EFFECTS OF NUTRITION ON MILK PRODUCTION AND REPRODUCTION OF DAIRY COWS

A dissertation presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Veterinary Science

at Massey University, Manawatū, New Zealand.

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2022

ABSTRACT

Sri Lanka is developing its dairy industry in a concerted effort to become self-sufficient in dairy products. Dairy farming in Sri Lanka occurs largely in small (≤10 cows) or medium (11-100 cows) scale farms, characteristically using diets that are based on tropical forages and various concentrate supplements. Despite this reliance on forages, little is known about their nutritive value or about the most appropriate ways of managing such forages in dairy rations. Consequently, per cow yields are generally low, and poor fertility is a significant limitation to the viability of the dairy industry.

The primary objective of this thesis was to examine the management of forages in Sri Lankan dairying systems and the consequences of that management upon dairy cow productivity and fertility. As there was a lack of systematic information regarding the nutritive value of forage-based diets, generating such data was the first focus of the study. These data were used to calculate the extent to which supplied diets met the energy and protein requirements of dairy cows. Direct observation of cows and blood-based measures of metabolic status were used to verify cows' nutritional status. As there was also a lack of systematic information on the fertility of Sri Lankan dairy herds, this was also evaluated.

The first study (Chapter 3) investigated the feeds and rations used in medium-scale dairy production systems of Sri Lanka. Metabolisable energy (ME) and crude protein (CP) content were assessed in the two most commonly used forages (Guinea grass ecotype A (*Panicum maximum*), hereafter referred to as Guinea grass, and Hybrid Napier CO-3 (*Pennisetum purpureum* * *P. americanum*), hereafter referred as CO-3 grass) and in the secondary forages Gliricidia (*Gliricidia sepium*) and maize stover (*Zea mays* L.). Mean ME and CP content of Guinea and CO-3 grasses were 7.8 and 9.3 MJ/kg dry matter (DM) and 8.0 and 8.8% DM, respectively. Total dietary intakes of ME and CP were calculated using these

values. Daily ME intake across the entire late-dry to mid-lactation period was consistently 7% lower than calculated requirements, whilst CP intake (13.5% DM) was below requirements in early lactating (16-18% DM required) but not dry cows (10-12% DM required). Metabolic profiling (serum albumin, urea, β-hydroxybutyrate and non-esterified fatty acid concentrations) over the same period confirmed the presence of widespread energy and protein deficiencies (Chapter 4). However, body condition scores (BCS) were consistent over the lactation at 4.3-4.5 (1-10 scale). Insulin-like growth factor-1 (IGF-1) concentrations (Chapter 6) were higher during Days 1-14 of lactation than during Days 43-57 (60.8 ± 2.6 ng/mL *vs.* 72.3 ± 3.6 ng/mL), and also in cows with higher post-calving BCS, but were not clearly related to calculated energy status. The major conclusion from Chapters 3 and 4 was that nutritional deficiencies in Sri Lankan dairy cows could largely be attributed to poor control of stage of maturity of harvested forages.

Reproductive performance of cows (Chapter 5) was assessed using farm records (including farmers' observations of oestrus) and serum progesterone assays over Days 43-120. Progesterone profiles showed that 61.6% of cows resumed ovarian cycles by Day 120; but only 42% of these were observed in oestrus. Further, of the cows that progesterone assay had not shown resumed cyclicity by Day 120, 20.9% had been considered by farmers to have displayed oestrus. Pregnancy rates were 12.7% by Day 120 and 59% by Day 400, average intervals from calving to conception were 196.1 ± 8.9 days, and from calving to first artificial insemination (AI) were 116.0 ± 2.5 days, with 3.05 ± 0.2 inseminations required per conception. The high proportion of anoestrous cows and low conception rates were largely attributed to inadequate nutrition during the transition and early lactation periods, compounded by inaccurate oestrus detection. Parity (1^{st}) and breed (Holstein-Friesian, HF) of cows were both risk factors for delayed resumption of oestrous cycles. Concentrations of IGF-1 were largely unrelated to reproductive outcomes, except those values on Days 43-57 were negatively related to the interval between calving and first AI.

Chapter 7 investigated the nutritional composition of ryegrass-white clover pastures in New Zealand that were grazed with different rotation lengths, on the hypothesis that longer rotations lengths would result in more mature swards, with lower quality herbage. Farms were selected with longer (winter ~35 days, spring ~25-30 days) or shorter (winter ~30 days, spring ~20-25 days) grazing rotations. Both ME and CP were reduced by higher pre-grazing DM and rotation length, whilst post-grazing DM and residual height were positively correlated ($r^2 \geq 0.8$) with pre-grazing DM. Thus, the quality of the forage from long grazing rotations was poorer, and, therefore, the amount of DM consumed by grazing cows may be affected.

Many cow-related factors significantly affected the reproductive performance (Chapter 8) of dairy cows in New Zealand. Cows with >85% of HF genes had lower pregnancy rates in the first three (PR21) and six (PR42) weeks from the start of breeding compared with those with ≤85% HF genes. Cows of parity 1-4 had higher PR21 and/or PR42 than those of parity ≥5. Pregnancy rate to first service (PREG1) was lower (odds ratio (OR): 0.7) in cows with higher BCS change (-0.5 to -1 units: 1-10 scale) between calving and the BCS nadir. Cows that calved in July had higher PR21, and PR42 (OR:1.96, and 2.51, respectively) than those that calved in September, while cows that calved in August had higher submission rates in the first three (SR21) and six (SR42) weeks from the start of breeding (OR: 2.39, 3.21, respectively) than those that calved in September. Higher fat production and total milk solids production were associated with significant negative energy balance (NEB) (≥-30 MJME/day), but NEB was less (or was positive) in cows with lower milk yields. Therefore, it appears that breed, BCS, parity, energy balance, calving month and milk composition are all useful indicators of future reproductive efficiency.

Taken together, these studies for the first time, characterise the quality of dairy forages in Sri Lanka and the adverse effects of inadequate nutrition upon cow productivity. The studies in New Zealand confirmed that forage quality and intake were adversely affected by over-maturity of pasture, driven primarily by rotation length, and these findings supported conclusions made regarding forages in Sri Lanka. Characterisation of reproductive performance of Sri Lanka dairy cows showed that it was at a level that undermines the viability and productivity of the dairy industry. Reproductive performance is not only impaired because of the adverse effects of nutrition, but also due to the inadequacy of farmers' reproductive management. Whilst these results are ostensibly depressing, they point to some relatively easy-to-implement interventions (e.g. improving forage management, better ration formulation, better education of farmers) to boost the future of the Sri Lankan dairy industry.

ACKNOWLEDGEMENTS

Undertaking this valuable PhD has been a truly life-changing experience for me and I accepted all the challenges positively as a part of the learning program. I am so proud of myself as I was able to reach this milestone; however, it wouldn't have been possible for me to finish the PhD without the support and the guidance received from many different people. Thank you all for this greatest support during my academic journey and the day-to-day life.

A heartfelt gratitude to my primary supervisor Prof. Danny Donaghy, Professor of Dairy Production Systems. Thank you for believing in my abilities to grow into a successful PhD candidate. I really enjoyed your invaluable discussions in formulating the research objectives and methodologies. To Prof. Richard Laven, Professor of Production Animal Health and Welfare, you provided me with the scientific knowledge and statistic tools that I needed to choose the right directions and successfully complete my dissertation. To Prof. Tim Parkinson, Professor in Veterinary Reproduction, your insightful feedback always pushed me to bring my work to a higher level which could help me to sharpen my scientific knowledge on dairy nutrition and reproduction. Tim, your exceptional knowledge and experience in the field of cattle reproduction have always been an inspiration during my PhD. To Dr. Jimena Yapura, Senior Lecturer in Anatomy and Physiology for making time to be available when I needed extra support and for keeping me on track. To Prof.Anil Pushpakumara, Professor in Veterinary Reproduction and Obstetrics for your continuing support and elaborate guidance throughout the PhD journey.

My special thanks go to farm managers and owners in both Sri Lanka and New Zealand for their prompt support in providing farm records and time spent in lengthy discussions throughout the research to come up with very good sampling protocols to complete this study. And I also would like to thank farm labourers who gave immense support to handle animals during the blood sampling for metabolic assays. I am also grateful to some farm families for their kindness, friendship, and the warmth they extended and for always making me feel so welcome.

I am highly indebted to Mik Harford, former project manager, Fonterra brands and Iroshana Kulathunga, former veterinary surgeon, Fonterra brands who supported in numerous ways the selection of farmers for the study from the Fonterra farming pool. I also wish to express my sincere gratitude to Manoj Jayasundara and Sanjaya Maduranga, Field extension officers, Fonterra brands for co-ordinating and extending their kind support for me in collecting farm records and feed samples. I would also like to thank Dr. Manoj Sanjeewa, Veterinary Surgeon, Wellard group; Dr. Upul Ranasinghe, Alltech Biotechnologies for their invaluable support during sample collection and processing in Sri Lanka.

I also thank for Prof. Nicolas Lopez-Villalobos, Professor of Cattle Breeding and Genetics for spending his valuable time to explain necessary statistical analysis of this study. Your insight into the development of the regression models using SAS was invaluable.

My heartfelt thanks go to Mrs. Deepika Talpearachchi and Miss Chandima Mellawa; Technical Officers, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya for giving me their generous and timely support to perform laboratory investigations. I owe a particular debt of gratitude to Dr. Chandika Gamage, Senior lecturer, Medical Faculty, University of Peradeniya for his great support for providing laboratory facilities to conduct my ELISA testing. And I also would like to thank Dr. Gamage's laboratory team for their great support by providing all necessary facilities to conduct ELISA works without any difficulties.

I wish to express my gratitude to the Totally Vets group in New Zealand for their numerous supports provided for me to find farmers in Manawatu region. Special thanks go to

Lindsay Rowe and Peter Aitken who helped me in selecting ideal farmers across the pastoral production systems. Thank you for making time to be available when I needed extra support. I was also able to learn a lot about dairy farming systems, veterinary care and dairy nutritional and reproduction management systems from those Totally Vets experts. I would also like to thank all farm managers, assistant managers and farm assistants who helped me in pasture sampling and cow data collection.

To my fellow postgraduate students at Massey University for engaging in scientific discussions, and for fun and adventures outside of PhD life. To lecturers in Veterinary and animal sciences who provided some information on New Zealand dairy farming when I needed it. And thank you for library team at Massey for providing scientific knowledge though out the PhD journey.

I wish to acknowledge the Massey University Doctoral scholarship program for providing the funding support to assist with living costs. I wish to acknowledge further funding supports I received during my PhD to assist with university fees, and the cost for blood and forage sampling and analysis. The funding I received were kindly granted by my supervisors' (Richard Laven and Danny Donaghy) Research accounts; Animal Science Postgraduate Fund; Veterinary Science Postgraduate Fund; Massey Foundation Grant and Catherine Baxter Postgraduate Scholarship.

A huge thank to my loving wife Thilini Ramanayake who always believed in me. You took the big responsibility to manage our kids Sanulya Hirudini and Nuhansa Hasmini, allowing me more time to engage with my studies, and always encourage me to finish studies on time. Thank you for being a best wife who creates the platform for a good family and for having lot of patience during this study.

Finally, the most affectionate gratitude is offered to my loving parents for being so generous. I wouldn’t have been able to come this position without having your proper guidance, parenting and influence. Thank you for your unconditional love and support though out my life journey. To all the friends, colleagues whose names were not mentioned for all the cheering up and again, thanks to you all.

LIST OF PUBLICATIONS/MANUSCRIPTS

While Chapters 3 and 7 were published/accepted in a peer-reviewed scientific journal, Chapters 4, 5, 6 and 8 have been submitted or are ready to be submitted to different peer-reviewed scientific journals.

Chapter 3: Kumara, S. N., Parkinson, T. J., Laven, R., Pushpakumara, P. G. A., & Donaghy, D. J. A nutritional investigation of major feed types and feed rations used in medium-scale dairy production systems. (Accepted for publication in *Animals*, August 2022)

Chapter 4: Kumara, S. N., Parkinson, T. J., Laven, R., Yapura, J., Pushpakumara, P. G. A., & Donaghy, D. J. Metabolic profile testing to assess the nutritional status of dairy cows in medium-scale dairy farms. (Submitted to *The Veterinary Journal*, July 2022)

Chapter 5: Kumara, S. N., Parkinson, T. J., Laven, R., Yapura, J., Pushpakumara, P. G. A., & Donaghy, D. J. Resumption of ovarian activity and factors influencing conception rates in postpartum dairy cows. (Submitted to *Research in Veterinary Science*, July 2022)

Chapter 6: Kumara, S. N., Parkinson, T. J., Laven, R., Yapura, J., Pushpakumara, P. G. A., & Donaghy, D. J. Relationship between serum IGF-1 concentrations and reproductive performance of postpartum dairy cows. (Prepared for submission to *Animals*, August 2022)

Chapter 7: Kumara, S. N., Parkinson, T. J., Laven, R., & Donaghy, D. J. (2022). The Influence of Rotational Length, along with Pre-and Post-Grazing Measures on Nutritional Composition of Pasture during Winter and Spring on New Zealand Dairy Farms. *Animals*, 12(15), 1934-1950. https://doi.org/10.3390/ani12151934

Chapter 8: Kumara, S. N., Parkinson, T. J., Laven, R., Yapura, J., & Donaghy, D. J. The influence of energy balance, milk production, and body condition on reproductive performances of spring-calved postpartum dairy cows in pasture-based systems in New Zealand. (Prepared for submission to *Journal of Dairy Science*, August 2022)

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COMMON ABBREVIATIONS

The abbreviations are defined at the first use and then without definition throughout this dissertation. Abbreviations are re-defined in each chapter.

µl	Microlitre
ADF	Acid detergent fibre
ADMD	Apparent dry matter digestibility
AI	Artificial insemination
AOAC	Association of Official Agricultural Chemists
ATP	Adenosine triphosphate
BCG	Bromocresol green
BCS	Body condition score
BHBA	β-hydroxybutyrate
BMPT	Blood metabolic profile testing
BUN	Blood urinary nitrogen
BW	Body weight
CCI	Calving to conception interval
CFSI	Calving to first service interval
CI	Confidence interval
CI	Calving interval
CL	Corpus luteum
CM	Calving month
CoA	Coenzyme A
CP	Crude protein
CPSM	Calving to planned start date of breeding interval
CR	Conception rates
DIM	Days in milk
DM	Dry matter
DMD	Dry matter digestibility

DMI	Dry matter intake
DOMD	Digestible organic matter digestibility
EB	Energy balance
EE	Ether extract
ELISA	Enzyme-linked immunosorbent assay
FSCI	First service to conception interval
FSCO	First service to successful service interval
FSH	Follicle-stimulating hormone
GH	Growth hormone
GL	Gestation length
HF	Holstein-Friesian
IGF-1	Insulin-like growth factor-1
IVD	*In vitro* digestibility
IVDMD	*In-vitro* dry matter digestibility
J	Jersey
JS	Jersey-Sahiwal
LH	Luteinizing hormone
LS	Leaf regrowth stage
LSM	Least squares mean
MMR	Manually mixed ration
ME	Metabolisable energy
MPT	Metabolic profile testing
MS	Milk solid
MY	Milk yield
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre digestibility
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NIRS	Near-infrared spectroscopy

NPD	Non-pregnant dates
NSC	Non-structural carbohydrate
OMD	Organic matter digestibility
P	Parity
P_NP	Pregnant- non pregnant
Peak-Fat%	Fat% per cow at peak milk production
Peak-MS	Milk solids per cow at peak milk production
Peak-Protein	Protein% per cow at peak milk production
PGDM	Pre-grazing dry matter
PGH	Pre-grazing height
PR21	Pregnancy rate in the first 3 weeks
PR42	Pregnancy rate in the first 6 weeks
PREG1	Pregnancy rate to first service
PSMC	Planned start date of breeding to successful service
r^2	Coefficient of correlation
RDP	Rumen degradable protein
RT	Rotation length
SD	Standard deviation
Se	Sensitivity
SEM	Standard error of the mean
SPC	Services per conception
SR21	Submission rate-first 3 weeks of the breeding season
SR42	Submission rate-first 6 weeks of the breeding season
TDMD	True dry matter digestibility
TP	Total protein
TP	Time period
VFA	Volatile fatty acids

CHAPTER 1. GENERAL INTRODUCTION

The dairy industry has enormous potential for contributing to the economies of many communities around the world, and demand for dairy products is increasing rapidly. To cater for this increasing demand, various pathways to improve the productivity of dairy cows have been investigated, mainly targeting cows' diets (Roche et al., 2006; Chagas et al., 2007). Effective management of cow breeding also significantly affects cow productivity (Hossein-Zadeh, 2013) and longevity (Essl, 1998), minimising cow replacement and fertility treatment costs can be minimized in the herd (Essl, 1998). Therefore, good management of nutrition and reproduction plays a significant role in a sustainable dairy industry (Chagas et al., 2007).

Nutrition of dairy cows in New Zealand predominantly depends upon pasture-based diets (Verkerk, 2003). However, this is less common in tropical countries, where by-products and concentrates are used to make mixed rations, particularly when there is a shortage of pasture during dry months (Mendieta-Araica et al., 2011; Yanti & Yayota, 2017). On the other hand, whilst the use of concentrates allows lactation to be extended into a greater part of the year, greater use of concentrates can negatively affect cows' health, milk production and fertility (Chen et al., 2012, 2015).

Increasing the use of non-forage feeds in the dairy industry can also cause the cost of production to increase significantly. Therefore, to be economically sustainable, dairy cow diets are generally adjusted to maximise milk production at minimum cost (VandeHaar & St-Pierre, 2006). Nutrition/feeding recommendations such as those of the National Research Council (NRC) (2001) or Moran (2005) are widely used to balance dairy diets for optimal production. Even so, the use of such recommendations in formulating rations on a day-to-day basis has to take into account the high level of variability in the dry matter, metabolisable energy, crude protein and crude fibre contents of feeds. Thus, frequent monitoring of the nutritional composition of the dairy feeds, including roughages and concentrates, is essential to maintaining the uniformity of mixed rations (Calberry et al., 2003). However, in tropical and/or developing countries, farmers generally do not monitor their dairy rations regularly and, thus, the majority of the dairy cows are at risk of poor nutrition (Moran, 2005).

Providing diets that are balanced in energy and protein to meet nutritional requirements is challenging, especially if cows are not grouped according to their physiological status, as nutritional demands vary widely depending on production and pregnancy status. Grouping is a rare practice in most tropical dairy regions due to a lack of knowledge regarding nutritional requirements, resulting in protein and energy imbalances. These in turn lead to impairments in production and fertility: Ferguson & Chalupa (1989), for example, noted that low energy diets negatively influence ovarian function and reduce follicular development.

The transition period (i.e. from 3 weeks prepartum to 3 weeks postpartum) of the cow is a critical period during which many physiological and behavioural changes take place (Drackley, 1999). Energy balance (EB) during this particular period is one of the key factors in determining both performance and risk of disease (Bauman & Currie, 1980). In transition cows, greater demand for energy combined with low dry matter intake (DMI) can lead to a state of negative energy balance (NEB; Hayirli et al., 2002). The severity, magnitude, and duration of NEB are closely associated with metabolic disorders (Kim & Suh, 2003), reproductive failure (Buckley et al., 2003), and also body condition and milk production. Analysis of blood metabolites is useful to detect presence of NEB, to avoid metabolic disorders during the transition period, and to thereby improve reproductive performance in the next breeding cycle. For this purpose, analysis of blood metabolites such as non-esterified fatty acids (NEFA), β-hydroxy butyrate (βHB), urea, albumin, globulin, glucose, calcium, magnesium and phosphorous are extremely useful (Ndlovu et al., 2007; Chapinal et al., 2012; LeBlanc et al., 2005). Furthermore, metabolic profile testing (MPT) combined with body condition scoring are valuable monitoring tools in large dairy herds (Van Saun & Wustenberg, 1997).

Postpartum reproductive failure is a common problem in dairy cows, resulting in long intervals between successive calvings. Postpartum anoestrus, poor heat detection and poor conception rates are the major issues. Postpartum anoestrus is the major impediment to maintaining the calving pattern required in dairy cows under pasture-based seasonal production systems such as those used in New Zealand (Smith et al., 2005). Anoestrus is mainly driven by genetics and nutrition (Lucy, 2019), especially in high-yielding cows. Thus, the duration of the anoestrus varies depending on nutritional levels at the pre-calving and post-calving stages, through which ovarian follicular development is altered and oestrus is delayed (Smith et al., 2005). Uterine impairments such as endometritis impair fertility both through prolonging the anoestrus period (i.e. through maintaining corpora lutea for an extended period thus inhibiting the hypothalamic-pituitary-ovarian axis: McDougall, 1994; Scott et al., 2011; Dahiya et al., 2018) and through damage to the endometrium that results in worsened embryo survival (Ayalon, 1984; Shelton et al., 1990). Poor oestrus detection due to lack of experience by farmers and cows that do not show overt signs of oestrus at the time of ovulation extend the interval between calving and re-breeding, and contribute to low submission rates (Diskin & Kenny, 2014). Poor conception rates are largely associated with incorrect timing of artificial insemination (AI) (often related to oestrus detection), poor AI techniques, and early embryonic death related to impairment of the pre-ovulatory follicle, failure of maternal recognition of pregnancy, or a poor uterine environment. However, although the pathophysiology of reproductive failure is well-researched in temperate dairy systems, causes are largely unidentified in Sri Lankan dairy production systems (Boettcher & Perera, 2007).

In summary, nutrition is the most significant limiting factor for reproductive efficiency, particularly in pastoral dairy systems. However, for tropical dairy systems in general, and for that of Sri Lanka in particular, there is a significant lack of basic information regarding the nutritive value of available forages and, hence, little information on the nutritive value of the diets provided to dairy cows. Likewise, basic data on the reproductive performance of dairy cows in Sri Lanka are largely absent. Hence, creating a basic database of this information is an essential prerequisite for understanding and identifying deficiencies in the feeding regimen that need correction to maximise milk production and enhance fertility. The studies in this thesis therefore focus primarily on the investigation of the forage components of cows' diets, including the effects of pasture maturity upon its nutritive value; and determining the relationship of these with parameters of milk production and reproduction.

CHAPTER 2. LITERATURE REVIEW

The literature databases searched for this review were Google scholar (https://scholar.google.com) and Web of Science (http://wokinfo.com). Peer-reviewed and non-peer-reviewed scientific articles, conference proceedings, thesis (Ph.D. and masters), and scientific abstracts written in English were considered. Web-based searched terms were dairy, cow(s), postpartum, prepartum, nutrition, reproduction, blood, metabolite(s), reproductive hormone(s), management, feeding, nutrition model(s), interaction, transition, tropical, temperate, Sri Lanka and New Zealand. The literature review focuses on nutrition and its interactions with reproduction in dairy cows managed under tropical (Sri Lanka) and pasture-based (New Zealand) production systems.

2.1 DAIRY MANAGEMENT

2.1.1 Dairy management and management systems in Sri Lanka

Dairy farming is becoming increasingly popular in Sri Lanka as it provides a regular income to farmers and nutritious fresh food to the local communities. The majority of dairy farmers operate small-scale operations (Ibrahim et al., 1999). These farms contain about 70% of the estimated 1.6 million dairy cattle in the country (DAPH, 2017), although, the number of medium-scale dairy operations have increased during the past few years in parallel with the import of cattle from Australasian countries (Desk, 2017; Foresight, 2020; Samaraweera et al., 2020).

Sri Lanka had a target for the dairy industry to increase milk production to achieve up to 50% self-sufficiency by 2015 (Ranaweera, 2011). Nevertheless, although milk self-sufficiency increased rapidly for a few years, from 15-20% in 2009 (Ranaweera, 2011), to 35% in 2015 (Vernooij et al., 2015), and 40% in 2016 (Pathumsha, 2016), Sri Lanka is still behind the goal set for 2015. The main constraint behind this unmet target is lack of knowledge and/or understanding of reproductive and nutritional management by small-scale dairy farmers (Bandara, 2000; Vyas et al., 2020), which translates into poor reproductive performance and the consequential effects on the total milk production per annum.

Feeding systems in Sri Lanka mainly depend on the agro-climatic zones, namely: wet, intermediate and dry zones (Figure 2.1). Large-scale and medium-scale farmers employ more intensive farming practices, whereas the majority of smallholder farmers run semi-intensive or extensive management. Many farmers rear cattle as an integral part of their land-use system, with the animals used to provide fertilizer for crops and to control weeds (Subasinghe & Abeygunawardena, 2013).

Most dairy farms in Sri Lanka have year-round calving, i.e. calving can occur at any time of year. This situation makes it challenging for farmers to monitor their freshly calved cows for reproductive abnormalities during the postpartum period, making it difficult to identify puerperal uterine pathologies such as retained fetal membranes, delayed involution, or endometritis which can result in prolonged calving to conception intervals (Sheldon et al., 2006). Further, providing nutritionally balanced diets for dairy cows in any stage of lactation, especially in the small and medium scale levels, is a considerable challenge due to the limited availability of good-quality forages and/or concentrates.

2.1.2 Dairy management in Kurunegala district

Kurunegala is the biggest district in the intermediate zone, where the main agricultural enterprises include coconut plantations, rice paddy lands and dairy farming (Subasinghe & Abeygunawardena, 2013). Feed resources used for dairy farming are pasture, fodder, crop by-products and formulated cattle feeds. Pasture harvested from uncultivated lands and roadsides, mainly Guinea grass (*Panicum maximum*), along with tree fodders such as *Gliricidia sepium*, are common roughage sources for small-scale dairy farming in this district, although most medium-scale farmers cultivate pastures, mainly hybrid Napier grass (*Pennisetum purpureum*Pennisetum americanum*), on their own land (Premaratne & Premalal, 2006). Feeding concentrates such as brewers-grains, rice bran and formulated cattle feed are also commonly practiced in the Kurunegala district.

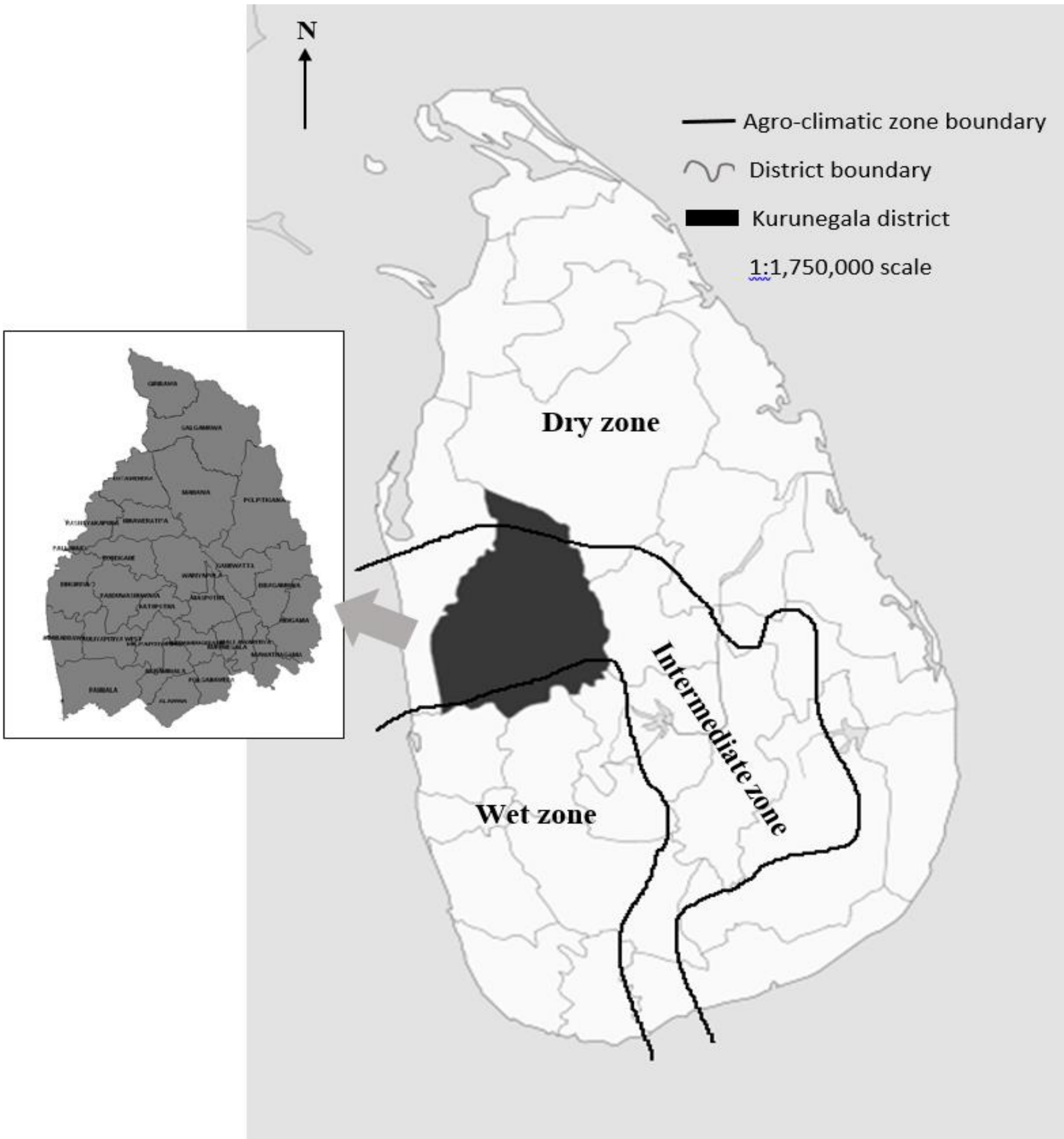

Figure 2.1. Agro-climatic zones in Sri Lanka (Sirisena & Suriyagoda, 2018); magnified area: Kurunegala district.

The Kurunegala district is the main milk-producing region of Sri Lanka (DAPH, 2017). The average total milk production for the district in 2017 was 109,878 L/day, with an average of 2.02 L/cow/day from 46,688 cattle across 22,890 dairy farms (DAPH, 2017). The genotypes of many of the cattle are European crosses (38.54%), Indian zebu and their crosses (21.20%) and locally bred animals (40.24%) (DAPH, 2017). Few cattle herds carry purebred cows or cows with >75% of exotic blood (Ibrahim et al., 1999).

Feeding of dairy cows with off-farm fodder grasses is popular among dairy farmers (Ibrahim et al., 1999), but the priority is given to feeding quantity, irrespective of quality. Indeed, minimum attention is given by farmers to maintaining the quality of forages through proper forage harvesting intervals (Premaratne, 1993). This is exacerbated by the practice of many farmers collecting their dairy forage requirements from different sources, mainly roadsides, with no scheduled rotational cuttings.

There are several limitations to the improvement of dairying in Sri Lanka. Poor nutrition in dairy cows has been identified as the major limitation to increasing milk production (Serasinghe, 2008). Extensive research has been carried out to improve the production of quality pasture among dairying communities; however, it has not been successful in the past due to poor reception by farmers, low awareness of improved forage technology and poor animal production and unavailability of land. Moreover, dairy farming has been further constrained by seasonal fluctuation in the quality and quantity of forages available, depending on the distribution of rainfall (Subasinghe & Abeygunawardena, 2013). However, there is potential for improved pasture and fodder utilization on medium and large dairy farms. Enhancement of a quality fodder supply is the most important issue to be addressed in order to increase domestic milk production (Vernooij et al., 2015).

2.1.3 Dairy management and management systems in New Zealand

The New Zealand dairy industry is an integral part of the country's economy, which contributed $7.8 billion (3.5%) to the total Gross Domestic Product (Ballingall & Pambudi, 2017). New Zealand exports over 95% of the milk it produces, as the country needs less than 5% to be self-sufficient (Stafford & Prosser, 2017). New Zealand is also a key player of the international dairy market, accounting for 30% of the dairy products traded annually (Stafford & Prosser, 2017).

New Zealand milk production is predominantly based on outdoor grazing of mixed pastures (DairyNZ, 2017), and farmers are renowned as "low cost" milk producers (Uribe, 1995). Dairy management depends on the seasonality of pasture production, in which pasture growth during a calendar year (Figure 2.2) is aligned with the nutritional requirements of cows at the different stages of the lactation cycle. In that way, the cost of production can be minimized by transforming as much herbage as possible into milk (DairyNZ, 2017). The pasture utilization is maximized/manipulated by stocking rate, calving date and the drying-off dates of dairy cows, to ensure that feed demand at the different stages of lactation coincides with period/s of the pasture surplus and/or deficits (Uribe, 1995).

Calving

Figure 2.2. Seasonal pasture production (DM/ Kg ha), feed demand and supplementation (Bozinviya, 2019)

Pasture quality and quantity vary seasonally (Machado et al., 2005). Pasture yields are slightly below feed requirements when cows start calving in late winter, therefore, supplemental feed during the calving season is needed to meet nutrient requirements (DairyNZ, 2017). In spring, the quantity and quality of pastures are generally better than in other seasons, and the nutritional needs of lactating cows in peak milk production are matched by grass growth. During late spring, pasture growth exceeds nutritional requirements, and the pasture quality starts to decline. Therefore, under typical pasture feeding systems, the peak milk production of cows is limited to a 3-4 week period. In summer, milk production starts to decline further, falling to ~10 L/day during the late autumn in unsupplemented cows. Cows are dried off in the late autumn, at a date which depends on pasture availability and BCS (Verkerk, 2003). Although spring calving is the most commonly practised system, some production systems have adapted to autumn calving or split (spring and autumn) calving (DairyNZ, 2017). Winter milking followed by autumn calving helps farmers to obtain premium prices for milk, as milk companies pay comparatively more for milk solids, however, the cost of production is high with the use of high levels of supplements during the winter.

New Zealand dairy farmers can be categorized into five production systems (DairyNZ, 2017) according to the levels of feed supplementation and dry cow management (Table 2.1).

Table 2.1. The five production systems; dairy farming in New Zealand

System	Imported feed	Supplements	Dry cows
1	0	Harvested from home farm: fed to milkers only	Managed on home farm
2	4-14%	Harvested from and/or off home farm: fed to milkers	Wintered off home farm
3	10-20%	Harvested from and/or off home farm: fed to milkers	Wintered off home farm
4	20-30%	Harvested from and/or off home farm: fed to milkers	Wintered off home farm
5	≥25-40%	Harvested from and/or off home farm: fed to milkers	Wintered off home farm

2.2 IMPORTANCE OF NUTRITION AND REPRODUCTION MANAGEMENT

2.2.1 Nutrition

A quality-based nutrition management system is essential for modern dairy farming and providing a correctly balanced diet is vital for good health and wellbeing of animals. The diet provides energy, protein, fat, vitamins and minerals for maintenance, growth and production. The amount of nutrients in a diet depends on the quality of the raw materials used. The quality is mainly influenced by the stage of harvesting, the prosperity of the soil and seasonal patterns. In tropical dairy farms, it is difficult to meet the nutritional requirement of dairy cows, as the nutritional inputs are usually of either poor or unregulated quality. However, in temperate regions, milk production has been significantly increased with improved pastures and concentrates.

The types of forages and concentrates used in dairying differ between tropical, subtropical and temperate regions. Legume and tree forages are abundant in the tropics and subtropics, whereas pasture is the main source of feed in temperate countries. In general, the quality of temperate forages is greater than tropical forages, mostly due to lower levels of fibre, and associated higher digestibility. In the tropics, legume and tree forages are not available all year-round, and the time of harvesting differs across management systems, with consequential effects upon digestibility, fibre content, crude protein (CP), and metabolisable energy (ME). The common practice of delaying harvesting time to achieve high yields of forage, a common practice in the tropics (Van Man & Wiktorsson, 2003) results in reduced digestibility, CP, ME and increased dietary fibre.

Several studies have investigated different strategies to feed cows by grouping them according to their stage of lactation, level of milk production and herd size (Chalupa et al., 1996; Contreras-Govea et al., 2015; Cabrera & Kalantari, 2016; Kalantari et al., 2016). Dairy cows in the herd are divided into dry cows, cows in late pregnancy (“close-up” cows), high production, mid-production and low production groups (Chalupa et al., 1996), which helps to optimize their feed intake as nutritional requirements vary between groups. For example, cows in close-up groups and high lactation groups require more feed with a higher nutritive density, than cows in the other groups, as they are utilizing more nutrients for fetal development and milk production, respectively. Diets with a higher nutritive value tend to be more expensive than conventional diets (Chalupa et al., 1996); however, net profit varies with milk production. The essence of good farm management is to provide sufficient nutritional inputs to the dairy cows according to the stage of lactation, whilst also ensuring the cost-effectiveness of the diet in terms of lactational yield and income from milk sales.

2.2.2 Reproduction

Reproductive performance is critical to sustainable pasture-based dairy production systems, such as those which predominate in New Zealand (Parkinson et al., 2019), as calving has to be synchronised with the start of the pasture growth cycle in order to maximise the economic output of milk production (Roche et al., 2000). Maintaining a short, synchronous calving period requires that there is a mean calving interval of ~365 days, which, in turn, means that cows have conceived at an average of 85 days after the last calving (DairyNZ, 2017). This in turn requires that cows are detected in oestrus, presented for insemination and conceive early in the breeding season: targets are that 90% of animals are presented for insemination within three weeks of the start of breeding, 60% conceive to first insemination, and 78% of the herd are pregnant within six weeks of the start of breeding (DairyNZ, 2017).

Achieving these targets requires that cows are well managed during the pre- and immediate post-calving periods. Prevention of metabolic diseases and either prevention or prompt treatment of peri-partum uterine pathology are, of course, essential. (Roche et al., 2000). However, the most important aspect of the management of cows during this period is nutrition. During the postpartum transition, most cows have a decreased dry matter intake (DMI) (Roche *et al*., 2000), at the time when energy demand for lactation is increasing. Moreover, feed availability is limited during the transition period (McCall & Smith, 1998), and the quality of over-wintered pasture is relatively poor. Hence, on forage-based rations, body condition score (BCS) characteristically decreases during early lactation. Furthermore, both the depth and duration of BCS loss negatively affect the interval between calving and the resumption of oestrous cycles (DairyNZ, 2017). Managing feeding over this period of time, particularly managing the duration and extent of loss of BCS, is therefore critical to the sustainability of the annual calving pastoral dairying system. There are, of course, other factors that affect the sustainability of the annual calving cycle: individual cow factors such having a late calving date (McDougall, 2010), BCS at drying off or at calving or anoestrus extending into the breeding season (Rhodes et al., 2003); and, superimposed over all of these is the effect of breed, whereby genetically high-yielding cows are predisposed to extended postpartum anoestrus.

There have been no studies conducted in Sri Lanka on the relationship between energy balance (EB) during the transition period and fertility levels of dairy cows. However, a study conducted by Kollalpitiya et al. (2012) found that mean calving to first service intervals of Jersey, Friesian, Ayrshire and crossbred cows were 83, 104, 89 and 87 days, respectively. The authors also reported that mean calving intervals (CI) of the above breeds were 418, 451, 420 and 443 days, respectively; therefore, calving to first service intervals and CI are relatively high compared to the New Zealand pasture-based system (75 days and 365.2 days; Grosshans et al., 1997).

Proper oestrus detection and timing of artificial insemination (AI) are important for successful breeding. Effective oestrus detection is needed to maintain submission rates during the mating season at an adequate level (Lucy, 2001). There are a number of techniques and equipment available to identify the signs of oestrus in large dairy herds and it is well recognised that oestrus detection rates are higher in systems with concentrated calving patterns (e.g. the NZ pastoral system) than in systems in which calving occurs over a protracted period (Macmillan et al., 1990). However, in Sri Lanka, oestrus detection is poor, mainly due to farmers' lack of education and experience, particularly in the smaller dairy herds.

2.3 TRANSITION AND EARLY LACTATING DAIRY COWS

2.3.1 Negative energy balance

Negative energy balance (NEB) occurs during the first 8-10 weeks postpartum when energy demands for maintenance and lactation exceed the energy intake from the diet (Butler 2005). Cows lose BCS and body weight (BW) during this period since they are utilizing internal body reservoirs (mainly fat deposits) to produce energy for maintenance and milk production. Although this NEB has negative effects on fertility and disease risk (Roche et al 2011), the utilisation of the body energy stores during NEB is a key component of efficient milk production, with selection for milk production resulting in a cow that readily mobilises energy stores to support lactation (Roche et al 2007; Xu et al., 2020).

2.3.2 Transition period

The transition period is recognized as the period from three weeks before to three weeks after calving (Wankhade et al., 2017). The transition period is considered to be a critical physiological stage of dairy cows, because it is marked by the metabolic, nutritional, hormonal, and immunological changes associated with the onset of lactation, and also influences the occurrence of metabolic and infectious diseases (Loiselle et al., 2009; Sundrum, 2015). Good health during the transition period is one of the key determinants of subsequent milk production (Holcomb et al., 2001) and reproductive performance. Cows in the transition period are at a high risk for developing ketosis and mastitis as 'extreme outcomes' of poor transition (Chagunda et al., 2006), with consequent severe economic losses.

Cows in the last three weeks of their pregnancy (also called "close-up dry"), are provided with a feed regime to prepare them for lactation. Energy and protein feeding during the prepartum transition is crucial for fetal and mammary gland development. Transition management is also strategically important to minimise the risk of metabolic and infectious diseases following parturition (Mulligan & Doherty, 2008).

The synthesis of colostrum and milk, and the reconstruction of the uterine tissue following parturition result in a high demand for nutrients, even though at the same time, dry matter intake during this period is considerably lower than later in lactation (Bertics et al., 1992; Grummer et al., 2004). When this combination of factors leads to NEB, the response of the cow is to stimulate the mobilisation of fat reserves, hence, cows lose BCS and BW during this period since they are utilizing body reserves to produce energy for maintenance and milk production (Roche et al., 2007). The effect of NEB on milk production is complex, with milk yield and fat-corrected milk being non-linearly associated with NEB, but milk fat% being positively correlated (Roche et al 2007). In contrast, in relation to subsequent fertility outcomes most of the evidence points to a proportionate effect of NEB, such that the greater or the more prolonged the NEB the greater the negative effects (Roche et al., 2011).

Negative energy balance results in either the presence of increased blood concentrations of non-esterified fatty acids (NEFA) (Doepel et al., 2002), or, if the supply of gluconeogenic substrates is also impaired, the accumulation of β-hydroxybutyrate acid (BHBA) in the blood (Butler, 2005; Nydam et al., 2013). Various metabolic and infectious disorders can occur when dairy cows fail to adapt to these metabolic changes. Dairy cows during transition are vulnerable to metabolic diseases such as hypocalcaemia, ketosis, fatty liver syndrome and displacement of the abomasum (Melendez & Risco, 2005; Wankhade et al., 2017). Managing calcium flows is important as hypocalcaemia can result in significant morbidity or mortality of affected animals (Bindari et al., 2013). Prevention of hypocalcaemia can be relatively straightforward: decreasing the calcium intake in close-up cows assists the parathyroid gland to activate the mechanisms responsible for maintenance of blood calcium concentration, but requires that farmers understand the condition well enough to institute preventive measures (Abdelhameed, 2016). Ketosis and fatty liver syndrome are less common conditions during early lactation, in which low glucose concentrations and high amounts of ketone bodies in the circulation are caused by increased fat mobilization, usually in over-conditioned cows. Starvation ketosis occurs when emaciated animals are continually underfed as residual tissue stores are mobilised.

2.3.3 Early lactation

During early lactation (i.e. the first 100 days of lactation), nutritional requirements, DMI, BW and milk production change significantly (Figure 2.3).

Figure 2.3. Phases of lactation, changes in body weight, milk production and dry matter intake of lactating dairy cows (The Cattle Site, 2020)

The ability to partition available energy for milk production in early lactation has made EB a key determinant of milk production, reproductive performance and occurrence of disease (Bauman & Currie, 1980; Kim & Suh, 2003). Likewise, NEB has adverse effects on metabolic diseases, reproductive performance and immune function (Cooper, 2014) as, for example, cows with greater NEB have prolonged anovulatory periods, which progress to lower pregnancy rates during the breeding season (Lucy et al., 1992). Conversely, Patton et al. (2006) found that earlier resumption of cyclicity was associated with increased DMI during early lactation. Therefore, feeding management for improving EB should increase both fertility and farm profitability.

2.4 NUTRITIONAL REQUIREMENTS OF DAIRY COWS

2.4.1 Protein requirements

Protein requirements of dairy cows are influenced by body size, level of milk production, body growth and the stage of pregnancy (Moran, 2005). The protein content of a cattle diet can be estimated as dietary feed CP (CNCPS, 2016). Crude protein, which is obtained from the total quantity of N in the diet, is a simplistic estimate of the protein content of the diet, since it is a combination of non-protein nitrogen (NPN; e.g. nitrate, urea), rumen degradable protein (RDP) and rumen undegradable protein (RUP) (NRC, 1989). Rumen degradable protein, as its name implies, is degraded in the rumen to ammonia and carbon-skeletons, and the nitrogen (together with nitrogen from NPN) is then used by rumen microbes for microbial protein synthesis. Rumen undegradable protein is not degraded in the rumen but is passed to the intestines for digestion into amino acids. Additionally, the proteins synthesized by rumen microbes are also passed to the intestine for digestion (Tedeschi et al., 2015; Putri et al., 2019). For high-yielding cows, the RDP: RUP ratio (NRC, 1989, 1996; CNCPS, 2016) is important for calculating their true protein requirements (Savari et al., 2018), but for animals with low or modest yields, in which protein is unlikely to be the limiting nutrient, CP is adequate. The CP requirements for dry and lactating cows as a percentage of the diet are 10-

12% and 14-18% respectively (NRC, 2001; Moran, 2005), whereas high yielding cows require that the overall protein content, and the ratio of RUP to other protein sources, is higher.

2.4.2 Energy requirements

Lactating dairy cows need energy for maintenance, activity, milk production, pregnancy and gaining body condition (Moran, 2005). During the prepartum period, energy is required for maintenance, fetal growth and mammary tissue development. After the onset of lactation, the cow requires energy primarily for maintenance and milk production (Remppis et al., 2011). However, feed intake decreases around parturition (NRC, 2001), so, providing more energy (or greater energy density) during the period between the late dry period and early lactation is essential for the establishment of an effective lactation.

Energy needs for maintenance includes general homeostasis (Moran, 2005), including the balance between body heat production and heat loss due to environmental conditions (Fox et al., 1995). Maintenance energy requirements are influenced by milk production, body weight, ambient temperature and the tissue and external insulation (Fox et al., 1995). Energy allowance for activity includes grazing and eating and walking to grazing from the milking parlour (Moran, 2005). Energy needs for fetal development in pregnant animals depends on the stage of pregnancy, as advanced pregnancies need substantially more energy than do mid or early pregnancies (NRC, 1989; Fox et al., 1995). Energy requirements for milk production are proportional to the volume of milk production, and fat and protein composition of the milk (NRC, 1989, 2001; Fox et al., 1995). Energy for growth is considered to be important for heifers, but not for mature cows as they have already achieved the maximum size. Thus, the stage of growth, weight, and rate of gain are all influencing factors when calculating energy requirements for growth (Fox et al., 1995). On the other hand, mature cows need energy for regaining body condition, which in turn, depends on the body fat reserves, stage of lactation and milk production (Fox et al., 1995). Finally, an additional amount of energy (~10% of the energy requirements for maintenance) is needed for cows reared in South-East Asian countries to cover energy loss for climatic/heat stress (Moran, 2005).

2.4.3 Dry matter requirements

The energy expenditure of lactating cows' peaks at 4-8 weeks postpartum, whereas DMI does not peak until ~10-14 weeks. Dry matter intake varies between 2.5% and 4% of a cow's BW over lactation. It is a function of body size and milk yield such that, at peak lactation, there is a good approximation between energy intake and requirements. The lag between intake and requirements in early lactation represents the period when rumen size and functional capacity are increasing, and liver function is becoming maximal (Jouany, 2006). To meet the energy requirements for early lactation, therefore, either the energy density of the ration has to be increased, or the cow has to mobilise body fat (and other energy stores) (Komaragiri et al., 1998; Weber et al., 2013). The energy density of the cows' ration can be predicted using the ratio of required net energy for lactation (NEL) (in which NEL includes energy requirements for maintenance, milk yield and replenishment of lost BW: NRC, 2001) and the DMI. During the first three weeks postpartum, DMI is suppressed by up to 9% below predicted values (i.e. from BW and lactation yield alone); and is further suppressed by 0.02 kg DMI/100 kg of BW for each 1% increase in moisture content of diets above 50% when fermented feed is provided. An increase in DMI of 1 kg/day can support an additional 2 to 2.5 kg of milk, therefore, higher DMI is desirable to enhance milk production and improve body condition (McNamara et al., 2003). Thus, feed intake is a key factor in sustaining high milk production and ensuring adequate energy intake. Strategies therefore have to be taken to maximize the DMI during early lactation. The NRC recommends feeding early lactation cows *ad libitum* during the first six to eight weeks postpartum, followed by feed based on the calculated energy requirement for lactating dairy cows. Changes of DMI over lactation are shown in Figure 2.4.

Figure 2.4. Changes in dry matter intake of lactating dairy cows (NRC, 2001)

2.4.4 Fibre requirements

Dietary fibre is important for effective rumen function and cow health. Fibre requirements are assessed based on the neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions of the final diet. The minimum requirements of NDF and ADF for dairy cows are 25% and 19% of dry matter (DM), respectively (NRC, 2001; Moran, 2005). Allen (1996) stated that the optimal level of NDF that can maximize the energy intake of early lactating dairy cows ranges from 25-35% of DM. The fibre requirements differ between pasture-based systems and total mixed ration based or highly supplemented systems. DairyNZ (2017) recommended a minimum NDF content of 35% and 27% (DM basis) for New Zealand dairy cows grazing high-quality pastures or diets high in starch feeds, respectively. Less dietary fibre and/or high starch diets can cause subacute rumen acidosis (Moran, 2005; DairyNZ, 2017), which may progress to displaced abomasum and/or lameness (Allen, 1996). Effective neutral detergent fibre (eNDF) is the term used to describe the NDF fraction that is most effective at stimulating rumination and saliva production (DairyNZ, 2017), therefore, feeds with high eNDF are important for maintaining rumen pH and increasing DMI (Allen, 1996). Effective neutral detergent fibre can be increased by increasing the forage proportion of the diet and/or increasing forage chopping length (Yang & Beauchemin, 2009).

2.5 ASSESSMENT OF NUTRITIONAL AND /OR FERTILITY STATUS OF DAIRY COWS

2.5.1 Body Condition Score

Body condition score is a useful management tool to assess nutritional status of dairy cows (López-Gatius et al., 2003). Condition scoring is commonly practised during the transition period and in early lactation when dairy cows are most prone to nutritional challenges. Further, BCS allows farmers to identify sentinel cows in early stages of BCS change, so that effective nutritional management can be applied to mitigate the losses, making the application of BCS a useful management tool for dairy farmers (Roche et al., 2004).

There are multiple different BCS systems (Bewley et al., 2010). In the United Kingdom and United States, BCS scales are usually 1-4 or 1-5 (Lowman et al., 1976; Mulvany, 1981; Wildman et al., 1982; Edmonson et al., 1989), whereas 1-8 and 1-10 scales are used in Australia and New Zealand (Roche et al., 2009). However, irrespective of the range of scores, all BCS systems are designed to reflect the distinction between thin and fat, regardless of the size of the cow (Stockdale, 2001). The amount of body fat over the rib cage, backbone, hips, around the base of the tail, and the transverse processes of the lumbar vertebrae (short ribs) are determined during BCS assessment (Vasseur et al., 2013). There is a strong positive relationship ($r^2 = 0.86$) between BCS and the proportion of physically dissected fat in Friesian cows (Stockdale, 2001). Cows that are thin during the early postpartum period do not have enough energy reserves to support high milk production (De Vries & Veerkamp, 2000), whereas cows that are fat at parturition are vulnerable to metabolic disease and infertility during the next cycle. Therefore, BCS as an indicator of fat reserves in the body helps to monitor cows for better milk production and fertility.

2.5.2 Metabolic profile testing

The metabolic profile test (MPT) was first established in early 70's (Payne et al., 1970) and was widely practiced thereafter in the UK for assessing the nutritional status of dairy cows. The MPT is a useful tool for the diagnosis of metabolic and nutritional diseases in dairy cows on a herd basis before the onset of any clinical manifestation of those impairments (Puppel & Kuczynska, 2016; Madreseh-Ghahfarokhi et al., 2018). Metabolic profile testing can be used to improve feeding management in dairy cows and thereby optimize milk production. The MPT, in conjunction with body condition scoring and ration evaluation, is an extremely useful tool for nutritional assessment of dairy herds (Van Saun & Wustenberg, 1997). Under intensively managed farming systems, the MPT is a useful tool to check that diets are correctly balanced and to predict future milk production and fertility outcomes. However, the use of MPT is not common in developing countries due to scarcity of laboratory facilities.

Cows experience metabolic disorders when there is a shortage of energy precursors or major minerals in the body, such that most metabolic disorders therefore occur during the first 3-4 weeks postpartum (LeBlanc et al., 2006; Vergara et al., 2014). Hypocalcaemia, fat cow syndrome, ketosis, subacute rumen acidosis and displaced abomasum are (sub)clinical disorders which peak during this particular time period (Vergara et al., 2014). Thus, subclinical or clinical hypocalcaemia is a disorder in very early lactation or/and peak lactation (Neves et al., 2018); fat cow syndrome occurs in over-conditioned cows at the time of calving (Epperson, 2005); subclinical ketosis is a common disorder in early lactating cows when DMI is inadequate; subacute rumen acidosis is very common where the ME:NDF ratio of the ration is inappropriate; and hypoproteinaemia is common where either dietary protein or energy is inadequate.

2.5.2.1 *Non-esterified fatty acids (NEFA)*

Non-esterified fatty acids are directly associated with energy imbalance in dairy cows and are an effective measurement to assess the level of NEB and metabolic diseases in early postpartum cows (Leslie et al., 2004; Oetzel, 2004).

Fat in adipose tissues of early postpartum dairy cows is mobilized in an attempt to balance overall energy demands. Ruminants mobilize fat by de-esterification of triglyceride stores rather than by the release of triglycerides into the blood. The amount of adipose tissue breakdown is thus directly reflected by the concentration of NEFA. Excessively high NEFA concentrations can be associated with NEB or fatty infiltration of the liver, which, in turn, are both associated with a higher incidence of metabolic diseases. Circulating NEFA is absorbed and metabolised by the liver and other tissues via mitochondrial oxidation, which provides the ATP needed for gluconeogenesis (Bell, 1995). An alternate pathway for hepatic oxidation (mitochondrial oxidation) of NEFA occurs in peroxisomes (Drackley, 1999). Further, peroxisomal oxidation may play a role as an overflow pathway to oxidize fatty acids during extensive NEFA mobilization (Drackley, 1999).

The distribution of NEFA into the blood stream provides energy to tissues throughout the body; however, in excess, NEFA itself may become toxic (Emery et al., 1992). When the capacity of the liver to metabolize NEFA into triacylglycerol (TAG) is limited, the excess NEFA is oxidized or exported as very low-density lipoprotein (VLDL). Further, TAG accumulates in the liver once its ability to metabolise TAG is reached. Consequently, acetyl CoA produced from oxidation of fatty acids cannot be utilized in the tricarboxylic acid cycle (TCA; Figure 2.5) to produce citrate. Thus, metabolism becomes directed towards the production of ketone bodies such as acetone, acetoacetate and BHBA (Nelson & Cox, 2004). Further, metabolic effects of NEB due to an imbalance in C2/C3 nutrient ratio result in low plasma glucose and insulin concentrations and high concentrations of plasma NEFA, BHBA, acetone, acetoacetate and liver TAG (Van Knegsel et al., 2007). Under such circumstances, NEB can become self-perpetuating (Remppis et al., 2011).

Figure 2.5. Hepatic lipid metabolism; Gluconeogenesis (A), tricarboxylic acid (TCA) cycle (B) and ketogenesis (C) (Reece & Rowe, 2017)

Plasma NEFA concentrations start to increase a few days before parturition, are high around parturition, and become elevated above prepartum values during the first two weeks postpartum (Drackley, 1999). Thereafter, NEFA serum concentrations tend to decrease as lactation progresses beyond its peak (Adewuyi et al., 2005; Cozzi et al., 2011). Non-esterified fatty acids concentrations are relatively low in mid lactation because EB becomes positive and cows restore the mobilized tissue reserves (Walters *et al*., 2002).

Although a certain amount of NEFA and BHBA are normal during early lactation, excessive amounts can lead to increased risk of disease, and decreased reproductive and productive performances (Ospina et al., 2010). The correlation of serum NEFA with EB is strongest in the post-calving transition period. Moreover, controlling energy intake during the dry period might be advantageous for the energy status of dairy cows after calving, whereas energy restriction in early lactation leads to metabolic stress (Urdl et al., 2015). Blood NEFA values ≥0.7 mmol/L are considered indicative of severe NEB (Whitaker, 2004).

2.5.2.2 β-hydroxybutyrate acid (BHBA)

β-hydroxybutyrate acid is one of the measures indicating EB of transitional and early lactating dairy cows. When the cow is in significant NEB, concentrations of BHBA in blood increase, indicating metabolic stress (Li et al., 2016). β-hydroxybutyrate, acetone, and acetoacetate are intermediate metabolites of oxidation of fatty acids. Increased mobilization of body tissues in early lactation is generally associated with an increased production and concentration of ketones (Ingvartsen & Andersen, 2000). Blood ketone concentrations are elevated in association with poor carbohydrate status only when concurrently associated with NEB. Measuring BHBA concentration is most useful within the first month post-calving, particularly in the first two weeks postpartum (Ingvartsen & Andersen, 2000).

Suggested concentrations of BHBA vary, for example, Whitaker et al. (1983) suggested that concentrations should be <1.00 mmol/L and <0.60 mmol/L for early lactation and close-up dairy cows, respectively, while Kraft & Durr (2005) suggested that values should be <0.85 mmol/L in lactating cows. Raised concentrations of ketones can cause reduced DMI and milk production (Goff et al., 1987). McNamara, et al. (2003) suggested that BHBA may be a more useful indicator of energy balance and also of BCS and BW loss than either glucose or NEFA.

2.5.2.3 Blood Urea

Non-protein nitrogen and most protein in ruminant diets are digested in the rumen to produce ammonia, which can subsequently be incorporated into microbial protein. The maximum quantity of microbial protein production takes place when the ratio of available energy to protein is optimized. A surplus of nitrogen relative to energy in the rumen results in high rumen ammonia concentrations (Abdoun et al., 2006). Excess rumen ammonia then passes across the rumen wall into the bloodstream before being converted to urea in the liver. Deamination of amino acids during post-rumen digestion and systemic protein turnover also results in elevated concentrations of urea in the liver. The urea is excreted in the urine or re-enters the rumen by diffusion or via the saliva (Hammond, 1997), or equilibrates with milk.

Urea concentration is a good indicator of long-term intake of dietary protein (Kida, 2003). The highest blood urea concentrations can be seen several hours after feeding (Hammond, 1983), and changes in milk urea follow changes in blood urea concentration by about one to two hours (Gustafsson & Palmquist, 1993). Blood urea concentration is also considered as an indirect measure of rumen ammonia concentration. Thus, valuable information in relation to dietary protein content and utilization can be derived from herd urea determinations (Shapiro & Lusis, 2001).

In the United States where much of the research on urea has been conducted, urea concentration is often reported as urea nitrogen concentration. The average blood urea nitrogen (BUN) concentration of lactating dairy cows in the Unites States is 15 mg/dL (equivalent to~5.4 mmol/L urea) (Roseler et al., 1993). When the BUN of high-producing dairy cows is <15 mg/dL, it indicates a relative deficiency of dietary protein (Hammond, 1997), with BUN concentrations of >20 mg/dL indicating high dietary protein intakes, which has, in turn, been associated with alterations to the uterine environment and reduced fertility (Butler et al., 1996). However, not all studies have shown an effect of high protein intakes on fertility (Carroll et al., 1988), with Roche et al (2011) concluding that, despite pasture-based dairy cows having much higher plasma urea concentrations than housed cattle, "current data do not indicate a reproduction benefit to reducing dietary protein in pasture-based systems".

Urea concentrations in normal cattle generally reflect the absorption of ammonia from the breakdown of nitrogen-containing compounds in the rumen. Increased RDP will lead to increased plasma urea concentrations (Roseler et al., 1993; Baker et al., 1995). However, the relationship is not exact as other dietary parameters such as dietary amino acid composition, and dietary carbohydrate intake also determine the rate of ammonia release and capture (and thus plasma urea concentration) , as well as animal factors including liver and kidney function and rate of muscle tissue breakdown (Eicher et al.,1999).

2.5.2.4 Albumin

Albumin is closely related to the amino acid availability in the blood. Albumin has a relatively short half-life, so can reflect protein deficiency problems over a period of a month or two (Van Saun, 2008). Albumin concentrations have been associated with postpartum diseases in dairy cows and, according to Van Saun (2004), albumin may be used to predict disease risk in close-up dry and fresh lactating cows. Fresh cows with ≥35 g/L serum albumin are less likely to have postpartum disease, while serum albumin concentrations ≤32.5 g/L in close-up dry cows were associated with a three-fold higher risk of postpartum disease (Van Saun, 2008). However, albumin concentrations have to be interpreted with respect to the stage of lactation and with respect to urea concentrations: fresh cows generally have low urea and albumin concentrations (Van Saun, 2008; Van Saun & Davidek, 2008), but relatively low plasma urea concentrations (<7.1 mmol/L) can be accompanied by normal albumin concentration (>35 g/L) in the early dry period when a dairy herd has protein deficiency.

2.6 BLOOD HORMONAL ASSAYS; USEFUL TOOLS TO DETECT FERTILITY STATUS OF DAIRY COWS

2.6.1 Insulin-like growth factor-1

Insulin-like growth factor-1 (IGF-1) was first identified by Rinderknecht & Humbel (1978). It is primarily produced in the liver, in response to hypothalamic growth hormone (GH). Insulin-like growth factor-1 stimulates the uptake and utilization of glucose in adipose and muscle tissues, which results in decreased blood glucose concentrations (Perez-Martin et al., 2003). However, just prior to calving, IGF-1 production goes down rapidly as a response to GH receptor inhibition in the liver (Zulu et al., 2002). Thus, the negative feedback on GH secretion of IGF-1 is reduced, allowing GH concentrations to rise. Consequently, gluconeogenesis and lipolysis are increased in the liver and adipose tissues (Zulu et al., 2002).

Insulin-like growth factor-1 is a key regulator for growth and lactation in cows (Renaville et al., 2002). It also acts as an intra-ovarian autocoid, such that ovarian IGF-1 and its binding proteins are local regulators of follicular growth, having direct effects and mediating responses to gonadotrophins (Perez-Martin et al., 2003). Thus, IGF-1 influences and amplifies the effect of follicle stimulating hormone (FSH) and luteinising hormone (LH) on the growth and differentiation of ovarian follicles into tertiary or dominant follicles (Lucy, 2000). Much of the IGF-1 in the follicular fluid of the ruminants is derived from the circulation, but in many other species, it is derived from follicular granulosa cells synthesis (Pushpakumara et al., 2002). Concentrations of IGF-1 during the mating period are associated with fertility, such that if the IGF-1 concentrations during the postpartum period are low (e.g. due to poor nutrition), animals tend to have impaired ovarian function (Taylor et al., 2004). Spicer et al. (1990) demonstrated a positive correlation between EB and IGF-1 concentrations, and between EB and progesterone concentration. Luteal activity during the postpartum period (when NEB is ~7 MJ/day) is reduced when the IGF-1 concentration is low (Spicer et al., 1990). Furthermore, FSH itself is unable to support normal follicular recruitment when IGF-1 concentrations in the systemic circulation are low (Schoppee et al., 1996).

2.6.2 Progesterone

Progesterone is the main reproductive hormone of pregnancy. Progesterone is initially produced solely in the corpus luteum (CL). After maternal recognition of pregnancy occurs, the CL remains active in the ovary for the duration of pregnancy, but luteal sources are supplemented by placental sources of progesterone until late in gestation. The CL breaks down at the end of the luteal phase of the oestrous cycle and at the onset of parturition, in response to uterine prostaglandin $F_{2\alpha}$ (Wiltbank et al., 2014; Robinson & Noakes, 2018). Progesterone concentrations are high between Days 4 and 18 of the normal oestrous cycle (Gomes & Erb, 1965; Forde et al., 2011; Robinson & Noakes, 2018). One key reproductive problem in cattle is premature luteolysis (before day 16), which usually results in early embryonic death. Interestingly, high yielding cows are more likely to experience such premature luteolysis than animals with lower yields: it has been argued that this occurs due to either inadequacy of the pre-ovulatory follicle or to inadequate IGF-1 support of the CL (Wathes et al., 2003; Robinson et al., 2008). Persistence of the CL is also an important reproductive problem. If the CL does not regress at the end of the luteal phase as, for example, when endometritis impairs $PGF_{2\alpha}$ secretion, the CL can be maintained for a long period, resulting in anoestrus (Kumar et al., 2014).

Exogenous progesterone can be administered in different ways (injections, intra-vaginal devices, implants) for treating irregularities of the oestrous cycle. It can be used in combination with other hormone treatments (e.g. FSH, $PGF_{2\alpha}$) as a means of synchronising oestrus as a tool for reproductive management. Furthermore, progesterone supplementation can be used to augment progesterone concentrations (Van Cleeff et al., 1991) as a means of decreasing fetal loss: presumably by enhancing embryo development and thus reducing premature luteolysis prior to maternal recognition of pregnancy.

2.7 INTERACTION BETWEEN NUTRITION AND REPRODUCTION IN DAIRY COWS

Poor fertility of dairy cattle has many causes (managemental, environmental, specific or nonspecific infectious or non-infectious disease) (Ferguson, 1991; Roche, 2006; Roche et al., 2011); however, good nutrition is crucial for optimal reproductive performance (Ferguson, 2005; Roche, 2006; Roche et al., 2012). Many nutritional factors have been identified as interfering with the reproductive performance of dairy cattle. Of these, insufficient energy and/or protein and/or poor feeding management are the most important (Ferguson, 1991; Roche, 2006), with deficiencies of specific trace elements and vitamins, deficiencies of specific nutrients (e.g. amino acids) and toxins all being less important (Ferguson, 1991) (Table 2.2).

Table 2.2. Nutrient deficiencies and excesses and reproduction failure (Ferguson, 1991)

Reproductive failure	**Deficiencies**	**Excesses**
Anoestrus Reduced oestrous expression	Energy Crude protein Vitamin A Iodine Manganese Phosphorus	Fluoride
Low conception Early embryonic death	Energy Crude protein Manganese	
Increase peri-parturient diseases leading to delayed involution Retained fetal membranes	Selenium Vitamins A, D, E Phosphorus Magnesium	Excess body reserves Calcium
Abortion Stillbirth Weak calves	Energy Vitamins A, D, E Crude protein Selenium Calcium Phosphorus	

Animal nutritionists and geneticists have focused on increased nutrition and genetic capability, respectively, for improving milk production. However, increased milk production has also been associated with infertility (Butler, 1998; Butler, 2000; Roche et al., 2011), to the extent that Butler (2003) considered that the conception rate of Holstein Friesian (HF) dairy cows had become inversely proportional to their milk production (Figure 2.6). The causes of this relationship have long been argued. On one hand, better feeding can reduce the occurrence of NEB during early lactation, which should improve fertility. Indeed, many studies in the mid-20th century showed parallel increases in fertility with improved feeding (Beever, 2006; Thatcher et al., 2006; Grummer, 2007). On the other hand, high yield that is unmatched by improved feeding has the opposite effect, due, at least in part, to the effects of NEB (noting also genetic effects upon the IGF-1 axis). The adverse effects of NEB are probably mediated through the impairment of LH secretion that occurs in animals that are either hypoglycaemic or hypoinsulinaemic. Hypoinsulinaemia is itself promoted by the massive drain of glucose into the udder for milk synthesis. Hypoinsulinaemia and insulin resistance are also promoted through the raised concentrations of NEFA that occur during NEB. Furthermore, insulin itself is a cofactor for follicular development. It is thus clear that NEB impairs fertility outcomes through several routes. The consequence is that both pulsatile LH sectreation and ovarian responsiveness to LH are impaired by NEB, resulting in dominent follicles that fail to ovulate. Some of these become non-ovulatory dominant or cystic follicles (Butler, 2000, 2003). These conditions may delay the first ovulation to 40-50 days postpartum (Butler, 2000), which can severely affect the time to resumption of oestrous cycles. Since the EB of dairy cows cannot be directly measured in the field, the importance of using diagnostic tools such as BCS or MPT is emphasised in the effective management of the postpartum period (Butler, 2000).

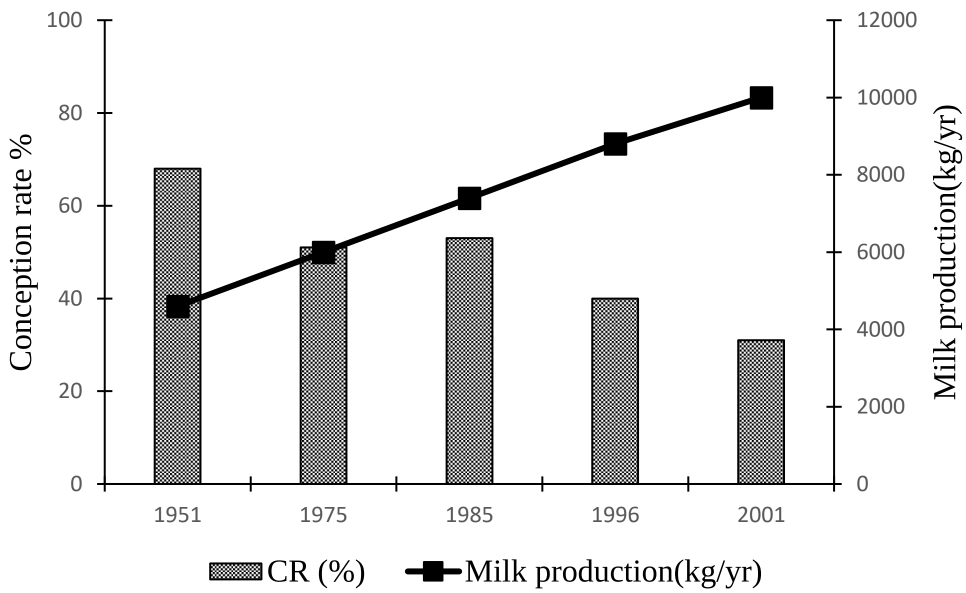

Figure 2.6. The relationship between conception rate (CR%) and annual milk production of HF cows in New York (Butler, 2003)

2.8 NUTRITIONAL MODELS AND THEIR USE IN DAIRY CATTLE

Nutritional models were developed to integrate knowledge of feed quality, feed intake, digestion and microbial growth efficiency in order to better estimate animal requirements (Tedeschi et al., 2005). These models are commonly used for prediction of milk yield and/or growth under different production systems (e.g. management, climate, breeds and feed rations) across the world (Alderman et al., 2001; Tedeschi et al., 2013). These models have principally been developed for high input systems where they are used to maximise milk production based on purchasing feed. In lower input systems, such as those which predominate in tropical/semi-tropical systems and low-income countries, the focus of modelling is less on maximising milk production, but more on optimising milk production by making best use of available feed sources (Oosting et al., 2014).

The codification of nutrient allowances for dairy cattle commenced in 1945 with the publication by the National Research Council (NRC) of the Nutrient Requirements of Dairy Cattle (NRC, 1945) which had detailed descriptions of nutritional requirements for dairy cows. The latest version of these recommendations (NRC, 2001), is still a primary source for data on nutritional requirements of dairy cattle. Ration adequacy is evaluated by comparing the nutrients supplied by the diet with the nutrient demands for milk production, body maintenance, growth, activity and pregnancy.

These collated requirements have been used to develop models which can be applied to predict productivity given the current feeding regime and to formulate rations for a required level of production. A large number of such models have been developed, such as the Large Animal Nutrition Model (LRNS), Cornell Net Carbohydrate and Protein System (CNCPS) and Ruminant (Fox et al., 1992; Russell et al., 1992). The benefit of these programmes is that they can be updated with new information on a regular basis (Eastridge et al., 1998).

For the research covered by this thesis, the most important of such models is that developed by Moran (2005). This model was designed as a feeding management and dairy advisory guide for smallholder dairy farmers in the humid tropics. It was aimed at creating balanced diets for dairy cattle using topical forages and a limited amount of supplementary feed. It is widely used as a feeding guide across South-East Asia as it discusses cost effective feeding and the benefits and the drawbacks of various feed components (Moran, 2005, 2017).

2.9 IMPROVING THE NUTRITION OF SRI LANKAN DAIRY CATTLE USING NUTRITIONAL MODELS

Tropical roughages play a crucial role in the diet of Sri Lankan dairy cattle. However, the quality of roughages is extremely variable because of variability in harvest times, with many forages harvested at an overly mature stage, adversely affecting NDF, ADF, CP and ME concentrations and, hence, DMI (Kuoppala et al., 2009). Different fodder species perform differently according to the soil conditions, therefore, selecting the best fodder species in terms of quality and the high DM yield per cutting is important for sustainable dairy production systems (Tessema et al., 2010). Nevertheless, developing a nutritionally balanced diet with existing feed materials is challenging. Nutritional modelling can be used to create optimal diets based on tropical forages and supplements which meet daily nutritional requirements, thus support milk production and reproduction of dairy cows.

2.10 SUMMARY AND SCOPE OF THE INVESTIGATION

It is clear from the literature review that the nutrition of dairy cows is a critical factor determining productivity and reproductive performance of dairy cows. Cow, farm and management-related factors influence energy, protein and DM intakes, so the impact of these changes on milk production and reproduction needs to be assessed to optimise cow productivity.

The review also indicates that extant research on dairying in Sri Lanka is limited, and that the effects of nutrition on fertility of dairy cows in Sri Lankan medium-scale farms have yet to be investigated. Current milk production per cow is clearly suboptimal, largely due to inadequate and/or low-quality feeding and poor reproductive management.

To address these gaps in knowledge, this thesis focused on the quality and usage of feeds (forages and supplements) that are currently used in Sri Lanka and, thereafter, to interpret the effectiveness of these feeds to meet animal requirements for production and reproduction. Data are also lacking on the effects of pasture herbage management on the herbage quality and consequent DMI, energy balance, milk production and reproductive outcomes in the context of (sub)tropical dairy production. As circumstances precluded studying this directly in Sri Lanka, parallel evaluations were conducted in New Zealand, based on the general hypothesis that cow productivity would be affected by pasture herbage management and also energy balance.

The main objectives of this thesis were to:

1) Investigate nutritional composition of major feed types in Sri Lanka, and the practice of feeding manullay mixed ration (MMR); with particular reference to the extent to which such rations met cows' DMI, CP and ME requirements (Chapter 3)
2) Investigate nutritional status and its interaction with lactation using metabolic profiles in cows over the prepartum transition to the mid-lactation periods, with particular reference to the effectiveness of MMR feeding regimens in meeting cows' nutritional requirements (Chapter 4)
3) Investigate factors influencing reproductive performances of dairy cows over the postpartum transition to mid-lactation, using progesterone and IGF-1 profiles, with particular reference to the effectiveness of using cows' hormones profiles to monitor reproductive efficacy (Chapter 5 and 6)

4) Investigate factors affecting pasture herbage quality across dairy farms in New Zealand having varying pasture management, thereafter, the effect of DMI and ME intakes that are achieved by dairy cows on EB, and subsequently on milk production, BCS and reproduction (Chapter 7 and 8).

CHAPTER 3: A NUTRITIONAL INVESTIGATION OF MAJOR FEED TYPES AND FEED RATIONS USED IN MEDIUM-SCALE DAIRY PRODUCTION SYSTEMS IN SRI LANKA

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Accepted for publication in *Animals*, August 2020

3.1 ABSTRACT

Little information is available regarding the feeding of dairy cattle in Sri Lanka or the impact of feeding on productivity. Nutritional quality, digestibility and the chemical composition of major feed types; and the use of these feeds in rations by medium-scale dairy farmers in the Kurunegala district of Sri Lanka were studied. Nine dairy farms were visited fortnightly over a 5-month period to identify feeds in regular use. All farms operated under a stall-feeding system, in which a manually mixed ration (MMR) was fed 2-3 times daily. Four forages; Guinea grass ecotype A (*Panicum maximum*): Guinea grass, Hybrid Napier CO-3 (*Pennisetum purpureum* × *Pennisetum americanum*): CO-3 grass, Gliricidia (*Gliricidia sepium*) and maize stover (*Zea mays* L.), along with three other supplementary feeds (maize silage, barley distillers' by-products, and commercially formulated cattle feed) were identified. These feeds were subjected to proximate analysis and *in vitro* digestibility analysis. Metabolisable energy (ME) of forages ranged from 7.5-10.0 MJ/kg dry matter (DM), with the ME of Guinea grass and CO-3 grass (7.5 and 8.0 MJ/kg DM, respectively) being lower than that of Gliricidia (10.0 MJ/kg DM). The neutral detergent fibre (NDF) of Guinea grass and CO-3 grass (both 72% DM) was also much higher than that of Gliricidia (47% DM). Crude protein (CP) was higher in Gliricidia (17.5% DM) than in either Guinea grass or CO-3 grass (8.0 and 8.8% DM, respectively). The ME of the supplementary feeds varied between 11.0 and 12.8 MJ/kg DM, and CP varied between 15.0 and 24.0% DM. The daily ME intake of cows was consistently 10% lower than their calculated daily energy requirement; for dry cows the mean deficit was 90 MJ/cow/day supplied vs. 101 MJ required, and for cows in early lactation the mean deficit was 126 MJ/cow/day supplied vs. 140 MJ required. The average CP intake of lactating cows (13.5% DM) was inadequate (requirements: 16 to 17.5% DM), but the average CP intake of dry cows (11.8% DM) was satisfactory (requirements: 11 to 12% DM). The current study shows that the majority of feed types used in these medium-scale dairy farms

provide insufficient ME or CP to meet the requirements of either lactating or dry cows and that, irrespective of the quantity of feed provided, cows would be unable to achieve their nutritional requirements.

Keywords: cows, forages, energy, protein, tropical dairy farming

3.2 INTRODUCTION

Rapidly increasing population and burgeoning per capita demands for animal protein in Sri Lanka has resulted in escalating demands for livestock products, especially dairy products (FAO, 2002). Most dairy farmers (70%) in Sri Lanka run small-scale dairy operations (1-10; Ibrahim et al., 1999; DAPH, 2017), although medium-scale dairying (11-100 cows; Kumara, 2017) is becoming more common. Medium-scale dairy operations are generally conducted under a more intensive feeding system (i.e. higher reliance on concentrate feed and use of cultivated forage crops) than those of small-scale farms. Nonetheless, although some farmers grow some forages themselves, the majority depend on forages that are harvested from roadsides, paddy fields, and crop lands (Weerasinghe, 2019).

In terms of forage quality, energy density is the most crucial factor for milk production, although protein, vitamins and minerals are also important (Ball et al., 2001). The quality of harvested forages compared to other available feed resources (animal and/or plant by-products, formulated concentrates, silage, etc.) varies considerably, as they are subjected to different climatic and management practices (Hughes et al., 2013). For example, the quality of harvested forages is generally low, characterised by high neutral detergent fibre (NDF; >60% on a dry matter (DM) basis), low digestibility (<50%), low metabolisable energy (ME; 6-9 MJ/kg DM), and low concentrations of soluble sugars and starches (<100 g/kg). Management practices such as regular addition of fertiliser, watering, and appropriate harvest intervals, all of which affect forage quality (Premaratne & Premalal, 2006; Ullah et al., 2010), are not widely known or implemented in medium-scale dairy systems in Sri Lanka. Consequently, the quality of harvested forage varies widely. As forages are the main component of the diet, forage quality has a significant impact upon the ability of the diet to meet the nutrient requirements at different stages of the lactation cycle.

Kurunegala district is the biggest district in the intermediate zone and the main milk-producing region of Sri Lanka (DAPH, 2017). Feeding dairy cows with off-farm fodder grasses is popular amongst its dairy farmers; however, priority is given to the quantity that is fed, regardless of the quality of the feed (Vyas et al., 2020). Only a small range of tropical forages are commonly used as dairy feeds. These include Guinea grass ecotype A (*Panicum maximum,* hereafter referred to as 'Guinea grass'), Hybrid Napier CO-3 (*Pennisetum purpureum* × *P. americanum,* hereafter referred to as 'CO-3 grass'), Gliricidia (*Gliricidia sepium)* and maize stover (*Zea mays L.; i*.e. the residual plant mass after harvesting of the cobs/grain). Guinea grass is a fast growing, leafy and quite hardy perennial grass that is suitable for a range of climates (Feedipedia, 2009). It has two types: a tall/medium tussock (>1.5 m at flowering, 1.5-3.5 m tall), and a short tussock (<1.5 m at flowering, 0.5-1.5 m tall) (Cook et al., 2005). Guinea grass ecotype A is the shorter type and the most common forage source for farmers in Sri Lanka, as it can be easily harvested from roadsides, alongside railway lines, natural grasslands and/or scrubland at low and mid-elevations (Weerasinghe, 2019). CO-3 grass has become the second most widely-distributed forage type, since its overall yield, crude protein (CP) and ME levels are regarded as being higher than many other tropical forage types (Weerasinghe, 2019). A number of development projects (e.g. Livestock Breeding Project under the Ministry of Agriculture and Livestock) have provided support and training to small holder farmers in the management of CO-3 grass, such that the risk of feed shortages can be mitigated during the dry season or when outsourced feed is unavailable. Gliricidia is a leguminous tree that is also a useful forage for a dairy production system and, further, it can be used as an alternative protein source to replace more costly concentrate feeds (Shem et al., 2003).

The nutritive value of feeds can be measured in different ways, and validation of values is crucial in situations where there are only limited feed libraries available for the given forage type. *In vivo* digestibility analyses, together with proximate analyses, have been carried out in the past to determine the chemical composition of feed types, however, these techniques have been partially replaced with in *vitro* digestibility studies since they are cheap, rapid and cost-effective laboratory methods (Mould, 2003). In most developed countries, the Near-Infrared Spectroscopy (NIRS) technique is commonly used for feed analysis since it is more accurate and cost effective than most other methods (Batten, 1998). However, this method is not commonly available in developing countries and, indeed, even proximate analysis and other 'wet' chemical methods are not readily available. Consequently, it has been exceedingly difficult to develop feed bank data for the tropical forages that are commonly used in Sri Lanka.

There is currently limited information about the use or the nutrient composition of forages, supplementary feeds and concentrate types in medium-scale dairy farms in Sri Lanka. Therefore, the main objective of the current study was to identify the forages and supplementary feeds that are most commonly available to medium-scale dairy farms, alongside investigating the ME and CP content of those feeds. The second objective of the study was to investigate the total energy and protein supplied through manually mixed ration (MMR; i.e. the diet manually prepared by mixing chopped forages and supplements) diets to dairy cows, and to determine whether these met calculated requirements for dairy cows according to the stage of their lactation.

3.3 MATERIALS AND METHODS

3.3.1 Animals and farms

Nine medium-scale (11-100 dairy cows) dairy farms in the Kurunegala district, Sri Lanka, were selected on the basis of management type (stall-feeding), feeding practice (MMR), housing system (loose barn), breed/s (Jersey, Jersey × Holstein-Friesian, and Jersey × Sahiwal) and farming experience (>2 years). Cows were fed 2-3 times daily. A total of 398 cows were enrolled into the current study from these farms. Farms were visited every two weeks over the period of May to September 2018.

Each of the farms had a year-round calving pattern, so at each visit cows were classified into dry (up to 14 days before calving), fresh (calving to 30 days in milk), early-lactation (31-100 days in milk), mid-lactation (101-200 days in milk), and late lactation (201-300 days in milk) to estimate their DM intake (DMI), CP and ME intakes.

Each farmer was asked to provide a list of forages and supplements that they regularly used. From these lists, four forages (Guinea grass, CO-3 grass, Gliricidia and maize stover), along with three other feeds (maize silage, barley distillers' by-products, and commercially formulated cattle feed) were identified for further examination. Lactating cows were additionally provided with a calcium mineral mix (60-90 g/cow/day).

3.3.2 Forages and supplements

Triplicate samples of the aforementioned four forages and three supplements were collected at 2-month intervals from each of the nine farms. At each sampling event, a representative sample (~8-10 plants) of each forage type was collected from a harvested bulk supply. These were then sub-sampled (~2-3 plants) to get a smaller representative portion for chemical composition analysis. Samples of supplementary feeds were randomly collected for chemical composition analysis from bulk stores.

Representative samples (n ~10) of forages were put on a flat surface for measurements of leaf length, leaf count (live and dead) and stem diameter at the base. Wet weights of forages were then determined. Forages were then chopped into 2-4 cm long pieces and oven dried at 60^{o}C for 2-3 days until they reached a constant weight. Dried samples were milled into 1 mm particles using a Thomas Hammer Mill at the Veterinary Research Institute (VRI), Sri Lanka. Ground samples were then sent to the Alltech laboratory, Bangalore, India and VRI, Sri Lanka for *in vitro* digestibility (IVD) assays and CP estimation, respectively.

3.3.4 Feed nutritive characteristics

The DM content of all feed types (on a % basis) was calculated as the difference between the wet (prior to chopping) and dry (after oven drying) weights. Since feed ingredients and the dietary proportions in the MMR differed from farm to farm, the amount of fresh matter of each feed type fed to cows was recorded. These data were used to calculate the total DM offered at the different stages of the lactation cycle, and then to estimate the total DMI of each cow.

Total nitrogen (N) was determined by the Kjeldahl method (AOAC, 1990; ID 984.13; Messman & Weiss, 1993), from which CP was calculated as N×6.25. Results were recorded as % CP on a DM basis. Ash and ether extract (EE) were determined by AOAC 938.08 and AOAC 945.16 methods, respectively. The NDF and acid detergent fibre (ADF) were measured by the filter bag technique described by Tilley and Terry (1963).

3.3.5 Feed digestibility

In-vitro true dry matter digestibility

The true DM digestibility (TDMD) was measured using the method of Kirby et al. (1978). *In-vitro* DM digestibility was determined for all seven feed types in triplicate in an artificial rumen (*Daisy^{II}* incubator; Ankom Technology®, Macedon, NY, USA), following the approach of Tilley and Terry (1963) as modified by Goering and Van Soest (1970). The artificial rumen consisted of a thermostatic chamber (maintained at 39^{o}C) with four rotating jars.

In-vitro neutral detergent fibre digestibility

The NDF digestibility (NDFD) was estimated using the following formula:

$$\%\ \mathrm{NDFD} = \frac{100 - (\mathrm{aNDF_{feed}} - \mathrm{aNDF_{res}}) \times 100}{\mathrm{aNDF_{feed}}}$$

Where; aNDFfeed =amount (g) of NDF incubated

aNDFres = amount (g) of NDF measured on the residue of fermentation

Apparent dry matter digestibility

The apparent DM digestibility (ADMD), commonly known as DM digestibility (DMD), was calculated as TDMD minus microbial biomass. The microbial biomass (% DM) for dairy cows under total mixed ration management system was estimated using ADMD and TDMD data published by Sherasia et al. (2017), thus, the optimum inclusion level of microbial biomass was detected as 5% DM for better digestibility. Therefore, ADMD was estimated using the following formula:

ADMD = TDMD -5

The ME (MJ/kg DM) of forages and supplements was derived using calculated ADMD data and estimated EE values. The following formulas were used to calculate the ME (CSIRO, 2007).

Forages: ME = 0.172 DMD-1.71

Supplement: ME = 0.134 DMD + 0.235 EE + 1.23

3.3.6 Estimation of DM, ME and CP intakes of dry and lactating cows

The mean fresh matter intake (per cow per day) was measured to calculate mean cow DMI. The DMI of each group of cows (dry and lactating) were separately calculated and averaged to provide group individual DMI. The mean cow ME and CP intakes were calculated as outlined below:

$$\text{ME intake}_{\text{(per cow)}} = P_{n=1} \times DMI \times ME_{n=1} + \ldots\ldots\ldots + P_{n} \times DMI \times ME_{n}$$

$$\text{CP intake}_{\text{(per cow)}} = P_{n=1} \times DMI \times CP_{n=1} + \ldots\ldots\ldots + P_{n} \times DMI \times CP_{n}$$

Where; P = Ingredient proportion in the MMR (Table 3.1)

n = ingredient

DMI = Dry matter intake

ME = Metabolisable energy concentration of the ingredient

CP = Crude protein concentration of the ingredient

3.3.7 Calculation of nutritional requirements of dry and lactating cows

The nutritional guidelines published by NRC (2001) and Moran (2005) were used to calculate ME, CP, and DMI requirements of dairy cows at different stages of their lactation. The energy partition for maintenance, milk production, pregnancy, body condition, activity, and climatic stress were calculated to assess the total ME requirements. Dairy cows in the present study were managed in free stalls with zero grazing, therefore, energy partitioning for grazing and walking were not considered for the calculation of total energy requirements.

The average body weight (kg), milk fat (%), protein (%), and lactose (%) used for energy calculations were 450, 4.6, 3.6, and 4.85, respectively. The average milk production at fresh, early, mid-, and late lactation were 15, 17, 12, and 10 L/cow/day, respectively.

3.3.8 Statistical analyses

The mean values and standard deviation (SD) for proximate chemical components, predicted chemical components, predicted ME and the measures of digestibility were calculated using a Microsoft Excel 2016 (Microsoft Corp., Redmond, WA) spreadsheet.

3.4 RESULTS

3.4.1 Forage characteristics

The descriptive parameters of harvested forages such as length, leaf count (live and dead) and stem diameter are shown in Table 3.1. The results indicated that the CO-3 grass had the longest stems (220 cm), whereas Maize forage had the shortest stems (70 cm). The average cutting interval was only reported for CO-3 and maize forages. The number of dead leaves recorded for maize stover was high (>40%), as dairy farmers were presented with maize forage from which the cobs had been harvested. Dead material was recorded between 10-20% for CO-3 and Guinea grasses. The stem diameter ranged from 18.3 ± 2.1, 15.8 ± 1.3 and 7.3 ± 0.6 mm in maize stover, CO-3 grass, and Guinea grass, respectively. Average length of CO-3 grass and maize stover increased with cutting interval, and stem diameters increased with average lengths (Figures 3.1 and 3.2).

Table 3.1. Harvested length (mean ± SD, range), leaf count (green, dead), stem diameter (mean ± SD, range) and presence of inflorescence of forages used in the selected nine dairy farms in the Kurunegala district, Sri Lanka

Form	**Cutting interval (days)**	**Length of harvested forage** (cm)		**Leaf count** (n)		**Stem diameter** (mm)		**Presence of inflorescence**
		Mean (SD)	Range	Green (range)	Dead (range)	Mean (SD)	Range	
Guinea grass								
Fresh	NA	115.5 ± 7.0	75-155	2-5	1-2	7.3 ± 0.6	4-10	N (2), Y (1)
CO-3 grass								
Fresh	45 (1), 50 (1), 60 (1)	157.5 ± 11.3	110-220	9-12	1-3	15.8 ± 1.3	13-20	N
Gliricidia								
Fresh	NA	NA	NA	NA	NA	13.3 ± 2.5	10-16	N (2), Y (1)
Maize stover								
Harvested	95 (1), 100 (1), 110 (1)	122.9 ± 3.9	70-165	4-6	6-8	18.3 ± 2.1	14-22	Y

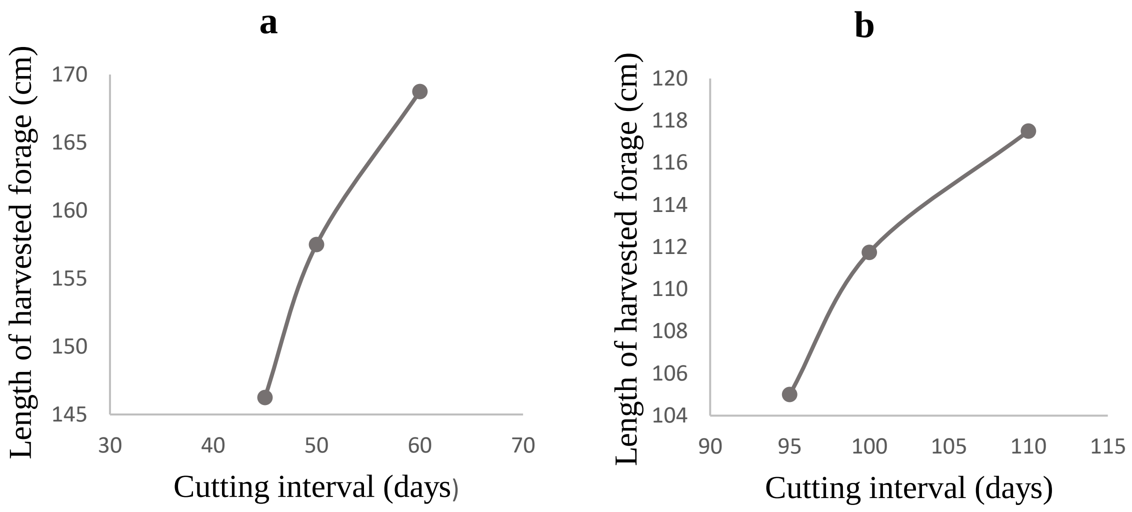

Figure 3.1. Average length of harvested forages changes over the cutting intervals a. CO-3 grass (n=3); b. Maize (n=3)

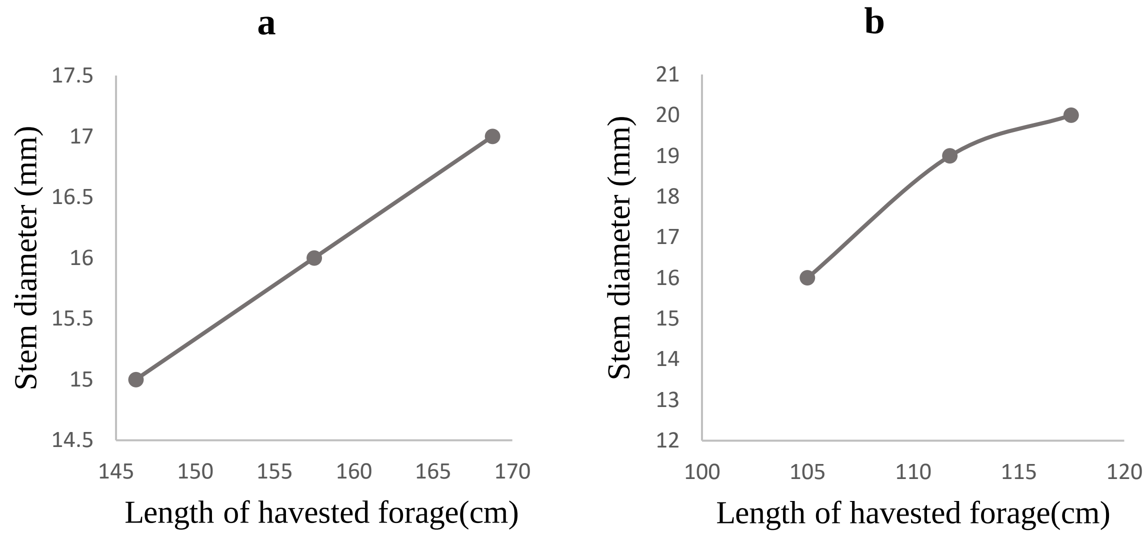

Figure 3.2. Average stem diameter changes over the length of harvested forages a. CO-3 grass (n=3); b. Maize (n=3)

The chemical composition and *in vitro* digestibility parameters of all forage species are presented in Tables 3.2 and 3.3, respectively. Gliricidia had more CP (17.7% DM) and EE (3.6% DM) than other grass species. The fodder grasses were all higher in NDF concentrations than Gliricidia (>64% DM vs. 47% DM, respectively. Additionally, the NDFD of fodder grasses was higher (>37% NDF) than Gliricidia (25.7% NDF). The TDMD values of all forages were similar and of average at around 60%. With respect to the ME of forages, Guinea grass had the lowest value, at 7.5 MJ/kg DM and Gliricidia had the highest value of 10.0 MJ/kg DM. Among the feed sources other than the forages, formulated cattle feed had the highest ME level (12.8 MJ/kg DM). The ME of maize silage and barley distillers' by-products were both ~11 MJ/kg. The CP content of fodder grasses was very low (8-8.8% DM).

Table 3.2. Dry matter (DM; % as fed), ash, crude protein (CP; % DM), ether extract (EE; % DM), neutral detergent fibre (NDF; % DM) and acid detergent fibre (ADF; % DM) composition (mean ± SD) of feed sources available at the medium-scale dairy farms of the Kurunegala district, Sri Lanka

Feed stuff	**DM %** as fed	**Ash** (% DM)	**CP** (% DM)	**EE** (% DM)	**NDF** (% DM)	**ADF** (% DM)
Forages						
Guinea Grass	23.6 ± 1.0	11.6 ± 1.4	8.0 ± 0.3	2.2 ± 0.4	71.7 ± 4.3	41.6 ± 2.8
CO-3 grass	18.8 ± 0.9	9.1 ± 0.9	8.8 ± 0.7	2.9 ± 0.6	71.6 ± 4.3	38.4 ± 3.7
Gliricidia	26.0 ± 1.2	10.0 ± 1.0	17.7 ± 1.2	3.6 ± 0.6	47.1 ± 4.4	33.7 ± 4.2
Maize stover	27.7 ± 1.4	8.1 ± 0.1	8.7 ± 0.8	1.9 ± 0.1	65.0 ± 4.0	32.8 ±3.3
Supplementary feeds						
Maize silage	29.1 ± 1.8	6.9 ± 0.7	7.5 ± 0.9	2.4 ± 0.2	52.0 ± 2.4	26.7 ± 2.0
Barley distillers' by-products	26.1 ± 2.0	4.0 ± 0.4	24.2 ± 2.4	7.2 ± 0.9	51.6 ± 5.4	20.5 ± 2.1
Formulated cattle feed	89.0 ± 1.8	8.3 ± 1.2	15.7 ± 1.9	6.0 ± 0.4	28.1 ± 1.4	NA

Table 3.3. Neutral detergent fibre digestibility (NDFD; % NDF), true dry matter digestibility (TDMD, % dry matter (DM)), apparent dry matter digestibility (ADMD, % DM) and metabolisable energy (ME; MJ/kg DM) of feed sources available at the medium-scale dairy farms of the Kurunegala district, Sri Lanka

Feed stuff	**NDFD** (% NDF)	**TDMD** (% DM)	**ADMD** (% DM)	**ME[a]** (MJ/kg DM)	**ME[b]** (MJ/kg DM)	**ME[c]** (MJ/kg DM)
Forages						
Guinea Grass Eco type A (*Pannicum maximum*)	37.7 ± 3.1	58.4 ± 3.4	53.4 ± 3.4	7.5 ± 0.6	7.9 ± 0.7	6.8
Hybrid Napier-CO-3 (*P. purpureum* × *P. americanum*)	43.3 ± 2.7	61.2 ± 3.8	56.2 ± 3.8	8.0 ± 0.5	8.2 ± 1.1	7.7
Gliricidia (*Gliricidia sepium*)	25.7 ± 4.0	73.0 ± 6.6	68.0 ± 6.6	10.0 ± 1.1	9.3± 1.6	NA
Maize stover (*Zea mays* L.)	45.0 ± 3.1	66.5 ± 4.1	61.5 ± 4.1	8.9 ± 0.5	9.6 ± 0.7	NA
Supplementary feeds						
Maize Silage (*Zea mays* L.)	50.7 ± 1.6	74.0 ± 1.3	69.0 ± 1.3	11 ± 0.2	10.8	NA
Barley distillers' by-products	36.8 ± 3.1	67.5 ± 4.6	62.5 ± 4.6	11.3 ± 0.4	11.3	NA
Formulated cattle feed	40.2 ± 3.9	81.0 ± 3.3	76.0 ± 3.3	12.8 ± 0.4	NA	NA

Values from *in vitro* digestibility are expressed as mean ± SD (standard deviation)

[a] Estimated ME from predicted ADMD and EE values

[b] ME values obtained from gas production (https://www.feedipedia.org)

[c] ME values obtained from published literature, Sri Lanka (Weerasinghe, 2019)

3.4.2 Feed supply and requirements

The diets that were fed to lactating and dry cows on each of the nine farms are presented in Table 3.4. Guinea and CO-3 grasses were the main fodder species used in all diet formulations, and composition ranged from 22-45% and 12-60%, respectively. Cows were fed with freshly harvested and/or ensiled mature maize stover. Leguminous trees such as Gliricidia are not abundant in the Kurunegala district, so Gliricidia was included in the diet of dairy cows to a maximum of 23% at all lactation stages. Barley distillers' by-products and formulated cattle feed were used to balance the ME and CP levels of the final diet. The majority of the farmers used a high amount of supplements, in ratios of around 40% compared with 60% forages.

The calculated nutritional requirements/recommendations for dairy cows based on Moran (2005) and NRC (2001) are presented in Table 3.5. Estimated DM, CP and ME intakes were then compared to those recommendations. This comparison is presented in Table 3.6. According to NRC (2001), cows in all stages of lactation experienced a shortage of CP, while dry cows experienced an excess of CP. Dry cows and lactating cows at all stages experienced low ME intakes according to calculations based on Moran (2005), however, according to NRC (2001), fresh cows and cows in late lactation had an excess intake of ME. The DMI of cows at dry and lactating stages were lower than recommended by Moran (2005). However, based on NRC (2001) the DMI of transition and late lactation cows exceeded recommendations, while the DMI of early and mid-lactation cows was lower than recommended.

Table 3.4. Feed composition (%) of the manually mixed rations given to dry and lactating dairy cows in the selected nine dairy farms of the study

Ingredient/s	Dairy farms - Manually mixed rations																	
	1		2		3		4		5		6		7		8		9	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
Forages																		
Guinea grass	0.0	0.0	45.5	22.2	47.6	23.3	35.7	23.3	45.5	37.5	32.7	0.0	30.3	23.8	30.3	31.8	0.0	0.0
CO-3 grass	60.6	40.0	30.3	27.8	47.6	46.5	47.6	46.5	30.3	37.5	58.2	40.0	48.5	12.7	48.5	12.7	40.0	40.8
Gliricidia	15.2	13.9	0.0	13.9	0.0	18.6	11.9	23.3	15.2	12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maize	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.0	0.0	12.0	0.0	0.0	40.0	15.1
Supplementary feeds																		
Maize silage	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.1	0.0	10.8	0.0	14.8	0.0	15.1
Barley distillers' by-products	15.2	22.2	15.2	22.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.1	9.1	18.1	9.1	18.1	12.0	10.8
Formulated cattle feed	9.1	13.9	9.1	13.9	4.8	11.6	4.8	7.0	9.1	12.5	9.1	14.5	12.1	22.6	12.1	22.6	8.0	18.2

D dry cows; L lactating cows

Table 3.5. A comparison of the amount of metabolisable energy (ME; MJ/kg dry matter (DM)), crude protein (CP; % DM), and DM intake (DMI; kg DM) of dairy cows in the current study, compared to guidelines published by NRC (2001) and Moran (2005)

Stage	**ME (MJ kg DM)**			**CP (% DM)**			**DMI (kg DM)**		
	Moran 2005	NRC 2001	Current	Moran 2005	NRC 2001	Current	Moran 2005	NRC 2001	Current
D	101.4	93.7	90.4	10-12	10.8-12.4	11.8	10.1	9.37	9.84
F	126.4	112.1	125.6	16-18	15.9-18.7	13.6	12.6	11.2	12.4
E	139.6	138.1	126.1	16-18	16.1-17.6	13.6	13.9	13.8	12.4
M	149.3	128.6	125.6	14-16	16.1-17.6	13.5	14.9	12.9	12.4
L	137.5	121.7	125.6	12-14	13.5-14.8	13.5	13.8	12.2	12.4

D: Dry cows (-14 days to calving); F: Fresh cows (1-30 days in milk); E: Early lactation (31- 100 days in milk); M: Mid lactation (101-200 days in milk); L: Late lactation (201-300 days in milk).

Table 3.6. Calculated deficit and/or excess of metabolisable energy (ME; MJ/kg dry matter (DM)), crude protein (CP; % DM), and DM intake (DMI; kg DM) of dairy cows in the current study, compared to guidelines published by NRC (2001) and Moran (2005)

Stage of lactation	**ME (MJ kg DM)**		**CP (% DM)**		**DMI (kg DM)**	
	Moran 2005	NRC 2001	Moran 2005	NRC 2001	Moran 2005	NRC 2001
D	-10.9	-3.24	+0.80	+0.2	-0.3	+0.47
F	-0.84	+13.6	-3.42	-3.72	-0.25	+1.18
E	-13.4	-11.9	-3.4	-3.4	-1.53	-1.38
M	-23.8	-3.03	-1.55	-2.55	-2.49	-0.42
L	-11.9	+3.83	+0.45	-0.55	-1.32	+0.26

D: Dry cows (-14 days to calving); F: Fresh cows (1-30 days in milk); E: Early lactation (31- 100 days in milk); M: Mid lactation (101-120 days in milk)

3.5 DISCUSSION

The present study represents the first time that the feeding practices for dairy cows in medium scale dairy farms in Sri Lanka have been examined with respect to the nutritive values of the forage and non-forage feeds available, and the ability of those diets to meet the nutritional demands of late dry and early- to-late lactation cows.

Forages are either acquired on an opportunistic basis (e.g. cut and carted from verges; collected from post-harvest maize stems) or are intentionally grown as feeds. Farmers have more control over the feed characteristics of the forages that they grow themselves through management of the cutting cycle, but, generally, the ME and CP of forages is low and the NDF is high. Significant contributions to energy come from supplementary feeds (e.g. brewers' grains, maize silage) and proprietary/formulated cattle compound feeds. However, even with these supplementary feeds, we found that the diet was unable to meet the full dietary needs of most cows at most stages of lactation. Hence, perhaps unsurprisingly, the majority of milking cows are primiparous, with multiparous cows being relatively rare.

3.5.1 Cow diet composition, shortage/excess of nutrients

The nutritional requirements for dairy cows in the current study were calculated based on both Moran (2005) and NRC (2001). The preferred guideline used to interpret the shortage and/or excess of ME, CP and DM of given feed rations was that of Moran (2005) as this was specifically developed to assess tropical dairy production systems (Moran & Brouwer, 2014; Saadiah et al., 2019). The required energy values for maintenance and pregnancy based on Moran (2005) were higher than those recommended by NRC (2001) (Table 3.7), and therefore, the calculated total energy requirements for all stages of the lactation were higher than supplied.

The CP concentration of all forages in the current study was higher than the critical CP intake (8% DM) required for unrestricted feed intake (Minson, 1981). Nonetheless, according to CP requirements from both Moran (2005) and NRC (2001), dairy cows in the fresh, early- and mid-lactation stages had CP concentrations (<14% DM) that were lower than recommended (14-18% DM). Low CP is characteristically associated with a negative effect upon milk protein production (Colmenero & Broderick, 2006), but not milk volume (Kalscheur et al., 1999). Broderick (2003) reported that by increasing the CP in the diet from 15.1% to 18.4%, milk protein and milk fat yields increased by 3% and 4%, respectively. Sinclair et al. (2014) noted that low CP levels had no significant effect on milk production, or on animal health, and fertility, but their definition of 'low CP' (14-15% DM) was higher than the CP values seen in the forages in the current study. There may be a self-perpetuating element in the low dietary CP, however, inasmuch as dietary CP level can affect DMI. Broderick (2003) found that increasing dietary CP was associated with an increase in DMI, whilst Kalscheur et al. (1999) reported that compared to low (13%) CP diets, high (23%) CP diets were associated with an increase in both DMI and body weight. Given that DMI values were lower than those recommended in the present study, it was possible that the cows on the study farms were also adversely affected by the low CP diets provided.

Based on the DMI and ME requirements from Moran (2005), all cows in the current study (dry and lactating) experienced deficits of DMI and ME, therefore, there is clear evidence that cows were at a mild-to-moderate negative energy balance (NEB) during the transition period, and that energy imbalance was evident until the end of lactation. Inadequate DMI during the transition and early postpartum increases BCS loss and BW loss, and reduces both milk production and fertility (Roche et al., 2007; Roche et al., 2011).

Table 3.7. Energy (MJ/d), protein (% dry matter (DM)) and DM (kg) requirements for dairy cows at the late pregnant, fresh, early lactating, mid-lactating and late-lactating stages

Activity	Late pregnant (2 weeks to calving)		Fresh (Calving to 30 DIM)		Early lactating (31 -100 DIM)		Mid-lactating (101-200 DIM)		Late-lactating (201-300 DIM)	
	Moran 2005	NRC 2001	Moran 2005	NRC 2001	Moran 2005	NRC 2001	Moran 2005	NRC 2001	Moran 2005	NRC 2001
Energy requirements										
Maintenance + Activity (MJ/d)	49	44	49	32.7	49	32.7	49	32.7	49	32.7
Pregnancy (MJ/d)	20	19.1	0	0	0	0	0	0	0	0
Milk production (MJ/d)	0	0	88.5	84.1	100.3	90.9	70.8	73.8	59	66.9
BC (gain/loss) (MJ/d)	27.5	23.9	14	20.15	14	8.25	22	14	22	14
Climatic stress (MJ/d)	4.9	6.54	4.9	6.54	4.9	6.54	4.9	6.54	4.9	6.54
Energy requirement (MJ/d)	101.4	93.7	128.4	103.2	140.2	138.4	146.7	127.1	134.9	120.2
Protein Requirement (% DM)	10-12	10.8-12.4	16-18	15.9-18.7	16-18	16.1-17.6	16-18	16.1-17.6	12-14	13.5-14.8
DM requirement (kg DM)										
If feed; 8 MJ/kg DM)	12.7	11.7	16.1	12.9	17.5	17.3	18.3	15.9	16.9	15.1
If feed; 10 MJ/kg DM)	10.1	9.37	12.8	10.3	14.1	13.8	14.7	12.7	13.5	12.1

DIM: days in milk; MJ/d: megajoule/day; % DM: percentage of dry matter; BC: body condition

*Formulas, values and assumptions for Moran, 2005 calculations; energy loss for activity: zero for inhouse cows; energy for pregnancy: maximum energy utilization at the late pregnancy; BCS: gain (0.5kg/day) during late pregnancy, mid- and late-lactation, loss (0.5kg/day) during fresh and early lactation; energy for climatic stress: 10% of the maintenance requirement.

* Formulas, values and assumptions for NRC, 2001 calculations; energy requirement for maintainance:0.080Mcal/kg body weight; energy loss for activity: zero for inhouse cows; energy for pregnancy: NRC (2-18); energy for milk production: NRC (2-16); BCS: NRC (2-23,2-24, 2-25, Table 2-4, 2-5); energy for climatic stress: 20% of maintenance requirement.

*Milk production: Average values of 15, 17, 12, and 10 L/day for fresh, early, mid, and late lactation were used to calculate energy requirements for milk production.

* Average body weight: 450Kg; average fat content: 4.6%; average protein content: 3.6%.

3.5.2 Forage and supplement analyses

The DMD and ME values, as well as CP, for the forages in the present study depended upon the proportion of leaf and the maturity of the forage at harvest. The number of leaves per plant is a useful parameter for calculating growth, DM yield and nutritive values of fodder species (Maleko et al., 2019). On the other hand, the number of leaves, leaf length and the plant height of fodder grasses increase with maturity or stage of harvesting (Sarmini & Premaratne, 2017), so the proportion of green:dead leaf is as important as the total leaf mass in determining the nutritive value of the forage. In the present study, CO-3 grass and post-harvest maize stems contained more leaves than Guinea grass, whilst the number of dead leaves increased with longer cutting intervals for green forages and the lowest green:dead leaf ratio was in maize stover. Waghorn & Clark (2004) stated that forage maturation increases fibre content and reduces CP and carbohydrate contents. This reduction in forage quality with maturation results in a decline in the forage digestion rate, CP intake and DMI of cows (Ball et al., 2001).

In the current study, the stem diameter of harvested CO-3 grass and maize stover increased with the stem length (Figure 3.2), and the stem length increased with the cutting interval (Figure 3.1) of both grasses. These results are consistent with the findings of Wangchuk et al. (2015) who reported that the basal circumference of Napier grass is positively correlated with its height, and height is similarly positively correlated with cutting interval, with an average height of 151, 218, and 256 cm being seen at 40, 60 and 80 days cutting intervals, respectively. Height is a critical factor in quality, Bernard et al. (2004) reported that the height to which maize is allowed to grow significantly influences the nutrient density, nutrient digestibility, and the DMI of dairy cows. In addition, letting plants grow has long term consequences for future quality. Orodho (2006), reported that cutting CO-3 grass to a low level (i.e. leaving a 10-15 cm stump) positively influences subsequent yield quality and plant regrowth. In other words, whilst letting forage grasses grow tall and cutting them low may maximise the mass collected at that harvesting, it impairs both the nutritive value of the material harvested, and the subsequent regrowth of the crop (Kabi & Bareeba, 2008). The practical implications of the results from the current study, backed up by the historical literature, are that Sri Lankan dairy farmers should select forages containing a high proportion of live leaves, and a minimum of stem.

Total NDF concentration and NDFD of the forages are major factors in determining forage quality (Oba & Allen, 1999). The high NDF concentration (>70% DM) of Guinea and CO-3 grasses in the current study resulted in low DMI during the dry and mid-lactating periods. The effects of advancing maturity upon forage digestibility, with concurrent adverse effects upon DMI, have been described by Ball et al. (2001) and Waghorn & Clark (2004) and also by Allen (2000), who showed that increasing NDF concentration in the final diet significantly reduced DMI and milk production. Whether this decrease in DMI is solely due to the concentration of NDF is, perhaps, debatable, since Jung & Allen (1995) concluded that rumen fill (and, hence, DMI) is also affected by factors such as NDFD, particle size and the chemical composition of the feed, whilst Oba & Allen (1999) found that DMI and milk production of dairy cows decrease with low NDFD of forages. In the current study, the NDFD was low at <45% in major forages (Gliricidia: 25.7%, Guinea grass: 37.7%, CO-3 grass: 43.3%) which would likely have contributed to the reduced DMI. Due to the lack of land availability, growing of maize was considered not to be economically feasible for dairy farmers. In contrast, buying the mature forage remaining after sweet corn harvest was feasible as it was abundantly available (and therefore cheap) during the sweet corn harvesting season. However, the nutritive value of maize declines markedly from the wet to the post-harvest dry stages (Khan et al., 2015) in terms of higher NDF and ADF, so the practice of using post-harvest maize forages undoubtedly affects the quality of the cow diet. As an aside, there has been debate about the NDFD of the legume Gliricidia. Hoffman et al. (2001) reported that legumes generally have low NDF concentrations and low NDFD compared to the grasses, whereas Aregheore et al. (2006) reported a far higher NDFD (43.9%) for Gliricidia. It is possible that the lower NDFD value obtained in the current study was due to the use of more mature Gliricidia leaves and stems, as digestibility reduces with maturity of forages (Hoffman et al., 2001), although no data were collected in the present study regarding the maturity of Gliricidia.

The CP concentration of Guinea grass (8.0 ± 0.3% DM) and maize stover (8.7 ± 0.8% DM) were similar to values that have been previously reported (Sarmini & Premaratne, 2017; Pavithra et al., 2019; Weerasinghe, 2019), although the CP concentration of CO-3 grass (8.8 ± 0.7% DM) was lower than the values reported by Weerasinghe (2019) and Pavithra et al. (2019), which might be due to either location-specific variation, and/or differences in maturity of the forage. The CP concentration of Gliricidia was twice that of fodder grasses, which was expected as it is a legume. The CP concentrations of formulated cattle feed and barley distillers' by-products were double and triple that of maize silage, respectively; however, low inclusion (<15% DM) of these feeds in the overall ration meant that their net contribution to total dietary CP was relatively limited.

The fat concentration (EE) of all forages, along with silage and supplementary feeds, ranged between 1.9 to 7.2% DM. The fat content of fodder grasses in the current study ranged from 1.9 to 3.6% DM which is comparable to the values recorded by Warly et al. (2004) for tropical grasses (2.7-3.9% DM). The fat concentration of maize stover was the lowest (1.9% DM) because mature maize stems contains very low amount of fatty acid (Khan et al., 2011).

3.5.3 In vitro digestibility and ME content of forages and supplementary feeds

The DMD for forages ranged from 51-65% DM, which was similar to the range reported by Warly et al. (2004) (49.9-62.2). Within the fodder grass species, Gliricidia had the highest TDMD, ADMD, and ME values, which were consistent with published values (Feedipedia, 2009). The highest TDMD and ADMD overall were reported for the formulated cattle feed, as expected.

With respect to the ME values of forages, both Guinea and CO-3 grasses contributed the least ME to the diet across all stages of lactation. The use of a high proportion of both these forage types (15-45% of Guinea grass and 30-60% of CO-3 grass) in the diet of cows in the current study resulted in an energy shortage during the dry period and mid-lactating periods. Farmers usually feed the highest level of supplements during early lactation due to a high milk response, then less supplementation later in lactation. However, poor nutrition and/or a lack of supplementation during early to mid-lactation may result in low body condition gain, which has a negative effect on milk production. Since the transition period is the most challenging time for dairy cattle, the level of energy intake during the period immediately pre-calving can have a significant influence on post-calving metabolic diseases, milk production (Law et al., 2011; Huang et al., 2014) and fertility (Drackley & Cardoso, 2014).

3.6 CONCLUSION

When the ME, CP and DMI requirements for different stages of lactation were calculated, dry cows and mid-lactating cows were shown to have a shortage of ME and DMI, and all lactating cows had a shortage of CP. Overall, the quality of Guinea and CO-3 grasses was low compared to the leguminous tree (Gliricidia) and maize stover. Ensiling maize resulted in ME values of 11 MJ/kg DM, with NDF value of 52% DM and NDFD value of 50.7% NDF, which are all adequate to maintain milk production. The DMD of forages was higher than the minimum required to be classified as ‘good quality’ forage; however, the NDFD of Gliricidia was below the required minimum. Alternative feeds, such as barley distillers’ by-products and formulated cattle feeds are higher quality supplements, and they provide a comparatively higher amount of ME and CP for the final diet but are more costly than tropical forages.

Overall, therefore, it is clear that the forage component of the diets of the cows in this study was the key limiter upon productivity. Improvements to the time of harvesting key grasses would clearly improve ME as well as digestibility and CP content; and, because of the limiting effect of these upon DMI, an overall increase in DMI (and hence of the key energy and protein indicators of the diet) would probably eventuate. Whilst the limitations imposed by poor-quality forages can be ameliorated to some extent by higher quality supplementary feeds, it is clear that the key to improving the productivity of Sri Lankan dairy cows lies primarily in the management of the quality and quantity of its forages.

CHAPTER 4: METABOLIC PROFILE TESTING TO ASSESS THE NUTRITIONAL STATUS OF DAIRY COWS IN MEDIUM-SCALE DAIRY FARMS IN SRI LANKA

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Submitted to *The Veterinary Journal*, July 2022

4.1 ABSTRACT

Milk production and fertility of dairy cows in tropical regions can be significantly compromised by nutrition due to the widespread use of low-quality forages. Metabolic profile testing (MPT) was undertaken on nine medium-scale dairy farms in the Kurunegala district, Sri Lanka, to investigate the relationship of metabolic parameters with production parameters (stage of lactation, body condition score (BCS), body weight (BW), breed, parity) and calving month; and for comparison with metabolisable energy (ME) and crude protein (CP) intakes. Farms were visited every two weeks between May 2018 and September 2018 to collect blood from all eligible cows (-14 to +120 days relative to calving). Cows were grouped into 7 time periods (TP): 1 (-14 to 0d), 2 (1 to 14d), 3 (15 to 28d), 4 (29 to 42d), 5 (43 to 60d), 6 (61 to 90d) and 7 (91 to 120d). Body weight and BCS were determined at each blood sampling, and ME and CP intakes were calculated. Parity (primiparous *vs.* multiparous), breed and calving month data were collected from farm records. Concentrations of albumin and urea were measured in all TPs, non-esterified fatty acids (NEFA) from TP 1 to 5, and β-hydroxybutyrate (BHBA) from TP 1, 2 and 4. Data were analysed using linear mixed, repeated-measures models with metabolite concentrations as the outcome variables. Albumin concentrations were lower at TP 2 and 3 than at other times; and varied with parity and month of calving. Urea concentrations were highest at TP 1 to 3 and showed a TP×parity interaction. Concentrations of BHBA were higher at TP 2 and 4 than at TP 1 and were also affected by parity and breed. Concentrations of NEFA were lower at TP 1, 4 and 5 than in early lactation. A significant negative correlation was observed between urea and BHBA (r^2 = 27%), but there were no other significant correlations between blood metabolites. The dry matter and ME intakes of dry cows and lactating cows at fresh, early, and mid-lactation were below predicted requirements. The CP intakes of fresh, early and mid-lactation stages were below predicted requirements, but were in excess at the prepartum transition. The MPT results closely reflected these dietary

changes. Hence, under Sri Lankan conditions MPT can be used for early identification and correction of nutrition constraints/excesses in the diet of dairy cows managed in semi-intensive medium-scale dairy farms.

Keywords: cows, metabolic, blood, nutrition, tropical dairy

4.2 INTRODUCTION

Maintaining optimum nutritional status in dairy cows is crucial for maintaining productivity and optimizing farm profitability. Optimizing nutrition is especially important over the interval between the mid-dry period and mid-lactation, when cows undergo major physiological and metabolic changes. Significant under-nutrition during this period can result in economically-important reductions in productivity and reproductive performance (Block, 2010).

Simply monitoring intakes, especially at the group level, is often insufficient to determine whether nutrition is adequate, because of the variability between cows' intakes and the variability between feeds. On the other hand, the concentrations of metabolites in the blood reflect the status of energy, protein and other nutrient intakes of individual animals (Ndlovu et al., 2007) and, hence, can be used to estimate their nutrient balance (Cronje & Pambu Gollah, 1996). Thus, measurement and analysis of these biochemical parameters can provide an animal-focused method of assessing nutritional status at the herd level: a process known as metabolic profile testing (MPT) (Payne et al., 1970). When used appropriately, MPT can be used to ascertain nutritional status, to identify dietary causes of disease and low milk production, and to indicate potential causes of (and to predict) poor reproductive performance (Lee et al., 1978). A wide range of blood metabolites have been used in metabolic profiles (Lee et al., 1978; Bjerre-Harpoth et al., 2012; Soca et al., 2014; Madreseh-Ghahfarokhi et al., 2018), but indicators of energy and protein status are presently the most widely used.

Non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) are the most commonly-used metabolites to assess energy, as their concentrations can be used to detect body fat mobilization and negative energy balance (NEB) in dairy cattle (Grummer, 1993). Increased blood concentrations of NEFA and BHBA are used to indicate potential negative impacts of dietary inadequacies on milk production and animal reproductive performances (Ospina et al., 2010). Glucose is usually measured alongside these parameters as, although of limited diagnostic value *per se*, it provides a useful triangulation to the assessment of the lipid parameters.

In regard to protein, urea and albumin are the most commonly-used indicators in dairy cattle (Ndlovu et al., 2007). The interpretation of these parameters requires a degree of care: at a simple level, blood urea concentration reflects dietary nitrogen content (as a proxy for protein content) (Shapiro & Lusis, 2001), while albumin concentration reflects hepatic production of that protein and thus amino acid availability for protein synthesis (Zhou et al., 2016). However, urea concentrations also vary with the proportion of non-protein *vs.* protein nitrogen, as well as with the level of deamination of protein either within the rumen or during metabolism. Similarly, whilst albumin concentrations undoubtedly reflect protein synthesis, this itself is as much a measure of energy status as it is of protein status. Finally, total protein concentration is also commonly measured as, again whilst of limited value of itself, it provides a low-cost estimation of globulin concentrations.

Changes in body weight (BW) and/or body condition score (BCS) can be used alongside MPT as an additional means of assessing the protein-energy status of dairy cattle (Kida, 2003; Roche et al., 2009). Finally, milk volume and composition are valuable indicators of dietary status, with clear relationships between milk fat and protein with dietary energy, protein and effective fibre.

Metabolic profile testing has been used on a very limited basis in Sri Lanka to determine nutritional status of dairy cows. The first published use of MPT on Sri Lankan dairy farms was by Whitaker et al. (1999), as part of a project to evaluate the use of MPT in dairy cattle on smallholder dairy farms in tropical and subtropical countries. In that study, ~40 cattle were tested for BHBA, urea, albumin, globulin, inorganic phosphate and haemoglobin concentrations, 1-2 weeks before calving and between 10–20 days and 2-3 months after calving. In addition, BCS (1-5 scale; (Edmonson et al., 1989)) and BW (heart girth; weigh band) were also measured. The concentrations of BHBA were generally high, with ~50% of cows having BHBA concentrations that were higher than recommendations (>0.6 mmol/L pre calving; >1.0 mmol/L post calving) at each sample point. This was associated with a marked increase in weight loss and decrease in BCS after calving. Urea concentrations were low (<3.6 mmol/L) (~35% of cows had low urea concentrations 2-3 months post-calving), as were albumin concentrations (<30 g/L) (~15% and ~25% of cows less than recommendations pre-calving and one-month post-calving, respectively).

Three other studies in Sri Lanka (Nishany et al., 2013; Herath et al., 2018; Ranaweera et al., 2020) have used MPT to evaluate energy and/or protein balance of feeds on production and/or reproductive performances of late dry and lactating dairy cattle. Nishany et al. (2013) measured serum NEFA and milk BHBA concentrations of dairy cattle from 5 days prepartum to 100 days postpartum on one large commercial dairy farm in central Sri Lanka. They found that high concentrations of BHBA (≥200 µmol/L) at five days prepartum were significantly associated with increased calving to first insemination interval (>75 days) and days open intervals (>150 days). Higher serum NEFA concentrations at 5 days prepartum (0.69 mEq/L) and 10 days postpartum (0.54 mEq/L) periods were associated with lower milk production.

Herath et al. (2018) evaluated serum NEFA, BHBA, urea and albumin concentrations in 15 cattle at transition, and early and mid-lactation on one medium-scale farm in central Sri Lanka. Mean NEFA and urea concentrations were above upper critical limits (NEFA: 0.52 mmol/L, urea: 27 mg/dL) at all three stages of lactation, however, BHBA and albumin concentrations remained within the upper and lower limits (BHBA: 0.3-1.5 mmol/L, albumin: 2.8-3.9 g/dL) at all three stages of lactation. The feed analysis identified that the crude protein (CP) content of the diet was adequate, but that metabolisable energy (ME) content was inadequate (NRC, 2001), such that the cows were experiencing NEB and energy-protein imbalance in the diet. Ranaweera et al. (2020) conducted a study in two dairy farms in the dry zone (tropical cattle) and intermediate zone (temperate crossbred cattle) in Sri Lanka, using 15 transition-period cows from each farm. Serum NEFA and BHBA concentrations exceeded the upper threshold levels (NEFA: 0.52 mmol/L, BHBA: 1.18 mmol/L) for temperate and tropical breed cows, respectively, indicating that, irrespective of breed, cows were suffering from NEB.

All of the aforementioned studies were limited in the number of farms and timing of the samples relative to calving. More information from MPT across a wider range of dairy feeds, production parameters, and different stages of the lactation cycle of dairy cows is needed to better understand energy and protein imbalances in lactating dairy cows in Sri Lanka. Therefore, the primary objective of the current study was to evaluate the nutritional status of close-up dry, fresh, early, and mid-lactation dairy cows on medium-scale dairy farms, using an MPT approach to assess serum NEFA, BHBA, albumin, and urea concentrations.

4.3 MATERIALS AND METHODS

The Animal Ethics Committee (Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka) approved all animal procedures (VER-2018-002).

4.3.1 Selection of farms, dairy cows and their management

The study was conducted on nine semi-intensive medium-scale dairy farms (10-100 milking cows/farm) in the Kurunegala district of Sri Lanka (7.48°N, 80.36°E) (Table 4.1). The cows were purebred Jersey and crossbreds (Holstein-Friesian (HF)×Jersey; Jersey×Sahiwal). Cows were principally fed two or three times daily with manually mixed rations (MMR) containing forages [Guinea grass ecotype A (*Panicum maximum*), Hybrid Napier CO-3 (*Pennisetum purpureum*Pennisetum americanum*), Gliricidia (*Gliricidia sepium), and* maize (*Zea mays L.*)] and supplements (maize silage, barley distillers' by-products, and commercially formulated cattle feed) (Table 4.2). All cattle were milked twice daily, and daily milk production varied between 7-15 L/cow.

Table 4.1. Details of the farms used in the current experiment

Data	No of cows/farm	No of cows selected	% Multiparous cows in selected cohort	Breed/s*	Milking set-up
Farm A	41	18	44	HFJ, JS, J	Portable
Farm B	22	8	0	HFJ	Portable
Farm C	10	2	0	HFJ	Portable
Farm D	11	2	100	JS, J	Portable
Farm E	12	2	50	HFJ, J	Portable
Farm F	12	3	67	HFJ, J	Portable
Farm G	95	24	0	HFJ	Herringbone
Farm H	97	15	0	HFJ	Herringbone
Farm I	98	22	0	HFJ	Herringbone

*HFJ = Holstein-Friesian × Jersey; JS = Jersey × Sahiwal; J = Jersey.

Table 4.2. Mean (range) of composition of diets provided to dry and lactating cows

Forages	**Mean (range) percentage of dry matter in diet**	
	Dry	Lactating
Guinea Grass Ecotype A	24.9 (0-45.9)	12.6 (0-29.0)
Hybrid Napier CO-3	32.3 (20.0-48.8)	23.4 (14.78-33.0)
Gliricidia	4.5 (0-14.4)	8.0 (0-22.6)
Maize	4.2 (0-38.2)	1.2 (0-10.9)
Supplementary feeds		
Maize Silage	0	5.5 (0-15.9)
Barley distillers' by-products	6.1 (0-14.4)	10.3 (0-24.4)
Formulated cattle feed	27.7 (17.1-36.7)	39.8 (23.1-52.7)
Minerals		
Calcium	0.2 (0-0.7)	0.6 (0.3-0.9)

4.3.2 Cow grouping, blood sampling, body condition score and body weight estimation

Eligible cattle were those between 14 days before calving and 120 days postpartum. Each herd was visited every two weeks between May and September 2018, and, on each occasion, blood samples were collected from each eligible animal. Cows were grouped into 7 time periods (TP): 1 (-14 to 0 days), 2 (1 to 14 days), 3 (15 to 28 days), 4 (29 to 42 days), 5 (43 to 60 days), 6 (61 to 90 days) and 7 (91 to 120 days) (Figure 4.1). Blood samples were collected after morning milking by coccygeal venepuncture into evacuated collections tubes (no anticoagulant: Greiner Bio-One, UK). After clotting for 2h at room temperature, serum was separated by centrifugation at 3000 g for 10 minutes. Serum was thereafter stored at -20^{o}C until assay. The BCS (10 point scale; (Macdonald & Roche, 2004; Roche et al., 2004)) was also determined for each cow, and bodyweight (BW) was estimated using a dairy cow weight tape (cattle weight tape, Valley Vet, New Zealand).

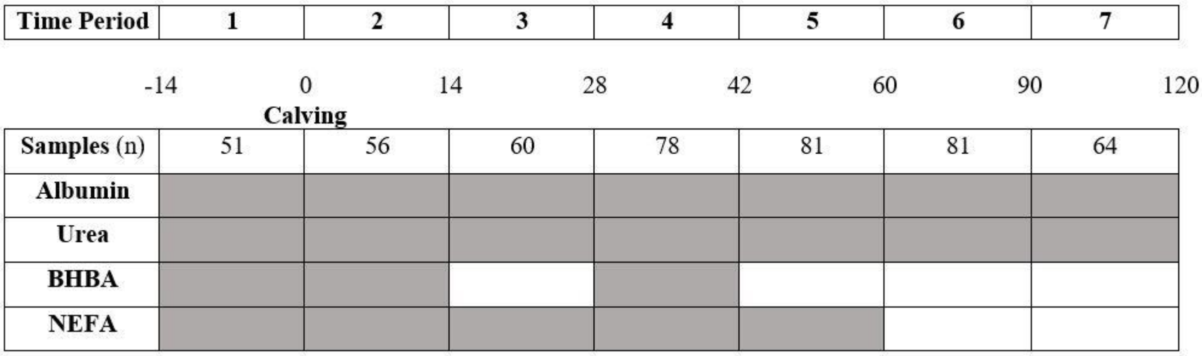

Figure 4.1. Samples collected from cows over each time period between 14 days prepartum to 120 days postpartum; Shaded boxes indicate that samples were assayed for that metabolite

4.3.3 Determination of serum metabolic profile

Serum concentrations of BHBA, urea, NEFA and albumin were all measured using commercial kits (Randox Laboratories, Antrim, UK), on a semi-automated biochemical analyser (Chem 7, 340-670nm, Erba Diagnostics Mannheim, Germany) at 37^{o}C. Randox calibration serum (catalog no. CAL2351) were used as daily quality control. Inter-assay coefficients of variations for albumin and urea were 3% and 8%, respectively. Intra- and inter-assay coefficients of variations for BHBA and NEFA were <4 and 6%, respectively. The minimum detectable levels for albumin, urea, BHBA and NEFA were 0.2 g/L, 0.1 mmol/L, 0.1 mmol/L and 0.072 mmol/L respectively, according to the manufacturer's instructions.

4.3.4 Metabolisable energy and crude protein calculations

The forage and concentrate samples were collected and analysed for nutritional composition (as described in Chapter 3). From the mean dry matter intakes (DMI) and composition of the diets, average ME and CP consumptions/intakes were calculated. These were compared with published recommendations for daily DMI, ME, and CP of dairy cows in tropical conditions (Moran, 2005) to determine whether cows were in positive or negative ME and/or CP balance at difference stages of lactation.

4.3.5 Statistical analyses

Data were first checked for normal distribution using the Kolmogorov-Smirnov test and q-q plots. Non-normally distributed data were log (ln) transformed before analysis. Data were thereafter subject to analysis by repeat measures linear mixed models, in which metabolite concentration was the outcome variable. Categorical predictor variables were parity (first = 1, subsequent =2), breed and month of calving. Time before and after calving was the repeated-measures variable and BCS the covariate.

The linear mixed model developed was:

$$\boldsymbol{y_{ijklmn} = \mu + P_i + B_j + CM_k + BCS_l + Time_k + e_{ijklmn}}$$

Where y is the metabolite concentration (albumin, urea, BHBA or NEFA), μ is the mean, P is the parity, B is the breed, CM is the calving month, BCS is the body condition score, time is time periods before and after calving and e is the random error term.

For all pairwise comparisons, the SIDAK correction was used to account for multiple comparisons. The spearman rank order correlations were also tested between each blood metabolites. All analyses were undertaken using SPSS 26 (SPSS INC., IBM statistics, USA). Marginal means (±SE) and lower and upper bound of serum metabolites concentrations under the adjusted 95% confidence interval (CI) were estimated.

Finally, for each of the four metabolites, thresholds from published literature (upper and lower threshold values for lactating and dry cows (as shown in Table 4.3) were used to categorise concentrations as high, normal or low for albumin and urea; and high or normal for NEFA/BHBA.

Table 4.3. Upper and lower concentration thresholds for serum metabolites in dry cows and lactating cows

Metabolite*	**Optimum concentration of metabolites**				**Reference/s**
	Dry cows		Lactating cows		
	Lower threshold	Upper threshold	Lower threshold	Upper threshold	
Albumin (g/L)	21	36	21	36	(Constable et al., 2016)
Urea (mmol/L)	2	9.6	2	9.6	(Constable et al., 2016)
BHBA (mmol/L)	-	0.6	-	1.0	(Whitaker, 2004)
NEFA (mmol/L)	-	0.4	-	0.7	(Whitaker, 2004; Ospina et al., 2010)

*BHBA beta-hydroxy butyric acid; NEFA non-esterified fatty acids

4.4. RESULTS

Data were collected from 96 cows (83 primiparous and 13 multiparous) with 51, 56, 60, 78, 81, 81 and 64 serum samples for TP 1, 2, 3, 4, 5, 6 and 7 respectively. There were 471, 462, 171 and 292 measurements available for albumin, urea, BHBA and NEFA, respectively.

4.4.1 Basic reproductive, BW and BCS data

Basic data for reproductive performance, BW, and BCS are shown in Table 4.4. Over the duration of the study, 40/96 cows (41.7%) conceived, mostly (34/40) after Week 19 postpartum. However, many cows (51/96) were inseminated during the first 18 weeks postpartum, but only 6 (11.8%) of these conceived to these inseminations.

Table 4.4. Reproductive performances, body weight (BW) and body condition score (BCS) of early, mid-, and late lactation pregnant and non-pregnant dairy cows

Variable	Stage of lactation					
	Early (7-15 weeks)		Early-mid (16-18 weeks)		Mid to late (19-40 weeks)	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
No. of cows	92	4	90	2	56	34
Proportion of cows (%)	95.8	4.2	97.8	2.2	62.2	37.8
Calving-conception (d)	-	74 ± 3	-	105 ± 4	-	209 ± 7
Calving- 1st service (d)	114 ± 3	74 ± 2	114 ± 2	94 ± 7	-	-
Average BW (kg)	435.1 ± 9.1	459.4 ± 37.8	436.6 ± 9.22	369.5 ± 64.5	-	-
Average BCS (1-10 scale)	4.3 ± 0.03	4.5 ± 0.01	4.3 ± 0.03	4.5 ± 0.01	-	-

BCS body condition score; BW body weight; Data are shown as mean ± SE except where stated

Table 4.5. Metabolic profiles of early and mid-lactation dairy cows

Stage of lactation	Time period	Days in milk	**Albumin** (g/L)	**Urea** (mmol/L)	**BHBA** (mmol/L)	**NEFA** (mmol/L)
			Mean (CI)	Mean (CI)	Mean (CI)	Mean (CI)
Transition-prepartum	1	-14-0	31.6[a] (30.1-33.0)	5.5[a] (5.0-6.0)	0.48[b] (0.39-0.57)	0.22[b] (0.17-0.28)
Transition-postpartum	2	1-14	27.3[b] (25.9-28.7)	5.3[a] (4.8-5.8)	0.63[a] (0.53-0.72)	0.35[a] (0.29-0.40)
Early lactation	3	15-28	28.3[b] (27-29.7)	4.5[b] (4.0-5.0)	-	0.40[a] (0.35-0.46)
Early lactation	4	29-42	32.5[a] (31.3-33.7)	3.8[cd] (3.4-4.2)	0.60[a] (0.50-0.70)	0.31[b] (0.26-0.37)
Early lactation	5	43-60	32.6[a] (31.4-33.8)	2.3[e] (1.8-2.7)	-	0.30[b] (0.24-0.36)
Early lactation	6	61-90	30.9[a] (29.7-32.1)	2.9[d] (2.5-3.4)	-	-
Mid-lactation	7	91-120	31[a] (29.7-32.3)	2.4[e] (1.9-2.9)	-	-

[a-e] Values within columns with different superscripts are significantly different from each other (LSD, $P<0.05$); BHBA beta-hydroxy butyrate; NEFA non-Esterified Fatty Acid; CI lower and upper bound values for 95% adjusted confidence interval

Average BCS showed little variation (4.3 to 4.7) over the duration of the study, and, in non-pregnant cows, BW also showed little variation (433 to 439 kg). Body weight was apparently lower in cows that conceived by the end of mid-lactation than in those that did not, but numbers of pregnant animals were small (n=2). The lack of variation of these variables over the duration of the study is confirmed by their low coefficients of variation: 19.9% for BW and 8.2% for BCS.

4.4.2 Metabolic data

Data for serum concentrations of albumin, urea, NEFA and BHBA are summarised in Table 4.5. Albumin concentrations (Table 4.5, Figure 4.2a) varied with TP, parity, and month of calving. Data were separated into 2 homogenous subsets, such that TP 2 and 3 did not differ from each other (mean difference: 0.11 g/L (adj. 95% CI: -1.5 to 3.7 g/L)) but were lower than all other time periods (lowest mean difference TP 2 vs. TP 6: 0.36 g/L (adj. 95% CI: 0.07 to 0.64 g/L). All the other TP (1, 4, 5, 6, 7) formed one homogenous subset (largest mean difference (5 vs. 6): 0.17 g/L (adj. 95% CI: -0.05 to 3.95 g/L). Thus, albumin concentrations of primiparous cows were higher during the late gestation period (14 days pre-calving to calving), and then decreased during the postpartum transition period (mean difference: 0.41 g/L (adj. 95% CI: 0.11 to 0.71 g/L)). Concentrations thereafter increased as lactation progressed, reaching a plateau from TP 5 to TP 7 (Figure 4.2a). Albumin concentrations of primiparous and multiparous cows were generally similar to each other, although values in multiparous cows were significantly lower than in primiparous animals at TP 3 (14-28 days post-calving: mean difference: 0.61g/L (adj. 95% CI: -0.81 to 2.03g/L)).

There was an effect in the model of calving month, therefore, the results were compatible with pairwise comparisons showing a difference for TP 5 and TP 7, in which the mean difference was 0.34 g/L (adj. 95% CI: 0.12 to 0.57g/L).

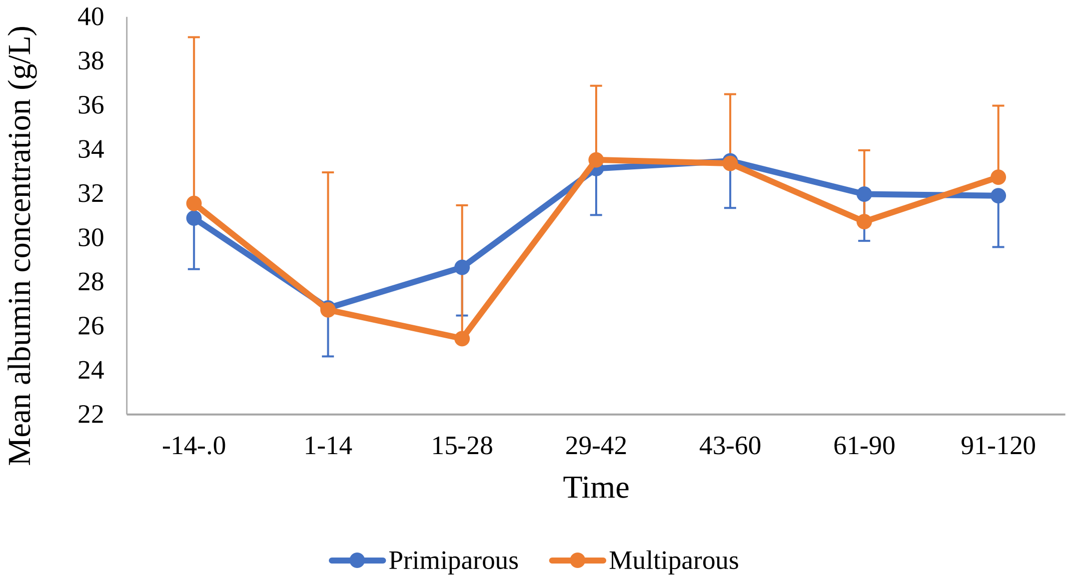

Figure 4.2a. Mean (± adj. 95% confidence interval (CI)) concentrations of albumin in primiparous and multiparous cows during Days -14 to 120 of lactation (see Figure 4.1 for description of time periods)

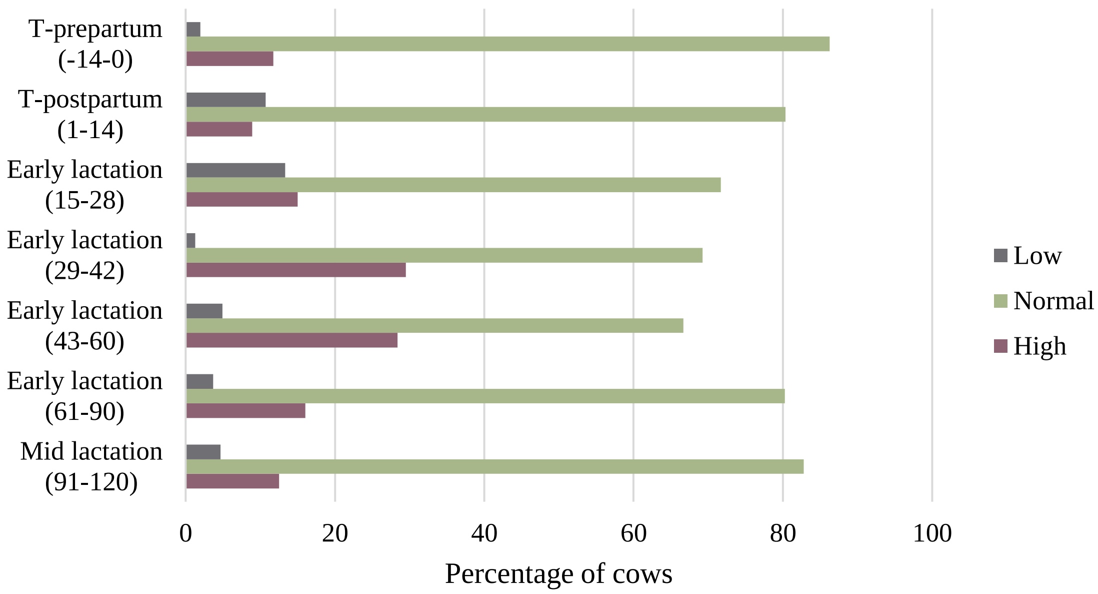

Figure 4.2b. Proportions of cows with albumin concentrations in the low, normal, and high ranges during various stages of lactation

Albumin concentrations were above the reference value for more than 25% of cows blood sampled during TP 4 and TP 5 (Days 29 to 60 postpartum) and above the reference for between 10-20% of cows tested during TP 3 (Days 15-28) and TP 6 (Days 61-90) (Figure 4.2b).

Overall, mean serum urea concentrations (Table 4.5, Figure 4.3a) were higher before calving than at all other times, with values progressively decreasing over the duration of the study. Urea concentrations were related to time after calving and TP×parity interaction. Urea concentrations were separated into 5 homogenous subsets. Time periods 1 and 2 were not separated from each other (mean difference: 1.3 (adj. 95% CI: -3.5 to 6.2 mmol/L)) and were higher than at all other time periods (smallest mean difference (TP 2 vs. 3): 4.9 mmol/L (adj. 95% CI: 0.07 to 9.7 mmol/L)). Conversely, data from TP 5 and TP 7 were not separated from each other (mean difference: 0.76 mmol/L (adj. 95% CI: -2.8 to 4.3 mmol/L)) and were lower than all other periods (smallest mean difference (TP 4 vs. TP 7: 8.4 mmol/L (adj. 95% CI: 2.6 to 14.2 mmol/L)). Data from the remaining time periods were intermediate between those from TP 1, 2, 5 and 7. Urea concentrations were substantially above the reference value (9.6 mmol/L) for lactating dairy cows at TP 3 (days 15-28), 4 (days 29-42) and 6 (days 61-90); however, figures were highest (>40% of cows blood sampled) at TP 3 (days 15-28). The results also showed that whilst ≥20% of cows during TP 5, 6 and 7 had urea concentrations <2 mmol/L, the majority of cows (>90%) had normal concentrations during TP 1 and 2 (Figure 4.3b).

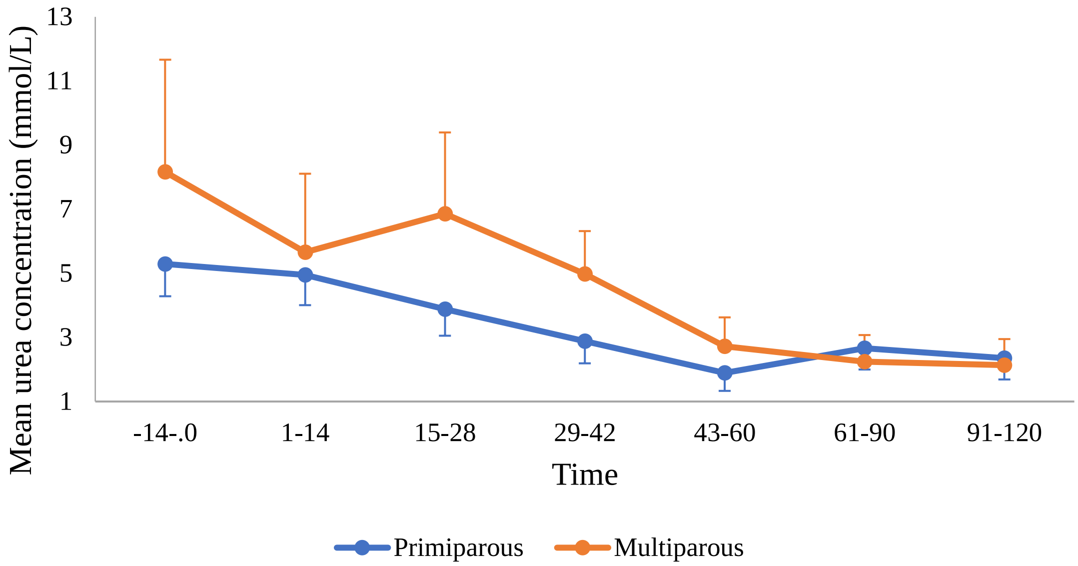

Figure 4.3a. Mean (± adj. 95% confident interval (CI)) concentrations of urea in primiparous and multiparous cows during days -14 to 120 of lactation (see Figure 4.1 for description of time periods)

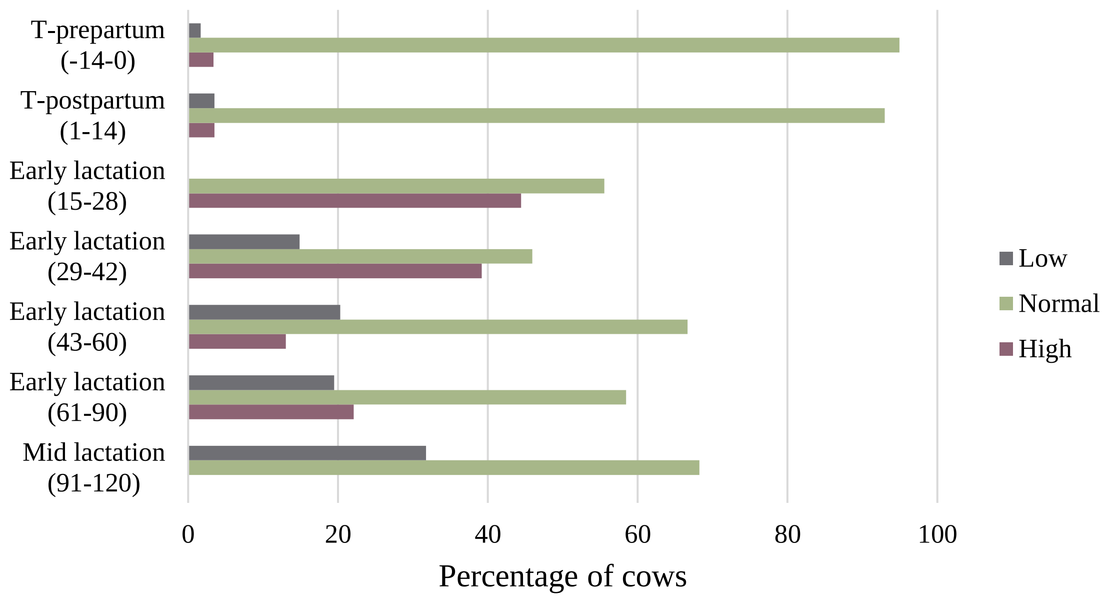

Figure 4.3b. Proportions of cows with urea concentrations in the low, normal, and high ranges during various stages of lactation

Mean urea concentrations were lower at every TP in primiparous compared with multiparous cows until 60 d postpartum, but particularly at TP 3 and 4: mean (back transformed) differences: 2.96 mmol/L (adj. 95% CI: 0.41 to 7.1 mmol/L) and 2.1 mmol/L (adj.

95% CI: 0.71 to 3.7 mmol/L) respectively. Urea concentrations were also affected by month of calving. Mean urea concentration by month of calving ranged from 3.28 mmol/L (adj. 95% CI: 2.54 to 4.11 mmol/L) in June, to 4.55 mmol/L (adj. 95% CI: 3.54 to 5.69 mmol/L) in July. The mean (back transformed) difference between June and July was 1.27 mmol/L (adj. 95% CI: 0.23 to 2.45 mmol/L) and between June and May was 0.87 mmol/L (adj. 95% CI: -0.03 to 1.87 mmol/L).

Concentrations of BHBA (Table 4.5, Figure 4.4a) were affected by parity, breed, and TP. Overall mean concentrations (primiparous and multiparous combined) were significantly lower at TP 1 than TP 2 (mean difference: 0.15 mmol/L (adj. 95% CI 0.02 to 0.28 mmol/L)) and also at TP 1 than TP 4 (mean difference: 0.12 mmol/L (adj. 95% CI: -0.04 to 0.28 mmol/L)). Values at TP 2 and 4 did not differ significantly from each other (mean difference: 0.027 mmol/L (adj. 95% CI: -1.09 to 0.16 mmol/L)). Mean BHBA concentrations were lower in primiparous than in multiparous cows at all three TP: mean differences were 0.47 mmol/L (adj. 95% CI: 0.05 to 0.83 mmol/L); 0.56 mmol/L (adj. 95% CI: 0.19 to 1.18 mmol/L) and 0.07 mmol/L (adj. 95% CI: -0.13 to 0.44 mmol/L) for TP 1, 2 and 4, respectively.

The overall mean of BHBA for each breed ranged from 0.28 mmol/L for Jersey×Sahiwal to 0.62 mmol/L for HF×Jersey crossbreeds. Jersey was intermediate at 0.50 mmol/L. All pairwise comparisons were compatible with no effect of breed except for Jersey×Sahiwal vs. HF×Jersey crossbreeds (mean difference 0.35 mmol/L (adj. 95% CI 0.07 to 0.49 mmol/L).

The results also showed that ≥10% of cows considered during TP 1, 2 and 4 had BHBA concentrations >1 mmol/L, However, the majority of cows (>80%) had BHBA concentrations in the normal range (Figure 4.4b).

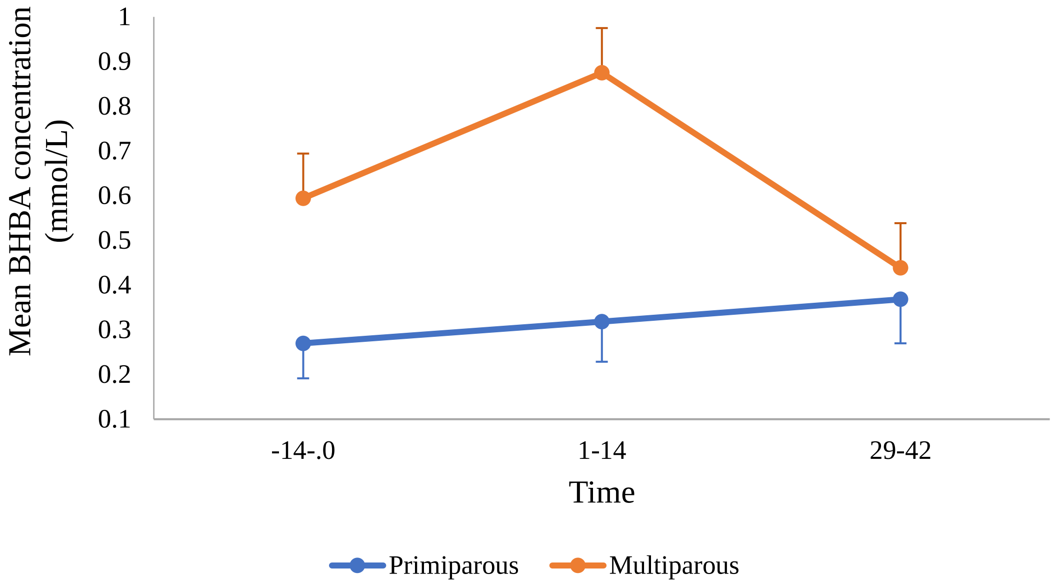

Figure 4.4a. Mean (± adj. 95% confident interval (CI)) concentrations of BHBA of primiparous and multiparous cows during Days -14 to 120 of lactation (see Figure 4.1 for description of time periods)

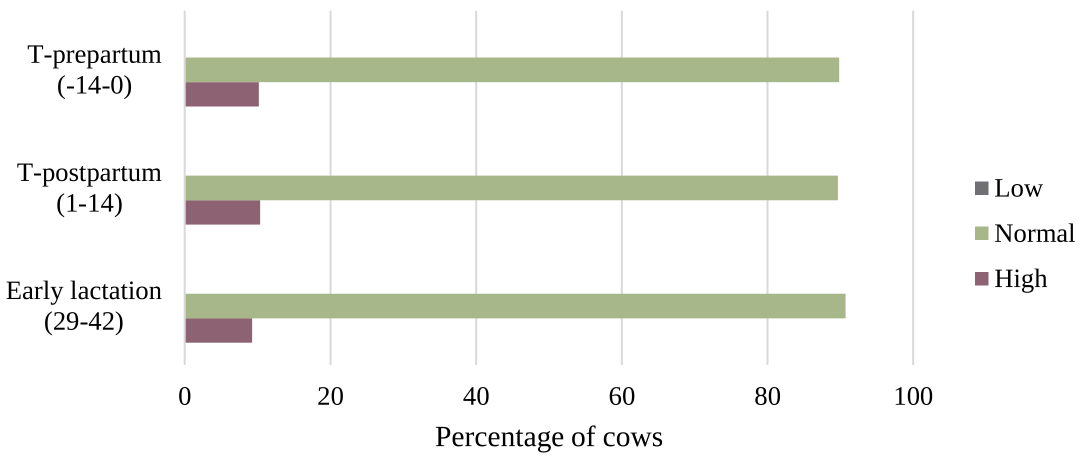

Figure 4.4b. Proportions of cows with BHBA concentrations in the low, normal, and high ranges during various stages of lactation

Analysis of NEFA concentrations (Table 4.5, Figure 4.5a) showed that the mean value of prepartum cows (TP 1) was lower than for early postpartum cows (TP 2 and 3) (mean difference: 0.12 mmol/L (adj. 95% CI: 0.02 to 0.23 mmol/L) and 0.18 mmol/L: (95% CI: 0.07

to 0.30 mmol/L), respectively), but there were no other significant differences between TP (adjusted p >0.1). The mean NEFA concentrations of primiparous cows remained modestly elevated as lactation progressed; however, it was lower during the postpartum transition of multiparous cows, and then increased during TP 3 (mean difference: 0.11 mmol/L (adj. 95% CI: 0.06 to 0.21 mmol/L).

The results showed that 10% of cows during TP 2 had NEFA concentrations > 0.7 mmol/L, however, the proportion of cows having high concentrations of NEFA gradually reduced towards mid-lactation and all cows had normal concentrations during TP 7 (Figure 4.5b).

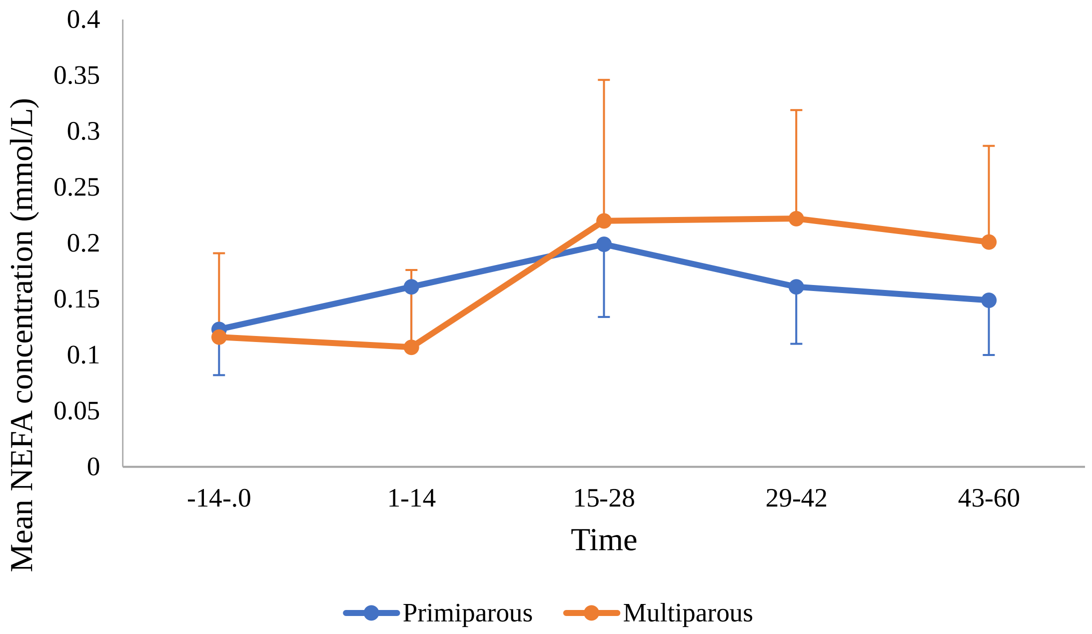

Figure 4.5a. Mean (± adj. 95% confident interval (CI)) concentrations of NEFA of primiparous and multiparous cows during Days -14 to 120 of lactation (see Figure 4.1 for description of time periods)

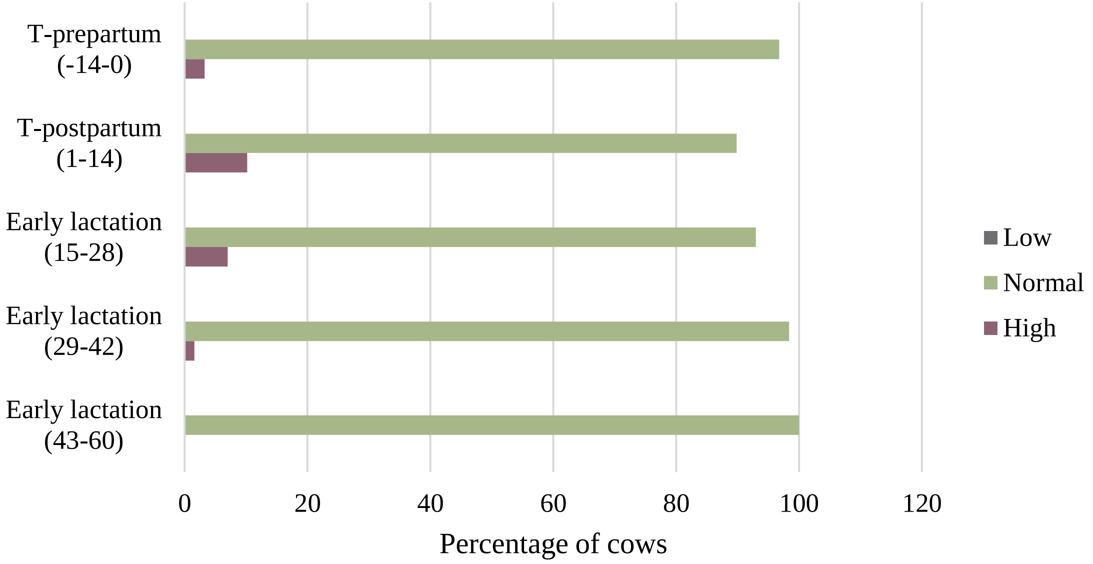

Figure 4.5b. Proportions of cows with NEFA concentrations in the low, normal, and high ranges during various stages of lactation

Phenotypic correlations between serum metabolites, corrected for fixed effects (TP, breed, calving month), showed only a significant ($p<0.05$) negative correlation between urea and BHBA (r^2 = 27%), however, all other blood metabolites were not significantly correlated to each other.

4.4.3 Dry matter intake, metabolisable energy and crude protein balances of dairy cows

Balances of DMI, ME and CP were calculated according to the methodology of Moran (2005) from the current feeding levels. These data are presented in Table 4.6. Lactating cows in fresh, early, and mid-lactation stages were deficient in CP, however, dry cows had an excess of CP. The DM and ME intakes of dry cows and lactating cows at fresh, early, mid-, and late lactating stages were below recommendations.

Table 4.6. Dry matter intake (DMI), metabolisable energy (ME), and crude protein (CP) requirements, intake, and deficit/excess of dairy cows at dry, fresh, early, and mid-lactation

Stage	DMI (kg DM)			ME (MJ kg DM)			CP (% DM)		
	Cal*	Intake	D/E	Cal*	Intake	D/E	Cal*	Intake	D/E
D	10.1	9.84	-0.3	101.4	90.4	-10.9	10-12	11.8	+0.8
F	12.6	12.4	-0.25	126.4	125.6	-0.84	16-18	13.6	-3.42
E	13.9	12.4	-1.53	139.6	126.1	-13.4	16-18	13.6	-3.4
M	14.9	12.4	-2.49	149.3	125.6	-23.8	14-16	13.5	-1.55

D: Dry cows (-14 days to calving); F: Fresh cows (1-30 days in milk); E: Early lactation (31-90 days in milk); M: Mid-lactation (91-120 days in milk); DMI dry matter intake; ME metabolisable energy; CP crude protein; Cal*: calculated requirements using Moran, 2005 Tropical nutrient requirements of dairy cattle; D/E: deficit/excess

4.5 DISCUSSION

The dairy industry has been identified as the most important subsector of agriculture in Sri Lanka (Vyas et al., 2020), providing fresh milk and milk products to growing local communities (Ranaweera, 2011). The milk self-sufficiency target level in Sri Lanka was set to 50% by 2015 (Ranaweera, 2011). However, this had not been achieved by 2020 (Vyas et al., 2020), even though the size of the national dairy herd appears to be ample to serve that purpose, which implies that farmers are facing major challenges to achieve adequate productivity in their cows. Factors such as increasing herd size, feed quality and availability, and decreased labour inputs appear to be leading to problems in maintaining the productivity, health and reproductive performance of dairy herds. The use of MPT programs, in dairies of any scale, may help to alleviate some of these problems, particularly through giving an early warning of undernutrition and/or nutrient imbalances. Macrae et al. (2006) noted that MPT has been used in UK dairy farms for 25 years to identify major nutritional constrains on dairy cow health and the productivity, and, in doing so, has underpinned the development of effective preventive medicine programs. Importantly, even though Whitaker et al. (1999) highlighted that although the use of MPT in herd monitoring programs in temperate countries is characteristically associated with high-producing dairy cows under considerable nutritional stress, the method is no less relevant to dairy cows under other circumstances. For example, the majority of cows in tropical countries produce relatively low amounts of milk, which is associated with moderate to severe nutritional stress (Whitaker et al., 1999) due to poor quality and management of forages. Moreover, in the absence of well-managed nutritional programs, dairy cows, at whatever level of production, can experience severe energy constraints during the transition and early lactation periods during the onset and peak of milk production.

Blood concentrations of albumin, urea, BHBA and NEFA are commonly used in MPT to estimate nutritional deficiencies and/or excesses (Macrae et al., 2006; Herath et al., 2018; Madreseh-Ghahfarokhi et al., 2018), and standard normal and abnormal values for blood metabolites have been progressively refined from experimental data and practical experience (Whitaker et al., 1993; Pushpakumara et al., 2003; LeBlanc et al., 2005; Macrae et al., 2006). For example, NEFA optimum values were defined as 0.7 mmol/L and 0.4 mmol/L for lactating and dry cows, respectively, in UK dairy systems (Whitaker, 2004; Macrae et al., 2006). Even so, such established values need to be interpreted in relation to the stage of lactation, type of farm management and the expected performances of cows in their lactation (Macrae et al., 2006). Further, cow age, parity, level of production, BCS, BW, amount of feeding and/or quality of feeding should also be considered when assessing the metabolic profiles.

In the present study, albumin concentrations were lowest in the postpartum transition period, with 10% of cows having concentrations of <21 g/L. Concentrations recovered thereafter, returning to pre-calving values of ~31 g/L during TP4 (>29 days postpartum). The proportion of cows having high concentrations was also greatest over the period between 29 and 60 days postpartum. Fresh lactating cows generally have low albumin concentrations (Van Saun, 2008; Van Saun & Davidek, 2008), whilst concentrations are generally higher in prepartum and late lactation cows than at other times of the lactation cycle, depending on the adequacy of the overall energy intake in the diet (Whitaker et al, 1999). The low albumin concentrations found in early lactation in the present study and the higher concentrations in mid-lactation are therefore largely as expected but have not previously been reported for Sri Lankan dairy cows. Low serum albumin concentrations are often associated with an increased incidence of postpartum diseases, and can be used to predict the disease risk in close up dry and fresh lactating cows (Van Saun, 2004). There was no formal collection of data relating to peri-partum diseases in the present study, although many farmers anecdotally noted high proportions of metabolic diseases, mastitis, and lameness. Future research in the Sri Lankan dairy industry could focus on establishing the prevalence of such diseases, and possibly exploring a role for serum albumin concentrations as a predictor of disease risk.

Urea concentrations were high in TP 1 and TP 2, and declined thereafter, with lowest mean values in the latter stages of the study. The proportion of cows with high urea concentrations was greatest over the period between 15 and 60 days postpartum, although mean concentrations were declining over that period. Low and high serum urea concentrations are generally interpreted to indicate insufficient or excessive intakes of dietary protein, respectively (Luke et al., 2019). Herath et al. (2018) found that cows received adequate amounts of dietary proteins during early to mid-lactation whereas, in the present study, CP was only barely adequate by mid-lactation. In the context of the present study, these blood metabolite results correlate well with the calculated deficiencies/excesses of dietary CP over the equivalent periods of lactation, reinforcing the notion that such measurements can be used to provide an objective assessment of acute dietary deficiencies. Moreover, the low albumin and urea concentrations at different stages of lactation indicated that lactating dairy cows were fed a protein-deficient diet. Deficiencies of CP can lead to a reduction in rumen microbial protein synthesis and have direct adverse effects upon milk production (Waghorn & Wolff, 1984; Luke et al., 2019).

Overall NEFA concentrations increased from the prepartum to postpartum periods, reaching maximal values at TP 3 (1-14 days), thereafter decreasing until 60 days postpartum. The overall BHBA concentrations during the two-week period centred on parturition increased by 4-10% but were similar thereafter until 42 days postpartum. Those figures reiterate that a considerable proportion (~10%) of cows developed significant NEB during the transition period and had a continuing energy imbalance towards the middle of the early lactation. These data reinforce the notion that dietary energy is the primary constraint of productivity compared to protein. Such an interpretation agrees with published literature from Sri Lanka (Whitaker et al., 1999; Nishany et al., 2013; Herath et al., 2018). When cows are in NEB during the transition period, body fat reserves are mobilized which lead to excess production of NEFA. The liver is the organ responsible for metabolizing circulating NEFA; however, NEFA cannot be completely oxidized for energy production when it is in excess in circulation. The excess NEFA is partially oxidized by the hepatocytes, leading to increases of BHBA and other ketone bodies in the blood and body secretions (Rukkwamsuk et al., 1999; Reynolds et al., 2003; Bicalho et al., 2017). The BHBA concentrations in the current study indicated that the risk of sub-clinical ketosis was perhaps greater during the transition period and up to 42 days postpartum, although no cows had concentrations that were representative of pathological elevation. The present results agree in principle with the findings reported by Nishany et al. (2013), although their absolute values of BHBA concentrations were three times (>200μmol/L; >30% of cows) higher than in the current study.

The regression analysis indicated that parity, breed, and TP in relation to calving were more strongly associated with serum BHBA concentrations than calving month and BCS, however, none of those measures were associated with changes in NEFA concentrations. Primiparous cows represented the majority (>80%) of the study population and they had greater concentrations of BHBA and NEFA during the transition period than did multiparous animals; moreover, NEFA concentrations were higher for multiparous than primiparous cows during early lactation. Primiparous cows usually represent a largely unculled population in a herd (Humer et al., 2015) and they tend to have poorly-regulated metabolism (Nasrollahi et al., 2017). Alternatively, the higher BHBA of multiparous cows in the current study could simply be a reflection of their greater fat stores.

Changes in BCS during the transition period may also help to identify nutritional deficiencies. However, frequent determinations of BCS are essential to provide reliable information and, moreover, there is a significant time lag between the onset of tissue catabolism during NEB and the presence of detectable changes in BCS (Roche et al., 2009). To try to improve the reliability of the BCS data in the present study, repeated observations were made according to the recommendations of Whitaker et al. (1999). Underfed cows usually use tissue reserves to provide energy for lactation (and/or uterine involution and body maintenance) during the early postpartum period. The time at which the nadir of NEB occurs is dependent upon the energy density of the ration and the milk yield of the animal (Gross et al., 2011); however, in most cases, the energy balance eventually becomes positive as DMI rises and milk yield ceases to rise. In the present study, the majority of cows reached nadirs of BCS and NEB in the early postpartum period as the ME intakes were below the calculated ME requirements. On the other hand, in the regression analysis, BCS change did not closely reflect blood metabolic profiles during the prepartum and postpartum periods. Perhaps this merely indicates that there is a temporal disconnect between the timing of observable BCS changes and the metabolic changes that are consequent upon underfeeding/tissue catabolism.

Consideration of DMI may help to understand the relationships outlined in the previous paragraph. For example, DMI during the dry period was 5% less than total DM recommendations. The dry period is characteristically considered as a period during which cows regain BCS in preparation for the next lactation, but, in the close-up transition period (i.e. the period assessed in the present study), DMI can be reduced by up to 40% below predicted values (Bertics et al., 1992; Hayirli & Grummer, 2004). The reasons for this depression in DMI have not been fully elucidated (Hayirli & Grummer, 2004), but probably include restriction of abdominal (i.e. rumen) space due to the rapid expansion of the fetus, and may involve direct suppression of appetite as the animal's fat:lean ratio changes. Periparturient cows in the present study received 11% less ME than the calculated total requirements, although CP was adequate. Therefore, given that there were increases of 4-10% in NEFA concentrations and 10% in BHBA concentrations and, given the calculated ME and DMI deficits, there is clear evidence that cows had moderate NEB in the peripartum period, even before changes in BCS became evident. Therefore, providing an energy-balanced diet based on DMI recommendations during the close-up dry period can overcome some of the undesirable effects related to feeding a low-energy diet (Bell, 1995; Douglas et al., 2006; Schoenberg & Overton, 2011), resulting in reduced BHBA concentrations in prepartum cows (Mann et al., 2015).

Dry matter intakes of fresh, early, and mid-lactation cows were below those calculated according to the methodology of Moran (2005), probably due to inadequate feeding. Intakes of ME and CP were correspondingly low compared to calculated requirements. Postpartum cows at transition and early lactation received up to 10% less ME than the calculated requirements, and this was associated with increases of NEFA and BHBA concentrations of >10%, indicating that cows were under moderate NEB until mid-lactation. The study conducted by Herath et al. (2018) reported an 18% ME deficit during all stages of lactation of dairy cows, however, those cows received adequate amounts of CP from MMR diets. Inadequate DMI during the transition and early postpartum periods triggers fat mobilisation (Grummer et al., 2004), resulting in either increased NEFA or impairment of the supply of gluconeogenic substrates and hence, the accumulation of BHBA in the blood. Consequently, postpartum dairy cows lose BCS and BW during this period since they are utilizing body reserves to produce energy for maintenance and milk production (Roche et al., 2007). Several studies have been carried out to investigate the effect of NEB on peak milk production and the subsequent fertility outcome, and most of the evidence concludes that the effect of NEB on milk production and fertility is related to both its magnitude and its duration (Roche et al., 2007, 2011).

Postpartum cows in transition, and at early and mid-lactation received 10-20% less dietary CP than calculated requirements, which resulted in decreases of blood urea concentrations by 15-30% and blood albumin concentrations by 5% from 29 to 120 days postpartum. It is therefore clear that cows in the present study were under a negative protein balance. Some studies have shown that low CP concentrations in the diets of early lactating dairy cows do not significantly affect either milk production (Holter et al., 1982; McCormick et al., 1999) or reproductive performance (Carroll et at., 1988). When cows are deficient in metabolisable protein, muscle mobilization and breakdown of protein reserves are triggered to satisfy the demands of the mammary gland for glucose and amino acids (Ji & Dann 2013). In contrast, Chew et al. (1984) considered that low CP intake during prepartum significantly lowered DMI and milk yield during early lactation but did not affect reproductive efficiency, and Cressman et al. (1980) and Forster et al. (1983) both stated that milk production increases when feeding high CP diets; however, high CP may negatively affect reproductive performance

(Jordan & Swanson, 1979; McCormick et al., 1999; Roche et al., 2000). Nevertheless, both Kalscheur et al. (1999), and Ji & Dann (2013) stated that feeding high CP diets is essential to maximize milk production and milk protein yields since meeting metabolisable protein requirements is limited by DMI during early lactation. The mechanisms by which protein intakes affect fertility of dairy cows remains unclear (Roche et al., 2000). However, it is clear that it is a complex issue with confounding factors such as energy intake, undegradable protein intake, age, and uterine health influence the responses to variations in protein supply (Ferguson & Chalupa, 1989). Therefore, formulating diets balanced for CP according to the stage of lactation may minimize economic impacts of insufficient and/or excess feeding of CP on milk production and reproduction.

The patterns of NEFA and BHBA, together with those of albumin and urea, taken in the context of changes in BCS and calculated dietary surpluses (or deficits) of ME and CP, show that cows were under varying degrees of NEB throughout most of the study period, although this improved as lactation progressed. Such nutritional deficits, even in the presence of very low metabolic demands for lactation, will have significant effects on both milk production and reproduction (Duffield, 2000; Roche et al., 2000). Therefore, under Sri Lankan conditions MPT can be applied to detect such nutritional problems at any time of the animals' lactation and/or non-lactating stages, and it can be helpful to prevent future productive and reproductive impairments (Macrae et al., 2006).

4.6 CONCLUSION

The use of MPT indicated that dairy cows are generally deficient in energy and protein throughout their lactation on medium-scale dairy farms in Sri Lanka. Application of MPT more widely in the Sri Lankan dairy industry could be helpful to protect animals from deficiencies and/or metabolic diseases. Assessing BCS is also a useful tool, when combined with MPT results at the farm decision-making point. Therefore, regular MPT and BCS should be instigated as part of a dairy management development program to improve the productivity of lactating dairy cows in Sri Lanka. Further studies are required to better understand the epidemiology of metabolic diseases at different stages of lactation among dairy cows in medium-scale herds in Sri Lanka.

CHAPTER 5. RESUMPTION OF OVARIAN ACTIVITY AND FACTORS INFLUENCING CONCEPTION RATES IN POSTPARTUM DAIRY COWS IN MEDIUM-SCALE DAIRY FARMS IN SRI LANKA

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Submitted to *Research in Veterinary Science*, July 2022

5.1 ABSTRACT

Little is known about the reproductive performance of dairy cows in semi-intensively managed medium-scale dairy farms in Sri Lanka. This study was undertaken to characterize the timescale, and factors affecting postpartum resumption of ovarian cycles, and conception rates in Sri Lankan dairy cows. Between May 2018 and September 2018, 112 cows from 8 dairy farms in the Kurunegala district of Sri Lanka that were between 43 and 120 days postpartum had blood samples collected every 2 weeks for progesterone and metabolic profile analyses. Final data for reproductive outcomes were collected in May 2019. Cows were grouped based on days postpartum: (1) 43-56, (2) 57-70, (3) 71-84, (4) 85-98 and (5) 99-120 days. Calving to first service interval (CFSI), calving to conception interval (CCI), first service to conception interval (FSCI), services per conception (SPC), and non-pregnant dates (NPD) were calculated from farm records. Parity, age, body condition score (BCS), body weight, days in milk and any postpartum disorders were also recorded. Cows were considered to be cycling when they had low (<1 ng/ml), moderate (1-4 ng/ml) and high (>4 ng/ml) progesterone concentrations, in any sequence. Cows with low progesterone concentration in all samples were considered acyclic/anoestrus. Progesterone concentrations were elevated in 62% (69/112) of cows during the first 120 days postpartum (i.e. they had resumed ovarian cycles) but 45% (n=31) of those cows were not observed in oestrus. Conversely, 42% of cows (n=47) were deemed to have been in oestrus (and were presented for artificial insemination, AI) between Days 42 and 120, but only 81% (n=38) were cycling according to the progesterone assay. Many cows (58%: n=65) were first inseminated after Day 120: the overall incidence of return to oestrus after AI was 73%. Only 41% (n=46) of cows became pregnant during the 400 days of the study. Mean (± SEM) CFSI, CCI, SPC, FSCI, and NPD were 116.0±2.5, 196.1±8.9, 3.1±0.2, 86.4±7.9 and 302.8±7.3 days, respectively. The CCI of cyclic cows (179.2±12.4 days) was significantly shorter than CCI of acyclic cows (225 ±8.0 days). Multiparous cows were more likely (odds ratio (OR): 8.3; $P<0.05$) to have resumed oestrus cycles before 120 days postpartum than primiparous cows. Pregnancy rate of Jersey cows was higher (OR: 9.9; $P<0.01$) than Jersey × Holstein-Friesian cows. In conclusion, delayed postpartum cyclicity and high incidence of conception failure to AI have a major impact on the productivity of postpartum cows. Efforts should be focused on improving postpartum fertility in dairy cows by improving feed management and quality, establishing good management practices, and minimising environmental challenges.

Keywords: Cows, progesterone, postpartum, anoestrus, fertility, tropics, Sri Lanka

5.2 INTRODUCTION

Dairy farming is becoming increasingly widespread in Sri Lanka as it provides regular income to farmers and nutritious fresh food to the local communities. The majority of farmers operate small-scale dairy operations, typcially with 5-15 cows (Ibrahim et al., 1999), which represents about 75% of the estimated 1.11 million cattle (Vidanarachchi et al., 2019) in the country. The number of medium-scale dairy operations has increased during the recent past, in parallel with imports of dairy (mainly Holstein-Friesian x Jersey) cattle from Australasia. The typical medium scale dairy farm has 10-100 dairy cows (Kumara, 2017), predominantly crossbred (Zebu x Sahiwal, Zebu x Jersey, or Holstein-Friesian (Perera & Jayasuriya, 2008)). Some farmers have a few pure-breed cows such as Jersey and Friesian to increase fat percentage and milk volume, respectively. Crossbred dairy cows produce ~10-15 L of milk daily, but have poor reproductive performance (Kollalpitiya et al., 2012).

The Sri Lanka government's target for the dairy industry was to increase milk production to achieve 50% self-sufficiency by 2015 (Ranaweera, 2011). Even though milk self-sufficiency has increased dramatically in recent years (up to 15-20% in 2009 (Ranaweera, 2011), 35% in 2015 (Vernooij et al., 2015), and 40% in 2016 (Pathumsha, 2016), these figures are still behind the goal that was set for 2015. One of the main constraints behind the failure to meet this target is a lack of knowledge and/or understanding of animal reproductive and nutritional management amongst dairy farmers (Vyas et al., 2020), which translates into poor reproductive performance and a significant detriment on the total milk production per annum. Therefore, expansion of extension services in livestock management, health and breeding management (Perera & Jayasuriya, 2008), as well as the introduction of new dairy technologies, could significantly contribute to improving the productivity of the milk industry in Sri Lanka.

In the three weeks preceding and following parturition (known as the transition period), cows undergo many physiological and physical changes, which creates a negative energy balance (NEB) (Grummer, 1995; Drackley, 1999; Esposito et al., 2014), resulting in changes in body weight (BW) and body condition score (BCS) and, potentially, in metabolic disorders (Heuer et al., 2001; Pushpakumara et al., 2003). Since NEB during the first three weeks postpartum is correlated to the interval between calving and first ovulation (Butler, 2000), NEB has a negative impact upon fertility. Since BCS change is related to the severity and duration of NEB, animals losing more BCS during the first 65 days postpartum are more likely to be acyclic at the end of the voluntary waiting period, thus worsening the interval from calving to conception and reducing conception rates per artificial insemination (AI) (Santos et al., 2009; Bisinotto et al., 2012). Negative energy balance can be catagorized by blood metabolic profile changes in postpartum dairy cows, noteably increases in urea, β-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) concentrations (Bell, 1995).

In addition, the uterus undergoes involution after parturition to reorganize the tissue to its pre-pregnancy state (Elliott et al., 1968; Gier & Marion, 1968; Wathes et al., 2007). Specifically, effete tissue has to be eliminated from the endometrium and caruncles, the endometrium has to be restored and excess tissues from the remainder of the uterus has to be eliminated (Gier & Marion, 1968). Cows that have stillbirths or abortions, retained fetal membranes, endometritis or metritis characteristically have a prolonged uterine involution (Fourichon et al., 2000; Wathes et al., 2007) and, thus, delayed postpartum return to cyclicity. Regardless of the main contributing factor, the culling rate increases when the cows do not show regular oestrus signs or are not inseminated at the right time, negatively impacting the dairy farm profitability (Rounsaville et al., 1979; Inchaisri et al., 2010).

Calving to conception interval (CCI) and calving interval (CI) are important indexes of reproductive performance in cows. For an efficient pasture-based dairy production system with a 365-day CI, CCI should not be more than 80-85 days (Ball & Peters, 2004); however, CCI of 80-130 days and CI of 365-420 days may be more effective for total mixed rations' based dairy production systems in tropical countries (Lyimo et al., 2004; Yifat et al., 2009; Gillah et al., 2012). The majority of cows under good management and nutrition systems resume ovarian cycles in the first month postpartum (Lyimo et al., 2004). For cows under Sri Lankan dairy production systems, the average CCI and CI have been reported to be ~194 days (Abeygunawardhana & Alexander 2001) and ~450 days (Kollalpitiya et al., 2012), respectively; and an even higher CI (505 days) has been observed in dairy cows in other tropical countries such as Pakistan (Sattar et al. 2005). The CCI of postpartum cows is influenced by postpartum energy balance/deficit (Macmillan et al., 1996), and oestrus detection and AI (Hay et al., 2019), and other factors such as uterine disorders (e.g: endometritis, retained fetal membranes) (Roche, 2006; LeBlanc, 2008) and metabolic diseases (e.g: hypocalcaemia) (Hay et al., 2019). Conception failure can prolong the dry period, whilst having more non-pregnant cows at the end of the breeding season increases the culling rate, which increases the expenses associated with reproductive management and reduces total milk production of the farm (Roche, 2006). Nevertheless it may be beneficial to keep non-pregnant cows for longer when it is necessary to maintain herd size and/or those cows have been shown to have a genetic advantage in milk production in previous lactation cycles (Bertilsson et al., 1997).

Progesterone is a key hormone regulating the oestrous cycle (Robertson, 1972). The corpus luteum (CL) produces progesterone throughout pregnancy, and is responsible for maintaining the gestation to term (Estergreen et al., 1967; Robertson, 1972; Fields & Fields, 1996). Progesterone concentrations can be measured in serum or milk, which can be used as an aid for monitoring the postpartum resumption of ovarian cycles, and pregnancy status (Walsh et al., 2011). When progesterone concentrations are assessed in parallel to data collection on BCS, parity, metabolic disorders, and uterine impairments, it provides useful information on the current reproductive status of the whole productive system (Aungier et al., 2014). Moreover, progesterone enzyme linked immunosorbent assay (ELISA) using milk samples can also be used to detect cyclicity in cows as they are commmecially available at a relatively low cost (Bajema et al., 1994).

Little is known about the postpartum return to cyclicity, oestrus detection efficiency, the number of inseminations per conception, pregnancy rates and/or days open in the medium scale dairy farming systems of Sri Lanka. Therefore, the objectives of the present study were to characterize the resumption of postpartum cyclicity and evaluate risk factors associated with cyclicity and conception rates (CR) in semi-intensively managed medium scale dairy farms in Kurunegala, Sri Lanka.

5.3 MATERIAL AND METHODS

Ethical approval was obtained from the Ethics Review Committee, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka (VER-18-002). Further, all sampling procedures were approved by the Massey University Animal Ethics Committee, New Zealand (MUAEC-18/32). Informed consent was obtained from dairy farmers prior to recruiting their cattle into this study.

The study was conducted in the Kurunegala district, situated in the intermediate zone of Sri Lanka (7.48°N, 80.36°E), at an altitude of 116 m above sea level. The annual rainfall ranges from 1750 to 2500 mm, with two seasonal peaks (the north-east monsoon and south-west monsoon) during May-September and December-February. The temperature ranges from 25^{o}C to 35^{o}C during the daytime, with an average temperature of 28.5^{o}C (http://www.meteo.gov.lk/). The mean annual relative humidity (RH) ranges from 65% to 90%, with an average annual RH of 84%.

5.3.1 Farms and their management

In Sri Lanka, calving takes place year round, which imposes challenges to the selection of animals in a particular stage of lactation in a given farm. From 15 farms visited for the initial recruitment of cows, eight medium-scale dairies were selected to participate in the study, based on management type (semi-intensive), feeding practice (manually mixed ration; MMR), housing system (loose barn), temperate breeds (Jersey and Jersey-Friesian crosses), breeding practice (AI) and farming experience (>2 years). Cows were fed two or three times daily with MMR containing the forages Guinea grass ecotype A (*Panicum maximum*), Hybrid Napier CO-3 (*Pennisetum purpureum* × *Pennisetum americanum*), Gliricidia (*Gliricidia sepium*) and maize stover (*Zea mays* L.), along with the supplementary feeds maize silage, barley distillers' by-products, and commercially formulated cattle feed. The amount of forages and supplements (kg fresh matter) given to the cows was recorded. Fresh clean drinking water was available *ad libitum*. Cows were milked twice daily using either portable or herringbone milking machines. Cows were observed for signs of oestrus from 60 days postpartum, and were bred by AI, following the AM/PM insemination rule (Trimberger, 1948; Graves *et al*, 1997).

5.3.2 Animals and their measurements

Eligible cattle were those between 42 and 120 days postpartum. Animals were mainly crossbred (Jersey × Holstein Friesian, Jersey × Sahiwal) and pure-bred Jersey cows, between 2.5 to 10 years of age. Multiparous cows had a mean parity of 3, with a range of 2 to 6. Cows were grouped for data analysis into Time Periods (TP): (1) 43 to 56 days, (2) 57 to 70 days, (3) 71 to 84 days, (4) 85 to 98 days, and (5) 99 to 120 days after calving.

Each herd was visited every two weeks between May 2018 to September 2018 for BCS assessment and BW measurements. Body condition score was estimated according to the 1-10 scale used in New Zealand (LIC, 1993). Bodyweight was recorded using a dairy cow weigh-band (Coburn Company, Inc., Whitewater, WI 53190 USA). The average BW was 416 ± 8 kg (mean ± standard error of mean (SEM)).

5.3.3 Reproductive parameters

Farm records for all eligible cows were collected until May 2019 to assess reproductive outcomes. The occurrence of postpartum reproductive impairements (retained fetal membranes, endometritis, metritis, pyometra) were recorded. Calving dates, insemination dates, and pregnancy diagnosis dates were also recorded to calculate reproductive indices. Pregnancy was confirmed by ultrasonography and/or rectal palpation ~50 days after the last insemination.

5.3.4 Blood sampling and progesterone assay

Eligible cows within each of the 5 categories were sampled every two weeks from 42 days postpartum to at least 120 days postpartum. Cows were sampled a maximum of five times. Blood samples were collected after morning milking via coccygeal venepuncture into evaculated collection tubes (Vacutainer, Jinsha North Road, Liuyang, Hunan, China) with no anticoagulant. After clotting for 2 h at room temperature, serum was separated by centrifugation at 3000 g for 10 minutes at 4^{o}C. Serum samples were then stored at -20^{o}C until assay.

Serum progesterone concentrations were measured by ELISA (Ridgeway Science, UK), (Sauer et al., 1986; Groves et al., 1990). The assay was further validated by measuring concentrations in blood samples obtained from animals known to be in oestrus, mid-luteal phase or pregnant. The inter- and intra-assay coefficients of variation were 13.6% and 7.8%, respectively, and the sensitivity (defined as the least detectable concentration) was 0.04 ng/mL. The highest concentration used in the standard curve was 20 ng/mL.

Characterisation of progesterone profiles was undertaken according to the definition given by Opsomer et al. (2000), with modifications. Concentrations of <1 ng/mL were characterised as low, 1-4 ng/mL as moderate and >4 ng/mL as high. Cows were considered pregnant when progesterone concentrations remained high or moderate for all samples taken after insemination. Cows were considered to be cycling when low, moderate, and high concentrations were measured, in any sequence (Wahab et al., 1990; Lyimo et al., 2004). Cows with low progesterone concentration in all samples (Lyimo et al., 2004) were considered acyclic/anoestrus.

5.3.5 Metabolic profiles

Serum samples were analyzed for albumin, urea, NEFA, and insulin-like growth factor-1 (IGF-1) concentrations. Albumin and urea concentrations were measured for all groups, while NEFA and IGF-1 were measured only in Group 1. Urea, albumin and NEFA concentrations were measured usuing commercially available kits (Randox Laboratories, Antrim, UK; catalogue nos. UR446, AB362 and FA115, respectively). Randox calibration serum (CAL2351) was used as a daily quality control. Inter-assay coefficients of variations for urea, albumin and NEFA were 8%, 3% and 6%, respectively. Insulin-like growth factor-1 concentrations in serum were measured in duplicate by ELISA (AL-121 TOTAL IGF-1 ELISA, ANSH Labs, Webster, USA). Intra- and inter-assay coefficients of variation for IGF-1 were 1.5% and 12.8%, respectively.

5.3.6 Analysis of data

Reproductive performance was assessed based on four outcome variables (I) cyclicity (cyclic or acyclic: binary), defined based on the progesterone concentrations measured in consecutive serum samples; (II) pregnancy (pregnant or non-pregnant: binary) status, defined based on the outcome of trans-rectal pregnancy diagnosis/ ultrasonography, (III) CCI (days), defined as the number of days between calving and breeding which resulted in pregnancy; (IV) services per conception (SPC), defined as the number of breedings that a cow or heifer required to become pregnant. For statistical analysis, the CCI was ascribed values of either 1 (≤200 days) or 2 (>200 days). Values of SPC were ascribed values of 1 (≤3 services) or 2 (>3 services). Additionally, calving to first service interval (CFSI, days: defined as the number of days between calving and first breeding), first service to conception interval (FSCI, days: defined as the number of days between the first breeding and the last breeding that resulted in pregnancy), and non-pregnant days (NPD, days: defined as the number of days a cow was unable to conceive even after multiple services and those cows were managed with other cows in the herd) were identified as other important reproductive indices (Figure 5.1).

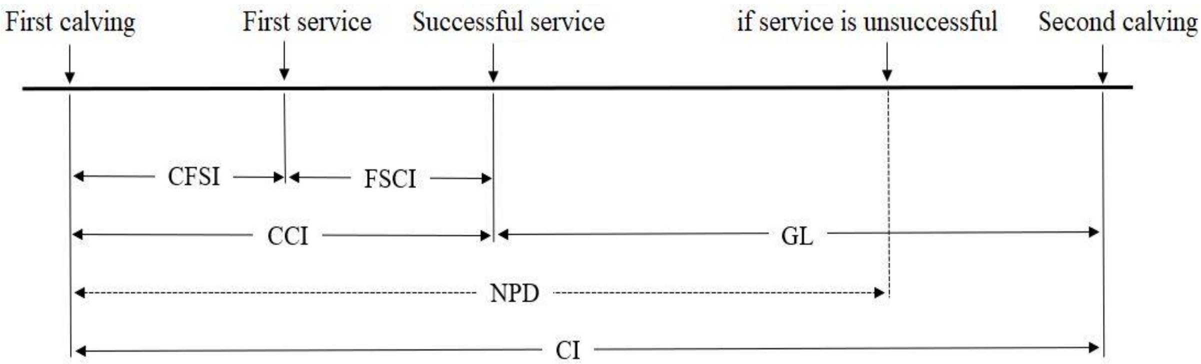


Figure 5.1. Graphical representation of the fertility indices included in the present study. CFSI calving to first service interval; FSCI first service to conception interval; CCI calving to conception interval; GL gestation length; NPD non-pregnant days; CI calving interval

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Multivariable logistic regression was performed using the GLIMMIX procedure to model binary outcome variables: cyclicity, pregnancy, CCI and SPC. Reproductive parameters were seperately used as the dependent variables. The explanatory independent variables were calving month (Calv_month), breed (1=Holstein Friesian×Jersey, 2=Sahiwal×Jersey, 3=Jersey), parity (1=primiparous and 2=multiparous) and the lowest BCS recorded during the mating period. All possible two-way interactions between the independent variables were initially included in models, but non-significant ($P \geq 0.05$) interactions were removed from the final models. Days in milk (DIM) was used as the covariate.

Relationships between blood metabolites (albumin, urea, NEFA and IGF-1) vs. cyclicity were assessed by logistic regression models using the GLIMMIX procedure. Blood metabolites and DIM were used as independent variables and covariate in each model, respectively. Cow number was used as a random effect for the analysis. The Kaplan-Meier survival analysis was conducted to determine the hazard of pregnancy for cyclic vs. acyclic cows.

5.4 RESULTS

5.4.1 Basic cow data

Data were collected from 112 postpartum cows (91 primiparous and 21 multiparous) with 64, 61, 57, 55, and 50 serum samples for TP 1, 2, 3, 4, and 5, respectively. The mean BCS during TP 1, 2, 3, 4, and 5 were 4.3 ± 0.04, 4.3 ± 0.04, 4.3 ± 0.04, 4.4 ± 0.04, and 4.4 ± 0.04, respectively.

5.4.2 Progestone profiles of postpartum dairy cows

The mean ± SEM, minimum and maximum progesterone concentrations for cyclic cows were 2.8 ± 0.19, 0.14, and 10.18 ng/ml, respectively. Similarly, values for acyclic cows were 0.5 ± 0.02, 0.12 and 0.98 ng/ml, respectively.

Progesterone concentration profiles of dairy cows were plotted against time over the 120 days of the postpartum period (Table 5.1 and Figure 5.2). In summary, 31% of animals had regular oestrous cycles, and 5% had irregular cycles, between Days 42 and 56 postpartum. In a further 19.6% of animals the onset of oestrous cycles was delayed to Day >84.

Table 5.1. Description and incidence of progesterone profiles of postpartum dairy cows.

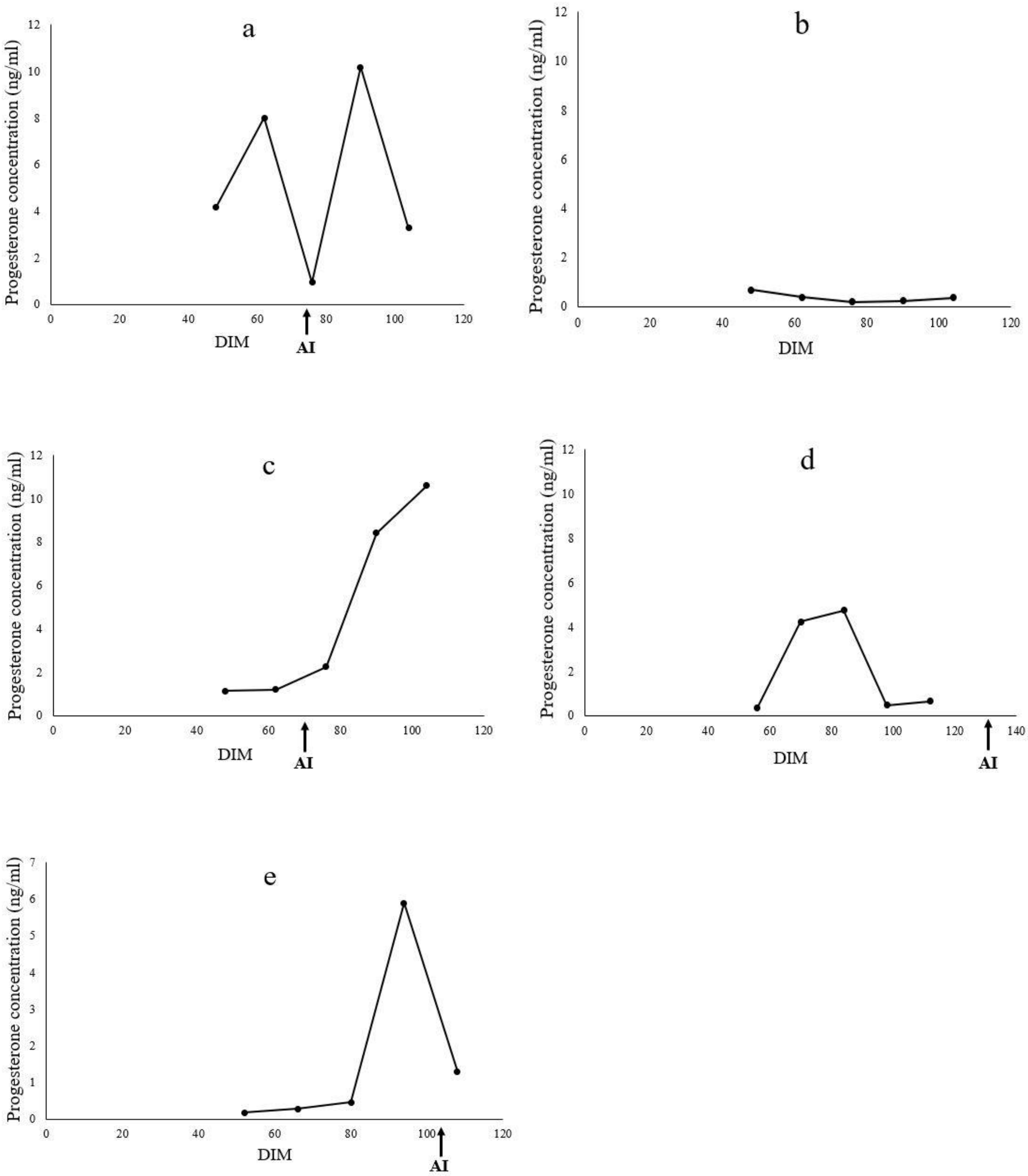
Profile	Description	N (%)
Regular cyclic (Figure 5.2a)	First rise in progesterone between 43-56 days postpartum and low, medium and high progesterone concentrations regularly, in any sequence	35 (31.3)
Acyclic (Figure 5.2b)	Low progesterone concentrations all time points or low progesterone after 57 days postpartum	43 (38.4)
Pregnant (Figure 5.2c)	Initial normal cyclicity and then high progesterone concentrations for 4-6 weeks consecutively	6 (5.4)
Irregular cyclic (Figure 5.2d)	Irregular pattern of low, medium and high progesterone profiles	6 (5.4)
Delayed 1st ovulation (Figure 5.2e)	First rise in progesterone after 84 days postpartum	22 (19.6)

N number of cows

Table 5.2. Cyclicity of dairy cows from 42 to 120 days postpartum, based on observed standing heat and progesterone concentrations in repeated serum samples taken fortnightly

	Number of cows displaying oestrus		
Cyclicity as determined by progesterone profile	Yes	No	Total cows
Cyclic progesterone	38	31	69
Acyclic progesterone	9	34	43
Total	47	65	112

Cyclic progesterone: progesterone concentrations >1 ng/ml in at least two samples;
Acyclic progesterone: progesterone concentrations <1 ng/ml in all samples.



Figures 5.2. Serum progesterone profiles illustrative of the different presentations found in the study cows. a. progesterone profile of a regular cyclic cow; b. progesterone profile of acyclic cow; c. progesterone profile of a pregnant cow; d. progesterone profile of an irregularly cyclic cow; e. progesterone profile of a cow presented with delayed 1st ovulation. Cows received first artificial insemination (AI) on 75, 153, 70, 134, and 102 days after calving, respectively

5.4.3 Reproductive performance

Of the 112 cows recruited in the study, seven were reported with retained fetal membranes and four with metritis. Of those animals, three with retained fetal membranes and four with metritis received veterinary care and treatment. Following successful treatment, cows were returned to their original herds.

The total number of cows considered to be in oestrus based on visual behavioral observation and classified as cyclic/acyclic based on repeated progesterone assays, are shown in Table 5.2. Artificial insemination was implemented after detected standing heat following the AM/PM insemination rule (Trimberger, 1948; Graves et al., 1997). From 112 cows, 42% (n=47) were observed in oestrus and presented for AI between 42 and 120 days postpartum; however, only 80.9% (n=38) of those cows were classified as cyclic according to the progesterone assay. The remaining 58% (n=65) of cows were not observed in oestrus during the first 120 days postpartum; however, 47% (n=31) of those cows were classified as cyclic according to the progesterone assay.

Progesterone concentrations were indicative of cyclicity in 61.6% (n=69) of cows during the first 120 days postpartum; however, 44.9% (n=31) of those cows were not observed in oestrus by the farmers and therefore not presented for AI during that period. Acyclicity was detected in 38.4% (n=43) of cows based on progesterone concentrations, of which 20.9% (n=9) were recorded as showing oestrus signs during the first 120 days postpartum and were, therefore, inseminated.

The reproductive performance of all farms during the first 400 days postpartum is summarised in Table 5.3. Pregnancy rates were 12.7% (n=6/112) and 41% (n=46/112) during the first 120 and 400 days postpartum, respectively. The minimum CCI was 68 days for cows which conceived at the first round of insemination, and the maximum CCI was 321 days at the sixth attempt of insemination.

Table 5.3. Summary statistics for calving to first service interval (CFSI), calving to conception interval (CCI), services per conception (SPC), first service to conception interval (FSCI) and non-pregnant/open days (NPD) in lactating dairy cows (as diagnosed up to 400 days postpartum) in medium-scale dairy operations in Sri Lanka

Variable	N	Mean ± SEM	CoefVar	95% confidence interval	
				lower	upper
CFSI, d	112	116.0 ± 2.5	23.1	96.5	133.8
CCI, d	46	196.1 ± 8.9	30.9	150.3	239.5
SPC, n	46	3.1 ± 0.2	45.8	3	6
FSCI, d	46	86.4 ± 7.9	61.6	42	125.3
NPD, d	66	302.8 ± 7.3	19.3	275.5	340.5

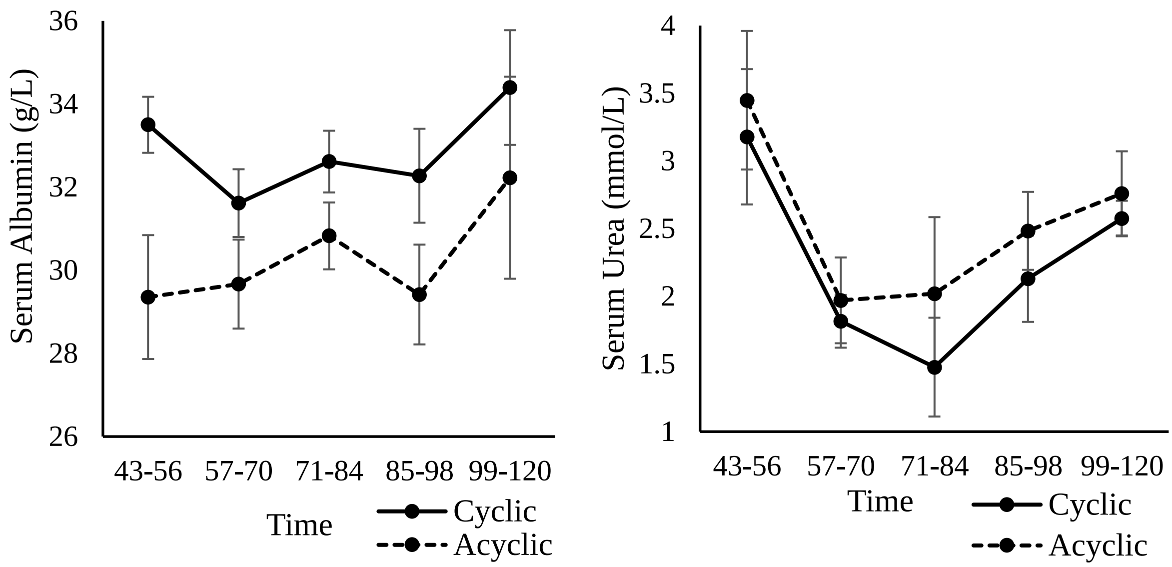
d days; n- counts; N total observations; SEM standard error of the mean; CoefVar coefficient of variation.

Based on the farm records, a total of 479 inseminations were performed during the first 400 days postpartum, resulting in a total of 46 confirmed pregnancies. From all cows inseminated, only six conceived to the first AI, resulting in a CR for first AI and an overall CR of 5.4% and 9.6%, respectively.

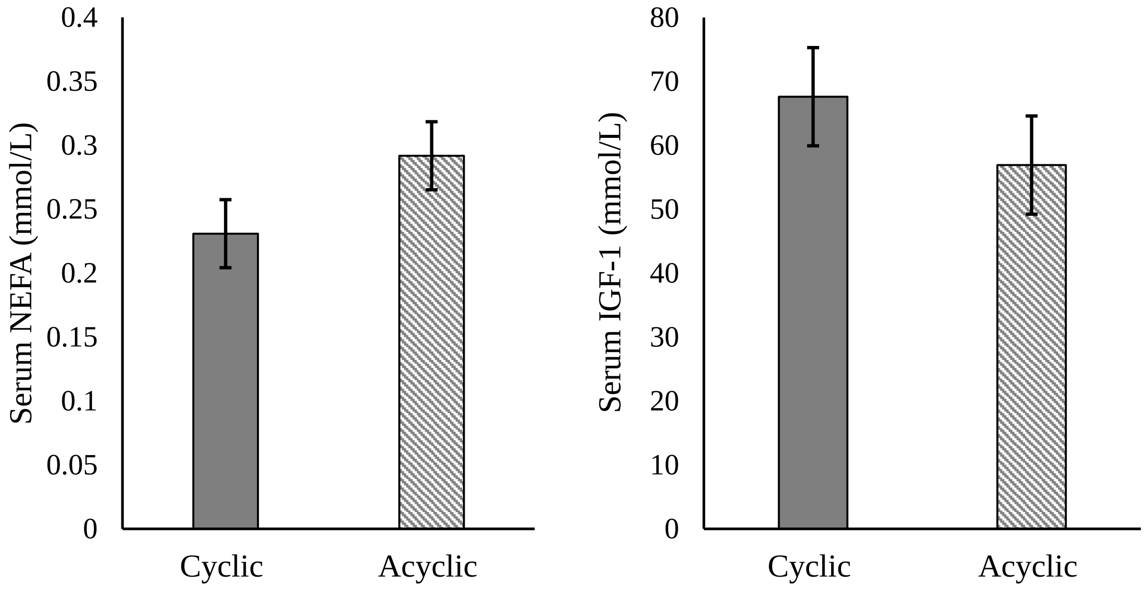
5.4.4 Blood metabolites concentrations

The mean concentrations of blood metabolites and IGF-1 of cyclic and acyclic cows during all five TP are shown in Figures 5.3 and 5.4. Cyclic cows had higher and lower concentrations of albumin and urea than acyclic cows during all TP, respectively, however, those relationships were not statistically significant (P>0.05). Similarly, cyclic cows had numerically higher and lower concentrations of IGF-1 and NEFA than acyclic cows during 43-

56 days postpartum, respectively. The NEFA concentration of cyclic cows was 16% lower than in acyclic cows, whilst IGF-1 concentration was 21% higher in cyclic cows than in acyclic cows during the first (43-56 days) postpartum period (Figure 5.4).



Figures 5.3. Mean (± SEM) concentrations of albumin and urea in cyclic and acyclic cows from 43 to 120 days of the lactation cycle



Figures 5.4. Mean (± SEM) concentrations of non-esterified fatty acids (NEFA) and Insulin-like growth factor-1 (IGF-1) in cyclic and acyclic cows from 43 to 56 days of the lactation cycle

5.4.5 Cyclicity status and pregnancy outcomes up to 400 days postpartum

The association between cyclicity status of cows between 43 and 120 days postpartum and pregnancy diagnosis up to 400 days postpartum is depicted in Table 5.4. Only 41.1% (n=46/112) of cows became pregnant during the first 400 days postpartum. From those cows diagnosed pregnant, 63% (n=29/46) were diagnosed as cyclic during the 43 to 120 days postpartum period on the basis of progesterone profiles.

Table 5.4. Pregnancy (as diagnosed up to 400 days postpartum) by cyclicity status between Days 43 and 120 postpartum in dairy cows in medium-scale dairy operations in Sri Lanka

Cyclicity status from 43-120 days postpartum	**Pregnancy (<400 days postpartum)**		**Total**
	Pregnant	Non-pregnant	
Cyclic	29	40	69
(%)	(25.9)	(35.7)	(61.6)
Acyclic	17	26	43
(%)	(15.2)	(23.2)	(38.4)
Total	46	66	112
(%)	(41.1)	(58.9)	(100.0)

The risk of pregnancy relative to CCI for cyclic and acyclic cows is depicted in Figure 5.5. The risk of pregnancy in cyclic cows increased rapidly as the CCI increased, and at 150 days CCI, the risk of pregnancy in cyclic cows was around 0.2, while the risk was less than 0.05 for acyclic cows. Cyclic cows were diagnosed pregnant in early lactation (~70 days DIM), while in acyclic cows, positive pregnancy diagnosis did not occur until the middle of lactation (~150 days DIM). Pregnancy rates drastically increased in acyclic cows between 210 and 240 days postpartum, while cyclic cows maintained a gradual and sustained increase in pregnancy during the 70-160 days and again between 210-240 days postpartum periods. In cyclic cow, CCI (179.2 ± 12.41) was significantly lower than in acyclic cows (225 ± 7.97).

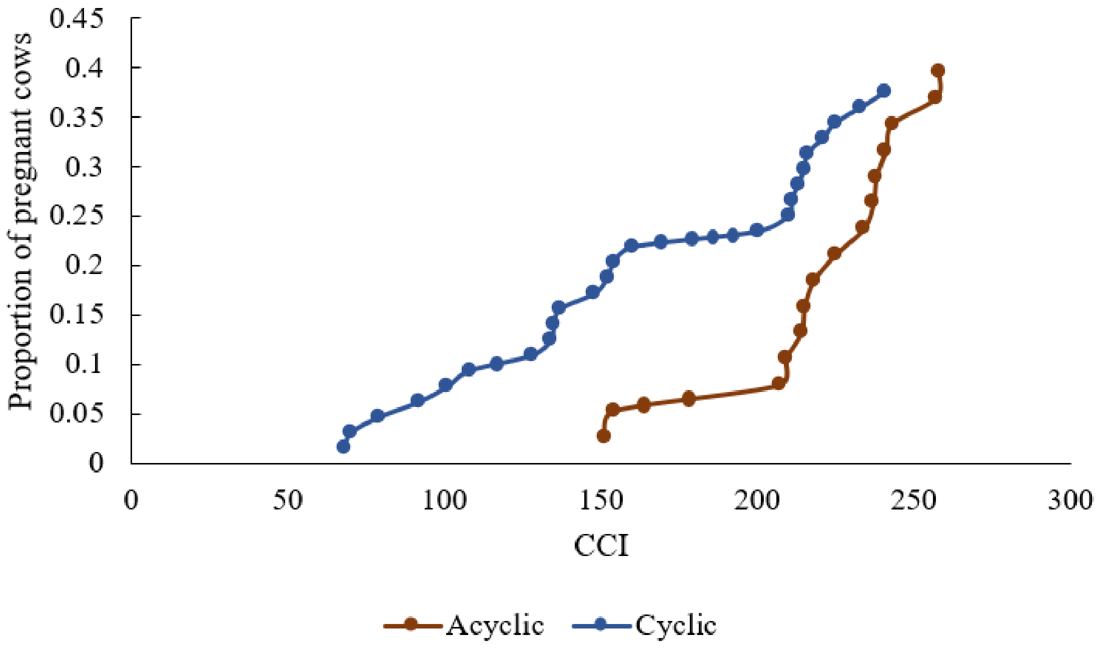


Figure 5.5. Kaplan- Meier survival analysis for the hazard of pregnancy against calving to conception intervals (CCI) of cyclic and acyclic lactating dairy cows

5.4.6 Logistic regression-multivariable analysis

Results from the multivariable logistic regression of the entire study cohort are shown in Table 5.5. Parity had a significant effect on cyclicity and CCI of postpartum dairy cows. Multiparous cows were more likely to be cyclic than primiparous cows (odds ratio (OR): 8.3; P<0.05); however, the majority of cows (91/112; 81%) included in the study were primiparous. Calving to conception intervals of multiparous cows were more likely to be lower than primiparous cows (OR: 8.3; P<0.05); however, the majority of cows (33/46; 71.7%) that conceived in the study were primiparous. The proportion of cows having oestrous cycles and CCI were not significantly influenced by breed, calving month and BCS.

Breed and the calving month had a significant effect on pregnancy rates and SPC. Pregnancy rate of Jersey cows was more likely to be higher than that of Jersey × Holstein Friesian (OR: 9.9; P<0.01). Cows that calved in May had greater OR for a higher pregnancy rate than cows that calved in June (OR: 7.67; 95% CI: 1.84-36.4; P<0.05). Number of SPC of

Jersey cows was more likely to be higher than Jersey×Holstein Friesian (OR: 9.9; P<0.01). Cows that calved in April (OR: 0.14; P<0.05) and June (OR: 0.1; P<0.01) had lower OR for SPC than cows that calved in May. Pregnancy rates and SPC were not related to parity or BCS.

Table 5.5. Logistic regression model with resulting odds rations, lower and upper 95% confidence interval (CI) for cyclic vs. acyclic, pregnant vs. nonpregnant status, calving to conception interval (CCI), and services per conception (SPC) with parity, breed and calving month effects of postpartum dairy cows

Reproductive parameter	**Influencing factor**	**Class**	**N**	**Odds ratio**	**95% confidence interval** (Lower-upper)
Cyclicity	Parity	Primi	91	0.12*	0.1-1.06
		Multi	21	Ref.	
Pregnancy	Breed	J × F	89	0.1**	0.08-0.39
		J × S	10	0.39	0.07-3.36
		J	13	Ref.	
	Calving month	March	12	2.33	0.16-33.8
		April	33	1.14	0.14-9.43
		May	52	7.67*	1.27-36.4
		June	15	Ref.	
CCI	Parity	Primi	33	0.12*	0.1-1.13
		Multi	13	Ref.	
SPC	Breed	J × F	89	0.1**	0.07-0.35
		J × S	10	0.55	0.06-5.48
		J	13	Ref.	
	Calving month	March	12	0.24	0.03-1.38
		April	33	0.14*	0.04-0.64
		May	52	Ref.	1.27-36.4
		June	15	0.1**	0.02-0.36

Primi: primiparous; Multi: multiparous; J × F Jersey - Holstein Fresian crossbred; J × S Jersey - Sahiwal crossbred; J Jersey purebred. **P<0.01; *P<0.05. Ref. Referent.

5.5 DISCUSSION

Lack of knowledge and/or understanding of reproductive and nutritional management by dairy farmers in Sri Lanka (Vyas et al., 2020), coupled with the scarcity of data summarising the current status of reproductive performance in medium-scale dairy farms, impose significant challenges to the improvement of dairy farm profitability. The present study used progesterone profiles and recorded data to characterize the postpartum return to oestrous cycles and to derive key reproductive indices, and thereafter identify risk factors associated with resumption of postpartum oestrous cycles and conception. As expected, the resumption of oestrous cycles and oestrus detection rates were critical to the reproductive outcomes of dairy herds, with the very significant delays to these events after calving appearing to be the limiting factor to reproductive success.

Serum progesterone profiles have been widely used to classify reproductive activity in postpartum dairy cows. Cows can be identified through progesterone profiling as having regular or irregular oestrous cycles, being anoestrous, or being pregnant (Intraraksa et al., 1990). Deciding on the frequency of sampling is challenging, particularly for the present study. On the one hand, the more frequently samples are collected, the more accurate the characterisation of ovarian activity (Savio et al., 1990), but, on the other hand, the logistical difficulties of collecting samples frequently can make such a sampling regimen impractical. Hence, the sampling regimen has to represent a compromise between the need for complete data *vs.* the practical limitations of frequent sample collection. In the present study, this was resolved by sampling cows at 2-week intervals over the period between 43 and 120 days postpartum. Whilst this frequency will not characterise oestrous cycles in detail, it is sufficiently frequent to identify when cows are anoestrous, are having normal or abnormal oestrous cycles, or are pregnant (or, at least, when they have an extended duration of luteal activity).

Data from the progesterone profiles showed that only around 60% of cows resumed oestrous cycles during the first 120 days postpartum. Moreover, of those cows, 45% were not detected in oestrus by behavioural observation. A study conducted by Lyimo et al. (2004) in the tropics found a higher proportion (>95%) of cows resuming oestrous cycles during the first 120 days postpartum than in the present study, although the oestrus detection rate was similar. It was suggested that subtle oestrus manifestation due to heat stress and/or occurrence of ovulation without behavioural manifestation of oestrus (silent heat) possibly interfered with the oestrus detection efficiency in that study (Stevenson, 2001; Lyimo et al.,, 2004; Aggarwal & Upadhyay, 2013). The situation in Sri Lanka is compounded by suboptimal facilities for the detection of behavioural oestrus (Vyas et al., 2020), making this an even greater challenge for dairy farmers. The problems of using behavioural signs for the detection of oestrus are, of course, well recognised, particularly in high-yielding cows (Stevenson, 2001; Gordon, 2011; Mottram, 2016; Reith & Hoy, 2018); so various aids to oestrus detection (e.g. heat-mount detectors, teaser animals, milk or serum progesterone tests, podometry, chin ball markers, tail paint, and activity monitors) are commonly used to improve the efficiency of detection (Foote, 1975; Firk et al., 2002; Durkin & DeLaval, 2010; Palmer et al., 2010; Kumar et al., 2013). Whether any of those technologies would be beneficial for enhancing oestrus detection in the Sri Lanka dairy system is unclear. On the one hand, the use of oestrus detection aids would probably increase either the proportion of cows detected in oestrus, or more accurately identify the onset of oestrus (or both), allowing more cows to be inseminated with better outcomes. On the other hand, even the simplest technology for improving oestrus detection comes at a cost, and it may be that the returns from milk sales would be insufficient to cover such costs.

Conversely, getting more cows pregnant in a timely fashion would improve milk outputs (and, hence, income), provided that there were adequate sources of feed to cover the needs of those cows.

Many factors influence the resumption of oestrous cycles of postpartum dairy cows, such as breed, parity, BCS, metabolic diseases, uterine abnormalities and nutritional status (Zain et al., 1995; Butler, 2001; Shrestha et al., 2004; Walsh et al., 2007a; Crowe, 2008, 2014). In the present study, breed, parity, calving month and BCS were considered as potential risk factors affecting the reproductive performance of dairy cows. The effects of breed are of particular interest in this context. It is generally accepted that crossbreeding increases fertility of dairy cows (Buckley et al., 2014). However, in the present study, Jersey cows performed better (higher pregnancy rates and lower SPC) than crossbred cows. Perhaps this is a reflection of the crosses that were used in this study- i.e., that neither Holsteins nor Sahiwal are particularly fertile breeds; an effect compounded by the poor tolerance of Holstein cows to tropical conditions. The effects of parity in the present study were also interesting. According to the logistic regression model, multiparous cows were more likely to be in oestrus before 120 days postpartum than primiparous cows. In contrast, the studies conducted by Tanaka et al. (2008) and Zhang et al. (2010) found that primiparous dairy cows were more likely to return to cyclicity than multiparous cows during the postpartum period. Conversely, there is also good evidence that primiparous dairy cows in pasture-based systems are at jeopardy of poor reproductive performance when their nutritional needs are not being met (McDougall, 2006). So there is a fascinating paradox in the present study: it is difficult to draw a definitive conclusion about the effects of parity due to the low proportion of multiparous cows that were included, but this low proportion reflects the population of the dairy herds. In other words, the population of cows presented for the present study indicates a poor survival of cows from primiparous to multiparous. This is also consistent with the very poor reproductive performance of primiparous animals in general (Buaban et al., 2015). Regarding the effect of calving month on fertility, pregnancy rates were higher and SPC were lower for cows that calved in May than for other times of the year. It is interesting to speculate whether this relates to the onset of the monsoon season: although there is no published literature to support this observation in Sri Lanka, data from elsewhere in tropical/subtropical regions support the notion that reproductive performance is better when feed availability is higher and thermal stress is reduced (Asimwe & Kifaro, 2007).

The cows included in the present study were poorly fed during lactation. Feed was of low quantity and poor quality (see also Chapter 3), being deficient in both energy and protein in the finished diet. These are recurrent observations in dairy herds of Sri Lanka (Herath et al., 2018; Ranaweera et al., 2020; Vyas et al., 2020). Many studies have shown that nutrition is a key factor contributing to the delay in the postpartum resumption of ovarian activity (e.g. Peters, 1984; Butler, 2000), and that CCI dramatically worsens when cows receive inadequate nutrition (Patton et al., 2007; Vyas et al., 2020). The high energetic demand of the postpartum period and the resultant NEB that occurs in the face of undernutrition causes downregulation of hypothalamic activity, resulting in disruption of normal patterns of gonadotrophin secretion which, in turn, can result extended periods of ovarian inactivity (anovulatory anoestrus) (Butler, 2000; Roche, 2006). Oocyte quality can also be directly impaired in cows in NEB, given that NEB can result in hypoinsulinaemia and that insulin in a critical cofactor for oocyte/follicluar growth (Britt, 1994; Leroy et al., 2008; Bisinotto et al., 2012). Further, increased concentrations of NEFA and BHBA in follicular fluid during NEB can adversely affect oocyte quality/maturation (Leroy et al., 2004; Matoba et al., 2012). Taken together, NEB, such as the cows experienced in the present study, can both impede resumption of ovarian cyclicity (Walsh et al., 2007b; Ospina et al., 2010) and the quality (viability) of any oocytes that are ovulated.

Curiously, therefore, the BCS of cows in the present study was not related to the time to resumption of oestrous cycles, nor to pregnancy rate, CCI, or SPC. This could be due to the low ($\leq$1) BCS change recorded for the majority of cows between calving and mating. Some other studies have reported that neither absolute BCS nor BCS change affected time to first observed oestrus and CCI (Ruegg & Milton, 1995; Walsh et al., 2007a). More generally, however, BCS at calving has an impact on postpartum health and the duration of anoestrus (Shrestha et al., 2005; Bewley & Schutz, 2008; Crowe, 2008). Shrestha et al. (2005) noted that a decrease in BCS of $\geq$1 unit (1-5 scale) at seven weeks after calving increased the occurrence of delayed first ovulation, therefore, it is advisable to restrict the BCS loss to <0.5 unit (1-5 scale) to optimize resumption of postpartum ovulation (Crowe, 2008). Body condition score provides useful information on the nutritional status of cows (Hady et al., 1994; Garnsworthy, 2006; Roche et al., 2009), thus it is a useful tool for informing management decisions regarding the quality and/or quantity of feeds, but it has limitations as a direct measure of reproductive performance.

The process of uterine involution is usually completed 4-5 weeks after an uncomplicated calving (Kindahl et al., 1999; Robinson & Noakes, 2018). The first ovulation is expected after 42 days postpartum and the first mating between 60-90 days postpartum (Falvey & Chantalakhana, 1999). However, the mean CFSI interval of the farms included in the present study was 116 days, mainly due to delayed resumption of ovarian activity and inefficient oestrus detection. A study conducted by Alexander et al. (1998) reported similar mean CFSI (111.2 ± 74.2 days) in hill country dairy farms in Sri Lanka. However, such values are not uncommon for dairy farms in the tropics, with studies reporting mean CFSI of 153.4 ± 81.3

days in Bangladesh (Siddiqui et al., 2013) and 205 ± 154 days in the Amazon for European × Zebu crossbred cows (Garcia et al., 1990). The CCI in the present study was 196.1 ± 8.9 days, which was similar (~200 days) to the study conducted by Garcia et al. (1990). However, the CCI reported herein is not acceptable according to the tropical dairy guidelines (optimum CCI <85 days; acceptable CCI <115 days; Falvey & Chantalakhana, 1999) and thus should be improved. Similarly, SPC recorded in the present study (3.05 ± 0.2) was higher than the value considered acceptable (<1.8) by the tropical dairy guidelines (Falvey & Chantalakhana, 1999).

Whether the poor reproductive performance of cows in the present study was also due to the presence of repeat breeder cows is worth considering. The repeat breeder syndrome has been defined as clinically normal cows that do not become pregnant after three consecutive inseminations (Yusuf et al., 2010). According to this definition, the incidence of repeat breeders in the present study population was 73.2%. Of these cows (82/112) only 16 (20%) conceived within 400 days postpartum. This incidence of repeat breeders is much higher than is generally reported, at least for well-managed cattle in temperate climates (Bulman & Lamming, 1978; Gustafsson & Emanuelson, 2002). The repeat breeding syndrome is classically associated with many factors (Kimura et al., 1987; Gustafsson & Emanuelson, 2002; Moss et al., 2002) such as improper insemination techniques (Morrell, 2006), poor oestrus expression, poor oestrus detection (Heuwieser et al., 1997) in cows with normal oestrous cycles, and with endocrine disorders (Lopez-Gatius et al., 2004), ovulation failure (Kimura et al., 1987; Silvia, 1994), uterine infections (Moss et al., 2002), and early embryonic death (Villarroel et al., 2004) in cows with abnormal cycles. In the present study, there was little evidence of early embryonic death, at least not as manifested by cows that returned to oestrus after luteal phases of longer duration than normal dioestrus (Santos et al., 2004). On the other hand, it is clear that there was widespread failure to detect oestrus (both missed and mis-diagnosed oestrus periods) and, probably, incorrect timing of insemination in the present study, so it seems reasonable to equate the apparently high incidence of repeat breeders with the more general syndrome of anoestrus/inadequate oestrus detection that was clearly prevalent throughout the study cows. The low incidences of cows with retained fetal membranes and clinical endometritis suggest that these were unlikely to be contributory factors.

The CR is an important indicator of reproductive performance of postpartum dairy cows. The overall CR in the present study was considerably lower (9.6%) than values reported in 2019 at district levels (29.2%; 60 calves from 205 AI procedures; Jayathilake et al., 2019) and also in 1996 at the national level of Sri Lanka (16.7%; 18,193 calves from 109,008 AI procedures; Abeygunawardhana & Alexander, 2001). Anzar et al. (2003) and Potdar et al. (2020) reported that overall CR rates in Pakistan and India were three times (29%) and five times (47.3%), respectively, higher than the present study. Several factors can influence the CR including cyclicity status, heat stress, parity, milk production, energy balance, and disease (Hansen & Areechiga, 1999; Lucy, 2001). Furthermore, insemination failures related to wrong insemination technique, inadequate timing of AI, and low semen motility at the time of AI also negatively impact the CR (Diskin, 2018). According to the published literature, heat stress and poor nutrition are the most significant factors negatively impacting CR of dairy cows in Sri Lanka (Abeygunawardena et al., 2001; Jayathilake et al., 2019). The biggest impact of low CR is associated with an increased culling rate (Ball & Peters, 2004), which detrimentally impacts total milk production for the farm.

5.6 CONCLUSION

The present study characterized the cyclic or acyclic status in primiparous and multiparous dairy cows from 43 to 120 days postpartum in eight medium-scale dairy farms, and attempted to identify risk factors associated with postpartum resumption of oestrous cycles and reproductive indices.

Low oestrus detection rate and prolonged postpartum anoestrus have a major impact on the productivity of postpartum cows in Sri Lanka. Parity of cows was a major risk factor for resumption of oestrous cycles and CCI, with breed and calving months as major risk factors for pregnancy rates and SPC. The present findings emphasise the importance of monitoring ovarian activity of postpartum dairy cows as an aid to identifying risk factors and possible causes of low CR. Despite the contribution of these various factors, it is clear that nutrition was the leading cause of subfertility. Correcting nutritional deficiencies, together with improving oestrus detection efficiency (by providing training on proper visual oestrus detection, using oestrus detection aids, and management of heat stress) and properly timed AI, may enhance pregnancy rates of postpartum dairy cows in the medium-scale dairy farms in Sri Lanka.

CHAPTER 6. RELATIONSHIP BETWEEN SERUM IGF-1 CONCENTRATIONS AND REPRODUCTIVE PERFORMANCE OF POSTPARTUM DAIRY COWS IN MEDIUM-SCALE DAIRY FARMS IN SRI LANKA

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Prepared for submission to *Animals*, August 2022

6.1 ABSTRACT

This study aimed to determine whether there was a relationship between concentrations of insulin-like growth factor-1 (IGF-1) in early postpartum cows and reproductive outcomes, in semi-intensively managed medium-scale dairy farms in Sri Lanka. Postpartum primiparous cows from four different dairy farms in the Kurunegala district of Sri Lanka were sampled fortnightly between May and September 2018. Cows were grouped by time period (TP) into TP1 (Days 1 to 14 postpartum) and TP2 (Days 43 to 57), all cows sampled in TP1 were re-sampled in TP2. Reproductive outcome data were collated in May 2019: calving to first service interval (CFSI), calving to conception interval (CCI), first service to conception interval (FSCI), services per conception (SPC), and non-pregnant dates (NPD). Body condition score (BCS) was assessed at the time of blood sampling. Overall mean IGF-1 concentrations were 66.5 ± 2.3 ng/mL (range: 22.8-133.2 ng/nL). Mean concentrations during TP1 and TP2 (60.8 ± 2.6 ng/mL and 72.3 ± 3.6 ng/mL) differed significantly from each other. Overall mean IGF-1 concentrations varied significantly between farms. Concentrations were not influenced by calving month or BCS at the time of sampling but were greater in animals with higher postpartum BCS. Higher IGF-1 concentrations were associated ($P<0.05$) with shorter CFSI, but IGF-1 concentrations were unrelated to CCI, FSCI, NPD and SPC. In conclusion, circulating IGF-1 concentrations during the postpartum period were related to CFSI, but were not related to final pregnancy outcomes. However, since delayed return to oestrus, which can be very prolonged, is a critical determinant of the poor reproductive performance of cows in medium-scale farms of Sri Lanka, the possibility of improving postpartum fertility in dairy cows by improving feed management and quality is emphasized, and the possibility of using IGF-1 concentrations as an indicator of the nutritional status is suggested.

Keywords: dairy cows, IGF-1, postpartum, fertility

6.2 INTRODUCTION

Sri Lanka set a target for its dairy industry to achieve 50% self-sufficiency in milk by 2015 (Ranaweera, 2011). However, although milk production has increased dramatically in recent years (15-20% in 2009, 35% in 2015 and 40% in 2016: Ranaweera, 2011; Vernooij et al., 2015; Pathumsha, 2016), the country is still behind that 50% goal. One of the main constraints behind the failure to meet this target is a lack of knowledge and/or understanding of reproductive and nutritional management amongst dairy farmers (Vyas et al., 2020), which translates into poor reproductive performance and a significant detriment to total annual milk production. Expansion of extension services in livestock management, health and breeding management (Perera & Jayasuriya, 2008), as well as the introduction of new dairy technologies, could significantly contribute to improving the productivity of the milk industry in Sri Lanka.

In the three weeks preceding and following parturition (transition period), cows undergo many physiological and physical changes which create a negative energy balance (NEB) (Grummer, 1995; Drackley, 1999; Esposito et al., 2014), changes in body weight, and body condition score (BCS), and metabolic disorders (Heuer et al., 2001; Pushpakumara et al., 2003). The extent of NEB during the first three weeks postpartum is highly correlated to the interval between calving and first ovulation (Butler, 2000). Hence, animals that are in severe or prolonged NEB, and also losing more BCS, particularly during the first 65 days postpartum, are more likely to be acyclic at the end of the voluntary waiting period, with an inevitable worsening of the interval from calving to conception and reduced conception rates (Santos et al., 2009; Bisinotto et al., 2012) with a consequential impairment of overall reproductive outcomes.

Insulin-like growth factor-1 (IGF-1: Rinderknecht & Humbel, 1978) has hormonal and autocoidal effects upon many aspects of systemic metabolic activity and ovarian function (Zhou et al., 1997; Taylor et al., 2004; Velazquez et al., 2008). It is mainly produced by the liver in response to growth hormone (GH) (Sjogren et al., 1999; Lucy, 2001; Velazquez et al., 2008), but is also produced by the granulosa cells of the follicle (Pushpakumara et al., 2002) and the corpus luteum (CL) (Perks et al., 1999; Wathes et al., 2003). Systemically, IGF-1 stimulates the uptake and utilization of glucose in adipose and muscle tissues, which results in decreased glucose concentration in blood (Perez-Martin et al., 2003). However, just after calving, IGF-1 production declines rapidly via inhibition of the GH receptor in the liver (Radcliff et al., 2003). Low blood concentration of IGF-1 alters the negative feedback on GH secretion, and therefore, blood GH concentrations rise (Veldhuis et al., 2001). Consequently, gluconeogenesis and lipolysis are increased in the liver and in adipose tissues providing the substrates to support the onset of copious milk production (Bell, 1995; Renaville et al., 2002; Radcliff et al., 2003).

Within the follicle, IGF-1 affects the growth and differentiation of tertiary and dominant ovarian follicles (Lucy, 2000) both directly and through influencing/amplifying the effect of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Furthermore, FSH itself is unable to support normal follicular recruitment where there are low concentrations of IGF-1 in the systemic circulation (Schoppee et al., 1996). IGF-1 also affects luteal activity, both directly and via the development of the pre-ovulatory follicle (Gallo & Block, 1991; Lucy et al., 1994; Yung et al., 1996), such that luteal activity is impaired in the face of significant NEB (Spicer et al., 1990). Thus, positive correlations between energy balance and IGF-1 concentration, and between energy balance and progesterone concentrations have been demonstrated (Spicer et al., 1990), such that inadequate luteinisation, especially of a small pre-ovulatory follicle, increase the risk of both inadequate progesterone production and premature luteolysis. These effects have been associated with poor expression of oestrus, low fertilisation rates and increased early embryonic death (Bisinotto et al., 2010; Santos et al., 2016).

Concentrations of IGF-1 during the breeding period are therefore associated with the fertility of dairy cows (Zulu et al., 2002), both through its general effects upon metabolism and its specific effects upon follicular growth and gonadotrophin-induced differentiation in the ovaries (Funston et al., 1996; Perez-Martin et al., 2003). Thus, where IGF-1 concentrations are low during the postpartum period, due to either genetic or nutritional effects, animals tend to lose BCS, which directly impairs both milk production and ovarian function. Likewise, poor nutrition and/or BCS loss during early lactation suppresses IGF-1-mediated oocyte development (Snijders et al., 2000), which may extend the follicular phase and resumption of ovulation, and luteal activity, which may impair embryonic survival (Lonergan, 2011)..

It may be that, if IGF-1 were to be measured in parallel to BCS, alongside data on parity, breed, and calving months, it could provide useful information on the current reproductive status of cows that are under metabolic stress. However, no studies appear to have been undertaken to estimate the reproductive efficacy of postpartum dairy cows using IGF-1 hormone profiles in medium-scale production systems in Sri Lanka. Therefore, the main objective of the current study was to investigate IGF-1 concentration changes of postpartum dairy cows during the transition period and two weeks before starting breeding. The aim was to examine the potential of using IGF-1 concentrations as a predictive indicator of the fertility of dairy cows in semi-intensively managed medium-scale dairy farms.

6.3 MATERIALS AND METHODS

Ethical approval was obtained from the Ethics Review Committee, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka (VER-18-002). Further, all sampling procedures were approved by the Massey University Animal Ethics Committee, New Zealand (MUAEC-18/32). Informed consent was obtained from dairy farmers before recruiting their cattle in this study.

6.3.1 Study area

The study was conducted in four dairy farms in the Kurunegala district, situated in the intermediate zone of Sri Lanka (7.48^{o}N, 80.36^{o}E), at an altitude of 116 m above sea level. The annual rainfall ranges from 1750 to 2500 mm, with two seasonal peaks, called the north-east monsoon and south-west monsoon, during May-September and December-February, respectively. The temperature in the Kurunegala district ranges from 25^{o}C to 35^{o}C during the daytime, with an average temperature of 28.5^{o}C (http://www.meteo.gov.lk/). The mean annual relative humidity (RH) ranges from 65% to 90%, with an average annual RH of 84%.

6.3.2 Farms and their management

In Sri Lanka, calving takes place year-round, which imposes challenges to the selection of animals in a particular stage of lactation in a given farm. Four medium-scale (11 to 100 lactating cows/farm) dairies were selected to participate in the current study, based on management type (semi-intensive), feeding practice (manually mixed ration: MMR), housing system (loose barn), temperate breeds (Jersey × Holstein-Friesian), breeding practice (artificial insemination, AI) and farming experience (>2 years). Cows were fed two or three times daily with MMR containing the forages (Guinea grass ecotype A (*Panicum maximum*) and Hybrid Napier CO-3 *(Pennisetum purpureum* * *P. americanum*)), and post-harvest maize residuals (*Zea mays* L.), along with three other feeds (maize silage, barley distillers' by-products, and commercially formulated cattle feed). Fresh clean drinking water was available *ad libitum*. Cows were milked twice daily using either portable or herringbone milking machines. Oestrus detection was undertaken from 60 days postpartum, and cows were bred by AI after signs of oestrus were detected.

6.3.3 Animals and their measurements

The study was conducted between May 2018 and May 2019. Fifty-six primiparous dairy cows, between 1 and 57 days postpartum, were selected for the study. Blood samples were collected from selected cows for IGF-1 assay between May 2018 and September 2018, and cows were followed through until May 2019 to collect farm records on reproductive outcomes. The animals included were Jersey × Holstein-Friesian crossbred cows between 2.5 to 3.5 years of age. Cows were grouped based on days from calving into two categories: time period (TP)1: 1 to 14 days postpartum, and TP2: 43 to 57 days postpartum.

Body condition score in each group of cows was estimated every two weeks according to the 1-10 scale used in New Zealand (LIC, 1993).

6.3.4 Reproductive parameters

Calving dates, insemination dates, and pregnancy diagnosis dates were collected from farm records to calculate reproductive outcomes, as illustrated in Figure 6.1. Pregnancy was confirmed by ultrasonography and/or rectal palpation ~50 days after the last insemination. Calving to conception interval (CCI) and services per conception (SPC) were calculated using calving dates and AI dates.

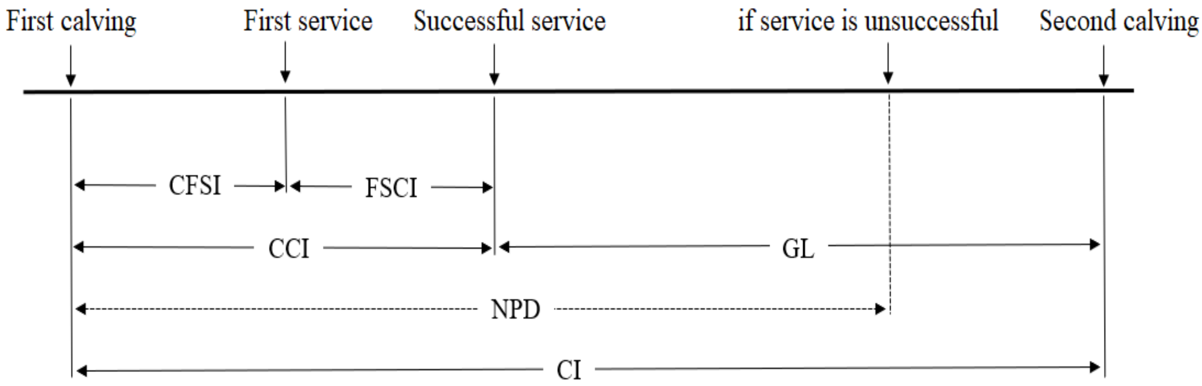

Figure 6.1. Graphical representation of the fertility indices included in the study, where CFSI = calving to first service interval, FSCI = first service to conception interval, CCI = calving to conception interval, GL = gestation length, NPD = non-pregnant days (cows not pregnant and remaining in the herd after being inseminated several times), and CI = calving interval

6.3.6 Blood sampling regimen

Blood samples were collected after morning milking via coccygeal venepuncture into evacuated tubes (Vacutainer, Jinsha North Road, Liuyang, Hunan, China) with no anticoagulant. After clotting for 1-2 h at room temperature, serum was separated by centrifugation at 3000 g for 10 minutes at 4°C. Serum samples were then stored at -20°C until assayed. Additional samples were collected during TP2 for cows that had previously been sampled during TP1.

6.3.7 Serum IGF-1 assay

Serum IGF-1 concentrations were measured in duplicate 20 µl aliquots by ELISA, using the AL-121 TOTAL IGF-1 ELISA (ANSH Labs, Webster, USA) according to manufacturer’s instruction. This is a highly specific, one-step sandwich-type immunoassay, whose primary use is for the measurement of total IGF-1 concentrations (i.e. bound and unbound) in human samples. The primary antibody is directed against human IGF-1 and has negligible cross-reactivity for IGF-II and IGF-binding proteins (IGFBP) 2, 3, 4, and 5, and has a marginal cross-reaction with human IGF-1/IFGBP complex.

The assay was validated for use with bovine serum by demonstrating parallelism between serial dilutions of two bovine serum samples (high: late dry, medium: early lactation) to dilutions of recombinant human IGF-1. The least detectable dose (LDD) was 1.49 ng/mL. Across 5 assays, the intra-assay coefficients of variation (CV) were 0.9-2.1%, and the inter-assay CV were 9.4% (2.5 ng/mL calibrator) and 16.2% (9.6 ng/mL calibrator). Full details of the assay process and the validation of the assay are provided in Appendix 3.

6.3.8 Statistical analyses

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc. Cary, NC). Data were first tested for normal distribution using the Kolmogorov-Smirnov test and q-q plots. Data were analysed using repeated-measures linear mixed models using the PROC MIXED function. In the model, IGF-1 concentration was the outcome variable and month of calving (1=May, 2=June, 3=July) was the categorical predictor variable, TP (1=1-14 days, 2=43-57 days) the repeated measure, and BCS (1= 3 to ≤4, 2= >4 to 5.5) the covariate. Time period × BCS interaction was also considered in the model. Cow number and farm identification (farmID) were set as the random effects.

The IGF-1 data were further analysed in separate models by binary logistic regression using the GLIMMIX procedure, to determine the association between IGF-1 as the predictor variable and pregnancy (pregnant or non-pregnant), calving to first service interval (CFSI; 1= ≤90 days, 2= >90 days), first service to conception interval (FSCI; 1= ≤85 days, 2= >85 days), CCI (1= ≤200 days, 2= >200 days), non-pregnant days (NPD; 1= ≤300 days, 2= >300 days) and SPC (1= ≤3 services, 2= >3 services) as explanatory individual output variables. Days in milk (DIM) was used as the covariate.

Mean IGF-1 comparison between farms (A-D) was undertaken using the PROC GLM function.

6.4 RESULTS

6.4.1 Basic cow data

Data were collected from 56 primiparous cows, with 56 and 55 serum samples for TP1 and TP2, respectively. The mean BCS during TP1 and TP2 were 4.7 ± 0.3 (95% confidence interval (CI): 4.5 to 5.0) and 4.3 ± 0.2 (95% CI: 4.0 to 4.5), respectively. The overall mean (± standard error of the mean (SEM)) IGF-1 concentration (ng/mL) during TP1 was 60.8 ± 2.6 (95% CI: 49.4 to 72.6) ng/mL and TP2 was 72.3 ± 3.6 (95% CI: 50.5 to 92.5) ng/mL. The mean ± SEM, minimum, and maximum IGF-1 concentrations for postpartum dairy cows were 66.5± 2.3, 22.8, and 133.2 ng/mL, respectively.

Corrected mean serum IGF-1 concentrations for each TP are plotted in Figure 6.2. Estimated means of IGF-1 concentrations were significantly ($P<0.05$) increased from the postpartum transition to mid-period of early lactation.

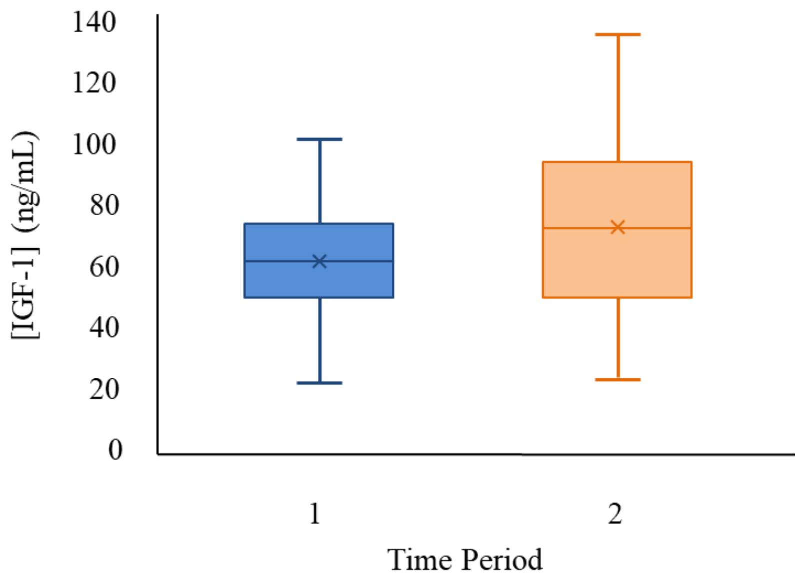

Figure 6.2. Boxplot represents differences in concentrations of insulin-like growth factor-1 (IGF-1) between TP1 (1-14 days postpartum) and TP2 (43-57 postpartum)

Serum IGF-1 concentrations (mean ± SEM) of Farms A, B, C and D were 76.1 ± 7.4, 72.5 ± 4.1, 60.7 ± 4.6, and 60.5 ± 3.3 ng/mL, respectively (Figure 6.3). Serum IGF-1 data were separated into two homogenous subjects, such that IGF-1 concentrations of Farms C and D were not different from each other (mean difference: 0.15 ng/mL (adj. 95% CI: -11.8 to 12.1 ng/mL)) but were lower than those of Farms A and B (highest mean difference Farm A and D: 17.6 ng/mL (adj. 95% CI: 1.1 to 34.1 ng.ml)). Serum IGF-1 concentrations of Farms A and B formed one homogenous subset (mean difference: 5.6 ng/mL (adj. 95% CI: -10.7 to 21.9 ng/mL)).

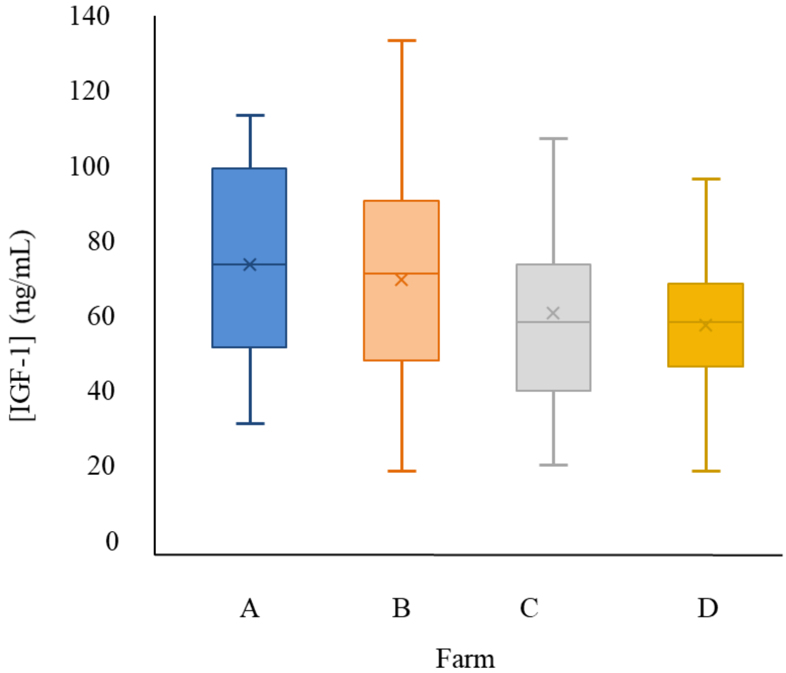

Figure 6.3. Boxplot represents mean Insulin-like growth factor-1 (IGF-1) concentrations (ng/mL) variations between four dairy farms in the Kurunegala district, designated as Farms A, B, C and D

The reproductive performance of dairy cows during the first 400 days postpartum is summarised in Table 6.1. The mean CFSI and CCI were above 100 and 200 days, respectively. Four cows conceived after the first insemination (5.4%) and 17 cows after the second or later inseminations (30.4%), whereas 35 cows did not become pregnant within 400 days postpartum (62.5%).

Table 6.1. Summary statistics for calving to first service interval (CFSI), calving to conception interval (CCI), services per conception (SPC), first service to conception interval (FSCI), and non-pregnant days (NPD) of primiparous postpartum cows in medium-scale dairy farms in Sri Lanka

Variable	**N**	**Mean ± SEM**	**95% CI**	
			lower	upper
CFSI, d	56	113.2 ± 3.3	53.0	161.0
CCI, d	21	202.2 ± 12.5	68.0	274.0
SPC, n	21	2.8 ± 0.3	1	6
FSCI, d	21	89.9 ± 12.3	0	221
NPD, d	35	300.8 ± 10.7	110.0	406.0

d days; n counts; N total observations; SEM standard error of the mean; 95% CI: 95% confidence interval

6.4.2 Model 1 (Repeated measure mixed model)

Results from mixed linear regression including all 56 cows are shown in Table 6.2. Serum IGF-1 concentrations were significantly ($P<0.05$) influenced by time after calving, with IGF-1 concentrations during TP2 being higher than the IGF-1 concentration during TP1 (mean difference: 15.9 ng/mL (adj. 95% CI: 8.1 to 23.7 ng/mL)). Serum IGF-1 concentrations were not influenced by calving month and BCS, however, IGF-1 concentration in cows in BCS group 2 (3 to ≤4) was numerically higher compared to the IGF-1 concentration in cows in BCS group 1 (>4 to 5.5) (mean difference: 9.1 ng/mL (adj. 95% CI: -1.4 to 19.5 ng/mL))

Table 6.2. Linear regression, least square means (SEM) of main effects on insulin-like growth factor-1 (IGF-1) concentration

	Independent variables									
	Calving month				BCS+			Time (TP)		
	1 (May)	2 (June)	3 (July)	SEM	1 ≤4	2 >4	SEM	1 (1-14)	2 (43-57)	SEM
IGF-1	68.1	67.2	56.1	7.8	59.2	68.3	6.6	55.8[b]	71.7[a]	3.9

+BCS body condition score; [a,b]value with different superscripts in the same row are different from each other (LSD, P<0.05)

6.4.3 Model 2 (Simple linear regression model and binary logistic regression)

According to the binary logistic regression, CFSI was inversely (P<0.05) related to IGF-1 concentrations. The CCI, FSCI, NPD, SPC and pregnancy status were not related to IGF-1 concentrations. Mean IGF-1 concentrations were numerically lower in pregnant cows than non-pregnant cows at TP1 (mean difference: -2.6 ng/mL (adj. 95% CI: -11.9 to 8.6 ng/mL)); however, mean IGF-1 concentrations were higher in pregnant cows than non-pregnant cows at TP2 (mean difference: 4.6 ng/mL (adj. 95% CI: -19.2 to 28.7 ng/mL)) (Figure 6.4).

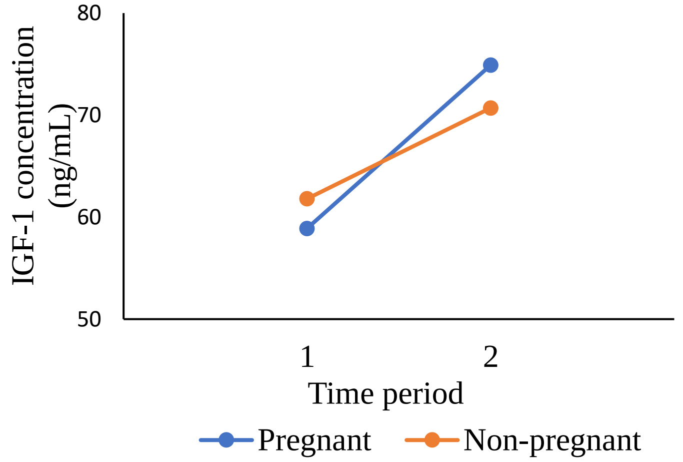

Figure 6.4. Insulin-like growth factor-1 (IGF-1) concentrations of pregnant/non-pregnant primiparous cows. Time period 1 (1-14 days postpartum); Time period 2 (43-57 days postpartum)

6.5 DISCUSSION

The present study has, for the first time, started to characterize the IGF-1 profiles of cows in medium-scale dairy farms in the sub-tropical environment of Sri Lanka. Insulin-like growth factor-1 is a key regulator of ovarian function, so measurement of its concentrations during the postpartum period may assist with the prediction of the reproductive performances of dairy cows (Taylor et al., 2004; Patton et al., 2007).

The overall reproductive performance of cows in the present study was poor, with an average CFSI interval of 113.2 ± 3.3 days. This period, which is well in excess of recommended figures for low-yielding cows (80-85 days, Nava-Trujillo et al., 2010; Kim & Jeong, 2019), is likely to be due to prolonged delays to the recovery of ovarian activity and the low efficiency of heat detection. A study conducted by Alexander et al. (1998) reported that such values are not uncommon in dairy farms in tropical/subtropical environments, and other studies in tropical regions have reported a mean CFSI of 153.4 ± 81.3 days in Bangladesh (Siddiqui et al., 2013) and 205 ± 154 days in the Amazon for European × Zebu crossbred cows (Garcia et al., 1990).

The CCI in the present study was 202.2 ± 12.5 days, which was similar to the studies conducted by Garcia et al. (1990) and Abeygunawardhana & Alexander (2001), but it exceeded the average (156 days) reported by Alexander et al. (1998). For an efficient pastoral dairy production system in which pastures grow seasonally and calving occurs annually, the mean CCI should be no greater than 80-85 days to achieve a mean 365 day CI (Ball & Peters, 2004); however, in tropical environments, CI of ~400 days have been recommended (Sandhu et al., 2011; Wondossen et al., 2018). Even so, according to the tropical dairy industry guidelines (Falvey, 1999), the SPC (3.05 ± 0.2) and the CCI reported in the current study were well above the acceptable limits (i.e. for tropical dairy farming the CCI should be <115 days and SPC <1.8 days). Higher CCI and SPC increase the number of non-pregnant cows at the end of the breeding period, which increases the expenses associated with reproductive management due to the increased culling rate.

The mean serum IGF-1 concentrations of primiparous cows during the early lactation were within the accepted range and were compatible with previously reported values (Taylor et al., 2004; Falkenberg et al., 2008). The overall IGF-1 concentrations increased significantly as lactation progressed. Nonetheless, greater changes between TP1 and TP2 were expected, inasmuch as previous studies have shown major increases in IGF-1 concentrations over the transition/early lactation period (Lucy et al., 1992), due to both the effects of lactation and of the nutrient balance that the cow experiences (Gobikrushanth et al., 2018). For example, Falkenberg et al. (2008) showed that IGF-1 concentrations in very early lactation are lower than in the later postpartum period in relation to the degree of NEB that the cows experienced over that time. In the current study, the range of IGF-1 concentrations at TP2 was larger (minimum and maximum concentration difference: 108.9 ng/mL) than during TP1 (minimum and maximum concentration difference: 76.8 ng/mL), which may reflect the developing effects of nutritional status, lactational yield, and BCS as lactation advanced, as well as the effects of individual variation.

In well-managed, high-yielding dairy cows, IGF-1 concentrations are influenced by days in lactation, such that higher concentrations are present around the start of the mating period (Taylor et al., 2004; Velazquez et al., 2008). Oestrous cycles characteristically recommence at 20-40 days postpartum (Bruinjé et al., 2017; Gobikrushanth et al., 2018), in the face of gradually rising IGF-1 concentrations. In temperate climates, the majority of cows under good management and nutrition systems resume ovarian cyclicity within ~3 weeks of calving (Butler, 2001; Crowe, 2008) and, even in tropical systems, can do so within the first month postpartum (Lyimo et al., 2004; Guaqueta et al., 2014). This resumption of ovarian activity represents the re-activation of the gonadotrophic axis from the long-term negative feedback effects of progesterone on pregnancy, during which follicular growth should be rapidly re-initiated in response to rising FSH concentrations (Crowe, 2008; Peter et al., 2009). Resumption of the capability for ovulation is more complicated, requiring both the resumption of pulsatile LH secretion, responsiveness of the follicle to LH and, finally, regeneration of the LH surge mechanism (Crowe, 2008). In these processes, the effects of NEB upon follicular development are mediated through impairment of LH secretory mechanisms (Bisinotto et al., 2012), the direct effects of circulating IGF-1 upon the granulosa cells, and interactions of other components of the metabolic endocrine system (notably insulin) with both neuroendocrine and intra-follicular activity. The extent to which NEB affects intrafollicular IGF-1 secretion is less well characterised (Lucy et al., 1992; Butler et al., 2004; Bisinotto et al., 2012), whilst it is also feasible that NEB affects the distribution/activity of the many IGF-1 binding proteins that are present in the base of antral follicles.

All of the above effects are likely to have pertained to the present study. Firstly, it was found that IGF-1 concentrations were greater in cows during early lactation with acceptable BCS than in cows with low BCS. When BCS is high, cows are more likely to have high concentrations of IGF-1 (Pushpakumara et al., 2003; Meikle et al., 2004; Gobikrushanth et al., 2018), although both decline during early lactation (Roche, 2007). Moreover, as also shown in previous studies, excessive loss of BCS at the beginning of lactation and poor BCS at the beginning of the breeding season can predict poor fertility (Domecq et al., 1997; Buckley et al., 2003). In other words, low concentrations of IGF-1 during the transition period (i.e. late dry to early lactation periods) and before the start of the mating period may compromise the reproductive system and, thereby, result in lower conception rates (Taylor et al., 2004). In the context of high-yielding dairy cows in temperate environments, Taylor et al. (2004) suggested minimum IGF-1 concentrations for multiparous postpartum cows during early lactation that would be likely to result in conception to the first service of >25 ng/mL and >50 ng/mL at one week after calving and at mating, respectively. It is difficult to relate such figures to the present study, however, as mean concentrations were 60.8 ± 2.6 ng/mL in TP1 and 72.3 ± 3.6 ng/mL in TP2 (i.e. at least 1.5 times higher than these recommendations), yet only 37.5% (21/56) of primiparous cows included in the current study were pregnant. This could, perhaps, merely reflect differences between the circulating IGF-1 concentrations that are present in multiparous cows *vs.* primiparous cows (inasmuch as concentrations in primiparous cows are significantly higher than in multiparous cows (Taylor et al., 2004)), such that minimum IGF-1 concentration for primiparous cows that are likely to conceive to the first service might be higher than suggested minimum IGF-1 concentrations for multiparous cows. It may also, perhaps, be worth noting that the animals used in the present study had the genetic capability to be high yielding (Holstein Friesian and Jersey), an ability that is typically associated with high levels of GH secretion that are consequent upon modifications to the GH-IGF axis that ensure low circulating concentrations of IGF. Surprisingly, therefore, the IGF-1 concentrations were higher than expected for genetically high-yielding cows in early lactation, and for animals that were not under a high metabolic load. The significance of these findings is unclear but may well warrant further investigation.

Relationships between reproductive outcomes and IGF-1 concentrations in the present study were not clear-cut, inasmuch as the only associations were the inverse relationships between CFSI and IGF-1 concentrations. Insulin-like growth factor-1 concentrations were unrelated to the FSCI, CCI, NPD and SPC indices. Beam & Butler (1999) reported that low IGF-1 concentrations during early postpartum resulted in longer time to resume ovarian cyclicity, with a consequential increase in calving to the first mating interval. Other reproductive parameters were unrelated to IGF-1 concentrations, perhaps merely due to the low pregnancy rate (37.5%, n=21/56) and to the limited number of cows included in the study. However, it may equally represent the lack of a cow-level effect, given that Wathes et al. (2007) and Gobikrushanth et al. (2018) considered that the lack of association between circulating IGF-1 and ovarian cyclicity of primiparous cows might be attributable to the partitioning of nutrients mainly toward growth and milk production instead of other biological functions such as reproduction. The present results are therefore consistent with the published literature on the relationship between IGF-1 and reproductive parameters (Roberts et al., 1997; Taylor et al., 2004; Thatcher et al., 2006); although Falkenberg et al. (2008) stated that cows with high serum IGF-1 concentration resume oestrous cycles earlier and get pregnant sooner than cows with low IGF-1 concentration.

Whether the measurement of IGF-1 concentrations is a worthwhile predictor of fertility in cows under the management and environmental circumstances of the present study is an intriguing question. There was undoubtedly a relationship between IGF-1 concentrations and CFSI, although IGF-1 concentrations were not, *per se*, predictive of that index. Understanding the relationship between IGF-1 and reproductive outcomes probably requires a better understanding of the factors that regulate IGF-1 secretion in cows under the conditions of nutritional and thermal/humidity stress that pertain to low latitudes, including reasons for unexpectedly high concentrations in genetically high-yielding cows. Thus, the results of the present study provide a preliminary understanding of the relationships (and the lack thereof) between postpartum circulating IGF-1 concentrations and reproductive performance in dairy cows in Sri Lanka. Undoubtedly, however, more extensive studies, involving more frequent measurements, greater experimental numbers, and controlled nutritional circumstances would be more effective in achieving this understanding, yet, however, desirable such experiments might be, the logistical and practical difficulties of undertaking them under Sri Lankan conditions are substantial.

6.6 CONCLUSION

This is the first study estimating reproductive performances using IGF-1 hormone profiles of postpartum primiparous cows in medium-scale dairying in Sri Lanka. The study confirmed that CFSI was negatively correlated with IGF-1 concentrations. Predictive thresholds for IGF-1 concentrations associated with reproductive performance of high-yielding dairy cows in temperate climates were not borne out, due to the relatively high overall IGF concentrations and lack of relationship of IGF-1 concentrations with pregnancy outcomes found in the present study. These preliminary results may be important for the dairy industry in Sri Lanka since infertility and/or sub-fertility are prominent reproductive failures. Finally, as IGF-1 concentrations during postpartum are closely associated with the cow's nutritional status, it is suggested that improvement of nutrition after calving may enhance subsequent reproductive performance.

CHAPTER 7: THE INFLUENCE OF ROTATIONAL LENGTH, ALONG WITH PRE- AND POST-GRAZING MEASURES ON NUTRITIONAL COMPOSITION OF PASTURE DURING WINTER AND SPRING ON NEW ZEALAND DAIRY FARMS

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Published in *Animals*, 2022, 12, 1934-50.

https://doi.org/10.3390/ani12151934

7.1 ABSTRACT

Inconsistencies in quality (nutritional composition) of pasture herbage can potentially affect the lactational performance of dairy cows. The quality of ryegrass-clover pasture was investigated between August (winter: start of calving) and November (spring: end of breeding) on pasture-based dairy farms (>85% of total feed from pasture) that had short (n=2, Farms A and B; winter ~30 days, spring ~20-25 days) or long (n=2, Farms C and D; winter ~35 days, spring ~25-30 days) grazing rotations, to determine whether quality was affected by grazing rotation length (RT). Weekly assessments of pasture growth and herbage quality were made using a standardized electronic rising plate meter, and near-infrared spectroscopy, respectively. Data were subjected to repeated measure mixed model analysis, in which herbage quality was the outcome variable.

The highest pre-grazing dry matter (PGDM) and height, post-grazing dry matter (DM) and height, and number of live leaves per tiller (leaf regrowth stage, LS) were present in late spring. Neutral detergent fibre (NDF) and acid detergent fibre (ADF), and metabolisable energy (ME) and organic matter digestibility (OMD) were positively correlated to each other ($r^2 \geq 0.8$) whilst ADF and lipid, and ADF and OMD were negatively correlated ($r^2 \geq -0.8$; $P<0.01$). Metabolisable energy content was negatively correlated with ADF and NDF ($r^2 = -0.7, -0.8$, respectively), and was inversely related to PGDM. Metabolisable energy was higher ($P<0.05$) in farms with shorter (overall mean: 11.2 MJ/kg DM) than longer (10.9 MJ/kg DM) RT. Crude protein was also inversely related to PGDM and was higher with shorter (23.2% DM) than longer (18.3% DM; $P<0.05$) RT. Pre-grazing DM affected the amount of pasture that was grazed and, hence, the amount of DM remaining after grazing (post-grazing DM or residual), such that PGDM was correlated with post-grazing height and residual DM ($r^2 = 0.88$ and 0.51, respectively; both $p<0.001$).

In conclusion, RT, LS, and PGDM during winter and spring influenced herbage quality, therefore, better management of pastures may enhance the productivity of dairy cows. This information can be helpful for dairy farmers to manipulate pasture management to improve herbage quality and optimize the cows' performances.

Key words: Nutritional composition, pasture, rotational grazing, energy, protein

7.2 INTRODUCTION

Pasture herbage quality and pasture mass (dry matter (DM), kg/ha) are key factors when calculating the nutritional requirements of dairy cows in pasture-based dairy production systems (Moller et al., 1996; Verkerk, 2003; Dillon, 2007). In New Zealand, herbage quality and pasture mass both vary seasonally (Machado et al., 2005) and are affected by rotational grazing management (Frame et al., 2002), soil condition/nutrients (Clark et al., 2007; Cullen et al., 2008), irrigation (Cullen et al., 2008) and fertilizer application (Saul et al., 1999). Grazing management has a significant effect on herbage quality, through influencing seasonal productivity, digestibility, protein/energy balance and level of rumen undegradable protein (Woodfield & Easton, 2004). Herbage quality is therefore of high significance to pasture-based dairy production, as it critically affects the DM intake of cows, and consequent milk production, animal health and reproduction.

In New Zealand, low and medium-input farming systems, where pasture provides >80% of the total diet, are common (Chapman et al., 2009; Ma et al., 2018). Perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) are the predominant pasture types for more than 90% of dairy farms (Waghorn & Clark, 2005; Hodgson et al., 2019), even in those whose total reliance upon pasture is <80% of the total diet.

Adjusting the grazing rotation length (RT) according to pasture growth and stocking rate is important to maximize pasture utilization (McMeekan & Walshe, 1963), allow post-grazing targets to be met (DairyNZ, 2019) and minimize any physical damage to the paddock and/or pasture (Macdonald & Roche, 2016). The stocking rate can alter through the season as pasture growth and, hence, pasture availability for grazing stock varies with climatic conditions (Fales et al., 1995; Kemp, 1999; Macdonald et al., 2011). For example, low, moderate and high pasture availability are prominent during winter, summer and spring, respectively, and stocking rates are commonly changed between seasons to maintain effective supply of pasture to grazing stock (L'Huillier, 1987).

Decades of breeding and selection have improved the digestibility, DM yield, non-structural carbohydrate content and condensed tannin levels of forage species (Woodfield & Easton, 2004), all of which can have a positive influence on milk production. However, long RT can result in feeding mature pasture with high fibre content, which leads to reduced digestibility and lower nutritive value (e.g. lower levels of metabolisable energy (ME) and crude protein (CP)), resulting in lower DM intake of dairy cows (Curran et al., 2010).

The present study reports variations in herbage quality (nutrient profiles) of pre-grazing herbage samples collected from four spring-calving dairy farms over a period of four months, covering calving to the end of the first six weeks of the breeding program. The relationships between pasture quality parameters were explored, and pasture variables were modelled.

7.3 MATERIALS AND METHODS

Observational studies were conducted in low-input dairy farms (n=4; nominated A-D) in the Manawatu region of the North Island of New Zealand (Figure 7.1). Farms were managed under Production System 1 or 2 (DairyNZ, 2017), where pasture and supplements (imported feeds) were >85% and ~10% of the final diet, respectively. Dairy farms were chosen in consultation with local veterinarians and consultants that worked with a range of clients and who were asked to nominate farmers who had different grazing management (RT, pre- and post-grazing management). Therefore, Farms A and B were selected as managing short RT, and Farms C and D were selected as managing long RT. The study was conducted between August (start of calving) and end of November 2019 (end of first six week of breeding). Farms A, B, C, and D had effective land areas of 257 ha, 222 ha, 336 ha, and 68 ha, respectively, and were all stocked at 3 and 3.5 cows/hectare during winter and spring, respectively. Rotational grazing was practiced in all farms and RT are shown in Table 7.1.

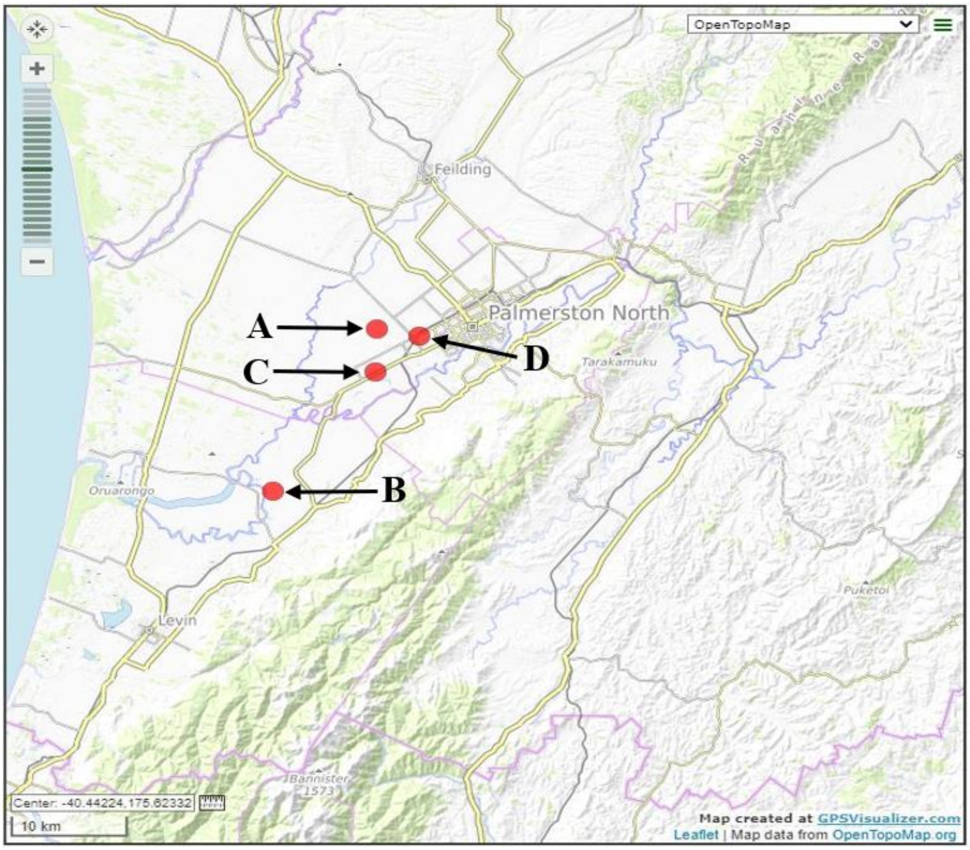

Figure 7.1. Study locations (highlighted in red) in the Manawatu region, close to the city of Palmerston North (https://www.gpsvisualizer.com/)

Table 7.1. Grazing rotation (days between grazing) of four dairy farms from August to November 2019

Farm	**Grazed pasture**	**Grazing rotation (days)**			
		Winter	Spring		
		August	September	October	November
A	Perennial ryegrass-white clover	30	20-25	20-25	20
B	Perennial ryegrass-white clover	30	25	20-25	20
C	Perennial ryegrass-white clover	35	30	25-30	25
D	Perennial ryegrass-white clover	35	30	30	25
	tall fescue-white clover	40	35	35	30

7.3.1 Pasture types and rotation managements

The predominant pasture was perennial ryegrass-white clover, however, Farm D also managed tall fescue (*Festuca arundinacea*)-white clover pasture (represented ~40% of grazing paddocks of the farm). Grazing rotation management on each farm was matched to expected seasonal daily pasture growth rates, with faster rotations matching faster growth rates (e.g. spring), and slower rotations matching slower growth rates (e.g. winter).

7.3.2 Pasture measurements

Pre-grazing pasture masses (PGDM; kg DM/ha) and pre-grazing pasture height (PGH; cm) were measured weekly using a standardized electronic rising plate meter (Tru-Test EC-10, New Zealand), with >50 readings taken following a 'W' or 'S' pattern across the grazing paddock to cover a representative area. The plate meter was programmed with the following calibration equation:

Pasture mass (kg DM/ha) = (140 × mean compressed height) + 500 (L'Huillier & Thomson, 1988; Hodgson & White, 1999)

Farm maps were used to identify the paddock numbers and calculate the grazing intervals. On each weekly visit to each farm, a paddock that was about to be grazed was selected for pre-grazing measurements, and a paddock which had been grazed a day previously, was selected for the post-grazing pasture measurements. Additionally, the perennial ryegrass leaf regrowth stage (LS; 10 measurements/paddock) was measured for each pre-grazing paddock, following the method outlined in McCarthy et al. (2015).

7.3.3 Pasture sampling and processing

Pre-grazing pasture samples were collected from >25 random locations in the paddock in duplicate using the hand-plucking method (De Vries, 1995). Samples were collected into polythene bags and transferred to the laboratory within 30-60 minutes. Each pasture sample was thoroughly mixed, and representative sub-samples (n=4) were taken. Sub-samples were dried at 60-65ºC for between 48 and 72 hours in a forced-draught oven until a constant mass was achieved. An electric balance was used to measure the mass of pasture before and after oven drying, to determine DM %. The dried samples were ground and sieved (1 mm) for chemical composition analysis.

7.3.4 In vitro analysis

Chemical composition of the pasture samples was assessed using near-infrared spectrophotometry (NIRS) (Corson et al., 1999). The DM, CP, acid detergent fibre (ADF), neutral detergent fibre (NDF) and ash content were estimated using the methods of the Association of Official Agricultural Chemists (AOAC) 930.16, AOAC 968.06, AOAC 973.18, AOAC 2002.04 and AOAC 942.05, respectively. Lipid and non-structural carbohydrate (NSC) contents were also measured by NIRS. Pasture samples were analysed for *in-vitro* organic matter digestibility (OMD) and digestible organic matter digestibility (DOMD) according to Roughan & Holland (1977). The ME content of pastures was calculated using DOMD (0.163 × DOMD MJ/kg DM) (Roughan & Holland, 1977).

7.3.5 Statistical analysis

Data were tested for normal distribution using the Kolmogorov-Smirnov test and q-q plots. $\text{Log}_{(\ln)}$ transformation was undertaken where data were not normally distributed. Scatter plot analyses of herbage-related variables were used to visualise the relationships among variables. Correlation analyses were used to determine the strength and directions of relationships between variables. Comparisons of each nutritional component were undertaken both within and across months during late winter to the end of spring.

Data were thereafter subjected to analysis by a repeated measure mixed model in which herbage quality parameters (CP, NDF, ADF, lipid, NSC, ash, OMD, ME) were the outcome variables. The categorical predictor variable was the farm, and time (week) within farms were the repeated measures. The analyses were undertaken using the using PROC MIXED function of SAS.

The linear mixed model developed was:

$$\boldsymbol{y_{ij} = \mu + F_i + Time_j + F_i \times Time_j + e_{ij}}$$

Where y is the pasture DM or herbage quality parameter (CP, NDF, ADF, lipid, NSC, ash, OMD, ME), μ is the mean, F is the farm, time is time periods during winter and spring, and e is the random error term.

Further, linear regression models were applied to determine the association between pasture DM and herbage quality parameters (CP, NDF, ADF, lipid, NSC, ash, OMD, ME) as outcome variables and PGDM yield, PGH, RT and LS (number of live leaves per tiller) as the explanatory individual variables. The analyses were undertaken using the PROC MIXED function of SAS.

The linear models developed were:

$$y_{ijkl} = \mu + PGDM_i + PGH_j + RT_k + LS_l + PGDM_i \times RT_k + GH_j \times RT_k + e_{ijkl}$$

Where y is the pasture DM or herbage quality parameter (CP, NDF, ADF, lipid, NSC, ash, OMD, ME), β_0 is the intercept e is the random error term and other factors as shown in Table 7.2.

Table 7.2. Factors used in linear regression models to determine the association between pasture dry matter (DM) and herbage quality (nutrients)

Factor	**Abbreviated term**	**Ascribed level**	
		1	2
Pre-grazing DM (kg/ha)	PGDM	≤3000	>3000
Pre-grazing height (cm)	PGH	≤9	>9
Rotation length winter	RT	30 days	35 days
Rotation length spring	RT	20-25 days	25-30 days
Leaf regrowth stage at grazing	LS	≤3 leaves/tiller	>3 leaves/tiller

Regression models were developed using 95% confidence intervals and 0.05 error. All analyses were undertaken using Statistical Analysis Software (SAS 9.4, SAS Institute Inc. Cary, NC, USA). Pearson and Spearman correlations were used to estimate the correlation of coefficients (r^2) of normally distributed and non-normally distributed data, respectively.

7.4 RESULTS

Herbage quality values are summarised in Table 7.3. Compared to reference values (Machado et al., 2005; Pullanagari et al., 2013; DairyNZ, 2017), average herbage quality values were at acceptable levels. The coefficient of variation between months was highest for NSC (13%) followed by ADF (11%). Ash, ADF and NDF contents were significantly ($P<0.05$) higher during the spring months of October and November than at other times, with CP showing a similar, non-significant trend. Lipid and ME contents were lower ($P<0.05$) during October and November than at other times.

The NDF data separated into two homogenous subsets, such that mean NDF during August, October, and November were not different ($P>0.05$) from each other (lowest mean difference August and October: -1.54% DM (adj. 95% CI: -3.16 to 0.06% DM)) but were higher than mean NDF during September (lowest mean difference September and October: -2.17% DM (adj. 95% CI: -3.67 to -0.68% DM)). The mean NDF during August, September, and November separated into another two homogenous sub-subjects, such that mean NDF during August and September were not different ($P>0.05$) from each other (mean difference: 0.62% DM (adj. 95% CI: -0.98 to 2.24% DM)) but differed from mean NDF during September to November (mean difference: -1.49% DM (adj. 95% CI: -3.1 to 0.11% DM)).

The ADF data separated into two homogenous subjects, such that mean ADF during August and September were not different ($P>0.05$) from each other (mean difference: 0.5% DM (adj. 95% CI: -0.62 to 1.63% DM)) but were lower than other months (lowest mean difference August and October: -1.13% DM (adj. 95% CI: -2.25 to -0.01% DM)). Mean ADF during October to November formed one homogenous subset (mean difference: -1.03% DM (adj. 95% CI: -2.15 to 0.09% DM)).

The ME data separated into two homogenous subjects, such that mean ME during August and September were not different (P>0.05) from each other (mean difference: 0.09 MJ/kg DM (adj. 95% CI: -0.15 to 0.33 MJ/kg DM)) but were higher than other months (largest mean difference August and October: 0.4 MJ/kg DM (adj. 95% CI: 0.16 to 0.64 MJ/kg DM)). Mean ME during October and November formed one homogenous subset (mean difference: -0.05 MJ/kg DM (adj. 95% CI: -0.29 to 0.18 MJ/kg DM)).

According to the Pearson correlation, strong positive relationships (r^2 >0.8) were observed between NDF and ADF, and between ME and OMD (Table 7.4). Similarly, strong negative relationships (r^2 >0.8) were observed between ADF and lipid, and between ADF and OMD. The ME content was negatively correlated with both ADF and NDF. The CP was positively correlated with ash, lipid, OMD and ME; however, it was negatively correlated with DM, NSC, NDF, and ADF. Lipid contents were positively and negatively correlated with NDF and ME, respectively.

The highest PGDM and PGH, post-grazing DM and height, and LS were achieved in late spring (Table 7.5). There were significant differences observed between months for PGDM, PGH, post-grazing DM, residual/post-grazing height, and LS. The highest (32%) and lowest (8%) variabilities between months were observed for LS and post-grazing DM.

According to the Spearman correlation, the most significant positive relationships (r^2 >0.5) were observed between PGDM and residual height, and PGDM and post grazing DM (Table 7.6). There were no negative relationships observed between parameters.

Table 7.3. Herbage quality (nutritional composition; mean ± 95% confidence interval) over the entire study period (winter-spring)

	Norms*	**Mean**	**August**	**September**	**October**	**November**	**SEM**	**r^2**
DM (DM %)	12-20	17.5	17.2^{b} (16.4-17.9)	18.51^{a} (17.9-19.2)	16.1^{c} (15.4-16.7)	18.4^{a} (17.7-19.2)	0.48	0.25
Ash (% DM)	8-12	9.95	9.44^{b} (9.05-9.82)	9.73^{b} (9.4-10.1)	10.3^{a} (9.97-10.6)	10.3^{a} (9.91-10.7)	0.25	0.13
Lipid (% DM)	3-6	4.13	4.47^{a} (4.3-4.64)	4.44^{a} (4.29-4.58)	3.88^{b} (3.73-4.02)	3.7^{b} (3.53-3.86)	0.11	0.4
NSC (% DM)	7-25	11.8	12.1^{a} (11-13.3)	12.3^{a} (11.3-13.3)	10.7^{b} (9.7-11.7)	12.2^{a} (11-13.3)	0.73	0.06
CP (% DM)	15-30	21.7	21.3 (19.3-22.5)	21.6 (19.9-22.6)	22.2 (20.9-23.7)	22.3 (20.6-23.8)	1.01	0.02
NDF (% DM)	35-45	42.5	42.0^{ac} (40.8-43.3)	41.4^{bc} (40.4-42.5)	43.6^{a} (42.5-44.6)	42.9^{ab} (41.7-44.1)	0.78	0.08
ADF (% DM)	20-30	21.4	20.8^{b} (19.9-21.6)	20.3^{b} (19.5-21)	21.9^{a} (21.2-22.7)	22.9^{a} (22.1-23.8)	0.54	0.19
OMD (% DM)	65-85	78.8	79.2^{ab} (78.2-80.3)	79.3^{a} (78.4-80.3)	78.5^{ac} (77.6-79.5)	77.9^{bc} (76.8-79.0)	0.69	0.04
ME (MJ/ kg	10.5-12.5	11.2	11.4^{a} (11.2-11.6)	11.3^{a} (11.2-11.5)	11.0^{b} (10.9-11.2)	11.1^{b} (10.9-11.2)	0.12	0.13

NSC non-structural carbohydrates; CP crude protein; NDF neutral detergent fibre; ADF acid detergent fibre; OMD organic matter digestibility; ME metabolisable energy; SEM standard error of mean

$^{a\text{-}c}$Means with different superscripts within rows indicates significant differences between months ($p<0.05$)

*(Machado et al., 2005; Pullanagari et al., 2013; DairyNZ, 2017)

Table 7.4. Correlation matrix for pasture dry matter (DM) and herbage quality (nutrients (n = 112)

	DM	Ash	Lipid	NSC	CP	NDF	ADF	OMD	ME
DM	1								
Ash	-0.37****	1							
Lipid	-0.23*	0.13	1						
NSC	0.37****	-0.62****	-0.06	1					
CP	-0.49****	0.79****	0.43****	-0.69****	1				
NDF	0.30**	-0.05	-0.60****	-0.13	-0.41****	1			
ADF	0.35***	-0.21*	-0.82****	0.05	-0.53****	0.84****	1		
OMD	-0.45****	0.33***	0.64****	-0.08	0.49****	-0.59****	-0.81****	1	
ME	-0.28**	-0.01	0.64****	0.01	0.38****	-0.72****	-0.80****	0.83****	1

NSC non-structural carbohydrates; CP crude protein; NDF neutral detergent fibre; ADF acid detergent fibre; OMD organic matter digestibility; ME metabolisable energy; *P <0.05, **P <0.01, ***P < 0.001, ****P <0.0001

Table 7.5. Pre- and post-grazing pasture measurements (mean ± 95% confidence interval)

	Mean	August	September	October	November	SEM	r^2
Pre-grazing DM (kg DM/ha)	3001	3007^{ab} (2850-3164)	2912^{b} (2776-3049)	2951^{b} (2815-3088)	3179^{a} (3021-3336)	101	0.06
Post-grazing DM (kg DM/ha)	1707	1707^{ab} (1650-1764)	1639^{b} (1590-1689)	1723^{a} (1673-1772)	1775^{a} (1718-1832)	36.3	0.11
Pre-grazing height (cm)	8.93	8.95^{ab} (8.39-9.52)	8.61^{b} (8.13-9.1)	8.75^{b} (8.27-9.24)	9.57^{a} (9-10.13)	0.66	0.1
Residual-height (cm)	4.31	4.31^{ab} (4.11-4.52)	4.07^{b} (3.9-4.25)	4.37^{a} (4.19-4.54)	4.56^{a} (4.35-4.76)	0.13	0.11
Leaf regrowth stage (no. of leaves per tiller)	2.29	1.90^{c} (1.74-2.05)	2.20^{b} (2.07-2.34)	2.28^{b} (2.15-2.41)	2.81^{a} (2.66-2.97)	0.10	0.41

DM dry matter; SEM standard error of mean

^{a,b}Means with different superscripts within rows are significantly different ($p<0.05$) from each other

Table 7.6. Correlation matrix for pasture measurements

	Pre-grazing DM	Post-grazing DM	Pre-grazing height	Residual height	Leaf stage
Pre-grazing DM (kg DM/ha)	1				
Post-grazing DM (kg DM/ha)	0.51****	1			
Pre-grazing height (cm)	0.88****	0.16	1		
Residual height (cm)	0.51****	0.96****	0.16	1	
Leaf regrowth stage (leaves/tiller)	0.40****	0.26***	0.40****	0.26**	1

DM dry matter; *P <0.05, **P <0.01, ***P < 0.001, ****P <0.0001

Table 7.7. Least square means of main effects upon herbage quality (PGDM/RT interaction between pre-grazing dry matter and grazing rotation length; PGH/RT interaction between pre-grazing height and grazing rotation length; LS leaf regrowth stage at grazing; DM dry matter; NSC non-structural carbohydrates; CP crude protein; NDF neutral detergent fibre; ADF acid detergent fibre; OMD organic matter digestibility; ME metabolisable energy)

	PGDM (kg DM)			**PGH (cm)**			**RT (days)**			**LS**			**Interactions**	
	≤3000	>3000	SEM	≤9	>9	SEM	1	2	SEM	≤3	>3	SEM	PGDM/ RT	PGH/ RT
DM (DM %)	19.8^{a*}	17.6^{b*}	0.7	18.1	18.8	0.7	17.5^{b*}	19.3^{a*}	0.7	17.6^{b*}	19.2^{a*}	0.6	S*	S*
Ash (% DM)	9.8	10.2	0.3	10.1	9.8	0.3	10.5^{a**}	9.4^{b**}	0.3	9.8	10.2	0.2	NS	NS
Lipid (% DM)	3.7	3.9	0.2	3.68	3.9	0.2	4.0^{a**}	3.4^{b**}	0.1	4.1^{a**}	3.5^{b**}	0.2	NS	NS
NSC (% DM)	11.8	11.9	0.9	11.4	12.2	1.0	11.5	12.1	0.8	11.9	11.5	0.9	NS	NS
CP (% DM)	21.4^{a*}	19.0^{b*}	1.1	21.7^{a*}	19.4^{b*}	1.2	22.2^{a**}	18.3^{b**}	1.1	21.1	20.4	0.7	S*	S**
NDF (% DM)	42.8	43.8	0.9	43.0	43.5	0.9	42.0^{b*}	44.6^{a*}	0.8	42.5	44.1	0.7	NS	NS
ADF (% DM)	22.1	22.9	0.6	22.3	22.7	0.6	21.2^{b**}	23.8^{a**}	0.5	21.3^{b**}	23.7^{a**}	0.6	NS	NS
OMD (% DM)	78.7^{a*}	76.6^{b*}	0.8	77.7	77.6	0.8	79.1^{a**}	76.3^{b**}	0.6	78.8^{a*}	76.6^{b*}	0.7	NS	NS
ME (MJ/ kg DM)	11.2^{a*}	10.9^{b*}	0.1	11.1	11.0	0.1	11.2^{a*}	10.9^{b*}	0.1	11.2^{a*}	10.9^{b*}	0.1	NS	NS

^{a,b}Values with different superscript in the same row are different from each other (LSD, **P<0.001; *P<0.05); SEM standard error of mean

7.4.1 Description of models

Dry matter contents (Table 7.7, Figure 7.2) were affected by time period (week), farm, and by week × farm interaction (P<0.05). There were interactions between PGDM and RT, and PGH and RT for DM of pasture (P< 0.05, Table 7.7). The mean DM of RT2 (19.3% DM) was higher than that of RT1 (17.5% DM; P<0.05). Similarly, mean DM of PGDM1 (19.8% DM) was higher than PGDM2 (17.6% DM; P<0.05). The highest DM contents were reported during time periods 4 and 11 in farm D.

Crude protein contents (Table 7.7, Figure 7.3) were affected by time period (week), farm, and by week × farm interaction (P<0.01). There were interactions between PGDM and RT, and PGH and RT for CP of pasture (P<0.05, Table 7.7). The mean CP of RT1 (23.2% DM) was higher than that of RT2 (18.3% DM; P<0.05). Similarly, mean CP of PGDM1 (21.4% DM) was higher than PGDM2 (19.0% DM; P<0.05).

Neutral detergent fibre contents (Table 7.7, Figure 7.4) were affected by time period (week), farm, and by week × farm interaction (P<0.01). The NDF was influenced by RT and mean NDF content of RT2 (44.6% DM) was higher than RT1 (42.0% DM).

Acid detergent fibre contents (Table 7.7, Figure 7.5) were affected by time period (week), farm, and week × farm interaction (P<0.01). The ADF was influenced by RT and LS (P<0.05). Mean ADF content of RT2 (23.8% DM) was higher than RT1 (21.2% DM) and the mean ADF content of LS2 (23.7% DM) was higher than LS1 (21.3% DM).

Metabolisable energy contents (Table 7.7, Figure 7.6) were affected by time period (week), farm, and by week × farm interaction (P<0.01). The ME was influenced by PGDM, RT, and LS. Mean ME content of PGDM1 (11.2 MJ/kg DM) was higher than PGDM2 (10.9 MJ/kg DM). Similarly, the mean ME content of RT1 (11.2 MJ/kg DM) was higher than RT2

(10.9 MJ/kg DM), and also the mean ME content of LS1 (11.2 MJ/kg DM) was higher than LS2 (10.9 MJ/kg DM).

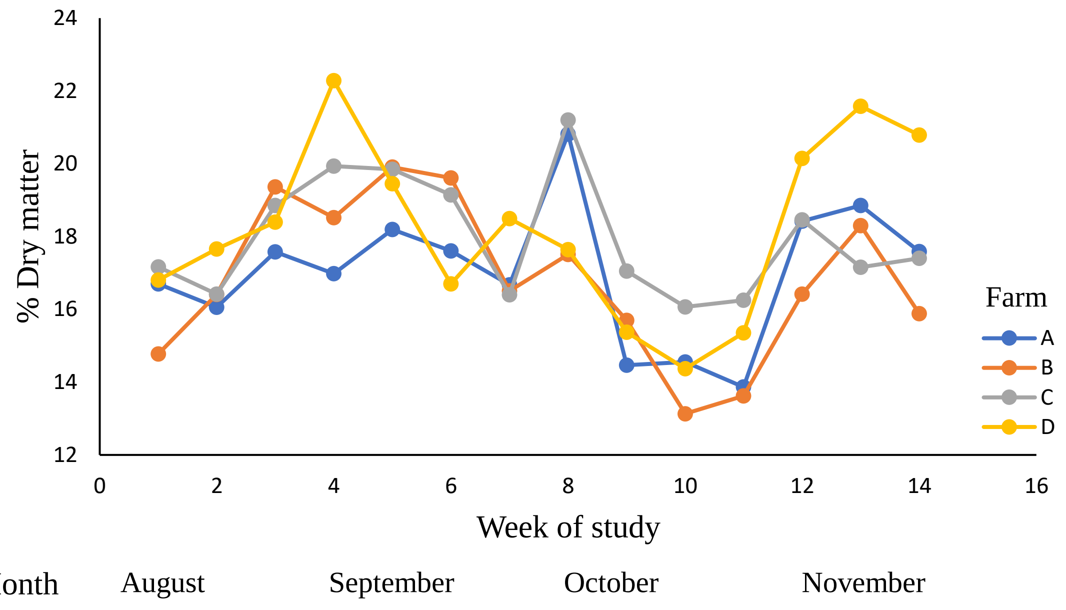

Figure 7.2. Variations in dry matter (DM) content (%) of pasture across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

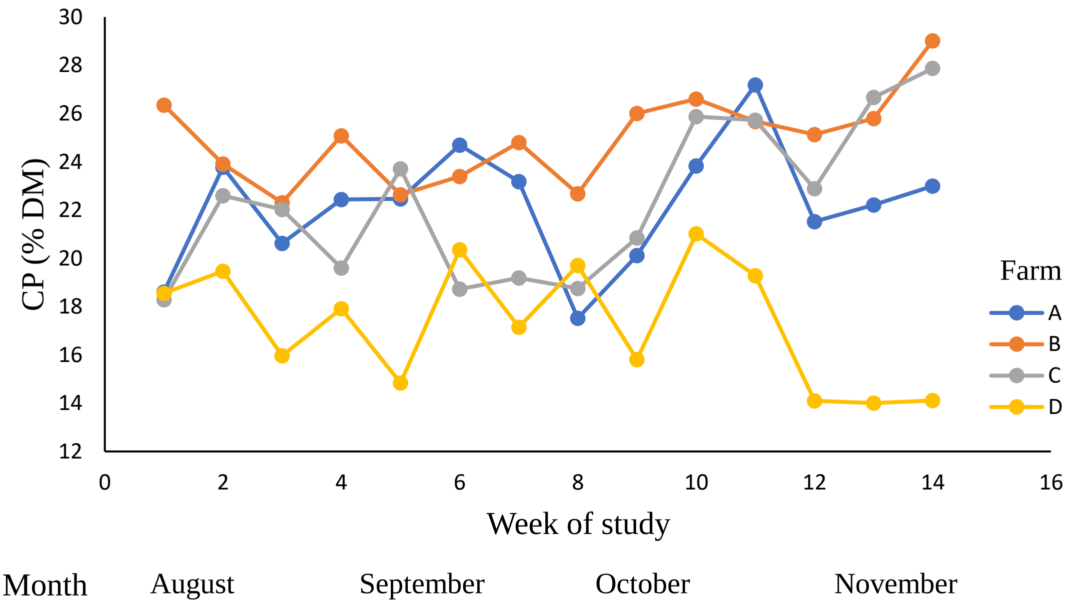

Figure 7.3. Variations in crude protein (CP) content (% dry matter (DM)) across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

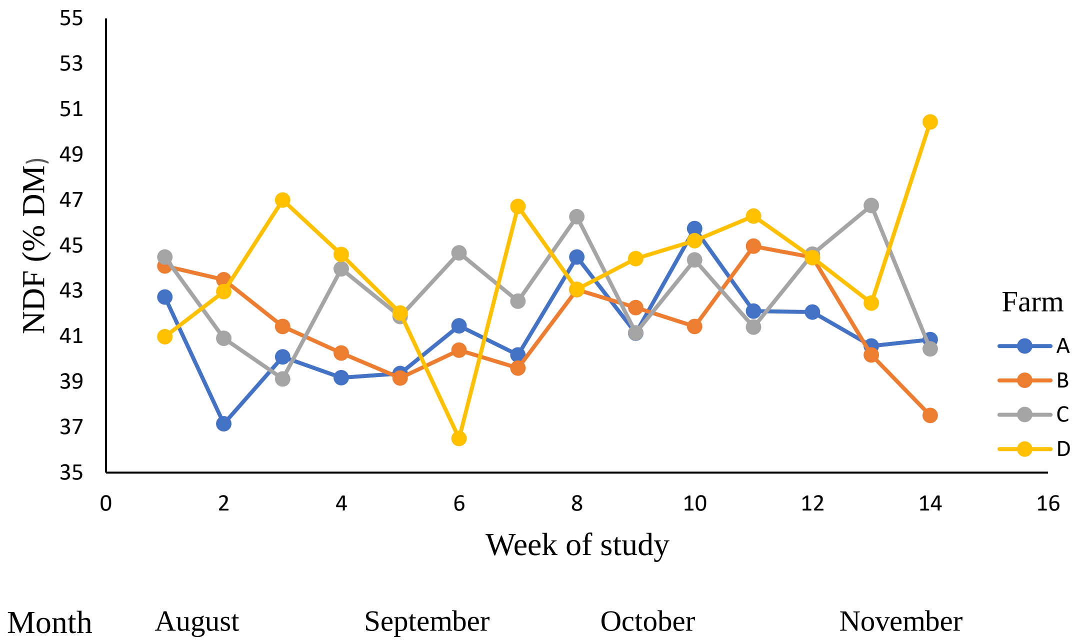

Figure 7.4. Variations in neutral detergent fibre (NDF) content (% dry matter (DM)) across dairy farms during winter and spring; 0-14 weeks (August to November 2019)

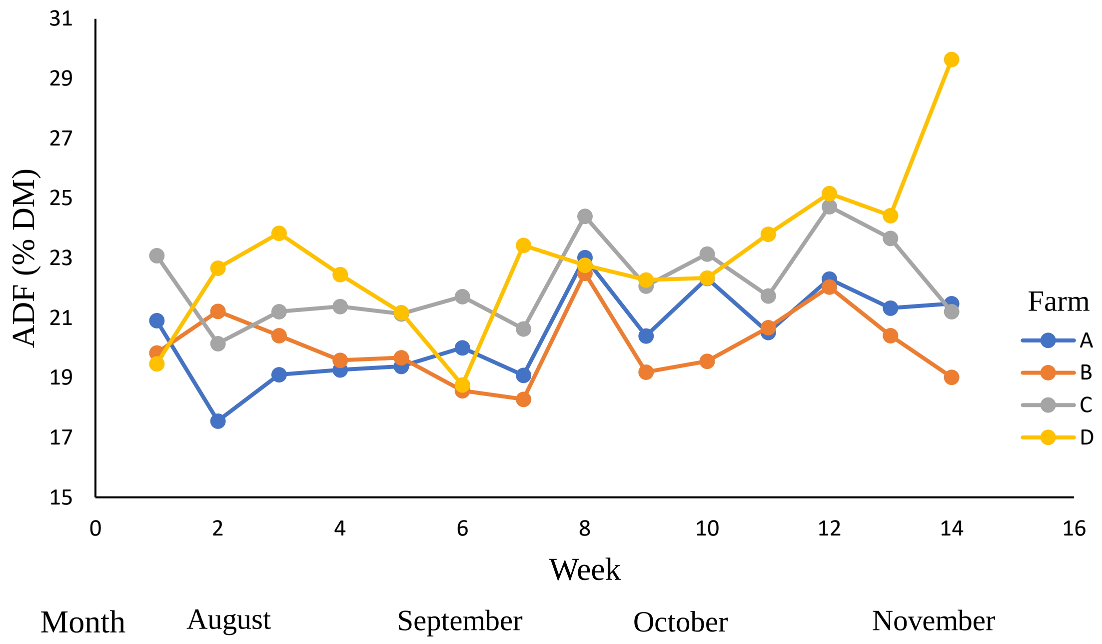

Figure 7.5. Variations in acid detergent fibre (ADF) content (% dry matter (DM) across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

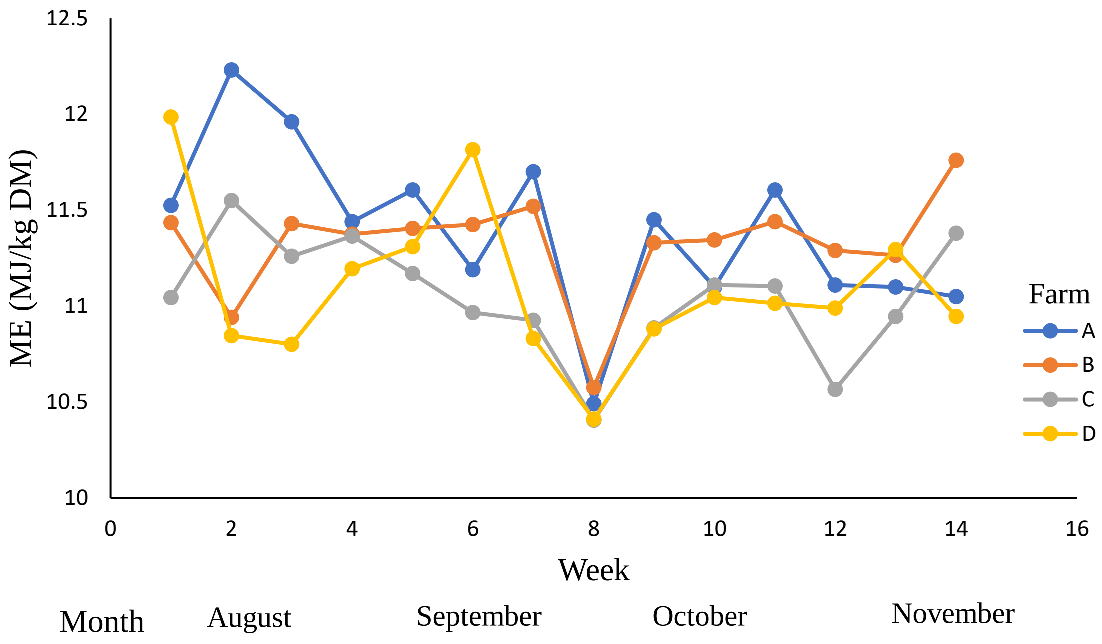

Figure 7.6. Variations in metabolisable energy (ME) content (MJ kg dry matter (DM)) across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

7.5 DISCUSSION

Pasture herbage quality varied over the study period [from August (winter: start of calving) to November (spring: end of the first 6 weeks of the breeding period)], however, herbage quality parameters were at acceptable levels (or at least 'good') according to the DairyNZ (2019) guidelines. Herbage quality was significantly related to RT, PGDM, PGH and LS. Importantly, ME and CP were lower when PGDM was high (>3000 kg DM, Table 7.7) than when PGDM was low, and were also lower with longer rather than shorter RT. Moreover, NDF and ADF contents were also increased with longer RT. Common pasture management practice is that RT is adjusted, sometimes according to different LS or otherwise based on pasture growth rates, to maintain optimum pasture quality and quantity (Curran et al., 2010; Donaghy et al., 2021). Leaf regrowth stage is primarily affected by temperature, and, to a lesser extent, soil moisture (Fulkerson & Donaghy, 2001; Rawnsley et al., 2007; Donaghy et al., 2021), which can be monitored weekly or monthly. All dairies selected in the current study were managed under the optimal LS stage (2-3 live leaves/tiller; Fulkerson & Donaghy, 2001) during late winter and early spring; however, LS went above the optimum (>3 leaves/tiller) during the period in late spring, where maximum pasture growth occurred (Howse et al., 1996). When LS increases beyond the optimum, dying leaves increase fibre levels and this, along with advanced reproductive development, decrease digestibility (Donaghy & Fulkerson, 2001; Donaghy et al., 2021). Therefore, harvesting surplus of grass for conservation at peak growth is essential to maximize pasture utilization and quality (Howse et al., 1996; Fulkerson et al., 2005; Lee et al., 2008; Beukes et al., 2019). In agreement with results from studies by Fulkerson & Donaghy (2001) and Turner et al. (2006c), the present study also found that as LS increased above 3, both ME and CP contents were lower. Therefore, optimum LS during the spring season and adjusting/lowering RT accordingly may directly enhance the pasture quality (McKenzie et al., 2006), by decreasing fibre content and increasing digestibility and nutrient density (Turner et al., 2006a, b). In that context, it was clear that RT was immediately changed if a farmer considered that either LS and/or PGDM were unsatisfactory. Hence, these results highlight the adaptability of ryegrass-clover pastures (Kemp et al., 2000; Lee et al., 2012), where quality does not exhibit drastic changes between seasons, such that ME contents of pasture generally meet the average requirements for lactating dairy cows (~11.0 MJ/ kg DM).

Positive and negative correlations of herbage quality traits (CP, ME, NDF and ADF) were similar to published data from New Zealand (Machado et al., 2005; Roche et al., 2009; Duranovich et al., 2020). However, relationships between CP and both NDF and ADF were stronger (r^2 >0.4) than previously published data (r^2 ~0.2-0.3). Metabolisable energy and CP were negatively correlated with both NDF and ADF, confirming that pasture maturity or longer RT lower the herbage quality. The negative relationship between ME and NDF in the present study (r^2 = -0.72) was less strong than that between ME and ADF (r^2 = -0.8) (see Table 7.4), which raises an important question of whether the cellulose and lignin fractions of fibre (as measured by ADF) had a greater effect upon pasture ME contents (Van Soest, 1994, Roche et al., 2009) than the hemicellulose fraction that is reflected in the NDF content (Van Soest & Mertens, 1985). In support of this, the inverse relationship between ADF and OMD (r^2 = -0.81) was stronger than that between NDF and OMD (r^2 = -0.59). Furthermore, OMD was highly correlated with CP (r^2 = 0.49), as Roche et al. (2009) also noted. Those authors considered that this occurs because digestibility and CP contents are both greater under optimum (2-3 leaves/tiller) rotational grazing management.

Contents of CP were positively correlated with lipid (r^2 = 0.43) and negatively with NSC (r^2 = -0.69), similar to the results published by Roche et al. (2009) but higher than those published (r^2 = 0.27 and r^2 = -0.14, respectively) by Machado et al. (2005). Fatty acid synthesis takes place within the chloroplasts of plant cells, where more plant protein is also stored as ribulose biphosphate carboxylase (rubisco) enzyme: consequently, a greater number of chloroplasts in the plant cells is associated with greater protein content and also lipid/fat production (Nelson & Cox, 2004; Roche et al., 2009). The negative relationship between CP and NSC is well documented (Machado et al., 2005) and due firstly to the stimulatory effect of N on plant growth (which causes an increase in herbage CP levels, but reduces NSC through increased growth and respiration), and secondly to the fact that plants use water-soluble carbohydrates (WSC) to convert nitrates that are taken up by the roots, into amino acids in the plant.

In the present study, plant CP content was highest during spring in which weather conditions (temperature, light, and water) combined to increased plant growth and development (Van Soest, 1994; Roche et al., 2009); however, NSC contents were considerably lower during spring as plants produce more structural carbohydrate (cellulose and lignin) to provide rigidity for the plant during its rapid growth (Van Soest, 1996). Therefore, conversion of WSC to structural materials corresponds with decreased NSC and increased ADF and NDF, confirming a negative relationship between NSC and ADF and/or NDF.

Crude protein content exceeded the average requirements for lactating dairy cows (18% DM: DairyNZ, 2019), as also found by Moller (1997). Cressman et al. (1980) and Forster et al. (1983) stated that milk production increases when feeding high CP diets, but high CP content can negatively affect reproductive performance (Jordan & Swanson, 1979; McCormick et al., 1999; Roche et al., 2000). Kalscheur et al. (1999) and Ji & Dann (2013) considered that feeding high CP diets is essential to maximize milk protein yields, since DMI limits the meeting of metabolisable protein requirements during early lactation. Importantly, high CP intake increases ammonia production in the rumen and, to slow down ammonia passing into the blood (Ferguson et al., 1993; Roseler et al., 1993; Godden et al., 2001). Cows use energy (Danfaer et al., 1980) to convert the toxic ammonia to less-toxic urea in the liver (Visek, 1968; Visek, 1984). Interestingly, cows that are provided pastures with high CP content appear able to quickly adapt to the consequential higher blood urea concentrations, apparently obviating most of the toxic effects of high blood ammonia that are reported in the literature (Laven et al, 2007).

In the current study, pasture residual height was positively correlated ($r^2 > 0.5$) with PGDM. Residual height can, in turn, be affected by decisions relating to pasture allowance that are based upon the pre-grazing DM yield and/or stocking density/rate (Lee et al., 2008). Residual height is also affected by the quality of pasture, as the amount of the plant that remains after grazing increases when the pasture contains more stem and dead material (Lee et al., 2007; Stakelum & Dillon, 2007). According to the DairyNZ (2019) guidelines, farmers should be able to manage residual heights to between 3.5 to 4 cm, equating to 1500-1600 kg DM/ha targeted post-grazing DM. In the current study where average residual height and post grazing

DM exceeded the DairyNZ (2019) guidelines, there can be a positive effect upon pasture regrowth, and negative effects upon pasture wastage and pasture intakes at the next grazing (Donaghy & Fulkerson, 2001; Donaghy et al., 2021). This appears to have been the case in the present study, inasmuch as although pre-grazing DM was maintained between 2800-3200 kg DM/ha during the study period (which was within the DairyNZ (2019) guidelines), the high post-grazing DM implies that pasture allocation exceeded requirements and, therefore, stocking rates should have been increased for more effective grazing management.

Contents of NDF in the current study were higher (>40%) than some of the values previously reported in New Zealand dairy systems (Machado et al., 2005). Dairy cow rations should have at least 25% of DM as NDF to ensure that cows maintain rumen function (NRC, 1989) and, in pastoral systems, there should be a minimum of 35% of DM as NDF to maximize voluntary feed intake (DairyNZ, 2019). Conversely, excess dietary NDF limits DMI due to slow passage through the rumen and intestines (Oba & Allen, 1999; Mertens, 2010).

The current study showed that intensive rotational grazing is crucial for optimizing pasture quality, and hence, sustaining the nutritional value of the cows' diet (Hodgson, 1990). Hodgson & Brookes (1999) considered that grazing pasture at appropriate time intervals prevents the development of structural tissue and slows the decline of digestible cell contents, resulting in increased (or, at least, sustained) ME and CP contents. Appropriate management of grazing rotations in relation to the given nutritional requirements of grazing stock, along with the implementation of appropriate stocking rates, are important to reduce pasture wastage and increase milk production (Macdonald et al., 2010).

7.6 CONCLUSIONS

Different grazing RT, leaf regrowth stage at grazing and pre-grazing DM yield during winter and spring influence the quality of pasture, however, the 5–10 day variations on RT among dairy farms in the present study did not have a significant influence on the pasture herbage quality. The length of the grazing rotation was adjusted based on the pasture availability, seasonal variations, and growth rate of different pastures. The NDF, ME, and CP are key nutritional factors that can be used to determine the quality of pasture, therefore, more attention on pasture monitoring, fertilizer application, irrigation, and maintenance of appropriate stocking density may be helpful to optimize the quality of pasture between seasons irrespective of the length of grazing rotations. Proper rotational grazing management improves the quality of pasture, and this directly affects cows' health, milk production and reproduction.

CHAPTER 8: THE INFLUENCE OF ENERGY BALANCE, MILK PRODUCTION, AND BODY CONDITION ON REPRODUCTIVE PERFORMANCES OF SPRING-CALVED POSTPARTUM DAIRY COWS IN PASTURE-BASED SYSTEMS IN NEW ZEALAND

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Prepared for submission to *Journal of Dairy Science,* August 2022

8.1 ABSTRACT

Reproductive performance is an important determinant of dairy production efficiency in pasture-based seasonal production systems. Relationships of cow-related factors and reproductive performance of seasonally calving, pasture-based dairy cows were investigated in dairy farms (n=4) in the Manawatu region of New Zealand. The farms operated under Type 1 and 2 production systems, in which the main (>80%) feed was perennial ryegrass/white clover pasture. Individual cow records (parity, breed, calving date, milk production and reproduction) were collected from the national database, MINDA. Feed intake was estimated by weekly measurements of pre- and post-grazing dry matter of pastures, plus energy provided by supplementary feed. Reproductive indices (submission rate in the first 3 (SR21) and 6 (SR42) weeks of the breeding season, pregnancy rate in the first 3 (PR21) and 6 (PR42) weeks, and pregnancy rate to first service (PREG1)) were calculated from MINDA data. Body condition score (BCS) was visually assessed every two weeks. Data were analysed by logistic and linear regression models. Peak milk yield, fat% and protein% were influenced by farm, parity and breed. Cows with ≥85% Holstein-Friesian (HF) genes had lower PR21 and PR42 than those with <85% HF genes. Pregnancy rate to first service was higher (odds ratio (OR): 1.57) in cows with 65-85% HF genes than those with >85% HF genes. Cows of parity 1-4 had higher PR21 and/or PR42 than those of parity ≥5. Pregnancy rate to first service was lower (OR: 0.7) in cows with higher BCS change (-0.5 to -1 units: 1-10 scale) between calving and the BCS nadir. Cows that calved in July had higher SR21, SR42, PR21, and PR42 (OR: 2.43, 2.65, 1.96, and 2.51, respectively) than those that calved in September, while cows that calved in August had higher SR21, SR42, and PR42 (OR: 2.39, 3.21, 1.95, respectively) than those that calved in September. Negative energy balance (NEB) of ≥-30 MJ ME/day was associated with higher peak total milk solids (Peak-MS) and peak fat% than in animals with NEB of <-30 to 0 MJ ME/day. Animals producing Peak-MS ≤1.8 kg/day had lower SR21 (OR: 0.41) and SR42 (OR:

0.25) than those producing >2.4 kg/day. Cows with peak milk protein <3.5% had lower PR21, PR42, and PREG1 (0.56, 0.44, and 0.6, respectively) than those with >3.7%. Submission and pregnancy rates were better on Farm B than on others. Duration and level of NEB varied between farms: Farms A, B, and D remained in NEB until 60-80 days after the start of calving, but Farm C returned to a positive energy balance after 80-90 days. Energy balance in early lactation was also significantly affected by breed, parity, and calving month. Therefore, breed composition, parity, calving month, milk yield, fat%, protein%, BCS and NEB are useful indicators of reproductive efficacy.

Keywords: Dairy, pasture, milk, reproduction, regression, energy balance

8.2 INTRODUCTION

New Zealand dairy production is predominantly based on outdoor grazing of mixed pastures (Daly et al., 1996; Woodward et al., 2013) in low-cost production systems (Uribe, 1995; Jiang & Sharp, 2014). The predominant pasture species are perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) (L'Huillier & Aislabie, 1988; Waghorn & Clark, 2004), or, less commonly, tall fescue (*Festuca arundinacea*) and clover. Production costs are minimized through dairy management systems that depend on the seasonality of pasture production, in which pasture growth during a calendar year is aligned with the nutritional requirements of cows at the different stages of the lactation cycle (Dillon et al., 1995; Macdonald et al., 2008; MacEvoy et al., 2008; Ramsbottom et al., 2015; Webster, 2017), such that pasture typically accounts for more than 80% of the final diet (DairyNZ, 2020). At the farm level, pasture utilization is maximized and manipulated via stocking rate, calving date, and the drying-off dates of dairy cows (Uribe, 1995).

Pasture herbage quality and quantity vary between seasons (Roche, 2017). Cows start calving in late winter when pasture production is less than the feed demand, so that feed supplementation is required to meet nutritional requirements (Roche, 2017). Pasture quantity and quality are maximal during spring, but pasture herbage quality starts to decline in early summer (Roche et al., 2009). Both quality and quantity of pasture decline during autumn, however, quality of pasture is high with a nadir of growth in the winter (Roche et al., 2009). Cows are therefore usually dried off in late autumn, based on the pasture availability and the body condition score (BCS) of the herd (Verkerk, 2003). Pasture herbage quality is a key factor for cow production, health, welfare, and reproduction and, in turn, appropriate grazing rotation management is a significant determinant of the seasonal productivity and quality of pasture (including digestibility, protein/energy balance, and rumen undegradable protein content: Woodfield & Easton, 2004).

Reproductive performance is one of the main drivers of profitability in such systems, inasmuch as the synchronization of calving with the pasture production cycle determines both milk production *per se* and the unit cost of milk production (Galvao et al., 2013). Hence, the main objective of breeding is to achieve the highest number of pregnancies in a confined breeding period (~9-12 weeks), with a set starting date, in order to achieve the necessary concentrated calving pattern in the following season (Garcia & Holmes, 1999; Lindley & Willshire, 2020). Concentration of the calving season allows dairy farmers to utilize their pasture effectively, whilst also enabling the milking herd to be managed as a single group. Achieving such a concentrated period of breeding depends upon the timing of resumption of oestrous cycles which, in turn, is critically affected by the duration and extent of negative energy balance (NEB) during early lactation (Butler & Smith, 1989; Senatore et al., 1996; Buckley et al., 2003; Patton et al., 2007). Negative energy balance is therefore considered a key regulator of the reproductive performance of dairy cows (Chilliard et al., 2000; Butler,

2014) and is related to submission rates, pregnancy rates, and conception rates at first service (Patton et al., 2007) in seasonally-calving herds.

Many studies (e.g. Madsen, 1975; Laben, et al., 1982; Hillers, et al., 1984; Butler & Smith, 1989; Harrison et al., 1990; Nebel & McGilliard, 1993; Pryce & Veerkmp, 2001; Lucy, 2007) showed that high milk yields are phenotypically and/or genetically associated with reduced reproductive performance. Many factors are involved in this relationship. Voluntary feed intake during early lactation (or at least during the postpartum transition period) is generally lower than daily requirements (Nebel & McGilliard, 1993), therefore, energy requirements for maintenance and milk production are met through a combination of dietary energy intake and mobilization of body reserves (Butler & Smith, 1989). Negative energy balance triggers tissue mobilisation to a greater extent in high- than in low-yielding cows, however, the primary driver of NEB appears to be deficiencies of feed energy intake rather than milk yield *per se* (Villa-Godoy et al., 1988). High milk yields are associated with higher growth hormone concentrations, but blood insulin concentrations are low. Low insulin concentrations decrease both luteinizing hormone (LH) pulsatility and the ovarian response to LH, resulting in delayed ovulation (Butler & Smith, 1989; Nebel & McGilliard, 1993). Finally, insulin-like growth factor-1 (IGF-1) concentrations are genetically lower in high- than low-yielding cows during the early postpartum period (Zulu et al., 2002; Taylor et al., 2004), and, since IGF-1 is a critical determinant of follicular activity through its modulation of the ovarian response to LH (Wathes et al., 2007; Lucy, 2008), low IGF-1 activity is associated with delayed resumption of oestrous cycles, reduced conception rates and increased early embryonic loss (Wathes et al., 2003).

The objectives of the current study were: (1) to describe reproductive traits/indices which relate to the seasonal production conditions in New Zealand; (2) to identify associations of cow factors such as parity, calving month, breed, BCS, and energy balance (EB) with reproductive performance during early lactation; and (3) to identify whether reproductive parameters were further associated with milk yield, fat %, protein %, and EB during peak lactation.

8.3 MATERIALS AND METHODS

Observational studies were conducted in low-input dairy farms (n=4) in the Manawatu region, New Zealand, from the first week of August 2019 to the end of November 2019. This study period represents the beginning of calving to the end of the first six weeks of the breeding program. Farms A, B, C, and D had effective areas of 68 ha, 336 ha, 222 ha, and 257 ha, respectively, and had average stocking rates of 3:1 and 3.5:1 dairy cows per hectare during the winter and spring seasons, respectively.

All farms were Production System 1 or 2 low input[1] farms (DairyNZ (2017), where pasture and concentrate feeding comprised ≥85% and <15% of the final diet, respectively. Rotational grazing was practiced by all farmers; however, the rotation lengths were varied during the winter and spring seasons. Farms A and B practised long rotations of ~35 days and ~25-30 days during winter and spring, respectively, whereas Farm C and D practiced short rotations of ~30 and ~20-25 days during winter and spring.

[1] **System 1: All grass self-contained, all stock on the dairy platform:** No feed is imported. No supplement fed to the herd except supplement harvested off the effective milking area and dry cows are not grazed off the effective milking area.

System 2: Feed imported, either supplement or grazing off, fed to dry cows Approximately 4 - 14% of total feed is imported. Large variation in % as in high rainfall areas and cold climates such as Southland, most of the cows are wintered off (DairyNZ, 2017).

8.3.1 Dry matter intake

Pre- and post-grazing pasture masses (kg dry matter (DM)/ha) of perennial ryegrass and white clover mixed sward were estimated weekly. Samples of pre-grazing pasture and supplements were collected weekly and monthly, respectively for DM estimation and chemical composition analysis (crude protein (CP), metabolisable energy (ME)) by near-infrared spectrophotometry. The total DM intake (DMI) of the herd was calculated as the difference between pre- and post-grazing DM values, and the effective area of the paddock. Farm maps were used to accurately identify the area of each grazing paddock, and the DMI of individual cows was then calculated by dividing the total DMI by the number of cows that had grazed that paddock:

$$DMI_{pasture}\ (cow) = \frac{(\text{Pre-grazing DM kg/ha - post-grazing DM kg/ha}) \times \text{paddock size (ha)}}{\text{number of cows grazed}}$$

Daily supplemental feeding (pasture silage, Palm Kernel Extract (PKE)) amounts were recorded to calculate daily DMI of supplements, thus total DMI was calculated as the sum of pasture and supplement intakes.

8.3.2 Cow body condition score measurements and productive parameters

Body condition scoring was undertaken using a 10-point scale (1= emaciated, 10= obese: Macdonald and Roche, 2004; Roche et al., 2004) fortnightly from 2 weeks after the start of calving until the end of the breeding period.

Milk production (total milk solid (MS; non-water components of milk-protein, fat, lactose and minerals)) and composition (weight and percentage of fat and of protein) data for individual cows were collected from herd milk test reports on four occasions per season (September/October, November/December, February, and April: corresponding to the start and end of breeding, mid-lactation, and late lactation), using the national dairy-herd database, MINDA (LIC, Hamilton, NZ).

8.3.3 Energy balance during early and mid-lactation

Daily EB was calculated for all postpartum dairy cows during early to mid-lactation. Energy balance was estimated as the difference between energy intake (MJ ME) and the sum of energy requirements (MJ ME) for maintenance (including activity/walking), liveweight change (growth) and milk production (NRC, 2001; Patton et al., 2007; DairyNZ, 2017; Correa-Luna et al., 2020).

8.3.4 Reproductive indices

Cow breeding and pregnancy diagnosis reports were collected from MINDA records (LIC, Hamilton, New Zealand) to calculate the following reproductive indices (Figure 8.1): (I) planned start date of breeding to first service interval (PSMF, days), defined as the number of days between start date of breeding to first service; (II) first service to successful service interval (FSCO, days), defined as the number of days between first service to the service which resulted in pregnancy; (III) planned start date of breeding to successful service/conception interval (PSMC, days), defined as the number of days between start date of breeding to successful service which resulted in pregnancy; (IV) calving to planned start date of breeding interval (CPSM), defined as the number of days between calving to planned start of breeding; (V) calving to first service interval (CFS, days), defined as the number of days between calving to first service; (VI) calving to conception interval (CCI, days), defined as the number of days between calving and the breeding which resulted in pregnancy; (VII) gestation length (GL, days), defined as the number of days from the estimated date of conception until birth; and (VIII) calving interval (CI days), defined as the number of days between two successive calvings.

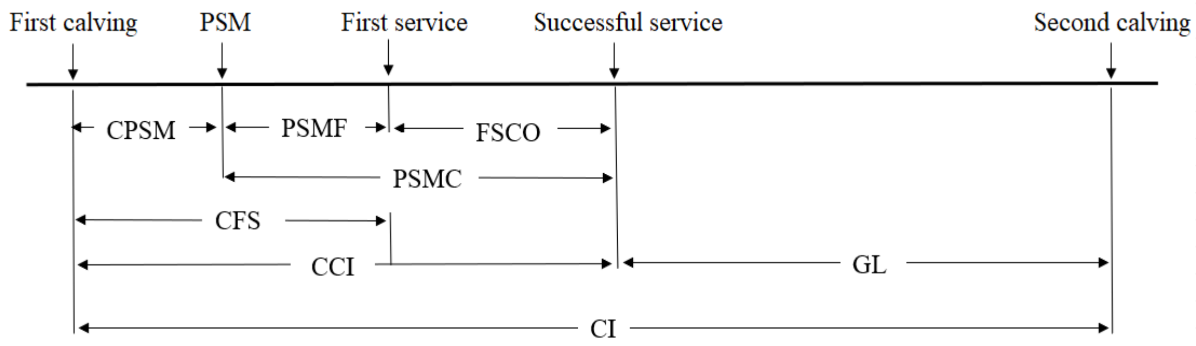

Figure 8.1. Graphical representation of the fertility indices; PSM planned start of breeding; CPSM calving to the planned start date of breeding interval; PSMF planned start date of breeding to first service interval; FSCO first service to successful service interval; PSMC planned start date of breeding to successful service/conception interval; CFS calving to first service interval; CCI calving to successful service/conception interval; GL gestation length; CI consecutive calving interval (Grosshans, et al., 1997)

From these data, additional reproductive indices were calculated: three-week (SR21: %) and six-week (SR42: %) submission rates (defined as percentage of cows presented for first breeding within 21 or 42 days, respectively, from the start of breeding); three-week (PR21: %) and six-week (PR42: %) pregnancy rates (defined as percentage of postpartum cows that conceived within 21 or 42 days, respectively, from the start of breeding). Pregnancy rate to first service (PREG1) and services per conception (SPC) were also calculated.

8.3.5 Statistical analyses

In total, 5073 records each of fat %, protein %, and milk solids (kg), respectively, and 5793 records of BCS, were included in the statistical analyses. Analyses were performed using Statistical Analysis Software (SAS 9.4, SAS Institute Inc. Cary, NC, USA). Regression models were developed with 95% confidence interval and 0.05 error. Raw data and/or $\log_e$ transformed data were tested for normal distribution using the Kolmogorov-Smirnov test and q-q plots, however, data were sorted numerically by each continuous individual variable and categorized into groups as reproduction data were right-skewed with respect to the standard curve.

Firstly, least-square means were calculated, and a simple linear regression model was applied to determine differences in DMI, milk yield, milk composition, ME intake, EB, and BCS between farms during the early and/or entire lactation.

Associations between reproductive outcome variables and explanatory individual variables were examined using a multivariable logistic regression model (Model 1). The explanatory independent variables are shown in Table 8.1. The explanatory outcome variables were SR21, SR42, PR21, PR42, PREG1, FSCO (1 = ≤21 days, 0 = >21 days), PSMF (1 = ≤14 days, 0 = >14 days), CCI (1 = ≤85 days, 0 = >85 days), and SPC (1 = 1 service, 0 = ≥2 services). The categories of farm, parity, breed, calving month, change in BCS (ΔBCS), and EB nadir were set as fixed effects and least-square means were calculated for fixed effects. A reference category was selected for each continuous independent variable, which was described as an odds ratio (OR) of 1. An increased likelihood is implied by an OR of >1, whereas a reduced likelihood is implied by an OR of <1. The analyses were undertaken using the generalized linear mixed model (GLIMMIX) function of SAS.

Table 8.1. Cow-related explanatory independent variables used in Model 1

Factor	**1**	**2**	**3**
Calving month	July	August	September
Breed (1/16 in 3-generation pedigree)	HF2-HF10	HF11-HF13	HF14-HF16
Parity	1, 2	3, 4	≥5
Difference of BCS between calving and nadir (points: 1-10 scale)	-1 to -0.5	-0.5 to 0	
EB at nadir (MJME/cow/day).	<-30	-30 to 0	

HF Holstein-Friesian; BCS body condition score; EB energy balance

Farm identity: A=1, B=2 etc.

Associations between milk production variables and the cow-related explanatory variables were assessed using general linear regression analysis (Model 2). Milk production parameters in the model included Peak-Fat (fat% per cow at peak milk production), Peak-Protein (protein% per cow at peak milk production), and Peak-MS (milk solids per cow at peak milk production). Farm, parity, breed, and EB nadir were set as fixed effects, with days in milk (DIM) as the covariate. Least-square means were calculated for fixed effects. The analysis was performed using the PROC GLM function of SAS. The linear regression model was:

$$\boldsymbol{y_{ijkl} = \mu + F_i + B_j + P_k + EBn_l + e_{ijk}}$$

Where, y is the milk production parameter (Peak-Fat%, Peak-Protein%, Peak-MS), μ is the mean, F is the farm, B is the breed, P is the parity, EB at nadir is the lowest energy balance recorded during early lactation, and e is the random error term.

Table 8.2. Lactation-related explanatory independent variables used in Model 3

Factor	**1**	**2**	**3**	**4**
Peak-MS (kg)	<1.8	1.8-2.09	2.1-2.4	>2.4
Peak-Fat%	<3.8	3.8-4.29	4.3-4.4	>4.4
Peak-Protein%	<3.5	3.5-4.59	3.6-3.7	>3.7
∆BCS (points, 1-10 scale)	-1 to -0.5	-0.5 to 0		
EB at nadir (MJ ME/cow/day)	<-30	-30 to 0		

EB energy balance; ∆BCS change in body condition score between calving and nadir; MS milk solids

Associations between binary reproductive outcome variables and lactation-related explanatory individual variables (Table 8.2) were examined using logistic regression (Model 3). The explanatory outcome variables were SR21, SR42, PR21, PR42, and PREG1. Milk yield (Peak-MS), Peak-Fat%, Peak-Protein%, ∆BCS, and EB nadir were set as fixed effects and least-square means were calculated for fixed effects. Analyses were undertaken using the GLIMMIX function of SAS. As previously, a reference category was selected for each continuous independent variable, defined as having an OR of 1.

Finally, the association between EB and explanatory individual variables (parity, breed, and calving month) were examined using general linear regression (mixed model) using the PROC MIXED function of SAS, where cow number and farm identification were set as random effects and BCS as a covariate

8.4 RESULTS

8.4.1 Basic data

There were 196, 439, 392, and 461 postpartum dairy cows included in the current study from Farms A, B, C, and D, respectively. Data for milk production are summarised in Table 8.3, and data for DMI, ME intake and BCS are summarised in Table 8.4. Reproductive data are summarised in Tables 8.5 and 8.6.

Mean pasture DMI, total DMI, milk composition, peak and annual milk production per cow, ME intake, ME requirements, EB, and BCS differed significantly between farms. The highest pasture DMI (kg/cow/day) and lowest total DMI (kg/cow/day) were observed for Farms D and A, respectively. Highest fat %, protein %, MS, and net energy (lactation) during peak lactation were observed for Farm C, which also had the highest ME requirements and ME intakes.

Overall means of SR21, SR42, PR21, PR42, and PREG1 were 82, 92, 51, 69, and 59%, respectively, however, there was a wide range between farms (67-89, 84-96, 32-54, 64-75 and 50-66% for SR21, SR42, PR21, PR42, and PREG1, respectively). The reproductive indices differed significantly ($p<0.05$) from each other, such that Farm B had the highest SR21, SR42, PR21, PR42, PREG1, and lowest PSMC and SPC compared to the other three farms.

Table 8.3. Milk production data for four pasture-based dairy farms in the Manawatu region of New Zealand

Parameter	Farm			
	A	B	C	D
Peak lactation yield, kg/cow/day				
Fat	0.90^{d} (0.88-0.92)	0.97^{c} (0.96-0.98)	1.25^{a} (1.24-1.27)	1.15^{b} (1.36-1.16)
Protein	0.75^{d} (0.74-0.76)	0.88^{c} (0.87-0.89)	1.04^{a} (1.03-1.05)	0.94^{b} (0.93-0.95)
Milk solids	1.67^{d} (1.65-1.69)	1.9^{c} (1.88-1.92)	2.30^{a} (2.28-2.31)	2.09^{b} (2.07-2.1)
Milk composition at peak lactation %				
Fat	4.63^{b} (4.56-4.71)	4.56^{b} (4.51-4.61)	4.97^{a} (4.92-5.12)	4.36^{c} (4.31-4.41)
Protein	3.79^{ab} (3.75-3.83)	3.76^{b} (3.74-3.79)	3.76^{b} (3.74-3.79)	3.82^{a} (3.79-3.84)
Net energy for lactation ($NE_{L)}$ at peak lactation, MJ/cow/day	133.8^{d} (131.9-135.8)	152.4^{c} (150.9-153.8)	183.6^{a} (182.1-185.1)	167.3^{b} (165.8-168.7)

Data are presented as mean (95% confidence interval, lower-upper)

$^{a\text{-}d}$Means within a row that do not share a common superscript are significantly different from each other (P<0.05)

Table 8.4. Dry matter intake (DMI) and metabolisable energy (ME) intake, ME requirement, energy balance (EB) and body condition score (BCS) of lactating dairy cows in pasture-based dairy farms in the Manawatu region of New Zealand

Parameter		**Farm**			
		A	B	C	D
DMI from pasture (kg/cow/d)					
Weeks	1-6	12.3^{b} (11.9-12.6)	12.0^{b} (11.7-12.3)	15.2^{a} (14.9-15.6)	15.7^{a} (15.3-15.9)
	7-12	13.9^{c} (12.9-14.9)	13.3^{c} (12.3-14.3)	16.0^{b} (15-17)	17.7^{a} (16.7-18.7)
	13-18	16.7^{b} (16.3-17.1)	15.4^{c} (14.9-15.8)	16.6^{b} (16.2-17)	19.8^{a} (19.4-20.3)
Total DMI (kg/cow/d)					
Weeks	1-6	12.3^{c} (11.9-12.6)	18.0^{b} (17.7-18.3)	19.2^{a} (18.9-19.6)	19.7^{a} (19.3-19.9)
	7-12	13.9^{d} (13.1-14.7)	18.4^{c} (17.6-19.3)	20.0^{b} (19.2-20.8)	21.9^{a} (21.1-22.8)
	13-18	16.7^{d} (16.3-17.1)	19.2^{c} (18.8-19.6)	20.6^{b} (20.2-20.9)	23.8^{a} (23.5-24.2)
ME intake (MJ ME/cow/day)					
Weeks	1-6	137.5^{d} (137.2-137.7)	197^{c} (196.8-197.2)	226.1^{a} (225.9-226.2)	212.9^{b} (212.7-213.1)
	7-12	148.5^{d} (147.9-148.9)	192.7^{c} (192.4-193.1)	224.6^{a} (224.2-224.9)	207.8^{b} (207.5-208.1)
	13-18	181.3^{d} (180.8-181.7)	209.5^{c} (209.2-209.8)	256.9^{a} (256.6-257.2)	229.6^{b} (229.3-229.9)
ME requirements (MJ ME/cow/day)					
Weeks	1-6	-	-	-	-
	7-12	173.5^{d} (169.5-177.4)	193.6^{c} (190.7-196.4)	237.6^{a} (234.7-240.5)	222.3^{b} (219.5-225.1)
	13-18	171^{d} (168.6-173.5)	203.1^{c} (201.4-204.8)	249.2^{a} (247.5-250.9)	217.3^{b} (215.5-219.1)
EB (MJ ME/cow/day)					
Weeks	1-6	-	-	-	-
	7-12	-25.2^{a} (-28.6- -21.8)	-6.1^{c} (-8.7- -3.6)	-15.1^{b} (-17.6- -12.5)	-14.7^{b} (-17.1- -12.3)
	13-18	2.03^{c} (-0.3-4.4)	5.84^{b} (4.2-7.4)	7.6^{b} (6.0-9.2)	12.1^{a} (10.5-13.8)
BCS (1-10 scale)					
Weeks	1-6	4.1^{b} (4.1-4.2)	4.2^{a} (4.2-4.2)	4.2^{a} (4.2-4.2)	4.2^{b} (4.1-4.12)
	7-12	4.0^{b} (4.1-4.2)	4.1^{a} (4.1-4.1)	4.1^{a} (4.1-4.1)	4.1^{a} (4.1-4.1)
	13-18	4.1^{c} (4.0-4.1)	4.07^{bc} (4.1-4.1)	4.1^{a} (4.1-4.1)	4.1^{b} (4.1-4.1)

Data are presented as mean (95% confidence interval, lower-upper)
$^{a-d}$Means within a row that do not share a common superscript are significantly different from each other ($P<0.05$)

Table 8.5. Indices of reproductive performance for four pasture-based dairy farms in the Manawatu region of New Zealand obtained directly from New Zealand national database (MINDA) records

	Norms*	Farm A	Farm B	Farm C	Farm D	SEM
CFMI (days)	77-84	80.9^{b}	80.7^{b}	72.8^{c}	85.1^{a}	0.52
		(64-90)	(70-94)	(62-88)	(75-97)	
CCI (days)	95-100	82.3^{c}	88.7^{b}	81.9^{c}	94.0^{a}	0.47
		(65-97)	(79-100)	(69-97)	(81-108)	
PSMF (days)	10-13	19.1^{a}	10.3^{b}	10.2^{b}	13.6^{a}	0.21
		(9-26)	(3-15)	(3-16)	(6-18)	
SPC (n)	1.5	1.4^{b}	1.2^{d}	1.3^{c}	1.5^{a}	0.01
		(1-2)	(1-2)	(1-2)	(1-2)	
CPSM (days)	60-70	61.8^{c}	69.9^{b}	62.0^{c}	71.5^{a}	0.44
		(44-74)	(60-84)	(51.2-78)	(63-84)	
FMCI (days)	16-27	5.9^{b}	4.3^{c}	6.9^{b}	8.7^{a}	0.29
		((0-11)	(0-12)	(0-18)	(0-21)	
PSMC (days)	21-25	25.5^{a}	13.4^{d}	17.2^{c}	21.4^{b}	0.33
		(15-33)	(5-19)	(4-25)	(8-31)	

CFMI calving to first breeding interval; CCI calving to conception interval; PSMF planned start of breeding to first service interval; SPC services per conception; CPSM calving to planned start of breeding interval; FMCI first breeding to conception interval; PSMC planned start of breeding to conception interval; Data are presented as mean (95% confidence interval, lower-upper);
*values were derived from Grosshans, et al., 1997; Macmillan et al., 1997; MacDougall, 2006;
$^{a-d}$Means within a row that do not share a common superscript are significantly different from each other (P<0.05)

Table 8.6. Calculated indices of reproductive performance for four pasture-based dairy farms in the Manawatu region of New Zealand

	Norms*	**Farm A**	**Farm B**	**Farm C**	**Farm D**	**SEM**
SR21 (%)	>80	67.0^{c}	89.3^{a}	85.7^{b}	84.2^{b}	-
SR42 (%)	>90	84.5^{c}	95.9^{a}	91.1^{b}	95.2^{a}	-
PR21 (%)	46-47	42.5^{d}	63.8^{a}	47.7^{c}	51.1^{b}	-
PR42 (%)	72-74	66.5^{b}	75.2^{a}	64.8^{bc}	68.9^{b}	-
PREG1 (%)	>50	56.2^{b}	66.2^{a}	57.9^{b}	55.7^{b}	-

SR21 three-week submission rate; SR42 six-week submission rate; PR21 three-week pregnancy rate; PR42 six-week pregnancy rate; PREG1 Pregnancy rate to first service; SEM standard error of the mean;

*values were derived from Grosshans, et al., 1997; Macmillan et al., 1997; MacDougall, 2006;

$^{a-d}$Means within a row that do not share a common superscript are significantly different from each other ($P<0.05$)

8.4.2 Energy balance vs. days in milk (DIM)

Dry matter intakes were calculated from calving to six-weeks after the start of breeding, therefore, EB was calculated until 112 days postpartum for Farm A and 126 days for Farms B-D, respectively. Energy balance data are illustrated in Figure 8.2. The most substantial NEB (~38.5 MJ/cow/day) occurred within 14 days after calving at Farm D. Negative energy balance at Farms A, B, and D continued until ~Day 60-80 postpartum; however, cows at Farm C returned to positive energy balance only after 80-90 days. Non-availability of milk records precluded the calculation of NEB during the immediate postpartum transition. In mid-lactation, EB gradually increased in all cows.

According to the mixed model analysis, breed, parity, and calving month were all significantly ($P<0.001$) related to EB during the early lactation period. Data for EB vs. breed separated into 2 homogenous subsets, such that Breed Codes 2 (Holstein Friesian (HF); HF_{11}-HF_{13}) and 3 (HF_{14}-HF_{16}) were similar to each other (mean difference: -0.71 MJ/day (adj. 95% CI: -2.77 to 1.35)) but were higher (more negative) than Breed Code 1 (HF_{2}-HF_{10}; highest mean difference: 4.88 MJ/day (adj. 95% CI: 2.57 to 7.2). Similarly, data for EB vs. calving month separated into 2 homogenous subsets, such that NEB during early lactation for cows that calved in August and September were similar to each other (mean difference: 2.66 MJ/day (adj. 95% CI: -0.29 to 5.61)) but were higher (more negative) than cows that calved in July (highest mean difference: 8.62 MJ/day (adj. 95% CI: 5.62 to 11.6). Energy balance differed for each parity code, such that highest and lowest mean differences were observed between 3 and 1 (34.8 MJ/day (adj. 95% CI: 32.5 to 37.2), and 3 and 2 (7.7 MJ/day (adj. 95% CI: 5.61 to 9.78)), respectively.

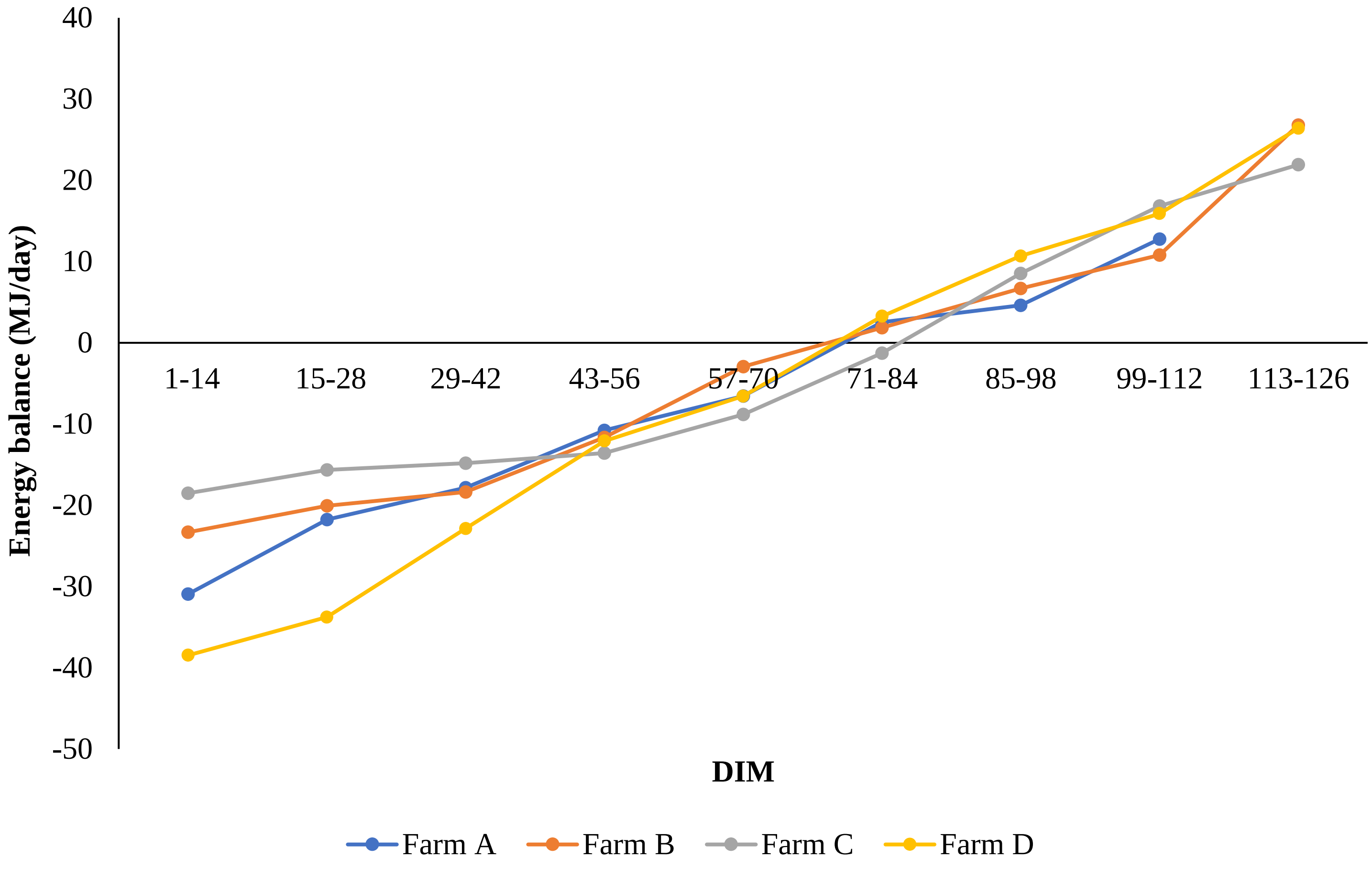

Figure 8.2. Energy balance [ME intake- ME requirements; MJ/day] relative to DIM of postpartum dairy cows

8.4.3 Body condition score (BCS) vs. days in milk (DIM)

Body condition score relative to DIM is shown in Figure 8.3. Overall mean BCS was lowest on Farm A throughout the study period. Body condition score declined significantly between calving and peak milk production at 43-70 days postpartum, and thereafter, BCS gradually increased on Farms C and D, but remained relatively static on Farms A and B.

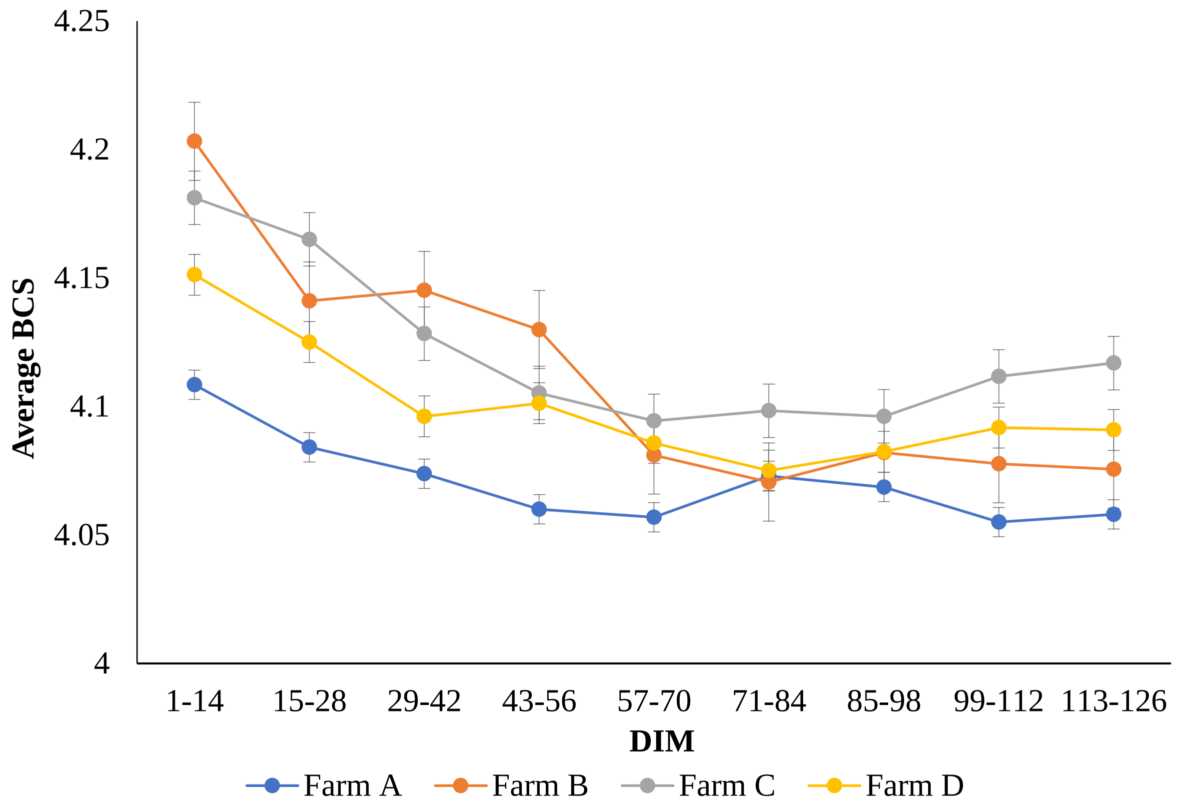

Figure 8.3. Mean BCS (± adj. 95% CI) of postpartum dairy cows during Days 1 to 126 of lactation

8.4.4 Milk production

Average milk production (kg MS) and fat:protein ratio relative to herd testing dates (n=4) are shown in Figure 8.4 (a and b). Peak yields occurred at 58±17 DIM: highest values occurred on Farms C (2.3 kg MS/day) and D (2.09 kg MS/day) compared to Farms A (1.67 kg MS/day) and B (1.87 kg M S/day). Average milk production declined significantly ($P<0.0001$; $r^2=38.7$) towards late lactation: at the end of the lactation, yields had declined to 1.69, 1.41, 1.04, and 0.91 kg MS/day in Farms C, D, B, and A, respectively. Fat:protein ratio increased significantly ($P<0.001$; $r^2 = 17.8$) from early to late lactation. The average fat:protein ratio was similar (1.2±0.02) across all farms at the peak lactation, however, it differed significantly between farms in late lactation (A: 1.35, B: 1.27, C: 1.45, D: 1.31). The milk production during the entire season was inversely proportional ($P<0.01$; $r^2 = 0.07$) to the fat:protein ratio across all farms.

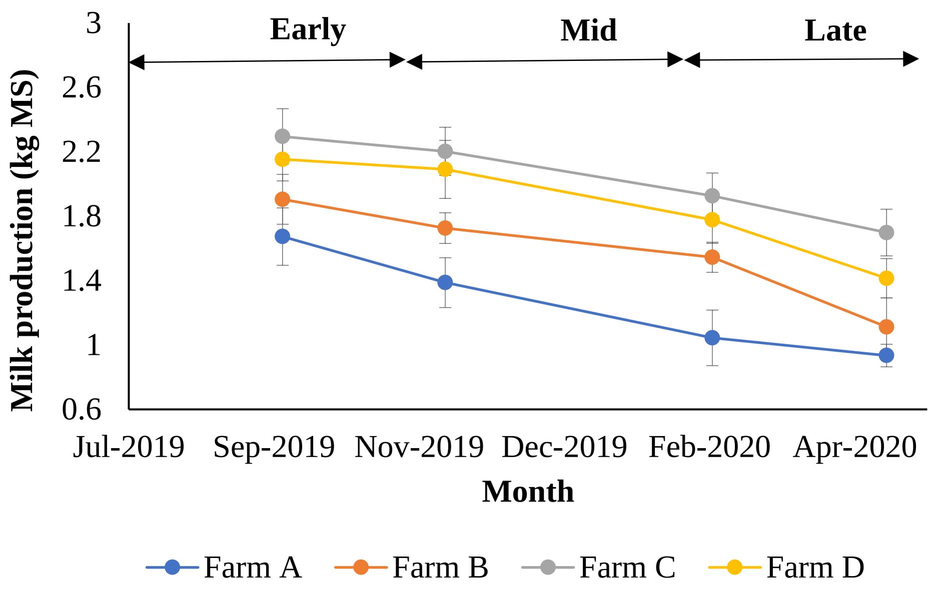

Figure 8.4a. Mean (± standard error of mean (SEM)) milk production (kg milk solids (MS)) of postpartum dairy cows during 2019-2020 lactation for four pasture-based dairy farms in the Manawatu region of New Zealand

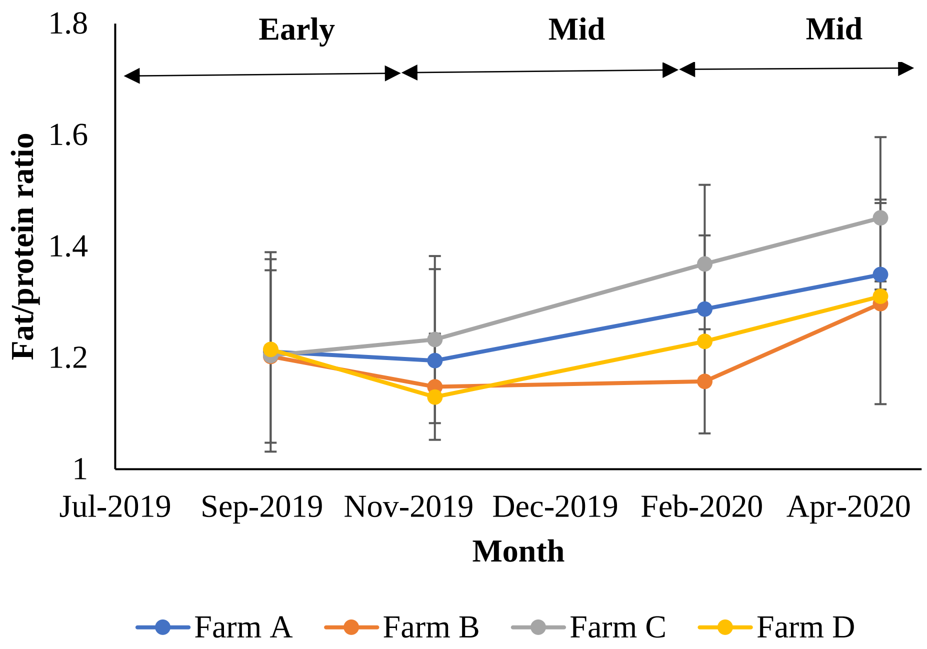

Figure 8.4b. Mean (± SEM) fat:protein ratio of postpartum dairy cows during 2019-2020 lactation for four pasture-based dairy farms in the Manawatu region of New Zealand

8.4.5 Cow factors associated with reproduction (SR21, SR42, PR21, PR42, and PREG1)

The multivariable logistic regressions for the explanatory individual variables; (i.e. farmID, parity, breed, calving month, ΔBCS, and EB nadir) and the reproduction variables (SR21, SR42, PR21, PR42, and PREG1) are presented in Tables 8.7-8.10. The farm variable was significantly associated with all outcome variables. Calving month was not associated with PREG1, however, it was significantly associated with all other dependent variables. Parity was associated with PR21 and PR42, and cattle breed was associated with PR21, PR42, and PREG1, however, neither fixed variable was significantly associated with SR21 and SR42. Body condition score and EB were associated with PREG1 and PR42, respectively, but not with SR21, SR42, and PR21.

Cows with <65% and 65-85 % of HF genes had a higher PR21 and PR42 than the reference category [high proportion of HF (>85%) genes], respectively, however, there were no differences for SR21 and SR42 between cows that had low (<85%) HF genes and the reference category. Cows of Parity 1-2 and/or 3-4 were more likely to have a successful PR21 and/or PR42 than those with ≥5 (reference category).

Cows that calved in July had higher SR21, SR42, PR21, and PR42 than those that calved in September (reference category), however, cows calved in August only had higher SR21, SR42, and PR42 than those calved in September. These indices were also higher in cows that calved in July than those that calved in August. Reproductive outcomes were significantly different between farms, with the highest SR21, PR21, and PREG1 being observed in Farm B. Cows that had <-30 MJME/day during EB nadir had a lower PR42 than those which had a higher EB nadir (reference category). Low BCS change (reference category) between calving to BCS nadir was significantly associated with increased PREG1 of postpartum dairy cows.

Table 8.7. Logistic regression for factors associated with submission rate in the first three weeks of the breeding season (SR21) and submission rate in the first six weeks of the breeding season (SR42) for four (A-D) pasture-based dairy farms

Reproductive parameter	Variable	Level	N	Odds ratio	95% CI (Lower-upper)	*P*-value
SR21	Farm ID	A	194	0.6	(0.26-1.36)	NS
		B	439	3.39	(1.56-7.35)	0.002
		C	392	1.33	(0.76-2.33)	NS
		D	461	Ref.		
	Calving month	July	634	2.43	(1.12-5.28)	0.02
		August	604	2.39	(1.12-5.12)	0.02
		September	248	Ref.		
SR42	Farm ID	A	194	0.35	(0.1-1.38)	NS
		B	439	0.99	(0.29-3.4)	NS
		C	392	0.35	(0.14-0.86)	0.02
		D	461	Ref.		
	Calving month	July	634	2.65	(0.89-7.92)	0.05
		August	604	3.12	(1.05-9.26)	0.04
		September	248	Ref.		

N number of cows; Ref. referent; NS (not significant) = *P*-value > 0.05; CI confidence interval

Table 8.8. Logistic regression model for factors associated with pregnancy rate in the first three weeks of the breeding season (PR21) for four (A-D) pasture-based dairy farms

Variable	Level	N	Odds ratio	95% CI (Lower-upper)	*P*-value
Farm ID	A	194	0.7	(0.36-1.37)	NS
	B	439	2.3	(1.45-3.64)	0.004
	C	392	0.58	(0.39-0.86)	0.01
	D	454	Ref.		
Parity	1-2	465	1.47	(0.88-2.46)	NS
	3-4	541	1.52	(1.05-2.2)	0.03
	≥5	468	Ref.		
Breed	HF<65%	354	1.65	(1.08-2.54)	0.02
	HF 65-85%	436	1.47	(0.99-2.18)	0.05
	HF>85%	654	Ref.		
Calving month	July	629	1.96	(1.05-3.65)	0.03
	August	603	1.46	(0.79-2.7)	NS
	September	247	Ref.		
ΔBCS	-1 - -0.5	465	0.89	(0.64-1.24)	NS
	-0.5 - 0	547	Ref.		
EB nadir	< -30	519	0.83	(0.59-1.17)	NS
	-30 -0	502	Ref.		

ΔBCS change in body condition score between calving and nadir; EB nadir lowest energy balance recorded; HF Holstein-Friesian; N number of cows; Ref. referent; NS (not significant) = *P*- value > 0.05; CI confidence interval

Table 8.9. Logistic regression model for factors associated with pregnancy rate in the first six weeks of the breeding season (PR42) for four (A-D) pasture-based dairy farms

Variable	Level	N	Odds ratio	95% CI (Lower-upper)	P-value
Farm ID	A	194	1.43	(0.62-3.32)	NS
	B	439	2.15	(1.17-3.96)	0.01
	C	392	0.47	(0.31-0.73)	0.001
	D	454	Ref.		
Parity	1-2	465	1.76	(1.17-2.67)	0.02
	3-4	541	2.29	(1.16-4.49)	0.01
	≥5	468	Ref.		
Breed	HF<65%	354	1.65	(0.99-2.74)	0.05
	HF 65-85%	436	1.59	(0.99-2.55)	0.05
	HF>85%	654	Ref.		
Calving month	July	629	2.51	(1.3-4.84)	0.01
	August	603	1.95	(1.02-3.72)	0.04
	September	247	Ref.		
ΔBCS	-1 - -0.5	465	0.86	(0.58-1.28)	NS
	-0.5 - 0	547	Ref.		
EB nadir	< -30	519	0.63	(0.42-0.94)	0.02
	-30 -0	502	Ref.		

ΔBCS change in body condition score between calving and nadir; EB nadir lowest energy balance recorded; HF Holstein-Friesian; N number of cows; Ref. referent; NS (not significant) = *P*- value > 0.05; CI confidence interval

Table 8.10. Logistic regression model for factors associated with pregnant to first service (PREG1) for four (A-D) pasture-based dairy farms

Variable	Level	N	Odds ratio	95% CI (Lower-upper)	P-value
Farm ID	A	194	0.69	(0.35-1.36)	NS
	B	439	2.24	(1.36-3.67)	0.001
	C	392	0.6	(0.4-0.89)	0.01
	D	4561	Ref.		
Parity	1-2	465	1.44	(0.83-2.49)	NS
	3-4	544	1.19	(0.82-1.74)	NS
	≥5	472	Ref.		
Breed	HF<65%	354	1.46	(0.94-2.27)	NS
	HF 65-85%	438	1.57	(1.04-2.36)	0.03
	HF>85%	659	Ref.		
Calving month	July	634	1.65	(0.89-3.06)	NS
	August	604	1.18	(0.64-2.17)	NS
	September	248	Ref.		
ΔBCS	-1 - -0.5	468	0.7	(0.5-0.99)	0.04
	-0.5 - 0	548	Ref.		
EB nadir	< -30	519	0.75	(0.53-1.06)	NS
	-30 -0	502	Ref.		

ΔBCS change in body condition score between calving and nadir; EB nadir lowest energy balance recorded; HF Holstein-Friesian; N number of cows; Ref. referent; NS (not significant) = *P*- value > 0.05; CI confidence interval

8.4.6 Parity, breed, energy balance, and milk production

The multivariable linear regressions between the explanatory individual variables (farm, parity, breed, and EB nadir), and milk production variables (Peak-Fat%, Peak-Protein%, and Peak-MS) are presented in Table 8.11. During peak lactation, all production variables were influenced by farm, parity, and breed factors. More severe NEB <-30 MJ ME/day was associated with higher mean Peak-Fat% and Peak-MS.

Table 8.11. General linear model, least-square means (95% confidence interval, lower-upper) of main effects (farm, parity, breed, energy balance (EB)) on milk production variables of early lactating dairy cows in four pasture-based dairy farms in the Manawatu region of New Zealand

Variable	Farm				Parity			Breed			EB nadir (MJME/cow/day)	
	A	B	C	D	1-2	3-4	≥5	HF_2-HF_{10}	HF_{11}-HF_{13}	HF_{14}-HF_{16}	<-30	-30 - 0
Peak milk production												
Peak-Fat (%)	4.35[a]	4.13[b]	4.37[a]	4.44[a]	4.37[a]	4.26[b]	4.33[ab]	4.43[a]	4.31[b]	4.24[b]	4.39[a]	4.25[b]
	(4.23-4.47)	(4.06-4.20)	(4.30-4.44)	(4.37-4.51)	(4.29-4.46)	(4.20-4.32)	(4.26-4.40)	(4.35-4.50)	(4.24-4.37)	(4.18-4.30)	(4.33-4.45)	(4.20-4.31)
Peak-Protein (%)	3.61[b]	3.72[a]	3.51[c]	3.60[b]	3.60[b]	3.60[b]	3.64[a]	3.65[a]	3.59[b]	3.60[b]	3.61	3.62
	(3.57-3.66)	(3.69-3.75)	(3.48-3.54)	(3.57-3.63)	(3.56-3.63)	(3.58-3.63)	(3.61-3.67)	(3.62-3.68)	(3.56-3.62)	(3.57-3.62)	(3.58-3.63)	(3.60-3.64)
Peak-MS (kg)	1.79[c]	2.16[b]	2.50[a]	2.17[b]	2.06[c]	2.18[b]	2.23[a]	2.13[b]	2.17[a]	2.17[a]	2.34[a]	1.98[b]
	(1.74-1.85)	(2.13-2.19)	(2.47-2.53)	(2.14-2.20)	(2.02-2.09)	(2.15-2.21)	(2.21-2.26)	(2.10-2.16)	(2.14-2.20)	(2.15-2.20)	(2.31-2.36)	(1.95-2)

HF Holstein-Friesian genetic lines; EB nadir lowest energy balance recorded; [a-d] values with different superscript in the same row are different from each other (LSD, P<0.05);

8.4.7 Associations between milk production at peak lactation and reproduction

The logistic regression between independent variables (Peak-Fat%, Peak-Protein%, Peak-MS, ΔBCS, and EB nadir), and the reproduction variables (SR21, SR42, PR21, PR42, and PREG1) are presented in Table 8.12. Production of ≤1.8 MS kg at peak lactation was associated with lower SR21 (OR: 0.41; 95% CI: 0.18-0.96; P=0.04) and SR42 (OR: 0.25; 95% CI: 0.1-0.83; P=0.02) compared to cows producing >2.4 MS kg. Peak milk protein of <3.5% at peak lactation was associated with lower PR21, PR42, and PREG1 than in animals producing >3.7%. Cows that produced 1.8-2.09 kg MS at peak lactation were more likely to have a higher PREG1 than the reference category (>2.4 kg MS). Cows that had an EB of <-30 MJ ME/day during the EB nadir had lower SR42 and PR42 than those that had less severe NEB. Body condition score loss of ≤0.5 between calving to BCS nadir was associated with higher PREG1 than in animals with greater BCS loss. Fat (%) during the peak lactation was not associated with any reproductive parameter.

Table 8.12. Association between milk production variables (Peak-MS, Peak-Fat%, Peak-Protein%), change in body condition score between calving and nadir (ΔBCS), lowest energy balance (EB nadir), and reproductive performances of lactating dairy cows at the peak milk production (resulting odds ratio and 95% lower-upper bound confidence interval (CI))

Reproductive parameter	Factor	Level	N	Odds ratio	95% CI	P-value
SR21	Peak-MS (MS kg)	<1.8	336	0.41	(0.18-0.96)	0.04
		1.8-2.09	317	0.69	(0.32-1.47)	NS
		2.1-2.4	302	1.1	(0.54-2.22)	NS
		>2.4	278	Ref.		
SR42	Peak-MS (MS kg)	<1.8	336	0.25	(0.1-0.83)	0.02
		1.8-2.09	317	0.52	(0.17-1.64)	NS
		2.1-2.4	302	1.21	(0.39-3.73)	NS
		>2.4	278	Ref.		
	EB nadir	< -30	519	0.39	(0.16-0.94)	0.03
		-30 -0	502	Ref.		
PR21	Peak-Protein (%)	<3.5	265	0.56	(0.33-0.95)	0.03
		3.5-3.59	452	0.66	(0.37-1.16)	NS
		3.6-3.7	236	1.26	(0.74-2.17)	NS
		>3.7	293	Ref.		
PR42	Peak-Protein (%)	<3.5	265	0.44	(0.24-0.81)	0.01
		3.5-3.59	452	0.64	(0.32-1.28)	NS
		3.6-3.7	236	1.91	(0.9-3.05)	NS
		>3.7	293	Ref.		
	EB nadir	< -30	519	0.59	(0.37-0.92)	0.02
		-30 -0	502	Ref.		
PREG1	Peak-MS (MS kg)	<1.8	336	1.32	(0.71-2.45)	NS
		1.8-2.09	317	1.62	(0.96-2.75)	0.05
		2.1-2.4	302	1.07	(0.69-1.67)	NS
		>2.4	278	Ref.		
	Peak-Protein (%)	<3.5	265	0.6	(0.35-1.03)	0.05
		3.5-3.59	452	0.82	(0.45-1.49)	NS
		3.6-3.7	236	1.33	(0.75-2.36)	NS
		>3.7	293	Ref.		
	ΔBCS	-1 - -0.5	468	0.74	(0.53-1.03)	0.05
		-0.5 - 0	548	Ref.		

SR21 submission rate in the first three weeks of the breeding season; SR42 submission rate in the first six weeks of the breeding season; PR21 pregnancy rate in the first three weeks of the breeding season; PR42 pregnancy rate in the first six weeks of the breeding season; PREG1 pregnant to the first service; N number of cows; Ref. referent; NS (not significant) = *P*-value > 0.05

8.5 DISCUSSION

Achieving high reproductive performance over a condensed breeding season is essential to achieve the 365-day, seasonal, calving pattern that is needed to sustain seasonal pasture-based dairy farming (Dillon et al.,1995; McDougall, 2006; McDougall et al., 2014). The reproductive performance achieved in the present study generally exceeded the minima (Grosshans, et al., 1997; Macmillan et al., 1997; MacDougall, 2006) that are needed to maintain this calving pattern in the published literature on dairy farming in New Zealand, although PREG1 for Farms A, C and D was poorer than target (Grosshans et al., 1997). Many factors, including breed, parity, BCS and energy status, influence the resumption of ovarian activity and the ability of cows to re-conceive (Walsh et al., 2007a, 2011). Several of these factors were also identified in the present study, such that, when adjusted for ΔBCS and EB nadir, there were significant effects of farm, parity, breed and calving month on reproductive performance. The effect of farm upon the present results is of interest: all were selected to be low-input, high-pasture systems, with heavy reliance upon perennial ryegrass and white clover pastures for feed supply. Nonetheless, it is likely that differences between reproductive performance between farms reflected minor variations in management, of which the amount of supplemental feeding (variation from ~5-14% of total feed) between farms may have been the most important (Richards et al., 1986; Walker et al., 2004).

Multivariable logistic regression showed poorer PR21 and PR42 in animals of parity ≥5, and that animals in their 3rd-4th lactations had better reproductive performance than both older (parity ≥5) and younger (parity 1-2) animals. These findings generally correlated well with previous findings that reproductive performance of dairy cows decreases as the parity increases (Macmillan et al., 1996; Lee & Kim, 2007; Tanaka et al., 2008; Zhang et al., 2010). Some of this parity effect may be related to NEB as the present study showed that NEB in early lactation increased as parity increased, consistent with previous findings in New Zealand dairy cows (Macmillan et al.., 1996). More severe, NEB has multiple effects on reproductive performance including delaying the return to oestrus, by suppressing the hypothalamic secretion of gonadotropin-releasing hormone, thereby delaying follicular development (Nebel & McGilliard, 1993; Wathes et al., 2007).

In the present study, PR21, PR42, and PREG1 were all lower in cows that had >85% HF genes compared to the crossbred HF cows (HF x Jersey; HFxJ) that had 12-85% HF genes. These findings reflect those of Grosshans et al. (1997), who showed that in HFxJ crossbreds, PR21 was higher for cows that had <65% HF genes compared to cows that had 65-85% HF genes. Since all the crossbred HF cows in the present study consisted of a certain amount of Jersey genetics, there is likely to be reduced expression of HF characteristics proportionate to the level of non-HF (in this case, Jersey). Whether the poorer result of the more pure-bred HF reflects a better ability of cows with more Jersey genes to initiate ovarian and oestrous cyclicity earlier after calving (e.g. Silva et al., 1992; Vance et al., 2013), or whether the difference can be attributed to the effects of heterosis (Harris & Kolver, 2001), is unclear from the present study. However, the present results align with those of Lateef et al. (2008), who showed that breeding efficacy is significantly higher for Jersey cows compared to HF cows. However, pure HF cows are capable of greater milk production than Jersey cows (Lateef et al., 2008), so the effects of HF genetics *per se* are likely to be aliased with those of milk yield. The present analysis showed that, compared with cows with <65% HF genes, cows with >85% HF genes had greater NEB in early lactation. Given that NEB has a substantial effect upon the resumption of oestrous cycles and the ability of postpartum dairy cows to conceive to first artificial insemination (AI; Butler, 2003; Wathes et al., 2007), it is interesting that, in the present study, the effects of breed upon PR21 was significant, whilst the effects upon SR21 and SR42 were only non-significant trends in the same direction.

Cows that calved in July and/or August had higher SR21, SR42, PR21 and PR42 compared to later-calving cows. Early calved cows had longer CPSM intervals, such that the cows that calved earlier in the calving season had a longer postpartum period in which to re-establish ovarian cycles (Evans et al., 2006). Other studies have shown that cows that calve later in the calving season (which thus have shorter CPSM intervals of <60 days) are at greater risk of culling due to poor reproductive performance (Berry et al., 2005; Evans et al., 2006). Resumption of oestrous cycles usually occurs at around the postpartum nadir of NEB (Butler et al., 1981; Canfield & Butler, 1991). There is considerable evidence that the number of oestrous cycles that a cow has had between calving and first AI are related to the chance of conception to that insemination (Santos et al., 2009). Similarly, inseminations before ~Day 40 postpartum are substantially less likely to result in conception that those later in the postpartum period (Bulman & Lamming, 1978; Schindler et al., 1991: Wathes et al., 2007). Further, the interval between the nadir of EB and first ovulation are highly correlated (Canfield & Butler, 1990), whilst the rate of loss of energy stores/body condition during the early postpartum period is also highly correlated with the interval to the resumption of oestrous cycles (Wathes et al., 2007; Crowe et al., 2014). Thus, as found in the present study, reproductive efficiency is better in cows that calve early in the season than in those that calve later.

In the current study, parity, breed and the depth of the EB nadir during early lactation were related to milk yield and milk composition during peak lactation. Fat and protein concentrations were higher for cows with ≥5 parities compared to 1-2 and/or 3-4 parities. Cows with <65% of HF genes produced higher fat% and protein% during the lactation peak, but their MS yields were lower, than cows that had ≥65% of HF genes. Previous research (e.g. Schutz et al.,1990; Vance et al., 2013) broadly agrees that fat and protein yields increase at different rates with increasing parity, and that breed differences significantly influence milk yield and milk composition.

Energy balance during peak lactation was significantly associated with fat and MS production, such that higher NEB (<-30 MJ ME/cow/day) was related to higher per cow fat and total MS production than lower NEB (-30 to 0 MJ ME/cow/day). Higher milk production was therefore associated with more severe NEB which would have led to increase loss of body fat reserves (Roche, 2006), increasing the potential for metabolic and endocrine changes to affect fertility (Veerkamp et al., 2003). In pasture-based systems, the mobilization of body reserves can be more extreme (Clark et al., 2005), especially in cows that have a high proportion of HF genes (Kolver & Muller, 1998; Verkerk et al., 2000) which promote growth hormone (GH) mediated utilisation of body stores for lactation (Lucy, 2008; Lucy et al., 2009).

In the current study, mean protein percentages (3.79%) during early lactation were much higher than average values reported during early lactation for New Zealand dairy cows (2.97%) (Auldist et al., 1998), which may be reflected in PR21 and PR42, which were both higher than target minima (see Tables 8.3 and 8.6). Curiously, the literature is equivocal on the relationship between milk production and reproductive traits. Thus, Buckley et al. (2003) showed a positive relationship between milk production and conception rates in pasture-based dairy production systems, whereas Hansen et al. (1983) and Villa-Godoy et al. (1988) did not. In the present study, PR21, PR42, and PREG1 were lower in animals producing <3.5% protein at peak lactation that in those with higher protein production. Severe and prolonged NEB is associated with decreased milk protein % (Fulkerson et al., 2001) and, hence, poor reproductive performance (Buckley et al., 2003), while increased fertility has been associated with high milk protein concentrations (Morton et al., 2017). Auldist et al. (1998) found that milk protein %

varies with DMI, whilst Kalscheur et al. (1999) and Colmenero & Broderick (2006) found that it varies with dietary crude protein intake. Therefore, adequate levels of dietary crude protein intake may enhance milk protein % and reproductive performances of lactating dairy cows.

In the current study, the nadir of BCS was not below 4.0 (1-10 scale) at any stage of early lactation, and the maximum ΔBCS of most postpartum cows was no greater than -1 unit (mean BCS at calving was 5). However, PREG1 of postpartum dairy cows was decreased where BCS loss between calving and the nadir was >0.5 unit. In the literature, pregnancy rates were lower when cows experienced severe BCS loss (Garnsworthy & Webb, 1999). A study by Gillund et al. (2001) found that cows that experienced losses of ≥1.25 units BCS (1-5 scale) during the postpartum period were half as likely to conceive at their first insemination, compared with cows that experienced losses of ≤0.5 units in body condition. Consequently, BCS loss during the postpartum period was associated with prolonged CCI and increased SPC (Gillund et al., 2001). Therefore, it is advisable to aim to minimise BCS loss to <0.5 unit (1-5 scale) during early lactation (Crowe, 2008) to advance the resumption of oestrous cycles and timely achievement of pregnancy (Loeffler et al., 1999).

8.6 CONCLUSION

Cow DMI influences milk production and EB during early lactation. Energy balance is the most important factor for milk production, BCS, and reproductive performance of postpartum dairy cows, however, other associated factors such as DMI, breed composition, parity, and calving month must be considered and controlled if fertility is to be improved. High-yielding and highly fertile postpartum cows are essential for a pasture-based production system, therefore, dairy cow management together with pasture and reproductive managements are crucial for a sustainable production system.

CHAPTER 9. GENERAL DISCUSSION

9.1 INTRODUCTION

The expanding dairy industry in Sri Lanka has made significant advances over the past decade but is still struggling to meet national self-sufficiency for milk consumption. Nonetheless, dairy farming has become an integral part of most local communities in Sri Lanka, providing fresh dairy products and creating income and employment. Per-cow yields are relatively low at about 10-15 L/cow/day in medium-scale farms (Kollalpitiya et al., 2012), and generally lower in small-scale farms (Bandara, 2000). Consequently, domestic milk production still provides less than half of the country's requirements (Ranaweera, 2011; Vyas et al., 2020): thus, in order to meet national demands, substantial quantities of milk powder are imported. These imports drain a considerable amount from the national budget (~NZ$30 million/annum: DAPH, 2019), which could be obviated if domestic milk production were improved.

The Sri Lankan government has invested significantly in improving the national dairy herd as a means of increasing the domestic milk supply (Vyas et al., 2020). Crossbreeding of local cattle with exotic gene lines across the national dairy herd means that many cows now have the genetic potential to produce substantial quantities of milk. The failure to achieve these potential yields appears to be mainly due to inadequate feeding and poor reproductive management, with the two factors being likely to be linked. Farmers' lack of knowledge of appropriate cattle husbandry practices, coupled with low yields of forages, a scarcity of land for quality fodder production, inadequate provision of extension services, alongside poor nutritional and reproduction management (Perera & Jayasuriya, 2008; Vyas et al., 2020), all prevent Sri Lankan dairy cows from achieving their genetic potential for lactation.

9.2 CONTEXT OF THE PRESENT STUDIES

There is a nucleus of literature on the management of, and the constraints to, the productivity of dairy cows in Sri Lanka (Bandara, 2000; Perera & Jayasuriya, 2008; Vyas et al., 2020). Whilst this literature is far from complete, it is clear that improving the quality of feed and management of reproduction are likely to be pivotal to achieving an improvement in national, whole-of-farm and per-cow milk productivity. A key deficit of the literature is the lack of systematic study of the feedstuffs (especially forages) available to dairy farmers, as well as of the utilisation of such feeds by cows. In the broader literature, the importance of nutritional management in sustainable dairy production has been extensively reviewed (e.g. Zemmelink et al., 1999): In particular, whilst the genetic potential of dairy cows is a key factor that affects milk production and reproductive performance, this genetic potential can only be expressed where nutrition is adequate (Vyas et al., 2020). There is a dearth of nutritional studies in Sri Lanka, in terms of either the available feeds or the ability of those feeds to meet the needs of lactating dairy cows. Hence, the studies in this thesis focused primarily upon the feeding (including the relationships between feeding, lactation and reproductive performance) of Sri Lankan dairy cows.

It was initially planned that the entire study would be conducted in dairy farms in Sri Lanka. Phase 1 was to identify feed resources available for dairy production, and to interpret the effectiveness of these feeds to meet animal requirements for production and reproduction through examination of blood metabolic and hormonal profiles. Phase 2 was planned to be an exploration of appropriate forage management to achieve high-quality herbage, and then the development of feed rations incorporating forages and other feed types to optimize the productivity of Sri Lankan dairy cows, using the gaps identified in Phase 1. However, Phase 2 could not be undertaken due to the sudden imposition of travel restrictions between Sri Lanka and New Zealand, due firstly to terrorist activity in Sri Lanka, and thereafter the COVID pandemic. Consequently, Phase 2 was redeveloped to explore forage quality in relation to grazing management, and to monitor production and reproduction outcomes in New Zealand dairy farms, and by doing so, provide a contrast to the Sri Lankan data from Phase 1. It was also originally intended that studies in Phase 1 would extend across small- and medium-scale farms in Sri Lanka; however, due to concerns over the reliability of data from small-scale farms, studies were confined to medium-scale farms.

Studies of the medium-scale dairy industry in Sri Lanka were therefore undertaken on several farms to investigate the quality and quantity of common feed materials, and to explore the relationships between feeding and cow body condition and reproductive performance during early to mid-lactation. Information on cows' nutritional and reproductive status was provided by measurement of circulating concentrations of metabolites and hormones (albumin, urea, BHBA, NEFA, IGF-1, progesterone). Thus, in Phase 1, the studies of medium-scale dairy farming of Sri Lanka (Chapters 3-6) evaluated available feeds (Chapter 3), metabolic profiles of cows in early lactation (Chapter 4), and reproductive performance and its relationship with nutritional parameters (Chapters 5 and 6). Evaluation of feeds (Chapter 3) determined, for the first time on a comprehensive basis, the availability of feed types, their nutritional composition, and the practice of feeding MMR; with particular reference to the extent to which such rations met cows' DMI, CP and ME requirements. Nutritional status and its interaction with lactation and reproductive performance were thereafter further investigated using metabolic profiles (Chapter 4), IGF-1, and progesterone concentrations (Chapter 5 and 6) in cows over the prepartum transition to the early and mid-lactation periods; again, with particular reference to the effectiveness of MMR feeding regimens in meeting cows' nutritional requirements.

There is a comprehensive literature relating to the nutritional management of pastoral dairy cows in New Zealand, which was used to support the design of Phase 2 of this thesis. The studies undertaken in New Zealand initially focused upon the effects that pasture herbage management have upon DMI, lactation and reproductive performance, based on the general hypothesis that pasture herbage quality (nutritional composition) would be affected by pasture herbage management. Thereafter the focus shifted to DMI and energy balance, on the basis that nutritional effects on reproductive performance that were attributed to poor quality forage in Sri Lankan dairy farming could also be present in New Zealand dairy farms, even though the scale of 'poor quality' was expected to be different between the two systems.

The factors affecting the nutritional composition of forages (Chapter 7) were studied across a group of farms selected as having varying pasture management. Thereafter, the DMI and ME intakes that were achieved by cows grazing these pastures during the transition period and early and mid-lactation stages were compared with calculated nutritional requirements to estimate ME balance. These data were, in turn, used to investigate the relationships between milk production, BCS and reproduction (Chapter 8).

9.3 FEEDING AND BREEDING OF DAIRY COWS IN MEDIUM-SCALE FARMS IN SRI LANKA

Phase 1 was undertaken to redress the dearth of information regarding the forages and supplements that are commonly used on medium-scale dairy farms in Sri Lanka. This process of cataloguing the availabilities, quantities and composition of feeds, and the feeding regimens in which they were used, was required as a prerequisite to calculate dietary ME and CP intakes, and, thence, to investigate any shortfalls in dietary ME and CP. The ME and CP contents of the abundantly-used (representing >50% of the final cow diet) forages of Guinea and CO-3 grasses were generally low (7.5-8.0 MJ/kg DM, 8.0-8.8% DM, respectively), and were lower than that of legumes (i.e. Gliricidia: 10.0 MJ/kg DM, 17.7% DM, respectively). The NDF values of Guinea and CO-3 grasses were significantly higher (≥72% DM) than found in other forages [e.g. Gliricidia (47% DM) and maize residuals (65% DM)]. The consequence of these low nutritive values of these forages, and of farmers' consistent overestimation of their quality, was that the actual daily ME intake was consistently 10% lower than the calculated daily energy requirements. For dry cows, this was an average of 91 MJ/cow/day supplied vs. 101 MJ/cow/day required, and for early lactating dairy cows, it was an average of 126 MJ/cow/day supplied vs. 140 MJ/cow/day required. The CP intake of lactating cows (13.5% DM) was also inadequate to meet their requirements (16-18.5% DM), although the CP intake of dry cows (11.8% DM) did adequately meet their requirements (11-12% DM). It is clear that the these limiting nutritional factors would significantly adversely affect milk production of these dairy cows (Vyas et al., 2020).

These conclusions were borne out through MPT (Chapter 4), which investigated the relationship of metabolic parameters (albumin, urea, NEFA, and BHBA) with production parameters (stage of lactation, BCS, BW, breed, parity) and calving month. In terms of direct measures of energy status, BHBA concentrations were significantly higher during early lactation than during the prepartum transition period, whilst concentrations of NEFA were lower during the prepartum transition period and mid-lactation (Days 29 to 60 postpartum), than in early lactation. Albumin concentrations were lower at the onset of lactation (Days 1 to 28 postpartum) than during mid-lactation (Days 29 to 120), whilst urea concentrations were higher before calving than all stages postpartum. The data for urea and albumin are interesting in comparison to those for BHBA and NEFA: while albumin can be taken as a measure of protein status (Caldeira et al., 2007; Roche et al., 2013), it can also be taken to reflect a net deficiency of protein in the diet. The low urea concentrations in the postpartum period indicated an overall deficiency of CP in the diet. Whether these data can also be taken as an indication of the quality of protein in the diet is unclear, as the urea concentrations were too low to be taken as categorical evidence of deamination of low-quality protein or excessive NPN in the diet (Westwood et al., 1998). Taken together with the results of Chapter 3, these data provide clear evidence of deficiencies of ME and CP during the late dry and early lactation stages. Further, the results of the current study suggest the opportunity for using MPT for early identification and correction of nutrition constraints in the diet of dairy cows. Given the importance of an adequate plane of nutrition during the transition/early lactation periods upon subsequent reproductive performance, it would be expected that those animals which are in a state of energy and protein deficiency would have compromised reproductive performance. This notion was studied in detail in the next two chapters of the thesis.

The next stage of the investigation was to characterize the reproductive performance of cows in medium-scale farms, and to identify the factors that might affect oestrous cycles and conception. Serum progesterone profiles were chosen as the definitive measure of oestrus in preference to visual observation of oestrous behaviour, as it obviated stockmen's errors of observation and the confounding effects of small herd sizes upon oestrous behaviour (Stevenson, 2001). Progesterone concentrations were measured over the period between Days 43 to 120 postpartum. The relatively late start date for measurement was chosen, as the voluntary wait period was never less than 42 days postpartum, so it was deemed that there was no value in an earlier start. Just over half (61.6%) of cows resumed ovarian cycles during the first 120 days postpartum, however, 44.9% of those cows were not visually observed in oestrus and therefore not presented for AI. A substantial proportion of cows (58.4%) were not inseminated at all until Day 120, as they did not show oestrus signs until after that day. However, a comparison of stockmen's observations with progesterone profiles clearly indicated that there was widespread failure to detect the onset of oestrus (both missed and mis-diagnosed oestrus periods) and, probably, incorrect timing of insemination. During the period to Day 400 postpartum, only 41.1% of cows were diagnosed pregnant. Further, 73.2% of cows returned to oestrus after insemination, a figure which is higher than generally expected for tropical dairying systems (Yusuf et al., 2012; Khair et al., 2013), so it seems reasonable to equate apparently high incidence of returns to oestrus with the more general syndrome of anoestrus/inadequate oestrus detection that was clearly prevalent throughout the study. Further, it is also well-recognised that Holstein-Friesian cows are genetically less fertile than less high-yielding breeds (Vance et al., 2013; Buckley et al., 2014), and that they are more susceptible to the detrimental effects of heat stress upon the expression of oestrus than tropical-adapted breeds or Jerseys (Jordan, 2003). The effects of thermal stress upon early embryonic mortality are also well established, with high temperatures being particularly detrimental to the tubal stages of the early embryo (Rensis & Scaramuzzi, 2003; Hansen, 2009; Saint-Dizier et al., 2020). It has been argued that Holstein-Friesian cattle are also more sensitive to such effects than other breeds, although the data supporting that contention are not clear-cut (Tarabany & El-Tarabany, 2015). Taken together, it appears that the poor nutritional state of the animals, together with suboptimal reproductive management within the herds, created the situation of very low pregnancy rates and, hence, of high culling rates (and therefore the presence of very few multiparous animals in the herds).

On the basis that poor nutrition appeared to be a key limitation to reproductive performance (Cardoso et al., 2013; Drackley & Cardoso, 2014), it was postulated that examining a key metabolic indicator such as IGF-1 concentration might be of value as a means of estimating likely nutritional limitations upon reproductive outcomes (Zhou et al., 1997; Velazquez et al., 2008). This was undertaken by collecting blood samples from primiparous cows at Days 1 to 14 postpartum and then and again at Days 43 to 57. These sampling times were selected on the basis that the two key times that IGF-1 have been shown to be related to reproductive outcomes are over the immediate postpartum period (primarily in terms of the resumption of oestrous cycles: Taylor et al., 2004; Gobikrushanth et al., 2018), and at the early/mid-lactation transition (primarily in terms of conception and the establishment of pregnancy: Thatcher et al., 2006). Mean concentrations were lower on Days 1-14 (60.8 ± 2.6 ng/ml) than on Days 43-57 (72.3 ± 3.6 ng/ml), which was largely as would be predicted from studies of primiparous animals in temperate climates (Taylor et al., 2004) and reflect the nutritional fluxes that animals experience during the establishment of lactation (Taylor et al., 2004). It was interesting to note that IGF-1 concentrations were related to the CFSI. It is generally held that IGF-1 concentrations in very early lactation are related to the regulation of the resumption of oestrous cycles (Formigoni & Trevisi, 2003; Taylor et al., 2004; Konigsson et al., 2008; Mullen et al., 2011; Nicolini et al., 2013; Gobikrushanth et al., 2018), but, probably, in the present study, the overall poor nutritional status of the cows precluded the more localised effects of IGF-1 (i.e. as an intra-ovarian autocoid or modulator of gonadotrophin actions upon the follicle: Ferguson, 2005) at that early stage of lactation. Nonetheless, there would probably be limited value in routinely measuring IGF-1 concentrations in Weeks 6-10 of lactation as a means of estimating the resumption of oestrous cycles. Indeed, if any hormone were to be measured, progesterone would probably have a better cost-benefit ratio, given the high proportion of oestrus that stockmen failed to detect.

9.4 GRAZING, PASTURE HERBAGE QUALITY AND BREEDING IN NEW ZEALAND

The second part of the thesis was targeted to dairy production systems in New Zealand. The initial hypothesis for Chapters 7 and 8 was that grazing rotation length is inversely proportional to the nutritional composition, such that changes in grazing management would affect the herbage quality with consequent effects upon DMI, milk production and reproduction. The studies in Chapters 7 were undertaken to characterize the quality of mixed pasture herbage and to investigate the effect of grazing management (i.e. PGDM, PGH, RT and LS) on the nutritional composition of dairy pastures.

In general, dairy cows were fed on a rotation of between the 2 to 3 LS of perennial ryegrass during spring; however, during late winter and late spring, LS were <2 and >3, respectively, which could directly affect the ME intake of dairy cows. The WSC concentrations were low when pasture was not mature (<2 LS) and ME and CP declined when the pasture became over-mature (as also reported by Fulkerson & Slack, 1994). Mean ME (11.0-11.4 MJ/kg DM) and CP (20.9-22.2% DM) concentrations during winter and spring seasons were within the average ranges (11.0-12.5 MJ ME/kg DM, 15-30% DM, respectively) published by DairyNZ (2019). Pasture herbage quality was significantly affected by time, farm, and time × farm interaction. Importantly, ME was influenced by PGDM, RT, and LS; however, CP was only influenced by PGDM, PGH and RT. This information can be helpful for dairy farmers to manipulate pasture management to optimize the cows' performance. It also helps to underscore the data obtained for Sri Lankan forages, inasmuch as ME and CP also declined in NZ pastures with increasing plant maturity and, importantly, with increasing grazing rotation length.

Moving on from this basic characterisation of pastures under different rotation management systems, the next study (Chapter 8) investigated the relationship between cow-related factors [breed, BCS, parity, feed intake, energy balance and calving month and milk composition] and reproductive performance. Cows with >85% of HF genes had lower PR21, PR42 and PREG1 rates than those with ≤85% HF genes. Cows in all four farms experienced severe NEB (~-40-60 MJ ME/cow/day) during early lactation, which was affected by breed composition and parity. Higher fat production and total MS production were associated with significant NEB (≥-30MJ ME/day), but NEB was less (or was positive) in cows with lower MS yields. Therefore, it appears that breed composition, parity, and energy balance are useful indicators of reproductive efficacy. Again, these data underscore the observations in Sri Lanka, where low BCS and/or bodyweight over the postpartum period were associated with delayed return to oestrus and low pregnancy rates. It is also interesting to note that, in Chapter 8, breed (i.e. >85% HF genetics) affected fertility independently of yield, and that a similar trend was also evident in the studies in Sri Lanka. Much has been written (Faust et al., 1989; Grosshans et al., 1997; Harris & Kolver, 2001; Pryce et al., 2004; Royal et al., 2000) about the decline in fertility in HF cows and of the difficulty in meeting the heavy nutritional demands of such animals from pasture-based diets (Kolver & Muller, 1998; Kolver, 2003; Kolver et al., 2007): these effects have been well characterised in the pasture-based dairying systems of New Zealand and the Republic of Ireland, and it is probably also worthwhile doing so in Sri Lanka in the face of the added difficulties caused by a tropical climate.

9.5 LIMITATIONS OF THE CURRENT STUDY

Recruitment of dairy farms - In Sri Lanka, the main obstacle was primarily related to the poor reliability of data from small-scale farms. Hence, although the initial intention was to study both small and medium-scale farms, the decision was made to eliminate the small-scale farms from the study. Secondly, there was a considerable diversity of feeding and reproductive management of dairy cows in medium-scale farms; therefore, from many farms visited for initial recruitment, only nine farms were eventually selected as meeting the criteria required to provide a level of consistency among farms. This undoubtedly limited the range of farms available to the study but did result in a manageable number of farms that were operating reasonably similarly to each other.

The principal difficulty of recruiting farms in New Zealand was to identify System 1 or 2 farms that consistently utilised long or short grazing rotations for several years. With small cohorts of farms in each category, there was a considerable risk that the effects of grazing rotation would be commingled with other management factors. Further, as the managers of farms in both categories were experienced in the regulation of pasture supply, adverse effects of grazing rotation upon pasture herbage quality or quantity were rapidly mitigated.

Herbage analysis - Chemical composition analysis for herbage can be undertaken using different techniques, e.g. 'wet' chemistry, NIRS, high performance liquid chromatography (HPLC), and/or gas chromatography (Weiss & Hall, 2020), however, these techniques are not commonly available for tropical forages in Asian countries. The NIRS was used to analyse pasture samples for chemical composition as it has been proven to be an accurate, cost effective, and rapid research tool that can assist farmers making decisions about their cows' diets. This technology can be used in Sri Lanka; however, calibrating the NIRS with known tropical forages is essential to increase the accuracy of testing. The NIRS is a well-established method in New Zealand pasture laboratories where it is calibrated with known high numbers (100-500) of analysed samples, that enhance the accuracy of testing.

Blood sampling regimens - The blood collection protocols of the present studies had to consider a number of constraints that exist in dairy production systems in Sri Lanka. Firstly, year-round calving patterns are commonly practiced, so the number of eligible cows (14 days prepartum to 120 days postpartum) per farm over the study period was confined to <30% of the total herd. Secondly, farms that were appropriate for the present study were geographically separated, therefore, practical collection of large numbers of blood samples from many farms according to the selection criteria over a short period of time was not feasible. Therefore, the number of farms selected for the study was determined based on the minimum number of blood samples required for detection of statistically significant differences between each time period.

9.6 CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

Conclusions - This thesis has provided clear evidence of the association between poor quality forage and the deficiency in total ME and CP intakes by dairy cows at different stages of lactation (Chapter 3) in medium-scale dairy farms of Sri Lanka. The overall quality of major forages (Guinea grass and Hybrid Napier CO-3 grasses) of cow rations was low (in terms of low ME and CP, and of high NDF and ADF) resulting in low forage DMI. Consequently, significant amounts (30-40% of MMR) of supplements and/or concentrates were included in the ration, yet milk yields were only of the order of 10-15L/cow/day. The high cost of supplementary feeds, particularly with respect to the poor yield response, posed a significant risk to the economic viability of milk production.

The regular use of MPT could become a tool to improve the nutritional management of dairy cows; however, given the evidence of gross feed deficits and very poor forage management, perhaps efforts (and expenditure) would be better used in that direction in the first instance, keeping the MPT program for farms that have started to institute a broader suite of management improvements. The current study did not directly address metabolic diseases during the pre- and postpartum transition period; it is interesting to speculate what the incidence of these might be and whether this could be affected by the use of MPT. More important is the effect that poor nutrition had upon culling rate. Data from Chapter 4 showed that more than 80% of animals in dairy herds were primiparous, meaning that the survival rate from 1st to 2nd lactation is very low indeed. This poor survival rate is undoubtedly a reflection of the animals' nutritional status, which directly affects their viability, their lactational yields and, arguably most importantly, their ability to re-conceive and calve a second time. Poor survival in underfed/poorly managed primiparous animals has long been a key target to improve overall farm management (Wathes et al., 2014), inasmuch as the fertility of primiparous animals determines the age structure of the herd (and, *ipso facto*, its culling rate) and through the greater ability of older cows to lactate.

Low oestrus detection rates and prolonged postpartum anoestrus had major impact on the fertility of postpartum cows in Sri Lanka. Circulating concentrations of progesterone served as an aid for monitoring the postpartum return to oestrous cycles, oestrus or anoestrus, and the pregnancy status of cows that have been inseminated. When progesterone assays are undertaken in parallel to data collection on BCS, parity, metabolic disorders, and uterine impairments, they provide useful information on the current reproductive status of the whole dairy production system.

Concentrations of IGF-1 during the postpartum transition period and start of the mating period are associated with the fertility of the dairy cows (Chapter 6). IGF-1 concentrations are low during the postpartum period whilst animals tend to lose BCS, which impairs ovarian function, indicating a longer period to the resumption of the ovarian activity. Perhaps the use of IGF-1 assays during early lactation could be used to provide information on the current reproductive status of dairy cows that are under metabolic stress in medium-scale production systems. More usefully, perhaps, measurements such as IGF-1 could be used to support research on the inter-relationships between nutritional adequacy and the recrudescence of reproductive activity in the postpartum period.

The studies conducted in New Zealand have provided clear evidence of an association between the length of grazing intervals and nutritional composition of fresh perennial ryegrass-white clover pastures (Chapter 7). Pasture herbage quality differed significantly between dairy farms where short and long RT were practised during winter and spring. The length of the grazing rotation is adjusted based on the pasture availability, seasonal variations in yield, and growth rates. The current studies have also clearly justified that cow DMI was influenced by the parameters of pasture herbage quality that were affected by RT, with consequential effects upon influences milk production and EB during early lactation. Energy balance is the most important factor for milk production, BCS and reproductive performance; however, other associated factors such as DMI, breed composition, parity and calving month must be considered to improve the fertility (Chapter 8).

Recommendations and future research - In order to improve current medium-scale dairy practices in Sri Lanka, dairy extension services must first be strengthened to educate farmers on proper husbandry practises, forage management, feeding, record keeping, and reproductive management, with a greater emphasis on oestrus detection. The level of farmer educational programs provided by the government need to be intensified, with all organisations including field veterinary services, livestock training services and agricultural services working in close collaboration to educate/advise farmers by regular interventions.

The ME and CP contents of the most abundant forage types (Guinea grass and CO-3 grass) were below the acceptable ranges (Feedipedia, 2009), most likely due to extended harvesting/cutting intervals. Therefore, adjusting of cutting intervals into ~4-6 week blocks may increase the digestibility of forage at a predetermined height, which includes more green leaves and less dead leaves and stem. For example, harvesting of forages before seed heads appear would improve digestibility. From an economic perspective, improving the nutritive values of forages would allow for adjustments to the cow rations with higher forage:concentrate ratios; and, this would also have the benefits of effective utilization of quality forages and subsequently increase milk production. Further, forecasting of forage supply depending on forage availability during each month of the year may help to feed cows effectively and can also satisfy their nutritional requirements. Additionally, nutritional models such as the Cornell Net Carbohydrate and Protein System (Lanzas et al., 2007) could be used in the future for better feed management and forecasting of milk production outcomes.

Dairy cows are vulnerable to metabolic impairments especially during the prepartum transition and early lactation periods. In this context, frequent estimation (at least monthly intervals) of BCS and BW are helpful to interpret cows' nutritional deficiencies and/or excesses and can be used as effective tools for monitoring nutritional status. Metabolic profile testing may also be a useful addition or educational tool to support such observations. Farmers should focus more on postpartum reproductive management which includes timely examination of the uterus and ovaries for postpartum pathology and activity. Abnormal conditions of the reproductive organs can extend the calving to insemination intervals and affect the conception rates of the dairy herd. Improving reporting/recording of onset of heat signs in the postpartum could facilitate the timing of breeding effectively. Progesterone and/or IGF-1 assays could be used to improve the detection of resumption of cyclicity of postpartum dairy cows, however, IGF-1 is more expensive and probably of less diagnostic value than the progesterone assay.

Seasonality, RT and the LS of pasture during grazing were three important factors affecting the changes of pasture herbage quality in New Zealand. Based on these considerations, the diet of dairy cows is adjusted according to their nutritional needs. This approach helps dairy farmers in New Zealand to overcome energy and protein deficiencies that directly affect milk production and reproductive performances in postpartum cows. These management aspects cannot be directly applied to the dairy industry in Sri Lanka since there is no seasonal pattern and grazing rotation management due to scarcity of land for pasture/fodder production. On the other hand, processes for optimising the stage of harvesting can be implemented to maximize quality of harvested fodder and thereby improve the quality of the cows' diet. Regular observations and analyses of feed ingredients may help to determine the herbage quality. In New Zealand, evaluation of reproductive data using milk production per lactation, BCS, and cow individual records (parity, breed, calving month) are essential to optimize reproductive performances of postpartum dairy cows during the breeding season and to thereby minimise culling rates. This is of direct relevance to tropical dairy systems, managing the production and reproduction records of as many dairy cows as possible.

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APPENDICES

APPENDIX 1. CHEMICAL COMPOSITION AND DIGESTIBILITY ANALYSES, AND PREPARATION OF MANUALLY MIXED RATION OF DAIRY COWS (CHAPTER 3)

Neutral detergent fibre determination

The NDF was measured by the filter bag technique described by Tillery and Terry (1963). The cleaned filter bags were air-dried and then weighed (W_1). Then triplicate samples of each feed type (0.45-0.5 g) were loaded into a filter bag and individual weights were recorded (W_2). Samples were uniformly spread inside the filter bags by shaking and flicking. Each filter bag was then placed in the bag suspender tray and then it was placed in the fibre analyser vessel. A neutral detergent solution was then added (1900-2000 ml) to the vessel. Feed samples were heated and agitated for 75 minutes. The drain valve of the vessel was opened to exhaust the hot solution and then closed. The lid of the vessel was opened, and then hot (70-90°C) water (1900-2000 ml) and alpha-amylase were added and rinsed twice. The solution was again heated and agitated for 5 minutes and then rinsed. The rinse procedure was repeated 3 times. After rinsing was finished, filter bags were removed from the vessel and excess water was gently pressed out. Filter bags were then placed in a 250 ml beaker filled with acetone for 3-5 minutes. Bags were removed from the breaker and air dried prior to oven drying at 102°C for complete drying (2-4 hours). Filter bags were then taken from the oven and cooled to ambient temperate and weighed (W_3). The following formula was used to calculate the NDF %:

$$\%\ \mathrm{NDF\ (DM\ basis)} = \frac{100 \times \left(\mathrm{W}_3 - (\mathrm{W}_1 \times \mathrm{C}_1)\right)}{\mathrm{W}_2\ \times \mathrm{DM}}$$

Where W1 = Filter bag tare weight

W2 = Sample weight

W3 = Dried weight of bag with fibre after extraction process

C1 = Blank bag correction final oven-dried weight/original blank bag weight

Acid detergent fibre determination

The acid detergent fibre (ADF) was measured by filter bag technique described by Tillery and Terry (1963). This procedure is similar to the NDF extraction method except that acid detergent solution (1900-2000 ml) was added to the fibre analyser vessel instead of neutral detergent solution for incubation. Feed samples were heated and agitated for 75 minutes. The rinsing procedure was undertaken without alpha-amylase. The following formula was used to calculate the ADF%:

$$\% \text{ ADF (DM basis)} = \frac{100 \times \left(W_3 - (W_1 \times C_1)\right)}{W_2 \times \text{DM}}$$

Where W1 = Filter bag tare weight

W2 = Sample weight

W3 = Dried weight of bag with fibre after extraction process

C1 = Blank bag correction final oven-dried weight/original blank bag weight

In-vitro true dry matter digestibility estimation

The true DM digestibility (TDMD) was measured using the method of Kirby et al. (1978).

Preparation of filter bags and samples

The filter bags (F57) were pre-rinsed in acetone for 3-5 minutes. The cleaned filter bags were air-dried and then weighed (W_1). Then triplicate samples of each feed type (0.25g) were loaded into the filter bag and weight was recorded (W2). Filter bags were then heat sealed and evenly distributed on both side of the digestion jar. A sealed blank bag was included in the study to determine the correction factor (C_1).

Preparation of (combined) buffer solution

There were two buffer solutions (A and B), and a neutral detergent solution was prepared as follows:

(a) Buffer solution A:	***g/litre***
$KH2PO_4$	10.0
$MgSO_4.7H_2O$	0.5
NaCl	0.5
$CaCl_2.2H_2O$	0.1
Urea (reagent grade)	0.5
(b) Buffer solution B:	***g/litre***
Na_2CO_3	15.0
$Na_2S.9H_2O$	1.0

(c) Neutral detergent solution

Both A and B buffer solutions were pre-warmed to 39^{o}C, and then mixed in a 1:5 ratio (A=266ml; B= 1330 ml). The exact amount of A to B was adjusted to obtain a final pH of 6.8 at 39^{o}C. The mixed solution was then added to the digestion jar. The digestion jars with samples and buffer solution were then placed in the *Daisy^{II}* incubator and the heat and agitation switches were turned on. The temperature of digestion jars was allowed to equilibrate for at least 20-30 minutes.

Preparation of rumen fluid (inoculum)

Rumen fluid (~2000ml) was collected from a rumen-fistulated buffalo cow and placed in a glassware thermos which was pre-heated to 39^{o}C. The fibrous mat from the rumen (2 fistfuls) was also collected in the thermos, in order to have an adequate microbial population to ensure digestion. The collected rumen fluid and fibrous mat were then blended in a CO_2 gas purged pre-heated blender at a high speed for 30 seconds. The blending action served to dislodge microbes that are attached to the fibrous mat and secured a representative microbial population for the in-vitro fermentation. Four hundred ml of the blended digesta was filtered through four layers of cheesecloth into a digestion jar which had been pre-heated with warm water to 39°C. The digestion jar was kept continuously purged with CO_2 during this process.

Incubation

The tightly closed digestion jars were then placed in the *Daisy*^{II} incubator and incubated for 48 hours at a temperature of 39.5°C ± 0.5°C. The jars were removed at the completion of incubation and drained of fluid. Filter bags were thoroughly rinsed with cold tap water until the water was clear. Finally, all rinsed bags were placed in the ANKOM^{200} fibre analyser to remove microbial debris and any remaining soluble fractions. The post in-vitro NDF weight was taken as W_3.

The following formula was used to calculate the TDMD:

$$\%\ \text{TDMD (DM basis)} = 100 - \frac{(W_3 - (W_1 \times C_1) \times 100}{(W_2 \times DM)}$$

Where W1 = Filter bag tare weight

W2 = Sample weight

W3 = Final bag weight after in vitro digestion

C1 = Blank bag correction final oven-dried weight/original blank bag weight

Preparation of manually mixed ration (MMR) of dairy cows

Harvested forages were chopped into 2-4 cm particles using a grass chopper machine, and then supplements and chopped grass were manually mixed according to the daily nutritional requirements of dry and lactating dairy cows (Supplementary figure 1).

Supplemental Figure 1. Preparation of manually mixed ration (MMR); A grass chopper machine; B weighing chopped forage; C mixing chopped forage and supplements; D feeding of manually mixed ration

APPENDIX 2. DETERMINATION OF SERUM METABOLIC PROFILE (CHAPTER 4)

The content of NEFA, BHBA, Urea and albumin were measured using a semi-automated biochemical analyzer (Chem 7, 340-670nm, Erba diagnostics Mannheim, Germany). The calibration serum level 3 (Catalog number CAL 2351, Randox laboratories Limited, United Kingdom) was used as a quality control.

Non-esterified fatty acids (NEFA)

The serum non-esterified fatty acid level was *in-vitro*, quantitatively measured by colorimetric method using NEFA test kit (Catalog No. FA 115, Randox Laboratory Limited, United Kingdom).

Procedure

The test conditions (Incubation time- 10 minutes, incubation temperature- 37^{0}C, wavelength 550 nm and aspiration volume 480 µL) were inserted and saved as NEFA test in semi-automated biochemical analyser. The saved NEFA test was selected to carry out the serum sample analysis. Double distilled water mixed with the reagent was used as the blank test. The quality control serum level 2 (CAL no. HN 1530) and serum level 3 (CAL no. HE 1532) were used as calibrators and the measured NEFA level of the calibration serum samples were compared with the reference value (1.02 mmol/ml) before carrying out the sample analysis.

The preparations of solutions and mixing of samples and the reagents were done according to the manufacturer’s instructions and reading was taken as the serum NEFA levels in mmol/L unit.

β-hydroxybutyrate (BHBA)

The serum BHBA level was *in-vitro*, quantitatively measured according to UV method using BHBA test kit (Catalog No. RB 1007, Randox Laboratory Limited, United Kingdom).

Procedure

The test conditions (Incubation time – 60 seconds, incubation temperature – 37^{0}C, delay 1 and 2 minutes, wavelength 340 nm and aspiration volume 480 µL) were inserted and saved as BHBA test in semi-automated biochemical analyser. The saved BHBA test was selected to carry out the serum sample analysis. Double distilled water mixed with the reagent was used as the blank test. The quality control serum level 2 (CAL no. HN 1530) and serum level 3 (CAL no. HE 1532) were used as calibrators and the measured value for the calibration serum sample was compared with the reference value of 1.05 mmol/L, before carrying out the sample analysis.

The preparations of reagents, mixing of samples and the reagents were done according to the manufacturer's instructions. The mixed sample and reagent were incubated for 60 seconds. Then the first reading was taken, read again after 1 and 2 minutes. The mean absorbance change per minute was determined and the recorded the BHBA value given in mmol/L units.

Albumin

The quantitative *in vitro* determination of albumin in serum by bromocresol green (BCG) method was carried out by albumen test kit (Catalog No. AB 362, Randox laboratories limited, United Kigndom).

Procedure

The test conditions (Incubation time – 10 minutes, incubation temperature – 37^0C, wavelength 630 nm and aspiration volume 480 µL) were inserted and was saved as albumin test in semi-automated biochemical analyser. Double distilled water mixed with the reagent was used as the blank test. The calibration serum level 3 (CAL no. 2351, Randox laboratories Limited, United Kingdom) was used as a calibrator and the reading of given for the calibration serum was compared with the reference value of 3.46 g/dL, before carrying out the sample analysis.

The samples and the reagents were mixed according to the manufacturer's -instructions. The relevant sample and the reagent were mixed accordingly, then, samples were incubated for 10 minutes, and reading was taken as the serum albumin levels in g/dL unit.

Blood Urea

The urea content of serum was carried out *in-vitro* by enzymatic kinetic method using urea test kit (Catalog no. UR 446, Randox laboratories limited, United Kingdom).

Procedure

The test conditions (wavelength – 340 nm, incubation time – 1 minute, delay 30 seconds, aspiration volume 480 µL and incubation temperature – 37^0C) were inserted to the bio analyzer. The reagent and the respective samples were mixed according to the manufacturer's instruction. After mixing, the initial absorbance was taken after 30 seconds, read again after one minute. The urea value of the sample was recorded in mg/dL units. The BUN value was calculated using the formula; BUN (mg/dl) = Urea (mg/dl) * 2.14

APPENDIX 3. IGF-1 ASSAY PROCESS AND VALIDATION (CHAPTER 6)

Insulin-like growth factor-1 concentrations in serum were measured in duplicate 20 µl aliquots by enzyme-linked immunosorbent assay, using the AL-121 TOTAL IGF-1 ELISA (ANSH Labs, Webster, USA). This is a highly specific, one-step sandwich-type immunoassay, whose primary use is for the measurement of total IGF-1 concentrations (i.e. bound and unbound) in human samples. The primary antibody is directed against human IGF-1 and has negligible cross-reactivity for IGF-II and IGF-binding proteins (IGFBP) 2, 3, 4 and 5, and has a marginal cross-reaction with human IGF-1/IFGBP complex. The antibody has a significant cross-reaction with rat IGF-1. The test kit standards consisted of 0, 0.9, 2.0, 6.2, 13.8, and 39.0 ng/mL IGF-1 synthetic calibrators. Low (3.2 ±0.2 ng/mL) and high (9.2 ±0.3 ng/mL) quality control samples were also provided.

Pre-treatment of serum samples

Serum samples stored at -20°C were thawed to room temperature (~25^{o}C). Culture tubes (12 ×75 mm) were pre-treated by adding 240 µl of IGF-1 Sample Buffer I. Aliquants (20 µl) of unknown serum samples were pipetted into the appropriated pre-labelled tubes, after which they were shaken (300-400 rpm) at room temperature for 30 minutes. IGF-1 Sample Buffer II (240) was then added into each tube, vortexed, and incubated at room temperature for 10 minutes. Following incubation, samples were vortexed thoroughly and refrigerated at 4^{o}C.

Assay Procedure

All specimens and reagents (ANSHLabs, Webster, USA) were thawed to room temperature, and all were mixed thoroughly by gentle inversion. In the assay, 50 µl of calibrators, controls, and pre-treated unknown serum samples were pipetted in duplicate into the appropriately labelled micro-titration wells coated with an IGF-1 antibody.

The plate was incubated with 100 µl of the horseradish peroxidase-labelled IGF-1 antibody-enzyme conjugate-RTU for 60 minutes on an orbital microplate shaker (Grant Bio PSM 1000i, UK) set at 600 rpm. The plate was manually washed five times with 350 µl of wash solution. The wells were thereafter treated with 100 µl of TMB chromogen substrate solution and incubated for 10 minutes on an orbital shaker set at 600 rpm. Stopping solution (100 µl) was finally added to each well to stop the reaction and the plate was read within 10 minutes, using a microplate reader with Gen5 microplate reader software (Biotek, Vermont, USA) set to 450nm with background wavelength correction at 620nm.

Validation for IGF-1 ELISA

The AL-121 IGF-1 ELISA for measuring the total IGF-1 concentrations was validated for precision through intra- and inter-assay coefficients of variation, dilution linearity, and the recovery of IGF-1 in spiked serum samples. In addition, parallelism was demonstrated for standards diluted in assay buffer and known concentration of serum samples. The serum samples were diluted in 0 ng/mL IGF-1 standard to test the dilution linearity for a calibrator (39 ng/mL), and two selected high and medium concentrated IGF-1 bovine serum samples collected from close-up dry and early lactating dairy cows, respectively. The dilution linearity of the assays for high concentration (Serum Sample A, a range of 1.9-174.9 ng/mL) and medium concentration (Serum Sample B, a range of 1.9-135.6 ng/mL) of bovine serum samples, was well within the accepted range of the mean measured/expected IGF-1 ratios of 100% and 113.86%, respectively. However, this was observed only when the serial dilution was at 1:16, 1:32, and 1:64, but not at 1:4, 1:8, and 1:128 (Supplemental Table 2). The dilution linearity of the assay for the highest concentration calibrator (39 ng/mL) was within the accepted range of mean measured/expected IGF-1 ratios of 109.6% when the sample diluted up to 1:32. Therefore, the dilution linearity and the parallelism studies of the Anshlabs TOTAL

IGF-1 ELISA test kit show that it can be used to measure concentrations of bovine IGF-1 at dilutions of 1:16 to 1:64.

Intra-assay coefficients of variation (CV) were calculated for each assay on the basis of the mean variance of the concentrations estimated in each sample duplicate. Inter-assay coefficients of variation were calculated from the means variance of high and low QC samples in each assay. Each serum sample was duplicated in each assay (Supplemental Table 1).

Supplemental Table 1. IGF-1 ELISA performance characteristics for the use in bovine serum

Characteristic	**Measurement**
Analytical sensitivity	0.025ng/mL
Least detectable dose (LDD)	1.49 ng/mL
Precision Intra-assay CV Inter-assay CV, over 5 assays	 0.9 - 2.1% (2.5-9.6 ng/mL) 9.4-16.2% (2.5-9.6 ng/mL
Linearity in IGF-1 calibrator (Dilution up to 1:32)	Mean 109.6% (Range 103.9-183.0%; 2.2-40.5 ng/mL)
Linearity in bovine serum with high starting concentrations (dilution between 1:16-1:128)	Mean 100% (Range 86.7-182.8%; 1.9-174.9 ng/mL)
Linearity in bovine serum with medium starting concentrations (dilution between 1:16-1:128)	Mean 113.9% (Range 92.9-182.811.9-135.6 ng/mL)

CV, coefficient of variation

Supplemental Table 2. Dilution linearity of bovine serum and a calibrator of the IGF-1 ELISA

Sample	Dilution factor	Expected IGF-1 concentration (ng/mL)	Measured IGF-1 concentration (ng/mL)	Measured/expected IGF-1 concentration
Calibrator F	Neat	39	40.5	103.9
(39ng/mL)	1:2	19.5	17.6	**90.3**
	1:4	9.8	8.2	**83.9**
	1:8	4.9	4.1	**83.6**
	1:16	2.4	2.8	**112.7**
	1:32	1.2	2.2	**183.0**
	1:64	0.6	2.1	341.8
Bovine serum	Neat	-	174.9	-
sample A	1:4	43.7	17.4	39.8
	1:8	21.9	38.0	174.0
	1:16	10.9	13.4	**122.8**
	1:32	5.5	4.7	**86.7**
	1:64	2.7	2.5	**90.5**
	1:128	1.4	1.9	139.1
Bovine serum	Neat	-	135.6	-
sample B	1:4	33.9	20.3	59.9
	1:8	16.9	40.7	240.1
	1:16	8.5	12	**141.6**
	1:32	4.2	3.9	**92.9**
	1:64	2.1	2.3	**107.1**
	1:128	1.1	1.9	182.8

With respect to the accuracy of the estimates of IGF-1 concentrations, bovine serum IGF-1 concentrations were assessed by estimating the recovery of IGF-1 from serum samples of known concentration (n=2) that were each spiked above the upper limits of quantification with a serial dilution of 39 ng/mL of human IGF-1 calibrator (Supplemental Table 3). The recovery of IGF-1 from the spiked sample of serum was calculated as follows (Obese et al., 2008);

$$\% \text{ recovery} = \frac{\text{Observed concentration of IGF-1 in a spiked sample}}{\text{Expected concentration of IGF-1 in that sample}} \times 100$$

The mean recoveries of IGF-1 from the spiked serum 1 and serum 2 samples were 121.4% and 131.2% respectively. These values are within acceptable ranges for apparent recoveries from spiked samples.

Supplemental Table 3. Percentage of recovery of IGF-1 concentrations in spiked samples of bovine serum

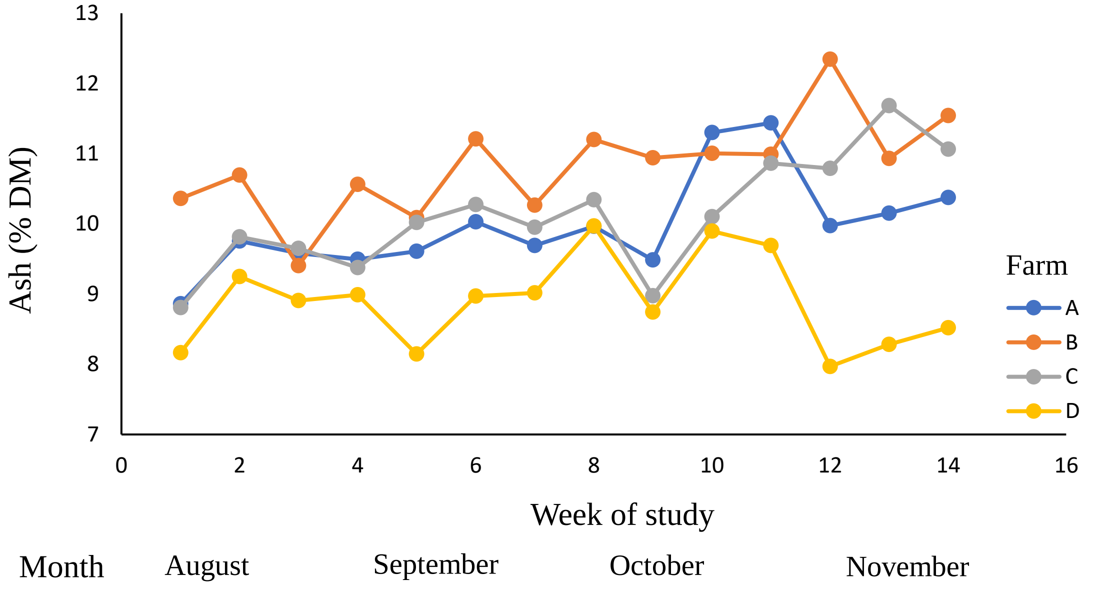
Serum sample	**Dilution factor of standard (39 ng/mL)**	**Observed[1] (ng/mL)**	**Expected[2] (ng/mL)**	**% Recovery[3]**
Sample 1 + 0 ng/mL	-	95.6	97.5	98.0
Sample 1 + 9.75 ng/mL	4	212.1	97.5	117.5
Sample 1 + 4.88 ng/mL	8	188.5	97.5	143.3
Sample 1 + 2.44 ng/mL	16	138	97.5	116.5
Sample 1 + 1.22 ng/mL	32	117.8	97.5	108.3
Sample 2 + 0 ng/mL	-	78.5	72.5	108.3
Sample 2 + 9.75 ng/mL	4	284.1	72.5	257.3
Sample 2 + 4.88 ng/mL	8	161.8	72.5	115.8
Sample 2 + 2.44 ng/mL	16	115.5	72.5	125.7
Sample 2 + 1.22 ng/mL	32	93.5	72.5	112.1

[1]Measured IGF-1 concentrations in serum after spiking with serial dilution of 39 ng/mL calibrator
[2]Expected IGf-1 concentration
[3]% Recovery, the ratio of observed and expected amount of IGF-1 × 100

APPENDIX 4. DESCRIPTION OF MODELS (CHAPTER 7)

Ash

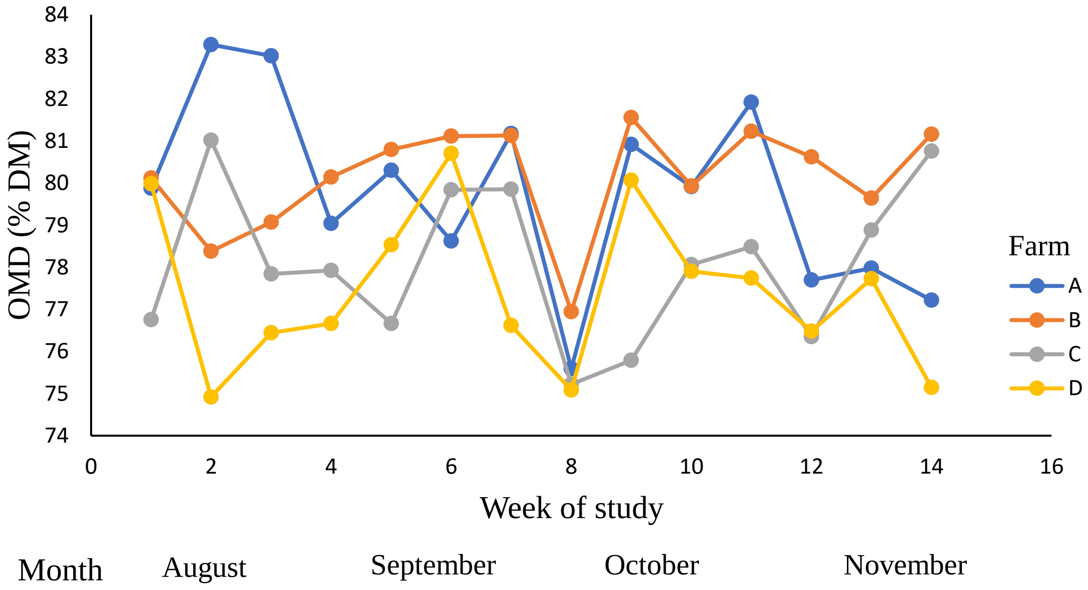
Ash contents (Table 7.7, Supplemental Figure 2) were affected by time period (week), farm and week × farm interaction (P<0.01). The ash was influenced by RT. Mean ash contents of RT1 (10.5% DM) was higher than RT2 (9.41% DM). The lowest Ash contents were reported in Farm D during all time periods.



Supplemental Figure 2. Variations in Ash content (% dry matter (DM)) across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

Organic matter digestibility

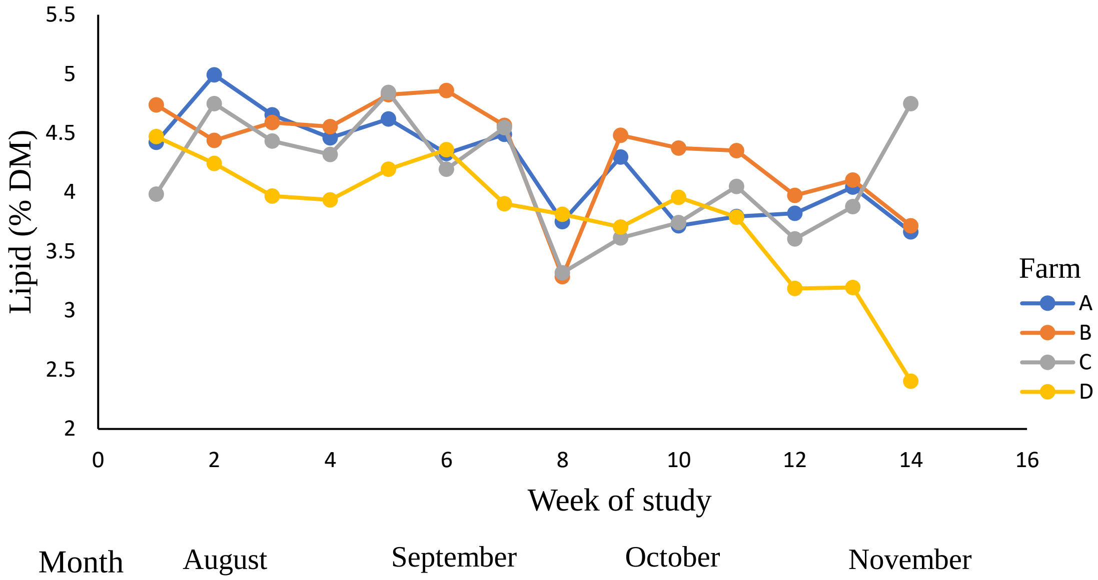
Organic matter digestibility (Table 7.7, Supplemental Figure 3) was affected by time period (week), farm and by week × farm interaction (P<0.01). The OMD was influenced by PGDM, RT, and LS. Mean OMD contents of PGDM1 (78.7% DM) was higher than PGDM2 (76.6% DM). Similarly, the mean OMD content of RT1 (79.1% DM) was higher than RT2 (76.3% DM), and also the mean OMD content of LS1 (78.8% DM) was higher than LS2 (76.6% DM).



Supplemental Figure 3. Variations in organic matter digestibility (OMD) content (% dry matter (DM)) across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

Lipid

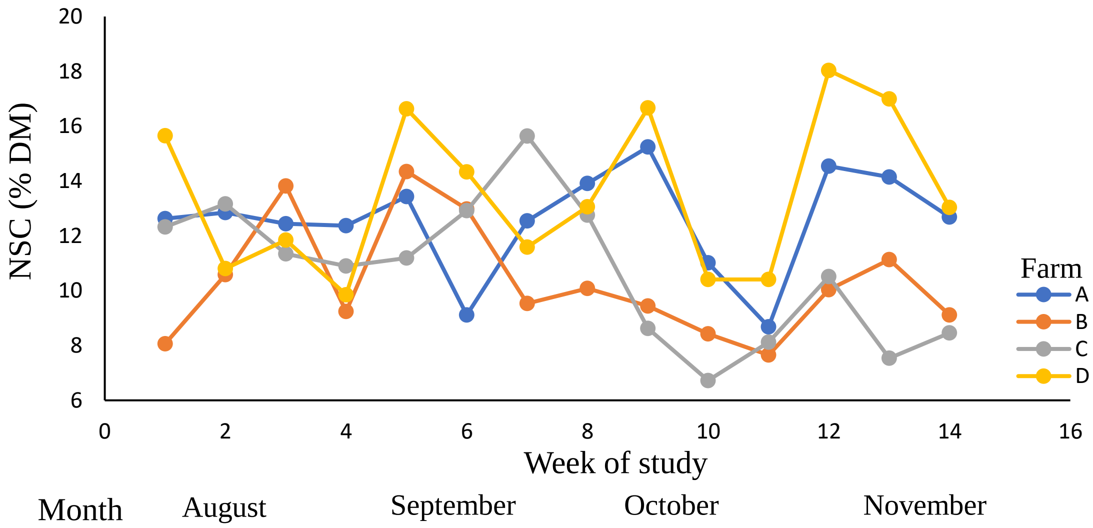
Lipid contents (Table 7.7, Supplemental Figure 4) were affected by time period (week), farm, and by week × farm interaction (P<0.05). The lipid was influenced by RT and LS. Mean lipid content of RT1 (4.03% DM) was higher than RT2 (3.57% DM), and the mean lipid content of LS1 (4.13% DM) was higher than LS2 (3.46% DM).



Supplemental Figure 4. Variations in lipid content (% dry matter (DM)) across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

Non-structural carbohydrates

Non-structural carbohydrates contents (Table 7.7, Supplemental Figure 5) were affected by time period (week), farm, and by week × farm interaction (P<0.01). The NSC was not influenced by PGDM, GH, RT, or LS.



Supplemental Figure 5. Variations in non-structural carbohydrates (NSC) content (% dry matter (DM)) across dairy farms (A-D) during winter and spring; 0-14 weeks (August to November 2019)

APPENDIX 5. COW FACTORS ASSOCIATED WITH REPRODUCTION (CHAPTER 8)

FSCO, PSMF, CCI, and SPC

Farm and calving month were significantly related to FSCO, PSMF, and CCI; however, reproductive indices were not influenced by breed (Supplemental Tables 4-7). Cows of Parity 1-2 and 3-4 had higher FSCO than those of Parity ≥5. None of other effects of parity were statistically significant. Cows at Farm A had higher PSMF than those at Farm D (OR: 0.23; 95% CI:0.1-1.07; P= 0.05). Cows at Farms B, and C had significantly lower CCI than those at Farm D. The FSCO, PSMF, and SPC outcome variables varied between farms, such that the highest FSCO, PSMF, and SPC were likely reported in Farm B compared to the referent Farm D. Cows that calved in July and August were had a lower CCI and higher PSMF than those that calved in September, respectively. Changes in BCS and EB nadir were not related any of the reproductive measure.

Supplemental Table 4. Logistic regression model (resulting odds ratio and 95% lower-upper bound CI) for factors associated with first service to successful service interval (FSCO) for four (A-D) pasture-based dairy farms

Variable	Level	N	Odds ratio	95% CI (Lower-upper)	P-value
Farm ID	A	194	1.53	(0.69-3.43)	NS
	B	439	2.81	(1.55-5.09)	0.001
	C	392	0.48	(0.31-0.72)	0.001
	D	450	Ref.		
Parity	1-2	464	2.4	(1.46-5.53)	0.002
	3-4	539	1.69	(1.13-2.52)	0.01
	≥5	467	Ref.		
Breed	HF<65%	354	1.5	(0.93-2.44)	NS
	HF >65-<85%	435	1.42	(0.91-2.24)	NS
	HF>85%	651	Ref.		
Calving month	July	628	1.89	(0.98-3.62)	0.05
	August	601	1.33	(0.7-2.54)	NS
	September	246	Ref.		
ΔBCS	-1 - -0.5	462	0.85	(0.58-1.24)	NS
	-0.5 - 0	546	Ref.		
EB nadir	< -30	519	0.78	(0.53-1.14)	NS
	-30 -0	502	Ref.		

ΔBCS change in body condition score between calving and nadir; EB nadir lowest energy balance recorded; HF Holstein-Friesian; N number of cows; Ref. referent; NS (not significant) = *P*- value > 0.05

Supplemental Table 5. Logistic regression model (resulting odds ratio and 95% lower-upper bound CI) for factors associated with planned start of breeding to first service (PSMF) for four (A-D) pasture-based dairy farms

Variable	Level	N	Odds ratio	95% CI (Lower-upper)	P-value
Farm ID	A	189	0.69	(0.35-1.35)	NS
	B	422	2.32	(1.45-3.71)	0.001
	C	362	1.88	(1.23-2.87)	0.004
	D	456	Ref.		
Parity	1-2	453	0.96	(0.57-1.61)	NS
	3-4	522	1.26	(0.85-1.85)	NS
	≥5	454	Ref.		
Breed	HF<65%	340	0.98	(0.63-1.54)	NS
	HF >65-<85%	422	0.9	(0.6-1.36)	NS
	HF>85%	632	Ref.		
Calving month	July	616	3.25	(1.72-6.15)	0.003
	August	583	3.37	(1.79-6.35)	0.002
	September	230	Ref.		
ΔBCS	-1 - -0.5	455	1.04	(0.74-1.48)	NS
	-0.5 - 0	530	Ref.		
EB nadir	< -30	494	0.98	(0.69-1.39)	NS
	-30 -0	488	Ref.		

ΔBCS change in body condition score between calving and nadir; EB nadir lowest energy balance recorded; HF Holstein-Friesian; N number of cows; Ref. referent; NS (not significant) = *P*- value > 0.05

Supplemental Table 6. Logistic regression model (resulting odds ratio and 95% lower-upper bound CI) for factors associated with calving to conception (CCI) for four (A-D) pasture-based dairy farms

Variable	Level	N	Odds ratio	95% CI (Lower-upper)	P-value
Farm ID	A	154	0.32	(0.13-0.78)	0.01
	B	351	2.53	(1.48-4.33)	0.001
	C	281	3.48	(2-6.04)	0.0001
	D	391	Ref.		
Parity	1-2	381	0.98	(0.52-1.86)	NS
	3-4	449	1.39	(0.86-2.23)	NS
	≥5	344	Ref.		
Breed	HF<65%	280	1.34	(0.78-2.33)	NS
	HF >65-<85%	353	1.49	(0.91-2.43)	NS
	HF>85%	515	Ref.		
Calving month	July	553	0.28	(0.1-1.24)	0.001
	August	481	0.3	(0.1-1.63)	0.001
	September	143	Ref.		
ΔBCS	-1 - -0.5	372	1.22	(0.81-1.84)	NS
	-0.5 - 0	471	Ref.		
EB nadir	< -30	427	1.19	(0.79-1.82)	NS
	-30 -0	436	Ref.		

ΔBCS change in body condition score between calving and nadir; EB nadir lowest energy balance recorded; HF Holstein-Friesian; N number of cows; Ref. referent; NS (not significant) = *P*- value > 0.05

Supplemental Table 7. Logistic regression model (resulting odds ratio and 95% lower-upper bound CI) for factors associated with service per conception (SPC) for four (A-D) pasture-based dairy farms

Variable	**Level**	**N**	**Odds ratio**	**95% CI (Lower-upper)**	**P-value**
Farm ID	A	154	0.72	(0.35-1.48)	NS
	B	351	2.21	(1.29-3.79)	0.004
	C	281	0.81	(0.51-1.29)	NS
	D	388	Ref.		
Parity	1-2	381	1.05	(0.58-1.89)	NS
	3-4	449	1.1	(0.72-1.71)	NS
	≥5	344	Ref.		
Breed	HF<65%	280	1.37	(0.83-2.27)	NS
	HF >65-<85%	353	1.38	(0.88-2.19)	NS
	HF>85%	512	Ref.		
Calving month	July	551	1.16	(0.54-2.48)	NS
	August	480	0.81	(0.38-1.7)	NS
	September	143	Ref.		
ΔBCS	-1 - -0.5	371	0.69	(0.47-1.01)	NS
	-0.5 - 0	469	Ref.		
EB nadir	< -30	425	0.73	(0.49-1.08)	NS
	-30 -0	436	Ref.		

ΔBCS change in body condition score between calving and nadir; EB nadir lowest energy balance recorded; HF Holstein-Friesian; N number of cows; Ref. referent; NS (not significant) = *P*- value > 0.05

APPENDIX 6. DRC 16 FORMS

DRC 16

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Animals

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Research of Veterinary Scienc

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Kumara, S. N., Parkinson, T. J., Laven, R., & Donaghy, D. J. (2022). The Influence of Rotational Length, along with Pre-and Post-Grazing Measures on Nutritional Composition of Pasture during Winter and Spring on New Zealand Dairy Farms, Animals, 12(15), 1934.

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