics of LD2 diet
Total gas production	65.1
[1]a	4.36
[2]b	64.6
[3]*GPp*	68.9
[4]c (%/hour)	7
[5]*GPe*	49.6

[1]a = initial gas production at first hour of incubation; [2]b = gas produced from slowly degraded substrates; [3]*GPp*= potential gas production; [4]c = rate of gas production; [5]*GPe* = effective gas production.

4.3.1. Supplied and predicted nutrients in LD1 and LD2 diet

Nutrient concentration (% in diet) observed and expected in LD1 diet are shown in Table 4.5. Observed concentration of metabolizable protein (MP = 5.7%), rumen degradable protein (RDP = 4.28%) and rumen undegradable protein (RUP = 1.43%), metabolizable energy (ME =1.9%) and non-fibre carbohydrate (NFC =19.5%) were consistently lower ($p<0.01$) in LD1 than expected MP = 10.9%, RDP = 8.2%, RUP = 2.7%, ME = 2.73% and NFC = 33%. Conversely,

concentration of neutral detergent fibre (NDF = 57.6%); total forage in ration (TFR = 68%) and; forage NDF (fNDF = 78%) observed were higher than expected NDF = 50.1%, TFR = 60.1% and fNDF = 68.9%).

Table 4.6 indicates observed and expected daily nutrient intake (kg/cow) of LD1 diet for cows at 1-30 d postpartum period. A higher ($p<0.0001$) intake of CP (1523 g/cow/day) than expected value (214 g/cow/day) was observed. A daily DMI of 11.2 kg/cow was observed in cows and higher than expected (9.9 kg/cow). A lower ($p>0.05$) DMI as a percentage of BW (2.6) was observed compared to the expected value of 2.7. A higher ($p>0.05$) forage intake expressed as a percentage of BW (1.8) in cows compared to expected value of 1.6. Mean daily intake of MP (638 g), RDP (478.5 g), RUP (159.5 g) and ME (21.5 Mcal) per cow were lower ($p<0.05$) than expected (MP = 1084 g; RDP = 813 g; RUP = 271 g; ME = 27 Mcal). Intake of NDF (57.6 % in diet) was higher ($p<0.05$) than expected (50.1%).

Table 4.5 - Comparison of observed and expected nutrient concentration in LD1 diet

	Nutrient concentration of [1]LD1 diet		
Parameters (%)	Observed	Expected	*p*-value
Crude protein	13.6	13.1	0.33
Metabolizable protein	5.7	10.9	0.004
Rumen degradable protein	4.28	8.2	0.001
Rumen undegradable protein	1.43	2.7	0.002
Metabolizable energy (MCal)	1.9	2.73	0.01
Neutral detergent fibre	57.6	50.1	0.001
Total forage in ration	68	60.1	0.002
Forage [4]NDF	78	68.9	0.03
Non-fibre carbohydrates	19.5	33	0.001

[1]LD1= Lactating diet for cows at 1-30 d postpartum period

Table 4.6 - Comparison of the observed and expected daily nutrient intake of LD1 diet

Nutrient intake of 1-30 d postpartum cows			
Parameters	Observed	Expected	*p*-value
Dry matter intake (kg /d)	11.2	9.9	0.09
Dry matter intake (% body weight)	2.6	2.7	0.08
Crude protein (g/d)	1523	1214	0.04
Metabolizable protein (g/d)	638	1084	0.0001
Rumen degradable protein (g/d)	478.5	813	0.0001
Rumen undegradable protein (g/d)	159.5	271	0.0001
Metabolizable energy (Mcal/day)	21.5	27	0.04
Neutral detergent fibre (kg/d)	5.76	5.01	0.02
Forage (% of body weight)	1.8	1.6	0.06

Table 4.7 represent estimates of observed and expected nutrient concentration in LD2 diet at 31-60 d postpartum period. There are variations in the concentration of the evaluated nutrients in the observed and expected (as required) by lactating cows at this stage of lactation. The concentration of CP (14.2%) in diet was observed and lower ($p<0.013$) than expected (15.5%). The concentration of MP (5.7%), RDP (4.31%), RUP (1.44%), ME (2.0 Mcal) and NFC (21%) observed in diet were lower ($p<0.05$) than expected values of MP (12.43%), RDP (9.32%), RUP (3.11%) and ME (3.0 Mcal) and NFC (38%). The results also showed a higher ($p<0.05$) fibre concentration in the observed values of NDF (54.7%), TFR (66%) and fNDF (79.2%) as compared to the predicted values of NDF (42.9%), TFR (52.3%), and fNDF (62.3%).

Table 4.8 shows observed and expected daily nutrient intake of LD2 diet for the lactating cows at 31-60 postpartum period. Observed intake of CP (1647 g/d), DMI (11.6 kg/d) and DMI as percentage of BW (3.2) were significantly higher ($p<0.05$) than expected values (CP = 1428 g/d; DMI = 9.2 kg/d; DMI as a percentage of BW (2.5). Equally, mean daily intake of MP (667 g), RDP (500 g), RUP (187 g) and ME (23.2 Mcal) as observed for cows at this lactation stage were significantly lower ($p<0.05$) than expected intake values (MP = 1144 g; RDP = 857 g; RUP = 286 g; ME = 28.1 Mcal). A higher ($p<0.05$) intake of NDF (5.5 kg) and forage intake as a percentage of BW (1.7) in LD2 diet were observed compared to expected (NDF = 4.3 kg; forage as a percentage of BW = 1.4).

Table 4.7 - Comparison of observed and expected nutrient concentration (%) in LD2 diet.

Parameters	Nutrient concentration of [1]LD2 diet		
	Observed	Expected	*p*-value
Crude protein	14.2	15.5	0.06
Metabolizable protein	5.75	12.42	0.003
Rumen degradable protein	4.31	9.32	0.002
Rumen undegradable protein	1.44	3.11	0.0001
Metabolizable energy (MCal)	2.0	3.05	0.0001
Neutral detergent fibre	54.7	42.9	0.0001
Total forage in ration	66	52.3	0.001
Forage [2]NDF (% of diet NDF)	79.2	62.3	0.03
Non-fibre carbohydrates	21	38	0.001

Table 4.8 - Comparison of observed and expected daily nutrient intake of cows at 31-60 d postpartum period

Parameters	Nutrient intake of [1]LD2 diet		
	Observed	Expected	*p*-value
Dry matter intake (kg[2]DM)	11,6	9.2	0.02
Dry matter intake (% body weight) (kg)	3.2	2.5	0.01
Crude protein (g)	1647	1428	0.0001
Metabolizable protein (g)	667	1143	0.0001
Rumen degradable protein (g)	500	857.	0.0001
Rumen undegradable protein (g)	167	286	0.0001
Metabolizable energy (MCal/day)	23.2	28.1	0.01
Neutral detergent fibre (kg)	5.5	4.3	0.014
Forage (% of body weight)	1.7	1.4	0.01

LD2= lactation diet for 31-60 d postpartum cows; [2]DM = dry matter

4.3.2. Energy and protein balance of lactating cows

The calculated daily energy supplied (available) and requirement for lactating cows at 1-30 d postpartum period are shown in Table 4.9. Differences were found between supplied and required protein and energy for the cows. A lower ($p<0.04$) daily energy intake of 20.5 Mcal/cow was observed compared to daily energy requirement of 27 Mcal/cow. This resulted in negative daily balance of -6.5 Mcal/cow of energy requirements. Similarly, the estimates of daily protein supplied (638g) to cows was significantly lower ($p<0.001$) than requirement (1084 g) resulting in high negative protein balance of -446g/d and a supply of only 59% of protein requirements.

However, NRC (2001) recommends 110 and 125% minimum requirement of ME and MP respectively for lactating cows.

Table 4.9 - Mean daily energy and protein balance in 1-30 d postpartum cows

Parameters	Energy and protein balance in 1-30 postpartum cows				
	Required	Supplied	Balance	Supplied (% of requirement)	*% recommended
Energy (Mcal/d)	27.0	20.5	-6.7	80	133 (NRC, 2001)
Protein (g/d)	1084	638	-446	59	125 (NRC, 2001)

*Minimum requirement

Daily required and supplied energy and protein for 31-60 d postpartum cows are depicted in Table 4.10. Cows had lower ($p<0.01$) daily energy supplied of 23.2 Mcal/cow than requirement (28.1 Mcal). Difference was found in supplied and required energy which resulted in 17.4 % negative balance (-5.6 Mcal) of daily requirements. Energy supply constituted 83% of the requirement and lower than NRC recommendation. Mean daily protein supplied (667 g/cow) at this lactation period was lower ($p<0.001$) than the required 1143g/cow. Cows fed LD2 diet had high negative balance of -476 g constituting 42% of daily protein requirement of lactating cows.

Table 4.10 - Mean energy and protein balance in cows at 31-60 d postpartum period

Parameters	Energy and protein balance in 31-60 d postpartum cows				
	Required	Supplied	Balance	Supply (% of requirement)	*% recommended
Energy (Mcal/d)	28.1	23.2	-4.9	83	171
Protein (g/d)	1143	667	-476	58	110

4.3.3. Performance of lactating cows

Table 4.11 indicates mean BW and BCS of lactating cows at 1-30 d postpartum period. Cows lost 2.6 kg of BW and 0.23 unit of BCS. Fat and protein contributed 3.95 and 20 kg respectively to body condition loss. It was estimated that 1-30 d postpartum cows would mobilize 149 Mcal of energy reserves within this period to lose 0.23 unit of body condition.

Figure 4.3-2 shows serum concentration of TP, AL, GL, CR and BUN in lactating cows at 1-30 d postpartum period. Mean concentration of TP was 72 g/L; below the reference range of 76.3-93.7 g/L. Concentration of AL and GL was 34 g/L and 37.1 g/L, respectively, and were below the

reference value range. Cows had lower ratio of AL: GL (0.95) indicating a higher GL concentration than AL. However, the ratio was within the reference value range of 0.8 - 1.0. Blood urea nitrogen in cows was 4.4 mmol/L) and within reference value of 4.8 mmol/L. Cows had CR concentration of 115.5 µmol/Lwhich was higher than the mean reference value of 99.7 µmol/L.

Mean milk yield and composition of lactating cows are presented in Table 4.12. Mean milk production for cows at 1-30 d postpartum period was 9.3 ± 2.1 kg/cow. The fat, protein and lactose content of milk produced was 3.5 ± 0.4, 3.2 ± 0.41 and 4.2 ± 0.3 % respectively. The fat, protein and lactose composition of the milk were lower than the reference values for Jersey cow. Somatic cell count (SCC) in milk and MUN concentration was 121 ± 13 x 10^3/ml and 12 ± 2.7 mg/dl respectively. Calculated energy corrected milk and feed efficiency in 1-30 d postpartum cows was 9.5 and 0.85 respectively.

Table 4.11 - Mean body weight and condition score of cows at 1-30 d postpartum period

Parameter	1-30 d postpartum cows
Body weight (kg)	356 ± 20
[1]ΔBW (kg)	-2.6
Body condition score	2.33 ± 0.64
[2]ΔBCS	-0.23
Body fat reserves mobilized (kg)	3.95
Body protein reserves mobilized (kg)	20
Body energy reserves mobilized (Mcal)	149

[1]ΔBW = Change in body weight; [2]ΔBCS= Change in body condition score.

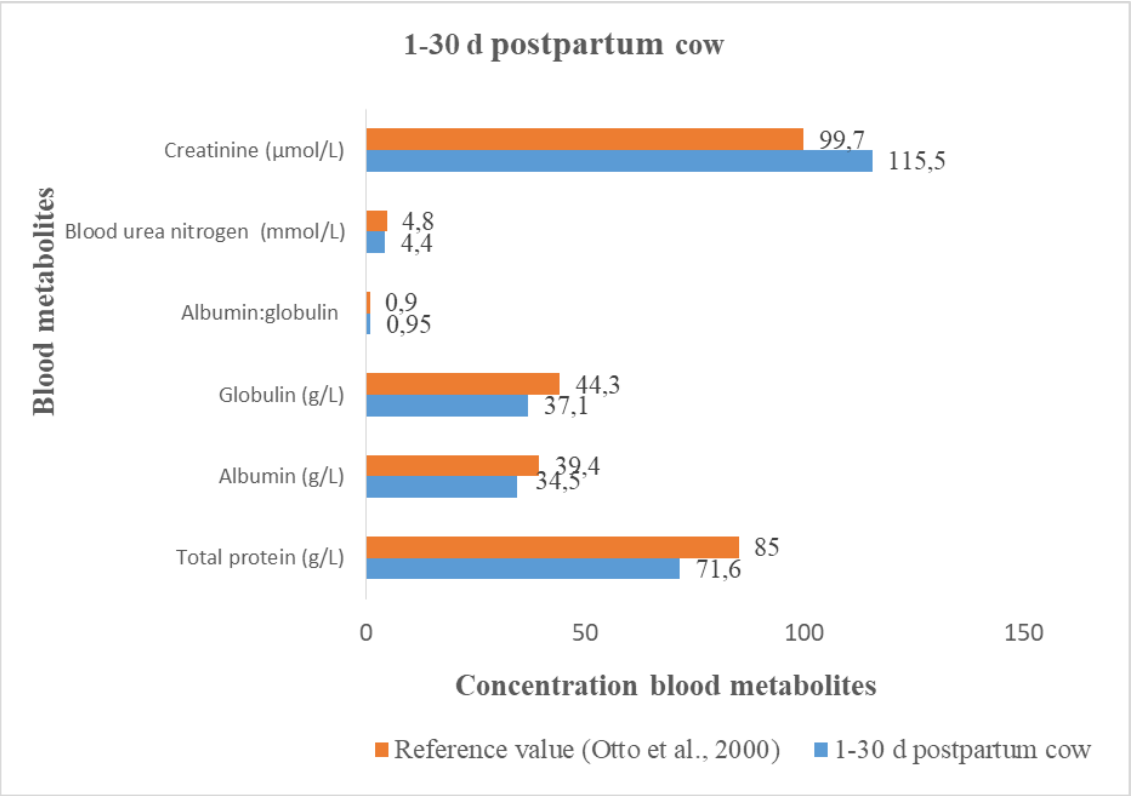

Figure 4.3-3 - *Serum concentration of proteins of 1-30 d postpartum Jersey cows. Reference value range (Otto et al., 2000): TP = 76.3 - 93.7 g/L; AL = 36.4 - 42.2 g/L; GL = 39.7 - 48.9 g/L; AL: GL = 0.9 - 1.2; BUN = 4.2 - 5.4 mmol/L; CR = 83 - 116.2 µmol/L.*

Table 4.12 - Mean daily milk yield, milk composition and production efficiency of cows at 1-30 d postpartum

Parameters	LD1 diet for 1-30 d postpartum cows
Dry matter intake (kg)	11.2
Milk yield (kg)	9.30
Fat (kg)	0.33
Protein (kg)	0.28
Energy corrected milk	9.50
Production efficiency (*ECM/DMI)	0.85
Milk composition	
Fat (%)	3.5 ± 0.4
Protein (%)	3.2 ± 0.41
Lactose (%)	4.2 ± 0.3
Somatic cell count (x10^3 cells/mL)	121 ± 13
Milk urea nitrogen (mg/dl)	12 ± 2.7

*ECM/DMI = Energy corrected milk; DMI = Dry matter intake

Body weight and BCS of lactating cows at 31-60 d postpartum period are presented in Table 4.13. Mean BW and BCS was 349 ± 27 kg/cow and 2.31 ± 0.72 respectively. Cows had loss of 7.13 kg in BW and 0.073 in the unit of BCS. Depletion of body reserves constituted 1.57 and 79 kg of fat and protein respectively. An estimate of 440 Mcal of energy was mobilized from energy reserves for the period.

Serum concentration of TP, AL, GL, BUN and CR of cows in 31-60 d postpartum are shown in Figure 4.3-3 Mean concentration of TP in 31-60 d postpartum period was 67 g/L. Concentration of albumin and globulin was 35g/L and 37g/L respectively. However, these values were below reference range for lactating cows. Albumin and globulin ratio of 0.9 was within the reference range of 0.8-1.0. Blood urea nitrogen concentration (4.6 mmol/L) was within the reference value range. Concentration of creatinine (102.4 μmol/L) was at the high end of reference range.

Table 4.14 represents mean daily milk yield and composition and production efficiency of cows at 31-60 d postpartum period. Mean milk production was 10.2 ± 2.4 kg/cow. The composition of fat and protein in milk was 3.5 and 3.0% respectively. The composition of lactose in milk was 4.4% and somatic cell count (SCC) in milk was 97 ± 11 x 10^3/L. Milk urea nitrogen content was 10 ± 3 mg/dl during this period. The calculated energy corrected milk was 10.3 and production efficiency was less than 1 (0.89).

Table 4.13 Body weight and condition score of cows 31-60 d postpartum

Parameter	31-60 d postpartum cows
[1]BW (kg)	349 ± 27
[2]ΔBW (kg)	-7.13
[3]BCS	2.31 ± 0.72
[4]ΔBCS	-0.073
Body fat reserves mobilized (kg)	1.57
Body protein reserves mobilized (kg)	79
Body energy reserves mobilized (Mcal)	440

[1]ΔBW = change in body weight; [2]ΔBW = change in body condition score body.

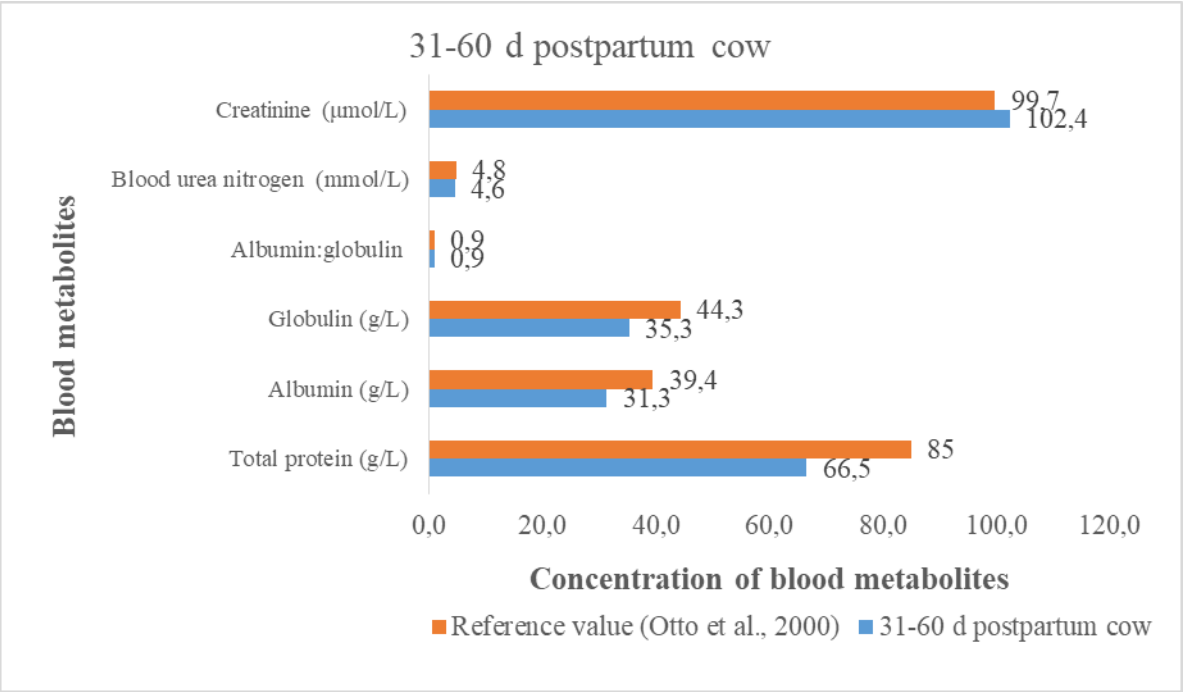

Figure 4.3-4 - *Serum concentration proteins of cows at 31-60 d postpartum period. Reference value range (Otto et al., 2000): TP = 76.3 - 93.7 g/L; AL = 36.4 - 42.2 g/L; GL = 39.7 - 48.9 g/L; AL: GL = 0.9 - 1.2; BUN = 4.2 - 5.4 mmol/L; CR = 83 - 116.2 µmol/L*

Table 4.14 - Mean milk yield, milk composition and production efficiency of cows 31-60 d postpartum

Parameters	LD1 diet 1-30 d postpartum period
Dry matter intake (kg)	11.6
Milk yield (kg)	10.2
Fat (kg/d)	0.36
Protein (kg/d)	0.31
Energy corrected milk	10.3
Production efficiency (*ECM/DMI)	0.89
Milk composition	
Fat (%)	3.5 ± 0.3
Protein (%)	3.0 ± 0.27
Lactose (%)	4.4 ± 0.4
Somatic cell count (x10^3 cells/mL)	97 ± 11
Milk urea nitrogen (mg/dl)	10 ± 3,

*ECM/DMI = ECM = Energy corrected milk; DMI = Dry matter intak

4.4. Discussion

4.4.1. Nutrient supply of LD1 and LD2 diets for lactating cows

The low DMD of lactating diets could be attributed to poor digestion of the highly lignified hay that formed 55% of LD1 and LD2 diets. Dietary forage content was within the range specified by Fellner (2002) although, on the high end. Lignification of plant tissue increases with plant maturity to enhance rigidity; however, the lignified structural carbohydrate components become less accessible (Nagadi *et al*., 2000). The NRC recommends 25-28% dietary NDF for lactating cows and 19% dietary NDF for 31-60 d postpartum lactation concentrate. In this study, diets were about 30 units higher than the NRC (2001) recommended levels. This implies that the proportion of slowly degradable carbohydrate fraction (B2) was high and non-structural carbohydrate such as starch and soluble organic fractions were low.

High SC and low NSC entails that proportions of propionate (C3 precursor for glucose production) would be lower relative to acetate (C_2) and butyrate (C_4) which are fat precursors. Glucose is the main precursor for lactose synthesis. The diets could not favour higher milk production since lactose content drives milk yield. The slowly degraded fraction plays a critical role as it induces longer mastication, which enables fractionation of the structural carbohydrate matrix, exposing sites for microbial attachment (Beauchemin, 2018), formation of biofilms and finally degradation by fibrolytic enzymes. Mastication improves saliva flow and rumen buffering as noted by van Soest (1991).

Optimum pH for rumen fermentation ranges between 5.7 and 7.2, as demonstrated in this study, promote the growth of both structural carbohydrates bacteria such as *Ruminococcus albus*, *Ruminococcus flavefasciens, Bacterioride saccinogene* and non-structural carbohydrate digesters such as *Bacteriodes ruminocola*, *Bacteriodes amylophilus*, *Streptococcus bovis* and *Succinomonas amylolytica* (Russell & Wilson, 1996). Higher degradability of dietary fibre was expected, however, the results were contrary. As noted above, complex lignification of the hay cell wall matrix and the physical characteristic of the forage fraction could have influenced rumen degradation. Leng (2014) reported that lignification reduces rates of tissue sloughing by planktonic bacteria at primary colonization of plant materials and hydrolytic bacteria and penetration by fungal zoospores lead to degradation of feed particle. Byskov *et al*. (2015) noted that rumination time varied with fibre source (hay vs silage).

The inaccessible and indigestible carbohydrate constituted 36.4% representing a high fraction in energy loss as methane (Cabezas-Garcia, 2017; Moss *et al*., 2000). Methanogens such as *Methanobrevibacter ruminantium* use hydrogen (H2) or formate with carbon dioxide (CO2) as the main substrates (McAllister *et al*., 1996). Non-structural carbohydrate bacteria produce N

compounds, which are essential for growth of SC bacteria. Aldrich *et al*. (1993) reported that 36% NSC improved bacterial N flow. Hoover *et al*. (1991) reported that 37% NSC supplied adequate energy for rumen microbial growth. The poor DMD could be linked to N starvation of SC bacteria due to insufficient dietary NSC (Chalupa *et al*., 1996; Russell *et al*., 1992). In this study, low DM degradability explains insufficient supply of energy from diets for microbial growth and the associated low flow of microbial N to the lower gut. Feeding primiparous cows with diets adequate in NSC is beneficial, as first-calf heifers require higher nutrient intake to attain mature growth and developing foetus (Coffey *et al*., 2006). Heifers usually calf when they are 85% mature (NRC, 1989).

It is important to feed glucogenic-rich diet such as starch and sucrose during early lactation as it increases the supply of nutrients for fibre digesting bacteria and decreased concentration of plasma NEFA and BHB (Pickett *et al*., 2003; Reist *et al*., 2002). Increased concentration of plasma NEFA and BHB is associated with NEB and results in increased incidences of diseases associated with peripartum period, decreased milk yield and poor fertility (Esposito *et al*., 2014; McArt *et al*., 2013; Seifi *et al*., 2011). Diets were poorly degraded and could not supply adequate microbial N to the small intestine which resulted in NEB, loss in body reserves (BW and BCS) and the overall poor lactation of cows in 1-30 d and 31-60 d postpartum lactation periods.

The low rumen degradation noted in this study entails poor outflow of rumen bacteria, and limited lower gut nutrient absorption. Metabolism in early lactation is hormeostatically regulated (Remppis *et al*., 2011). When bacteria that degrade soluble carbohydrates such as *Streptococcus bovis* are starved, energy supply to proteolytic and structural (cellulolytic) bacteria is reduced resulting in unbalanced and uncoupling of protein and energy degradation. Supplementation of lactating diets with readily degradable carbohydrate may enhance good utilization of nitrogen compounds including peptides, urea and amino acids and synchronization of carbohydrate and protein degradation (Zhao *et al*., 2015). The effects of NEB can be reduced by providing high-energy boosters such as propylene glycol during late pregnancy (Ayoub *et al*., 2015;) and antioxidant rich supplements like selenium and vitamin E at calving to support the functioning of the immune system (NRC, 2001). Cows are also supplemented with glucose to reduce hypoglycaemia. The proposed supplements are not possible in resource limited communal area environments hence, cows in this study had no additional nutrient support.

Rumen N balance was in excess (130%), which is an indicator of uncoupled protein: energy ratio; dietary energy was low and hence N utilization for bacterial protein synthesis was affected. Unused N is eventually lost as faecal or urinary N. Insufficient utilization of N could increase ammonia uptake from the rumen which may be converted to urea in the liver and secreted in milk (Useni, 2017). Microbial protein forms over 50% of the total non-ammonia N for lactating cows

(Clark *et al*., 1992; Fellner, 2002). The NRC, (2001) recommends 23% dietary CP and Owen, (2014) suggested 20% CP for lactating cows. *In vitro* nitrogen degradability was low reflecting inefficiency in rumen fermentation of CP in the diets. In LD1 diet, 28% of N was within the slowly degraded NDF fraction (NDFICP) while 25% was not available (ADICP0). Effectively, the diet had only 6.2% available protein and 37% of that component was within the slowly degraded NDF available fraction. The LD2 diet (31-60 d postpartum) had effectively 7.4% of CP as available protein. However, about 67% of the effectively available protein constitutes the fraction of soluble protein and the remaining 33% as the slowly degraded NDF.

In the current study, the amount of soluble protein in the diets was lower than the level of 50% suggested by Chalupa *et al*. (1996) for adequate provision of ruminal ammonia. According to Jusoh *et al*. (2014), ruminants require a minimum of 7.5% CP for effective function. The estimated dietary CP (% of DM) for effective function of the lactation diets were below minimum (maintenance). Proteolytic bacteria including *Butyrivibrio fibrisolvens, Prevotella ruminicola* and *Clostridium proteoclaticum* in the rumen of the cows were starved and bacteria growth was therefore compromised.

Dietary TDN was within the range stipulated for high milk producing cows (60-78%) (NRC, 2001). High dietary TDN improved intake and animal performance (Schrama *et al*., 2013). However, the effect of LD1 and LD2 diets would be lower as TDN was on the lower end of the suggested range. Non-structural carbohydrates determine TDN and decline as fibre components increase. The content of NSC was low and therefore energy concentration was low. During the transition phase, cows mobilized body reserved to support additional milk production. The NEm in 1st and 2nd lactation cows are higher due to growth requirements estimated at 20% and 10% higher respectively (NRC, 1989).

Cows were zero grazed, hence had low energy cost for exercising and more milk was expected. However, high body size and weight as well as requirement for high milk production as lactation progressed explain increased energy requirements for lactation. At least, one third of dietary nutrients were within the indigestible fractions as reflected by the low RFV; this translates to an equivalent loss in performance. The RFV was within the range for forage, crop residues and highly lignified herbage including *Seriphium plumosum*, *Sporobolus pyramidulis* and other perennial decreaser grasses such as *Themeda triandra*.

The LRNS program used in this study estimated supply and requirements and nutrient supplied for growth, pregnancy and lactation. Measuring animal performance responses to diets with different composition at different lactation stages was to quantify intestinal supply of MP and ME and requirements for maintenance, growth and production. Production of

microbial protein from the degradation of forage fraction in the rumen and the available amino acid fraction from dietary crude protein in the lower intestine were presented in the model.

The significant difference ($P<0.05$) between the observed and expected dry matter intake (DMI) of cows in fresh (0-30 d postpartum) and early lactating (30-60 d postpartum) explained the need for the cows in these groups to meet the metabolic demands for building muscle tissue, maintenance, The higher dry matter intake observed did not result in positive performance response of cows as loss in body reserves (BW and BCS) was observed in lactating cows at this stage (fresh) of lactation. The quality of the fed diets indicative of low dietary CP and the corresponding high dietary fibre may explain the observed performance. The risk of severe negative energy balance observed at 0-30 d postpartum is a common condition with cows after calving (Wathes *et al.*, 2007). However, the condition could be minimized with nutrition management that make provision for precision feeding essential to meeting energetic demands and reduce incidence of metabolic disorder in cows. It is expected that the cows used in this study as first-calf heifers are yet to attain the mature body weight. Therefore, prioritization of energy demands for growth (tissue building) usually placed demands on increased energy changes instead of milk synthesis (Litherland, 2009).

In the current study, the performance of lactating cows reflected the intake of digestible energy (DE), metabolizable energy (ME) and metabolizable protein (MP) and differences in diet digestibility significantly influenced nutrient supply. It became important that forage-concentrate interaction of diets fed to lactating cows provides a positive associative influence on performance response as concentrate provide limiting nutrients that are deficient in the forage fraction of diets. Similar reports from the study of Tahir, (2012) indicated positive associative effects of concentrate-forage resulted in higher ME intake compared to the expected such as found with diet supplementation of protein or digestible fibre. The lower ($P<0.05$) concentrations of MP and ME observed in LD1 and LD2 diets fed to cows at 0-30 d postpartum and 31-60 d postpartum, respectively, may reflect inadequate degradable carbohydrate and crude protein in the diets. This is consistent with the reports of Suharti *et al*. (2011) and Kumar *et al*. (2013) who found a relationship between ME in ruminants and the phycochemical characters of fed diet in which high NDF content resulted in high rumen fill, low degradation and the ensued low ME.

The total diet MP constitutes rumen degradable protein (RDP) from microbial origin and rumen undegradable protein (RUP) from dietary crude protein that bypass rumen degradation. It was recommended that the fraction of RDP be ≥ 50% of fed diet (Fox *et al*., 2003). The observed concentration of RDP fraction of 47.9 and 50% respectively in LD1 and LD2 diet seems low and may be explained by the physico-chemical characteristics of the grass hay fraction of diets. Insufficient supply of RDP in lactating diets as observed at both fresh and early lactation period appeared to have resulted in depressed ruminal microbial growth and possible reduction in microbial protein synthesis. This may result in reduced benefits of the lactation diets to the lactating cows hence, the observed loss in body reserves and low milk yield. In agreement with these results, Das *et al*. (2014) revealed that adequate supply of RDP in diets of lactating cows is crucial to efficient microbial fermentation and the resultant improved microbial protein yield required for optimal benefits to the host animals.

4.4.2. Energy and protein balance in lactating cows

Evaluation of energy and protein content of lactation diets at 1-30 d postpartum and 31-60 d postpartum period respectively is crucial to assessing the nutritive value of diets and possible benefits to the cows. Negative balance of nutrients in diets indicates their insufficient availability in meeting cows' metabolic demands for maintenance, lactation and further growth as cows are in first lactation. Low dietary protein and energy in diets may explain low availability of these nutrient relative to requirements for optimal lactation. Overall negative energy and protein balance occurred as intake of energy and protein lagged behind requirements in cows. Cows had tissue depletion, hence the observed decreased BW and BCS of cows. Continuous and higher demand for nutrients at 31-60 d postpartum period as milk production increases explains higher negative protein balance commonly associated with early lactation. High-energy and protein requirements for milk production increase as lactation progresses to peak and production usually exceeds available metabolizable energy intake resulting in negative energy balance (Prendiville *et al*., 2011). However, during summer, grasses and herbaceous legumes are of good quality, hence cows could derive better nutrition from grazing that would support them on hay and poor quality concentrate. The cows might regain body reserves post-peak.

Furthermore, the high structural carbohydrate fraction of LD1 and LD2 diets indicating high dietary fibre content may have slowed down microbial activity. To this, availability of limiting nutrients including energy and amino acids are compromised, resulting in the observed NEB with the lactating cows during this study. The nutritional status of the cows at close-up period could also contribute to the poor performance of cows post-calving. The results indicate adverse

metabolic status such as low BW and BCS in close-up period, negatively influencing postpartum performance including depletion of body reserves and low milk yield. The negative protein balance recorded in both lactation periods reflected insufficient availability of MP from microbial protein and dietary CP of diets. This could be because of low N supply either from dietary CP and microbial protein source. It is also possible that diets are not efficiently utilized, resulting in loss of nutrients.

Feeding low dietary CP to dairy cows has been found to result in nutrient waste and environmental challenges such as pollution (Kebreab *et al*., 2002). It appears there was no adequate supply of N from the lactation diets to support efficient microbial growth and the corresponding microbial protein synthesis. This could have adversely affected ruminal production of VFA, propionate to acetate ratio and energy requirements of the cows. Overall, most cows were hypoglycemic and the condition was more evident in cows at 31-60 d postpartum period. This was reflected in the amount of mobilized energy from body reserves at different periods of lactation. It would require high-energy boasters or inophores such as monensin to stimulate higher nutrient supply from the gut for the treatment of the prevalent hypoglycemia in dairy cows raised on smallholder farms.

4.4.3. Protein metabolism

Mean concentration of total protein (TP) in cows in both investigated lactating periods were below the reference value range for lactating cows. The effect was more pronounced for cows at 31-60 d postpartum period. Lower concentration signals low dietary CP resulting in low circulating blood amino acids that are usually taken up by mammary cells for milk protein synthesis hence, the observed low circulating TP. The low concentration of AL observed in cows indicates insufficient available protein in diets required for optimum milk synthesis. Insufficient dietary CP contributed to low AL concentration hence, the low lactation performance observed in this study. Kubkomawa *et al*. (2015) noted concentration of AL in the serum reflects protein status of the cow and the concentration decreased in the event of inadequate feeding of dietary CP. Increased blood circulating AL in cattle is associated with good nutritional standing and BCS (Coppo, 2004; Damptey *et al*., 2014). Low AL concentration of cows reflected poor nutritional condition hence, the consequential poor BCS of less than 2.5 in lactating cows in this study. Increased concentration of serum AL, AL: GL ratio increased with feeding higher levels of dietary CP (Amanlou *et al*., 2017).

Low concentration of GL in the cows indicated healthy status of cows and increased concentration of GL designated occurrence of disease condition (Whitaker *et al*., 1999; Ndlovu *et al*., 2007; Damptey *et al*., 2014). The within-range ratio of AL: GL in cows in the current study as lactation progresses may be explained by the healthy status of the animals. Although cows had higher

concentration of globulin than albumin during the investigated lactation periods, cows did not develop disease condition. The reason for this is not clear. However, it is possible that the low concentration of AL and high GL concentration indicates low dietary protein in diets for the lactating cows with no consequential incidence of disease condition. The parity level (primiparous) and moderate BW and BCS (not overconditioned) may lower the chances of the cows' susceptibility to infections thereby promoting longevity of cows, low rate of culling, better financial returns and sustainable dairy production.

Mobilization of body reserves such as protein to support milk production typical to early lactation may explain increased creatinine concentration in cows. This is consistent with Cozzi *et al*. (2011) who suggested that breakdown amino acids could have resulted from mobilized muscle protein from the body tissue. Furthermore, responses of cows to hot weather conditions and possible consequential body dehydration common to the production environment (semi-arid) investigated could explain the high circulating creatinine observed in the cows.

Increased concentration of BUN with increased dietary CP explains the higher BUN observed in cows fed at LD2 diet phase with higher dietary CP. Production environment (semi-arid) characterised by high temperature and low humidity may play a role in predisposing cows to heat stress. Demand for increased milk protein declines during which excess amino acids are oxidised and degraded resulting in increasing urea production and concentration in the blood as noted by Liu *et al*. (2003b).

4.4.4. Performance of lactating Jersey cows

Loss of BW and BCS around calving are common phenomena in lactating cows (Heinrichs *et al*., 2011) as cows mobilize body reserves to support milk production (Busato *et al*., 2002). Energy from reserves yields 0.82 Mcal/cow/day towards milk production. Based on this efficiency, energy from body reserve mobilization was about 7.0 Mcal/cow/day. This is typical during transition. Protein breakdown contributed 83% of the energy and fat contributed 17%. This indicates higher depletion of body tissue compared to fat. High blood creatinine immediately postpartum confirms muscle breakdown during which cows mobilise protein from endogenous tissues such as visceral tissue and skeletal muscle to provide amino acids essential for milk synthesis in the occurrence of negative energy and protein balances (Damptey *et al*., 2014)

Mean milk yield of 9.3 kg /cow/day entails that LD1 had supported maintenance and 3 kg/cow/day with no energy available for growth. The difference (6.3 kg of milk) was contributed from body reserves. This is expected as intake lags behind production hence, depletion of body tissues. This level of reserve mobilization is acceptable, as it would take 90 days for a cow to lose one BCS. Holstein cattle mobilizes up to 23 Mcal/day to support 20 kg milk production in early

lactation (NRC, 2001) relative to 7 Mcal/day for 9.3 kg milk observed in Jersey cows in the current study. Similar findings of Litherland (2009) indicated fat and multiparous cows experience more NEB. The need for higher energy density in early lactation should be emphasized. Cows had further decrease in BW even when diets were changed 4 weeks postpartum. About 98% of excess nutrients are depleted as protein and 2% as fat. Additional energy demand from reserves increased by 5 Mcal/day which means the new diet (LD2) did not support additional kilograms of milk produced as the lactation period progressed. This resulted in further decrease in the BW and BCS observed at this stage of lactation.

The low milk yield recorded in this study reflected the limitation of the fed diets. Feeding of less than16% dietary CP limits milk production as noted in this study. NRC (2001) recommended 15% dietary CP for a first-calf heifer at close-up prepartum period to support foetus growth and optimal early lactation performance. Milk production in primiparous cows in early lactation increased with high dietary CP compared to low level dietary CP prepartum (Santos *et al*., 2001). The low milk protein percentage observed in this study was lower than values reported in other studies that ranged from 3.6 to 3.8% (Bailley *et al*., 2005; Palladino *et al*., 2010) for Jersey cow reflected the low dietary NSC such as starch. Voigt *et al*. (2003) noted feeding of glucogenic diets increased percentage of milk protein. Other reports showed no effect of feeding starch on milk protein (Garnsworthy *et al*., 2009). Deficit of these energy metabolites could negatively influence partitioning of nutrients (Mackle *et al*., 1999). Besides, different responses of milk protein with supply of energy may possibly be related to glucogenic nutrients such as amino acids in the rumen (Hills *et al*., 2015). Supply of essential and non-essential amino acids to mammary cells is required for milk protein synthesis (Liu *et al*., 2003b). It was observed that milk protein percentage decreased with increased milk yield. The ratio of forage to concentrate in diets was higher than the suggested 40:60 by Sutton *et al*. (1985) to maintain a normal milk fat percentage. The physical characteristics of grass hay may have resulted in high dietary NDF which are slowly degraded, hence the low-fat content.

The higher content of lactose recorded for cows as lactation progresses indicates increased production of glucose and the ensuing lactose production. The amount of propionate produced as an end-product of fermentation serves as the main substrate for the production of glucose (gluconeogenesis) which in turn serves as a major source of energy for ruminants (Bodas *et al*., 2012). Blood glucose is the major precursor of lactose (Kittivachra *et al*., 2007). This may explain the increased lactose content of milk produced in 31-60 d postpartum period and increased milk yield with possible increased in blood glucose. Low milk yield and lactose content of milk in cows reflected possible low energy availability and low blood glucose. It is obvious that the milk

yield at different lactation periods was induced by the level of lactose synthesis as lactose constituted the major volume of milk (Sutton *et al*., 2003)

The low MUN concentration in cows indicates inadequate dietary CP, reduced activity of proteolytic bacteria such as *Clostridium spp* and less protein degradation (proteolysis) in diets. Jilek *et al*. (2006) and Abdouli *et al*. (2008) showed that the concentration of MUN decreased with increased lactation period. The reason for the inverse relation was not clear. Nevertheless, the low metabolic demands for lactation and the lower milk production of cows at 1-30 d postpartum period may partly explain the high concentration of MUN recorded compared to cows in subsequent lactation period. Cows in low lactation require less milk protein and hence CP was less efficiently utilized. In addition, the level of MUN could indicate imbalance in rumen ammonia utilization. Ruminal N was under-utilized resulting in the observed increased MUN. Diets high in NSC such as starch improve rumen ammonia usage as noted by Drackley *et al*. (2006). The level of urea nitrogen in milk produced by cows in this study relative to milk yield should support good fertility in cows as too high levels of MUN could be detrimental to reproductive performance in the cows. Milk urea nitrogen greater than 19 mg/dl (19% of milk produced) decreases fertility (Butler *et al*., 1995).

Somatic cell count (SCC) of $\leq 100 \times 10^3$ cells/mL indicates normal healthy cow while high level ($>400 \times 10^3$/mL) of SCC indicates incidence of infection such as mastitis (Bytyqi *et al*., 2010; Sharma *et al*., 2011). Cows had high SCC but decreased in latter lactation stage (31-60 d postpartum). This could be expected as preparation of inborn immune response for calving and improvement in the defence mechanism of the mammary gland may take precedence around calving. Cinar *et al*. (2015) reported a higher SCC of about 292×10^3 cells/mL 15 days post-calving which progressively decreased to about 93×10^3 cells/mL in three month postpartum with increased milk yield. In the present study, the SCC recorded later during lactation was within the reference value for healthy cows indicating cows are healthier as lactation progresses. Cows may have gradually recovered from possible infections or due to reduction in immune response of cows and the corresponding decreased presence of antigens, hence the low SCC observed as lactation progressed and milk yield increases. Measures to reduce SCC during lactation may reduce significant milk loss (Hand *et al*., 2012).

The low calculated yield of ECM and production efficiency for cows indicate low efficiency of feed conversion to milk. This implies that less milk is produced relative to the amount of feed intake. Overall, the physico-chemical characteristics of diets notably influenced the profile (level and type) of absorbed nutrients from the rumen or gastrointestinal tract into the bloodstream of the cows. This explains the corresponding effect on energy metabolism (Williams and Stanco,

2000; Garnsworthy *et al*., 2008) and the ensuing influence on milk yield and composition (Van Knegsel *et al*., 2005; Useni, 2017).

4.4.5. Conclusion

The intake of a high forage fraction exceeded NRC (2001) recommendations and resulted in low degradation and poor performance of lactating cows. Although, cow diets were supplemented with concentrates, their performance was similar to that of beef on natural grazing. The observed performance responses of cows at different periods of lactation reflected the nutritive value of the fed diets in terms of the rumen degradability and availability of potential nutrients for the benefit of the animals. The lower nutrient concentration and intake of lactation diets than predicted values from the in this study could be due to differences in the environmental climate, weather, humidity and temperature of the production sites, diet composition, and animal breed, feeding and animal management style between actual and predicted situations. Supplementing diets for cows that are in poor body condition reduces efficiency of nutrient utilization. Increased concentrate supplement at 31-60 d post calving produced a marginal increase in milk yield as supply of nutrients was limited in supporting substantial milk production. Gradual increase of concentrate supplement in cows'diets could enhance efficient utilization of dietary nutrients and ideal BC for lactation.

CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 General conclusion

Dairy production on smallholder farms could be a viable enterprise providing income and improve nutrition, health and income security for smallholder households. Nutrition of dairy cows on the smallholder farms was therefore investigated. On-farm observations and measurements were fitted into a nutrient modelling application, analysed and a nutrient narrative on the performance of transition cows (pregnant and early lactation dairy cows) on communal area farms in Limpopo province, South Africa was synthesised. The research focused on the nutrient supply and absorption of the most common diets fed pregnant heifers and during early lactation and their effects on milk production and growth. The quality of the forage fraction (grass hay) of diets, diet degradation and nutrient absorption in the lower gut were contained in the nutrition model applied in nutrient prediction studies on dairy nutrition These evaluations and overall effects of diet quality on the performance of the animals formed the basis of this research.

Diets were lower in nutrient value and deviated significantly from the recommended levels. Feeding of high levels of structural carbohydrate to lactating cows had negative effects on the effective microbial fermentation and degradation. The findings explained the complexity of the composition of the forage fraction of diets and inefficiency of nutrient supply. These could be linked to supply of more mature and lignified grass hay fraction in diets especially in the dry season (winter) on smallholder farms in Limpopo province.

A quality diet for dairy cow prepartum should contain sufficient degradable dietary carbohydrate and protein sources for efficient microbial protein synthesis and amino acids for the heifers' benefit. However, the concept of the improved feeding program of pregnant cows, particularly the first-calf heifers, aimed at providing the required nutrients for foetal and heifers growth, building of good body reserves that will minimize mobilization of body fat and other metabolic diseases in the early lactation stage. The program is worth implementing in the production system.

Results indicated diets fed in pregnancy had sufficient nutrients for maintenance but less nutrients for pregnancy and growth; heifers need nutrients to support both pregnancy and growth. The greatest impact was during early lactation due to reduced gut volume, low intake and added demand for galactopoeisis. Heifers and lactating cows may experience low secretion of insulin-like growth factor (IGF-1); a hormone secreted by the liver as a response to the stimulation of GH. The growth hormone (GH) stimulates IGF for the proliferation and differenciation of bone

and muscle cells promoting growth respectively in bone and muscle hence the observed low growth in heifers. The secretion of somatotropin, a polypeptide GH from the anterior pituitary positively affected postnatal metabolism and growth in skeleton and tissues. Glucose is usually released from the lower gut into the blood stream for absorption. Adequate glucose concentration in the blood is prerequisite to high milk yield in dairy cows. In this study, lactating cows raised on the smallholder farms were hypoclycemic due to low glucose concentration in the blood as evident in the NEB observed in early lactation. Blood stimulates secretion of glycogon and equinephrine hormones for the conversion of stored glycogen to glucose through the process of glycogenolysis. There was low production of glucose from diets to be utilised by cows for lactation. This resulted in the low availability of glucose and consistent loss of body reserves to support milk synthesis at lactation which was observed in early lactation. In addition, the low milk yield in the cows during early lactation could be linked to the low effect of GH in stimulating lactation in the cows hence, milk synthesis was hampered.

Meeting the unique nutrient requirements of dairy cows for smooth transitioning from gestation to lactation period could prevent metabolic disorder and improved postpartum performance and longevity in cows. Supplementing pregnant heifers' diets with glucose could supply additional energy around calving and improve performance. Although, the cost could be a concern, but the positive outcome could be worthwhile. It is known that administration of GH to lactating cow improves milk production without changing milk composition with higher positive effects on high producing cows. However, injection of GH to the lactating cows in the communal smallholder farms may not be feasible as production cost for resource and skilled personel limited sector may hamper such treatment.

The intake of a high forage fraction exceeded NRC, (2001) recommendations and resulted in low degradation and poor performance of lactating cows. Although, cows' diets were supplemented with concentrates, their performance was similar to that of beef on natural grazing. Supplementing diets for cows that are in poor body condition reduces efficiency of nutrient utilization. Increased concentrate supplement at 31-60 d post calving produced a marginal increase in milk yield as supply of nutrients was limited in supporting substantial milk production. Gradual increase of concentrate supplement in cows'diets could enhance efficient utilization of dietary nutrients and ideal BC for lactation. The tested hypotheses were therefore proven to be true as nutrient supply of standardized diets fed to dary cows on smallholder farms investigated in this study affect growth and lactation performance of dairy cows. However, accurate evaluation of feeding value of lactation diet at different lactation stages and nutrient requirements appeared to be core to achieving optimal growth, improved cows' health, feed efficiency and lactation performance of Jersey cows on smallholder farms

5.2 Recommendations

A quality diet for dairy cow prepartum and postpartum should contain sufficient degradable dietary carbohydrate and protein sources for efficient microbial protein synthesis and amino acids for the heifer's benefit and optimal lactation. However, the concept of improved feeding program for dairy cows aimed at providing the required nutrients for foetal and heifers growth especially in first-calf heifers; building of good body reserves that will minimize mobilization of body fat and other metabolic diseases in the early lactation stage. Improved feeding program is worth implementing in the smallholder dairy production system for increased procuction and enhanced sustainable dairy enterprise, and valuable contribution of the sector to the food security. This may include;

- Planting of high energy cereal crops such as maize and sorghum as a source of non-structural carbohydrate by smallholder farmers to supplement cow diets as diets were low in non-structural carbohydrate is recmmended. The results of this study showed that increasing NFC of diets for cows could improve growth and lactation, therefore, increasing the fraction of NFC including starch in lactation diets is suggested for enhanced diet feeding value, efficient nutrient utilization and increased benefits of diets to the cows.

- Planting of resilient shrubs and browsers as feed supplements to dairy cows around production environments during dry season is suggested. This may prevent inadequate nutrient supply typical to this season and to reduce cost of commercial concentrtes

- In the current study, it was noted that feeding of high structural carbohydrate to lactating cows had negative effect on effective microbial fermentation, degradation and adequate utilization of diet nutrient and restricted diet benefit to the cows. Therefore, there is a need to improve the quality of dietary forage by selecting non-lignified grass for improved degradation of diet nutrients and availability of energy and N required for growth and lactation in dairy cows.

- Improvement of dietary CP by increasing the level of concentrate in dairy diets may improve MP and ME of diets and should be considered on the smallholder farms..Low forage to concentrate ratio in diet and increased energy dense could adequately supply and make available limiting nutrients through efficient microbial fermentation of diets in the rumen. As a result, improved diet degradation and absorption of nutrients into the

lower gut and the subsequentlybetter production performance responses of fed cows should be expected. Therefore, maintaining sufficient dietary forage that will stimulate efficient microbial fermentation, enhance microbial protein synthesis and increased availability of amino acids is key to optimizing growth and lactation in dairy cows.

- Technically, training of smallholder farmers by appropriate development and support programs through an extention personnel on feeding management is suggested as management structure in this sector is very dynamic on feeding management is fragmented A well-managed transition period in dairy cows involves preparation of good BW and BCS of close-up pregnant heifers by meeting the cows' unique nutrient demands is prerequisite and crucial to maximizing postpartum performance.

- Future research is suggested to implement predicted requirements of nutrients in this study in dairy cow diet on smallholder farms and to further evaluate their production performance accordingly.

- There is need to evaluate the performance of dairy cows on smallholder farms from early gestation until late lactation to assess effects of low nutrient supply beyond transition period.

- Evaluation of nutritional status including energy and protein of dairy cow on smallholder farms over more than one lactation period and the long term effect on their production and reproduction performance.

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APENDICES

Appendix 1: Composition of the buffer solution used in the *in vitro* degradability and the gas production experiments.

Reagent	Quantity added
Rumen buffer solution	
Distilled water	I litre
NH_4CO_3	4 g
$NaHCO_3$	35 g
Dissolve 0.122 g resaruzin in 100ml (distilled water)	
Macro- mineral solution	
Distilled water	1 litre
NAH_2PO4	11.4 g (anhydrous)
KH_2PO4	12.4 g (anhydrous)
$MgSO4.7H_2O$	1.17 g
Micro- mineral solution	
Distilled water	100 ml
$CaCl_2.2\ H_2O$	13.2 g

$MnCl_2.4\ H_2O$	10.0 g
$CoCl_2.6\ H_2O$	1.0 g
$FeCl_2.\ 6H_2O$	8.0 g

Reducing agent (Cysteine sulphide)	
Beaker A	
Distilled water	50 ml
Cysteine hydrochloride	0.625 g
1 N NaOH	4 ml
Beaker B	
Distilled water	50 ml
$Na_2S.9H2O$	0.625 g
Mixed A & B solution	100 ml

Final buffer solution (per 4 litre)	
Distilled water	2 litre
Rumen buffer solution	1 litre
Macro-mineral solution	1 litre
Resaruzin 0.1% (w/v)	0.25 ml
Micro-mineral solution	0.25 ml
Tryptose	5 g

Final buffer solution + reducing solution	
Final buffer solution	4 litre
Reducing solution (mixed A & B solution)	100 ml

Appendix 2: Summary of LRN program for dry cows (1/42)

One Page Summary (VH DA1, LRN program (DRY COWS-JULY)., 2017-07-24)

Animal Inputs

Animal Type: Dry Cow
Breed: Jersey
Age: 22 months
Shrunk Body Weight: 350 kg
Days Pregnant: 220 days
Condition Score: 2,00

Age at First Calving: 24 months
Calving Interval: 13 months
Milk Production: 0 kg/d
Milk Fat: 5,2 (%)
Milk True Protein: 3,9 (%)
Days In Milk: 0 months

Previous Temp: 24 °C
Current Temp: 28 °C
Activity: Small Free-Stalls (< 200 Cows)

Diet Nutrient Balances

Requirements	ME (Mcal/d)	MP (g/d)	Ca (g/d)	P (g/d)	K (g/d)
Maintenance	16,19	545	0	0	0
Pregnancy	2,64	139	4	3	1
Lactation	0,00	0	0	0	0
Growth	2,66	53	2	1	0
nNet Required	21,49	737	12	14	39
Total Required	21,49	737	12	14	39
Total Supplied	15,98	502	18	26	160
Balance	-5,50	-235	7	12	122

Animal Performance

DMI - Actual: 9,2 (kg/d)
DMI - Predicted: 6,7 (kg/d)
ME Balance: -5,50 (Mcal/d)
MP Balance: -234,5 (g/d)
Daily Weight Change due to Reserves: -0,7 (kg/d)

Diet Summary

Feed/Mix Name	DM (kg/d)	As-Fed (kg/d)
Love grass hay	6,700	7,204
comm lactating diet	2,500	2,717

Diet Parameters

MP From Bacteria: 376 (g/d)
MP From Undeg. Feed: 127 (g/d)
MP% - Bacterial: 74,82 (%)

Methionine (%MP): 2,75 (%)
Lysine (%MP): 7,69 (%)

Ruminal N Balance: 107 (g/d)
Peptide Balance: 37 (g/d)
Urea Cost: 0,49 (Mcal/d)

Dry Matter: 93%
Crude Protein: 12,9 (%DM)
TDN : 0 (%DM)
ME: 1,74 (Mcal/kg DM)
NEm: 0,90 (Mcal/kg DM)
NEl: 1,12 (Mcal/kg DM)
DIP: 85%
peNDF: 45 (%DM)
Total Forage in Ration: 78 (%DM)
Total NFC: 18%
Cost per Animal/d : $ 0,00

Calcium: 0,20% D
Phosphorus: 0,28%
Magnesium: 0,11%
Potassium: 1,74%
Sodium: 0,01% DI
Chlorine: 0,02% D
Sulfur: 0,04% DM

Cobalt : 0,22 ppm
Copper : 14,84 pp
Iodine : 14,57 ppm
Iron : 69,33 ppm
Manganese : 123,3
Selenium : 0,00 pp
Zinc : 27,67 ppm
Vitamin A : 6700
Vitamin D : 16750
Vitamin E : 43550

Appendix 2: Summary of LRN program for lactating cows (1/42)

a

One Page Summary (VH L1A, LRN program (LACTATING COWS)., 2017-07-19)

Animal Inputs

Animal Type: Lactating Dairy Cow
Breed: Jersey
Age: 25 months
Shrunk Body Weight: 373 kg
Days Pregnant: 0 days
Condition Score: 2,00

Age at First Calving: 24 months
Calving Interval: 13 months
Milk Production: 8 kg/d
Milk Fat: 4,0 (%)
Milk True Protein: 4,0 (%)
Days In Milk: 30 months

Previous Temp: 26 °C
Current Temp: 31 °C
Activity: Small Free-Stalls (< 200 Cows)

Diet Nutrient Balances

Requirements	ME (Mcal/d)	MP (g/d)	Ca (g/d)	P (g/d)	K (g/d)
Maintenance	15,57	606	0	0	0
Pregnancy	0,00	0	0	0	0
Lactation	9,92	492	12	8	12
Growth	0,99	28	1	0	0
Net Required	26,48	1127	24	20	57
Total Required	26,48	1127	24	20	57
Total Supplied	21,31	638	19	32	168
Balance	-5,17	-489	-5	12	111

Animal Performance

DMI - Actual: 11,2 (kg/d)
DMI - Predicted: 8,0 (kg/d)
Inputted Milk Production: 8,0 (kg/d)
ME Allowable Milk: 3,8 (kg/d)
MP Allowable Milk: 0,1 (kg/d)

Daily Weight Change due to Reserves: -0,8 (kg/d)
Days to Lose 1 Condition Score : 67.50452
Milk/Feed: 0,7

Diet Summary

Feed/Mix Name	DM (kg/d)	As-Fed (kg/d)
Love grass hay	6,740	7,247
comm lactating diet	4,500	4,891

Diet Parameters

MP From Bacteria: 493 (g/d)
MP From Undeg. Feed: 146 (g/d)
MP% - Bacterial: 77,19 (%)

Methionine (%MP): 3,02 (%)
Lysine (%MP): 8,32 (%)

Ruminal N Balance: 131 (g/d)
Peptide Balance: 36 (g/d)
Urea Cost: 0,62 (Mcal/d)

Dry Matter: 93%
Crude Protein: 13,4 (%DM)
TDN : 0 (%DM)
ME: 1,90 (Mcal/kg DM)
NEm: 1,22 (Mcal/kg DM)
NEl: 1,22 (Mcal/kg DM)
DIP: 86%
peNDF: 37 (%DM)
Total Forage in Ration: 68 (%DM)
Total NFC: 23%
Cost per Animal/d : $ 0,00

Calcium: 0,17% D
Phosphorus: 0,29%
Magnesium: 0,11%
Potassium: 1,49%
Sodium: 0,01% D
Chlorine: 0,02% D
Sulfur: 0,06% DM

Cobalt : 0,18 ppm
Copper : 12,39 pp
Iodine : 11,99 ppm
Iron : 62,38 ppm
Manganese : 102,7
Selenium : 0,00 pp
Zinc : 22,79 ppm
Vitamin A : 6740
Vitamin D : 16850
Vitamin E : 43810