Investigations into the use of in-paddock technologies to study growth, supplement intake and metabolic responses of grazing beef cattle

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Acknowledgments

The present thesis probably looks like a manuscript with different tables, graphs and statistics; however, it is not. Conversely, I would say that it is a tremendous amount of effort, time, education, friendship and love from a group of people that decided to invest in this project.

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Declaration of Authorship

This thesis has been written in publication style. Chapters 3 to 7 are therefore stand-alone manuscripts, each with its own abstract, introduction, materials and methods, results, discussion, conclusion, acknowledgments and references. Chapters 4 and 5 have been published in peer reviewed journals and extracts of published versions are included in this thesis. Chapter 3 has been submitted to a peer review journal. Chapters 3, 6 and 7 are presented following general thesis guidelines. José A. Imaz is the first author on all chapters and the entire thesis. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and resources have been acknowledged, either in the author list at the beginning of each chapter/publication or in the acknowledgments section. The work presented in this thesis is, to the best of my knowledge and belief, original, except as mentioned in the text. I declare that I have not submitted this material, either in full or in part, for a degree at this or any other university or institution of tertiary education.
Research Work and Authorship

This thesis includes 2 original manuscripts published in a peer reviewed journal (Chapters 4 and 5) and 1 original manuscript that have been submitted to peer a review journal for consideration (Chapter 3). Those manuscripts accepted by peer review journals were formatted and presented as per journal guidelines. The research aims, organisation, analysis and writing of all chapters/publications in this thesis were the responsibility of the candidate, José Augusto Imaz, working under the supervision of Professor Luciano Adrián González and Professor Sergio García at The University of Sydney.
Publications

Peer-reviewed Journal Publications


Peer-reviewed Conference Proceeding Publications


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Abbreviations
The following abbreviated terms have been used throughout the thesis:

ADF: Acid Detergent Fibre
BHB: 3-hydroxybutyrate (Beta-hydroxybutyrate)
BS: Blood samples
BS1: Blood sampling 1
BS2: Blood sampling 2
BS3: Blood sampling 3
BS4: Blood sampling 4
BS5: Blood sampling 5
C: Concentrate
CP: Crude Protein
CV: Coefficient of variation
d: Day (s)
DM: Dry Matter
DMI: Dry matter intake
DOMD: Digestibility of organic dry matter
EF: Electronic feeder(s)
EID: Electronic identification
GNSS: Global Navigation Satellite System
GPS: Global Positioning System
h: Hour (s)
hh:mm: Hours: Minutes
ILW: length of the Interval between liveweight measures
kg: Kilograms
LDL: Low-density lipid
LH: Lucerne hay
LW: Liveweight
LWC: Liveweight change
ME: Metabolizable energy
Min: Minimum
Max: Maximum
MLB: Molasses-lick block (s)
m: Meters
m²: Metres squared
min: Minute (s)
n: Number of observations
NMR: Nuclear magnetic resonance
NSW: New South Wales
NDF: Neutral Detergent Fibre
OH: Oaten hay
OC: Oats crops
P: P-value
PCA: Principal component analysis
P+C: Winter pastures and concentrate
RFI: Residual feed intake
RFID: Radio frequency identification
SD: Standard deviation
SE: Standard error
SEM: Standard error of the mean
SG: Sudangrass
TAC: Tricarboxylic acid cycle
VLDL: Very low-density lipid
WOW: Walk-over-weighing stations
Summary

In the typical pasture- and rangeland-based beef production systems of Australia, management to enhance efficiency of gain is limited due to the lack of real time data on the liveweight (LW) evolution of the animals. Walk-over-weighing scales (WOW) and electronic feeders (EF) are in-paddock technologies to, respectively, measure LW and supplement intake of grazing beef cattle remotely, automatically and in near-real time. Metabolomics can also be combined with these data streams to understand the physiological and metabolic basis of differences in LW and supplement intake through the quantifications of the abundance of small metabolites in animal fluids. Thus, the integration of in-paddock monitoring, and metabolomics would provide new insights to study the variability in growth rate and supplement intake across seasons, types of feed and individual animals. The general objectives of this thesis were: a) to utilise in-paddock technologies to monitor, describe and understand the variability in LW, growth rate and intake of supplements of grazing beef cattle, and b) to combine in-paddock measurements with blood metabolomics to explore metabolic pathways driving such variability across the growth trajectory.

The specific objective of Chapter 3 was to determine the impact of the length of the interval between LW measures (ILW) on LW and daily liveweight change (LWC) calculations in different cattle categories as the later could largely differ in LW. Liveweight data were obtained using WOWs during 112, 224 and 1460 days (4 years) in calves, weaners and mature cows, respectively. These datasets were then subsampled to simulate different ILW with one LW record every: a) 1, 2, 4, 8 and 16 weeks for calves; b) 1, 2, 4, 8, 16 and 32 weeks for weaners; and c) 1, 2, 4, 8, 16, 26, 32, 52 (1 year) and 208 weeks (4 years) for cows. Summary statistics for LW and LWC were calculated for each animal and ILW to study potential loss of information [minimum, (Min); maximum, (Max); mean; standard deviation, (SD) and coefficient of variation, (CV)]. Minimum and Max LWC, and SD and CV of LW were affected by ILW in all animal categories (P < 0.05). Compared with daily data, the minimum frequency required to capture peaks and troughs (Min and Max) in LWC was 2 weeks for calves and weaners, and 8 weeks for cows. Additionally, ILW of 4 (calves and weaners) and 8 (cows) weeks was needed to measure similar temporal variability in LW and LWC compared to daily ILW through their SD and CV. This study contributed with practical knowledge on minimum frequencies of measurement needed to avoid losing key information for the different categories of animals.

Chapter 4 assessed the ability of WOW and EF to study the dynamic temporal relationships between LW, LWC, the intake of molasses-lick blocks (MLB), and feeding behaviour with forage type, quantity and quality. Fifty-two crossbred weaners (mean ± SD; initial LW = 178 ± 31.2 kg/hd) were rotationally grazed or fed for 254 days the following forages: sudangrass (SG), autumn pastures (Pastures), winter pastures with concentrate (P+C), oat crops (OC), lucerne hay (LH), and oaten hay (OH). Forage quantity and quality were measured on the day of entry and exit of each paddock grazed and on days of hay delivery, which consisted of days with high and low feed availability and quality, respectively. Results indicated that MLB intake was 111% higher (P < 0.05) at low compared to high quantity and quality of forages, which was reflected in
greater feeding frequency, duration and rate. Using WOW enabled the determination that MLB supplementation improved LWC during periods within SG, Pastures, or OH forages only (P < 0.05). It was concluded that WOW and EF could reflect the nutritional status of grazing cattle and capture the dynamic changes in forage type, quantity and quality. This allows understanding and quantifying the consequences of the complex interaction between forage type, quantity and quality remotely and automatically in grazing cattle.

The objective of Chapter 5 was to use WOW and EF to remotely measure and study the variability in MLB intake, LW and feeding behaviour between individual growing beef cattle. Supplement intake, LW and LWC of 27 MLB-supplemented animals (mean ± SD; initial LW = 190 ± 34.1 kg) were analysed. The dataset was from the trial presented in Chapter 4 and consisted of data collected throughout 220 days while animals were fed on Pastures, OC, P+C, LH and OH. A large variability between individuals was found in MLB intake ranging from 0 to 194 g/hd per day (P < 0.01). The intake of MLB was positively correlated with LWC (P < 0.05, $R^2 = 0.19$) and feeding frequency and duration over the entire study (P < 0.01; $R^2 > 0.86$) although these correlations were affected by the type of feed. Feeding behaviour of cattle was useful to predict MLB intake without the need of measuring MLB disappearance. The ability to monitor LW and feeding behaviour or feed intake of individual animals in a group could allow automatic individualised feeding of grazing cattle.

Chapters 3, 4 and 5 demonstrated the ability of in-paddock technologies to monitor animals daily and individually, quantify changes between and within animals, and the importance of data collected with high frequency. However, understanding the biological nature of such changes can further advance the future applicability of these in-paddock technologies, as the latter could enable to associate desirable performance traits of cattle with the concentration of metabolites in their blood stream. Thus, the information collected was then used in Chapters 6 and 7 to investigate associations between growth rate and MLB intake with the metabolome of these growing beef cattle. Blood samples were obtained from each animal at five time points during the long-term field study described above: Day 66 (BS1, Pastures), Day 116 (BS2, oat crops), Day 156 (BS3, lucerne hay), Day 185 (BS4, lucerne hay) and Day 219 (BS5, oaten hay). The relative abundance (RA) of blood metabolites were determined using proton nuclear magnetic resonance (NMR). Results from Chapter 6 indicated that LWC across sampling time points ranged from 0.127 to 1.287 kg/hd per day and MLB intake from 4.87 to 159.50 g/hd per day. A total of 27 metabolites were identified with the RA of 63 and 33% of them associated with LWC and MLB intake, respectively (P < 0.05). Five amino acids (valine, leucine, isoleucine, phenylalanine and tyrosine) were positively correlated with LWC (P < 0.001). Additionally, acetate, and 2- and 3-hydroxybutyrate were positively (P < 0.05), and creatinine was negatively (P < 0.001) correlated with LWC. The intake of MLB was negatively correlated with dimethyl sulfone and acetyl groups (P < 0.01) and positively with acetate (P < 0.01). The RA of metabolites involved in protein and energy metabolism was associated with LWC and supplement intake. Blood metabolomics offer potential to understand body growth metabolism of cattle varying their growth paths over time.
Chapter 6 studied the temporal changes and relationships between LWC, MLB intake and blood metabolites at the group level. In contrast, the objective of Chapter 7 was to study the relationship between LWC, MLB intake and blood metabolites within each time point across individual animals. Over the entire trial, LWC of individual animals ranged from -0.55 to 1.80 kg/hd per day and the individual intake of MLB from 0 to 780 g/hd per day. The greatest number of metabolites associated with LWC (P < 0.05) was found at BS2 (55% of metabolites identified) when the animals were probably in compensatory growth. On BS2 (OC), 3 lipid groups were positively associated with LWC (P < 0.01) whereas negative correlations were observed for 2- and 3-hydroxybutyrate, pyruvate, acetyl groups, citrate, valine, leucine and isoleucine (P < 0.01). Creatinine and creatine were negatively associated with LWC across all sampling points (P < 0.01) but BS3 (P > 0.05). Only 22% of the metabolites were correlated with MLB intake (P < 0.05) being negative with creatine (BS1 and BS2) and dimethyl sulfone (BS5). Together, Chapters 6 and 7 showed that blood metabolomics, in combination with the use in-paddock technologies, can be used to explore the metabolic profile of individual animals that perform better under the same conditions, opening up new areas for future research (e.g. genetics; nutrition; management).

Results from the present thesis suggest that in-paddock technologies have the ability to capture near-real time measurements of cattle production that are key to enhance management in livestock systems; and to investigate associations between desirable traits and the metabolome of grazing beef cattle. These technologies measure performance of individual animals with high frequency and remotely instead of averaging performance from ‘distant periods’ before the blood assessments. The present thesis adds value and integrates data collected by in-paddock technologies with metabolomics. The implementation of these novel technologies and approaches can be used to enhance animal productivity and welfare and may prove useful to automate activities of beef cattle managed extensively.
Chapter 1

Introduction

The Australian beef industry is largely based on pastures and rangelands production covering more than 79% of the total area of agricultural land (Greenwood et al., 2018). Each region of production greatly differs in climate, forage resources, breeds of cattle and cattle management. Southern beef farming has a predominantly temperate climate with winter dominating rainfall and *Bos taurus* herds predominantly of British (Angus, Hereford) and Continental breeds (Charolais, Limousin) fed on native and introduced temperate pastures (Millar and Badgery, 2009). Conversely, northern beef production is dominated by subtropical to tropical climates with marked wet and dry seasons in the rangelands dominated by pure *Bos indicus* (e.g. Brahman) or composites (e.g. Santa Gertrudis and Droughtmaster). Managing beef cattle grazing systems has been traditionally challenging due to the large variability in space and time as a result of countless biological processes and environmental conditions (O’Reagain et al., 2009, González et al., 2014). Understanding of and dealing with such variability is crucial to enhance management of beef grazing systems and optimise productivity and sustainability (O’Reagain et al., 2009).

Factors influencing beef cattle growth have been extensively studied with the aim of increasing productivity. Nevertheless, the intrinsic variability of beef grazing systems limits the understanding and management of animal growth (Coates and Penning, 2000, Henry et al., 2012). Such challenges have been mostly related to the limitation of quantifying the effects of different cattle management practices over time and for individual animals (González et al., 2018). For instance, cattle liveweight (LW) is seldom measured and, as a result, variations in growth rate or liveweight change (LWC) go undetected. Nutritional management of grazing cattle is frequently orientated to increase growth rate through, for example, the use of energy and protein supplementary feed (Poppi and McLennan, 1995, Caton and Dhuyvetter, 1997) and grazing management (Scanlan et al., 1994, Burns and Sollenberger, 2002). However, mustering and weighing cattle to measure growth rate is logistically challenging in commercial farms, requiring labour and negatively affecting animal welfare and productivity (Petherick et al., 2009). In addition, limited research exists on individual intake of the introduced supplements in grazing cattle because of the difficulty to measure intake in grazing animals supplemented as a group.

Nowadays, the use of advanced sensor and communication technologies offers potential to improve on-farm data collection (Handcock et al., 2009; Gonzalez et al., 2018). In-paddock automatic weighing, also known as walk-over-weighing stations (WOW), is one of these technologies which could provide LW of grazing cattle automatically and remotely in near-real time (Charmley et al., 2006, González et al., 2018). Additionally, remotely collected LW data could be integrated with other in-paddock technologies such as electronic feeders (EF) to
measure the intake of introduced supplements and feeding behaviour of individual animals (Reuter et al., 2017, Williams et al., 2018).

Pioneer studies on the use of in-paddock technologies such as WOW (Filby et al., 1979, Anderson and Weeks, 1989) and EF (Coppock, 1977; Collis, 1980; Broster et al., 1982) focused on their development and field implementation for the dairy industry (van Straten et al., 2009; Alawneh et al., 2011). However, only two studies continuously measured LW to predict calving date in breeding cows (Aldridge et al., 2017; Menzies et al., 2018) and only one study explored factors influencing the growth of pastured beef cattle (González et al., 2014). Similarly, EF have been used in beef cattle to deliver different feed types ranging from large amounts (i.e. kg/hd; Montanholi et al., 2013; Karisa et al., 2014; Hegarty et al. 2014; Williams et al. 2018; Ralston et al. 2005) to small (g/hd; Cockwill et al., 2000; Reuter et al., 2016; Reuter et al., 2017b; McCarthy et al., 2018; Pickett and Gunter, 2019). Interestingly, EF showed potential to study the consumption of ’self-fed’ supplements offered as free choice to cattle (Cockwill et al., 2000). This latter study used loose supplement with high salt content; however, molasses-lick blocks (MLB) are the most common self-fed supplements in Australia often containing protein, oil, minerals, feed additives and medications with the hardness being used to control the amount of MLB that animals can consume (Zhu et al., 1991). The integration of MLB intake data from EF with LW data offers new possibilities for advanced management of cattle that have not been explored yet.

Liveweight, LWC and supplement intake are critical measures to monitor productivity, welfare and sustainability of beef cattle production. The concentration of various chemical compounds is often measured to study animal physiology and metabolism to understand the biological mechanisms and responses of animals to nutritional and environmental factors. However, obtaining reliable measurements of the physiological and metabolic status of animals at different stages of growth under grazing conditions has been limited in the past by the lack of parallel data on LWC, which in turn can only be achieved in practice through adequate automatic in-paddock technologies. In-paddock weighing, and EF can be potentially combined with other disciplines such as metabolomics to understand the metabolic basis of the changes observed in LWC and supplement intake. Metabolomics can assess the presence and concentration of molecules with low molecular weight (i.e. metabolites) in body fluids including blood, urine and saliva. Thus, the combination of WOW, EF and metabolomics (in the future potentially derived from automated sampling of fluids) could be a novel approach to insightfully and remotely monitor and study grazing cattle linking metabolic responses to growth rate and supplement intake. This approach could improve animal management and understanding of metabolic pathways linked to changes in growth and supplement intake. Previous findings suggested that metabolomics can be used to describe metabolic profiles (i.e. presence and abundance of metabolites); however, this information needs to be associated with desirable traits to allow predictions from animals’ metabolome (Fontanesi, 2016; Goldansaz et al., 2017; O’Callaghan et al., 2018). For example, the metabolome of beef cattle was associated with feed efficiency and LWC in housed feedlot cattle (Karisa et al., 2014), carcass quality and animal welfare of steers...
(Carrillo et al., 2016; Connolly et al., 2019) and feed efficiency and developmental stage in grass-fed heifers (Consolo et al., 2018). However, to the author’s best knowledge, there are no studies exploring the association between the blood metabolome of individual grazing cattle with varying growth trajectories due to changes in the quantity and quality of feed available.

Based on current state of knowledge, in-paddock technologies such as WOW and EF, and metabolomics could provide critical information to manage and understand changes in growth rate and the effects of supplementary feed of beef cattle. However, there are limited published studies to evaluate the potential that these data sources could provide in combination.

The above-mentioned statements and gaps in knowledge heavily rely on and support the use of on-farm data collected with high frequency using new technology. However, the value and need of data collected with high frequency and the ability of WOW and EF technology to reflect animal responses to nutritional status have not been clearly demonstrated. Frequent LW data from WOW can also be used to simulate the length of interval between LW measurements to study the impacts on LW and LWC calculations. Such an effect could be different amongst animal categories that greatly differ in LW trajectories throughout the production cycle. For example, calves at foot are expected to grow steadily until weaning because they rely on the cows’ milk for growth and cows are expected to use nutrients and energy reserves to maintain milk production. Additionally, no studies have attempted to use both WOW and EF to monitor growth trajectories of animals for long periods of time (e.g. across seasons) while animals are fed different types of feed that affect LWC and supplement intake. The use of these technologies may also be beneficial to identify individuals with greater LWC and responses to introduced supplements at a particular time point and over the entire growing season. Additionally, EF enables measuring intake of supplements and feeding behaviour of individual cattle. Finally, near-real time monitoring of LW and supplement intake offer opportunities to assess the association between LWC, supplement intake and the metabolome of animals as LWC varies over time. This approach could also help to elucidate metabolic changes associated with high performing individuals or during periods of low or compensatory growth.

1.1 General objectives of the thesis
The general aims of the present thesis were to:

a) Utilise data streams obtained from in-paddock WOW and EF technologies to monitor, describe and understand the variability in LW, LWC, the intake of MLB and feeding behaviour of grazing beef cattle and;

b) Combine data from these technologies with the blood metabolome of animals to determine the association with LWC and supplement intake.

1.2 General hypothesis of the thesis
The general hypothesis of the present thesis was that in-paddock technologies have the ability to capture data reflecting the nutritional status and response to management of grazing beef cattle, and the combination of these data with metabolomics can help explain the metabolic
changes associated with growth rate and supplement intake. This approach could help develop better strategies to manage grazing beef cattle to improve productivity and efficiency of production through a reduction of the variability in growth rate over time and between animals. Furthermore, the generated knowledge could contribute to establishing the foundations for automation of nutritional management in extensive grazing conditions.

1.3 Objectives of each chapter

In line with these general aims and hypothesis, the specific experimental objectives addressed in each chapter of this thesis were:

1. To quantify the effects of the length of the interval between LW measures on LW and LWC calculations in three beef cattle categories (i.e. calves, weaners and breeding cows, Chapter 3).

2. To assess the dynamic relationship between LW, LWC, the intake of MLB, feeding behaviour of cattle, and forage type, quantity and quality (Chapter 4).

3. To assess the relationship between MLB intake, LW and feeding behaviour across individual growing beef cattle (Chapter 5).

4. To investigate the association between the relative abundance (RA) of blood metabolites, LWC and MLB intake of beef cattle grazing different types of feed over time affecting their growth trajectory (Chapter 6).

5. To investigate the association between the relative abundance (RA) of blood metabolites and LWC and MLB intake across individual animals within a point in time of their growth trajectory (Chapter 7).

1.4 Thesis outline

This thesis is composed of a review of the published literature on the topic of automatic measurement of LW and supplement intake, and the use of metabolomics to study metabolic profiles mostly of grazing beef cattle (Chapter 2), five experimental chapters (Chapter 3 to 7) and a general discussion and conclusion (Chapter 8). Each chapter is presented as a stand-alone scientific manuscript with abstract, introduction, materials and methods, results, discussion and conclusion.

The objective of the literature review (Chapter 2) was to determine the state of knowledge of the topics of the present thesis and the approach proposed regarding the use of in-paddock technologies and metabolomics to study grazing beef cattle. Gaps in knowledge are identified and recommendations for further research made throughout with some of these undertaken in the present thesis.

Chapter 3 explores the impacts of different interval length between LW measures on LW and LWC calculations of three cattle categories. This chapter highlights the critical information required to capture the greatest variability, and peaks and troughs in LW and LWC using automatic in-paddock weighing.
Based on results from Chapter 3, daily LW and LWC of MLB-supplemented and not supplemented animals was measured, capturing the highest variability in LW and MLB intake of group-fed cattle. Continuous monitoring of such variables was useful to determine the impacts of MLB supplementation on LWC and to investigate associations between MLB intake, feeding behaviour and available forage quantity and quality (Chapter 4).

Chapter 4 demonstrates the complex temporal changes of LWC, MLB intake, forage quantity and quality, and highlight the application of the findings to manage groups of grazing cattle.

Chapter 5 explores the relationship between LWC and supplement intake and feeding behaviour across individual animals consuming different forages.

Chapters 4 and 5 suggested that in-paddock technologies offer opportunities to remotely associate LW and supplement intake in near real-time so the response of animals can be assessed. Chapter 6 goes a step further integrating remotely collected data with blood metabolomics to explore the temporal association between LWC, MLB intake and metabolism.

Finally, Chapter 7 investigated the metabolic profile that explained differences in LWC and MLB intake between individual animals at a given point in time.

Therefore, Chapters 3 to 7 explored the variability in LWC, MLB intake and blood metabolites over time and between individual animals and assessed the correlation between them. Additionally, the potential of this approach is discussed with the application for the automation of activities in grazing beef cattle which could see commercial applications in the near future. Chapter 8 contains the general discussion and conclusions which bring together concepts described along with the new knowledge generated in the present thesis.

1.5 References


Consolo NRB, Munro JC, Bourgon SL, Karrow NA, Fredeen AH, Martell JE and Montanholi YR 2018. Associations of blood analysis with feed efficiency and developmental stage in grass-fed beef heifers. Animals 8, 133.


Chapter 2

Literature review

2.1 Overview

In-paddock technologies offer potential to measure, remotely and automatically, parameters closely associated with productivity and profitability of grazing cattle such as LW and supplement intake. Surprisingly, these technologies were developed decades ago but their application to study factors driving growth and metabolism of grazing beef cattle have been shallowly explored so far. The present chapter examines the literature to determine the latest research and development regarding the use of in-paddock automatic weighing, EF and options of integrating the information generated by such technologies with metabolomics in grazing beef cattle. Gaps in knowledge and potential use of these data streams to increase productivity and efficiency are identified and suggestions for future research are made.

2.2 Introduction

Constraints associated with measuring key parameters in cattle, such as LW and feed intake, have traditionally limited farm management because of the difficulties of performing such measurements with enough frequency on individual animals grazing as a group (González et al., 2014). The latter is one of the reasons limiting the monitoring of the temporal and inter-individual variability of productivity in grazing beef cattle, which then limits the development and adoption of more precise management options with potential to improve productivity, profitability, sustainability and animal welfare (O'Reagain et al., 2009; González et al., 2018). This can be particularly evident for extensive grazing beef cattle production where properties and herds largely differ in size, incidence of environmental factors and cattle management and nutrition (O'Reagain et al., 2009; Ash et al., 2015; Greenwood et al., 2018). Remarkably, beef farming covers more than 79% of the total agricultural land area in Australia (ABARES, 2019) pointing out the relevance of this industry. Nowadays, the development of in-paddock technologies offers the possibility to monitor cattle remotely, automatically and in near-time without the need of animal handling (González et al., 2018). In particular, the use of WOW and EF showed potential to collect frequent LW and supplement intake data in grazing cattle (González et al., 2018; Williams et al., 2018) but surprisingly, there is limited research that investigated their ability to capture critical information that can be used to manipulate growth trajectories of grazing cattle. In addition, other disciplines are required to understand metabolic mechanisms influencing animal growth. In this regard, advanced techniques able to analyse body fluids for metabolic profiling could be very useful for these purposes, a term referred to as livestock metabolomics (Goldansaz et al., 2017). Metabolomics is the measurement of a broad range of metabolites in body fluids with potential to be used for biomarker discovery of desirable traits such as performance and welfare, amongst others. Therefore, the purpose of this review is, firstly, to
briefly stress the importance of the beef cattle industry for the Australian economy. Secondly, to examine the current knowledge on the use of in-paddock technologies, particularly WOW and EF, to capture data to monitor individual cattle frequently and automatically. Third, to review the use of metabolomics to investigate metabolic changes associated with growth rate and supplementary feed intake by beef cattle. Additionally, the potential for the integration of in-paddock technologies and high throughput metabolic profiling was discussed.

2.3 Importance of the livestock industries in Australia

The Australian beef industry has an important role within the Australian economy. The gross value generated annually, which includes beef and cattle production and live cattle exports was $A 12.7 billion in 2016-2017 (MLA, 2018) representing approximately 20% of the total value of farm production in Australia (Greenwood et al., 2018). The national number of beef cattle has been around 25 million head in the past decade (Australian Farm Institute, 2015; Greenwood et al., 2018) and recently included 11.5 million beef cows and heifers (MLA, 2018) and 2.8 million head of dairy cattle. The Australian beef industry is largely pasture and rangeland-based (Greenwood et al., 2018) with feedlots comprising between 2-4% of the total Australian cattle population at any one time (ALFA, 2019). Depending on the year, Australia is amongst the world’s five top largest beef exporters with the predominant markets being Japan, USA, Korea and China whereas Indonesia, Israel and Malaysia are major markets for Australian live cattle exports (MLA, 2019).

Australian beef production consists of cow-calf systems (i.e. breeding herds) primarily pasture- and rangeland-based and finishing cattle either on pasture or in feedlots. The average herd size is 431 animals for southern Australia beef properties (MLA, 2015) and contributes to the domestic and export market in approximately equal volumes (Greenwood et al., 2018). Native and introduced temperate pastures are the most utilised forages to feed British breeds (Millar and Badgery, 2009) mainly Angus and Hereford (Bos Taurus) although Continental breeds like Charolais and Limousin are also common. Breeding or joining periods in cows are usually well defined, ranging between 6 and 12 weeks, to calve in autumn or spring depending on forage availability and rainfall.

Northern beef production systems are mostly rangeland-based with a marked difference between wet and dry seasons and rainfall events concentrating in summer. Climate conditions range from subtropical to tropical including in the state of Queensland, the Northern territory and in the northern parts of Western Australia. This region exports live animals and most of its slaughtered beef production. Farms in the Northern region have much larger herd sizes than the Southern region with an average herd size of 1580 animals (MLA, 2015). However, soil and climate result in generally a lower stocking rate compared to southern Australia (O’Reagain et al., 2009; Greenwood et al., 2018). The extensiveness of some properties led to improved farm handling facilities and, for example, the use of helicopters to muster animals. Brahman (Bos indicus) and Santa Gertrudis and Droughtmaster (Bos indicus x Bos Taurus) are the most predominant breeds in northern Australia due to tick resistance and heat tolerance.
Queensland is the largest beef producing state, accounting for almost half of the national stock (10.5 million; ABS, 2016) followed by New South Wales with approximately 5 million. It is also important to mention the relevance of feedlots in the recent years. Their location reflects similar trends of the total cattle stock with around 60% of feedlots in Queensland and 30% in New South Wales (ALFA, 2019). In 2016-2017, a total of 2.7 million grain fed cattle were slaughtered representing approximately 35% of adult cattle slaughtered in Australia (MLA, 2018). The feedlot industry is focussed on increasing beef quality for the domestic market and marbling for higher value export markets in North Asia (Greenwood et al., 2018).

2.4 The intrinsic variability of grazing beef systems

Efforts have been made to study and to enhance the productivity of beef grazing systems without losing focus on ecological and economical sustainability. Multidisciplinary research was conducted to investigate key factors influencing growth rate (Coates and Penning, 2000; Henry et al., 2012). Nevertheless, the large variability existing in climate, soils, vegetation and management across Australian regions challenges the comparison and understanding of growth in grazing beef cattle. This variability is, in part, due to total annual rainfall and its distribution across seasons, and temperature which then affects animals and vegetation type, quantity and quality of the feed available to them. It has been demonstrated that managing the high inter-annual variability in rainfall is one of the major challenges for long-term cattle production because this has marked effects on carrying capacity (Scanlan et al., 1994; O’Reagain et al., 2009). Furthermore, nutritional deficiencies due to extended low rainfall not only reduce cattle growth and reproduction but also increases mortality rates (Burns et al., 2010). To overcome such deficiencies producers feed cattle with different types of supplementary fodder including grain, hay, silage, grazing annual crops or a combination of these. However, feed supplementation in the rangelands and northern Australia is limited to loose and hard lick blocks mainly. In this context, animals constantly modify growth rate as a consequence of both the existing availability of natural resources (e.g. forage growth) and producers’ supplementary feed offered (e.g. hay supplementation).

Nutritional management in grazing cattle is frequently orientated to increase growth rate using energy and protein supplementation (Poppi and McLennan, 1995; Caton and Dhuyvetter, 1997; Teague et al., 2004) and grazing management (Scanlan et al., 1994; Burns and Sollenberger, 2002). However, quantifying the effect of these practices on commercial farms could lack accuracy because LWC of cattle is seldom measured (Azzaro et al., 2011; Hao Guo et al., 2019). Similarly, measures of individual feed intake of the introduced supplementary feed in grazing cattle are rarely performed in grazing animals (Bowman et al., 1995; Arthur et al., 2004). Novel technologies could be utilised to monitor grazing cattle providing frequent data related to animal LW, feed intake, herd and feeding behaviour. For instance, the use of frequent LW data would allow detection of negative changes in animal LWC and the risk of mortality at early stages and maximise animal growth using wise managerial practices. Finally, new research could be
performed challenging the existing knowledge by investigating novel topics using a technology-based approach.

Collecting data to monitor animal-dominated landscapes poses several challenges mostly explained by the large and frequent variability at both spatial and temporal scales (González et al., 2014). For instance, far from being constant, cattle change growth rate through the production period depending on the quantity and quality of forage which could largely change through different paddocks and parts of extensive properties (i.e. spatial scale). Fortunately, advanced sensor and communication technologies (Handcock et al., 2009) offer potential to address the challenge of data collection and to increase the understanding of beef cattle grazing systems. In particular, remote in-paddock weighing also known as walk-over-weighing stations (WOW), is one of these technologies which could provide weight of livestock at fine temporal and spatial scales in near real-time (Charmley et al., 2006; González et al., 2018). Additionally, data obtained from WOW could be integrated with other in-paddock technologies such as EF to measure supplement intake and feeding behaviour of individual animals (Reuter et al., 2017; Williams et al., 2018) and methodologies reflecting changes in the metabolic profile of animals, such as metabolomics (Fontanesi, 2016; Goldansaz et al., 2017).

Pioneers on the use of in-paddock technologies, particularly WOW and EF, were mostly focused on their development and errors as a result of field implementation (Coppock, 1977; Filby et al., 1979; Collis, 1980; Broster et al., 1982; Anderson and Weeks, 1989). Surprisingly, few studies have assessed these technologies’ ability to monitor grazing beef cattle (Cockwill et al., 2000; González et al., 2014; Aldridge et al., 2017; Reuter et al., 2017; Williams et al., 2018) but none has combined these data streams to explain temporal and individual variability in growth, supplement intake and possible metabolic responses correlated with performance. Throughout this literature review, an overview of technologies and analytical procedures with potential to frequently monitor animals and vegetation is provided with a focus on WOW, EF and metabolomics in grazing beef cattle.

2.5 Technologies and methodologies to monitor cattle performance

Technologies to monitor animals can directly and indirectly measure body dimension, behaviour, feed intake, reproduction and mortality. Some of the most commonly evaluated parameters include LW, body condition, and fat and muscle content (Coates and Penning, 2000). For instance, 2D imagery was utilised to measure these body traits in cattle and sheep (Khojastehkey et al., 2016; Ozkaya et al., 2016) and video recording from infrared cameras were useful to study the effects of stressful conditions on cattle health (Alsaaod et al., 2015). Additionally, Global Navigation Satellite Systems (GNSS) particularly Global Positioning System (GPS) have been embedded in cattle collars to describe spatial and temporal distribution of beef grazing cattle (González et al., 2014). Accelerometers embedded in collars or ear tags are also used to measure animal behaviour for research and commercial applications (Wolfger et al., 2015; Williams et al., 2018). Similarly, WOW are integrated scales providing cattle LW data remotely. Studies were reported in beef (Charmley et al., 2006) dairy cattle (Alawneh et al.,
and sheep (Brown et al., 2015) whereas only a few studies have used this technology to describe cattle growth in grazing beef cattle for long periods of time (González et al., 2014; Aldridge et al., 2017). In contrast, different types of EF have been more widely adopted by the dairy and pig industries (Azizi et al., 2009; Huzzey et al., 2014; Johnston et al., 2016; Thompson et al., 2017). However, the development and application of those EF to measure supplementary feed intake in beef grazing animals was relatively recent (Reuter et al., 2017; Williams et al., 2018). Particularly, EF with potential application in grazing beef systems can record feed disappearance and feeding behaviour (number and duration of feeding events) for individual animals tagged with electronic identification (EID). Conversely, similar technologies are widely used in the feedlot beef industry (Sowell et al., 1998; McAllister et al., 2000; Quimby et al., 2001). Finally, metabolomics is a discipline that can be defined as the comprehensive measurement of many metabolites with low molecular weight (<1500 Da) in fluids, tissue and tissue extract (Dona et al., 2014; Clish, 2015). These can be used to investigate the presence and abundance of metabolites that are directly related to the phenotype of organisms, which is the result of the interaction between the genotype and the environment (Goldansaz et al., 2017). Metabolomics is part of a group of ‘omics’ which also includes genomics, transcriptomics and proteomics depending on the target to identify (Psychogios et al., 2011). This is a brief overview of some of the most utilised technologies and methodologies to measure variables that could reflect animal performance and welfare. The following sections describe the use of WOW, EF and metabolomics in more detail.

2.5.1 Walk-over-weighing technologies

Automatic weighing was developed in the 1960s to obtain LW data remotely (Martin et al., 1967) and was later improved by combining LW with EID (Anderson and Weeks, 1989). Automatic weighing was first applied in 1979 in the dairy industry, when a commercially available weight crate was modified by Filby et al. (1979) to continuously weigh cattle. Originally, the primary issues with the system included multiple animals attempting to walk over the scale simultaneously and animals going over the scale too fast, resulting in inaccurate and unusable data (Filby et al., 1979). However, this pioneer study demonstrated the potential of WOW when used in conjunction with an individual animal identification to produce an average trend for each animal over time. Later, Peiper et al. (1993) successfully utilised a WOW system in a commercial dairy herd. Automatic weighing works by collecting LW data as animals walk (or traverse) a strategically placed weighing system. To be useful for measurements on individual animals, the WOW system must contain an EID reader, a weighing platform, a data processing and recording system and eventually a data transmission system. The most common system of identification in livestock is radio frequency identification (RFID). This system utilises low-frequency radio signals to transfer information between a transponder, containing the unique identification code, and an antenna that collects the signal and transfers it to a decoder (McAllister et al., 2000).

The resultant LW records obtained using WOW systems are then analysed by applying different algorithms (Alawneh et al., 2011), as well as the animal identification, date and time.
For example, a continuous averaging algorithm refers to an automatic process of calculation, usually performed with the aid of computers, to analyse data points by creating a series of averages. The animal weight and identification data can be sent wirelessly (e.g. central computer, mobile phone) or be stored in an attached computer enabling remote access. Although automatic weighing was developed in the dairy industry decades ago (Filby et al., 1979) its utility was demonstrated recently in beef, dairy and sheep (van Straten et al., 2009; González et al., 2014; Zachut and Moallem, 2017) whereas no studies have been reported in feedlot beef cattle. However, the application of WOW to study the complexity of factors associated with performance in grazing beef cattle is still at early stages. Firstly, studies should demonstrate potential advantages of recording continuous LW data for cattle monitoring (e.g. daily) compared to longer intervals of data collection traditionally obtained by bringing animals to central handling facilities. Secondly, the ability of WOW to quantify changes in LWC over long periods of time (e.g. across seasons) as animals graze different types of forages has not been demonstrated. Finally, LW and LWC data collected with these systems should be correlated with the physiology and metabolism of beef cattle such as the concentration of metabolites in blood.

The number of LW records obtained by the WOW could be affected by different factors including system setup, type of production (e.g. dairy, beef cattle), animal category (e.g. mature or young cattle) and nutritional management (e.g. grazing, feedlot cattle). However, literature is not abundant considering its development decades ago. A review of 7 studies (breeding cows, beef steers, sheep and dairy cattle) reveals that the number of records vary from 0.15 to 1.7 per animal and day (Peiper et al., 1993; Charmley et al., 2006; van Straten et al., 2008 and 2009; Alawneh et al., 2011; González et al., 2014; Aldridge et al., 2017; Menzies et al., 2018). Previous studies also reported that the precision of WOW could be increased with a greater number of LW records over the collection period (Peiper et al., 1993; Charmley et al., 2006) although this assumption was not tested statistically. The place of installation of the WOW has differed across studies to suit the production system. For example, the WOW has been installed at the entry of the milking facilities in dairy farms (Zachut and Moallem, 2017) or at the entry of a yard or placed in front of the trough in beef cattle (Charmley et al., 2006; González et al., 2014).

Another challenge to address with WOW is dealing with possible errors of the data recorded. The most common errors were associated to the miss-assignment of a LW record to an EID record, and the recording of biologically impossible weights or outliers (both high and low). These errors may be due to differences in animal size (e.g. cow length), walking-through velocity and crowding (i.e. two animals too close to each other) as described by Peiper et al. (1993). Furthermore, recording very small weights (usually less than 10 kg) were cited as a result of hardware and software problems (Aldridge et al., 2017) which could potentially limit their use on small animals (e.g. lambs). The authors attributed this to the configuration of the system, which tries to tare back to a zero weight while an animal is still on the scale.

The literature agrees that LW data obtained from WOW need to be analysed to identify and remove outliers before calculating LW at a given point or LWC. Firstly, records with missing EID are unusable because of the impossibility to match LW to individual animals (Alawneh et al.,
Depending on the potential application of LW data (e.g. studies assessing animal behaviour) or industry application (e.g. monitoring cattle numbers), this criterion could also be applied to delete records with missing date and time. Secondly, after removing records with missing information, outliers for LW should also be removed. Alawneh et al. (2011) classified outliers into two categories: (1) those that were biologically implausible for all animals in the herd, and (2) those biologically implausible for a given animal. The later study considered biologically implausible LW values to be those varying by more than 4 standard deviations from the LW mean of each animal. Onyiro et al. (2008) used the same approach (i.e. distance in SD of individual LW means) to identify and remove outliers in dairy cows. Aldridge et al. (2017) aimed to predict calving date using WOW in a beef herd and deleted records that were ± 60 kg from the LW mean of each individual animal. This value was selected based on a review of the estimated maximum weight loss due to the calf birth weight, placental tissue, and anatomic and allantoic fluids.

A different method based on smoothing data obtained using WOW was also implemented to understand fluctuations in cattle LW. However, a limited number of studies addressed this topic. Two studies in dairy cows used penalised cubic spline regression methods in grazing (Alawneh et al., 2011) and confined conditions (van Straten et al., 2008). In both cases, authors concluded that smoothing was successful to calculate LW changes and its associations with body condition score (BCS) for periods no longer than 150 days. Nevertheless, only a few studies have embraced outlier detection and data analysis using an integrated approach. Gonzalez et al. (2014) have analysed extreme weights for deleting biologically implausible outliers. Then, data were fitted to penalised B-spline (smoothing method) for each individual animal (Eilers and Marx, 1996). The final step included deleting LW outliers below or above 1.5 times of residuals obtained from the smoothed mean of each animal. After deleting these outliers, the penalised B-spline was fitted again to obtain the predicted LW for each individual animal. Previous studies described in this section clearly indicated that this technology has potential to detect changes in LW across different production systems (e.g. beef, dairy), animal categories (e.g. mature cows, weaners) and length of data collection periods. However, future studies would need to add value to this information linking LW changes to changes in feed quantity and quality. In addition, there is a need to build solid correlations between LW, LWC, feed intake, feeding behaviour and metabolic responses of animals.

Despite the advantages and potential applications of in-paddock weighing reported previously by different authors, the number of studies utilising WOW to increase the understanding of grazing beef systems is limited. In this regard, Aldridge et al. (2017) and Menzies et al. (2018) investigated the prediction of calving date in extensively managed breeding herds using WOW. In these studies, predictions of calving date ranged from 59 (n=232) to 63% (n=40) using plotted data of cows’ growth paths from late gestation to post-calving time. The later studies and other experiences in dairy cattle (van Straten et al., 2008 and 2009) suggested that monitoring the growth trajectories of animals could be crucial to study factors influencing LW changes in breeding cows around calving time but also during and across entire seasons (González et al., 2011; González et al., 2014; Brown et al., 2015).
van Straten (2009) used WOW to study associations between LW, body condition and reproductive performance in 2020 Holstein cows. The authors were able to accurately determine the percentage of post-calving body weight loss of individual animals and its relationship with their future reproductive performance in dairy cows. These previous studies suggest that a similar approach could be used in beef breeding herds to study the effects of severe LW loss on reproduction, compensatory growth and weaning rates. However, no similar studies were reported in beef cows. Conversely, González et al. (2014) reported the only study in the last 20 years showing the benefits of LW monitoring using WOW for grazing management in growing beef cattle. This latter study used 60 grazing Brahman and Belmont Red composite steers monitored for 341 days in Northern Queensland. The use of WOW enabled the researchers to associate changes in LW with environmental factors (e.g. rainfall), grazing management (e.g. paddock rotation) and changes in forage quality and quantity across seasons (e.g. wet to dry seasons). Nevertheless, no studies reported on the use of WOW to monitor the effects of supplementation and frequent changes in feed types. Equally unexplored is the maximum number of animals that could be monitored per weighing station which would be relevant to determine the suitability of WOW systems in commercial farms of different sizes.

2.5.2 Electronic feeders

Frequent measurements of individual intake and feeding behaviour of cattle are key to understand and manage beef grazing systems (Imaz et al., 2019, 2020; Chapters 4 and 5). The use of EF could offer an alternative to obtain such measurements with introduced supplementary feed. The development and use of EF for cattle were originally driven by the dairy industry in the 1980s (Coppock, 1977; Collis, 1980; Broster et al., 1982) and these efforts persisted until nowadays (Azizi et al., 2009; Huzzey et al., 2014; Johnston et al., 2016; Thompson et al., 2017). However, EF were more recently adopted by the beef industry (Cockwill et al., 2000; Ralston et al., 2005; Reuter et al., 2016; McCarthy et al., 2018; Williams et al., 2018; Wyffels et al., 2018). The use of EF aims to deliver different types of feed to cattle and, at the same time, records critical information regarding feed intake and feeding behaviour of animals. The later includes feeding frequency (number of visits to the EF) and feeding duration (time spent per visit). Electronic feeders recently adopted to monitor the supplement intake of grazing beef cattle are, in general, composed by the following parts: a) an electronic RFID reader to recognise individual animals visiting the feeder; b) a container to place the feed offered (varying their size and shape, frequently made of steel); c) cell bars linked to a data acquisition card or system to continuously measure and capture weight changes due to feed disappearance; and e) a powering system. It is important to note that EF could include a system to control access to the bin (e.g. pneumatic gate) or to deliver the targeted amount of feed. However, describing the ability of EF to tailor supplementation to individual cattle is out of the scope of the present literature review. Rather, the present literature review covers previous studies using EF to monitor supplement intake and feeding behaviour of animals, particularly in grazing beef cattle.
Electronic feeders have been used to deliver a wide range of feed types in beef cattle ranging from concentrates or total mixed rations (kg/hd) to minerals supplies (g/hd) that are consumed in different amounts. The EF were successfully used to measure feed intake (average 9.90 kg/hd per day, 140 days of feeding) in housed feedlot steers fed with a high moisture corn-based diet to study associations between residual feed intake and the abundance of blood metabolites (Montanholi et al., 2013; Karisa et al., 2014). Similarly, Hegarty et al. (2014) used EF to select feedlot cattle with lower residual feed intake (RFI), and correlate it with methane production, during a 70-day trial (grain-based diet of 75% barley, 12 animals per pen and an average intake of 11.31 kg/hd per day). In beef cattle grazing a dormant tallgrass prairie, Williams et al. (2018) used EF to assess the variability in responses to feed supplementation (80% soybean meal and 20% soybean hulls). Animals (n=32) were subjected to two methods of supplement delivery (hand-fed vs electronic feeder) for 52 days. In the later study, data obtained from EF was crucial to assess the individual and temporal variability of supplement intake, which ranged from 0 to 1.21 kg/hd per day and explained the lower LWC of animals consuming supplements as ‘free choice’ compared to conventional ‘hand fed’ cattle. Additionally, Ralston et al. (2005) used EF to study the impacts of creep feed intake by 51 grazing suckling beef calves. Diet was mostly composed of ground barley and the intake of calves ranged between 0.067 to 3.42 kg/hd per day while the attendance to the creep feeder ranged from 2 to 43% of the total number of animals over time. Authors concluded that feed intake of calves was too variable to be considered an effective method of delivering medications.

Electronic feeders have also been utilised to monitor the intake of supplements such as loose minerals and MLB that are consumed in small quantities in relation to total daily intake (Cockwill et al., 2000; Reuter et al., 2016; Reuter et al., 2017; McCarthy et al., 2018; Pickett and Gunter, 2019). Cockwill et al. (2000) performed a comprehensive study measuring mineral intake and feeder attendance by pastured (lucerne and brome grass) cow-calf pairs provided with free-choice access to a loose mineral formulation containing different types and levels of salt. Results revealed that mineral intake varied from 15.7 (calves) to 241.6 g/hd/day (cows) with the maximum attendance of 1.05 visit/day/hd registered by cows. However, mineral intake was only measured for a period of a week and longer-term studies are needed because forage type and quality, and environmental factors could have a large effect on consumption. In another experiment, Cockwill et al. (2000), measured feeder attendance and the intake of MLB by cows for 14 days, and heifers managed in a dry lot for 13 and 30 days. Molasses-lick-block intake varied across trials, animal categories and individuals ranging from 0 to 1650 g/hd per day with a maximum attendance of 90 and 84% of all animals in the herd for heifers and cows, respectively. In general, the above-cited studies indicated that EF could be used to measure feed intake in a range of situations involving large (kg) to small (g) quantities of feed consumption and frequency of feeder usage (i.e. cattle attendance). However, further research may need to explore the use of EF for longer periods of time in grazing cattle exposed to changes in their basal diet. Additionally, no studies were found integrating supplement intake and LW measures.
at the same temporal scale (e.g. daily) and correlating such measures between individuals fed as a group.

Studies suggest that EF could offer the potential to monitor supplement intake of individual grazing cattle for long periods of time, which could make it possible to draw on the relationships with desirable traits in cattle. These data streams seem to be more commonly used in the dairy industry in comparison with the beef industry. For instance, data obtained from EF were used to determine competitive interactions of dairy cows at the feed bunk suggesting that competitive behaviour could be automatically quantified from EF (Huzzey et al., 2014). Similarly, Johnston et al. (2016) reported that behavioural data from EF could help detect the incidence of bovine respiratory disease (BRD) in dairy calves with a 60% precision. Azizi et al. (2009) analysed the relationship between feeding behaviour, feed intake, milk yield, and day from parturition obtaining significant correlations between these factors and meal duration, daily meal time and meal size. Similarly, Thomson et al. (2017) used an interesting approach of data mining of on-farm electronic equipment to identify the minimum time that cows spent away from the pen to be milked. Surprisingly, limited number of studies reported the use of data from electronic feeding systems in grazing beef cattle to predict or correlate it with their performance. Particularly for grazing beef cattle, these correlations would be advantageous to associate changes in forage availability with variations in LWC and supplement intake.

2.5.3 Metabolomics to study cattle metabolism and physiology

The use of metabolomics as a phenotyping tool experienced a fast growth in the last decade, particularly linked to advances in human medicine and computational capabilities. This approach has been also used in agriculture as a tool to assist crop breeding and productivity (Simo et al., 2014; Mahdavi et al., 2015; Sumner et al., 2015; Mahdavi et al., 2016). In a recent review, Goldansaz et al. (2017) provided a clear picture of the stage of utilisation of metabolomics in livestock, showing that the highest number of studies have been conducted in cattle (50% of the articles selected) and focussed on animal health, nutrition and production. Studies involving pigs and sheep accounted for 28% and 12% of the selected articles, respectively.

Analytical procedures and technologies used in metabolomics include, but are not limited to, liquid chromatography coupled with single-stage mass spectrometry or tandem mass spectrometry, gas chromatography coupled to mass spectrometry, high or ultrahigh performance liquid chromatography coupled to ultraviolet or fluorescent detection and nuclear magnetic resonance (NMR) spectroscopy (Emwas et al., 2019). However, for the purposes of the present literature review, most of the descriptions will focus on NMR because it is a fast and simple technique requiring little pre-processing of blood samples which makes it suitable for their applications in livestock research. Advantages of using NMR, in comparison with other methodologies, were comprehensively described by Emwas et al. (2019) and Dona et al. (2014). Nuclear magnetic resonance (NMR) spectroscopy is non-destructive which requires little or no chromatographic separation and sample treatment. Recent efforts on developing NMR instrumentation associated to new technologies made NMR highly automatable and
reproducible enabling large-scale metabolomic studies. Additionally, NMR is particularly suitable for detecting and characterizing compounds with low molecular weight such as sugars, organic acids, alcohols, polyols and other highly polar compounds. Conversely, NMR proved to be less sensible to detect the presence and concentration of metabolites compared to other methodologies (Emwas et al., 2019). Nuclear magnetic resonance is based on the physical phenomenon of resonance transition which occurs in the atomic nuclei as a result of being exposed to an external magnetic field applying an electromagnetic radiation with specific frequency. The presence and abundance of metabolites can be detected according to the position, intensity and fine structure of resonance peaks as a resultant of the absorption of the electromagnetic radiation (Weljie et al., 2006; Psychogios et al., 2011; Dona et al., 2014; Emwas et al., 2019).

Particularly for cattle, metabolomics has been used to analyse blood, saliva and ruminal content (Goldansaz et al., 2017; O'Callaghan et al., 2018) but also meat and milk quality (Karisa et al., 2013; Melzer et al., 2013). Fontanesi (2016) and Goldansaz et al. (2017) comprehensively reviewed studies involving metabolomics in livestock. These authors suggested that metabolic profiles can insightfully describe the presence, number and abundance of metabolites. However, this information needs to be associated with desirable traits to allow their predictions from animals’ metabolome. The metabolome of cattle was associated with feed efficiency, residual feed intake and LWC in housed beef feedlot cattle (Karisa et al., 2014; Connolly et al., 2019), carcass quality and animal welfare in beef steers (Carrillo et al., 2016; Connolly et al., 2019), changes in the rumen and milk metabolome of dairy cattle (Melzer et al., 2013; O'Callaghan et al., 2018), metabolic variations between late pregnancy to early lactation in dairy cattle (Ceciliani et al., 2018), predictive biomarkers of disease state in transition dairy cows (Klein et al., 2012; Hailemariam et al., 2014) and feed efficiency and developmental stage in grass-fed beef heifers (Consolo et al., 2018). Remarkably, the existing number of studies in cattle associating the metabolome of animals with production traits and welfare practices would not reflect the relevance of this industry (Goldansaz et al., 2017) and studies on dairy cattle seem to be more abundant than beef cattle.

No studies were found investigating associations between LWC and the metabolome of beef cattle grazing different forages across seasons, which affects nutritional status and growth rate. However, studies on feedlot cattle emphasise the potential of metabolomics to investigate associations between the presence and abundance of metabolites and performance traits in cattle. Karisa et al. (2014) reported that several metabolites in the blood of beef steers (428 kg/hd of average LW) fed ‘in-doors’ with a total mixed ration were associated with RFI (i.e. glycine, creatine, hippurate, glutamate, hydroxyisobutyrate, formate) and LWC (i.e. glutamate, isoleucine) at three sample points throughout 10 weeks (i.e. week 2, 6 and 10). However, associations with LWC, which were calculated by averaging the days in between sampling periods, were only evident on week 6. Similarly, Connolly et al. (2019) reported associations between the metabolome, growth rate and carcass quality in beef feedlot cattle fed a high-energy diet (i.e. marbling score, rib fat, rump fat, carcass weight). In this later study, blood samples were
taken at early stages of the production cycle (ranging from 54 to 152 days of entering the feedlot) and the metabolome was correlated with carcass measurements at slaughter time after 393 to 435 days on feed. Metabolites positively associated with marbling score included propionate, acetate, histidine, creatine, isoleucine and 3-hydroxybutyrate whereas only creatine and 3-hydroxybutyrate were negatively correlated with LWC, which was calculated as an average over the entire feedlot period. Therefore, correlations between the blood metabolome and LWC measure at the exact same time may give meaningful results. Nevertheless, these were important findings because they suggest that the blood metabolome is associated with carcass traits measured 10 months after the measurements were made, which could be useful for early decision making and selection of animals. No similar studies were published in grazing beef cattle exploring the association between the metabolome and LWC over long-grazing periods (e.g. more than 6 months) while feeding a sequence of different feed types. Also, the combination of metabolomics with continuous LW measurements could be interesting to correlate the concentration of metabolites with the ‘current’ LWC of individual animals instead of averaging LWC calculated in between distant weighing points.

2.6 Potential applications of automatic weighing, electronic feeders and metabolomics to understand growth trajectories and the intake of supplements

Previous sections discussed the use of in-paddock technologies to automatically and continuously collect LW and supplement intake data on individual animals and its combination with metabolomics. In addition, it was stated that such data streams offer a large number of opportunities to study a range of topics linked with cattle productivity, philology and welfare. However, it was also stressed that the utilisation of generated data still remains in the early stages of development. Using in-paddock technologies in combination with metabolomics to understand key factors influencing temporal and individual variability of growth rate and supplement intake in grazing beef cattle can potentially contribute to such development. This is the focus of the following sections.

2.6.1 Improving frequency of liveweight data collection

Liveweight is a widely accepted proxy of the physiological and nutritional state of cattle and could indicate changes in growth rate, health, genetics, and reproductive performance in cattle (NASEM, 2016). Thus, weighing livestock regularly could be advantageous to monitor productivity, health and welfare. However, the lack of practical ways to weigh animals means that most producers rarely, if ever, weigh their cattle (Filby et al., 1979). Measuring LW in extensive farming conditions (e.g. grazing cattle in large properties) involves mustering the herd to central handling facilities sometimes for several km to individually weigh animals (i.e. traditional weighing). Subsequently, weighing events may occur as rarely as once or twice a year and are restricted to coincide with husbandry procedures, such as weaning, drenching or vaccinating. Conventional weighing increases labour, cost and can potentially have negative impacts on animal welfare depending on weighing procedures and animal management.
Poor handling can produce stress, pain and injury, leading to suffering and reducing carcass quality due to bruising, greater susceptibility to disease by immunosuppression, increase the prevalence of dark cutters, and lower reproductive rates (Fordyce et al., 1988). Automatic in-paddock weighing offers an alternative to measure cattle LW frequently without the need of mustering and handing animals. Nevertheless, it is assumed that increasing the frequency of LW measures could be beneficial to detect responses to various environmental and managerial factors. However, it is uncertain how often animals should be weighed to capture the desired change in LW for different applications. The number of studies assessing the impact of different frequency of LW data collection on LW and LWC calculations are limited (Currie et al., 1989). Therefore, the use of WOW could improve LW data collection and serve to investigate the minimum frequency of LW data required to capture critical information for decision making and animal research using growth rate calculations.

2.6.2 Measuring individual LW and supplement intake

In-paddock weighing, and electronic feeding systems enable identification of individual animals (González et al., 2018). This could be critical information for early detection of changes that differentiate performance among individuals. Abrupt changes in LW, feed intake or attendance to the WOW and feeder stations can be indicative of disease, reproductive status, nutrition or predator presence, to mention a few potential applications (Filby et al., 1979; Peiper et al., 1993; Burns et al., 2010; Roche et al., 2017). The WOW and EF could also be used to refine feeding strategies according to individual performance and forage conditions as these change throughout the year. Such implementation could be aided using auto-drafting gates attached to WOW systems to sort animals into different yards and to improve nutritional management of animals within a group. From a research viewpoint, these tools allow scientists to explore individual variability in LW and feed intake which can also be linked to physiological processes in the long-term (e.g. muscle and fat deposition) and short-term (e.g. within-day changes in ruminal fill; Gregorini et al., 2007). Additionally, the integration of data streams from different sources (e.g. WOW, EF and metabolomics) could allow detection of individuals with superior performance or prediction in the early stages of the production cycle. For instance, these inputs along with the use of auto-drafting gates could enhance future automation of activities such as detection of sick animals or sorting individuals with low performance to a different yard with supplementary feed.

2.6.3 Effects of self-fed supplements on cattle LWC and feeding behaviour

The use of feed supplements in cattle production has been studied extensively because of the effects on growth, reproduction, animal welfare (e.g. reducing mortality) and environmental footprint including greenhouse gas emissions (Poppi and McLennan, 1995; Caton and Dhuyvetter, 1997; McLennan et al., 2017). Most previous studies have been focused on protein and energy supplementation of loose supplements (e.g. concentrate, grain) fed with a wide range of basal diets (e.g. grazing pastures, dry-lot hay fed), animal categories (e.g. calves to
mature cows), frequency of delivery (e.g. daily or discontinuous) and amount of supplement (Caton and Dhuyvetter, 1997; Farmer et al., 2001). However, the use of ‘self-fed’ supplements have received less attention (Bowman and Sowell, 1997). Self-fed or free-choice supplements are usually offered without any restriction of quantity and access. Cooked and compressed molasses-lick blocks (MLB) are arguably amongst the most common self-fed supplements which often contain protein, oil, minerals, feed additives and medications. However, MLB could largely differ in composition and hardness which directly affect the amount of MLB that animals could consume (Zhu et al., 1991).

Previous research demonstrated that cattle fed low-quality forages (e.g. 4 to 6% crude protein, CP) responded to MLB supplementation by increasing forage intake and in-vivo digestibility (Löest et al., 2001). In general, most studies used MLB supplementation when low-quality feed was the main diet but less studies used MLB when feed quality was medium to high (Bowman et al., 1995). However, studies are not conclusive regarding growth responses to MLB supplementation, particularly when medium to high-quality forages are offered. In this regard, Titgemeyer et al. (2004) reported no effect of MLB on LWC of cattle fed lucerne hay whereas Brown et al. (1993) reported increased LWC in supplemented animals with liquid molasses when the CP of the basal diet was enriched with cotton seed meal. The digestibility of low-quality forages such as wheat straw (3.3 % CP) was improved with the inclusion of MLB in housed adult beef cattle (n=16) and led to similar growth rate compared to animals supplemented with grain (i.e. barley; Toppo et al., 1997). Similarly, beef steers housed in individual pens and fed with prairie hay (5.2 to 5.9 % of CP) showed an increase of both hay intake and in-vivo digestibility with ad-libitum MLB access (Löest et al., 2001, Titgemeyer et al., 2004). Bowman et al. (1995) published a comprehensive review of the use of molasses supplements (liquid and MLB) for cattle and sheep mostly fed low-quality forages. These authors concluded that the effects of molasses on supplemented animals were not conclusive because of the many confounding factors such as pasture condition, forage quantity and quality, individual animal variability and supplement delivery methods. Remarkably, only 4 out of 43 experiments reviewed measured supplement intake and feeding behaviour. Therefore, WOW and EF could be useful to fill these research gaps and assess the variability over time and between individual grazing animals. This could allow better understanding of the interactions between forage type, supplement intake and feeding behaviour.

### 2.6.4 Detecting periods of undernutrition and compensatory growth

Grazing animals may go through periods of undernutrition as forage in a paddock is depleted and mature. Compensatory growth may occur depending on age, animal category, and duration and severity of the feed deprivation (Ryan, 1990). During such periods of undernutrition, growth rate could range from a modest increase to severe weight loss and, if this restriction is maintained over time, animals may adapt to such a lower nutritive state by stunting (Ryan, 1990). Increasing the severity of feed restriction before re-alimentation is more likely to maintain LWC for longer periods rather than increasing LWC. In contrast, increasing the duration of the
restriction without altering the severity is likely to result in higher LWC compared to unrestricted cattle once the animals are re-alimented (Ryan, 1990). Integrating WOW and EF could offer applications to timely identify the effects of undernutrition and deliver supplementary feed only when it is required. Increasing the frequency of LW data collection would also allow determination of the duration and magnitude of compensatory growth in cattle. The biological mechanisms of feed restriction and compensatory growth have been studied from different angles including changes in body composition and fill of the gastrointestinal tract (Gregorini et al., 2007). However, no studies have undertaken a comprehensive assessment of body metabolism during feed restriction and compensatory growth. The use of metabolomics to understand the metabolic changes during compensatory growth could shed new knowledge of the mechanisms involved during this process.

2.7 Conclusions and research possibilities

Previous developments and the on-going refinement of in-paddock technologies and methodologies to monitor key performance and biological parameters in grazing beef cattle offer potential to advance scientific knowledge and improve animal production, welfare and sustainability. However, the utilisation of data generated by WOW, EF and metabolomics has not been attempted before. In-paddock weighing can measure cattle LW daily and EF can measure the intake of supplementary feed and feeding behaviour of cattle. In addition, studies in humans and livestock demonstrated that metabolomics offer several possibilities to measure the abundance of multiple metabolites from biological fluids. The advantages and applications of integrating WOW, EF and metabolomics has yet to be explored. However, the minimum frequency of LW data collection to capture the required variability in the growth trajectory of cattle using in-paddock weighing needs to be assessed before the results can be interpreted robustly and implications assumed. Also, the ability and value of data from WOW and EF to measure LW and supplement intake, respectively, in long-term grazing trials needs to be ascertained. This could include field trials to reflect common changes in the type of feed and growth variability under commercial conditions. Such variability would need to be understood at varying temporal scales and across individual animals. These technologies and the integration approach could be used to determine the correlation between LW, LWC, supplement intake, feeding behaviour and metabolites of grazing beef cattle. In addition, the approach could contribute to acquiring more detailed knowledge to explain the response of animals to environmental and managerial factors including supplementation and grazing management, and feed quantity and quality. One of the biggest challenges lies in the integration of data streams obtained from in-paddock technologies and metabolomics to understand physiological and metabolic changes driving animal performance. Therefore, the above research possibilities and challenges constitute the core of the research program presented in this thesis.
2.8 References


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Chapter 3

Using automated in-paddock weighing to evaluate the impact of the length of the interval between liveweight measures on growth rate calculations in beef cattle

3.1 Overview

Chapter 2 highlighted the importance of the frequency of liveweight data collection to timely detect changes in liveweight and growth rate. In general, it is assumed that increasing the frequency of liveweight data collection would enhance the description of the growth trajectories in cattle. However, this has not been tested previously because of the constraints of weighing cattle regularly (e.g. daily). The use of automated in-paddock weighing enables frequent liveweight data collection from different cattle categories (e.g. calves, mature cows). Cattle category could largely differ in terms of the trajectories in liveweight and growth rate over time and thus, the optimal frequency of data collection could differ among categories. Therefore, this chapter explores the use of remotely collected liveweight data obtained by using in-paddock weighing to determine the impact of the length of the interval between liveweight measures on liveweight and liveweight change calculations in calves, weaners and breeding cows.

3.2 Abstract

Animal liveweight (LW) data collection is key to monitor health, nutrition and reproduction of cattle. However, this is challenging using traditional technology due to the need of mustering animals into handling facilities with the required frequency. Such practical constraints make it difficult to gather frequent LW data to study the effects of different length of intervals between LW measures (ILW) to accurately describe the growth path of animals. However, nowadays, frequent LW data can be acquired remotely using in-paddock technologies without the need to handle the animals. Thus, the aim of this study was to quantify the impacts of ILW to capture LW and growth paths of three beef cattle categories (calves, weaners, and cows). Liveweight data were collected using in-paddock walk-over-weighing scales (WOW), placed before the access to the water trough. The lengths of continuous LW data records were 112, 224 and 1460 days (4 years) for calves, weaners and mature cows, respectively. These datasets were then subsampled to simulate different ILW with one LW record every: a) 1, 2, 4, 8 and 16 weeks for calves; b) 1, 2, 4, 8, 16 and 32 weeks for weaners; and c) 1, 2, 4, 8, 16, 26, 32, 52 (1 year) and 208 weeks (4 years) for cows. Daily LW change (LWC) was calculated as the difference between two consecutive LW observations divided by the number of days elapsed. The minimum (Min), mean, maximum (Max), standard deviation (SD) and coefficient of variation (CV) for LW and LWC were calculated for each animal and ILW. Minimum and Max LWC, and SD and CV of LW were affected (P < 0.05) by ILW in all animal categories whereas no effects (P > 0.05) were observed for the rest of the variables. The relationship between ILW and LW variability (SD, CV) was quadratic for calves and weaners but linear for cows (P < 0.05). In comparison to daily data, the minimum frequency required to capture Min and Max LWC was 2 weeks for calves and weaners, and 8 weeks for cows. In addition, an ILW of 4 (calves and weaners) and 8 (cows) weeks was needed to achieve similar SD and CV of LW and LWC compared to daily ILW. These results suggest that WOW could be used more strategically within and between farms, as LW data need to be captured at regular intervals but not necessarily daily.
3.3 Introduction

Monitoring cattle liveweight (LW) is critical to calculate daily LW change (LWC) and both parameters are directly linked to productivity, animal health and welfare (Alawneh et al., 2011; González et al., 2014). However, measuring LW often requires mustering cattle to central facilities to individually weigh animals. This labour-intensive task could produce adverse impacts on productivity and welfare (Petherick et al., 2009), rendering it impractical to frequently collect LW data. Additionally, mustering and handling could exacerbate variations in LW as a result of modifying their ruminal fill (Hoch et al., 2004). As a result, the frequency of LW data collection achievable by conventional weighing may not be enough to capture LW variability existing across different animal categories (e.g. calves, weaners, cows). Thus, decreasing LW data collection frequency would increase the length of the interval between LW measures (ILW) for each animal.

Nowadays, cattle LW data can be obtained remotely using digital technologies such as in-paddock walk-over-weighing scales (WOW) and then analysed to manage LW variations (González et al., 2014). Recent studies in sheep, beef and dairy cattle reported on the use of WOW to describe LW and LWC paths of cattle without human handling (Charmley et al., 2006; Alawneh et al., 2011; Brown et al., 2015). Thus, in-paddock weighing showed the ability to monitor LW paths of cattle with maximum level of detail (daily frequency) which can then be used to study the impact of ILW on LW and LWC calculations. Also, using this approach would help to assess the variability in LW and LWC using descriptive statistics for individual animals rather than averaging groups of animals. Otherwise, studying the effects of ILW of grazing cattle for long periods (e.g. years) could not be possible due to the impracticality and constraints of mustering and weighing cattle constantly.

Liveweight varies according with the animal category (e.g. calves, mature cows) and temporal scale (e.g. within and between days, months and seasons), possibly affecting the results of simulating different ILW. Detecting periods of minimum and maximum LW and LWC may be essential for timely management (e.g. introduce feed supplementation) and to detect large variations in LW (e.g. pre- and post- parturition in cows). Similarly, detecting deviations from the average LW and LWC (e.g. standard deviation, coefficient of variation) would allow managers to identify animals’ responses to environmental conditions and nutrition. To our knowledge, no studies have previously been published assessing ILW in different beef cattle categories using LW data from in-paddock weighing systems.

The aim of this study was therefore to quantify the effects of the length of the interval between LW measures (i.e. ILW) on LW and LWC calculations of individual animals in three cattle categories (calves, weaners and breeding cows). We hypothesised that ILW affects the ability to describe the paths of LW and LWC. The minimum ILW required to capture the highest variability in LW and LWC would depend on the ability of a given interval to detect extreme values (peaks and troughs) of the growth paths for each animal category.
3.4 Materials and methods

All experimental procedures were approved by the institutional Animal Ethics Committee from The University of Sydney (Approval 2014/615 and 2017/1162).

3.4.1 Experimental details

The study was conducted at John Pye Farm (Greendale, NSW The University of Sydney) where LW measurements were obtained using a WOW on three different cattle categories: calves, weaners (steers and heifers), and mature breeding cows.

3.4.2 Weaner cattle management

Forty-one weaners (24 steers and 17 heifers) between 6 to 7 months of age were tagged with electronic identification (EID) and fed with a sequence of forages for 224 days (from 12 April to 22 November 2017). Cattle were grazed rotationally on 24.7 ha of temperate pastures and oat crops divided into 18 paddocks. Concentrate supplementation was offered infrequently (Monday, Wednesday and Friday) at a rate of 1.25 kg/hd per day from 07 of August to 22 of November due to drought. Over the grazing period, animals were moved to a fresh paddock when forage availability to the base of 5 cm was approximately 1000 and 750 kgDM/ha for pastures and oat-crop paddocks, respectively.

3.4.3 Cow-calf herd management

Eighteen multiparous Charolais cows were tagged with electronic identification (EID) and grazed native pastures from 01 September 2014 to 31 August 2018 (1460 days, 4 years). Predominant forage species included kangaroo grass (Themeda australis) and weeping grass (Microlaena stipoides). Lucerne and oaten hay and silage were fed intermittently over this 4-year period to cover seasonal pasture deficits and because of drought. Cow EID, birth date and sex of the calf at calving were recorded. Twelve calves from the cows born within one season were selected for this study with an average birth weight of 48 ± 8.20 kg (mean ± SD) and the earliest birth was recorded on 19 of August 2017 and the latest on 27 September 2017. Calves were selected based on those showing the highest number of LW records and the lowest minimum interval between LW records during the period of study (112 days).

3.4.4 In-paddock measurements of liveweight

A central yard (15 m x 25 m) located at the sole water point was built for each of two herds (weaner herd and breeding herd which contained cows and calves). An in-paddock WOW station was placed at the entry of each yard to record LW, EID, date and time (Precision Pastoral Ltd, Alice Spring, Northern Territory, Australia for weaners and Tru-test Ltd, Auckland, New Zealand for cows and calves). The WOW consisted of a platform (0.8 width x 2.4 m length) placed over two load bars and mounted along steel and wooden panels (3m-long x 2m-height) on both sides. Spear gates were used at the entry of the WOW and each exit gate to allow animals to move in
only one direction. Animals were previously trained to use the WOW following the procedure proposed by González et al. (2014).

3.4.5 Data processing and statistical analysis

Data recorded by the WOWs were filtered for outlying data and then smoothed using penalised b-splines using the methods described by González et al. (2014). Briefly, Gonzalez et al. (2014) first deleted extreme weight outliers which were biologically implausible. Then, data were fitted to penalised B-spline for each individual animal and LW outliers were detected and deleted based on 1.5 times below or above of residuals obtained from the smoothed mean of each animal. After outliers’ deletion, the penalised B-spline was fitted again to obtain the predicted LW for each individual animal. Daily liveweight change (g/hd per day) was calculated from the smoothed data as the first derivative of the predicted LW curve. The resulting LW and LWC data were averaged by date for each animal if more than one measurement per day and animal existed. Table 3.1 shows that days with usable LW records represented 42, 69 and 51% of the full-length period for calves, weaners and cows, respectively. This resulted in average intervals between consecutive records (days) ranging from 1.45 (weaners) to 2.48 (calves). Days without records were interpolated considering a linear change in LW and LWC between the previous and the next record. This process originated a dataset with all days having LW and LWC measurements for all animals with calves, weaners and cows having records for 112, 224 and 1460 days, respectively. The first day considered for each calf was the date of the appearance of the first usable LW record after smoothing and deletion of outliers (González et al., 2014). This complete dataset was then used to create subsets of data simulating different ILW for each animal according to the length of the data collection period. Thus, from day 1 until the last day of the period considered, one LW record was selected for each animal every 1 (1W), 2 (2W), 4 (4W), 8 (8W) and 16 weeks (16W). The latter resulted in calves having 1 LW record at the beginning and 1 LW record at the end of the entire period. Because the period of data collection was longer for weaners and cows, records at 32 weeks (32W) apart were selected for weaners and cows. Cows also had 1 LW record selected every 26 and 52 weeks, and 1 LW record at the beginning and at the end of the entire 4-year period (208 weeks). Liveweight change was then re-calculated for each dataset as the difference between two consecutive LW observations divided by the number of days between both observations.

Table 3.1: Descriptive statistics (Mean ± SD) of liveweight (LW) data for each animal category.

<table>
<thead>
<tr>
<th>Items (days)</th>
<th>Calves</th>
<th>Weaners</th>
<th>Mature cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period length</td>
<td>112</td>
<td>224</td>
<td>1460</td>
</tr>
<tr>
<td>Days with valid LW records</td>
<td>47.1 ± 6.5</td>
<td>155.4 ± 11.7</td>
<td>755.2 ± 57.6</td>
</tr>
<tr>
<td>Average of the interval</td>
<td>2.48 ± 0.35</td>
<td>1.45 ± 0.11</td>
<td>1.94 ± 0.14</td>
</tr>
<tr>
<td>between valid LW records</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data from each animal and each ILW was then used to calculate the minimum (Min) LW and LWC; maximum (Max); standard deviation (SD) for LW and LWC and coefficient of variation (CV) for LW (the CV of LWC for cows was not analysed due to negative values). The objective of these calculations was to obtain extreme values or peaks and troughs (Min and Max) and measures of variability (SD, CV) over the entire period. Calculations of SD and CV for calves and weaners and Min LWC for weaners and cows were log\(^{10}\) transformed prior to the analysis. Data were analysed for each animal category separately using a linear model including ILW as a fixed factor and each summary statistic as response variables, i.e. Min, Max, SD and CV. In addition, linear and quadratic effects of increasing ILW data were tested for every response variable. Means were separated using Bonferroni adjustment for multiple comparisons. Statistical significance was declared at P < 0.05. All statistical procedures were done using SAS/STAT software (SAS Institute Inc., Cary, NC, USA).

3.5 Results

3.5.1 Calves

Increasing ILW resulted in a linear increase of the Min LWC measured (Table 3.2; P < 0.001). Maximum LWC decreased quadratically when increasing ILW (P < 0.001) because the largest drop in Max LWC was observed at 4W. Both the SD and CV of LWC decreased linearly with increasing ILW (P < 0.001). These results were reflected in Figure 3.1 which shows that the peaks and troughs of the LWC trajectory tend to disappear as ILW increases, whereas LW does not seem to be affected. However, SD and CV of LW increased quadratically with increments of ILW (P < 0.001).
Table 3.2: Average of the mean, minimum, maximum and SD of liveweight change (LWC) and liveweight (LW) of calves calculated at daily, weekly, fortnightly, 4-week, 8-week and 16-week intervals between liveweight measures (ILW).

<table>
<thead>
<tr>
<th>LWC (g/hd per day)</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>1022 ± 20.0 a</td>
<td>825 ± 35.9 b</td>
<td>1284 ± 26.3 a</td>
<td>126 ± 12.2 a</td>
<td>12 ± 1.3 a</td>
</tr>
<tr>
<td>1W</td>
<td>1025 ± 20.0 a</td>
<td>830 ± 35.9 b</td>
<td>1266 ± 26.3 ab</td>
<td>129 ± 12.2 a</td>
<td>12 ± 1.3 a</td>
</tr>
<tr>
<td>2W</td>
<td>1025 ± 20.0 a</td>
<td>851 ± 35.9 b</td>
<td>1221 ± 26.3 ab</td>
<td>120 ± 12.2 a</td>
<td>12 ± 1.3 a</td>
</tr>
<tr>
<td>4W</td>
<td>1025 ± 20.0 a</td>
<td>899 ± 35.9 ab</td>
<td>1156 ± 26.3 b</td>
<td>98 ± 12.2 ab</td>
<td>10 ± 1.3 ab</td>
</tr>
<tr>
<td>8W</td>
<td>1025 ± 20.0 a</td>
<td>967 ± 35.9 ab</td>
<td>1084 ± 26.3 cd</td>
<td>58 ± 12.2 b</td>
<td>6 ± 1.3 b</td>
</tr>
<tr>
<td>16W</td>
<td>1026 ± 20.0 a</td>
<td>1026 ± 35.9 a</td>
<td>1026 ± 26.3 d</td>
<td>0 ± 12.2 c</td>
<td>0 ± 1.3 c</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>1</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Linear</td>
<td>1</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1</td>
<td>0.26</td>
<td>&lt; 0.01</td>
<td>0.73</td>
<td>0.73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LW (kg/hd)</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>138 ± 5.1 a</td>
<td>82 ± 4.9 a</td>
<td>197 ± 5.3 a</td>
<td>33 ± 0.9 e</td>
<td>23 ± 0.8 e</td>
</tr>
<tr>
<td>1W</td>
<td>138 ± 5.1 a</td>
<td>82 ± 4.9 a</td>
<td>197 ± 5.3 a</td>
<td>36 ± 0.9 de</td>
<td>25 ± 0.8 de</td>
</tr>
<tr>
<td>2W</td>
<td>138 ± 5.1 a</td>
<td>82 ± 4.9 a</td>
<td>197 ± 5.3 a</td>
<td>39 ± 0.9 d</td>
<td>27 ± 0.8 d</td>
</tr>
<tr>
<td>4W</td>
<td>139 ± 5.1 a</td>
<td>82 ± 4.9 a</td>
<td>197 ± 5.3 a</td>
<td>45 ± 0.9 c</td>
<td>32 ± 0.8 c</td>
</tr>
<tr>
<td>8W</td>
<td>139 ± 5.1 a</td>
<td>82 ± 4.9 a</td>
<td>197 ± 5.3 a</td>
<td>57 ± 0.9 b</td>
<td>40 ± 0.8 b</td>
</tr>
<tr>
<td>16W</td>
<td>139 ± 5.1 a</td>
<td>82 ± 4.9 a</td>
<td>197 ± 5.3 a</td>
<td>80 ± 0.9 a</td>
<td>56 ± 0.8 a</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>0.99</td>
<td>1</td>
<td>1</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Linear</td>
<td>0.99</td>
<td>1</td>
<td>1</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.99</td>
<td>1</td>
<td>1</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
Figure 3.1: Means of liveweight (LW, discontinuous line) and liveweight change (LWC, solid line) of twelve calves calculated from daily observations or from observations every 1, 2, 4, 8 and 16 weeks. Dots indicate LW records selected.

3.5.2 Growing weaner cattle

All calculations except mean daily LWC, and the mean, minimum and maximum LW were affected by ILW (Table 3.3; P < 0.05). Variables affected by ILW showed a quadratic decrease (P < 0.05) except Min LWC which increased linearly from -104 to 530 g/hd per day as ILW increased from Daily to 32W (P < 0.05). For Min and Max LWC, 2W data were the longest ILW that did not differ from daily LWC (P > 0.05). Frequencies lower than 4W were not able to capture negative Min LWC. Graphical presentation of these results indicated that increasing ILW results in a dramatic effect on LWC calculations (Figure 3.2). In addition, max LWC detected by Daily ILW was 63% greater than 32W (P < 0.05). Standard deviation of LWC decreased significantly by 28% at 16W compared to Daily (P < 0.05) but no differences were found between Daily until 8W (P > 0.05). A similar reduction and statistical significance were observed for the CV of LWC.
however differences with Daily were noticed from 4W and beyond (P < 0.05). In contrast to LWC, only the variability of LW was affected by ILW (P < 0.05) with increasing SD and CV of LW as ILW increased with differences starting to differ from Daily at 4W (P < 0.05).

Table 3.3: Average of the mean, minimum, maximum and SD of liveweight change (LWC) and liveweight (LW) of growing weaners calculated at daily, weekly, fortnightly, 4-week, 8-week, 16-week and 32-week intervals between liveweight measures (ILW).

<table>
<thead>
<tr>
<th>LWC (g/hd per day)</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>531 ± 13.5 a</td>
<td>-104 ± 17.2 e</td>
<td>1439 ± 28.9 a</td>
<td>459 ± 10.2 a</td>
<td>87 ± 1.7 a</td>
</tr>
<tr>
<td>1W</td>
<td>529 ± 13.5 a</td>
<td>-88 ± 16.1 e</td>
<td>1436 ± 28.5 a</td>
<td>458 ± 10.2 a</td>
<td>86 ± 1.7 a</td>
</tr>
<tr>
<td>2W</td>
<td>529 ± 13.5 a</td>
<td>-56 ± 16.5 de</td>
<td>1370 ± 28.1 ab</td>
<td>446 ± 10.2 a</td>
<td>84 ± 1.7 ab</td>
</tr>
<tr>
<td>4W</td>
<td>529 ± 13.5 a</td>
<td>-2 ± 15.1 d</td>
<td>1253 ± 28.1 b</td>
<td>421 ± 10.1 ab</td>
<td>79 ± 1.7 b</td>
</tr>
<tr>
<td>8W</td>
<td>529 ± 13.5 a</td>
<td>83 ± 15.1 c</td>
<td>1041 ± 28.1 c</td>
<td>377 ± 10.1 b</td>
<td>70 ± 1.7 c</td>
</tr>
<tr>
<td>16W</td>
<td>529 ± 13.5 a</td>
<td>208 ± 15.1 b</td>
<td>852 ± 28.1 d</td>
<td>329 ± 10.2 c</td>
<td>62 ± 1.7 d</td>
</tr>
<tr>
<td>32W</td>
<td>530 ± 13.5 a</td>
<td>530 ± 15.1 a</td>
<td>530 ± 28.1 e</td>
<td>0 ± 10.1 d</td>
<td>0 ± 1.7 e</td>
</tr>
</tbody>
</table>

P-values

<table>
<thead>
<tr>
<th>Model</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.99</td>
<td>0.92</td>
</tr>
<tr>
<td>0.035</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>0.66</td>
<td>0.92</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LW (kg/hd)</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>233 ± 5.9 a</td>
<td>188 ± 5.3 a</td>
<td>308 ± 6.7 a</td>
<td>36 ± 1.2 e</td>
<td>16 ± 0.6 e</td>
</tr>
<tr>
<td>1W</td>
<td>233 ± 5.9 a</td>
<td>188 ± 5.3 a</td>
<td>308 ± 6.7 a</td>
<td>38 ± 1.2 e</td>
<td>16 ± 0.6 e</td>
</tr>
<tr>
<td>2W</td>
<td>234 ± 5.9 a</td>
<td>188 ± 5.3 a</td>
<td>308 ± 6.7 a</td>
<td>39 ± 1.2 de</td>
<td>17 ± 0.6 de</td>
</tr>
<tr>
<td>4W</td>
<td>235 ± 5.9 a</td>
<td>189 ± 5.3 a</td>
<td>308 ± 6.7 a</td>
<td>43 ± 1.2 d</td>
<td>18 ± 0.6 d</td>
</tr>
<tr>
<td>8W</td>
<td>237 ± 5.9 a</td>
<td>189 ± 5.3 a</td>
<td>308 ± 6.7 a</td>
<td>50 ± 1.2 c</td>
<td>21 ± 0.6 c</td>
</tr>
<tr>
<td>16W</td>
<td>236 ± 5.9 a</td>
<td>189 ± 5.3 a</td>
<td>308 ± 6.7 a</td>
<td>64 ± 1.2 b</td>
<td>27 ± 0.6 b</td>
</tr>
<tr>
<td>32W</td>
<td>248 ± 5.9 a</td>
<td>189 ± 5.3 a</td>
<td>308 ± 6.7 a</td>
<td>86 ± 1.2 a</td>
<td>34 ± 0.6 a</td>
</tr>
</tbody>
</table>

P-values

<table>
<thead>
<tr>
<th>Model</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.035</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>0.66</td>
<td>0.92</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P-values</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>0.93</td>
<td>0.02</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

57
3.5.3 Mature cows

The Min and Max LWC of mature cows were affected by ILW and quadratic effects were observed (Table 3.4, P < 0.05). Calculations from Daily and Weekly data showed that cows lost up to 1081 g/hd per day and such values of Min LWC were also captured using ILW up to 8W (P > 0.05). No differences were detected until 16W for SD of LWC which decreased quadratically (P < 0.05) by 23% and 77% from Daily to 16W and Year frequencies (Figure 3.3). Mean, Min, and Max LW were not affected by ILW (P > 0.05); however, SD of LW increased linearly with
ILW (Table 3.4, P > 0.05). Graphical representation of these data in Figures 3.3 and 3.4 demonstrates that increasing ILW reduces the ability to capture periods of high weight gain or loss such as during calving.

Table 3.4: Average of the mean, minimum, maximum and SD of liveweight change (LWC) and liveweight (LW) of beef cows calculated at daily, weekly, fortnightly, 4-week, 8-week, 16-week, 26-week, 1-year or 4-year intervals between liveweight measures (ILW).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LWC (g/hd per day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>134 ± 8.1 a</td>
<td>-1051 ± 54.1 d</td>
<td>1478 ± 149.6 ab</td>
<td>565 ± 25.5 a</td>
</tr>
<tr>
<td>Weekly</td>
<td>130 ± 8.1 a</td>
<td>-1081 ± 52.2 d</td>
<td>1575 ± 149.6 a</td>
<td>562 ± 25.5 a</td>
</tr>
<tr>
<td>2W</td>
<td>130 ± 8.1 a</td>
<td>-1023 ± 50.5 d</td>
<td>1685 ± 145.4 a</td>
<td>554 ± 25.5 ab</td>
</tr>
<tr>
<td>4W</td>
<td>131 ± 8.1 a</td>
<td>-981 ± 49.0 d</td>
<td>1457 ± 145.4 ab</td>
<td>539 ± 25.5 ab</td>
</tr>
<tr>
<td>8W</td>
<td>131 ± 8.1 a</td>
<td>-890 ± 47.6 d</td>
<td>1136 ± 145.4 ab</td>
<td>506 ± 25.5 ab</td>
</tr>
<tr>
<td>16W</td>
<td>131 ± 8.1 a</td>
<td>-622 ± 47.6 c</td>
<td>926 ± 145.4 b</td>
<td>437 ± 25.5 b</td>
</tr>
<tr>
<td>26W</td>
<td>131 ± 8.1 a</td>
<td>-340 ± 47.6 b</td>
<td>581 ± 145.4 c</td>
<td>296 ± 25.5 c</td>
</tr>
<tr>
<td>52W (1 year)</td>
<td>129 ± 8.1 a</td>
<td>-54 ± 47.6 a</td>
<td>275 ± 145.4 d</td>
<td>130 ± 25.5 d</td>
</tr>
<tr>
<td>208W (4 years)</td>
<td>129 ± 8.1 a</td>
<td>129 ± 47.6 a</td>
<td>129 ± 145.4 e</td>
<td>0 ± 25.5 e</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>0.99</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Linear</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Quadratic</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>LW (kg/hd)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>675 ± 9.5 a</td>
<td>562 ± 9.2 a</td>
<td>780 ± 13.5 a</td>
<td>52 ± 2.9 c</td>
</tr>
<tr>
<td>Weekly</td>
<td>675 ± 9.5 a</td>
<td>562 ± 9.2 a</td>
<td>780 ± 13.5 a</td>
<td>52 ± 2.9 c</td>
</tr>
<tr>
<td>2W</td>
<td>675 ± 9.5 a</td>
<td>562 ± 9.2 a</td>
<td>779 ± 13.5 a</td>
<td>52 ± 2.9 c</td>
</tr>
<tr>
<td>4W</td>
<td>674 ± 9.5 a</td>
<td>562 ± 9.2 a</td>
<td>778 ± 13.5 a</td>
<td>53 ± 2.9 c</td>
</tr>
<tr>
<td>8W</td>
<td>674 ± 9.5 a</td>
<td>562 ± 9.2 a</td>
<td>776 ± 13.5 a</td>
<td>55 ± 2.9 c</td>
</tr>
<tr>
<td>16W</td>
<td>673 ± 9.5 a</td>
<td>564 ± 9.2 a</td>
<td>770 ± 13.5 a</td>
<td>59 ± 2.8 c</td>
</tr>
<tr>
<td>26W</td>
<td>667 ± 9.5 a</td>
<td>564 ± 9.2 a</td>
<td>758 ± 13.5 a</td>
<td>61 ± 2.8 bc</td>
</tr>
<tr>
<td>52W (1 year)</td>
<td>662 ± 9.5 a</td>
<td>564 ± 9.2 a</td>
<td>756 ± 13.5 a</td>
<td>73 ± 2.9 b</td>
</tr>
<tr>
<td>208W (4 years)</td>
<td>661 ± 9.5 a</td>
<td>567 ± 9.2 a</td>
<td>755 ± 13.5 a</td>
<td>139 ± 3.3 a</td>
</tr>
<tr>
<td></td>
<td>P value</td>
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</tr>
<tr>
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<td>0.99</td>
<td>0.76</td>
<td>&lt; 0.001</td>
</tr>
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<td>0.59</td>
<td>0.11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.31</td>
<td>0.89</td>
<td>0.13</td>
<td>0.68</td>
</tr>
</tbody>
</table>
Figure 3.3: Means of liveweight change (LWC) of eighteen beef cows calculated from daily observations or from observations every 1, 2, 4, 8, 16, 26, 52 (1 year) and 208 weeks (4 years). Dots in Daily indicate calving time.
Figure 3.4: Means of liveweight (LW) of eighteen beef cows selected daily or from observations every 1, 2, 4, 8, 16, 26, 52 (1 year) and 208 weeks (4 years).
3.6 Discussion

The objective of the present study was to determine the minimum frequency required to monitor LW and LWC without losing critical information that may be important for timely management decisions (e.g. feeding, prevention of disease, reproduction management, calving). With this aim, we explored the effects of different ILW among cattle categories (calves, weaners and mature cows). Our results indicate that the minimum interval required to capture Min and Max LWC was 2 weeks for calves and weaners, and 8 weeks for cows, in comparison with daily data. Additionally, similar variability (SD and CV of LW and LWC) to that using daily LW data was detected with ILW of 4 weeks for calves and weaners and 8 weeks for cows.

Studies reporting on similar findings are limited. Currie et al. (1989) compared daily LW collected by WOW with LW data obtained by conventional weighing every 6 weeks over approximately 90 days. They concluded that LWC patterns calculated from conventional weighing data largely differed from those provided by continuous LW measurements in grazing beef steers. Other studies used daily LW from WOW but did not directly compare ILW. Working with dairy cattle, Alawneh et al. (2011) suggested the collection of daily LW for earlier detection of illness events or changes in feed management, which would not be possible using ILW longer than 1 week. Similarly, González et al. (2014) showed that nutritional management of grazing beef steers to avoid LW loss could not be achievable by using ILW longer than 4 weeks. The present study demonstrates graphically (Figures 3.1-3.4) and statistically (Tables 3.2-3.4) that the ability of monitoring LWC and LW was reduced as ILW increased, due to a progressive flattening of LWC patterns.

The quantification and detection of periods with relatively poor or good animal performance could improve management decisions. For example, monitoring LWC could help to identify periods of decreasing rates of positive LWC or severe weight loss which has implications on productivity, reproduction and survival. In the present study, the Min and Max LW and LWC throughout trials and animal categories demonstrated the ability of these measurements to identify and quantify the extent of periods of undernutrition and subsequent growth recovery when on-farm decisions may be needed. Similarly, SD and CV of LWC or LW could help in quantifying variability in the ability of animals to cope with changes in environmental and management factors over time. For example, both SD and CV of LWC within the trial period were smaller for calves, medium for weaners and largest for cows, which is also well reflected in the figures. In addition, SD and CV data are in alignment with our hypothesis, which states that the growth trajectory would be different for each animal category with calves showing the least variability in LWC, weaners intermediate and cows the largest variability. This is expected, as calves obtained most of their feed from their mothers (von Keyserlingk and Weary, 2007) which could explain the smaller variability observed on calves’ LWC. In addition, mature cows are likely to experience abrupt changes in LW and LWC due to gestation, calving, lactation, weaning and seasonal fluctuations on their nutritional status (Lake et al., 2006; Cooper-Prado et al., 2014).
In line with our hypothesis, results showed that ILW has a significant impact on the quantification and detection of Min and Max LWC and the variability (SD and CV) within a period for all animal classes. However, the mean LWC and LW were not affected by ILW in any animal class. It is important to note that, for example, the mean LWC may be arithmetically affected by ILW during periods of non-linear trajectories; however, it may not differ statistically. Additionally, increasing ILW significantly increased Min LWC and reduced Max LWC across all animal categories until both parameters were similar to each other at the highest ILW.

The impact of ILW was expected to be larger in animal categories that experience larger variability in LWC and LW, i.e. largest in cows and least in calves. For calves, decreasing ILW had a quadratic effect on Max LWC with the largest impact at 4W and longer intervals between LW measurements whereas Min LWC increased linearly with ILW. Findings confirm this hypothesis because the difference in Min LWC between Daily and at 16W was 201, 312 and 429 g/hd per day for calves, weaners and cows, respectively.

Our results indicate that weaners had a linear increase in Min LWC and a quadratic effect on Max LWC because the latter drops sharply at 2W and higher ILW. Results from the present study agree with Currie et al. (1989) who described the growth patterns of yearling steers for two summer periods using in-paddock and conventional weighing (i.e. static scales, three times per year). These authors suggested that growth rates calculated at long weighing intervals failed to describe the growth path over the entire season with enough accuracy to specifically identify points in time when live weight was levelling out, increasing or decreasing. However, Currie et al. (1989) did not test ILW and only descriptive LW data were presented in their paper.

In contrast to calves and weaners, decreasing ILW of cows resulted in a quadratic effect on both Min and Max LWC. Intervals between measurement up to 8 and 16 weeks were able to capture similar Min and Max LWC, respectively, compared to daily information. An adequate ILW for cows should be able to capture critical time periods affecting LW across seasons as a result of different philological stages related to the effects of pregnancy, parturition and LW recovery (Cooper-Prado et al., 2014). The present study indicates that cows lost approximately 1.05 kg/hd per day during the calving period whereas the 16W interval only captured an average of 0.62 kg/hd per day. It is possible to speculate that long periods between successive weighing events may have contributed to the lack of success in identifying sources of variability affecting the reproduction and growth of the dam, and its correlation with their offspring performance in a previous study (Osoro and Wright, 1992). However, further research is required to elucidate this.

Another important aspect to consider in animal research is the impact that ILW could potentially have on the interpretation of results. For example, looking at the effect of growth rate measured over long periods of time on variables measured from biological samples (e.g. blood physiology or faecal samples) taken at one point in time could lead to severe misinterpretation of results. An example with the weaner data from the present study indicates that LW measured on days 0 and 150 results in an estimated growth rate of 0.40 kg/hd per day; however, the instantaneous growth rate on day 150 was 1.20 kg/hd per day. Results and conclusions could be very different if measurements made on a blood sample obtained at day 150 are correlated
with estimated growth rates from long-interval LW measurements which, in this example, were 3-fold different. Therefore, it is recommended that studies correlating growth and LW with biological traits measure LW as frequently as possible; regarding this, our results provide specific guidance on minimum intervals required for each category.

The use of in-paddock LW measurement enables the collection of frequent data where conventional weighing procedures may not be feasible because it increases labour, reduces productivity and negatively affects animal welfare (Petherick et al., 2009). Furthermore, mustering and handling cattle could affect LW on a short-term basis by altering ruminal fill (Watson et al., 2013). In this regard, there have been multiple attempts to standardise static weighing procedures which included limiting feeding in the previous 3-5 days, weighing on 2 or more consecutive days (Watson et al., 2013) and restricting access to feed and water before weighing (Smith et al., 1982; Kirton et al., 2012). Remote automatic weighing could help farmers and researchers to overcome these constraints by collecting LW data more frequently but without handling animals. Then, data can be analysed and used to minimise LW variability (González et al., 2014) and data streams could be presented in real-time to manage LW and growth rate.

The findings of the present study could enhance cattle weighing procedures using either static scales or in-paddock technologies. For example, studies on ILW could aid to select the right frequency to muster animals to central yards while contemplating the ability to capture changes in LW and LWC and minimising labour required (Stock et al., 1983). In addition, results from the present study suggest that in-paddock weighing could be used discontinuously for collecting LW data during certain critical periods, although the ideal frequency would depend on each animal category. For instance, the same WOW system could be used to monitor different herds, or farmers could associate to purchase and use the equipment cooperatively as having WOW installed to acquire daily LW on each category of cattle can be too expensive or logistically impractical. However, further research is needed to assess if this option, which implies the use of data collected sporadically, has similar accuracy as the smoothing and outlier detection algorithms used in the present study from daily data points.

Determining the optimal ILW could enhance cattle management at different temporal scales and purposes. On a short-term basis, timely decision-making, including precision animal nutrition could be improved (González et al., 2018). For instance, quantifying the duration and extent of weight loss can be critical to determine the introduction of feed supplementation. Grazing management can also be enhanced by moving animals to another paddock based on both changes in feed availability and LWC. On a long-term basis (e.g. the entire production period), an accurate description of growth variability (SD, CV) would allow for the identification of performance boundaries while aiming to reduce such variability.

3.7 Conclusions

Remote in-paddock weighing offers a platform to study variability in cattle growth and LW patterns over time. The length of the interval between LW measures affects growth rate...
estimations and the ability to capture and quantify periods with low or high animal performance. Therefore, the present work provides first and specific guidance on minimum intervals required for each animal category (2 weeks for calves and weaners, and 8 weeks for cows). Selecting the appropriate frequency of LW data collection could enhance timely and accurate management interventions on animal nutrition and cattle operations.

3.8 References


Chapter 4

Real-time monitoring of self-fed supplement intake, feeding behaviour, and growth rate as affected by forage quantity and quality of rotationally grazed beef cattle

4.1 Overview

Chapter 2 stressed the need to assess the ability of in-paddock technologies to monitor cattle liveweight and supplement intake of cattle over long-term grazing periods (i.e. seasons) as forage conditions change. Additionally, Chapter 3 demonstrated the importance of the frequency of liveweight data collection on liveweight and liveweight change calculations. However, the ability and potential of integrating liveweight and supplement intake data measured continuously (i.e. daily) to capture the effect of changing forage quantity and quality over time has not been demonstrated in grazing young cattle. The aim of the present chapter was to study the relationship between liveweight, liveweight change, supplement intake and the quantity and quality of feed available to weaner cattle across different type of feeds and seasons.

Real-Time Monitoring of Self-Fed Supplement Intake, Feeding Behaviour, and Growth Rate as Affected by Forage Quantity and Quality of Rotationally Grazed Beef Cattle

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Simple Summary: In grazing systems, the use of novel technologies such as electronic feeders and automatic weighing systems enables collection of daily data of cattle feeding behaviour and growth. These technologies can be useful to study animal response to varying forage quantity and quality within and throughout grazing periods and seasons. The aim of this 254-day experiment was to investigate the effect of forage type, quantity, and quality on the consumption of a self-fed supplement (molasses-lick blocks (MLB)) and on the growth rate and feeding behaviour of grazing beef cattle. Results indicated that type and amount of forage affect MLB intake and feeding behaviour. Thus, when feed availability is low (e.g., forage on the paddock is depleted), animals increase consumption of and number and duration of visits to the supplementary feed. The use of MLB only improved growth rate of cattle when animals grazed sorghum and pasture or were fed oaten hay. Monitoring the feeding behaviour of animals around MLB reflects changes in forage quantity and quality.

Abstract: Supplement intake and liveweight (LW) data were collected daily and remotely by digital in-paddock technologies (electronic feeder (EF) and walk-over-weighing scale (WOW)) to study the effect of forage type, quantity and quality on the intake of a self-fed supplement (molasses-lick blocks (MLB)), LW, liveweight change (LWC), and feeding behaviour of grazing beef cattle. Fifty-two crossbred weaners were rotationally grazed or fed for 254 days on different forages: sudangrass (SG), autumn pastures (P), winter pastures with concentrate (P+C), oat crops (OC), lucerne hay (LH), and oaten hay (OH). Forage quantity and quality were measured on the day of entry (high feed availability) and exit (low feed availability) stages of grazing or hay delivery. The intake of MLB was 111% higher \( (p < 0.05) \) at low compared to high feed availability, and this was also reflected in the feeding behaviour of animals (e.g., greater feeding frequency and rate). Moreover, there was a large temporal variability of daily MLB intake (Coefficient of variation (CV) = 146.41%). Supplementing MLB improved LWC only with SG, P, or OH \((p < 0.05)\). The behaviour of animals around MLB reflects changes in forage quantity and quality and could be used to enhance cattle grazing and nutritional management in real time.

Keywords: technologies; data; supplementation; forage quantity; feeding behaviour; management
1. Introduction

Efficient and profitable pasture-based beef production systems rely on sustained animal growth rates over entire seasons. Animals are typically supplemented to cover true pasture deficits or to increase performance, but it is difficult to monitor temporal changes of supplement intake and liveweight (LW) of grazing cattle without human intervention in near real time [1]. However, recent technological advances like electronic feeders and in-paddock walk-over weighing scales (WOW) allow producers to overcome this limitation by monitoring individual animals and/or whole herd performance [2].

Electronic feeders can be also used to assess the feeding behaviour of animals (i.e., frequency and duration of single feeding events) associated with variations in supplement intake through different types of feedstuffs [3]. These technologies can be particularly important under grazing conditions, where complex interactions between forage quantity, quality, and characteristics of supplement drive intake and growth responses [4]. Additionally, feeding behaviour could be used to predict supplement intake of grazing animals. Simultaneous and frequent (e.g., daily) collection of data on supplement intake and LW can be useful for beef producers who have cattle grazing on different types of forages through the seasons, with the associated variability in liveweight gain (growth rate). Assessing cattle liveweight change (LWC) frequently (e.g., daily or weekly) and in real time could help with early interventions when changes in growth are detected. Timely management would allow producers to graze paddocks to optimise LWC, to introduce feed supplementation, and to adjust stocking rate. The WOW could also include gates for auto-drafting animals into different pens or yards, which would allow more precise feed supplementation of groups of animals for a target animal performance.

In practice, most supplements cannot be offered as “free-choice” or ad-lib due to animal health and economic reasons. However, molasses-lick blocks (MLB) are self-fed supplements which aim to control MLB intake through the block hardness [5] while offering protein, minerals, and feed additives (e.g., ionophores, urea, and oil). Previous research suggests that MLB intake and feeding behaviour of cattle can be affected by several factors, including forage quantity and quality and the availability of other feed supplements [6,7]. However, the effects of these factors have not been investigated measuring MLB intake and LWC of a group of animals with concurrent measurements of feed quantity and quality [8]. It is also known that molasses-based supplements could have positive impacts on LWC when consuming low-quality forages, potentially due to the improvements on feed digestion and dry matter intake [9,10]. However, none of these experiments measured animal responses frequently (i.e., daily or weekly LW) for a long period of time (i.e., throughout seasons) as forage quantity and quality change.

Therefore, the objectives of the present study were (a) to monitor the effects of MLB supplementation on cattle LW under grazing conditions using electronic feeder (EF) and WOW and (b) to assess the dynamic relationship between MLB intake; feeding behaviour; and forage type, quantity, and quality. We hypothesised that the intake of MLB and feeding behaviour of cattle was affected by changes in forage quantity and quality.

2. Materials and Methods

The experiment was conducted at John Pye Farm (latitude: 33°56’93’S, longitude: 150°40’47”E, Greendale, NSW, The University of Sydney). All experimental procedures were approved by The University of Sydney Animal Ethics Committee (Approval 2017/1162).
2.1. Experimental Details

Fifty-Two Charolais × Angus crossbred weaners (mean ± SD, initial LW = 178 ± 31.2 kg/hd; initial age = 187 ± 43 days) were tagged with electronic identification (EID), blocked by sex and LW, and randomly assigned to one of two treatments: (a) No MLB supplementation (NS) and (b) access to a single MLB (40 kg; 4 Season Co. Pty Ltd, Crestmead, Queensland, Australia) made available inside an EF throughout the experiment as free choice. The chemical composition of the MLB was (Dry Matter (DM) basis) Crude Protein (CP) = 8.9%, Neutral Detergent Fiber (NDF) = 2.83%, and Digestibility of organic dry matter (DOMD) = 71.5%, and the ingredient composition was 42% molasses, 9% salt, 3% urea, 3% vegetable oil, 1.3% phosphorus, 3% calcium, 4% magnesium, 15% cottonseed (Gossypium hirsutum) meal, 2% Lasalocid (Bovatec, Zoetis, Parsippany, New Jersey), 6% trace mineral mix (copper, cobalt, iodine, and zinc), and 11.7% water. A total area of 24.7 ha of pastures and annual crops was rotationally grazed for 254 days. The paddocks with annual crops (6.7 ha) were subdivided into cells of approximately 1 ha using electric fences. Total area with mixed pastures (18 ha) was subdivided into 9 paddocks, which ranged from 0.8 to 6.65 ha. The average grazing time on each paddock during the trial was 10.1 ± 5.3 days. Additionally, hay supplementation was introduced from 12 September to 22 November 2017. Total rainfall during the experiment was 239 mm, and monthly rainfall was 68.8, 10.0, 12.7, 58.0, 0.0, 22.4, 0.0, 53.7, and 13.0 from March to November, respectively. No irrigation was applied. Average stocking rate was 2.5 hd/ha ranging from 13.7 dry sheep equivalents (DSE)/ha to 23.2 DSE/ha.

Animals were grazed on temperate pastures and annual crops and were supplemented with hay and concentrate. These feed types were (a) Sudangrass (SG; Sorghum vulgare var. sudanense) grazed from day 1 (when animals were moved to the final trial location) to day 26; (b) autumn pastures (P) grazed from day 27 to 121; (c) oat crops (OC, Avena sativa) grazed from day 122 to 156; (d) winter pastures with concentrate supplementation (P+C) from day 157 to 185; (e) Lucerne hay (Medicago sativa, LH) offered from day 186 to 220; and (f) oaten hay (Avena sativa, OH) offered from day 221 to 254. It is important to stress that MLB-supplemented and NS animals co-grazed the paddocks and were sorted automatically into treatments at the entrance of the yard every time they wanted to access water (Figure 4.1).

Feed availability was classified as early (high feed availability) or late (low feed availability) during a paddock grazing which coincided with the highest or lowest forage availability per ha, respectively, or with the day when hay was delivered or not. Molasses-lick-block intake and feeding behaviour on high and low feed availability from P, OC, and P+C (for which grazing time per cell was greater than 10 days) was estimated by averaging as follows: (a) The first 2 days after entering to a fresh paddock (feed availability = high); (b) the last 2 days before leaving a paddock (feed availability = low). When paddock utilisation was shorter than 7 days, only the first and the last days were considered as high and low, respectively. Molasses-lick-block data from LH and OH supplementation was pooled from 14:00 h of the “feed delivery day” to 14:00 h of the next day (24 h period, feed availability = high). The remaining days were considered “no feeding day” until a new hay bale was delivered (14:00 h of the current day to 14:00 h of the next day; feed availability = low).

2.2. Walk-Over-Weighing and Electronic Feeder Setup

A WOW with an auto drafter gate (Precision Pastoral Ltd, Alice Spring, Northern Territory, Australia) and an EF (Smartfeed developed by C-lock Inc., Rapid City, SD, USA) were installed at the only central water point. The WOW recorded animal EID, date, time, and LW whereas the EF
recorded EID, time, date, and the weight of feed disappearing in each visit to the feeder. A description of dimensions and operation of each technology was reported by González et al. [2] and Reuter et al. [11] for WOW and EF, respectively.

A yard (15 m × 25 m) was built at a central location of the paddocks grazed in the experiment and subdivided into two equal-sized sections, each of them sharing a single water point (Figure 4.1). The yard had a single entry where the WOW was located, and each section of the yard had a single exit (Figure 4.1, numbers 5 and 6). Spear gates were used at the entry to the WOW and each exit, allowing animals to move in only one direction. The auto-drafter was placed immediately after the weighing station to allow animals to be automatically drafted into a supplemented (left) and not-supplemented (right) group. Additionally, the auto-drafter can enable all cattle to remain together as a group in the same paddock, offering equal grazing conditions. An EF was placed in the yard section of the supplemented animals on 10 April (day 28) aside the exit gate (Figure 4.1) and firmly anchored to the ground. Feeding events started to be recorded on 16 of April (day 34).

![Figure 4.1](image)

**Figure 4.1.** Image from a drone showing the water point enclosure with an in-paddock weighing station, auto-drafter gate, and electronic feeder to draft animals into a supplemented or unsupplemented group. Numbers refer to (1) enclosure entry; (2) locations of the weighing platform, auto-drafter gate, and solar powering equipment; (3) locations of the electronic feeder and solar powering equipment; (4) water point; (5) exit spear gate of supplemented animals; (6) exit spear gate of non-supplemented animals; and (7) internal enclosure to avoid electronic identification (EID) being recorded from animals inside the yard (not walking through the weighing platform).

Animals were gradually trained to use the WOW system and EF. During the first 4 weeks from weaning to the beginning of the experiment, animals could recognise and walk through a WOW following the training procedure proposed by Gonzalez et al. [2]. Finally, animals were moved to the
final trial location and randomly allocated to one of two treatments described above. The EF contained a pneumatic gate, which was left continuously open for 2 weeks, so the animals could recognise the feed bin as the feed container. At week 3, the pneumatic gate was set to close halfway (50% closed) and to open when an animal approached it to eat. Finally, the feeder gate was set to allow full closing on week 4. Feeding behaviour and MLB intake were recorded from the first day after installation. Animal attendance to the water point, the auto-drafter operation, and feeder usage were monitored daily, analysing the list of animals that have been read by both the WOW and EF. During the first 6 weeks of the experiment, supplemented animals had one available MLB outside the EF, placed in a central location of their yard, to expose all animals to the supplement. Disappearance of the MLB placed outside was recorded manually by weighing the block three times per week (Monday, Wednesday, and Friday at 10:00 a.m.).

2.3. Grazing and Supplementation Management and Measurements

Concentrate and hay supplementation were introduced due to reduced grass growth during the winter and due to drought and were delivered infrequently on Monday, Wednesday, and Friday. Pellets (CP: 16.2%; NDF: 35.3%; DOMD: 68.7%) and chopped lucerne-chaff (CP: 15.8%; NDF: 41.1%; DOMD: 54%), mixed in a proportion of 75:25, were offered from 07 August (day 147) to 12 September (day 183) at a rate of 1.25 kg as fed/hd per day. While feeding hay (periods E and F), animals also had a pasture paddock available under continuous grazing. Pre-grazing forage quantities were 1130 and 830 kg DM/ha to a base of 5 cm at the beginning of periods E and F, respectively. During these periods E and F, square bales were weighed prior to delivery (375 kg as fed for LH and 425 kg as fed for OH) and its availability was monitored daily by placing automatic camera traps taking a single photo of the hay every five minutes. Hay wastage was estimated on six bales per feed type by weighing the remaining hay immediately before offering a new bale and by correcting by the DM content of the collected material. The estimated values of hay utilisation efficiency were 94 and 78% for LH and OH, respectively. Additionally, two samples of 300 g of hay were taken on each feeding day and a weekly composite sample was obtained for chemical analysis after mixing.

The pasture mix contained perennial ryegrass (Lolium perenne), fescue (Festuca arundinacea), white clover (Trifolium repens), Cocksfoot (Dactylis glomerata) and Chicory (Chicorium intybus), Kangaroo grass (Themeda triandra Forsk syn australis), Paspalam (Paspalum dilatatum Poir.), Purple pigeon grass (Setaria incrassate cv. Inverell), Setaria (Setaria sphacelata var. seric), and Rhodes grass (Chloris gayana Kunth). Predominant species were Fescue, Cocksfoot, Paspalam, and Rhodes grass. Annual crops (SG and OC) were double cropped on the same location; previous fertilisation was with 50 kg/ha of nitrogen, 22 kg/ha of phosphorus, and 50 kg/ha of potassium before sowing for each crop. Animals were moved to a fresh paddock when forage quantities to the base of 5 cm were approximately 1500, 1000, and 750 kg DM/ha for SG, pastures, and OC, respectively. For SG, ten 0.5 m² quadrats were cut manually before and after each paddock was grazed. For pastures and OC, forage quantity was measured using an electronic plate meter (EC20, NZ Agriworks Ltd, Feilding, New Zealand). For this purpose, a calibration model between forage quantity (kg/DM per ha) and pasture height measured with the plate was done by manually cutting to the base of 5 cm fifty 0.25 m² quadrats for each forage type. Linear models were obtained and used to estimate forage DM quantity (R² = 0.73 for pastures and R² = 0.83 for OC). Forage sampling for chemical composition consisted of six cut samples taken to the base of 5 cm with a 0.25 m² quadrat before and after grazing of each paddock. The first two samples were used to determine DM content (%) and the next two
samples were used to analyse chemical composition. The last two samples were used to determine the proportion of green and dead material by separating both fractions manually prior to drying.

2.4. Chemical Analysis

The DM of forage samples were determined by drying at 60 °C for 72 h in a forced-air oven (Heraeus, D-63450 Hanau). Samples for chemical analysis were dried following the same procedure and ground through a 1-mm sieve screen prior to analysis (Restsch, SM 100). Chemical composition (NDF, Acid Detergent Fiber (ADF), CP, ash content, organic matter (OM), dry matter digestibility (DMD), and DOMD) of forage samples were estimated using near-infrared spectroscopy (The Feed Quality Service, Wagga Wagga Agricultural Institute, Department of Primary Industries, Wagga Wagga, Australia) using a Bruker MPA FT-NIR instrument in conjunction with OPUS ver. 7.5.18 (Bruker Optik GmbH, Ettlingen, Germany). Samples obtained from MLB and pellets were analysed for NDF and ADF in accordance with Reference [12] using an Ankom 220 model fiber analyser (ANKOM, Technology Macedon, NY, USA). Nitrogen concentration was determined using the DUMAS method according to AOAC Crude Protein 990.03 on a LECO Trumac combustion Analyser (LECO Corporation, Saint Joseph, Michigan, USA) and using a factor of 6.25 for conversion to CP. Dry matter digestibility was analysed using the Pepsin–Cellulase Method [13].

2.5. Statistical Analysis

The statistical analysis of forage data from grazed paddocks was performed using a mixed-effects linear regression model with paddock as random effect and feed type and feed availability 2-way interactions as fixed effects. Forage data during hay feeding were analysed separately using a mixed-effects linear regression model with feed type as fixed effect and week of sampling as repeated measures.

Daily MLB intake and feeding behaviour data from individual animals were averaged by feed type (P, P+C, OC, LH, and OH) and feed availability (high and low) and then analysed using mixed-effects linear regression models including feed type, feed availability, sex, and 2- and 3-way interactions as fixed effects with animal as random factor. Daily MLB intake, feeding frequency, and feeding duration data were transformed to log10 prior to analysis.

Liveweight data were analysed following the procedure proposed by Gonzalez et al. [2], which consists of deleting records containing missing EID, extreme weights, and fitting data to penalised B-Splines for each individual animal. Animal growth rate (LWC, kg/hd per day) was calculated as the first derivative of the predicted LW curve, and the resulting LW data were averaged by date for each animal if more than one measurement per day and animal existed. The statistical analysis of LW and LWC was done using linear mixed-effects models where MLB supplementation, sex, date, and 3-way interactions were fixed effects and animal was a random effect.

Data from EF were analysed following the next steps: (1) Records without EID were deleted (n = 457, 12%); (2) records with negative feed intake were deleted (n = 652, 17%); (3) a correlation model between time (sec) and MLB intake (g) was fitted, and residuals were calculated; and (4) records with residuals lower or higher than 3 were deleted (n = 20 adding up to 69.52 kg of MLB). Feeding records in the final dataset had a mean ± SD of 0.169 ± 0.213 kg, whereas deleted feeding events had 3.47 ± 2.17 kg. Resulting MLB intake data (n = 2661 visits) were summed by date for each animal (g/hd per day). Also, daily feeding frequency (visits/hd per day), feeding duration (min/hd per day), visit length (min/visit), and visit size (g/visit) were calculated. Feeding rate was calculated for each day
dividing MLB intake (g/hd per day) by total duration (min/hd per day). Daily MLB intake was transformed to log₁₀ and analysed using mixed-effects linear regression models including date, sex, and 2-way interactions as fixed effects with date as the repeated factor for each animal. Covariance structure was selected based on the lower Bayesian criterion (BIC). Least square means were calculated, and differences between means were corrected for multiple comparisons using Bonferroni test. Statistical significance was declared at \( p < 0.05 \). Coefficient of variation (CV) of daily MLB intake was calculated using values obtained from the linear model. All linear model procedures were done using SAS Software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Feed Quantity and Quality

Forage quantity and quality differed across feed types as cattle grazed different pastures throughout the seasons \( (p < 0.05; \text{Figure 4.2 and Table 4.1}) \). However, the interaction between feed type and feed availability \( (p < 0.001; \text{Figure 4.2a}) \) indicated that the difference between pre- and post-grazing was larger for SG compared to P and OC (Figure 4.2, \( p < 0.05 \)). Oats winter crop was grazed in an early vegetative stage, resulting in the highest proportion of green forage quantity both pre- and post-grazing \( (p < 0.01) \). Pre- and post-grazing forage quantity did not differ for P+C \( (p > 0.05) \). In contrast to forage availability, no feed type × feed availability interaction \( (p > 0.05) \) was observed for CP, NDF, ADF, DMD, and DOMD during these grazing periods (Table 4.1). However, forage quality was lower post- compared to pre-grazing for all feed types \( (p < 0.05; \text{Table 4.1}) \). Oaten hay had higher forage quantity \( (p < 0.05; \text{Figure 4.2b}) \) and lower quality (CP, NDF, ADF, and DMD) compared to LH \( (p < 0.05; \text{Table 4.2}) \).
Table 4.1. Chemical composition of grazed forages before (pre-) and after (post-) grazing by cattle offered supplementation with molasses-lick blocks or not.

<table>
<thead>
<tr>
<th>Items</th>
<th>Sudangrass (SG)</th>
<th>Pastures (P)</th>
<th>Oat Crops (OC)</th>
<th>Pastures + Concentrate (P+C)</th>
<th>p-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>CP (%)</td>
<td>12.6 ± 1.18</td>
<td>7.91 ± 1.182</td>
<td>5.91 ± 0.892</td>
<td>14.5 ± 1.53</td>
<td>7.18 ± 1.531</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>63.3 ± 1.56</td>
<td>66.6 ± 1.56</td>
<td>66.5 ± 1.18</td>
<td>71.5 ± 1.32</td>
<td>28.0 ± 2.54</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>33.5 ± 1.28</td>
<td>37.9 ± 1.28</td>
<td>38.2 ± 0.86</td>
<td>42.4 ± 0.96</td>
<td>14.6 ± 1.85</td>
</tr>
<tr>
<td>DM (%)</td>
<td>19.9 ± 3.28</td>
<td>23.7 ± 3.28</td>
<td>33.29 ± 2.59</td>
<td>46.2 ± 2.72</td>
<td>19.4 ± 2.99</td>
</tr>
<tr>
<td>OM (%)</td>
<td>89.7 ± 0.57</td>
<td>91.4 ± 0.57</td>
<td>91.7 ± 0.38</td>
<td>92.6 ± 0.42</td>
<td>94.0 ± 0.83</td>
</tr>
<tr>
<td>DMD (%)</td>
<td>59.2 ± 1.64</td>
<td>52.9 ± 1.64</td>
<td>55.7 ± 1.05</td>
<td>48.2 ± 1.35</td>
<td>92.8 ± 2.30</td>
</tr>
<tr>
<td>DOMD (%)</td>
<td>57.0 ± 1.40</td>
<td>51.7 ± 1.40</td>
<td>54.0 ± 0.90</td>
<td>47.6 ± 1.15</td>
<td>85.4 ± 1.94</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>10.2 ± 0.57</td>
<td>8.60 ± 0.571</td>
<td>8.30 ± 0.394</td>
<td>7.38 ± 0.427</td>
<td>5.97 ± 0.838</td>
</tr>
</tbody>
</table>

*ab Means ± SE with different letters statistically differ (P < 0.05); CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; DM = Dry Matter; OM = Organic Matter; DMD = Dry Matter Digestibility; DOMD = Digestibility of organic dry matter.
Table 4.2. Chemical composition of hay offered to cattle during supplementation with molasses-lick-blocks or not.

<table>
<thead>
<tr>
<th>Items</th>
<th>Hay Offer</th>
<th>p-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lucerne (LH)</td>
<td>Oaten (OH)</td>
</tr>
<tr>
<td>CP (%)</td>
<td>21.9 ± 1.39</td>
<td>7.38 ± 1.141</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>32.6 ± 1.91</td>
<td>63.2 ± 2.34</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>24.3 ± 2.20</td>
<td>34.9 ± 1.79</td>
</tr>
<tr>
<td>DM (%)</td>
<td>92.2 ± 2.53</td>
<td>93.5 ± 2.75</td>
</tr>
<tr>
<td>OM (%)</td>
<td>88.9 ± 0.94</td>
<td>93.3 ± 0.77</td>
</tr>
<tr>
<td>DMD (%)</td>
<td>71.2 ± 3.07</td>
<td>58.0 ± 2.50</td>
</tr>
<tr>
<td>DOMD (%)</td>
<td>67.1 ± 2.60</td>
<td>56.0 ± 2.13</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>11.1 ± 0.94</td>
<td>6.7 ± 0.772</td>
</tr>
</tbody>
</table>

Figure 4.2. Forage quantity during periods of grazing (a) and hay feeding (b) of cattle supplemented with molasses-lick blocks or not: Grazing periods include forage quantity before (pre) and after (post) in each paddock grazing period for each feed type. Hay-feeding periods include total feed availability (hay + forage from direct grazing) on days of hay delivery (Monday, Wednesday, and Friday). Different letters indicate significant differences (p < 0.05) between pre- and post-grazing (Figure 4.2a) and type of hay (Figure 4.2b). Asterisks indicate significant differences (*P < 0.05; ***P < 0.001).

3.2. MLB Supplement Intake

Herd average daily MLB intake was affected by date and sex (p < 0.05), but their interaction (p > 0.10) ranged on average from 0 g/hd per day during the pasture period to 705.50 while on OH, with a large variability through time (CV = 146.41%; Figure 4.3a). Average of MLB intake over the entire period was 74.04 ± 17.40 g/hd per day being greater (p < 0.05) in steers compared to heifers (78.54 ± 4.18 vs 67.80 ± 5.06 g/hd per day for steers and heifers, respectively). Feeder attendance varied from
0 to 77.80% of the total number of animals in the supplemented group (OH), and 3 animals never registered a visit to the feeder (Figure 4.3b). Average MLB intake among individual animals over the trial varied from 194.7 to 0 g/hd per day.

Herd averages of MLB intake, feeding rate, visit length, and visit size were affected by feed availability and feed type ($p < 0.05$, Table 4.3). These feeding parameters were lower at high compared to low feed availability ($p < 0.05$, Table 4.3). In addition, MLB intake and feeding rate were higher while on OH compared to the rest of the feed types ($p < 0.001$, Table 4.3). Additionally, the intake of MLB was greater ($p < 0.05$) with low compared to high feed availability for all feed types except for OH ($p > 0.05$; Figure 4.4a). Sex did not affect MLB intake or feeding behaviour ($p > 0.10$). Feeding frequency and duration were affected by feed availability × feed type interaction ($p < 0.05$, Figure 4.4). Feeding frequency and feeding duration were lower at high compared to low feed availability while on P, OC, P+C, and LH ($p < 0.05$, Figure 4.4) but not while on OH ($p > 0.10$, Figure 4.4b, c).

**Figure 4.3.** Daily molasses-lick-block (MLB) (a) intake (group average) and (b) feeder attendance (% of total herd) of cattle in a rotational grazing system: Animal attendance represents the number of animals consuming MLB within a day divided by the total number of animals of the herd. Feed types offered: P, Pastures; OC, Oat crops; P+C, Pastures with concentrate supplementation; LH, Lucerne hay; OH, Oaten hay.
Table 4.3. Feeding behaviour of cattle consuming molasses-lick-block supplement while grazing different types of forages. Means without a common letter differ (p < 0.05).

<table>
<thead>
<tr>
<th>Items</th>
<th>Feed Availability</th>
<th>Feed Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIGH</td>
<td>LOW</td>
</tr>
<tr>
<td>MLB Intake (g/hd per day)</td>
<td>53.7</td>
<td>112.8</td>
</tr>
<tr>
<td>Feeding Frequency (g/hd per day)</td>
<td>0.34</td>
<td>0.61</td>
</tr>
<tr>
<td>Feeding Duration (g/hd per day)</td>
<td>2.12</td>
<td>4.02</td>
</tr>
<tr>
<td>Feeding Rate (g/min)</td>
<td>19.6</td>
<td>24.0</td>
</tr>
<tr>
<td>Visit length (min/visit)</td>
<td>4.92</td>
<td>6.18</td>
</tr>
<tr>
<td>Visit Size (g/visit)</td>
<td>107.7</td>
<td>153.4</td>
</tr>
</tbody>
</table>

a,b,c,d Means with different letters statistically differ (P < 0.05).
3.3. Animal Performance

Overall, means LWC throughout the study were 435 ± 26 and 529 ± 25 g/day for NS and MLB groups (p < 0.05), respectively, representing an 18% increment with MLB. Liveweight change was not affected by sex or its interactions (p > 0.10). However, there was a significant interaction between MLB supplementation and time for LWC and LW (p < 0.05; Figure 4.5). Supplemented animals had greater LWC compared to NS animals only while on SG, P, and OH (p < 0.05, Figure 4.5). In addition, supplemented animals were heavier (+23.5 kg/hd) than NS animals.
animals at the end of the trial \((p < 0.05; \text{Figure 4.5})\). Liveweight was affected by sex being higher in steers compared to heifers \((p < 0.05; \text{data not shown})\).

![Figure 4.5](image.png)

**Figure 4.5.** Daily liveweight change (LWC) \((a)\) and liveweight (LW) \((b)\) of cattle supplemented with molasses-lick blocks (MLB; solid line) or not supplemented (NS; broken line). Feed types offered: P, Pastures; OC, Oat crops; P+C, Pastures with concentrate supplementation; LH, Lucerne hay; OH, Oaten hay). * Means differ \((* p < 0.05)\).

4. Discussion

The objective of the present trial was to quantify the dynamic relationship between MLB intake; cattle growth rate (group averages); cattle feeding behaviour; and forage type, quantity and quality by monitoring the effects of MLB supplementation on cattle LW under grazing conditions using EF and WOW. Continuous recording of LW and MLB intake enabled monitoring livestock throughout seasons and, therefore, to report the dynamic responses to type, quantity, and quality of feed available to them. One of the novel findings of the present study was that voluntary MLB intake and feeding behaviour are, on average, sensitive to changing types of forage, which can be useful to monitor and improve the nutritional management of animals. Such changes could go undetected or could be detected later without the combination of EF and WOW. To this effect, variations in MLB intake due to changes in forage quantity and quality could be detected by direct monitoring of block disappearance and MLB feeding.
behaviour (e.g., average daily feeding and duration). Moreover, results showed that LWC was highly variable across and within feed type because selective grazing decreases forage quantity and quality and that MLB supplementation was identified as one of the factors influencing LWC. In-paddock weighing combined with EF was shown to be useful to monitor the dynamic responses of cattle [1,3] in which actual consumption of the supplementary feed occurred only during certain times instead of the entire period. Remote monitoring of daily temporal variations of growth, liveweight, and supplement intake could help enhance the management of group-fed cattle.

Findings from the present trial suggest that feeding behaviour around MLB, including average feeding duration and frequency, reflect changes in MLB intake as a result of changes in feed quantity and quality. Therefore, remote monitoring of feeding behaviour around supplements could be useful to improve grazing management by reflecting such changes. To the best of our knowledge, this is the first study assessing the relationship between MLB feeding behaviour of individual animals and fed as a group and the quantity and quality of forage available measured over a long-term grazing study. Previous studies [14,15] also reported that the intake of molasses-based supplements can be influenced by forage availability but that feeding behaviour has not been adequately addressed in those studies [6]. Moreover, feeding rate was affected both across and within feed types, increasing at low feed availability even during short periods of rotational grazing (e.g., OC) or during hay supplementation. In addition, feeding rate increased when animals were consuming low-quality OH independently of forage availability. Previous studies reported that feeding rate is a robust indicator of feed characteristics, hunger, social competition, and feeding management [16]. Therefore, the greater MLB intake and feeding rate reported during low measurements in pastures and OH suggests animals were hungrier during such time periods due to low availability of nutrients.

However, feeding behaviour needs to be interpreted in relation to the type of supplement offered (MLB). The use of MLB aims to control intake and feeding rate through the block hardness, forcing animals to lick the supplement to consume it [5]. Thus, we expected that the ability of animals to increase feeding rate of MLB was limited compared to other supplements such as fodder or concentrates. However, licking rate per se was not measured and the results may also be affected by the composition of the block (i.e., % of CP, urea, and block hardness). Further studies are required to determine the ability of animals and the time required to change feeding rate according to changes in MLB composition. In line with this, Bowman et al. [6] concluded that the correlations between feeding duration, feeding frequency, and intake found with loose supplements could not be extrapolated to molasses supplements. Finally, feeding frequency and duration of MLB in the present study did not differ between high and low feed availability when animals were offered OH, suggesting that low-quality forages fed continuously could increase the basal attendance of animals to MLB. Thus, our results suggest that combining variations in feeding parameters of MLB could be useful, for example, as part of a procedure to automate the monitoring of forage quantity and quality.

An innovative aspect of this study is the simultaneous monitoring of LW and MLB intake, which has several implications for results interpretation. In this regard, MLB supplementation was one of the factors influencing LWC only during some periods of time. In addition, both MLB intake and the proportion of animals attending the feeders, which directly affects MLB intake, proved to be highly variable throughout the trial (within and across feed types). Variations in MLB could be a preliminary approach to future studies linking the increase of free-choice supplements with variations in LWC to anticipate further reductions, for example, by rotating animals to another paddock. Frequent and automatic data collection allows detecting periods when the effects of feed supplementation are more
accentuated. This could be a relevant aspect as infrequent LW measures at group level (e.g., months) were probably masking supplementation periods with no responses in previous studies [6,8].

Positive effects of MLB supplementation on LWC were observed during periods when low- to medium-quality forages (SG, pastures, and OH) were offered but not with high-quality forages (LH and OC). Similar responses to molasses-based supplements were reported previously [6,10] and linked to improved forage intake and digestion [9]. Nevertheless, these results should be interpreted with caution as LWC responses and MLB intake between individual animals could largely differ, as we show in a companion paper (unpublished data) [17]. In addition to the nutritional benefits of supplementing MLB, ionophores included in the blocks (Lasalocid sodium) could have been effective to control common diseases of young cattle, such as coccidiosis, while improving feed efficiency [18,19]. The availability of minerals from MLB could also influence LWC of weaners as minerals are usually deficient in most forages in comparison to the high requirements of young growing cattle [20]. However, intake of minerals may be affected by the lack of uniform consumption by animals when it is offered as a free choice [21]. Therefore, both nutritional and health impacts of MLB could explain the better performance of MLB-supplemented animals, particularly at the beginning of the present trial as young cattle were introduced to new feed types after weaning. However, further research is needed to establish and quantify cause–effect relationships, e.g., the incidence of coccidiosis in grazing animals fed MLB with Lasalocid.

Remote LW monitoring of a group of animals proved to be highly relevant to identify average changes in daily LWC, even within the same feed type. In this regard, the use of in-paddock weighing may offer a novel platform to better understand the seasonal complexity (forage quantity and quality) driving animal growth. It also reveals that managing nutrition to reduce LWC variability can be a challenging task, as animals could rapidly respond and adjust their LWC to factors modifying it. Additionally, monitoring the entire growth path of animals can be used to study the effects of previous growth on their current performance. For example, animals showing the same LWC may have different nutritional requirements and metabolic responses if they are experiencing an ascendant (e.g., OC and P+C) or descendant (e.g., LH) path of growth. Detecting these changes in LWC could also be crucial to understand periods of compensatory growth in cattle.

5. Conclusions

Average MLB intake of supplemented animals varied both between and within forage type. Such variations can be detected remotely in near real time using remote sensing, suggesting that continuous and simultaneous, automated monitoring of LWC and supplement intake could be useful to monitor changes in forage quantity and quality over time under grazing conditions.

Author Contributions: All authors listed (J.A.I., S.G., and L.A.G.) had participated in the data analysis, interpretation of results, and writing of the manuscript.

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6. References


Chapter 5

Application of in-paddock technologies to monitor individual self-fed supplement intake and liveweight in beef cattle

5.1 Overview
Chapter 4 demonstrated the ability of in-paddock technologies to continuously monitor liveweight and supplement intake over time reflecting changes in the quantity and quality of the feed available. In addition, the impact of molasses-lick-block supplementation on growth rate of group-fed growing cattle was demonstrated. However, supplement intake and growth responses to supplementation could largely differ among individual animals grazing as a group. It was hypothesised that in-paddock weighing and electronic feeders enable individual measurement of liveweight and supplement so the relationship between them could be explored over time. Therefore, there is a need to determine the ability of both technologies to capture the potential relationship between supplement intake and performance of individual animals, and of the type of forage on these relationships. The present chapter aims to measure the intake of a self-fed supplement, and liveweight and feeding behaviour of individual animals remotely to study their relationships.

Application of In-Paddock Technologies to Monitor Individual Self-Fed Supplement Intake and Liveweight in Beef Cattle

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Received: date; Accepted: date; Published: date

Simple Summary: Individual, daily and simultaneous measures of key variables to manage cattle have been traditionally difficult to achieve in grazing animals. However, nowadays, this could be achieved using technologies which are placed ‘in paddock’ such as automated weighing scales to measure liveweight (LW) and electronic feeders (EF) to measure supplement intake. We used both technologies to study the interplay between the intake of a self-fed supplement (molasses-lick blocks, MLB), growth, and feeding behaviour of individual animals fed a sequence of different feed types. We identified a large individual variability in MLB intake with some animals consuming supplement regularly while others not consuming supplement at all. Regular consumers tended to grow more rapidly. Additionally, our results indicate that animals’ MLB intake can be predicted using the number of visits to the EF and their duration. In-paddock technologies could aid to quantify key factors, such as individual variability of supplement intake and LW, that would otherwise remain undetected.

Abstract: The aim of this study was to assess the ability of in-paddock technologies to capture individual variability of self-fed supplement intake (molasses-lick blocks, MLB), feeding behaviour, and liveweight (LW) in grazing beef cattle. An electronic feeder (EF) and in-paddock walk-over-weighing system (WOW) were installed to measure, daily and simultaneously, individual MLB intake and LW. Cattle grazed (pastures and oat crops) and were fed (lucerne and oaten hay) during a 220-day trial. Over the entire period, we were able to quantify a large variability in MLB intake between individuals (p < 0.01; ranging from 0 to 194 g/hd per day). Liveweight change (p < 0.05, R = 0.44) and feeding behaviour (e.g., feeding frequency and duration, p < 0.01; R² > 0.86) were positively correlated with MLB intake over the entire period but these correlations seemed to be affected by the type of feed. The intake of MLB seems to be explained by the individual behaviour of animals rather than the entire group. The use of in-paddock technologies enabled remote measurement of variability in supplement intake and cattle growth. The ability to monitor LW and feeding behaviour of individual animals in a group could allow automatic individualised feeding of grazing cattle (amount and type of supplement) and managing low-performing animals under grazing conditions.

Keywords: technologies; individual; supplement intake; liveweight; monitoring; automate
1. Introduction

Managing the productivity of supplemented grazing beef cattle has been challenging. This is mostly because of the large variability in liveweight (LW) and supplement intake that exists between and within individual animals [1,2]. However, labour and time constraints have prevented the frequent measurement (e.g., daily, weekly) of these variables on individual animals without altering their normal feeding behaviour and welfare [3,4]. As a result, frequency of data collection was, in many cases, lower than that required to precisely describe individual variation of LW and supplement intake which were traditionally measured on group instead of individual basis [5]. The use of ruminal markers is impractical to measure individual supplement intake accurately for periods longer than 2 weeks [6,7]. Furthermore, the lack of individual data restrains correlating feeding and performance variables throughout seasons.

Nowadays, digital technologies such as electronic feeders (EF) and in-paddock walk-over-weighing scales (WOW) could be used to measure individual supplement intake and LW of cattle in near real-time and without human intervention [8]. Electronic feeders can also record feeding behaviour including frequency, duration, and size of every single feeding event [9]. This technology-based approach could enable the study of free choice (self-fed) supplement intake, such as molasses-lick-blocks (MLB). These MLB can provide energy, protein, minerals, medication, and additives while controlling supplement intake through the block hardness [10].

Only a few studies reported on feeding behaviour of self-fed supplements using EF [9,11] whereas no studies were found that reported the response of MLB-supplemented beef cattle fed with a range of forage types over time. Monitoring individual feeding behaviour might have the potential to predict MLB intake when measuring block disappearance is not possible. Integrating data streams obtained from EF and WOW could allow identification of poor performing animals and determine if their lower performance is related, among other factors, to supplement intake.

The lack of individual and simultaneous measures of supplement intake and LW had limited previous research on molasses-based supplements to explore differences between animals [5]. Previous research indicated that individual supplement intake of group-fed cattle can largely exceed the target amount in some animals while others do not consume any supplement [1]. Particularly, studies were not conclusive on whether molasses-based supplementation has positive effects on liveweight change (LWC) or not. Improved LWC was mostly reported while feeding low-quality forages, however, others have not demonstrated any positive effect of MLB in LWC of animals fed either low- or high-quality forages [1,5].

The aims of the present study were to: (a) measure individual intake of supplement (MLB) and liveweight remotely; and (b) integrate MLB intake, feeding behaviour (EF) and liveweight (WOW) data to monitor their relationships. We hypothesised that in-paddock feeding, and animal weighing systems could allow measuring the relationship between MLB intake and performance in real-time of individual animals feeding a sequence of feedstuffs for long periods of time.

2. Materials and Methods

All experimental procedures were approved by the institutional Animal Ethics Committee from The University of Sydney (Approval 2017/1162).

2.1. Experimental Details
A feeding trial was carried out for 220 days (from 16 April to 22 November 2017) using weaned beef cattle (Charolais × Angus crossbred) between 6 to 7 months of age (n = 27, 11 heifers and 16 steers; Initial LW = 190 ± 34.1 kg). Cattle were tagged with electronic identification (EID) and grazed rotationally on 24.7 ha divided into 18 paddocks of temperate pastures and oat crops at John Pye Farm (Greendale, NSW, Australia). Hay and concentrate supplementation were introduced due to drought. All animals were allowed to lick a single molasses-lick block inside an EF allowing for ad-libitum consumption during the entire trial period (40 kg block; 4 Season Co. Pty Ltd., Crestmead, QLD, Australia). Further details regarding the composition of pastures and nutritional value of supplements are reported later in this manuscript. The chemical composition of MLB (DM basis) was 8.9% of CP; neutral detergent fibre, NDF: 2.83%; and digestibility of organic dry matter, DOMD: 71.5%; and the ingredient composition was 42% molasses, 9% salt, 3% urea, 3% vegetable oil, 1.3% phosphorus, 3% calcium, 4% magnesium, 15% cottonseed meal, 2% Lasalocid (Bovatec, Zoetis, Parsippany, NJ, USA), 6% trace mineral mix (copper, cobalt, iodine, and zinc), and 11.7% water. Total rainfall during the trial was 170.2 mm.

2.2. In-Paddock Measurements and Yard Setup

A figure showing the layout of the yard setup is given in Imaz et al. [12]. A two-section yard centrally located to the paddocks (15 × 25 m) was built, enclosing the only water point, where both WOW and EF were located. An in-paddock WOW station with an auto drafter gate was placed at the entry of the yard to record LW, EID, date and time (Precision Pastoral Ltd., Alice Spring, Northern Territory, Australia). The WOW consisted of a platform (0.8 m width × 2.4 m length) placed over two load bars and mounted along wooden panels (3 m length × 2 m height) on both sides. Spear gates were used at the entry of the WOW and each exit gate to allow animals to move in only one direction. Animals were trained to use the WOW following the procedure proposed by [13] during the first 4 weeks after weaning and finally moved to the final trial location on 13 March.

An EF was installed, immediately before the exit gate, to record EID, time, date and MLB disappearance (Smartfeed developed by C-lock Inc., Rapid City, SD, USA). Only supplemented animals (n = 27) had access to the EF because animals were drafted (WOW gate) to either the right or left section of the yard depending on the treatment each animal was allocated. Animals that were not supplemented (NS, n = 25) were grazing together with the MLB-supplemented group but drafted to a different yard and results were reported in a companion paper [12]. A single MLB was placed inside the supplemented yard to expose all animals to the supplement from 13 March to 16 April when records started to be recorded. Animals were trained to use the EF leaving the pneumatic gate continuously open for 2 weeks. At week 3, the pneumatic gate was set to close half way (50% closed) and finally set to close fully on week 4. Up to our knowledge, this model of EF was not used before for measuring the individual intake of MLB by cattle. However, the same EF was recently used by Reuter et al. [9] and Williams et al. [11] to measure the individual supplement intake of cattle which ranged from 0 to 2.78 kg/hd per day. In the present study, the EF was calibrated twice a week on average by comparing the weight reported by the system with a known weight (10 kg). In addition, weights that were too high were identified using video cameras and found to be the result of animals hitting the bin resulting in abrupt changes of the weight recorded by the system. Similar observations of animals pushing on the feeder bin were reported by Reuter [9]. In addition, comparisons between MLB disappearance and the total intake calculated as the sum of the intake of all visits by each individual animal were carried out to monitor the accuracy of the system. For example, the total MLB
disappearance during a 4-week period (from 1 July to 28 July 2017) was 20.85 kg whereas the calculation of the sum of intake of all feeding events in the same period was 20.30 kg.

2.3. Nutritional Management

Animals grazed pasture and forage crops, and fed concentrates depending on forage availability, which resulted in the following distinct periods: (a) grazing autumn temperate pastures from day 1 to 87 (Pastures, 7.37% CP, 68.78% NDF, 52.01% DOMD; 16 April to 12 July); (b) grazing oat crops from day 88 to 121 (OC, *Avena sativa*; 10.83% CP, 34.60% NDF, 80.50% DOMD; 13 July to 16 August); (c) grazing winter pastures with concentrate supplementation from day 122 to 147 (P+C, 11.42% CP, 49.60% NDF, 68.45% DOMD; 17 August to 12 September); (d) fed lucerne hay from day 148 to 180 (LH, *Medicago sativa*; 5.5 kg/hd per day, 21.80% CP, 32.65% NDF, 67.15% DOMD; 13 September to 17 October); (e) fed oaten hay from day 181 to 220 (OH, *Avena sativa*; 7.6 kg/hd per day, 7.38% CP, 63.22% NDF, 56.00% DOMD; 18 October to 22 November). The predominant forage species of ‘Pastures’ and ‘P+C’ were perennial ryegrass (*Lolium perenne*), fescue (*Festuca arundinacea*), white clover (*Trifolium repens*), cockfoot (*Dactylis glomerata*), chicory (*Chicorium intybus*), kangaroo grass (*Themeda triandra* Forsk syn *australis*), paspalum (*Paspalum dilatatum* Poir.), purple pigeon grass (*Setaria incrassata* cv. Inverell), setaria (*Setaria sphacelata* var. seric), and Rhodes grass (*Chloris gayana* Kunth). Fescue, cockfoot, paspalum, and Rhodes grass were the most predominant species.

Under grazing conditions, animals were moved to a fresh paddock when forage availability to the base of 5 cm from the ground level was approximately 1000 and 750 kgDM/ha for pastures (P, P+C) and OC paddocks, respectively. Detailed information regarding pasture measurements are provided in Imaz et al. [12]. Briefly, forage quantity was measured using an electronic plate meter (EC20, NZ Agriworks Ltd., Feilding, New Zealand) which was previously calibrated based on the type of forages measured. Square bales of hay were offered each week on Monday, Wednesday, and Friday (LH = 350 kg/bale and OH = 425 kg/bale). Hay bales were first placed in a fresh pasture paddock, where animals remained until the end of the experiment, when forage availability to the base of 5 cm from the ground level was 1130 and 830 kgDM/ha at the beginning of LH and OH, respectively. Hay consumption and wastage was estimated by weighing six individual bales of each hay before delivery and then weighing the remaining hay, immediately before offering a new bale. Dry supplementation (C) was offered on Monday, Wednesday, and Friday in a separate feedbunk during period P+C at a rate of 1.25 kg/hd per day by mixing pellets (16.2% CP; 35.3% NDF; 68.7% DOMD) and chopped lucerne-chaff (15.8% CP; 41.1% NDF; 54% DOMD) mixed in a proportion of 75:25. Chemical composition analyses of forages offered, concentrate and MLB was performed following the procedures reported in a companion paper by Imaz et al. [12].

2.4. Statistical Analysis

Liveweight data, recorded by the WOW, were filtered for outlying data using methods described by González et al. [13]. Growth rate (i.e., LWC, g/hd per day) was calculated as the first derivative of each predicted LW over the entire period. Then, LW data were averaged by date for each animal if more than one measurement per day and animal existed. The total number of single visits recorded by the EF (n = 3790; including those records without EID and negative records) were analysed following the next steps: (1) Records without an EID were deleted (n = 457); (2) Records with negative intakes were deleted (n = 652); (3) The resulting feeding records (n = 2681), a correlation model between time (sec) and MLB intake (g) was fitted and studentized residuals calculated for each
feeding event; (4) Records with residuals lower than −3 or higher than 3 were deleted (n = 20; adding up to 69.52 kg of MLB). Feeding records (i.e., single visit of an animal to the EF) in the final dataset had a mean ± SD of 0.169 ± 0.213 kg/visit whereas feeding records that were deleted had 3.47 ± 2.17 kg/visit.

Supplement intake per visit (g/visit), number of visits per day and visit length (min/visit) were added up by date for each animal to obtain daily values (daily MLB intake, g/hd per day; daily feeding frequency, visits/hd per day; daily feeding duration, min/hd per day). Visit size (g/visit), visit length (min/visit) and feeding rate (g/min) were calculated from daily values. Data from WOW was also used to calculate the attendance to the water point (WOW attendance, visits/hd per day).

Liveweight and MLB intake data were also used to calculate average values for each animal throughout the entire trial period and a Pearson correlation analysis was performed. Coefficient of variation (CV) between animals and coefficient of determination ($R^2$) between MLB intake and feeding behaviour for each feed type were calculated (i.e., for each period with different basal diet). A value of zero was used for those animals which did not consume supplement for the variables MLB intake, feeding frequency, and feeding duration over the entire trial, or in a particular feed type. Additionally, CV between animals and $R^2$ between MLB intake and feeding behaviour were calculated for each of three different sub-periods within the feed type ‘Pastures’ (P1 = from day 1 to 30; P2 = from day 31 to 60; and P3 = from day 61 to 87). This was done to compare the repeatability of such measures within the same type of feed (Pastures). Molasses-lick-block intake and feeding duration were transformed to log to normalise the data before statistical analysis. The intake of MLB was analysed using a mixed-effects linear regression model including feed type and animal sex (Sex) as fixed factors and its interaction. In addition, for the relationship between MLB intake and feeding duration was analysed using a mixed-effects linear regression model with feed type as fixed factor and feeding duration as covariate. Statistical significance was declared at $p < 0.05$.

The relationship between LWC and MLB intake for each feed type was analysed using analysis of covariance. Firstly, LWC and MLB intake for each individual animal were averaged by feed type. These data were then analysed using a mixed-effects linear regression model including feed type and animal sex (Sex) as fixed factors, and MLB intake as a covariate and all possible interactions to investigate linear effects of MLB intake on LWC. Statistical significance was declared at $p < 0.05$. Covariance structure was selected based on the lowest Bayesian Information Criterion. All statistical procedures were done using SAS/STAT software (SAS Institute Inc., Cary, NC, USA).

3. Results

Individual MLB intake, feeding frequency, and feeding duration throughout the trial varied among animals ($p < 0.05$; Figure 5.1) and the latter two variables explained above 85% of the variability in individual MLB intake. Molasses-lick-block intake ranged from 0 to 194.70 g/hd per day (CV = 79.6%; Figure 5.1; panel a) with similar variability between animals also observed for feeding frequency and feeding duration.

Three animals never registered a visit during the entire trial period (Figure 5.1; panel b; 11.10% non-consumers) and only nine single records were taken between 20.00 hrs and 04.00 hrs. Walk-overweighing attendance showed a poor correlation with MLB intake (Figure 5.1; panel d; $R^2 = 0.05$) and the lowest CV among animals in comparison with feeding frequency and feeding duration (Figure 5.1; panel b and c). On average, individual animals attended the water point at least once per day through the entire trial period.
Figure 5.1. Molasses-lick-block (MLB) intake (a), feeding frequency (b), feeding duration (c), and walk-over-weighing (WOW) attendance (d) of individual animals (Mean ± SE) during a 220-day MLB supplementation trial. Animals (columns) are ordered on the x-axis in decreasing amount of MLB intake in all panels. Coefficient of determination ($R^2$) was calculated for each variable against MLB intake. Coefficient of variation (CV) was calculated among individual animals.

Average MLB intake per feed type was greater under OH compared to the rest of the feed types which did not differ between them ($p > 0.05$; Table 5.1). Supplement intake was not affected by sex ($p = 0.11$) or its interaction with feed type ($p > 0.10$). The CV between animals ranged from 144.82% for P to 75.25% for OH. The strength of the relationship between MLB intake and feeding frequency or duration changed with feed type. The lowest $R^2$ for feeding frequency and duration was observed while grazing OC, and these variables explained over 89% of the variability of MLB intake while animals were consuming P and P+C (Table 5.1). Within ‘Pastures’, feeding frequency explained 91%, 87%, and 93% of the variability in MLB intake for P1, P2 and P3, respectively (data not shown). Similarly, feeding duration explained 94%, 92% and 94% of the variability in MLB intake for P1, P2 and P3, respectively (data not shown). However, feeding duration explained a higher proportion of the variability in MLB-intake compared to feeding frequency while animals fed on LH and OH. Regression coefficients of MLB intake against feeding duration ranged from 16.53 (OC) to 27.12 (OH) g/hd per min whereas intercepts did not differ from 0 for all feed types.
Table 5.1. Molasses-lick-block (MLB) intake, coefficient of variation (CV) of MLB intake between individual animals, and coefficient of determination ($R^2$) to predict MLB intake from feeding frequency and feeding duration for each feed type.

<table>
<thead>
<tr>
<th>Feed Type *</th>
<th>MLB Intake (g/hd per day)</th>
<th>CV (%)</th>
<th>Frequency</th>
<th>Duration (min/hd per day)</th>
<th>$R^2$</th>
<th>$R^2$</th>
<th>$\beta$</th>
<th>SE</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pastures</td>
<td>44 ± 11.6 b</td>
<td>144.8</td>
<td>0.93</td>
<td>0.96 21.4 2.11</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>42 ± 14.7 b</td>
<td>99.5</td>
<td>0.66</td>
<td>0.66 16.5 3.12</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P+C</td>
<td>62 ± 15.7 b</td>
<td>128.1</td>
<td>0.89</td>
<td>0.94 23.2 2.39</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>71 ± 15.0 b</td>
<td>102.0</td>
<td>0.56</td>
<td>0.80 21.1 1.94</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OH</td>
<td>173 ± 14.8 a</td>
<td>75.25</td>
<td>0.72</td>
<td>0.83 27.1 1.36</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pastures, grazing autumn temperate pastures; OC, grazing oat crops; P+C, grazing winter pastures with concentrate supplementation; LH, lucerne hay; OH, oaten hay.

Table 5.2 shows the correlations between MLB intake amongst different periods of time or feed types. Results indicate that MLB intake of individual animals in one period was positively correlated with the intake in any other period ($p < 0.05$). However, the strength of the correlation was not consistent across feed types. For example, the amount of MLB consumed while grazing Pastures was moderately correlated ($R < 0.60$) with the amount consumed in other feed types. In contrast, the correlations between the amount of MLB consumed while on OC, P+C, LH, and OH were high ($R \sim 0.81$).

Table 5.2. Pearson’s correlation matrix between average molasses-lick-block intake (MLB, g/hd per day) of individual animals consuming different feed types. Values above the diagonal are correlation coefficients, $p$ values are below the diagonal.

<table>
<thead>
<tr>
<th>Feed Type *</th>
<th>Pastures</th>
<th>OC</th>
<th>P+C</th>
<th>LH</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pastures</td>
<td>1</td>
<td>0.521</td>
<td>0.470</td>
<td>0.584</td>
<td>0.536</td>
</tr>
<tr>
<td>OC</td>
<td>&lt;0.01</td>
<td>1</td>
<td>0.882</td>
<td>0.825</td>
<td>0.805</td>
</tr>
<tr>
<td>P+C</td>
<td>0.013</td>
<td>&lt;0.01</td>
<td>1</td>
<td>0.769</td>
<td>0.770</td>
</tr>
<tr>
<td>LH</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>1</td>
<td>0.830</td>
</tr>
<tr>
<td>OH</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>1</td>
</tr>
</tbody>
</table>

* Pastures, grazing autumn temperate pastures; OC, grazing oat crops; P+C, grazing winter pastures with concentrate supplementation; LH, lucerne hay; OH, oaten hay.

Liveweight change of individual animals over the entire period ranged from 416 to 719 g/hd day (Mean ± SE = 553 ± 43 g/hd per day). Additionally, the average LWC for each feed type, when MLB intake is zero, is indicated by the intercept; being P+C and OC the fastest and lowest growth, respectively. Liveweight change was affected by the interaction between feed type x MLB intake ($p < 0.05$). Thus, results are presented for each feed type (Table 5.3). Intercepts indicate a positive growth of animals across all feed types. The regression coefficients differed ($p < 0.05$) from zero for P+C and LH indicating a positive linear relationship between LWC and MLB intake during these periods. No relationship between LWC and MLB intake was found for the rest of the feed types (Table 5.3; $p > 0.05$).
Table 5.3. Prediction equations for liveweight change from molasses-lick-block intake (MLB) of weaner cattle consuming different feed types. Regression coefficient ($\beta$), intercept ($\alpha$), and $p$-value for the intercept and regression coefficient.

<table>
<thead>
<tr>
<th>Feed type *1</th>
<th>Intercept</th>
<th>Linear Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha$</td>
<td>SE</td>
</tr>
<tr>
<td>Pastures</td>
<td>236</td>
<td>42.1</td>
</tr>
<tr>
<td>OC</td>
<td>180</td>
<td>49.4</td>
</tr>
<tr>
<td>P+C</td>
<td>1037</td>
<td>44.1</td>
</tr>
<tr>
<td>LH</td>
<td>790</td>
<td>48.7</td>
</tr>
<tr>
<td>OH</td>
<td>804</td>
<td>58.6</td>
</tr>
</tbody>
</table>

*1 Pastures, grazing autumn temperate pastures; OC, grazing oat crops; P+C, grazing winter pastures with concentrate supplementation; LH, lucerne hay; OH, oaten hay.

Molasses-lick-block intake throughout the entire trial period was positively correlated with feeding frequency, feeding duration, visit length, and visit size (Table 5.4; $p < 0.05$) but not with feeding rate ($p > 0.05$). However, the strongest correlation coefficient ($R > 0.90$) was observed for feeding frequency and feeding duration. No correlations between WOW attendance and LWC, MLB intake and feeding variables were observed (Table 5.4; $p > 0.05$). Liveweight change was positively correlated with MLB intake, feeding frequency, feeding duration, and visit length (Table 5.4; $p < 0.05$; $R \sim 0.45$). Initial and final LW were positively correlated with MLB intake but only final LW was correlated with LWC (Table 5.4; $p < 0.05$).
Table 5.4. Pearson’s correlation matrix between molasses-lick-block intake (MLB, g/hd per day), feeding frequency (visits/hd per day), feeding duration (min/hd per day), liveweight change (LWC, g/hd per day), visit size (g/visit), visit length (min/visit), feeding rate (g/min), walk-over-weighing attendance (WOW; visits/hd per day), and initial and final liveweight (LW; kg/hd) during a 220 day MLB supplementation trial. Values above the diagonal are correlation coefficients, p-values are below the diagonal.

<table>
<thead>
<tr>
<th>Items</th>
<th>MLB Intake</th>
<th>Frequency</th>
<th>Duration</th>
<th>LWC</th>
<th>Visit Size</th>
<th>Visit Length</th>
<th>Feeding Rate</th>
<th>WOW Attendance</th>
<th>Initial LW</th>
<th>Final LW</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLB intake</td>
<td>1</td>
<td>0.928</td>
<td>0.950</td>
<td>0.444</td>
<td>0.479</td>
<td>0.700</td>
<td>0.111</td>
<td>0.217</td>
<td>0.461</td>
<td>0.598</td>
</tr>
<tr>
<td>Frequency</td>
<td>&lt;0.01</td>
<td>1</td>
<td>0.975</td>
<td>0.415</td>
<td>0.190</td>
<td>0.591</td>
<td>-0.033</td>
<td>0.257</td>
<td>0.366</td>
<td>0.502</td>
</tr>
<tr>
<td>Duration</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>1</td>
<td>0.447</td>
<td>0.280</td>
<td>0.722</td>
<td>-0.076</td>
<td>0.133</td>
<td>0.369</td>
<td>0.543</td>
</tr>
<tr>
<td>LWC</td>
<td>0.021</td>
<td>0.032</td>
<td>0.020</td>
<td>1</td>
<td>0.283</td>
<td>0.413</td>
<td>0.390</td>
<td>-0.012</td>
<td>0.008</td>
<td>0.451</td>
</tr>
<tr>
<td>Visit size</td>
<td>0.018</td>
<td>0.374</td>
<td>0.186</td>
<td>0.180</td>
<td>0.650</td>
<td>0.565</td>
<td>0.011</td>
<td>0.145</td>
<td>0.250</td>
<td></td>
</tr>
<tr>
<td>Visit length</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.045</td>
<td>&lt;0.01</td>
<td>1</td>
<td>0.065</td>
<td>-0.271</td>
<td>0.116</td>
<td>0.351</td>
</tr>
<tr>
<td>Feeding rate</td>
<td>0.605</td>
<td>0.878</td>
<td>0.723</td>
<td>0.060</td>
<td>0.004</td>
<td>0.762</td>
<td>1</td>
<td>0.321</td>
<td>0.019</td>
<td>0.114</td>
</tr>
<tr>
<td>WOW attendance</td>
<td>0.277</td>
<td>0.195</td>
<td>0.507</td>
<td>0.954</td>
<td>0.958</td>
<td>0.200</td>
<td>0.126</td>
<td>1</td>
<td>0.201</td>
<td>0.068</td>
</tr>
<tr>
<td>Initial LW</td>
<td>0.015</td>
<td>0.060</td>
<td>0.058</td>
<td>0.969</td>
<td>0.498</td>
<td>0.588</td>
<td>0.929</td>
<td>0.315</td>
<td>1</td>
<td>0.906</td>
</tr>
<tr>
<td>Final LW</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.021</td>
<td>0.251</td>
<td>0.101</td>
<td>0.603</td>
<td>0.742</td>
<td>&lt;0.01</td>
<td>1</td>
</tr>
</tbody>
</table>
4. Discussion

The aim of the present study was to assess self-fed supplement intake, feeding behaviour, and LW of individual animals to link these outcomes to individual animal daily LWC. In-paddock technologies were used to measure MLB intake and LW because these technologies offer the potential to collect data continuously and remotely [11,12]. Our results showed that these technologies enabled individual assessment of MLB intake and LWC across periods with different feed types and therefore examination of between- and within-animal variation. The MLB intake of individual animals was highly variable over the entire trial. The intake of MLB across the trial influenced LWC and this effect was only found while certain feed types were fed (P+C, LH). Therefore, the combination of WOW and EF technologies allowed establishing correlations between LW and feeding parameters both in the long-term across the entire trial and within shorter periods of time when animals consumed particular feedstuffs. This approach of combining EF and WOW could allow automated monitoring and management systems of grazing beef cattle.

The EF revealed a large variability of MLB intake (CV ranging from 75 to 128) among individual animals over the entire trial. Findings from the present study agree with previous work in beef cattle. For instance, Kendall et al. [7] reported a CV between animals of 57% and 82% for grazing heifers and steers, respectively, consuming MLB offered in individual pens. Graham et al. [6] observed even higher MLB-intake variability between individual steers, ranging from 77 to 488 g/hd per day, estimated by using markers over a six-day period. The variability in MLB intake of the present study can be attributed to a wide range from animals which did not consume any supplement (non-consumers, 11%) to animals that consumed 194 g/hd per day. Bowman and Sowell [1] reviewed 15 studies, including beef and sheep data and reported that the percentage of non-consumers varied from 0% to 50% with an average of 14.32%. However, none of these studies measured the amount of MLB intake individually in grazing animals. The most common intake measurements were done by using markers [14], total faecal collection of indoors animals [15], and identifying consumers by colouring them [16] for periods no longer than four weeks. Based on previous research and our results, high MLB intake variability and attendance to the blocks seems a consistent attribute of animals consuming MLB. However, MLB intake and LWC variations observed in different studies proved to be affected by block formulation (e.g., hardness) and composition such as urea content [1,5,10,17] and these factors could modify block intake patterns with potential impacts on performance [1,5]. Therefore, results from the present trial describing variations in MLB intake and LWC cannot be considered a general assumption and every situation may need to be monitored. A companion paper of the present study [12] has also demonstrated that multiple factors such as changes in forage quantity and quality affect the temporal variability in MLB intake and LWC. Therefore, the ability of in-paddock technologies to monitor animals can help understanding the variability in MLB intake and LWC which seems to be driven by complex interactions between the quality and quantity of the main feed available, block composition and formulation, and animal characteristics.

The use of EF could be useful to identify variability in supplement intake among individuals and to measure feeding behaviour of animals. In addition, feeding behaviour could be used to predict MLB intake when measures of MLB disappearance cannot be taken. Both feeding frequency (R ~ 0.93) and duration (R ~ 0.95) showed a strong positive correlation with MLB intake over the entire trial. Furthermore, these
associations across the entire trial were similar to those found within sub-periods of the same feed type, i.e., Pastures. However, these relationships can be affected by changes in feeding behaviour and social interactions. Wierenga and Hopster [18] reported that animals interacted with an EF by alternating rewarded (visits leading to supplement consumption) and unrewarded visits based on feed availability. In addition, prediction of MLB intake from feeding time and feeding frequency was affected by feed type of the main forage diet in the present study which may limit the application to feed types which were not part of the study. Results also suggest that feeding duration could be a better predictor of MLB intake than feeding frequency under varying feed types. Feeding rate, visit size, and visit length explained lower proportion of the variation compared to daily feeding frequency and duration, and thus these measures are less suitable to predict MLB intake. In addition, block hardness could affect intake [10] and the relationship between feeding behaviour and intake so these prediction equations are not universally applicable. Nevertheless, data on feeding behaviour could be useful to estimate supplement intake of individual animals and allow for the development of low-cost monitoring systems based on EID readers, without the need for weighing cells to measure feed disappearance.

The lack of simultaneous supplement intake and LW data has limited the study of cattle growth responses to molasses-based supplements [5]. Beef producers often feed a wide range of forages throughout seasons and the present study showed that associations between MLB intake and LWC between animals changed according to feed type. However, further research is needed to confirm the results obtained in the present study using a higher number of animals and manipulating the quantity and quality of the basal diet over the time. The MLB used in the present study was formulated for year-round supplementation providing both bypass protein (from cottonseed meal) and non-protein N (3% urea). Remote monitoring technologies helped to quantify the variability in performance across individual animals attributed to MLB intake during both the entire trial and while offering particular feed types. This was confirmed by the positive linear relationship between MLB intake and LWC only being evident while feeding P+C and LH. However, there were other factors not considered in the present study that could strongly influence individual LWC such as total individual feed intake (feed + MLB) and social dominance.

Bowman et al. [5] reported that LWC was mostly improved when feeding molasses with a high proportion of non-protein N (>10%) and low-quality forages. However, positive responses were also reported when the CP of the diet was increased [19]. In addition, across the entire period of the present trial, MLB intake was positively correlated with LW which could indicate that heavier animals were dominant at the feeder. Furthermore, the coefficient of correlation between MLB intake, expressed as percentage of body weight, and the LW of animals over the entire trial was 0.42 (p < 0.05, data not shown). These results confirm that heavier animals ate more MLB however the reason for these findings cannot be confirmed in the present trial. Social dominance may have played a role in these findings despite the fact that the EF was used for only 77.5 min/d and thus the feeder was unoccupied for the majority of the day to allow subordinate animals to consume MLB.

Integrating both EF and WOW data streams could enhance the study of factors involved on animals’ motivation to consume self-fed supplements. For example, MLB intake varied greatly amongst individual animals and feed types, and feeding frequency was lower than 1 visit/hd per day, even for frequent consumers. The feeding behaviour of MLB supplements does not seem to be driven by satiety and hunger mechanisms as reported in previous studies [20] where total mixed rations (TMR) and loose supplements
were fed to predict the probability of the animal beginning a meal, which increases with the time since the last meal. However, we did not find any evidence of such mechanisms in the present study (data not shown). Interestingly, WOW attendance was not associated with feeding behaviour, MLB intake, or LWC in the present study. In addition, cattle attendance to the water point was less variable than visit frequency of MLB suggesting that WOW attendance may be strongly influenced by the gregarious behaviour of the herd. Thus, WOW attendance may reflect the herd’s time of drinking preference when most animals of the herd go to the water point but not necessarily consume supplement. Further studies should be conducted to elucidate factors driving individual intake of self-fed animals which may offer potential to improve animal performance by reducing the variability of intake amongst animals.

5. Conclusions

The combined use of in-paddock technologies, such as electronic feeders and automatic weighing scales, allow to continuously monitor performance of individual animals. The analysis of data obtained from these technologies revealed associations between molasses-lick-block intake, feeding behaviour and growth rate in real-time which could be useful to quantify the impacts of MLB supplementation across and within different types of feed. The ability to capture these associations could improve nutritional management and supplement formulation and tailor these to the forage being consumed. Finally, the findings of the present study could contribute to the future automation of farming activities, such as timing, type, and amount of supplementation tailored to the requirements of individual grazing beef cattle.

Author Contributions: All authors listed (J.A.I., S.G., and L.A.G) had participated in the data analysis, interpretation of results, and writing of the manuscript.

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6. References

Chapter 6

Changes in the metabolome of grazing beef cattle reflects temporal variations in growth rate and supplement intake

6.1 Overview

The use of metabolomics to study metabolic pathways driving performance responses of grazing beef cattle remains in early stages. Chapters 3, 4 and 5 demonstrated the ability of in-paddock technologies to monitor daily the liveweight and supplement intake of individual cattle grazing in a group. Thus, the combination of these three data streams (in-paddock weighing, electronic feeders and metabolomics) may allow exploration of temporal relationships between growth rate, supplement intake and the abundance of blood metabolites in cattle grazing different type of forages across seasons. The present chapter investigated the associations between the relative abundance of blood metabolites and liveweight change and the intake of molasses-lick blocks in grazing weaners experiencing contrasting growth rate due to changes in the feed quantity and quality.

6.2 Abstract

The aim of the present study was to investigate associations of the blood metabolome of grazing cattle with growth rate and intake of molasses lick-blocks supplements (MLB). A 220-day trial was conducted feeding weaner cattle with a sequence of different feed types. Blood samples (BS) were obtained from each animal via puncture of the coccygeal vein on five time points: Day 66 (BS1, pastures), Day 116 (BS2, oat crops), Day 156 (BS3, lucerne hay), Day 185 (BS4, lucerne hay) and Day 219 (BS5, oaten hay). Liveweight (LW) and MLB intake was measured using an automated in-paddock weighing scale (i.e., walk-over-weighing scale, WOW) and electronic feeder (EF), respectively. The relative abundance (RA) of blood metabolites were determined using proton nuclear magnetic resonance (NMR). Liveweight change (LWC) varied across sampling time points from 0.127 to 1.287 kg/hd per day and MLB intake from 4.87 to 159.50 g/hd per day. Among 27 metabolites identified, the RA of 63 and 33% of them were associated with LWC and MLB intake, respectively (P < 0.05). A group of 5 amino acids (valine, leucine, isoleucine, phenylalanine and tyrosine), acetate, and 2- and 3-hydroxybutyrate were positively correlated with LWC (P < 0.001). In contrast, creatinine was negatively (P < 0.001) correlated with LWC. The intake of MLB was negatively correlated with dimethyl sulfone and acetyl groups (P < 0.01) and positively with acetate (P < 0.01). The RA of metabolites involved in muscle protein and energy metabolism was associated with LWC and supplement intake. These results suggest that blood metabolomics offer potential to understand the metabolism of body growth of grazing animals. In-paddock technologies seem to be crucial to understand the associations between performance and the metabolome of animals in near-real time or at a given point in time.
6.3 Introduction

Liveweight change (LWC) is one the most critical descriptors of beef cattle performance (Haley et al., 2005; Nkrumah et al., 2006). Cattle growth proved to be sensible to many factors including nutrition (NASEM, 2016), diseases (Marchesini et al., 2018), and welfare (Ceballos et al., 2018). However, metabolic pathways behind such responses are far from being fully understood (Goldansaz et al., 2017). The complexity of interactions between performance and metabolic responses might be particularly evident in grazing conditions where animals may consume various types of forages as they are rotated through different paddocks across seasons. Thus, LWC could change greatly over time reflecting variations in type, quantity and quality of the feed available (Burns and Sollenberger, 2002). However, the relationship between performance and the metabolome has not been previously investigated in grazing cattle to the authors’ best knowledge.

Metabolic pathways affecting cattle growth rate could highlight metabolites that are the end products of complex interactions between external (e.g. nutrition) and internal (e.g. genotype) factors expressing the phenotype (Fontanesi, 2016; Goldansaz et al., 2017). As a result, these metabolites of low molecular weight (e.g. sugars, lipids, amino acids) can be a powerful tool to understand and enhance nutritional management of animals (Xiao et al., 2012) and biomarker discovery of desirable economic traits (Karisa et al., 2014).

Nowadays, automatic and real-time monitoring of grazing cattle performance can be done remotely using digital ‘in-paddock’ technologies such as automatic weighing scales (WOW) to measure LW and LWC (González et al., 2014) and electronic feeders (EF) to measure individual intake of supplements (Williams et al., 2018; Imaz et al., 2019; Chapter 4 and 5). These technologies showed the ability to capture changes in animal performance and supplement intake with fine temporal detail and across individual animals reflecting forage type, quality and quantity (Imaz et al., 2019; Chapter 4 and 5). Therefore, these technologies could allow assessing the relationship between the blood metabolome, performance and supplement intake over time as animals graze different forages. The ability to measure LWC and MLB intake at a particular point in time allows to correlate the metabolome of animals on the same time scale instead of averaging LWC responses or MLB consumption over long periods of time. Previous studies in feedlot cattle reported that the metabolic profile was linked with feed efficiency (Karisa et al., 2014) and carcass quality (Connolly et al., 2019). However, the later studies correlated the metabolome of animals measured at a particular point in time with performance measured over longer periods of time. In addition, the relationship between temporal changes of blood metabolites, LWC and MLB intake in grazing beef cattle has not been studied.

The aims of the present study were a) to investigate the association between the relative abundance (RA) of blood metabolites and LWC of beef cattle grazing different forages which affected LWC; b) to quantify the effects of MLB intake on the RA of blood metabolites. We hypothesised that significant changes in the blood metabolome occur as animals grazed different forages, which affected animal performance and the amount of supplement consumed.
6.4 Materials and methods

All experimental procedures were approved by the institutional Animal Ethics Committee from The University of Sydney (Approval 2017/1162).

6.4.1 Study design

Fifty-two weaner cattle were blocked by sex (22 heifers and 30 steers), initial age (219 ± 50 days), and initial body weight (186 ± 35.1 kg/hd), and randomly assigned to either a control (n=25, not supplemented) or supplemented treatment (n=27). All animals grazed on temperate pastures for 220 days and were supplemented with hay when necessary at John Pye Farm (The University of Sydney, NSW). A two-section yard centrally located to the paddocks (15 m x 25 m) was built enclosing the only water point which contained an in-paddock WOW station with an auto drafter gate at the entry of the yard (Precision Pastoral Ltd, Alice Spring, Northern Territory, Australia). Animals were previously trained to use the WOW (González et al., 2014) and spear gates were used at the entry of the WOW and each exit gate to allow animals to move in only one direction as previously described by Imaz et al. (2019). The yard section where 27 supplemented cattle were automatically drafted contained an electronic feeder (EF) (Smartfeed, C-lock Inc., Rapid City, South Dakota, United States of America), in which a single molasses-lick block (MLB) was offered as free-choice. The feeder recorded electronic identification (EID) of the animal, date, time, MLB intake (g/hd per day) and duration (sec) of every visit. The chemical composition of MLB (DM basis) was 8.9% of CP; neutral detergent fibre, NDF: 2.83%, and digestibility of organic dry matter, DOMD: 71.5% and the ingredient composition was 42% molasses, 9% salt, 3% urea, 3% vegetal oil, 1.3% phosphorus, 3% calcium, 4% magnesium, 15% cottonseed meal, 2% Lasalocid (Bovatec, Zoetis, Parsippany, New Jersey), 6% trace mineral mix (copper, cobalt, iodine, and zinc) and 11.7% water. Further details about the experimental setup and results concerning factors affecting performance and MLB intake can be found elsewhere (Imaz et al., 2019 and 2020 or Chapters 4 and 5).

6.4.2 Feeding management

Feed types consumed during the study were: a) Autumn temperate pastures grazed from day 0 to 87 (Pastures, 7.37% CP, 68.78% NDF, 52.01% DOMD); b) Oat crops grazed from day 88 to 122 (OC; 10.83% CP, 34.60% NDF, 80.50% DOMD); c) Winter pastures with concentrate supplementation grazed from day 123 to 149 (P+C, 11.42% CP, 49.60% NDF, 68.45% DOMD); d) Lucerne hay fed from day 150 to 184 (LH; 5.5 kg/hd per day, 21.80% CP, 32.65% NDF, 67.15% DOMD); e) Oaten hay fed from day 185 to 220 (OH; 7.6 kg/hd per day, 7.38% CP, 63.22% NDF, 56.00% DOMD). Animals showed a large variability in LWC within and across these periods consuming different diets (Figure 6.1). Over the grazing periods, animals were moved to a fresh paddock when forage availability, to the base of 5 cm, was approximately 1000 and 750 kg DM/ha for pastures (P, P+C) and OC paddocks, respectively. However, from day 150 to the end of the trial, animals where kept on the same paddock while fed with lucerne and oaten hay because of lack of pasture due to drought. Large square bales of hay (LH ~ 350
kg/bale and OH ~425 kg/bale) were offered on Monday, Wednesday and Friday of each week. Additional details regarding pasture composition can be found in Imaz et al. 2019 and 2020 (Chapters 4 and 5, respectively).

Supplementation was offered discontinuously (Monday, Wednesday and Friday) in a separate and non-electronic feeder, during period P+C at a rate of 1.25 kg/hd per day by mixing pellets (16.2 %CP; 35.3 %NDF; 68.7 %DOMD) and chopped lucerne-chaff (15.8 %CP; 41.1 %NDF; 54 %DOMD) in a proportion of 75:25.

6.4.3 Chemical analysis of feed samples

Further details regarding chemical composition of samples obtained from different forages, MLB and pellets can be found in Imaz et al. 2019 and 2020 (Chapters 4 and 5, respectively).

6.4.4 Blood sampling

Five blood samples (BS) were obtained from each animal via puncture of the coccygeal vein using evacuated tubes (containing lithium heparin) on 21 Jun (day 66, BS1), 10 Aug (day 116, BS2), 19 Sep (day 156, BS3), 18 Oct (day 185, BS4) and 21 Nov (day 219, BS5) between 09:00 AM and 11:00 AM (Figure 6.1). Blood samples were immediately refrigerated at 4 °C for approximately 20 min, then centrifuged (2000 × g for 30 minutes); plasma was harvested and stored at -80 °C until metabolomics analysis.

![Blood sampling scheme](image.png)

**Figure 6.1:** Daily liveweight change (LWC) of cattle measured using automatic weighing and times of blood sampling (arrows, BS) to assess the metabolome. Feed types offered are indicated: P = Autumn temperate pastures; OC = Oat crops; P+C = Winter pastures with concentrate supplementation; LH = Lucerne hay; OH = Oaten hay.
6.4.5 Sample preparation for metabolome profiling

Sample preparation and analysis were performed following published methodology by Dona et al. (2014) as previously described by Conolly et al. (2019) at facilities of Sydney Analytical (The University of Sydney, Australia). The relative abundance of metabolites was determined using proton nuclear magnetic resonance (1H-NMR). An aliquot (350 µL) of plasma was mixed with 350 µL of aqueous (80% H2O:20% D2O) phosphate buffer solution including 0.075 M NaH2PO4, pH = 7.4 (KOH adjusted), 0.1% sodium azide, 1 mM 3-trimethylsilyl-1-[2,2,3,3,-2H4] propionate (TSP). Samples were vortexed for 30 sec and centrifuged at 6,000 × g for 10 min. Aliquots of the supernatants (600 µL) from each plasma sample were transferred into 5 mm NMR tubes.

Samples were analysed with a Bruker Advance III 600 MHz spectrometer equipped with a 5-mm TCI cryoprobe and then run under automation mode using a Sample Jet with all samples refrigerated at 278° K until just prior to acquisition. Data were collected at 310° K for a total of 20 min per sample. 1H NMR spectra were acquired using the noesygrpr1d and cpmgpr1d pulse sequences (32,000 scans collected for each experiment). Irradiation of the solvent (water) resonance is applied during pre-saturation delay (4.0 s) for all spectra and for the noesy also during the mixing time (0.01 s). The pulse sequence parameters, most notably the 90° pulse (~12 µs) are optimised for each sample. The data were collected with approximately 96 k (noesy) or 32 k (cpmg) real data points and processed with an exponential line broadening of 0.3 Hz prior to Fourier transformation.

Data were analysed using Matlab 7.0 Software (Matworks, Natick, MA). The spectra were aligned and normalised, automatically phased, baseline corrected and referenced to the α-C1H-Glucose doublet (5.233ppm). The residual water (2.42-3.14ppm) was truncated from the dataset to reduce analytical variability. The normalised spectra were then subjected to Standard Recoupling of Variables to obtain clusters (or components or features).

The cluster value for each sample is the area under the curve for each cluster (component or peak). These values are used as relative concentrations and were multiplied by 10^6 to reduce the number of decimal places before statistical analysis. In addition, Chenomx®, existing literature and the Livestock Metabolome Database (Nicholson et al., 1995; Weljie et al., 2006; Goldansaz et al., 2017) were used for metabolites’ identification from raw data. Finally, the relative abundance (RA) of every single metabolite identified was calculated by adding up the relative concentration of peaks belonging to the same metabolite.

6.4.6 Statistical analysis

The RA of metabolites from the NMR data for each animal and sampling day were matched with LWC and supplement intake obtained from the WOW station and EF to assess their relationship. The WOW data were filtered for outlying data points, averaged per day and LWC estimated using methods described by Gonzalez et al. (2014). Liveweight change and MLB intake was averaged from the 3 days prior to each BS to reduce daily LWC variability and reflect
the forages and MLB consumed prior to sampling (Hristov et al., 2003). Data from EF containing MLB intake were analysed as described by Imaz et al. (2019).

The RA of glutamine, formate, glycine, dimethyl sulfone and lactate were transformed to log10 prior to analysis to normalise their distribution. The RA of metabolites, LWC and MLB intake data were analysed using mixed-effects linear regression models including animal sex (Sex) as a fixed factor, time of BS as repeated measure, EID as the subject random factor, and the BS × Sex interaction. Spatial power was used as the covariance structure based on the lowest Bayesian Information Criterion which considered the distance in days between repeated measures. Differences between least square means were corrected for multiple comparisons using Bonferroni test. The association between the RA of metabolites, LWC and MLB intake were investigated with the above mixed-effects linear regression model including LWC or MLB as linear independent factors using analysis of covariance. All statistical analyses were done using SAS 9.4 (SAS Institute Inc., Cary, New Jersey, USA). Significant statistical differences were declared at $P \leq 0.05$.

Principal component analysis (PCA) were conducted using the RA of 27 identified metabolites as variables in the model with all possible PCs. Then, only those PC with eigenvalues > 1 were selected for the final PCA. Finally, PC1 and PC2 were to obtain loading and score plots to visualise the loading of each metabolite on each PC, correlation between metabolites, and potential clustering of animals according to blood sampling.

6.5 Results

6.5.1 Liveweight, liveweight change and molasses lick-block intake

Liveweight change and MLB intake were affected by BS with the greatest LWC and lowest MLB intake occurring on BS3 compared to the rest of sampling points (Figure 6.2 a and b; $P < 0.05$). In contrast, LWC was lowest while grazing Pastures (BS1) and MLB intake highest while fed LH at BS4 ($P < 0.05$). Interestingly, MLB intake tended to be positively associated with LWC ($P < 0.10$; data not shown) and it was affected by Sex being higher in steers than heifers ($P < 0.05$; data not shown). However, LW was affected by the BS × Sex interaction ($P < 0.05$) because steers were heavier than heifers on BS4 ($P < 0.05$) and tended to be heavier on BS5 ($P < 0.10$; data not shown) but not in BS1, BS2 or BS3 ($P > 0.05$). Animals increased LW over time (Figure 6.2 c; $P < 0.01$).
Figure 6.2: Liveweight change (LWC, panel a), Molasses lick-block (MLB) intake (panel b) and Liveweight (LW, panel c) of grazing beef cattle at the time of blood sampling when the metabolome was assessed (BS1, BS2, BS3, BS4 and BS5).

6.5.2 Temporal variation in the relative abundance of metabolites

The RA of all metabolites identified was affected by BS (Figures 6.3, 4 and 5; P < 0.05). In agreement with LWC, the RA was highest at BS3 for several amino acids including valine, leucine, isoleucine and tyrosine, and for dimethyl sulfone and 2-hydroxybutyrate (Figure 6.3 a, b, c, e, g and h). Conversely, creatinine showed the lowest RA at BS3 (Figure 6.3 f; P < 0.01) whereas creatine was lowest on BS1 (Figure 6.4 f; P < 0.01). Other metabolites including 3-
hydroxybutyrate (Figure 6.3 I; BHB), glucose, glutamine (Figure 6.4 c and e), and glycine and acetyl groups (Figure 6.5 a and g) showed their highest RA at BS2 (P < 0.05). In contrast, very low-density lipids (VLDL, Figure 6.4 g), unsaturated lipids, low density lipids (LDL) and choline were lowest at BS2 (Figure 6.5 b, d, c; P < 0.05). Acetate peaked at BS5 when OH was the main feedstuff (Figure 6.4 d; P < 0.05). Finally, the RA of glucose and acetate was affected by Sex, being greater in steers than heifers (P < 0.05) whereas choline was affected by the BS x Sex interaction (P < 0.05, data not shown).

Figure 6.3: Temporal changes in the relative abundance of blood metabolites of grazing beef cattle.
Figure 6.4: Temporal changes in the relative abundance of blood metabolites of grazing beef cattle.
Figure 6.5: Temporal changes in the relative abundance of blood metabolites of grazing beef cattle.

### 6.5.3 Association between the relative abundance of metabolites and LWC

A total of 27 metabolites were identified from the 1H NMR spectra and the RA of 17 metabolites were associated with LWC (Table 6.1; P < 0.05). However, these associations were strongest (P < 0.001) for 9 metabolites: valine, leucine, isoleucine, phenylalanine, tyrosine, acetate, 2-hydroxybutyrate, dimethyl sulfone and creatinine. All of these metabolites showed positive regression coefficients, except creatinine which showed a negative association with LWC (Table 6.1; P < 0.001). For those metabolites with P < 0.05, negative associations were
found between LWC and lactate, mannose and a VLDL, whereas positive regression coefficients were obtained for 3-hydroxybutyrate, glucose, alanine, glutamine and creatine.

Table 6.1: Analysis of covariance between the relative abundance of blood metabolites and liveweight change (LWC) of weaner cattle grazing various types of feed.

<table>
<thead>
<tr>
<th>Item (LWC)</th>
<th>Intercept Estimate</th>
<th>SE</th>
<th>P-value</th>
<th>Regression Coefficient Estimate</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>551.53</td>
<td>6.781</td>
<td>&lt; 0.001</td>
<td>65.41</td>
<td>8.262</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Leucine</td>
<td>129.65</td>
<td>1.835</td>
<td>&lt; 0.001</td>
<td>16.24</td>
<td>2.258</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>78.29</td>
<td>1.290</td>
<td>&lt; 0.001</td>
<td>15.49</td>
<td>1.635</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>37.96</td>
<td>0.496</td>
<td>&lt; 0.001</td>
<td>3.39</td>
<td>0.604</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>27.56</td>
<td>0.485</td>
<td>&lt; 0.001</td>
<td>4.99</td>
<td>0.615</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dimethyl sulfone</td>
<td>26.07</td>
<td>2.469</td>
<td>&lt; 0.001</td>
<td>44.30</td>
<td>3.171</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatinine</td>
<td>41.63</td>
<td>0.533</td>
<td>&lt; 0.001</td>
<td>-4.77</td>
<td>0.599</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2-hydroxybutyrate</td>
<td>146.73</td>
<td>1.748</td>
<td>&lt; 0.001</td>
<td>12.82</td>
<td>2.035</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Acetate</td>
<td>229.87</td>
<td>10.685</td>
<td>&lt; 0.001</td>
<td>64.67</td>
<td>13.962</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mannose</td>
<td>14.33</td>
<td>0.215</td>
<td>&lt; 0.001</td>
<td>-0.77</td>
<td>0.253</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Alanine</td>
<td>92.83</td>
<td>0.843</td>
<td>&lt; 0.001</td>
<td>2.26</td>
<td>0.910</td>
<td>0.01</td>
</tr>
<tr>
<td>Lactate</td>
<td>106.08</td>
<td>3.644</td>
<td>&lt; 0.001</td>
<td>-10.08</td>
<td>3.611</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatine</td>
<td>110.01</td>
<td>2.183</td>
<td>&lt; 0.001</td>
<td>6.04</td>
<td>2.579</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>3192.03</td>
<td>48.184</td>
<td>&lt; 0.001</td>
<td>146.15</td>
<td>59.949</td>
<td>0.02</td>
</tr>
<tr>
<td>Glutamine</td>
<td>234.67</td>
<td>2.971</td>
<td>&lt; 0.001</td>
<td>8.77</td>
<td>3.647</td>
<td>0.02</td>
</tr>
<tr>
<td>Lipid VLDL*1</td>
<td>242.44</td>
<td>5.274</td>
<td>&lt; 0.001</td>
<td>-16.19</td>
<td>6.665</td>
<td>0.02</td>
</tr>
<tr>
<td>3-hydroxybutyrate</td>
<td>317.49</td>
<td>4.742</td>
<td>&lt; 0.001</td>
<td>13.23</td>
<td>5.405</td>
<td>0.02</td>
</tr>
<tr>
<td>Glycine</td>
<td>173.19</td>
<td>4.355</td>
<td>&lt; 0.001</td>
<td>-10.58</td>
<td>5.641</td>
<td>0.18</td>
</tr>
<tr>
<td>Unsaturated lipid</td>
<td>141.11</td>
<td>4.150</td>
<td>&lt; 0.001</td>
<td>-6.89</td>
<td>5.277</td>
<td>0.19</td>
</tr>
<tr>
<td>Choline</td>
<td>86.68</td>
<td>1.163</td>
<td>&lt; 0.001</td>
<td>-1.70</td>
<td>1.438</td>
<td>0.24</td>
</tr>
<tr>
<td>Formate</td>
<td>8.59</td>
<td>0.260</td>
<td>&lt; 0.001</td>
<td>0.30</td>
<td>0.340</td>
<td>0.24</td>
</tr>
<tr>
<td>Citrate</td>
<td>162.89</td>
<td>3.086</td>
<td>&lt; 0.001</td>
<td>3.08</td>
<td>3.928</td>
<td>0.43</td>
</tr>
<tr>
<td>Threonine</td>
<td>75.86</td>
<td>1.109</td>
<td>&lt; 0.001</td>
<td>0.82</td>
<td>1.415</td>
<td>0.57</td>
</tr>
<tr>
<td>AcetylGroups</td>
<td>223.25</td>
<td>2.970</td>
<td>&lt; 0.001</td>
<td>1.80</td>
<td>3.553</td>
<td>0.61</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>55.29</td>
<td>0.883</td>
<td>&lt; 0.001</td>
<td>-0.42</td>
<td>0.888</td>
<td>0.64</td>
</tr>
<tr>
<td>Lipid LDL*2</td>
<td>138.90</td>
<td>1.630</td>
<td>&lt; 0.001</td>
<td>-0.77</td>
<td>1.949</td>
<td>0.69</td>
</tr>
<tr>
<td>Methylhistidine</td>
<td>28.61</td>
<td>0.400</td>
<td>&lt; 0.001</td>
<td>0.11</td>
<td>0.494</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*1 = very low-density lipids.
*2 = low-density lipids.

6.5.4 Association between the relative abundance of metabolites and MLB intake

The RA of 9 metabolites were affected by MLB intake (Table 6.2; P < 0.05). Dimethyl sulfone, formate, acetyl groups and tyrosine were negatively associated with MLB intake, whereas a
A positive association was found between MLB intake and acetate ($P < 0.01$), the 3 lipid groups and citrate ($P < 0.05$).

Table 6.2: Analysis of covariance between the relative abundance of blood metabolites and molasses-lick-block (MLB) intake of weaner cattle grazing various types of feed.

<table>
<thead>
<tr>
<th>Item (MLB intake)</th>
<th>Intercept Estimate</th>
<th>SE</th>
<th>P-value</th>
<th>Regression Coefficient Estimate</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulfone</td>
<td>54.25</td>
<td>2.051</td>
<td>$&lt; 0.001$</td>
<td>-43.85</td>
<td>15.147</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Formate</td>
<td>8.92</td>
<td>0.165</td>
<td>$&lt; 0.001$</td>
<td>-2.81</td>
<td>1.273</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>AcetylGroups</td>
<td>226.67</td>
<td>2.102</td>
<td>$&lt; 0.001$</td>
<td>-50.19</td>
<td>15.541</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Acetate</td>
<td>263.22</td>
<td>7.350</td>
<td>$&lt; 0.001$</td>
<td>164.18</td>
<td>59.120</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Lipid VLDL$^*$1</td>
<td>229.21</td>
<td>3.529</td>
<td>$&lt; 0.001$</td>
<td>68.30</td>
<td>26.544</td>
<td>0.01</td>
</tr>
<tr>
<td>Citrate</td>
<td>161.72</td>
<td>2.260</td>
<td>$&lt; 0.001$</td>
<td>34.85</td>
<td>15.540</td>
<td>0.02</td>
</tr>
<tr>
<td>Lipid LDL$^*$2</td>
<td>137.59</td>
<td>1.174</td>
<td>$&lt; 0.001$</td>
<td>18.24</td>
<td>8.257</td>
<td>0.03</td>
</tr>
<tr>
<td>Unsaturated Lipid</td>
<td>134.47</td>
<td>2.722</td>
<td>$&lt; 0.001$</td>
<td>45.44</td>
<td>21.308</td>
<td>0.03</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>30.97</td>
<td>0.373</td>
<td>$&lt; 0.001$</td>
<td>-5.56</td>
<td>2.758</td>
<td>0.04</td>
</tr>
<tr>
<td>PhenylAlanine</td>
<td>40.33</td>
<td>0.346</td>
<td>$&lt; 0.001$</td>
<td>-4.98</td>
<td>2.573</td>
<td>0.06</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>55.32</td>
<td>0.730</td>
<td>$&lt; 0.001$</td>
<td>-8.35</td>
<td>4.760</td>
<td>0.08</td>
</tr>
<tr>
<td>Glucose</td>
<td>3286.82</td>
<td>35.608</td>
<td>$&lt; 0.001$</td>
<td>-250.96</td>
<td>252.240</td>
<td>0.32</td>
</tr>
<tr>
<td>Creatine</td>
<td>113.31</td>
<td>1.712</td>
<td>$&lt; 0.001$</td>
<td>13.47</td>
<td>13.963</td>
<td>0.33</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.21</td>
<td>0.006</td>
<td>$&lt; 0.001$</td>
<td>0.03</td>
<td>0.051</td>
<td>0.58</td>
</tr>
<tr>
<td>Alanine</td>
<td>94.04</td>
<td>0.704</td>
<td>$&lt; 0.001$</td>
<td>-2.36</td>
<td>4.435</td>
<td>0.59</td>
</tr>
<tr>
<td>Lactate</td>
<td>100.63</td>
<td>3.366</td>
<td>$&lt; 0.001$</td>
<td>3.01</td>
<td>18.047</td>
<td>0.62</td>
</tr>
<tr>
<td>3-hydroxybutyrate</td>
<td>324.63</td>
<td>3.740</td>
<td>$&lt; 0.001$</td>
<td>10.36</td>
<td>23.960</td>
<td>0.66</td>
</tr>
<tr>
<td>Threonine</td>
<td>76.12</td>
<td>0.771</td>
<td>$&lt; 0.001$</td>
<td>2.25</td>
<td>5.735</td>
<td>0.69</td>
</tr>
<tr>
<td>Choline</td>
<td>86.14</td>
<td>0.710</td>
<td>$&lt; 0.001$</td>
<td>3.36</td>
<td>5.193</td>
<td>0.71</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>87.54</td>
<td>1.069</td>
<td>$&lt; 0.001$</td>
<td>2.92</td>
<td>7.853</td>
<td>0.71</td>
</tr>
<tr>
<td>2-hydroxybutyrate</td>
<td>154.47</td>
<td>1.381</td>
<td>$&lt; 0.001$</td>
<td>-3.06</td>
<td>9.239</td>
<td>0.74</td>
</tr>
<tr>
<td>MethylHistidine</td>
<td>28.76</td>
<td>0.291</td>
<td>$&lt; 0.001$</td>
<td>-0.62</td>
<td>2.043</td>
<td>0.76</td>
</tr>
<tr>
<td>Glutamine</td>
<td>239.98</td>
<td>2.177</td>
<td>$&lt; 0.001$</td>
<td>4.82</td>
<td>17.674</td>
<td>0.78</td>
</tr>
<tr>
<td>Creatinine</td>
<td>38.68</td>
<td>0.450</td>
<td>$&lt; 0.001$</td>
<td>0.85</td>
<td>3.037</td>
<td>0.78</td>
</tr>
<tr>
<td>Mannose</td>
<td>13.96</td>
<td>0.152</td>
<td>$&lt; 0.001$</td>
<td>-0.27</td>
<td>1.101</td>
<td>0.81</td>
</tr>
<tr>
<td>Valine</td>
<td>592.23</td>
<td>5.074</td>
<td>$&lt; 0.001$</td>
<td>6.44</td>
<td>37.273</td>
<td>0.86</td>
</tr>
<tr>
<td>Leucine</td>
<td>139.17</td>
<td>1.481</td>
<td>$&lt; 0.001$</td>
<td>-0.88</td>
<td>11.049</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*1 = very low-density lipids.
*2 = low-density lipids.
6.5.5 Principal Component Analysis with the relative abundance of metabolites

The score plot from PCA presented in Figure 6.6 show that samples clustered together depending on blood sampling. Samples from BS2 clustered in the bottom lower sector of the plot due to positive values for PC1 and negative values for PC2. Samples from BS1 showed negative values for PC1 but PC2 was both positive and negative. In contrast, BS3 samples clustered together with positive values for both PC1 and PC2. PC scores for samples on BS4 and BS5 clustered around zero.

![Score plot from principal component analysis of 27 blood metabolites of weaner cattle grazing various types of feed.](image)

The PC1 explained 26.34% of the variability in the RA of 27 metabolites and PC2 explained 17.85% of the variability (Figure 6.7). Interestingly, metabolites clustered in different regions of the plot. Lipid groups (Lipid VLDL, Unsaturated lipid and Lipid LDL) and Choline clustered on the upper-left sector because these had a negative loading on PC1 and positive loading on PC2. A group of amino acids (Methyl Histidine, Tyrosine, Leucine, Isoleucine, PhenylAlanine and Valine), Dimethyl Sulfone and 2-hydroxybutyrate clustered on the upper-right sector with positive loading on both PC1 and PC2. In addition, other metabolites such as Glutamine, Acetyl Groups, Glucose, Glycine and Pyruvate clustered in the lower-right region.
Figure 6.7: Loading plot from principal component analysis of 27 blood metabolites of weaner cattle grazing various types of feed.

6.6 Discussion
The aim of the present study was to investigate changes in the RA of blood metabolites in growing beef cattle throughout the seasons and their relationships with LWC and MLB intake. These relationships were explored at five time points during a grazing trial when LWC of animals was affected as a result of consuming different types of forages varying in quantity and quality. Far from being a fully controlled metabolic study of housed cattle, the novelty of the present study lies on detecting complex metabolic interactions occurring in grazing cattle. With this aim, the use of in-paddock technologies was critical in the present study to monitor cattle performance and enable the integration of the three data streams at a given point in time for individual animals grazing as a group. The associations were stronger between the RA of metabolites and LWC compared to those between the RA of metabolites and MLB intake as shown by the p-values and the fact that 63% of the metabolites were associated with LWC but only 33% with MLB intake. A positive association was found between LWC and the RA of certain amino acids (valine, leucine, isoleucine, phenylalanine and tyrosine) and important energy sources such as acetate, creatine, 2- and 3-hydroxybutyrate, and glucose. Conversely, negative associations were found between LWC and the RA of other metabolites involved in both carbohydrate and lipid metabolism such creatinine, lactate and very low-density lipids. These associations were
supported by results from PCA with the loading plot showing these three groups of metabolites clustering together in each quadrant. To the best of our knowledge, the present study is the first assessing the metabolome of grazing beef cattle over several time points and its correlation with LWC and MLB intake. Previous studies have indicated that correlations between the RA of blood metabolites and growth rate were affected by characteristic of the animals (breed, LW, age, beef or dairy production), feeding management (grazing pastures, feed lotting) and experimental design (e.g. blood assessments) (Sikka et al., 1994; Bruce et al., 2008; van der Drift et al., 2012; Pires et al., 2013; Carrillo et al., 2016; O'Callaghan et al., 2018; Connolly et al., 2019). The low supplement intake (up to 200 g/hd per day) may explain such lower correlations with the RA of metabolites than those observed for LWC, however, further studies are required on whether is possible or not to manipulate cattle metabolism by using self-fed supplements. It is important to point out that results for LWC and MLB intake should be interpreted with caution because metabolic responses are driven by complex interactions which were not fully considered in the present study including genetics and weather.

The RA of all metabolites, LW, LWC, and MLB intake changed over time reflecting changes in the nutritional status of animals as previously presented by Imaz et al. (2019). The effects of changes in the RA of metabolites in the blood stream of animals and its association with changes in LWC is reflected in the score plot of the PCA. The score plot from PCA grouped samples according to LWC, particularly BS1, BS2 and BS3 which showed increasing LWC. When animals experienced the lowest LWC in BS1, negative scores for PC1 were driven by high RA of lipid groups, lactate and creatinine. After this period of low growth rate, when animals were increasing LWC in BS2, high values in PC1 and low in PC2 were driven by metabolites involved in carbohydrate metabolism providing energy such as glucose, pyruvate, BHB and acetyl groups. Interestingly, the metabolome of animals with highest LWC in BS3 was characterised by a high RA of amino acids but also high RA of lipid groups. The experimental conditions of the present trial were similar to those in commercial farms where cattle consume different feed types through seasons, which affects LWC. A limited number of studies have investigated associations between the metabolome of animals and LWC. Connolly et al. (2019) found that BHB and creatine were negatively associated with growth rate in feedlot cattle. In contrast, the present longitudinal study found a positive association between LWC and BHB, creatine and glucose and may indicate that periods of fast growth rate are associated with greater concentration of these energy sources in the bloodstream required to tissue growth and synthesis. Thus, the present study addresses the need to identify metabolites that are susceptible to change with growth rate and maturity in grazing beef cattle (Goldansaz et al., 2017).

Dietary crude protein and bacterial cells in cattle supply amino acids to support a range of biological functions including muscle growth and deposition because these are the building blocks of proteins (Kung and Rode, 1996). Positive associations between amino acids and LWC observed in the present study suggest that periods with faster growth rate may be linked with higher RA of these amino acids, or that periods with lower growth rate showed lower RA of these amino acids in the blood stream. Several reasons for these findings may be plausible including
that CP in the diet which could subsequently limit or increase the absorption of amino acids from the gastrointestinal tract into the blood stream which could then affect growth rate. The PCA results also suggest that several amino acids had positive loading on both PC1 and PC2, which led to clustering of BS3 samples in the upper-right quadrant. The practical applications of these results are uncertain because there is limited research on the use of metabolomics for nutritional management. However, it be useful to investigate if supplementation with these amino acids can improve body growth and thus enhance nutritional management tailoring supplements to the type, quantity and quality of forage being consumed. This approach was explored by Xiao et al. (2012) to study metabolic changes in response to dietary glutamine supplementation in early weaned piglets. Xiao et al. (2012) demonstrated that supplemented piglets showed different metabolism of carbohydrates, proline, tyrosine and glycerophospholipids and tended to grow faster. However, further studies are needed to test this hypothesis in cattle and to elucidate the metabolic pathways in which amino acids are involved (e.g. protein, energy).

Metabolites involved in energy metabolism, such as acetate, 2- and 3-hydroxybutyrate, glucose, and creatine showed positive associations with LWC in agreement with previous research (Menahan et al., 1967; Smith and Crouse, 1984; Hanset and Michaux, 1986; Wallimann et al., 2011). Other studies suggested that greater abundance of creatinine was associated with intense mobilisation of muscle protein during periods of energy shortage in dairy cattle (Pires et al., 2013) and LW loss in beef cattle (Bruce et al., 2008). In the present study, creatinine was negatively associated with LWC and its RA was lowest when animals were growing fastest which does not agree with those observations in cattle under nutritional deficiencies.

The RA of dimethyl sulfone was positively associated with LWC in the present study. Previous research (O'Callaghan et al., 2018) reported that dimethyl sulfone was important to differentiate between forage-based diets (i.e. ryegrass, ryegrass and white clover, total mixed ration). Dimethyl sulphone is produced in the rumen during the catabolism of sulfur amino acids (i.e. methionine) which could be hydrolysed to dimethyl sulphide and then oxidised to dimethyl sulfone (Taylor and Kiene, 1989). Increased abundance of dimethyl sulfone in ruminal fluid was correlated with increased crude protein in the diet, particularly on pastures (O'Callaghan et al., 2018). Therefore, results from the present study suggest that the positive association between dimethyl sulfone and LWC may be due to higher crude protein concentration provided by LH when animals were at their fastest growth rate. Interestingly, the RA of dimethyl sulfone in the present study was low on BS4 when animals consumed LH but were growing at a slower rate. However, total forage availability (LH + forage from direct grazing) was probably limiting LWC on BS4. Additionally, PCA analysis revealed that dimethyl sulfone clustered together with amino acids and therefore seem to share similar metabolic pathway.

Acetate, propionate and butyrate are by-products of ruminal fermentation of dietary carbohydrates and provide approximately 65% of the energy used by ruminants (van Houtert, 1993). Acetate can be used by Coenzyme-A to form Acetyl-CoA (Wathes et al., 2012) which enters the tricarboxylic acid cycle (TAC) to produce energy by oxidation when animals
experience low energy balance, but it can also be used to synthetise fat when the animal is in positive energy balance (Preston and Leng, 1987). Acetate was positively associated with LWC in the present study, which may suggest it is involved in metabolic pathways of energy production or lipid synthesis. However, these are speculations and further studies are required to elucidate the metabolic pathways of acetate depending on energy balance, current growth rate and previous trajectory of growth. The fact that acetate clustered together with lipid groups in the PCA may suggest its participation with fat synthesis under the experimental conditions of the present study.

Beta-hydroxybutyrate is one of the main circulating ketone bodies and energy sources in ruminants (Zarrin et al., 2017). Its concentration in the blood stream increases in animals experiencing negative or positive energy balance (Zammit, 2009; Zarrin et al., 2017). The positive association between BHB and LWC found in the present study does not allow determining the metabolic pathways it contributes because the experimental design was not meant to respond that question. For instance, during negative energy balance, BHB originates in the liver from metabolism of lipids and then enters the TCA cycle to form acetyl-CoA and produce energy (van der Drift et al., 2012). Alternatively, BHB can be derived from the absorption of acetate and butyrate from the rumen independently of energy balance and then enter the TCA to produce energy (Hocquette et al., 2009). Under positive energy balance, BHB can be esterified to fatty acids, triacyl glycerides and phospholipids (Zammit, 2009). Thus, BHB seems to be a key metabolite to supply energy and precursors of fat during periods of fast growth rate. Additionally, results from the present study may suggest that 2-hydroxybutyrate is associated with energy metabolism required during growth but speculations are limited by the lack of research assessing 2-hydroxybutyrate in the blood stream of cattle. However, 2-hydroxybutyrate was associated with residual feed intake in beef cattle but the potential reasons for this were not discussed (Karisa et al., 2014). Further research is needed to investigate the relative contribution of BHB to energy required for growth or as building blocks for fat synthesis during growth.

Molasses lick-block intake was affected by the type of feed offered, showed large variation amongst individual animals, and it was positively associated with growth rate in the present study (Imaz et al., 2019). There were negative associations between dimethyl sulfoxide and acetyl groups with MLB intake. These associations were not expected because MLB provided by-pass protein, minerals and energy (molasses and vegetable oil). However, the positive association between MLB intake and the RA of lipids could reflect greater absorption of lipids in the intestine during periods of time when MLB consumption increased. Similarly, the positive association between acetate and MLB intake could be linked with the enhancement of the fermentation of carbohydrates in the rumen which are then absorbed into the blood stream through the rumen wall. In the present trial, MLB also contained Lasalocid which could increase acetate production by stimulating rumen fermentation (Ricke et al., 1984; Spears and Harvey, 1987).

Direct comparison of our results with previous studies is difficult due to the uniqueness of our experimental design. Firstly, in the present study, cattle metabolome was investigated over time in contrast to other studies which assessed it metabolome at a single point in time (Sikka et al.,
Additionally, we explored associations between the metabolic profile of animals and their LWC and MLB intake measured during the 3 days prior to sampling instead of using LWC values measured for longer periods of time. Thus, correlations between the RA of metabolites and performance should be interpreted accordingly. For instance, Conolly et al. (2019) assessed the correlation between the metabolome at a given point in time and the growth rate measured over a 450-day feedlotting period. In addition, other differences with previous studies include breed and age of the animals, growth rate and experimental design. Thus, Conolly et al. (2019) suggested that animals that grew faster had lower concentrations of BHB because they use more energy to grow which reduces its RA in blood (oxidise more BHB or use more to synthesise fat). In contrast, results from the present study may suggest that BHB increase during periods of faster growth rate, or that animals grow faster during periods of higher energy availability.

6.7 Conclusions
The relative concentrations of various amino acids and carbohydrates used as energy sources such as acetate, creatine, 2- and 3-hydroxybutyrate, and glucose were positively associated with growth rate and increased during periods of faster growth. In contrast, other metabolites involved in energy metabolism such as creatinine, lactate and very low-density lipids decreased and were negatively correlated with growth rate. Our findings contribute to understand links between metabolic pathways, cattle growth rate and metabolism which could be used to improve nutritional management and supplement formulation. In-paddock technologies proved to be useful to assess the associations between the metabolome, growth rate, and supplement intake.

6.8 References


Chapter 7

Metabolic profile of high performing individuals within a group of grazing beef cattle

7.1 Overview

Chapter 5 demonstrated the utility of data generated by in-paddock technologies to establish the relationships between liveweight, growth rate, supplement intake and feeding behaviour of weaner cattle at the individual animal level. Additionally, Chapter 6 studied temporal changes of and relationships between the metabolic profile, performance and supplement intake of animals grazing different forages throughout the year. The objective of the present chapter 7 was to study the metabolic profile associated with growth rate and intake of molasses-lick blocks between individual animals at each point in time.

Imaz JA, García S, González LA 2020. The metabolic profile of grazing beef cattle is associated with growth rate variability among individuals. Submitted for publication.
7.2 Abstract

We hypothesised in the present study that individual variability of liveweight change (LWC) and molasses lick-block intake (MLB) was associated with the relative abundance (RA) of blood metabolites in grazing beef cattle. A 220-day grazing trial was conducted with 52 weaner cattle (initial age: 219 ± 50 days, initial live weight: 186 ± 35.1 kg/hd) fed a sequence of different feed types that affected LWC. Blood samples (BS) were obtained from each animal at five time points: BS1 on day 66 while grazing pastures; BS2 on day 116 while grazing oat crops, BS3 on day 156 while fed lucerne hay; BS4 on day 185 at the end of the lucerne hay feeding period, and BS5 on day 219 while fed oaten hay. Liveweight (LW) and supplement intake were continuously monitored using an in-paddock walk-over-weighing scale (WOW) and electronic feeder (EF), respectively. The RA of metabolites in plasma were determined using proton nuclear magnetic resonance (NMR). Over the entire trial, LWC of individual animals ranged from -0.55 to 1.80 kg/hd per day and the individual intake of MLB from 0 to 780 g/hd per day. Analysis of covariance using a linear mixed-effects model was performed to assess the association between the RA of metabolites and LWC and MLB intake at each sampling point. The greatest number of metabolites associated with LWC (P < 0.05) was found at BS2 (15 out of 27 identified metabolites) when the animals were resuming growth after a period of nil to low growth, and the lowest number at BS1 and BS3 (1 metabolite; P < 0.05) when the animals were at the slowest and fastest LWC, respectively. At BS2, the RA of 3 lipid groups were positively associated with LWC (P < 0.01) whereas negative regression coefficients were obtained between LWC and 2- and 3-hydroxybutyrate, pyruvate, acetyl groups, citrate, valine, leucine and isoleucine (P < 0.01). Creatinine was negatively associated with LWC across all sampling points (P < 0.01) but at BS3 (P > 0.05). Similarly, creatine was negatively associated with LWC at BS2 and BS4 (P < 0.05). Only 22% of the metabolites were correlated with MLB intake (P < 0.05) being negative with creatine (BS1 and BS2) and dimethyl sulfone (BS5) and positively correlated with acetate (BS5). In addition, positive regression coefficients were obtained between MLB intake and two lipid groups at all sampling points. Blood metabolomics, in combination with the use of in-paddock technologies, offers potential to identify and describe associations between the metabolic profile, LWC and supplement intake of individual grazing cattle.
7.3 Introduction

The growth trajectory of grazing cattle has been traditionally studied at the group rather than at the individual animal level (Owens et al., 1993). However, a large variability in performance exists between animals within a herd and their response when resuming growth after a period of nil or low growth and feed supplementation (Ryan, 1990; Bowman and Sowell, 1997; Neave et al., 2018). The reasons for such variability between individual animals under the same conditions remain unclear but differences in their metabolism may exist to be expressed as a performance phenotype. In addition, the lack of technologies to measure liveweight change (LWC) and supplement intake of individual grazing cattle with high frequency has been a limitation. There is a need to measure LWC and supplement intake at a similar temporal scale as what the metabolome may reflect to allow an accurate assessment of the relationship between them. The integration of new technologies such as automatic weighing systems and electronic feeders with metabolomics offer unprecedented opportunities to improve the understanding of the physiological basis of such variability in performance between animals (González et al., 2014; Williams et al., 2018; Imaz et al., 2019, 2020). A previous study demonstrated the capability of this approach to study changes in the metabolic profile of beef cattle grazing a range of forages that affected LWC over time (Chapter 6). However, that study only focused on temporal changes in LWC, supplement intake and the blood metabolome to assess the metabolism of animals during periods of low and high growth rates. Nevertheless, the metabolic profile driving the differences in performance between individual animals under the same conditions deserves further research. This could help explain the reasons for the large variability in growth rate amongst individual animals fed in a group. For example, it is known that animals use metabolites such as glucose, 3-hydroxybutyrate, amino acids and acetate as energy sources or building blocks of proteins or fatty acids for muscle growth or fat deposition (Connolly et al., 2019; Chapter 6). Thus, animals with faster growth rate at a given point in time are expected to show a different metabolic profile compared to those with lower growth rate.

Metabolomics uses advanced analytical chemistry techniques to measure large numbers of small molecules or metabolites in cells, tissues and biofluids (Goldansaz et al., 2017). These metabolites could reflect interactions between the genotype and the environment, which results in the expression of the phenotype (Fontanesi, 2016). However, the metabolic profile of individual animals should be associated with desirable performance traits to add value to metabolomics data. Data from ‘in-paddock’ technologies could be integrated with metabolomics to understand the biology of animal growth and characterise the metabolic profile of high and low performing animals.

The cattle metabolome has been studied in relation to residual feed intake (Karisa et al., 2014) and carcass quality in feedlot cattle (Connolly et al., 2019), forage-based diets (O’Callaghan et al., 2018), risk of disease (Klein et al., 2012) and milk quality (Melzer et al., 2013). However, to the best of our knowledge, no studies have investigated the associations between the individual metabolic profile of grazing beef cattle and LWC and molasses lick-block (MLB) supplements using in-paddock technologies.
The aim of the present study was to characterise the metabolic profile of high and low performing animals at time points of low to high performance. This was achieved by investigating the associations between the relative abundance (RA) of blood metabolites with LWC and MLB intake of individual cattle grazing as a group at five time points of their growth trajectory. We hypothesised that the metabolic profile of individual animals was linked to LWC and MLB intake.

7.4 Materials and methods

All experimental procedures were approved by the institutional Animal Ethics Committee from The University of Sydney (Approval 2017/1162).

7.4.1 Study design and experimental animals

Fifty-two crossbred weaner cattle (Angus x Charolais) were blocked by sex (22 heifers and 30 steers, initial age: 219 ± 50 days, initial weight: 186 ± 35.1 kg/hd) and weight, and randomly allocated to one of two supplementation treatments (MLB and control). Cattle grazed temperate pastures for 220 days and were fed with hay when necessary at John Pye Farm (The University of Sydney, NSW) from 16 April to 22 November of 2017. A two-section yard enclosing the only water point and centrally located to the paddocks (15 m x 25 m) was built. The yard contained an in-paddock WOW station with an auto-drafting gate at the entry of the yard (Precision Pastoral Ltd, Alice Spring, Northern Territory, Australia). Animals were previously trained to use the WOW (González et al., 2014) and spear gates at the entry and exit of the yard allowed animals to move in only one direction. Twenty-seven animals were automatically drafted to one of the yard sections containing an electronic feeder (EF) where a single MLB was offered as free-choice (Smartfeed developed by C-lock Inc., Rapid City, South Dakota, United States of America). The EF was able to record electronic identification (EID) of the animal, date, time, MLB intake (g/hd) and duration (sec) of every visit. The rest of the animals (n=25) were drafted to a different yard without access to supplementation.

7.4.2 Blood sampling

Five blood samples (BS) were obtained from each animal via puncture of the coccygeal vein using evacuated tubes containing EDTA (Vacutainer BD, Becton Dickinson, Franklin Lakes and USA) on 21 Jun (day 66, BS1), 10 Aug (day 116, BS2), 19 Sep (day 156, BS3), 18 Oct (day 185, BS4) and 21 Nov (day 219, BS5) between 09:00 AM and 11:00 AM (Figure 7.1). Blood samples were immediately refrigerated at 4°C for approximately 20 min, then centrifuged (2000 × g for 30 minutes) and plasma was harvested and stored at -80°C until metabolomics analysis. Forages fed at the time of blood sampling (Figure 7.1) were pastures (BS1), oat crops (BS2), lucerne hay (BS3 and BS4) and oaten hay (BS5).

7.4.3 Feeding management

The main types of feed and the number of days offered are summarised in Table 7.1. A detailed description of grazing and feeding management and chemical composition of feed were
reported by Imaz et al. (2019). The chemical composition of MLB (DM basis) was 8.9 % of CP; neutral detergent fibre, NDF: 2.83 %, and digestibility of organic dry matter DOMD: 71.5 % and the ingredient composition was 42 % molasses, 9 % salt, 3 % urea, 3 % vegetal oil, 1.3 % phosphorus, 3 % calcium, 4 % magnesium, 15 % cottonseed meal, 2 % Lasalocid (Bovatec, Zoetis, Parsippany, New Jersey), 6 % trace mineral mix (copper, cobalt, iodine, and zinc) and 11.7 % water.

Table 7.1: Feeding management and chemical composition of feed types offered during the experiment.

<table>
<thead>
<tr>
<th>Feed types offered</th>
<th>Start (day)</th>
<th>End (day)</th>
<th>CP (%)</th>
<th>NDF (%)</th>
<th>DOMD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn temperate pastures (Pastures)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oat crops (OC)</td>
<td>88</td>
<td>122</td>
<td>10.83</td>
<td>34.60</td>
<td>80.50</td>
</tr>
<tr>
<td>Winter pastures with supplementation (P+C)</td>
<td>123</td>
<td>149</td>
<td>11.42</td>
<td>49.60</td>
<td>68.45</td>
</tr>
<tr>
<td>Lucerne hay (LH)</td>
<td>150</td>
<td>184</td>
<td>21.80</td>
<td>32.65</td>
<td>67.15</td>
</tr>
<tr>
<td>Oaten hay (OH)</td>
<td>185</td>
<td>220</td>
<td>7.38</td>
<td>63.22</td>
<td>56.00</td>
</tr>
</tbody>
</table>

* DOMD: digestibility of organic dry matter.

Chemical composition (NDF, ADF, CP, ash content and dry matter and organic matter digestibility: DMD and DOMD) of forage samples were estimated using near-infrared spectroscopy (NIRS). Chemical composition of samples obtained from MLB and pellets were analysed for NDF and ADF in accordance with Van Soest et al. (1991).

7.4.4 Sample preparation for metabolome profiling

Sample preparation and analysis for metabolome profiling were described in detail by Connolly et al. (2019) and a companion paper by Imaz et al. (Chapter 6). Briefly, we followed published methods (Dona et al., 2014) and proton nuclear magnetic resonance (NMR) to determine the relative abundance or concentration of metabolites using a Bruker Advance III 600 MHz spectrometer. Raw data were imported into Matlab 7.0 Software (Matworks, Natick, MA) and the spectra were aligned, automatically phased, baseline corrected, normalised and referenced to the α-C1H-Glucose doublet (5.233 ppm). The normalised spectra were then subjected to standard recoupling of variables to estimate start and end point of each feature or cluster and the relative abundance of each cluster determined from the area under the curve (component or peak). These values of relative concentrations were multiplied by 106 to reduce the number of decimal places. Finally, the raw spectra were imported into Chenomx® for the assignment of metabolites to these clusters. This was achieved by comparing 1H NMR spectra to the spectral library of Chenomx® NMR Suite Professional (Chenomx Inc., Edmonton, AB, Canada) as well as referencing from published literature and the Livestock Metabolome
Database (Nicholson et al., 1995; Weljie et al., 2006; Goldansaz et al., 2017). After identification, the relative abundance (RA) of every metabolite identified was calculated by adding up the relative concentration of all peaks belonging to the same metabolite.

7.4.5 Statistical analysis

Data from WOW were filtered for outlying data points, averaged per day and LWC estimated using methods described by Gonzalez et al. (2014). Data from EF containing MLB intake were analysed as described by Imaz et al. (2019). Liveweight change and MLB intake from the 3 days prior to each BS was averaged for each animal to represent that point in time and possible nutritional effects of forages and MLB consumed prior to the blood sampling (Hristov et al., 2003). These data of LW, MLB intake and the RA of each cluster and metabolite for each animal and time point were merged for analysis.

The RA of glutamine, formate, glycine, creatine, dimethyl sulfone and lactate were transformed to log_{10} prior to analysis to normalise their distribution. The association between the RA of metabolites, LWC and MLB intake at each point in time were investigated using mixed-effects linear regression models including BS and Sex as fixed factors, LWC or MLB as covariates and the interaction between the covariates and sampling date as random effect to test for the null hypothesis that the regression coefficient (β) between the RA of metabolites and LWC or MLB intake within a sampling date was equal to zero and not different between sampling dates. Blood sampling date was the repeated measure subjected to EID with spatial power covariance structure based on the lowest Bayesian Information Criteria and the need to accommodate unequal interval of time between measures. All statistical analyses were done using SAS 9.4 (SAS Institute Inc., Cary, New Jersey, USA). Significant statistical differences were declared at P ≤ 0.05.

7.5 Results

7.5.1 Variability of LWC and MLB intake between individual animals

The highest and lowest variability between animals measured through the CV was observed on BS1 and BS3 for LWC, respectively. However, the largest and smallest standard deviation was found for BS5 and BS1, respectively (Table 7.2).
Figure 7.1: Liveweight change (LWC) of cattle measured using automatic weighing stations. Continuous line indicates the average daily LWC across all animals during the entire trial. Dots indicates LWC of individual animals at five time points where blood metabolome was assessed (BS). Feed types offered are indicated: Pastures = Autumn temperate pastures; OC = Oat crops; P+C = Winter pastures with concentrate supplementation; LH = Lucerne hay; OH = Oaten hay.

Table 7.2 and Figure 7.1 show that LWC was positive across all time points on average, however, LWC from individual animals ranged from -0.55 to 1.80 kg/hd per day. No animals lost weight on BS3 whereas some animals lost weight on all other BS points. Although 93% of the LWC observations were positive, there were 8, 2, 0, 4 and 3 individual animals with negative LWC on BS1, BS2, BS3, BS4 and BS5, respectively. A large variability between individual animals was also observed in MLB intake at all time points as reflected by CV (Table 7.2). In addition, MLB intake was affected by BS being lowest on BS3 and greatest on BS4 (Table 7.2; P < 0.05).
Table 7.2: Descriptive statistics of daily liveweight change (LWC) and molasses lick-block (MLB) intake across five time points (BS).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LWC (kg/hd per day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS1</td>
<td>0.12</td>
<td>0.15</td>
<td>-0.36</td>
<td>0.44</td>
<td>129</td>
</tr>
<tr>
<td>BS2</td>
<td>0.48</td>
<td>0.25</td>
<td>-0.06</td>
<td>1.04</td>
<td>53</td>
</tr>
<tr>
<td>BS3</td>
<td>1.27</td>
<td>0.28</td>
<td>0.59</td>
<td>1.80</td>
<td>22</td>
</tr>
<tr>
<td>BS4</td>
<td>0.35</td>
<td>0.31</td>
<td>-0.28</td>
<td>1.30</td>
<td>88</td>
</tr>
<tr>
<td>BS5</td>
<td>0.84</td>
<td>0.43</td>
<td>-0.55</td>
<td>1.34</td>
<td>51</td>
</tr>
<tr>
<td><strong>MLB intake (g/day per hd)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS1</td>
<td>24</td>
<td>56</td>
<td>0</td>
<td>240</td>
<td>234</td>
</tr>
<tr>
<td>BS2</td>
<td>61</td>
<td>101</td>
<td>0</td>
<td>427</td>
<td>167</td>
</tr>
<tr>
<td>BS3</td>
<td>5</td>
<td>13</td>
<td>0</td>
<td>63</td>
<td>272</td>
</tr>
<tr>
<td>BS4</td>
<td>200</td>
<td>224</td>
<td>0</td>
<td>780</td>
<td>112</td>
</tr>
<tr>
<td>BS5</td>
<td>160</td>
<td>186</td>
<td>0</td>
<td>533</td>
<td>116</td>
</tr>
</tbody>
</table>

7.5.2 Associations between the relative abundance of metabolites and LWC

The RA of 16 out of 27 metabolites identified in the present study were affected by the BS x LWC interaction (Table 7.3; P < 0.05). Among these metabolites, the majority (56 %) of the significant associations between the RA and LWC were observed on BS2 (data not shown) when LWC was in an upwards trajectory after a period with low or nil growth rate during winter (Figure 7.1).

Table 7.3: P-values obtained for blood sampling (BS) as main factor, liveweight change (LWC) as covariate and their interaction (BS x LWC).
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>P-value 1</th>
<th>P-value 2</th>
<th>P-value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid LDL *2</td>
<td>&lt; 0.001</td>
<td>0.221</td>
<td>0.013</td>
</tr>
<tr>
<td>2-hydroxybutyrate</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>0.011</td>
</tr>
<tr>
<td>Citrate</td>
<td>&lt; 0.001</td>
<td>0.090</td>
<td>0.012</td>
</tr>
<tr>
<td>Creatine</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>0.021</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>&lt; 0.001</td>
<td>0.562</td>
<td>0.070</td>
</tr>
<tr>
<td>PhenylAlanine</td>
<td>&lt; 0.001</td>
<td>0.110</td>
<td>0.124</td>
</tr>
<tr>
<td>Dimethyl Sulfone</td>
<td>&lt; 0.001</td>
<td>0.037</td>
<td>0.790</td>
</tr>
<tr>
<td>Formate</td>
<td>&lt; 0.001</td>
<td>0.306</td>
<td>0.244</td>
</tr>
<tr>
<td>Lactate</td>
<td>&lt; 0.001</td>
<td>0.481</td>
<td>0.295</td>
</tr>
<tr>
<td>Glutamine</td>
<td>&lt; 0.001</td>
<td>0.681</td>
<td>0.325</td>
</tr>
<tr>
<td>Glucose</td>
<td>&lt; 0.001</td>
<td>0.190</td>
<td>0.416</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>&lt; 0.001</td>
<td>0.629</td>
<td>0.548</td>
</tr>
<tr>
<td>Threonine</td>
<td>&lt; 0.001</td>
<td>0.931</td>
<td>0.650</td>
</tr>
<tr>
<td>Mannose</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>0.455</td>
</tr>
<tr>
<td>Glycine</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.790</td>
</tr>
</tbody>
</table>

*1 = very low-density lipids.
*2 = low-density lipids.

Only results with a significant association between the RA of metabolites and LWC for each time point are shown (Table 7.4). Most of the associations between the RA of metabolites and LWC were negative, except for choline, lipids (very low-density lipids, VLDL; low-density lipids, LDL; and unsaturated lipids), pyruvate, acetate and alanine that were positively associated with LWC (Table 7.4; P < 0.05). Creatinine showed a negative association with LWC at BS1, BS2, BS4 and BS5 (Table 7.4; P < 0.05) whereas creatine showed a negative association with LWC at BS2 and BS4 (Table 7.4; P < 0.05). The amino acids valine, leucine and isoleucine showed negative β at BS2 (P < 0.05) whereas alanine had a positive association with LWC at BS5 (Table 7.4; P < 0.05). The RA of 2- and 3-hydroxybutyrate, pyruvate, citrate and acetyl groups were negatively associated with LWC at BS2 (P < 0.05). However, pyruvate showed a positive association with LWC at BS3 (P < 0.05) and acetate at BS5 (Table 7.4; P < 0.05).

The RA of 3 metabolites was associated with LWC as the main factor independently of BS (Table 7.3; P < 0.05). Dimethylsulfone was positively associated with LWC regardless of BS (β = 6.98 ± 3.38, P-value 0.037). Conversely, glycine (β = -19.05 ± 5.510, P < 0.01) and mannose (β = -1.40 ± 0.328, P < 0.01) decreased as LWC increased. The RA of 8 metabolites were affected by BS only (Table 7.3, P < 0.01), these being glutamine, lactate, glucose, threonine, tyrosine, formate, isoleucine and phenylalanine. Acetate, creatine, methyl-histidine and choline were affected by Sex or its interaction with BS (Data not shown; P < 0.05).
Table 7.4: Regression coefficient (β), intercept (α) and P-value for the intercept and regression coefficient between the relative abundance of blood metabolites and daily liveweight change at five time points (BS). Only significant metabolites affected by BS x LWC and where the regression coefficient was significant are shown (P < 0.05).

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Metabolite</th>
<th>α</th>
<th>SE</th>
<th>P-value</th>
<th>β</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS1</td>
<td>Creatinine</td>
<td>42.68</td>
<td>0.819</td>
<td>&lt;.0001</td>
<td>-11.65</td>
<td>4.265</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>BS2</td>
<td>VLDL lipid *1</td>
<td>118.29</td>
<td>9.359</td>
<td>&lt; 0.001</td>
<td>82.22</td>
<td>17.084</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Unsat. lipid</td>
<td>45.43</td>
<td>6.943</td>
<td>&lt; 0.001</td>
<td>70.57</td>
<td>12.629</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>LDL lipid *2</td>
<td>105.88</td>
<td>4.060</td>
<td>&lt; 0.001</td>
<td>22.75</td>
<td>7.371</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Choline</td>
<td>61.84</td>
<td>2.697</td>
<td>&lt; 0.001</td>
<td>20.21</td>
<td>4.894</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Methyl-Histidine</td>
<td>31.27</td>
<td>1.137</td>
<td>&lt; 0.001</td>
<td>-9.95</td>
<td>2.103</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>43.67</td>
<td>1.305</td>
<td>&lt; 0.001</td>
<td>-10.76</td>
<td>5.434</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>88.86</td>
<td>2.929</td>
<td>&lt; 0.001</td>
<td>-11.30</td>
<td>5.434</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Pyruvate</td>
<td>67.50</td>
<td>2.094</td>
<td>&lt; 0.001</td>
<td>-11.47</td>
<td>3.686</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>144.35</td>
<td>4.053</td>
<td>&lt; 0.001</td>
<td>-31.99</td>
<td>10.024</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>AcetylGroups</td>
<td>273.29</td>
<td>5.792</td>
<td>&lt; 0.001</td>
<td>-31.99</td>
<td>10.024</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>2-hydroxybutyrate</td>
<td>168.97</td>
<td>3.929</td>
<td>&lt; 0.001</td>
<td>-32.60</td>
<td>7.289</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Citrate</td>
<td>195.11</td>
<td>7.580</td>
<td>&lt; 0.001</td>
<td>-43.51</td>
<td>13.822</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Creatine</td>
<td>139.51</td>
<td>5.299</td>
<td>&lt; 0.001</td>
<td>-49.78</td>
<td>9.797</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>3-hydroxybutyrate</td>
<td>392.71</td>
<td>12.515</td>
<td>&lt; 0.001</td>
<td>-94.51</td>
<td>23.565</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>662.70</td>
<td>14.711</td>
<td>&lt; 0.001</td>
<td>-117.00</td>
<td>27.275</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BS3</td>
<td>Pyruvate</td>
<td>42.27</td>
<td>3.954</td>
<td>&lt;.0000</td>
<td>8.57</td>
<td>2.975</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>BS4</td>
<td>Unsat. lipid</td>
<td>159.36</td>
<td>4.841</td>
<td>&lt;.0001</td>
<td>32.63</td>
<td>10.754</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>39.51</td>
<td>0.893</td>
<td>&lt;.0001</td>
<td>-4.90</td>
<td>1.799</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Creatine</td>
<td>128.25</td>
<td>3.492</td>
<td>&lt;.0001</td>
<td>-18.45</td>
<td>7.625</td>
<td>0.025</td>
</tr>
<tr>
<td>BS5</td>
<td>Acetate</td>
<td>313.52</td>
<td>23.554</td>
<td>&lt;.0001</td>
<td>107.10</td>
<td>24.816</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Alanine</td>
<td>79.75</td>
<td>2.588</td>
<td>&lt;.0001</td>
<td>13.53</td>
<td>2.718</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>45.14</td>
<td>1.326</td>
<td>&lt;.0001</td>
<td>-3.92</td>
<td>1.390</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>AcetylGroups</td>
<td>255.05</td>
<td>5.853</td>
<td>&lt;.0001</td>
<td>-33.72</td>
<td>5.947</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

*1 = very low-density lipids.
*2 = low-density lipids.
7.5.3 Associations between the relative abundance of metabolites and MLB intake

Dimethyl sulfone, creatine and lactate were affected by the BS x MLB intake interaction (Table 7.5; P < 0.05) with negative regression coefficients for creatine in BS1 and BS2 (P < 0.05), lactate in BS1 (P < 0.05) and dimethylsulfone in BS5 (P < 0.05). In contrast, acetate was positively correlated with MLB intake on BS5 (Table 7.6; P < 0.05). Two lipid groups were affected by MLB intake as the main factor regardless of BS (Table 7.5; P < 0.05) where the RA of VLDL (β = 41.73 ± 18.00, P-value 0.021) and unsaturated lipids (β = 34.52 ± 13.41, P-value 0.011) increased as MLB intake decreased. Creatine was affected by Sex being greater in heifers than steers (P < 0.05; Data not shown).

Table 7.5: P-values obtained for blood sampling time (BS) as main factor, molasses lick-block (MLB) intake as covariate and its interaction (BS x MLB).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>BS</th>
<th>MLB</th>
<th>BS x MLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine</td>
<td>&lt; 0.001</td>
<td>0.260</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dimethyl Sulfone</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Acetate</td>
<td>&lt; 0.001</td>
<td>0.325</td>
<td>0.012</td>
</tr>
<tr>
<td>Lactate</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>0.026</td>
</tr>
<tr>
<td>3-hydroxybutyrate</td>
<td>&lt; 0.001</td>
<td>0.567</td>
<td>0.090</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>&lt; 0.001</td>
<td>0.794</td>
<td>0.110</td>
</tr>
<tr>
<td>Glutamine</td>
<td>&lt; 0.001</td>
<td>0.550</td>
<td>0.115</td>
</tr>
<tr>
<td>Lipid VLDL</td>
<td>&lt; 0.001</td>
<td>0.021</td>
<td>0.155</td>
</tr>
<tr>
<td>Unsaturated Lipid</td>
<td>&lt; 0.001</td>
<td>0.011</td>
<td>0.173</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>&lt; 0.001</td>
<td>0.287</td>
<td>0.284</td>
</tr>
<tr>
<td>Lipid LDL</td>
<td>&lt; 0.001</td>
<td>0.091</td>
<td>0.312</td>
</tr>
<tr>
<td>Glycine</td>
<td>&lt; 0.001</td>
<td>0.395</td>
<td>0.506</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>&lt; 0.001</td>
<td>0.328</td>
<td>0.568</td>
</tr>
<tr>
<td>Citrate</td>
<td>&lt; 0.001</td>
<td>0.969</td>
<td>0.583</td>
</tr>
<tr>
<td>Glucose</td>
<td>&lt; 0.001</td>
<td>0.923</td>
<td>0.598</td>
</tr>
<tr>
<td>Mannose</td>
<td>&lt; 0.001</td>
<td>0.760</td>
<td>0.629</td>
</tr>
<tr>
<td>Formate</td>
<td>&lt; 0.001</td>
<td>0.110</td>
<td>0.65</td>
</tr>
<tr>
<td>AcetylGroups</td>
<td>&lt; 0.001</td>
<td>0.087</td>
<td>0.656</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>&lt; 0.001</td>
<td>0.630</td>
<td>0.693</td>
</tr>
<tr>
<td>Leucine</td>
<td>&lt; 0.001</td>
<td>0.528</td>
<td>0.716</td>
</tr>
<tr>
<td>Valine</td>
<td>&lt; 0.01</td>
<td>0.916</td>
<td>0.809</td>
</tr>
<tr>
<td>Threonine</td>
<td>&lt; 0.001</td>
<td>0.618</td>
<td>0.874</td>
</tr>
<tr>
<td>MethylHistidine</td>
<td>&lt; 0.001</td>
<td>0.975</td>
<td>0.889</td>
</tr>
<tr>
<td>Creatinine</td>
<td>&lt; 0.001</td>
<td>0.578</td>
<td>0.912</td>
</tr>
<tr>
<td>2-hydroxybutyrate</td>
<td>&lt; 0.001</td>
<td>0.791</td>
<td>0.931</td>
</tr>
<tr>
<td>Alanine</td>
<td>&lt; 0.01</td>
<td>0.556</td>
<td>0.951</td>
</tr>
<tr>
<td>Choline</td>
<td>&lt; 0.001</td>
<td>0.803</td>
<td>0.963</td>
</tr>
</tbody>
</table>
Table 7.6: Regression coefficient ($\beta$), intercept ($\alpha$) and P-value for the intercept and regression coefficient of the relative abundance of blood metabolites against molasses-lick-block (MLB) intake at five time points (BS). Only significant metabolites affected by the BS x MLB intake interaction are shown (P < 0.05).

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Metabolite</th>
<th>Intercept</th>
<th>Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\alpha$</td>
<td>SE</td>
</tr>
<tr>
<td>BS1</td>
<td>Lactate</td>
<td>115.49</td>
<td>5.286</td>
</tr>
<tr>
<td></td>
<td>Creatine</td>
<td>91.20</td>
<td>2.541</td>
</tr>
<tr>
<td>BS2</td>
<td>Creatine</td>
<td>120.57</td>
<td>2.644</td>
</tr>
<tr>
<td>BS5</td>
<td>Dimethyl Sulfone</td>
<td>49.10</td>
<td>2.346</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>378.02</td>
<td>11.126</td>
</tr>
</tbody>
</table>

7.6 Discussion

We hypothesised in the present study that the variability in LWC and MLB intake between individual animals within a group at a given point in time was associated with the RA of blood metabolites. One of the most important findings of the present study was that the number of associations between the RA of metabolites and LWC changed with growth rate. Most noticeable was the large number of metabolites that were associated with LWC on BS2 when animals were coming out of a period with low growth rate after the winter. The metabolome is closer to the phenome than the genome because metabolites are the building blocks of body tissues and provide the energy required for growth and body functions (Fontanesi, 2016; Goldansaz et al., 2017). Therefore, the metabolism of animals under the same environmental and management conditions should reflect their performance. The RA of metabolites may indicate that the metabolite could either limit or enhance growth rate or be affected by the amount of supplement consumed (e.g. MLB) after the absorption of nutrients (Xiao et al., 2012). Nevertheless, the correlations between desirable traits and the abundance of metabolites are difficult to establish due to the complexity of metabolic responses and the low number of metabolomics studies in cattle under similar experimental conditions. In our study, these associations between the metabolome and LWC and MLB intake were explored across five time points in a grazing trial where cattle growth rate largely differed due to changes of the feed available as reported in related studies (Imaz et al., 2019, 2020). Our results showed significant associations between the RA of various metabolites and LWC, and MLB intake to a lesser extent, supporting the main hypothesis.

Interestingly, both the number and strength of these associations varied across time points (BS). Out of 27 metabolites identified in the present study, 74 and 22% of them were affected
by LWC and MLB intake, respectively. Furthermore, significant associations between the RA of metabolites and LWC were most evident at BS2 (52 % of the metabolites identified) when animals were on an ascendant curve of growth after a period of poor performance during winter (approximately 0.1 kg/hd per day). Thus, metabolic differences between individual animals at BS2, when animals may have experienced compensatory growth, seemed most important to affect growth rate. In contrast, differences in growth were poorly linked to the metabolic profile of animals during periods of low or high LWC, such as BS1 and BS3, respectively. This could have major implications for animal management and breeding. For instance, previous research has demonstrated that the metabolome is influenced by genetics and it is linked to animal performance and carcass traits (Connolly et al., 2019). Therefore, blood metabolomics at the time of resuming growth after a period of poor performance could be used to breed animals that respond better to an increased plane of nutrition after a period of undernutrition and to understand the physiological basis of growth recovery.

The metabolism of animals at BS2 was characterised by an increased number of metabolites associated with LWC. Animals with faster growth rate showed greater RA of lipids (LDL, VLDL and unsaturated lipid) and lower RA of creatine, creatinine, 2- and 3-hydroxybutyrate, pyruvate, and acetyl groups. The latter 4 metabolites can be oxidised after conversion to acetyl-CoA to produce energy in the tricarboxylic acid cycle (TCA) or be used to synthesise fatty acids as well, or both (Hanset and Michaux, 1986; Hocquette et al., 2009; van der Drift et al., 2012). Thus, these results may suggest that animals with faster growth rate are using these metabolites at a faster rate during active growth at BS2 resulting in lower RA in blood. Creatine and creatinine are also involved in energy metabolism in muscle and brain where the intermediary form is creatine phosphate serving as an energy store in a reversible reaction to recycle energy (Wyss and Kaddurah-Daouk, 2000; Wallimann et al., 2011). Plasma concentration of creatine and creatinine was negatively associated to LWC across most time points and thus these metabolites were more consistent compared to others. It is speculated that the ability of individual animals to produce and recycle energy via these compounds partly explains the greater performance of some animals compared to others of the same group. In addition, animals with greater performance seem to be able to uptake precursors and synthesise lipids at a faster rate compared to low-performing animals, which is reflected in greater RA of lipids in blood. These hypotheses maybe valid during active growth after a period of low growth, but more research is needed to confirm results of the present study and further understand the metabolism of body growth.

The negative association between BHB and LWC found on BS2 agrees with recent studies in feedlot cattle (Connolly et al., 2019). However, it is important to highlight that circulating BHB in cattle could reflect different metabolic pathways depending on whether animals are in positive or negative energy balance as described by Wathes et al. (2012) and supported by Connolly et al. (2019). In conclusion, multiple metabolites involved in energy, fat and muscle metabolism are associated with LWC during active growth at BS2 and metabolomics seems to be a promising tool to monitor energy balance and growth biology in cattle.
In contrast to metabolites involved in energy metabolism, fewer metabolites involved in protein metabolism such as amino acids showed significant associations with LWC in BS2. These results were unexpected considering that amino acids were most closely associated to temporal changes in LWC with sharp increases in the RA of amino acids on BS3 coinciding with the fastest LWC as reported in a companion study (Chapter 6). However, the objective of that study was completely different to the present study which looked at the metabolic profile of animals related to LWC within a sampling point. Therefore, amino acids do not seem to confer particular advantages in performance of one individual over another at a given point in time despite the fact that the RA of amino acids was higher in periods with high growth rate.

However, valine and leucine were negatively associated with LWC at BS2 in the present study. These are branched amino acids known to participate in metabolic pathways related to muscle growth, protein synthesis, lipogenesis and lipolysis in animals and humans (Zhang et al., 2017). Additionally, branched amino acids could also contribute to energy production in animals by entering the TAC. It has been previously reported that valine and leucine increased growth and milk production in pigs and cattle (Hultquist and Casper, 2016; Zhang et al., 2018). Thus, it is speculated that animals with increased growth rate use valine and leucine at a faster rate resulting in lower concentrations in blood, or that the RA of these amino acids in blood limited growth rate at BS2, when animals were possibly experiencing compensatory growth, because of deficiencies in the diet. However, these are just speculations because the concentration of amino acids in the diet was not measured. Connolly et al. (2019) suggested that branched amino acids may regulate fat deposition in feedlot cattle, however, no consistent relationships with growth rate were found in that study. In the later study, the growth rate of animals was less variable in comparison with the present study because animals were fed most of the production period with high-grain diets, which could possibly explain the lack of association between growth rate and branched amino acids reported by Connolly et al. (2019).

There were a lower number of associations between the RA of metabolites and MLB intake than between the RA of metabolites and LWC. This could be due to the low quantity of MLB consumed by animals in relation to their total feed intake. The MLB provide energy from molasses and vegetable oil, and by-pass protein from cottonseed meal. The positive association between lipids and MLB intake independently of sampling time may be due to the absorption of lipids into the blood stream coming from MLB. In addition, a negative correlation between MLB intake and creatine was observed at BS1 and BS2. The reasons for this finding are unknown and further research investigating the effect of supplementation on muscle energy metabolism is recommended. The MLB intake positively affected the RA of acetate in BS5 which could be due to MLB enhancing ruminal fermentation. The inclusion of Lasalocid provided by MLB could also stimulate acetate production as observed by Ricke et al. (1984) and Spears and Harvey (1987). Furthermore, negative associations between dimethyl sulfone and MLB intake were not expected as MLB contribute to enhance the protein supply of animals through cottonseed meal and positive correlations were observed between dimethyl sulfone and animals grazing enriched diets with crude protein (O'Callaghan et al., 2018).
Imaz et al. (Chapter 6) reported that various metabolites involved in energy metabolism had greater RA during periods of high LWC (BS3) such as 2- and 3-hydroxybutyrate, acetate and glucose. This seems to disagree with the present study where 2- and 3-hydroxybutyrate showed a negative association with LWC in BS2. These results suggest that temporal changes in the RA of metabolites and LWC were associated with the diet consumed. It is also important to highlight that the present study assessed the metabolic profile within a point in time across animals fed the same diet which was correlated to growth rate. Therefore, both analytical approaches differ markedly and indicate that the underlying biological processes involved in both hypotheses are different.

7.7 Conclusions
Findings from the present study indicate that the main metabolic differences explaining differences in growth rate between individual animals under the same conditions are most evident while resuming growth after a period of low gains. During such periods, animals with faster growth rate had greater relative abundance of circulating lipids and lower of 2- and 3-hydroxybutyrate, creatine, creatinine, pyruvate, and acetyl groups. On one hand, these results suggest that faster-growing animals use energy sources in blood at a faster rate and may use these precursors to synthesise lipids at a faster rate. On the other hand, this information contributes to the understanding of the metabolic profile of individual animals with greater growth rate, which could then be used to enhance the nutritional management of animals and biomarker discovery for use in breeding programs.

7.8 References


Chapter 8

General discussion and conclusion

The primary purpose of this final chapter is to integrate the knowledge generated in each individual chapter, highlight those areas that require further research, and outline the key conclusions of these investigations. The research undertaken in the present thesis explored the use of in-paddock technologies to generate near-real time data, which is then converted to information and this into knowledge of grazing beef cattle growth and intake of supplementary feedstuffs. The ability to monitor parameters linked with animals’ performance included LW, and subsequent calculation of LWC (Chapter 3), the intake of MLB and feeding behaviour of cattle at the group (Chapter 4) and individual animal basis (Chapter 5) during a long-term grazing trial. Additionally, performance data were used to build associations with the RA of metabolites and to investigate metabolic pathways involved with cattle growth and supplement intake. Therefore, the combination of these three data streams enabled quantification of the factors affecting LW, LWC, MLB intake and metabolism of grazing beef cattle throughout their entire growth trajectory (Chapter 6) and the metabolic profile of individuals at a given point in time (Chapter 7). Therefore, both statements of the general hypothesis of this research (that in-paddock technologies have the ability to capture data reflecting the nutritional status and response to management of grazing beef cattle, and the combination of these data with metabolomics can help explaining the metabolic changes associated with growth rate and supplement intake) are accepted. Additionally, Chapter 2 reviewed other studies which have been mostly focused on the development of technologies instead of their ability to capture relevant data and adding value to data through the integration of data streams, an approach often called data fusion (González et al., 2014). In this regard, the present thesis has generated knowledge utilising these data sources to explain the factors driving growth and supplement intake.

8.1 Utilisation of near real-time data to enhance farm management

In Chapter 4, continuous monitoring of LW and supplement intake enabled the estimation of LWC and feeding behaviour of animals on a daily basis. The practical application of these data open opportunities and it also has its challenges regarding the interpretation of these results. Firstly, Chapter 4 showed that daily LW data enable the detection of variations in LWC in real-time which could enhance decision making around nutritional and health management of cattle. In addition, daily MLB intake was associated with changes in forage quantity and quality available to the animals. This could lead to the development of more comprehensive tools to manage grazing based on both estimations of forage availability and cattle feeding behaviour around the blocks. This could potentially represent a step forward to automate grazing management based on a multi- measurement approach. For instance, the feeding behaviour of animals along with measures of feed available could offer a number of opportunities that could
be further improved through integration with other data streams such as satellite imagery. High-resolution satellite imagery can be used to calculate vegetation indexes, which correlate to standing biomass, growth and quality of pastures (Schaefer and Lamb, 2016). Thus, vegetation indexes could be combined with daily MLB intake and both could be used as a predictor of forage quantity and quality indicating the need of rotating animals to a fresh paddock remotely. Nevertheless, the appropriateness of other measures would require further research if the association between variables is weaker. For example, LWC and supplement intake showed a weak association which was also affected by type of forage being consumed (Chapter 4). This may be due to the fact that the impact of nutrient intake would be reflected on LWC later in time due to the need to metabolise them (Houtert, 1993; Kung and Rode, 1996). Thus, averaging responses from longer intervals of data collection than daily (e.g. weekly, fortnightly) maybe more suitable for these applications. However, these are just speculations and further research is needed. Although cattle performance can be measured daily, the interpretation and method of data analysis may need to be done according to the biological nature of variables involved.

From a research point of view, being able to continuously monitor cattle performance would also need to be supported by data from field observations. Due to the frequent and large datasets generated by in-paddock sensors, it would probably be a challenging task to perform. Therefore, new procedures of field data collection may need to be considered. For example, Chapter 4 indicates that changes in MLB intake over time would have been difficult to understand without the continuous pre- (High) and post (Low) field measurements of the forage quantity and quality. Therefore, the lack of automated and integrated approaches to match near real-time data with field data would avoid explaining the observed variability in MLB intake or growth rate. Furthermore, little research has been published on procedures to accustom animals to gradually use in-paddock technologies. Descriptions of methodologies and procedures from several studies were utilised in the present thesis to guide our research (González et al., 2014; Williams et al., 2018). However, most of these were described as part of studies focussed on animal performance and did not specifically test the effects of different procedures on animal welfare, behaviour and data acquisition.

Chapter 3 demonstrated the large impact of the length of the interval between LW measures (ILW) on LWC calculations. These effects were particularly evident on the calculations of maximum and minimum LWC across all animal categories investigated. Therefore, choosing the right ILW improves the value of LWC and minimise animal handling on beef farms using conventional weighing. Additionally, LWC calculated from different ILW could also be used as an input to develop prediction models to test their ability to predict other variables of interest, such as daily feed intake from individual measurements of LW. As reviewed in Chapter 2, this approach was used by González et al. (2014b) to predict methane emissions from grazing beef cattle, however, only calculations using daily LW data as input were explored. Thus, determining the effect of data collection frequency on the output of prediction models would be important. However, we speculate that prediction models developed using data collected at longer intervals
than daily or weekly may be not suitable to use with near-real time data. Therefore, prediction models may need to be re-built based on new datasets.

As noted in the introduction section of the present thesis, there is a need of identifying and deleting LW outliers to calculate LW and LWC of individual animals (Alawneh et al., 2011; González et al., 2014; Brown et al., 2015). However, such calculations could also be affected by the number of records and the length of the time period of data collection. Increasing the number of days with LW records could enhance the accuracy of calculations due to a reduction of interpolations to estimate LW and LWC on days with missing values. Nevertheless, the number of records within a day could affect LW calculations as they are subjected to variations in the gut fill of animals. Analysing the potential effects of gut fill on LWC calculations was out of the scope of the present thesis. However, this topic deserves further research because there are no previous studies using LW data obtained from WOW systems to show the within day variability of LW. The present thesis was focussed on understanding temporal and across-animal variability in LW and LWC and the within-day variability was reduced or eliminated using the smoothing technique described by Gonzalez et al. (2014). There were days without usable LW records and, when existing, variations in the number of records within a day ranged from 1 to 10 records per animal (data not shown). Additionally, individual total feed and water intake were not measured in the present experiment and therefore it is not possible to study the associations between LW and feed and water intake which are the main contributors to fill of the gastrointestinal tract in addition to defecation and urination. There is a need performing controlled studies to measure how variations in feed, water consumption, urination and defection are associated with changes in LW. Also, whether these variations can be detected by using LW data obtained remotely or not and describing the frequency of LW records needed. These datasets could be used to develop models and algorithms to account the effects of gut fill on LW and LWC calculations and used in combination with near real-time LW data to gain accuracy in LW estimations.

Chapter 2 reviewed the importance of the livestock sector for the Australian economy, particularly the beef industry. In this context, managing environmental and production variability in beef grazing systems remains as one of the top priorities for producers and scientists. Results from studies utilising WOW and EF demonstrated their potential utilisation for a range of management applications. For instance, stocking rate could be closely monitored and managed using WOW to adjust it during periods of forage shortage and to prioritise those categories with higher nutritional requirements or simply to sale stock under prolonged drought conditions. Variations in LWC could indicate the optimal time to start or stop feed supplementation. Supplementation management could include diet formulation according to performance where EF may offer potential to individualise type and quantity of supplements. Selecting animals through both improved LWC and reduced intake of supplementary feed may enhance feed efficiency and, therefore, economic returns to farmers. Nutritional management concepts described for growing cattle could apply for cow-calf systems where weight loss could be more pronounced and critical to detect. In addition, calves’ growth path could be monitored for timely
intervention, which is a novel approach in comparison with conventional methods recording only birth and weaning weight as described by González et al. (2018) and Chapter 3.

In-paddock technologies could assist scientists to build models and correlations between variables of interest to predict outcomes when direct measures are not practical or logistically impossible to perform. In Chapter 5, we used a similar approach to use feeding behaviour (feeding frequency and duration) to predict the intake of MLB across different feed types. These equations could be used to predict MLB intake without measuring supplement disappearance and potentially contribute to the development of low-cost feeders based on animal attendance to the feed stations. Similarly, in Chapter 5, we developed equations using MLB intake to predict LWC which was significant for two types of feed (P+C and LH). Total feed intake by animals is a critical measure directly related to both LW and LWC however methodologies to measure DMI are complex, impractical to implement on large herds (e.g. > 40 heads) and invasive with the potential to alter normal behaviour of animals. Individual and frequent measures of LW may offer a fresh angle to integrate such data to existing prediction models of animal growth to validate and predict total feed intake in grazing animals. This could potentially attain an acceptable accuracy due to the continuous data input generated by in-paddock technologies.

8.2 Individual measurements by in-paddock technologies and its application to automate cattle activities

As discussed across Chapters 2, 3, 4 and 5, the lack of continuous and individual measures of LW and the intake of supplementary feed was probably one of the most limiting factors to study grazing beef cattle. Several studies speculated that variability between individual animals probably affected experimental results, but individual assessments were not presented (Bowman et al., 1995; Bowman and Sowell, 1997). Findings from Chapter 5 and 7 support the hypothesis that the use of in-paddock technologies provide near-real time measures of LW and MLB intake individually to draw associations with changes in the metabolic profile of high-performing individuals. Chapter 5 indicated that growth rate of individual animals was affected by MLB over the entire period of study but then the effect was found to be significant while animals fed on winter pastures and lucerne hay but not on other types of feed. Interestingly, responses to MLB at the group level (Chapter 4) differed from those obtained from averaging individual values across feed types (Chapter 5). This may indicate the existence of other factors (e.g. social interactions, genetics, previous nutrition, health), in addition to the variability in MLB intake, to explain the variability in individual LWC between supplemented animals. This speculation is supported by a low correlation between individual MLB intake and LWC (Table 5.4; R = 0.44).

A thorough examination of the nutritional aspects of MLB was not the main aim of the present study. However, the limitations that exist in our experimental approach to explain effects of MLB supplementation on animal performance deserve discussion. Firstly, it is not clear which nutritional mechanisms enhanced the LWC of MLB supplemented animals at some stages of their growth path. Molasses-lick-block supplementation was a source of energy and protein but
also minerals and medications (i.e. Lasalosid). The intake of MLB does not seem to be providing sufficiently large amount of energy and protein to explain the extent of improved growth. Metabolizable energy (ME) provided by MLB from its measured intake, which was calculated by adding the ME contribution of different MLB ingredients, ranged from 0 to 6 MJ/hd per day. For instance, the average ME provided from MLB intake during the OH period was close to 2 MJ/hd per day. This represents a low contribution of MLB to total daily ME intake, which averaged 88 and 73 MJ/hd per day for MLB supplemented and control groups, respectively. Therefore, differences in energy intake from both groups (15 MJ/hd per day) is much higher than the estimated energy provided by MLB (2 MJ/d). This discrepancy could be explained by a positive effect of Lasalocid and salt and minerals on growth rate suggested in chapters 4 and 5. Proposed effects of Lasalocid include altered bacterial populations, increased propionate and decreased acetate and butyrate concentration, protein-sparing effects, increased diet digestibility and daily intake, rumen fill, and rate of passage (Golder et al., 2016). Also, it has been reported that MLB consumption could increase the intake of the forage base diet and, as a consequence, improving animal gain (Bowman et al., 1995). The latter speculation could be supported by the existence of ‘better doers’ which consume more MLB and have higher LWC and less incidence of diseases than ‘poor doers’. Nevertheless, these are speculations which may involve confounding factors and the present experimental design does not allow to ascertain the reasons for these finding because it was not the objective of the study. Future studies could explore whether the intake of supplements offered as a ‘free choice’ and the behaviour of frequent consumers is linked with superior health and performance of individual animals.

Finally, individual LW and supplement intake at particular time points were associated with the metabolome at that exact point in time instead of using average values of LWC and MLB intake of longer periods of time. These associations using daily data are expected to be more accurate compared to using values from longer time periods. Another example of the difference between individual and group data can be drawn comparing Chapters 6 and 7. At the group level, the RA of several amino acids (e.g. valine, leucine) were positively correlated with LWC; however, negative associations were observed between the RA of these amino acids and LWC when the data were analysed to explore these associations at an individual animal level within sampling points. Interestingly, the RA of amino acids was significant with promoting growth of animals during periods of high growth rate. However, the RA of amino acids of high-performance individuals within a time point (Chapter 7) had lower relevance than at group level (Chapter 6). In this regard, being able to explore individual data raises challenges to interpret such information. In the later example, findings from Chapter 6 suggest that, in general, an increase in the RA of amino acids could be associated with greater LWC whereas variability in the utilisation of amino acids at individual level may not strongly define high-performance of individuals.

In addition to the concept of individual measurements and cattle operations, the use of autodrafting gates at the entry of the yard contributed to individualise (or make more accurate) the treatments by drafting supplemented cattle to a different yard section. This also means that
animals grazed as a single group instead of using separate treatments and paddocks where differences between forage and other environmental conditions could override the effects of supplementation. Therefore, the combination of auto-drafting gates and algorithms to detect abrupt changes in LW and supplement intake could be potentially used, for example, to identify and automatically separate sick animals to a different yard for examination.

Chapter 2 reviewed that in-paddock technologies WOW and EF were originally developed for the dairy industry decades ago (Filby et al., 1979). However, their adoption was more recent for grazing beef cattle. Studies in dairy cattle (Alawneh et al., 2011) may not represent the extensive conditions of production that can be observed for grazing beef cattle. Dairy cattle come at least twice daily to handling facilities for milking which allow producers to supervise individual animals continuously. Conversely, beef producers monitor their animals less frequently and thus, technologies for grazing beef cattle should be self-powered and require low maintenance (e.g. twice a year). Furthermore, the ability of EF to deliver and measure supplement consumption individually should be tested with different feed types and number of animals using the feeder during long-term grazing studies. For example, the attendance to the EF was lower than 1 visit/hd per day even during periods of high MLB intake. However, the attendance and amount of supplement consumed can change by feeding supplements that are more attractive to animals. In this regard, results from chapters 4 and 5 revealed that the usage of EF was lower than 1 hour/day across all animals, indicating that the feeder was not being used for 96% of the day. The accuracy of the EF utilised in the present study should also be tested under scenarios of increased competence driven by feed to determine which number of feeding stations are required to deliver tailored supplementation and whether this can be possible or not. Additionally, further studies should also be conducted to determine the effects of animals’ feeding behaviour on the accuracy of measurements of feed disappearance. For instance, the EF utilised in the present study seems to be sensible to small variations in feed disappearance (i.e. +/- 5 g/visit). However, there were records not biologically possible (e.g. negative or too-high values) resulting from animals hitting the EF bin. Finally, a single EF for 27 animals may probed to be enough in our feeding conditions but it would not be economically feasible in extensive scenarios with many animals. There is a need of exploring management strategies to increase EF usage without compromising accuracy and feed delivery. Another approach described in the present thesis was the use of EF technology to estimate supplement intake indirectly from the equations presented in chapter 5 to estimate MLB intake from feeding duration. The latter approach could be used to develop affordable feeders based on animals’ attendance and feeding duration instead of measuring feed disappearance which may involve using a more costly equipment. However, eating or licking rate may be affected by MLB composition and hardness so equations should be tailored for each formulation.

Similarly, studies are needed to assess factors influencing the number of LW records obtained using WOW in different production systems (e.g. large vs small beef properties). Liveweight data from the present study enabled growth trajectories of different animal categories to be obtained during periods ranging from 112 days (Calves) to 4 years (Cows). However, the water point was
centrally located to the paddocks that were subjected to controlled rotational grazing most of the time and paddocks were small. The ability to capture complete growth trajectories in larger paddocks with sparse water points is unknown. Additionally, the ability of WOW to continuously monitor LW would require analysis of the distance to the water points and periods with high rainfall may reduce attendance. These conditions may lead to identify other incentives to modify voluntary traffic of cattle which could include the use of supplements, different WOW setup location (e.g. in between paddocks) or portable scales. Additionally, there is a need to investigate the maximum number of animals per WOW station which can be used without altering the number of records required to describe animals’ growth paths. The studies discussed in the introduction (Chapter 2) utilised up to 824 cows per WOW (dairy herd, van Straten et al. 2008); therefore, the number of animals described in those studies and the present thesis may not be directly comparable with larger farms. However, determining the correct number of animals per WOW maybe a complex task because it could be affected by multiple factors including feed type, quality and amount. For instance, animal attendance to the water point could be lower while grazing winter crops with high water content (e.g. oat cops) compared with low-quality dry pastures or fed with hay during the dry season. In the latter case, cattle may attend the water point several times per day being highly dependent of such source of water. However, these are just hypothesis and further research is required.

8.3 Integrating data from in-paddock technologies and metabolomics

Integrating LW and supplement intake data with the abundance of blood metabolites at particular time points was relevant in Chapters 6 and 7 to associate changes between the metabolome of animals and their near-real time performance. The variations in the RA of metabolites could indicate the use of particular metabolic pathways which would subsequently affect the phenotype of animals. It is important to notice that the relationship between LW data and metabolites will be affected by the time period in which each measurement was recorded. For example, both the blood metabolome and LWC were measured at a specific point in time and similar scale of time (daily). However, results may be completely different if LW for the calculation of LWC was measured further apart. The effect of ILW on the estimation of LWC of weaner cattle was discussed in Chapter 3 and is presented in Figure 8.1 below. Vertical arrows indicate two time points of blood sampling when animals were mustered to the yard (BS2 and BS3, Chapter 6 and 7). The LWC on BS3 across all animals estimated using daily WOW data was 1.25 kg/hd per day, however LWC calculated from LW measures on days 116 (BS2) and 156 (BS3) was 1.08 kg/hd per day. This represents 14% less compared to daily LWC on BS3 with animals experiencing an ascendant curve of growth. Additionally, horizontal arrows indicate the importance of continuously monitoring LW of cattle because the same value of LWC (i.e. 1.08 kg/hd per day) could be measured at a time when animals were experiencing an ascendant or descendant growth path. In addition, Table 8.1 presents the estimated LWC calculated between individual animals on BS2 using daily measures and increasing the time between LW measurements from 1 to 8 weeks before the blood assessment. Increasing ILW resulted in a
reduction of the estimated mean LWC from 0.52 to 0.11 kg/d, and the maximum and standard deviation. These results demonstrate the importance of the frequency of data collection to capture trends and trajectories in LWC and the large influence this could have on results using these data such as is the case with blood metabolites.

Figure 8.1: Liveweight change (LWC) of weaner cattle calculated from daily data on day 156 (BS3; 1.25 kg/hd per day; vertical arrow) and calculated from LW measures recorded between Day 116 (BS2) and 156 (BS3; 1.08 kg/hd per day; horizontal solid line).

Table 8.1 of this section conveys a clear picture of possible impacts of correlating LWC calculated from data collected at different ILW with the RA of metabolites measured at a particular blood sampling point. Increasing the ILW measurements had large implications on the correlation between LWC and the RA of metabolites at BS2 (Table 8.2). For example, the metabolite 2-hydroxybutyrate was significatively correlated (P < 0.05) with LWC measured daily or up until week 4 before the blood assessment (LWC4). However, this significance disappeared and became weaker with longer ILW (P-value increased from 0.08 for LWC5 to 0.70 for LWC8). Similarly, the coefficient of correlation between the RA of 2-hydroxybutyrate and LWC ranged from -0.47 for daily LWC to -0.05 for LWC8 (Table 8.3). However, the effect of ILW on the coefficient of correlation between LWC and the RA depended on the metabolite with some being more affected than other at different ILW (Table 8.2 and 8.3). On one hand, these results demonstrate the differences in results and conclusions that could be obtained depending on the ILW or time period in which each measurement is taken. On the other hand, these results highlight the need for more research in this space because these correlations may translate into different biological meanings of the associations between desirable traits and the metabolome of animals.
Table 8.1: Descriptive statistics of liveweight change (LWC) calculated between individual animals the day before blood sampling 2 (BS2, daily LWC) and from different intervals of time between liveweight (LW) measures ranging from 1 to 8 weeks.

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily LWC</td>
<td>0.517</td>
<td>0.261</td>
<td>-0.023</td>
<td>1.097</td>
</tr>
<tr>
<td>LWC1</td>
<td>0.396</td>
<td>0.262</td>
<td>-0.167</td>
<td>0.932</td>
</tr>
<tr>
<td>LWC2</td>
<td>0.290</td>
<td>0.240</td>
<td>-0.215</td>
<td>0.779</td>
</tr>
<tr>
<td>LWC3</td>
<td>0.194</td>
<td>0.160</td>
<td>-0.143</td>
<td>0.519</td>
</tr>
<tr>
<td>LWC4</td>
<td>0.167</td>
<td>0.149</td>
<td>-0.142</td>
<td>0.484</td>
</tr>
<tr>
<td>LWC5</td>
<td>0.145</td>
<td>0.128</td>
<td>-0.100</td>
<td>0.423</td>
</tr>
<tr>
<td>LWC6</td>
<td>0.129</td>
<td>0.108</td>
<td>-0.091</td>
<td>0.348</td>
</tr>
<tr>
<td>LWC7</td>
<td>0.119</td>
<td>0.094</td>
<td>-0.074</td>
<td>0.324</td>
</tr>
<tr>
<td>LWC8</td>
<td>0.115</td>
<td>0.086</td>
<td>-0.061</td>
<td>0.311</td>
</tr>
</tbody>
</table>
Table 8.2: P-values from Pearson’s correlation analysis between the relative abundance of 13 blood metabolites in weaner cattle (Chapter 7, BS2) and liveweight change (LWC) calculated with daily data (Daily LWC) and calculated from intervals of liveweight (LW) data collection ranging from 1 to 8 weeks. Colours indicate the degree of significance (Red, P < 0.01; Green, P > 0.10).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Daily LWC</th>
<th>LWC1</th>
<th>LWC2</th>
<th>LWC3</th>
<th>LWC4</th>
<th>LWC5</th>
<th>LWC6</th>
<th>LWC7</th>
<th>LWC8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.0003</td>
<td>0.0003</td>
<td>0.0036</td>
<td>0.0438</td>
<td>0.2201</td>
<td>0.4097</td>
<td>0.4461</td>
</tr>
<tr>
<td>2-hydroxybutyrate</td>
<td>0.0007</td>
<td>0.0006</td>
<td>0.0019</td>
<td>0.0019</td>
<td>0.0114</td>
<td>0.0777</td>
<td>0.3190</td>
<td>0.5844</td>
<td>0.6999</td>
</tr>
<tr>
<td>Valine</td>
<td>0.0014</td>
<td>0.0005</td>
<td>0.0010</td>
<td>0.0010</td>
<td>0.0038</td>
<td>0.0251</td>
<td>0.1187</td>
<td>0.2429</td>
<td>0.2975</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.0020</td>
<td>0.0015</td>
<td>0.0024</td>
<td>0.0024</td>
<td>0.0111</td>
<td>0.0517</td>
<td>0.1608</td>
<td>0.2737</td>
<td>0.3552</td>
</tr>
<tr>
<td>Creatine</td>
<td>0.0023</td>
<td>0.0063</td>
<td>0.0173</td>
<td>0.0173</td>
<td>0.0573</td>
<td>0.1877</td>
<td>0.4351</td>
<td>0.6647</td>
<td>0.7255</td>
</tr>
<tr>
<td>Unsaturated Lipid</td>
<td>0.0217</td>
<td>0.0095</td>
<td>0.0102</td>
<td>0.0102</td>
<td>0.0164</td>
<td>0.0376</td>
<td>0.0967</td>
<td>0.1653</td>
<td>0.2280</td>
</tr>
<tr>
<td>PhenylAlanine</td>
<td>0.0281</td>
<td>0.0416</td>
<td>0.0491</td>
<td>0.0491</td>
<td>0.0834</td>
<td>0.1900</td>
<td>0.4152</td>
<td>0.6476</td>
<td>0.8542</td>
</tr>
<tr>
<td>MethylHistidine</td>
<td>0.0312</td>
<td>0.0206</td>
<td>0.0248</td>
<td>0.0248</td>
<td>0.0566</td>
<td>0.1319</td>
<td>0.2637</td>
<td>0.3679</td>
<td>0.4450</td>
</tr>
<tr>
<td>Lipid VLDL</td>
<td>0.0477</td>
<td>0.0205</td>
<td>0.0169</td>
<td>0.0169</td>
<td>0.0237</td>
<td>0.0413</td>
<td>0.0779</td>
<td>0.1135</td>
<td>0.1579</td>
</tr>
<tr>
<td>AcetylGroups</td>
<td>0.0643</td>
<td>0.0801</td>
<td>0.1654</td>
<td>0.1654</td>
<td>0.4108</td>
<td>0.8561</td>
<td>0.7069</td>
<td>0.5530</td>
<td>0.6224</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.0747</td>
<td>0.0442</td>
<td>0.0362</td>
<td>0.0362</td>
<td>0.0398</td>
<td>0.0632</td>
<td>0.1012</td>
<td>0.1392</td>
<td>0.1612</td>
</tr>
<tr>
<td>Formate</td>
<td>0.0863</td>
<td>0.1562</td>
<td>0.1702</td>
<td>0.1702</td>
<td>0.2126</td>
<td>0.3339</td>
<td>0.5028</td>
<td>0.6108</td>
<td>0.6263</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.0921</td>
<td>0.0760</td>
<td>0.0861</td>
<td>0.0861</td>
<td>0.0847</td>
<td>0.1164</td>
<td>0.1895</td>
<td>0.2753</td>
<td>0.3115</td>
</tr>
</tbody>
</table>
Table 8.3: Pearson Correlation coefficient between the relative abundance of 13 metabolites in weaner cattle (Chapter 7) and liveweight change (LWC) calculated with daily LW data the day before the blood assessment (Daily LWC) and calculated from intervals between liveweight (LW) measures ranging from 1 to 8 weeks. Colours indicate the degree of correlation (Red, highly to moderate correlated; Green slightly to not correlated).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Daily LWC</th>
<th>LWC1</th>
<th>LWC2</th>
<th>LWC3</th>
<th>LWC4</th>
<th>LWC5</th>
<th>LWC6</th>
<th>LWC7</th>
<th>LWC8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>-0.53</td>
<td>-0.55</td>
<td>-0.50</td>
<td>-0.50</td>
<td>-0.41</td>
<td>-0.29</td>
<td>-0.18</td>
<td>-0.12</td>
<td>-0.11</td>
</tr>
<tr>
<td>2-hydroxybutyrate</td>
<td>-0.47</td>
<td>-0.48</td>
<td>-0.43</td>
<td>-0.43</td>
<td>-0.36</td>
<td>-0.25</td>
<td>-0.14</td>
<td>-0.08</td>
<td>-0.05</td>
</tr>
<tr>
<td>Valine</td>
<td>-0.44</td>
<td>-0.48</td>
<td>-0.46</td>
<td>-0.46</td>
<td>-0.40</td>
<td>-0.32</td>
<td>-0.22</td>
<td>-0.17</td>
<td>-0.15</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.43</td>
<td>-0.44</td>
<td>-0.42</td>
<td>-0.42</td>
<td>-0.36</td>
<td>-0.28</td>
<td>-0.20</td>
<td>-0.16</td>
<td>-0.13</td>
</tr>
<tr>
<td>Creatine</td>
<td>-0.42</td>
<td>-0.38</td>
<td>-0.34</td>
<td>-0.34</td>
<td>-0.27</td>
<td>-0.19</td>
<td>-0.11</td>
<td>-0.06</td>
<td>-0.05</td>
</tr>
<tr>
<td>Unsaturated Lipid</td>
<td>0.33</td>
<td>0.37</td>
<td>0.36</td>
<td>0.36</td>
<td>0.34</td>
<td>0.30</td>
<td>0.24</td>
<td>0.20</td>
<td>0.17</td>
</tr>
<tr>
<td>PhenylAlanine</td>
<td>-0.31</td>
<td>-0.29</td>
<td>-0.28</td>
<td>-0.28</td>
<td>-0.25</td>
<td>-0.19</td>
<td>-0.12</td>
<td>-0.06</td>
<td>-0.02</td>
</tr>
<tr>
<td>MethylHistidine</td>
<td>-0.31</td>
<td>-0.33</td>
<td>-0.32</td>
<td>-0.32</td>
<td>-0.27</td>
<td>-0.22</td>
<td>-0.16</td>
<td>-0.13</td>
<td>-0.11</td>
</tr>
<tr>
<td>Lipid VLDL</td>
<td>0.28</td>
<td>0.33</td>
<td>0.34</td>
<td>0.34</td>
<td>0.32</td>
<td>0.29</td>
<td>0.25</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td>AcetylGroups</td>
<td>-0.26</td>
<td>-0.25</td>
<td>-0.20</td>
<td>-0.20</td>
<td>-0.12</td>
<td>-0.02</td>
<td>0.05</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>-0.25</td>
<td>-0.29</td>
<td>-0.30</td>
<td>-0.30</td>
<td>-0.29</td>
<td>-0.27</td>
<td>-0.23</td>
<td>-0.21</td>
<td>-0.20</td>
</tr>
<tr>
<td>Formate</td>
<td>-0.25</td>
<td>-0.20</td>
<td>-0.20</td>
<td>-0.20</td>
<td>-0.18</td>
<td>-0.14</td>
<td>-0.09</td>
<td>-0.07</td>
<td>-0.07</td>
</tr>
<tr>
<td>Mannose</td>
<td>-0.24</td>
<td>-0.25</td>
<td>-0.25</td>
<td>-0.25</td>
<td>-0.22</td>
<td>-0.19</td>
<td>-0.16</td>
<td>-0.14</td>
<td></td>
</tr>
</tbody>
</table>
8.4 Using in-paddock technologies and metabolomics to anticipate events and predict future performance of cattle

Chapters 3 and 4 of the present thesis demonstrated that daily data provided by in-paddock technologies could be useful to rapidly detect changes in animal growth and supplement consumption. These temporal and individual changes in growth also showed correlations with the RA of blood metabolites. However, further studies should explore the potential of frequent data to anticipate future events. For instance, Chapter 4 indicated that changes in MLB intake and feeding duration could alert variation in the quantity and quality of forages under grazing conditions. Therefore, it could be possible to anticipate reductions in LWC of growing animals with enough lead time to manage factors influencing it (e.g. introduce feed supplementation, reduce stocking rate). In breeding cows, detecting the first appearance of individual records from calves and their growth trajectories are measures that could be used to individualise weaning based on both calf and cow nutritional status. These potential outcomes could be studied and used along with auto-drafting gates to anticipate and automate activities in the near future.

Findings from Chapters 6 and 7 and published studies described in Chapter 2 indicated that metabolomics is a promising tool to study cattle metabolism (Fontanesi, 2016; Goldansaz et al., 2017). Firstly, throughout Chapters 6 and 7 we identified several metabolites associated with growth rate at group level over time in weaner cattle. These results could improve the understanding of metabolic pathways driving nutrition of grazing animals as they are constantly subjected to changes in feed quantity and quality due to depletion of available forage and growth of new forage. Thus, nutritional management could be tailored to maintain the abundance of blood metabolites that consistently promote growth over long-feeding periods, for example by improving supplement formulation. In addition, metabolomics helped explain the large variability in LWC between animals subjected to the same management and nutrition (Chapter 7). The scope of the present thesis was orientated to cattle nutritional management, but it could be among pioneer studies attempting to anticipate long-term animal performance at early stages of the production cycle. The potential utilisation of this approach has been demonstrated in previous studies on feedlot cattle (Connolly et al., 2019) but no studies were found in grazing beef cattle. Further studies are needed to assess the consistency of correlations between the RA of metabolites and desirable traits at different time of the life of an animal and production systems. Similar studies could be done with heifers born from cows with high fertility to avoid future losses and maintaining less productive animals in the herd. Within this scheme, frequent in-paddock measures may take a critical place to confirm animal performance predicted using metabolomics.

8.5 Conclusions

The central aim of this thesis was to explore the ability of in-paddock technologies, specifically WOW and EF, to continuously collect data from individual grazing beef cattle, and to combine these with metabolomics to understand the physiological changes reflecting LWC and MLB intake. This novel approach enabled a better understanding of grazing cattle performance and
metabolism, which could promote the development of strategies to manage cattle and improve their productivity through a reduction of the variability in growth rate over time and between animals. This thesis has contributed with new, unique and original knowledge to the field of precision livestock production as follows:

1) The present research was the first to demonstrate the impacts of the length of the interval between liveweight measurements on liveweight and estimated liveweight change in different animal categories. This represents key information to monitor grazing cattle automatically and remotely, and to enhance procedures involved in conventional weighing as well.

2) A novel approach was used in this research using in-paddock LW monitoring to detect the effects of molasses-lick-block supplementation on daily growth rate of cattle over a long-term rotational grazing trial. In addition, this study is the first to assess the relationship between the intake of a self-fed supplement and the quantity and quality of forages available which could be used as an indicator to improve grazing management.

3) This thesis also demonstrated practical applications of in-paddock technologies to monitor and determine differences in growth rate and supplement intake among individual animals. The present research was the first to establish correlations between the intake of a molasses-lick-block supplement, performance and the feeding behaviour of animals. Feeding behaviour was shown to be accurate to predict supplement disappearance and to develop novel feeding methodologies and low-cost automatic feeders.

4) Finally, this is the first study integrating in-paddock technologies with metabolomics to understand and explore associations between the metabolic profile, growth rate and supplement intake of grazing beef cattle. Our findings indicated that metabolomics offer several opportunities to improve cattle performance over time as animals were fed a range of feed types. This approach could also be used to identify the metabolic profile of high-performing individuals.

Overall, the present research added value to the use and integration of data collected by in-paddock technologies with metabolomics. The knowledge generated can be used to enhance animal productivity and welfare, and to contribute to implement novel technologies and methodologies to anticipate and automate events in grazing beef cattle in the near future.

8.6 References


