Evaluation of the Behaviour of Angus Heifers Grazing Mixed Pasture and Cereal Crops

Submitted by

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List of Abbreviations

ADG	Average daily gain
ADF	Acid detergent fibre
ANOVA	Analysis of variance
BPF	Ban pass filter
BW	Body weight
DMD	Dry matter digestibility
DOM	Digestible organic matter
CP	Crude protein
CSV	Comma separated value
CTI	Centre for technology infusion
DM	Dry matter
DMI	Dry matter intake
DSE	Dry sheep equivalent
FOO	Feed on offer
GPS	Global Positioning System
GUI	Graphical user interface
HPF	High pass filter
LPF	Low pass filter
MAF	Moving average filter
ME	Metabolisable energy
MEMS	Micro-electro-mechanical system
ML	Machine learning
NDF	Neutral detergent fibre
NSW	New South Wales
NPN	Non protein nitrogen
PG	Pre-grazing
PSG	Post-grazing

PD	Peak detection
TMR	Total mixed ration
VFA	Volatile fatty acid
VM	Vector magnitude

Abstract

Feed requirements of ruminants grazing on perennial pastures cannot be fulfilled in winter owing to the low growth rate of grass. To supplement the insufficient feed produced on grasslands in winters (i.e., winter feed gap), cereal forage crops are often introduced to ruminants. Cereal crops have long been used in Australia to fill the winter feed gap. However, limited data exist on the behaviour of cattle grazing cereal crops compared with mixed pasture. The overall aim of this thesis was to evaluate the behaviour of Angus heifers while grazing mixed pasture, wheat and barley crops. Heifers (n = 79, aged 9 months) were fitted with halters having motion and proximity sensors (ActiGraph wGT3X-BT) and were grazed on mixed pasture, then moved to wheat crop followed by barley crop. Each pasture lasted 9 days; sensor outputs were collected for the last 5 days. At the end of each phase, heifers were weighed, blood samples collected and data from the sensors were downloaded using ActiLife software. Algorithms to classify cattle behaviour from the triaxial acceleration values were developed at the Centre for Technology Infusion, La Trobe University, with MATLAB software. In the classifier program, algorithms were based on the decision tree method and moving time-window. The report generator was designed for data visualising and generating summarised reports as tables, which were saved in comma-separated value format. After raw data validation using captured videos, sensor data were translated to feeding behaviour (grazing time; bite number, duration, and intensity) and rumination (duration and number of boluses). Proximity sensors were used to determine the number of daily visits by heifers to water troughs (in all pastures) and straw and salt licks (which were only provided in wheat and barley pasture). Rumination time was 357 ± 40.4 and 343 ± 82.5 min/day in mixed pasture and wheat pasture, respectively, dropping sharply in barley pasture to 192 ± 54.3 min/day. Grazing time in wheat pasture (231 ± 116.7 min/day) was significantly ($p < 0.05$) shorter than in mixed pasture (512 ± 53.5 min/day) and barley pasture (476 ± 93.5 min/day). However, most of the grazing occurred in daylight between 07:00 to 18:00 in all pastures. Bite intensity in barley pasture (0.15 ± 0.006 g; gravity = 9.8 m/s^2) was significantly ($p < 0.05$) lower than in mixed pasture (038 ± 0.02 g) and wheat pasture (0.22 ± 0.01 g). Moreover, the high nitrate level in lush barley (8,300 mg/kg dry matter) increased the nitrate level in plasma of heifers grazed on barley crop (22.3 ± 12.3 µmole/L). Visits to the water troughs and salt licks in barley crops doubled compared with that in mixed and wheat pasture, respectively. The results of this study should be interpreted with caution because animals had only 4 days acclimation period on each pasture. Transition from mixed pasture to cereal crops in a short period led to changing the behaviours of heifers, including increasing their visits to drinking water troughs, salt licks and straw. Also, a high level of nitrate can affect rumen functions consequently changing the behaviour of Angus heifers.

Keywords: Angus heifers, grazing, rumination, nitrate

Statement of Authorship

This thesis includes original results obtained by the author. Except where reference is made in the text of the thesis, this thesis contains no material published or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma. No other person's work has been used without due acknowledgement in the main text of the thesis. This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

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Chapter 1: Introduction and Literature Review

1.1 Background

Most pasture lands in Australia are covered by perennial grasses. In winter, the low growth rate of grasses results in failure to meet feed requirements of livestock, which is known as the feed gap. Unsuitable weather and soil conditions, such as inappropriate temperature, sunlight and soil moisture and nutrients can affect pasture growth, subsequently causing substantial feed deficits in winter. In the pasture-based mixed farming system in southern Australia, feed supplements are costly; thus, cereal crops or dual-purpose crops, such as wheat and barley, are introduced to livestock to fill the winter feed gap. Due to fertiliser practices on cereal crops, they might be high in nitrate levels; therefore, nitrate-nitrite toxicity may occur while cattle are grazing on cereal crops.

Despite the high quality of dual-purpose crops, the response of cattle is very variable, and the reason for this variability is yet to be determined. Monitoring cattle feeding behaviour on a large scale in the pasture-based system is challenging. Feeding behaviour and other activities while cattle graze on different pastures can be useful to evaluate the health and welfare of animals. The level of nitrate can be high in cereal crops under certain circumstances and cause nitrate-nitrite toxicity which could impact cattle behaviour. Recently, sensor technology has been implemented on research farms to determine the feeding behaviours and other activities of cattle while they graze on pastures. By using triaxial sensor technology, valuable data regarding beef cattle behaviour, such as grazing, bite count, bite intensity, rumination, drinking, walking and salt licking can be obtained. These data can be used to assess the productivity of each animal.

1.1.1 Hypothesis

The null hypothesis of this study was that the behaviour of Angus heifers does not differ while grazing on either mixed pasture or cereal crops.

1.2 Introduction

The world population is expected to reach 9.2 billion by 2050 and 12.6 billion by 2100 (Kc & Lutz, 2017), which will increase the demand for food and, in particular, animal products. Beef cattle production is a major agricultural industry in all territories and States of Australia, with 23% and 22% of the total gross value of farm production allocated to beef production and farm export, respectively. The beef industry is the most common farm business in Australia, and 57% of Australian farms carry beef cattle (Department of Agriculture and Water Resources, 2018). According to a 2016 Bureau of Statistics report, the total number of beef cattle in Australia is 25 million, with Victoria accounting for 3.5 million. More than 30 different breeds of beef cattle are raised in Australia, with Aberdeen Angus a popular breed, particularly in southern Australia.

This breed, mostly recognised for its high-quality meat, was introduced to Australia in 1840 from Scotland (Department of Environment and Primary Industries, 1996).

In Victoria, beef cattle farms are spread across the State, with most of them located in the Western District, Gippsland, Oven Murray and Goulburn regions. Beef production in Victoria, in 2013–2014, was the highest in terms of value at $1.0 billion, representing 44% of Victoria's meat export (Department of Economic Development, Jobs, Transport and Resources, 2014).

In Australia, the beef cattle industry is primarily pasture-based as it is the cheapest source of (cattle) feed. Further, annual pastures are the main source of feed for livestock, particularly in high-rainfall regions in the Australian pasture-based system to provide feed requirements for ruminants. However, because of the slow growth rate of pasture during winter, grazing animals often cannot obtain their feed requirement from the pasture; thus, providing them a feed supplement or an alternative source of feed (for grazing) during winter is necessary (Dove et al., 2015).

1.3 Pastures

Pasture lands are mainly covered by either native or introduced grass and legume species so that these can be grazed by livestock and/or used for conserved fodder production. Pasturelands are economically valuable since they are the fundamental element of animal production. They also promote carbon sequestration, preserve biodiversity, reduce soil degradation and loss and maintain water quantity and quality (Conant, Paustian, & Elliott, 2001; Hopkins & Alison, 2006).

Many varieties of grass and legumes are sown in pastures in Australia. Phalaris (*Phalaris acquatica*) and subterranean clover (subclover, *Trifolium subterraneaum*) are widely sown in western and southwestern Victoria. However, limited studies consider livestock behaviour when grazing on mixed pastures (especially for phalaris and subclover) in Australia during winter. In a study on the performance of ewes and lamb on different pastures in Australia, their liveweight gain was measured during different periods of the years in New South Wales (NSW). From June to November, the liveweight gains on sub-clover pasture and phalaris pasture were 21.6 kg (0.130 kg/d) and 20.4 kg (0.123 kg/d), respectively. Sheep performance from January to April and April and June on subclover was 1.4 kg (0.02 kg/d) and 3 kg (0.042 kg/d) and on phalaris was 2.3 kg (0.033 kg/d) and 3.1 kg (0.044 kg/d), respectively (Reed et al, Snaydon, & Axelsen, 1972).

Depending on the regional weather and soil conditions and low growth of pasture, grazing management in winter can be occasionally challenging to farmers. Several factors influence pasture growth rate, including sunlight, temperature and soil moisture and fertility. Among these factors, temperature has a significant effect. Low temperature can reduce dry matter (DM) production by 1 kg ha/d in winter (PROGRAZE, 2013). Pasture growth rates estimated mid-month in different regions within a year in Victoria and NSW are shown in Figure 1.1.

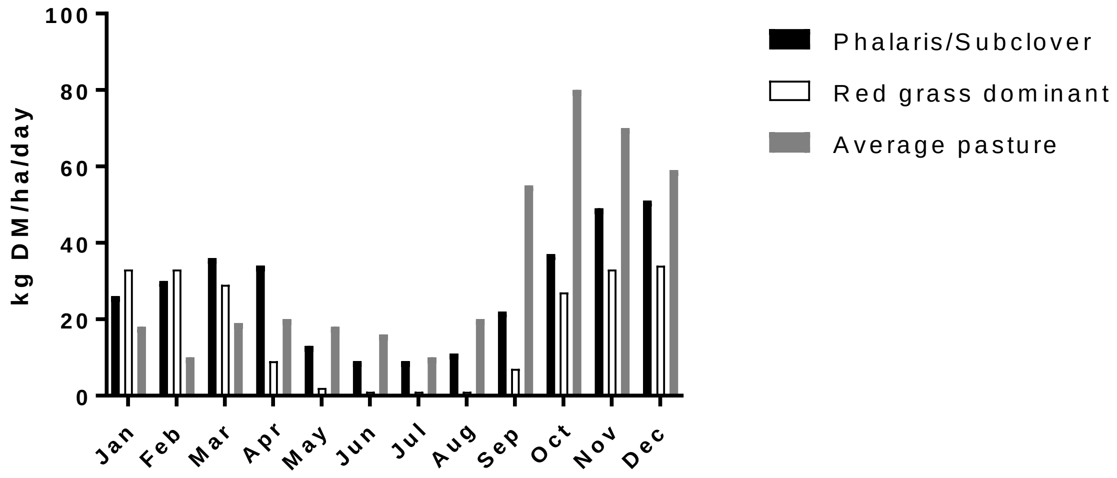


Figure 1.1: Estimated pasture growth rate (kg DM/ha/d) in different regions; phalaris and sub-clover are in Northern Tablelands, NSW (source: NSW PROGRAZE Manual, Appendix 4, NSW Agriculture); average pasture growth rate in Gippsland-Ellinbank, Victoria (source: Victoria PROGRAZE Manual; adapted from Figure 8)

The gap between the amount of forage produced in winter because of the low pasture growth rate and the amount of feed required by grazing livestock is known as the 'winter feed gap'. Typically, the concept of feed gap means that during a period, insufficient forage is supplied to livestock to meet their requirements either in terms of quality or quantity. Feed gap plays an important role in livestock production because feed shortage in winter can be a bottleneck for livestock production. Pasture management practices often significantly reduce the intensity and frequency of feed gap by rotating pastures in a certain period to maximise livestock efficiency. The severity of feed gap can only be recognised by the manager of the property based on production goals. However, feed gap conditions arise from an interaction between the variety of forages on the property, the intake of livestock on the grassland of that property and the manager's economic objectives. Since the livestock can easily be rotated between paddocks, feed gap is often considered a property-level phenomenon (Moore et al., 2009). One option to overcome the winter feed gap is to introduce livestock to cereal forage crops that provide sufficient energy and protein.

1.3.1 Stocking rate, feed on offer and sward height

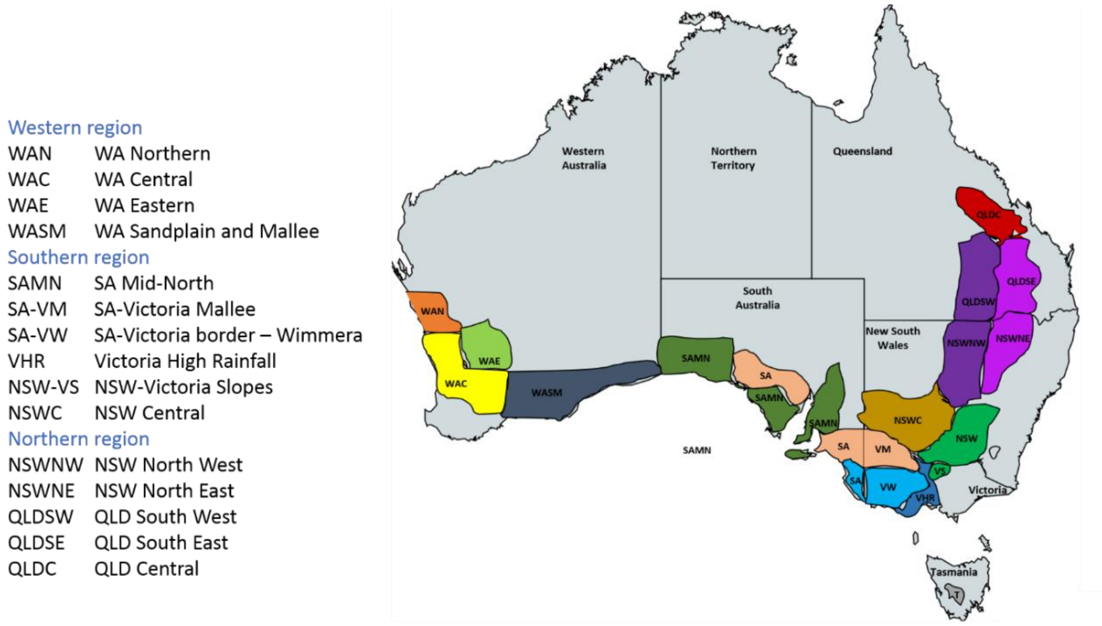
Stocking rate is an indication of the number of livestock grazing per unit area. In other words, it is simplified the relationship between herbage demand and supply which can be a useful management tool in pastoral farming (Animut et al., 2005). Stocking rate has a major influence on feed intake on pasture and subsequently herbage allowance per cow (Baudracco et al., 2010). The amount of available herbage or mass of pasture expresses in kg DM/ha is feed on offer (FOO). Both FOO and stocking rate are a valuable measurement for grazing management decision. Three levels of FOO are crucial when livestock graze on the paddock: 500, 1000 and 1500 kg DM/ha (Smith et al., 1972). When FOO is below 500 kg DM/ha, there are two major consequences; one is sheep would be in a negative energy balance and second pastures are vulnerable to damage from uprooting of seedlings by grazing. However, sheep can be in a positive energy balance if FOO stands between 500 to 1000 kg DM/ha. Also, the rate of pasture growth is higher than if it is removed by grazing. When the levels of FOO exceeds 1500 kg DM/ha, sheep are rapidly gaining weight (Smith et al., 1972). In a study on beef cattle, eating behaviour was assessed when cattle were offered 1900 and 3900 kg DM/ha. Cattle spent less time at feedlot (1.23 h) while they were on a pasture with higher FOO, on the other hand, cattle allocated more time (1.37 h) at feedlot when they were offered 1900 kg DM/ha (Lee et al., 2013). Ruminants would graze more when less feed is offered in order to meet their nutritional demands, whereas they can ingest more feed per bite by grazing less on pasture with higher FOO.

The amount of feed intake in the pasture-based system is regulated by two factors: FOO kg DM/ha and the composition of feed. Generally, high-quality feed takes less time to digest and can move through the animal’s digestive system more rapidly than low-quality feed. Hence, ruminants can consume more feed in a shorter time. Additionally, intake may also be limited by sward height. They compared feeding behaviour of cattle on pasture with 5 and 10cm sward surface height in multiple periods. On all occasions, steers spent less time grazing (7.9 h vs 10.8 h; 8.9 h vs 11.1h and 9.6 h vs 10.3 h, respectively) when they were on 10 cm compared to 5 cm pasture sward height. On the other hand, they ruminated more (8.4 h vs 6.8 h, 8.1 h vs 5 h and 7.4 h vs 8 h, respectively) when they were on 10 cm pasture sward height (Realini et al., 1999). The possible explanation would be an increased intake per bite on taller sward height. Sward height has a positive correlation with intake per bite. This means that decreased sward height minimises an intake per bite (Hodgson, 1981). Therefore, the differences between short and tall sward height resulted in different herbage intake and grazing behaviour, and consequently animals grazed on 10 cm sward height had 71% higher liveweight gain compared to grazing on 5 cm sward height pasture (Realini et al., 1999). All these three factors, stocking rate, FOO and sward heights, may affect feeding behaviour of ruminants.

1.4 Cereal Crops

Across Australia, around 22.3 million ha are annually allocated to the growing of cereal crops (Australian Export Grains Innovation Centre [AEGIC], 2016). Figure 1.2 displays Australian crops zones based on climate, weather patterns and soil type. Australian croplands are divided into two major grain production regions, northern and southern, and the country has two crop-growing periods; winter and summer. Geographically, the northern cropping region includes central and southern Queensland through to northern NSW (PROGRAZE) extending down to the Dubbo region. The main winter grain crops in this region are wheat, barley, oats, triticale, canola, safflower and linseed; the main summer crops include sorghum, sunflowers, maize, soybeans and cotton (AEGIC, 2016). Summer crops are sown during a long period, beginning from March in the Queensland Central region to July in NSW Central region and winter crops are sown from September to December.

Southern crop lands include central NSW (south of Dubbo) through to Victoria, Tasmania, South Australia and the southwest corner of Western Australia. These southern farmlands produce the most crops in Australia, since they account for a wide area of the country and have a Mediterranean rainfall pattern in winter. Their winter crops include wheat, barley, oats, triticale, cereal rye, lupins field peas, canola, chickpeas, faba beans, vetch, lentils and sunflower. Owing to the dry summer, crop production (particularly for rice and maize farms) in these southern regions requires irrigation. In Australia, wheat crop is considered the first major crop and barley the second (AEGIC, 2016).



Source: adapted from AEGIC (2016)

Figure 1.2: Australian agroecological zones

1.4.1 Dual-purpose crops

The ‘Grain and Graze’ programme has extensively explored the use of grain crops as nutritious forage sources for livestock and the way it contributes to the farm grazing system in extensive regions of Australia. Owing to feed shortage in winter, farmers in the mixed farming system use dual-purpose crops to reduce the grazing pressure on pastures as well as provide high-quality forage to livestock (Kirkegaard et al., 2008) and consequently eliminate the need for feed supplementation. The term dual-purpose means that such crops have two uses in the mixed farming system; that is, these crops are grazed by livestock and then used for hay, silage or grain production. These dual-purpose crops include the cereals (oats, wheat, triticale, barley) and canola (a brassica species) and they are widely grown across south-eastern Australia, with a total area of 300,000 ha sown to cereal crops in 2011. Of this, 130,000 ha were allocated to wheat, 90,000 ha to oats and 60,000 ha to triticale. Compared to oats and wheat, barley and triticale are less commonly cultivated as dual-purpose crops (Radcliffe et al, 2012).

Dual-purpose crops, sown early in autumn, are grazed in the vegetative growth stage to supply adequate feed in the winter feed gap period. The growth rate of cereal crops in the early stage are mainly linked to soil moisture and temperature. Additional factors that act like a trigger from the vegetative stage to the flowering stage are photoperiods and vernalisation (Li et al., 2013). Photoperiod is a response to spring (increasing day length), and vernalisation is a response to winter (cold temperature). Experiencing a cold temperature period, around 4 to 18 ºC, for 4 to 6 weeks is required for plants to trigger reproduction; the plant will remain vegetative if it does not experience this cold temperature (Ream et al., 2014). Seasonal conditions do affect the DM content, but generally, barley produces feed earlier than wheat in both winter and spring (Nicholson et al, 2016).

1.4.2 Grazing dual-purpose crops

Since the plants are anchored and grow secondary roots, the cropland can be grazed. Hence, the grazing usually starts around the three-leaf stage (Growth Stage/Score 13/21) of cereals. However, this time might be adjusted if a dry period occurs after initial germination (Keith, 2009). Preferably, to achieve suitable performance, the amount of feed produced on a farm should be about 500 kg DM/ha to 800 kg DM/ha for sheep production and 1,000 kg DM/ha for cattle production (Nicholson et al., 2016). Most cereal crops cannot reach this growth level to achieve the best performance of livestock; conversely, postponing the grazing time to obtain best performance may limit grazing opportunity and reduce grain yield. No definite recommendation exists for the minimum amount of biomass at grazing time. Radcliffe et al. (2012) suggested that plants are anchored when forage exceeds 1,000–1,500 kg DM/ha, while Virgona et al (2006) suggested that plants (wheat crops) at 500 kg DM/ha are well anchored, and therefore, ruminants cannot uproot the plants.

Sheep and cattle have been grazing wheat in many mixed farming systems because of its long vegetative phase (Virgona et al., 2006). The forage yield during the vegetative stage varies among cereal crops. Further, depending on the cultivar, grazing or cutting may or may not affect the forage yield. Thus, harvesting the crops is not recommended, owing to their low DM yield at boot stage (i.e., developing seedhead).

Barley is usually grown across a large geographic area accounting for 4 million ha across southern Australia. It is the largest crop after wheat in terms of volume. Its weak straw strength makes barley susceptible to logging, especially in high-moisture conditions. Moreover, it reaches its maturity stage much faster than other cereal crops. Hence, there is a short time-window for harvesting barley, which is often considered a disadvantage. It is estimated that the forage yield would be 5 t DM/ha when in the vegetative stage, which enables extension of grazing of this crop (Australian Bureau of Agricultural and Resource Economics and Sciences, 2018).

The major risk of grazing on croplands is its possible adverse effect on grain yield. Virgona et al. (2006) showed that grazing for a short period on wheat crops does not have any major effect on plant development and gain yield. In two different experiments with grazing sheep on wheat crops, they found that with grazing for 19 d the grain yield increased from 2.30 to 2.88 t/ha (in a season with dry early spring) but (in a second experiment) grazing for 15 d had no effect on grain yield. Grazing for 51 d reduced grain yield from 5.97 to 3.98 t/ha. If the grazing occurs after the beginning of stem elongation stage, it can lead to loss of grain yield. Farm economic performance was improved by allowing ewes to graze wheat crops before GS31. However, this improvement depends on the effect on grain yield alongside lambing time and stocking rate (McGrath et al., 2014).

1.4.3 Composition of dual-purpose cereal crops

Cereal crops are historically a high-quality feed source with more than 80% DM digestibility (DMD) and high in energy, with more than 12 MJ metabolisable energy (ME/kg DM; Kelman and Dove, 2007). Additionally, cereal crops have high crude protein (CP), which does not constrain growth or feed intake by ruminants (Masters & Thompson, 2016). If CP is below 100–120 g/kg digestible organic matter (DOM), it would be considered a constraint to growth of young animals (Weston, 2002). In a detailed review, Masters and Thompson (2016) estimated that 80% DMD corresponds to ~ 750 g DOM/kg DM, and thus, the minimum CP content of 212 g/kg DM (21.2% CP) equals 280 g CP/kg DOM. Several studies have determined DMD, CP and ME of various wheat varieties/cultivars. Dove and McMullen (2009) reported 87.6% DMD, 31.3% CP and 13.4 MJ ME/kg DM of the cultivar Mackellar winter wheat and 83.9% DMD, 29.4% CP and 12.7 MJ ME/kg DM for the cultivar Marombi.

Several studies have focused on the liveweight of livestock grazing on different cereal crops in winter, either in the long term or short term (Larry et al., 1995; Freer et al., 1997; Lippke et al., 2000; Phillips & Horn, 2008; Dove & McMullen, 2009). Generally, forages contain more K than the required level for sheep and cattle (Nutrient requirements of domesticated ruminants., 2007), and this amount of K is greater in wheat forage (Dove and McMullen, 2009). Considering the low level of Na in wheat forage, the K : Na ratio increases when sheep graze on wheat pasture. This adversely affects the absorption of Mg from the rumen (Dove and McMullen, 2009; Dove et al., 2016). Most of the studies emphasised that magnesium and sodium supplements (NaCl : MgO in 1:1 ratio) should be provided when sheep or cattle are grazing on cereal crops (especially wheat crops) to increase the liveweight gain by 15–60% (Dove & McMullen, 2009; Dove et al 2011). Providing mineral supplements may affect feed intake while ruminants are grazing cereal crops.

Dove and Kelman (2015) reported that ruminants feed intake were reduced on cereal crops if mineral supplementations were not provided to them. However, this reduction in feed intake is not well determined. This could be due to the changes in rumen transaction related to high dietary K concentration or a post-absorptive metabolic disturbance (Dove et al., 2016).

Cereal crops are categorised as plants that have the capability of accumulating high levels of nitrate (Baker and Tucker, 1971). Nitrogen (N) is deficient in soils in most agricultural regions of Australia. Several practices are adopted to increase N levels in soils, such as applying N-rich fertilisers (e.g., urea and sulphate of ammonia), to ensure its concentration in soil is adequate (McDonald, 1989). N fertilisers are often applied to crops at the sowing stage to ensure robust growth. High soil N level can lead to high nitrate concentrations in plants. Some other factors may also facilitate nitrate accumulation by plants, such as low soil sulphur and molybdenum deficiency, potassium application, drought, cloudy or cold weather and stage of maturity (Crawford et al., 1961; O'Hara and Fraser, 1975). Nitrate mostly accumulates in stems rather than leaves and concentrations tend to be highest in immature forage. High nitrate concentrations in forage and subsequent conversion to nitrite in the rumen can cause toxicity in grazing livestock, particularly under cool and cloudy conditions (Francis, Bartley, & Tabley, 1998). Therefore, the high nitrate levels in dual-purpose cereal crops used to fill the winter feed gap can potentially affect the feeding behaviour of the animals grazing these crops.

1.5 Nitrate

Nitrate (NO_3), at high concentration, is an undesirable compound in ruminant feed because it can induce methaemoglobinaemia (Bruning-Fann & Kaneene, 1993). Nitrate intake in ruminants is positively associated with its concentration in forage and drinking water.

1.5.1 Nitrate metabolism in rumen

Ruminants have a unique ability to convert non-protein nitrogen (NPN) to protein (Helmer and Bartley, 1971). Urea and nitrate (NO_3) are both considered as a valuable source of NPN for rumen microorganisms (Kertz, 2010; Hulshof et al., 2012), although nitrate can be added to cattle diet to reduce enteric methane emission (Lee and Beauchemin, 2014). Dietary nitrate as a source of NPN may affect DMI and rumen parameters. The sugarcane-based diet containing calcium nitrate reduced DMI, but the total concentration of volatile fatty acids (VFA) was not affected (Hulshof et al., 2012). However, due to electron sink properties of nitrate the main advantage is methane mitigation (Sar et al., 2005; Nolan et al., 2010; Hulshof et al., 2012).

In the rumen environment, nitrate is reduced to ammonia via nitrate reductase enzyme activity. Ruminal bacteria that reduce nitrate include *Selenomonas ruminantium*, *Veillonella parvula*, *Wolinella succinogenes*, *Campylobacter fetus* and *Mannheimia succiniciproducens* (Lin et al., 2013). Roughage is the main source of feed for ruminants and it is fermented by ruminal microbes. Predominant cellolytic bacteria in rumen are *Ruminococcus albus, Ruminococcus flavefaciens and Fibrobacter succinogenes* (Miron, Ben-Ghedalia, & Morrison, 2001). The composition of rumen microbes varies depending on the diet fed and thus rumen flora changes when animals consume nitrate-containing plants (Guo et al, 2009). Zhou, Yu and Meng (2012) reported that dietary nitrate inhibited the activity of *F. succinogenes* in rumen *in vitro* culture. In another study on steers, it was concluded that nitrate did not change the number but changed the composition of bacteria in the rumen (Zhao et al., 2015). They also reported that a diet that contained 1% of nitrate (low level) increased the amount of non-cellulose particles in rumen. The mechanism through which nitrate inhibits bacteria activity is unknown, and feed fermentation in the presence of nitrate requires more *in vivo* studies, since *in vitro* devices might not exactly reflect the rumen environment. However, Nolan et al. (2010) reported that the presence of dietary nitrate (4% KNO3) had no effect on DM digestibility in the rumen of sheep. The variation between the results of different studies could be due to the different acclimation period, different source of nitrate or variation between animals.

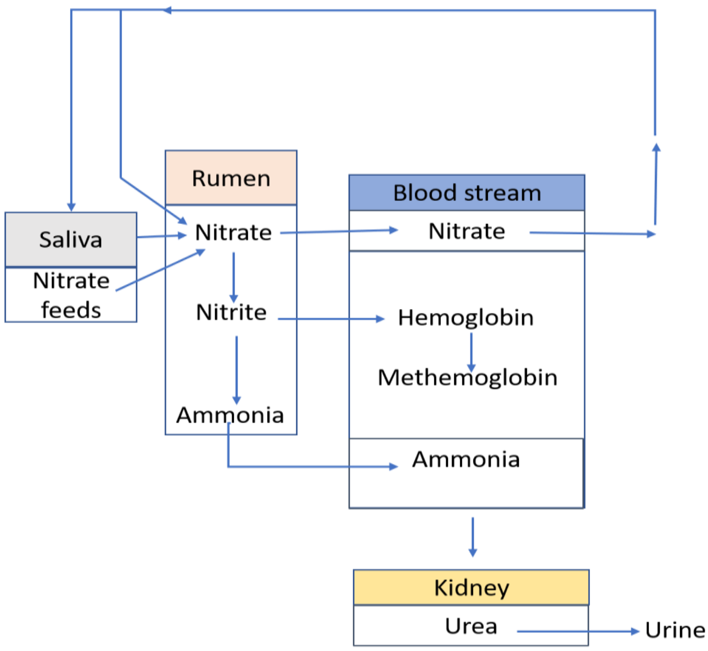
1.5.2 Nitrate toxicity

Under normal conditions, forage contains negligible amounts of nitrate, but in certain circumstances, it can accumulate high concentrations, leading to toxicity when consumed by ruminants. Several important factors can induce nitrate toxicity in ruminants as Yaremcio (1991) explained: (1) the rate of nitrate intake, which means the amount of feed and the speed at which the feed is consumed; (2) rate of conversion of nitrate to ammonia in the rumen; (3) rate of digestion of feed and release of nitrates; and (4) feed passage rate to lower compartments.

Consumption of high levels of nitrate by ruminants may cause toxicity (Bruning-Fann and Kaneene, 1993). Therefore, it is important to know the main factors affecting nitrate levels in plants. Apart from N fertilisation, nitrate accumulation in forage can be affected by many factors, such as maturity, sunlight, soil moisture and drought. The concentration of nitrate is higher in lush plant (actively growing) than in mature plant tissue. Lack of sunlight can cause lower activity of nitrate reductase, leading to limited photosynthesis (Pfister, 1988). Nitrate is not evenly distributed in plants. For example, nitrate content is much higher in stems than in leaves (greater nitrate reductase activity) and seeds (Fjell, Blasi, & Towne, 1991; Pfister, 1988). Soil moisture and temperature can also affect nitrate content in plants. Low rainfall influences the plant growth rate and consequently reduces nitrate reductase activity; therefore, nitrate accumulates in the plant (Pfister, 1988).

Both nitrate and nitrite can accumulate in the rumen and be absorbed into the bloodstream when concentrations exceed the capacity of rumen microorganisms to convert it to ammonia (Jones, 1972). However, nitrites are more toxic than nitrates. Absorbed nitrate is not toxic per se, but it can be recycled back to the rumen through saliva or intestinal secretions and then converted to nitrite, which is reabsorbed into the bloodstream and intensifies the problem (Bruning-Fann & Kaneene, 1993). The nitrite entering the blood binds to red cells and changes the ferrous (Fe_{2+}) form of haemoglobin to the ferric (Fe_{3+}) form that converts haemoglobulin to methaemoglobin. Methaemoglobin does not have the ability to carry oxygen molecules and it also increases the affinity of hemoglobulin to oxygen (Lundberg, Weitzberg, & Gladwin, 2008). The nitrate pathway in ruminants is illustrated in Figure 1.3.

Two types of toxicity caused by nitrate in feeds are chronic nitrate-nitrite toxicity and acute poisoning. Chronic toxicity is defined as a type of poisoning that does not result in visible clinical signs of disease. It mainly causes weight gain reduction, appetite depression, lower milk production and higher susceptibility to infections. With acute poisoning, clinical signs are evident, and animals might die within a few hours of initial ingestion (Yaremcio, 1991).



Source: adopted from Yaremcio (1991)

Figure 1.3: Nitrate pathway in ruminants

1.5.3 Feed intake and animal performance

Nitrate is fed to ruminants as a source of NPN to mitigate methane emission (Lee and Beauchemin, 2014) or it is accumulated in the plant (Yaremcio, B., 1991). The majority of studies investigated supplementary nitrate in the form of calcium nitrate (Velazco et al., 2014; Duthie et al., 2016) or calcium amonnium nitrate (Olijhoek et al., 2016; Capelari and Powers, 2017), whereas nitrate in the form of potassium nitrate (KNO_3) was found in plants (O'Hara and Fraser, 1975). Owing to its toxic properties, it is expected that nitrate can cause reduction in intake, weight gain and milk production when cows encounter mid-nitrate poisoning. When the level of nitrate exceeds 1% of DM, it adversely affects weight gain and feed intake in cattle, and when more than 3% of DM, it reduces feed intake and weight gain in sheep (Bruning-Fann & Kaneene, 1993). Gradual introduction of nitrate to the diet can allow the rumen microbes to adapt and increase their capability to reduce nitrite (Alaboudi & Jones, 1985). Nolan, et al (2010) showed that a diet supplemented with 3.04% nitrate did not affect feed intake and weight gain in sheep.

In another study, adding 2.6% nitrate did not have negative effects on sheep and decreased heat production by up to 7% (van Zijderveld et al., 2010). The period over which nitrate is introduced impacts on the level of nitrate that ruminants can tolerate. Van Zijderveld et al. (2010), used a 4week adaptation period to dietary nitrate, and methaemoglobinemia was not observed. Similarly, Nolan et al. (2010) used an 18-d adaptation period without the occurrence of methaemoglobinaemia. However, DM intake (DMI) and ADG significantly decreased in the case of steers supplemented with feed containing a high level of nitrate (0.45% NO_3^--N^3 in dietary DM) despite an 18-d acclimation period. By contrast, a low level of nitrate (0.25% NO_3^--N^3 in dietary DM) did not cause negative effects on animal performance (Miller et al., 2016). For example, Hulshof et al. (2012) and van Zijderveld et al. (2011) increased the nitrate concentration in the diet by 0.5% every 4 or 7 d to reach target levels, while Trinh et al. (2009), in their study with goats increased nitrate levels (0.2, 0.4, 0.8, 1.6, and 3.3%) weekly over the 5-week study period. However, a stepwise strategy is recommended to minimise the risk of nitrate poisoning (Lee and Beauchemin, 2014). Lee et al., (2015) concluded that when dietary nitrate containing more than 3% lowered feed intake and feed consumption rate. A standard acclimation procedure for nitrate cannot be recommended because different procedures can also affect animal feeding behaviour and performance It should be noted that the variation between animals could be a possible explanation in different studies. Nevertheless, there are a few studies that evaluated feed consumption rate and feeding intake when dietary nitrate added to the diet. To our knowledge, there has been limited studies to evaluate ruminant grazing and rumination time while they are grazing pasture with high nitrate concentration.

1.6 Cattle Behaviour

The behaviour of animals is an initial indicator of their welfare state and performance. Cattle show various behaviours, including grazing, ruminating, walking, resting, browsing, idling, loafing, sleeping, licking and scratching. However, beef cattle spend most of their time (95%) grazing, ruminating or resting and walking (Kilgour et al., 2012). One of the first cattle behaviour studies carried out by Hancock (1954) on dairy cattle investigated how different conditions, such as pasture quality and temperature, can influence their behaviours. Depending on the management system, the nutrition and climate can considerably influence cattle behaviours. The time spent grazing can potentially be reduced by increasing the temperature (Hejcmanová et al., 2009) or owing to a higher wind-chill temperature (without precipitation), following which time allocated to lying, feeding and rumination can be increased (Graunke et al., 2011).

Recently, sensor technology was used in many studies to monitor cow health and welfare in different circumstances. Marchesini et al. (2018) used activity and rumination as indicators to assess the health status of beef cattle. In a recent study, by using a neck-mounted mobile sensor system, feeding time spent was monitored to ascertain the onset of lameness in dairy cows (Barker et al., 2018).

1.6.1 Feeding behaviour

Grazing is defined as a group of complex actions to grip the herbage into the mouth, which involves searching and selecting suitable forage and consuming the feed available in the pasture (Gregorini et al., 2008). During the process of grazing, cattle wrap their tongues around grass to pull it up, which involves movement of their lips, teeth and tongues to move feed into their mouths (Church, 1988), after which they chew the grass, adding saliva to form a bolus, and then swallow it into the anterior rumen (Arnold & Dudzinski, 1978). The frequency and patterns of grazing are highly variable under different seasons and environments that interact with exterior conditions. Under temperate free-range conditions, cattle normally have three to five grazing regimes. For instance, cattle show different grazing events on short days (winter) and long days (summer); during short days, grazing events merge, leading to longer grazing duration. On long days, grazing events merge, resulting in longer and fewer grazing periods that mainly occur in cooler parts of the day (Gregorini, 2012). Most of the grazing occurs in the morning, with 60% between 07:00 and 15:00; additionally, on average, 85% of total grazing takes place in daylight and only 15% in dark hours (Albright & Arave, 1997). For example, when the temperature rises to extreme levels, cattle seek shelter and resume grazing when the temperature drops in the evening (Vallentine, 2001).

Thus, by changing the quality and quantity of pasture conditions, grazing time would be adjusted accordingly. Cattle are known as selective grazers, whereby they not only search the paddock for available biomass of the required height, but also for sufficient protein and energy to meet their nutritional requirements (Hardison et al., 1954). By understanding the grazing behaviour of cattle on different pastures and the factors influencing grazing behaviour, pastures can be managed more efficiently to maximise the performance of the animals and minimise negative environmental effects. Bite activity is a subcategory of grazing behaviour. When animals grasp the forage, the bite activity begins. Variation in bite numbers depends on several factors, mainly the quality and quantity of the pasture. For example, cattle in a lush, thick pasture ingest forage with a fewer number of bites but each bite contains more forage than a bite in a short pasture, in which the cattle also must take more bites (Soder et al., 2009).

1.6.2 Rumination

Rumination is a natural behaviour of ruminants to break down the size of feed particles. This process includes regurgitation of digesta in the rumen, remastication of digesta and then reswallowing it. The process begins with the extra contractions of the reticulum prior to contraction of the mixing cycle and negative pressure in the trachea because of intense contraction of the diaphragm (Church, 1988). During rumination, mastication is more consistent and slow-moving than during the eating period. When a bolus is regurgitated, some of its small particles and liquid are swallowed, but the remaining bolus is remasticated for about 30 to 70 sec before being re-swallowed. The next cycle of regurgitating the bolus starts 2 to 4 sec later (Beauchemin, 2018). The rumination process consisting of a series of boluses lasts from 30 sec to 2 h in a day. It is generally assumed that if rumination takes a longer time, the ruminating bouts take longer (r = 0.55) as well but are unrelated to meal size (Dado & Allen, 1994). However, the length of rumination bouts cannot be a potent predictor of rumination time because many distractions may halt rumination (Beauchemin, 2018). Generally, the rumination time is 10 to 12 h each day, but it may be longer in cows fed rations with a high neutral detergent fibre (NDF) content (De Boever et al., 1990). In a study, the rumination time of two diets with different levels of NDF was compared (Adin et al., 2009). The rumination time was shorter (54.3 min/d) for a diet included 35% roughage NDF than the diet with 18.7% roughage NDF. This could be due to the difference in physically effective NDF (peNDF) in their diets (12.4%) because of the relationship between rate of particle passage and rumination time. For example, a higher peNDF intake leads to higher rate of particle passage in the rumen and a lower rumination time. This indicates the negative correlation between peNDF intake and rumination time per kg of peNDF ingested. Schulze et al. (2014) showed that decreased rumination time per kg NDF intake with greater NDF intake per kg BW is negatively correlated (r= -0.58) with eating time. They believe when eating time is decreased, less mechanical rupture applied to forage particles and rumination time is increased to reduce forage particles size. NDF content has a low correlation with length of rumination, which is reported as less than 0.10 (White et al., 2017). Thus, the rumination time is highly correlated with NDF intake rather than NDF content. The correlation between rumination time and NDF intake in different studies was between 0.35 to 0.54 (Yang et al., 2001; Yang and Beauchemin, 2006, 2009).

By increasing the particle size of TMR ration, the rumination time increases as well; however, after a certain size of particles, rumination time does not increase further. It is very difficult to determine a precise threshold for particle size. Allen (1997) recommended that an average particle size greater than 10 mm cannot increase the rumination time; conversely, the rumination time substantially reduces when the average particle size is less than 5 mm.

Rumination mainly occurs during night hours while animals are resting; however, cattle ruminate in the daytime if not interrupted with feeding and milking (Paudyal et al., 2016). It is reported that rumination normally peaks four hours after feeding and is associated with duration of lying time

(Schirmann et al., 2016), but Stone et al., (2017) reported that in case of increase in rumination time, animals ruminate less at night while lying down. Several studies have reported a wide range of rumination times, and these can significantly differ because of many reasons, such as chemical and physical composition of the ration, measurement techniques and inherent variability among cattle. For example, White, Hall, Firkins and Kononoff (2017) found in a meta-analysis study (n = 179) that the average rumination time was 436 min/day with a range of 236 to 610 min/day, but Zebeli, Tafaj, Steingass, Metzler and Drochner (2006) studied early lactating dairy cows fed total mixed ration (TMR) and reported that the mean of rumination time was 434 min/d with a range of 151 to 630 min/d.

1.7 Methods to Determine cattle Behaviour

Monitoring animal feeding behaviour is an important factor to evaluate performance, health and welfare of animals. Feed intake is highly influenced by diseases such as diarrhoea, milk fever, ketosis, or hoof lesion in dairy cows (Bareille et al., 2003). In a study on dairy cows, a reduction in rumination time was used as a reliable predictor to evaluate the level of health and stress of animals (Herskin et al., 2004). Hence, monitoring feeding behaviour would be a beneficial parameter to detect emerging diseases in early stage.

Feeding behaviour may reflect the quality and quantity of the offered feed (Werner et al., 2018) and this can help to optimise grassland management decision to increase feed intake and reduce grass residuals (Arthur and Herd, 2008). However, regardless of the farming system, the accuracy of measuring feeding behaviour is important to optimise farm efficiency and profitability.

1.7.1 Manual observation

Manual observation can be implemented by either direct visual observation or video recording. The former is considered a traditional method to observe livestock behaviours and has been used over the past few decades. Determining animal behaviours using this method is laborious and leads to some errors, owing to factors such as observer tiredness, physical barriers, distance from animals (proximity), weather conditions and limited daytime (Valente et al., 2013). In particular, such observation in loose housing systems is costly; thus, consistent visual estimation of different behaviours can only be made via an automatic video recording system (Nielsen et al., 2010). However, apart from grazing behaviour, other activities such as rumination and bites might not be recorded accurately.

The activities of animals can be recorded continuously using video recorders through daylight and dark hours (using cameras with infrared recording technology). Although processing and interpreting video records is labour intensive, this method is considered more accurate than traditional visual observation methods (Mitlöhner et al., 2001).

In continuous video recording, the starting and ending time of feeding behaviours for each animal should be identified, which is a laborious task and can introduce some errors (Dong et al., 2018). Desnoyers et al., (2009) suggested that video monitoring is suitable to assess behaviours in the stall house or farm shed production system, but in free-range farming, it becomes less accurate in analysing feeding behaviours. Some other drawbacks of video monitoring system are the limitation in visualising behaviour at a distance, especially if there are obstacles, such as trees or hills, and challenges in continuous monitoring of individual animals.

1.7.2 Global positioning system

The global positioning system (GPS) is a common technology often used to track the ecological behaviours of free-range animals. These technologies can help researchers understand the interaction between the environment and animal (Handcock et al., 2009). GPS collars attached to animals can provide their spatial location in the pasture, which is useful in monitoring the herd remotely while the animals are engaged in behaviours such as walking, running and stopping. However, the cost of GPS systems often restricts large-scale application of this technology. Further, GPS-based devices have several limitations, including spatial accuracy, battery life, rate of data collection and loss of data owing to environmental factors (Swain et al., 2011). GPS devices can be employed to track the number of steps and estimate the energy expenditure (Brosh et al., 2006). Recently, GPS has been used in real-time tracking for improving livestock management. For example, in an extensive system, the manager might not be able to view the livestock even once a week. By real-time tracking of livestock, the manager can stay informed and act to rectify an issue (Bailey, Trotter, Knight, & Thomas, 2018).

1.7.3 Pedometer

Pedometers are often used to measure the distance that an animal travels by recording the number of steps. O'Callaghan, Cripps and Downham (2003) used pedometers to detect lameness in dairy cattle. They concluded that its use could help in understanding pain-related behaviours in cattle. Roelofs et al. (2005) concluded that pedometers can accurately detect oestrous and it is a reliable tool to predict ovulation. They also found that pedometers can be used to improve fertilisation rates. Umemura (2013) used a pedometer on a neck collar to monitor grazing behaviour and number of bites and was able to distinguish grazing from walking behaviour.

1.7.4 Accelerometer

The accelerometer is a high-accuracy sensor (depending on some factors, including type of accelerometer, time of monitoring, type of software algorithm and user interface) that predicts all types of behaviours and postures, and its performance outweighs that of other devices, such as pedometers (Trost et al., 2005). The method of detecting certain behaviours in cows using different kinds of accelerometers has been developed during the past 40 years (Rutten et al., 2013). Accelerometers containing electronic loggers have been attached on animals to provide information about their body posture and dynamism (Graf, Wilson, Qasem, Hackländer, & Rosell, 2015). This information can be provided as a transcription to define behaviours. Two types of accelerometers are available: uniaxial and multiple axis. It has been reported that the validity of the multiple axis type far outweighs that of the uniaxial type (Trost et al., 2005).

Accelerometers are suitable devices to measure animal movement in terms of acceleration. The extracted data can then be used to predict the intensity of physical activity over time. Most accelerometers are designed as piezoelectric sensors consisting of one to three orthogonal planes: anteposterior, mediolateral and vertical. This study was designed based on accelerometer sensors. Accelerometer device performance is based on the principle of force of gravity and acceleration of movement. As regards acceleration measurement, gravitational acceleration units (g; 1 g = 9.8 m/s^2) is employed (Chen & Bassett, 2005). Accelerometer devices should be embedded into the collar to fit onto the neck of animals.

The application of sensor technology to monitor ruminant behaviours has recently captured attention because it can be used on large herds and allows highly accurate, 24-h monitoring. Robert et al., (2009) monitored standing, lying and walking activities of 15 crossbred calves using accelerometer sensors attached to the lateral aspect of the right rear leg. The results predicted using these sensors were in excellent agreement with those from video recordings, for cattle that were lying and standing (99.2% and 98% respectively); however, these sensors were significantly less accurate in monitoring cattle that were walking (67.8%). The authors emphasised that accelerometer sensors can provide accurate measurements of behaviour. In another study, accelerometer sensors were employed to identify ill animals by monitoring changes in their feeding behaviour (Arcidiacono et al., 2017). Wearable sensors were attached onto the cow's collar to monitor feeding and standing behavioural activities. It was found that a threshold-based classifier for monitoring individual cows is a suitable method for real-time computing applications.

Chapter 2: Materials and Methods

2.1 Animals and Pastures

In this study, all the procedures and experimental protocols reported in this thesis were in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NHMRC, 2013) and approved by the La Trobe University Animal Ethics Committee (AEC14-31). This experiment was carried out without any interruption to commercial practice. The procedures and equipment utilised in this thesis are explained in detail in this chapter.

Field trials were conducted from July to August 2016 in collaboration with Murdeduke Agriculture Pty Ltd (38°12'36.9"S 143°55'05.6"E), which is a mixed farming property. In this trial, 79 Angus heifers (out of 150) with initial mean liveweight of 286 ± 3.01 kg (within the range of 219 kg to 336 kg) were allocated to this study due to the limited number of accelerometer sensors (79). However, power analysis test was run for 79 animals and the power was 99% ($p < 0.01$).

The heifers grazed in a 26-ha paddock on phalaris (*Phalaris arundinacea*) and subclover (*Trifolium subterraneum*) mixed pasture (phase 1). Then, they were moved to a 43-ha paddock to graze on actively growing (lush) wheat, as phase 2 and then finally moved to a 13-ha paddock of lush barley, as phase 3, in a 13-ha paddock. Also, straw and salt lick were offered as a routine by the farm manager when animals were grazing on wheat and barley crops. The Zadok growth scale of cereal crops was at the stem elongation according to the observation of the farmer.

The duration of each phase was 9 d; the first 4 d were the adaptation period and during days 5 to 9, raw data were collected from the sensors (see section 2.2). The heifers were weighed at the beginning and before moving off to the next paddock, in each phase. The timing of animal movements was dictated by commercial considerations. The ADG was measured by dividing the weight gain (calculated by subtracting final liveweight from the beginning liveweight) by number of grazing days (i.e., 9 d).

Figure 2.1: Rotational management of trials

2.1.1 Plasma sample separation

Blood samples (10 mL) were collected from tail vein using BD Vacutainer K2E (EDTA) on the last day of each phase and kept in an icebox at 4 ˚C. All the samples were delivered to Laboratory 320, Health Science 2, Bundoora, La Trobe University, Vic 3083, to separate plasma from blood samples. Blood samples were centrifuged immediately at 1,500 Relative Centrifugal Force (Sigma 3-16KL) at room temperature for 10 minutes. After centrifugation, the supernatant (plasma) was taken to two Eppendorf tubes and labels with the details of cows and phases affixed. Plasma samples were stored at −80 ˚C until analysis for nitrate concentration.

2.1.2 Feed analysis

Feed samples were cut at the ground level and collected from multiple different sites pre-grazing (PG) and post grazing (PSG) to estimate FOO for the three pastures. The locations of the collected samples in each pasture were depicted in figures 2.2, 2.3 and 2.4. The sites to collect samples were randomly chosen. Also, the sample size in each pasture was described in tables 2.1, 2.2 and 2.3. The longitude and latitude were shown at top right side of the map of each pasture in figures 2., 2.2 and 2.3.

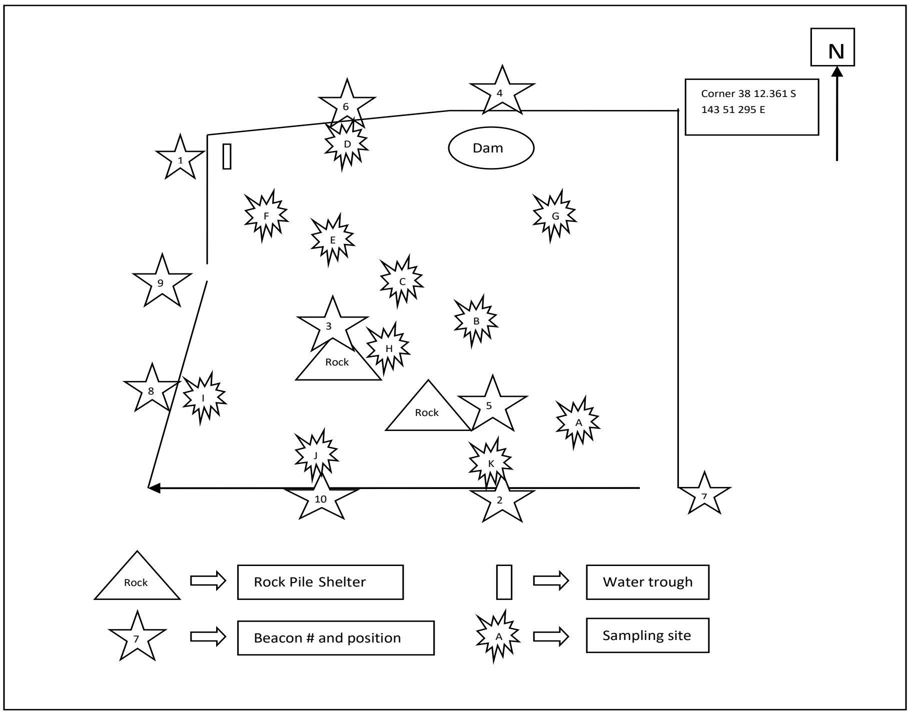


Figure 2.2: Mixed pasture map to point out the location of rock pile shelter, water trough, beacon positions and sampling sites. Sample collection sites and beacon positions were described with letters A to K and numbers 1 to 10, respectively.

Table 2.1: Sample size g/0.1m2 collected at different sites in mixed pasture. PG: pre-grazing; PSG: post grazing

Collection Site	Mixed pasture	
	PG	PSG
	g/0.1m^2	g/0.1m^2
A	92	65
B	60	50
C	80	54
D	25	8
E	32	18
F	60	34
G	56	60
H	80	60
I	26	14
J	41	24
K	22	12

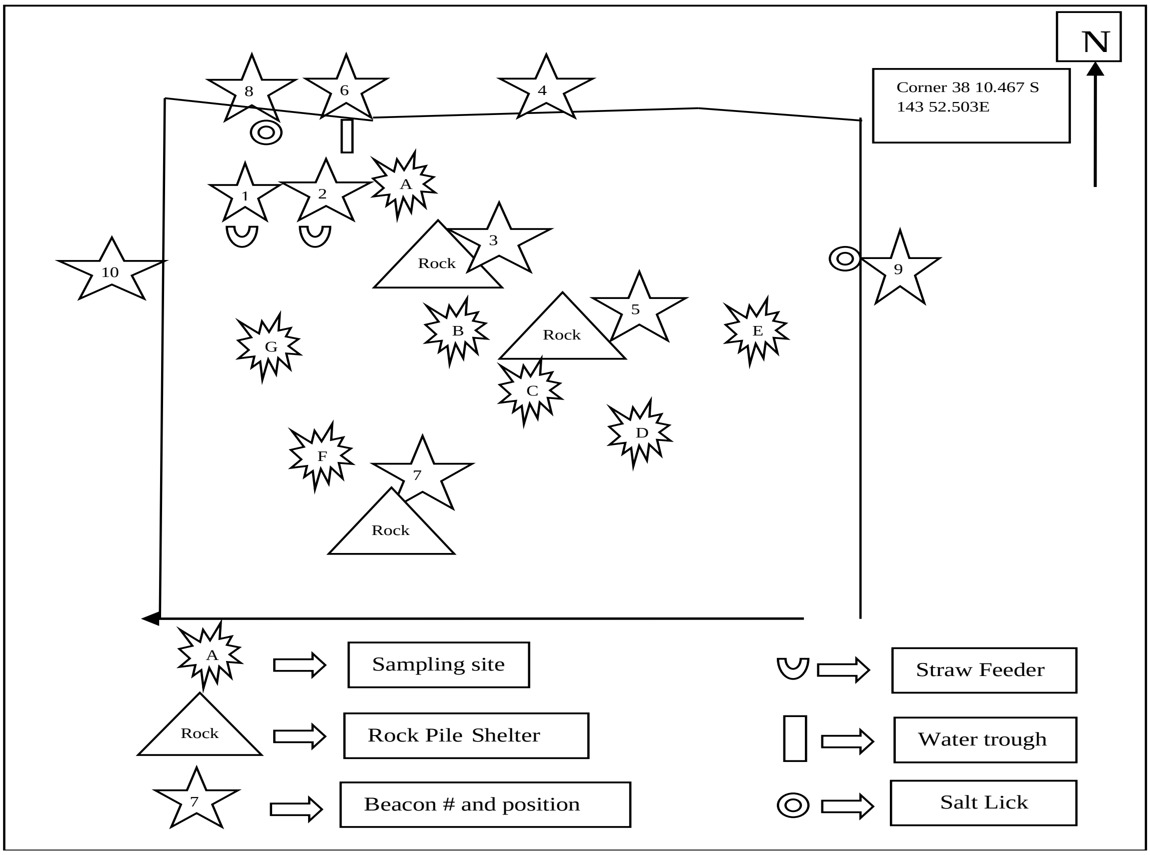


Figure 2.3: Wheat pasture map to point out the location of sampling sites, straw feeder, rock pile shelter, water trough, beacon numbers and positions and salt lick. Sampling collection sites and beacon number and positions were described with letters A to G and numbers 1 to 10, respectively.

Table 2.2: Sample size g/0.1m2 collected at different sites in wheat pasture. PG: pre-grazing; MG: mid grazing; PSG: post grazing.

Collection Site	Wheat pasture		
	PG	MG	PSG
	$g/0.1m^2$	$g/0.1m^2$	$g/0.1m^2$
A	182	256	208
B	250	222	200
C	196	148	156
D	194	128	104
E	314	216	288
F	266	90	130
G	138	108	134

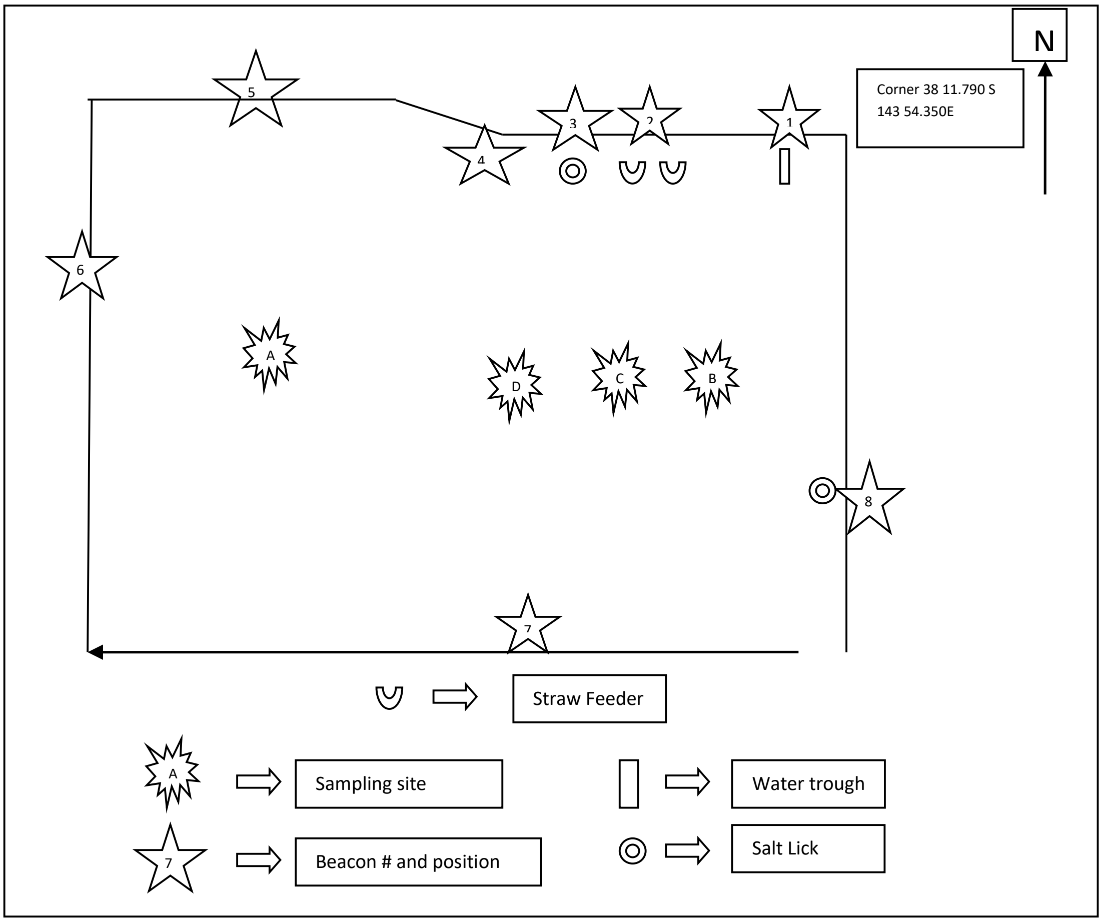


Figure 2.4: Barley pasture map to point out the location of sampling sites, straw feeder, water trough, beacon number and positions and salt lick. Sampling collection sites and beacon positions were described with letters A to D and numbers 1 to 8, respectively.

Table 2.3- Sample size g/0.1m2 collected at different sites in barley pasture. PG: pre-grazing; PSG: post grazing.

Collection Site	Barley pasture	
	PG	PSG
	g/0.1m^2	g/0.1m^2
A	148	74
B	201	99
C	195	93
D	160	100

Feed samples were sent to FeedTest (Agrifood Technology, Werribee, Vic 3030) to determine Ash, Dry Matter (DM), Crude Protein (CP), Digestible Dry Matter, Metabolizable Energy (ME), Acid Detergent Fibre (ADF), NDF and nitrate as an NPN. To analyse feed composition, the near infrared method was used since it enables accurate and rapid analysis.
Sward heights were measured using a sward stick at the same locations as sample collected before and after grazing. The average of sward heights in each pasture was provided to the authors.
The collection and analysis of pasture samples were carried out by Mr Simon Falkiner and results were provided to the author.

2.1.3 Feed on offer and stocking rate

The following calculation was used to determine FOO (Munn et al., 2009).

FOO (kg DM/ha) = ((g/0.1m^2) *DM)/100/(1000*10*10000)

Dry sheep Equivalent (DSE), which is a standard unit to assess feed requirement in paddocks, was used to calculate the stocking rate of each of the paddocks using the following equation (Jones and Sandland, 1974).

Stocking rate (DSE) = (Total animals grazing × DSE* rating)/paddock size

2.2 Sensor Specification and Attachment

Commercial triaxial accelerometer sensors equipped with Bluetooth technology (ActiGraph wGT3X-BT; ActiGraph, LLC, Pensacola, FL) were used.

2.2.1 Sensor equipment

The sensors attached on the Angus heifers were commercially available sensors from ActiGraph® (ActiGraph, LLC). The sensor specifications are indicated in Table 2.1.

The sensor captures and records data continuously with high resolution during physical activity. This sensor is based on both micro-electro-mechanical system (MEMS) and ambient light sensor. MEMS technology is used to combine nanoscale into nanoelectromechanical systems and nanotechnology. This technology is mainly used for motion microscopic devices. By carrying ambient light sensors, it offers light intensity values (in lux) every single minute during an activity (Figure 2.2).

Table 2.4: Specifications of ActiGraph wGT3X-BT sensors

ActiGraph wGT3X-BT specifications	
Dimension	4.6 cm × 3.3 cm × 1.5 cm
Weight	19 grams
Sample rate	30–100 Hertz
Dynamic range	+/− 8G
Battery life	8–10 d (shorter time with Bluetooth)
Memory	4GB
Data storage	180 d
Communication	USB, Bluetooth

Source: www.actigraphcorp.com

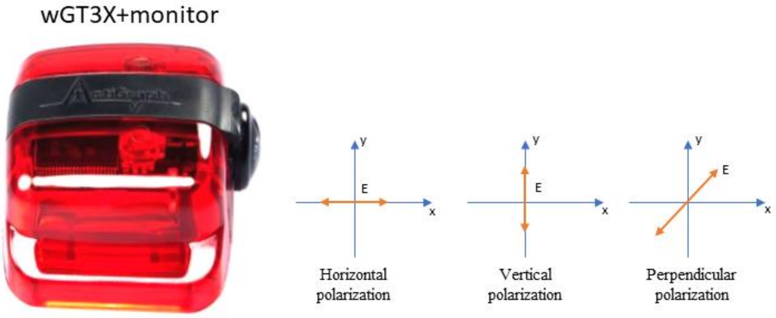


Figure 2.5: ActiGraph sensor device

2.2.2 Sensor details

The accelerometer sensor in the ActiGraph Sensor has 3-axis sensing capability:

- Vertical Axis Activity Acceleration Data (Axis 1)
- Horizontal Axis Activity Acceleration Data (Axis 2)
- Perpendicular Axis Activity Acceleration Data (Axis 3)

The sensor output sample rate was in the range of 30–100 Hz with a 12-bit analogue digital convertor. In this trial, the sensors were configured at a frequency of 30 Hz. Since the sensor was attached to a heifer, data were stored in the form of raw, non-filtered format units of gravity (g). The sensor has the ability to store activities up to 180 d, after which date can be transferred to a computer.

2.2.3 Water resistance

In this study, it was necessary to use a waterproof sensor because of the possibility of rainy days while heifers were grazing on a pasture-based system. GT3X+BT was water resistant in accordance with IEC 60529 IPX7, or immersion in 1 m of water for up to 30 min.

2.2.4 Sensor battery

ActiGraph wGT3X+BT devices contain a lithium-ion rechargeable battery. Once the battery was fully charged (within 3 h depending on the battery deployment), it should allow sensor operation for 10–20 d; however, because Bluetooth was enabled to track proximity location, the operational life of the ActiGraph Sensor unit dropped to 7–12 d (due to additional power consumption by the Bluetooth radio).

2.3 Sensor Attachment to Animals and Programming

ActiGraph wGT3X-BT MEMS accelerometers were initialised to collect signals at frequency of 30 Hz (30 data/sec) and were embedded in the halter (© The Farmers Mailbox, Whittlesea, Victoria 3757 Australia) with dimension of 25 mm width, noseband adjustable from 43 to 55 cm, head strap circumference adjustable from 43 to 58 cm and weight of 282 g (Figure 2.3).

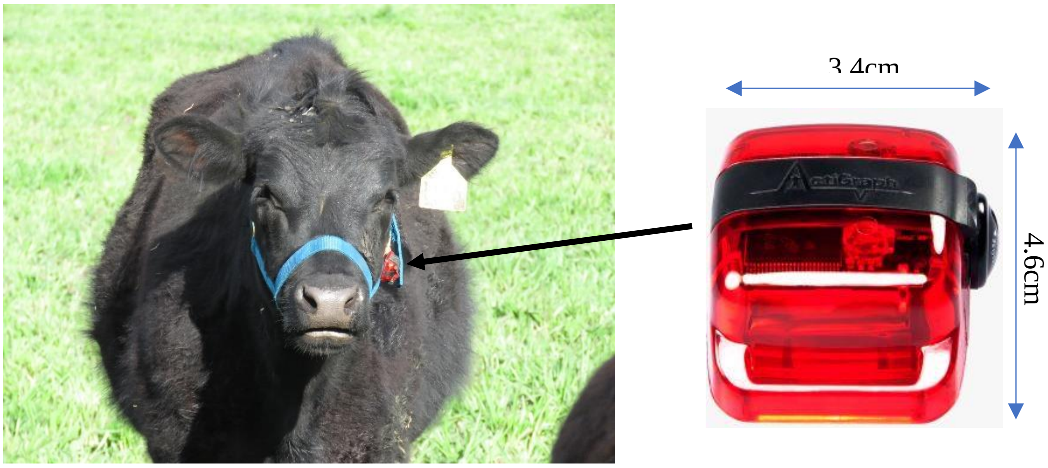


Figure 2.6: Sensor (ActiGraph wGT3X-BT) assembly embedded in the halter on the animal

Sensors were initialised a day before the commencement of each trial phase using ActiLife software (version 6.13.2) at a certain time and date at the Centre for Technology Infusion (CTI). Regardless of date and time, during initialising, sensors were categorised as beacons or receivers. The sensors configured as receivers allow tracking (counting) of the proximity events when sensors configured as beacons approach within close distance of the receivers. The beacons transmit their unique identification at a pre-configured rate, which allows the receivers to count proximity events indicating; for example, sensors attached to the heifer configured as a beacon and sensors placed at water troughs and salt licks configured as a receiver. The sensors were initialised to record the movement of heifers at different times, as shown in Table 2.2.

Table 2.5. The start time and end time of the sensors

Pastures	Start time (date–hour)	End time (date–hour)
Mixed pasture	19/07/2016–00:00:00	23/07/2016–23:59:59
Wheat pasture	29/07/2016–00:00:00	02/08/2016 at 23:59:59
Barley pasture	12/08/2016 at 00:00:00	16/08/2016 at 23:59:59

After removing the sensors from the cattle's neck at the end of each phase, the sensors were taken to CTI to download data to a computer via USB cables. A dictionary was created to organise the collected data, which was based on each heifer's unique radio frequency identification number and each unique sensor serial number to link files to the cattle correctly. Some videos were captured during each phase, when the heifers were engaged in activities such as grazing and ruminating, to validate the predicted behaviours from the recorded sensor data.

2.3.1 Sensor positioning and orientation on muzzle of heifers

The sensor assembly was attached to the halters near the jaw muzzle as shown in Figure 2.4. To ensure consistency of the data obtained from the 3-axis accelerometers (which allow easier and better identification of feeding activities, such as rumination and grazing), during the trial, the sensor was always placed near the jaw at a similar angle for all heifers. Inconsistency of the sensor position is undesirable.

Figure 2.7: Position and orientation of sensor near the jaw of the animal

Commercial accelerometer sensors (model wGT3X-BT, ActiGraph Corporation) with motion and proximity sensor functions were fitted onto the halter of heifers using cable ties. The halter–sensor assembly was replaced every 7 to 10 d because of the short battery life of the sensor units. Sensors were also positioned at various locations (i.e., water troughs, salt licks and straw) to monitor other feeding behaviours based on proximity of sensors attached to the infrastructure (receiver) and sensors attached to the heifers (beacon). The beacons were programmed to send signals at 30 Hz (30 times/sec). Video recordings were captured for validation purposes.

2.4 Sensor Calibration and Pre-Testing for Behavioural Activities

The samples were obtained at 30 Hz. The length of each phase recorded was approximately 9 consecutive days, but since the first 4 d were the adaptation period, the raw data for each cow in each phase consisted of 12,960,000 lines (30 Hz × 60 sec × 60 min × 24 h × 5 d). It is not possible to scan the large amount of this extracted data by manual/visual means for identifying (or predicting) unique behaviour signatures. The ActiLife software, provided with the Actigraph sensors, offers an option to export data in the comma separate value (CSV) format, which can be opened through Microsoft Excel. However, since the volume of data was very large, it was still impractical to open the CSV format through Microsoft Excel and interpret it in a meaningful way.

Therefore, a support team at CTI, La Trobe University, developed an algorithm in MATLAB to analyse each set of raw data after it was converted from CSV format to MAT format. To visualise raw data, a graphical user interface (GUI) was developed, which plots the input file in form of a graphical output (as shown in Figure 2.5). In the first step, to visualise the plot, the directory of raw data should be typed; then, the raw data related to each animal can be selected for the output graph. Since triaxial accelerometer sensors were used in this experiment, any of those three axes can be chosen to plot. Other processed data were derived from the raw data using MATLAB software program, including VM (vector magnitude), LPF (low pass filter), HPF (high pass filter), MAF (moving average filter), BPF (band pass filter) and root mean square.

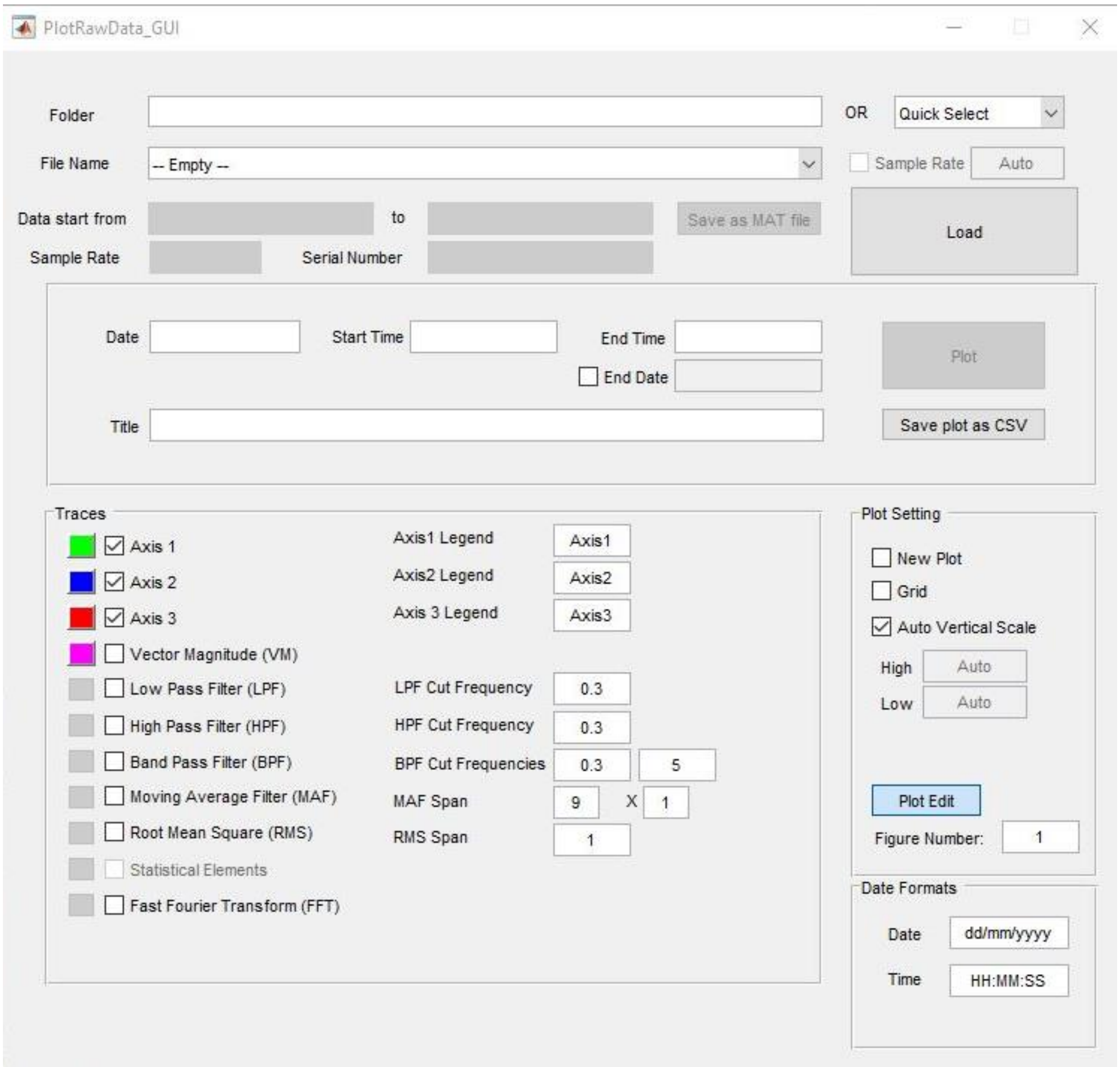


Figure 2.8: The Graphical User Interface (GUI)

For example, in Figure 2.6, a plot is generated for cow 218 in phase 2 on 31/07/2016 from 7 am to 12 pm (18,000 sec). Different activities of cow 218 based on the head and jaw movement were plotted in 3 axes. To predict the cow behaviour, the decision tree technique was implemented.

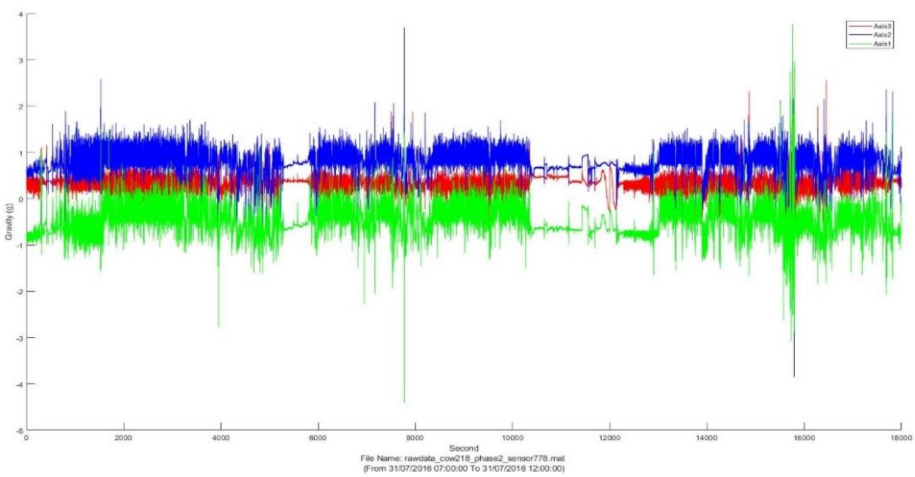


Figure 2.9: Generated plot in 3-axes within 18,000 sec

2.5 Decision Tree Method

Several techniques could have been used to classify animal behaviours in this study. The use of machine learning (ML) techniques (Charmley, Gowan, & Duynisveld, 2006) in animal behaviour studies offers supportive data modelling in classical statistics. Using ML approaches, several topics, such as collective behaviours, communication and welfare, could be addressed (Valletta, Torney, Kings, Thornton, & Madden, 2017). A variety of ML approaches could be applied, including the decision tree algorithm (Chou, 1991), support vector machine (Vapnik, 1995) and neural networks (Jain, Jianchang, & Mohiuddin, 1996). Among these, the decision tree algorithm as a supportive learning algorithm could be used to address regression and classification problems. Further, the decision tree algorithm is generally an instinctive predictive model (Hutchinson & Gigerenzer, 2005). Valletta et al. (2017) described a decision tree approach as (1) finding the yes/no rule that best split the data based on one of the features (2) producing the most homogenous groups by the best split and (3) repeating steps 1 and 2 to rectify classification of all data.

2.5.1 Behaviour analysis algorithm (flowchart)

An animal behaviour analysis algorithm was used to process the sensor data and it generated separate reports for grazing time, grazing bites counts, bite intensity and rumination. The algorithm was constructed from three code blocks for (1) feature extraction from data, (2) classifier and (3) report generator (Figure 2.7).

Feature extraction was the initial stage of processing raw sensor data to generate data features, such as high pass filtered (HPF) data, low pass filtered (LPF) data, vector magnitude (VM), peak detection (PD) and peak characterisation. All these features were necessary for detecting individual behaviour of animals. For instance, LPF was useful to observe base line, such as gravity of earth that was used in finding the angle of the animal’s head, while HPF outputs were used in identifying dynamic and static motion data (behaviours), such as idle from walking. In addition, each activity had its own specific pattern in HPF data, such as rumination and grazing. Moreover, PD is the best feature for bite detection and by characterising these peaks, more details, such as bite intensity, were extracted from the data.

The raw data consisted of triaxial acceleration values showing magnitudes (g) in specific directions in time domains that contained patterns of animal behaviours. In terms of bite detection, the vibration patterns of chewing and bite were detected in raw data. The methods of extracting biting details and other behaviours of the cows were slightly different from each other. To detect the bites, it was necessary to identify the peaks with specific minimum prominence to separate these from chewing. Moreover, each peak in the raw data (similar to mountain peaks) had a width that can be measured at the centre of the prominence. Accordingly, the area under the peak was estimated by multiplying the prominence by the width of the same peak, and the calculated area under the peak was used as coefficient of bite intensity (correlated to total energy of a bite).

In the classifier program, the algorithms were based on the decision tree and the moving time-window. The windows with variable width (different lengths of time) were moved on top of each data feature and compared with the specified constraints provided in an excel file called 'Activity Parameters'. Consequently, the classifier could associate each window of data features to an activity class, such as grazing, rumination and idle.

The report generator was designed for data visualising and generating summarised reports in the form of tables, which were saved in the CSV format. For instance, in data visualisation, each activity was marked on top of raw data waves. In addition, the summary of these results was saved in tables with timestamp and analytics details. The generated results were validated by comparing sensor-determined behaviours with observation and video recordings.

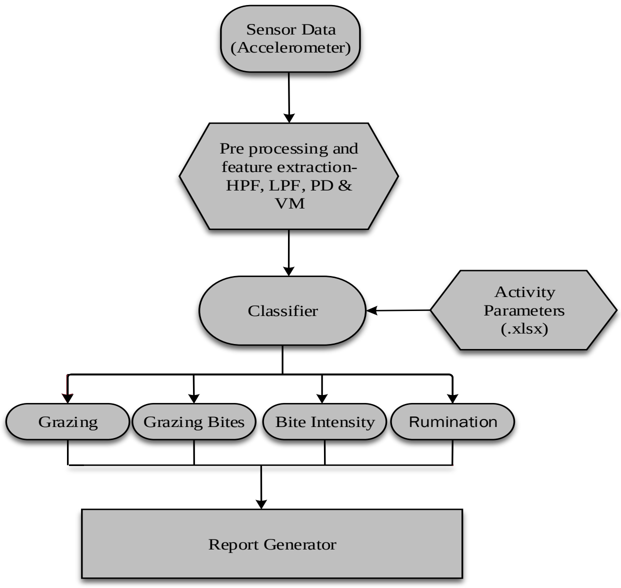


Figure 2.10: Block diagram of animal behaviour analysis

2.5.2 Analysing the record data by sensors

The software to implement the decision tree method was developed by the researcher at CTI in MATLAB software (Figure 2.8). First, in the setting sections, the specified algorithm must be selected and uploaded using 'Algorithm File' and 'Open' buttons (Figure 2.9). Then, the recorded data for each cow/phase should be selected and loaded using 'Select File(s)' and 'Load the File' buttons. In the next step, the date, hour and minutes should be set to determine the period of each phase. For instance, in the Length box, 60 × 24 × 5 is inserted to generate data in 5 days in the specific phase. In the ‘Select an Algorithm’ box, the category (grazing and rumination) and subcategory (number of bites, bite intensity and boluses) should be selected. If ‘time budget’ and ‘result table’ are ticked, data will be categorised hourly in the CSV format for analysis of the required information.

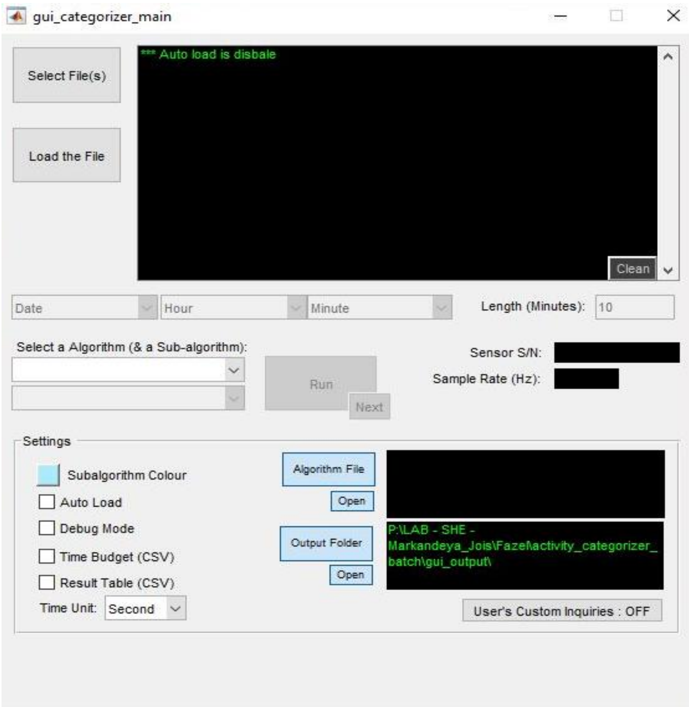


Figure 2.11: GUI-categoriser software developed by CTI in MATLAB

	A	B	C	D	E
1		Parameters	Main_Parameters	Sub_Parameters_1	Sub_Parameters_2
2	Amplitude	amp_axis1_min	0.06		
3		amp_axis1_max	0.3	0.05	
4		amp_axis2_min	0.06		
5		amp_axis2_max	0.4		
6		amp_axis3_min	0.05		
7		amp_axis3_max			
8	Level is mean	lvl_axis1_min			
9		lvl_axis1_max	-0.38		
10		lvl_axis2_min			
11		lvl_axis2_max			
12		lvl_axis3_min			
13		lvl_axis3_max			
14	Percentile range	prctl_axis1_min	5	5	
15		prctl_axis1_max	95	95	
16		prctl_axis2_min	2		
17		prctl_axis2_max	98		
18		prctl_axis3_min	2		
19		prctl_axis3_max	98		
20	Misc	window_size	30	0.5	
21		plot_title	Rumination Time Detection Re	Rumination Bolus Detection Result	
22		plot_legend	Rumination Time	Rumination Bolus	
23		plot_time_unit	Minute(s)	Bolus(es)	
24		plot_mark_stye	--- Not Used ---	om	
25	Amplitude HP and AC	plot_raw_enable	TRUE	FALSE	
26		plot_raw_figure_number	104	104	
27		plot_raw_range_min	-4	-0.2	
28		plot_raw_range_max	4	0.2	
29	Level LP and DC	plot_ac_enable	TRUE	FALSE	
30		plot_ac_figure_number	105	105	
31		plot_ac_range_min	-4	-0.2	
32		plot_ac_range_max	4	0.2	

AlgorithmList | Idle | Walking | Grazing_P1_P2 | Grazing_P3_P4 | RuminBolus_P1_P2 | RuminBolus_P3_P4

Figure 2.12: Implemented algorithm based on threshold of gravity of 3-axis sensor for rumination behaviour

The algorithms for associating the recorded data by sensors to different behaviours were configured after analysing several videos taken at the same time-window and validating the predicted behaviour plots. For example, in Figure 2.10, amplitude is the distance between the peak and bottom of the wave; for example, for rumination behaviour axis one (green colour), the acceleration must be in the range of 0.06 to 0.3. The level for each axis is the average centre of each window plot. Further, in this study the time-window size was 30 sec. However, in the presence of a spike in the window, if the spike was less than 5% (in axis 1) different than the acceptable range (0.06 to 0.3), the window was classified as rumination behaviour. There might be some interventions during the activities of an animal (e.g., rumination), and to address this, in the classification of behaviours, some short pauses were allowed in each time-window. HPF was calculated by subtracting Max and Min of amplitude in each window size, whereas LPF was the average of the base in that window, which was an indication of the level of each signal in that window. Further, some of the behavioural categories had subcategories (e.g., bite counts, and bite intensity were the subcategories of grazing).

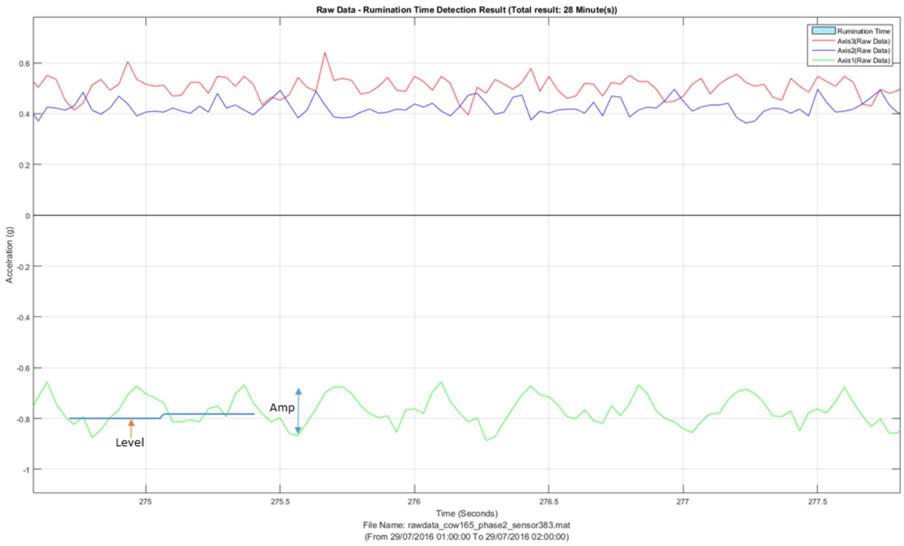


Figure 2.13: Description of amplitude (Kelln et al., 2011) and level for better understanding of algorithms

2.6 Proximity

Several sensors were placed at different locations at the farm to measure the number of visits of each animal to water trough and salt lick sites. The attached sensors to the animals were initialised as beacons (transmitters) and the sensors mounted at water trough, salt lick and straw sites were initialised as receivers. The ID of each transmitter was sent once per minute and the receivers were searching for beacons 4 times per second. The proximity feature of sensors was expected by the manufacturer to be able to record proximity events in a radius of 8–10 m, but in farm conditions, this was observed to be around 2–4 m owing to the different position of sensors attached on each animal. Using ActiLife software, the data were downloaded and transferred via cable to computer for further analysis. The number of visits of animals to water trough and salt lick sites in each phase was divided by the number of days to calculate the average visit per day.

2.7 Validation

Data were validated based on the videos captured during the trial. The accuracy of video validation was 79.2% for rumination behaviour and 64.6% for grazing. Rumination and grazing behaviour were also validated according to the generated patterns, which was 98.5% within 230 random samples in all three phases with a length of 10–20 min for each sample. In the first phase, 45 rumination and 45 grazing samples were validated; however, in phases 2 and 3, for rumination, 35 and 40 samples and for grazing, 35 and 30 samples, respectively, were validated.

The reason for the lower accuracy while validating data based on the captured videos was the short length of video clips. The algorithm was coded to scan the pattern more for a minimum of 2 min, and thus, when it was scanning for less than 2 min, it was generated with less accuracy.

2.8 Plasma Nitrate Concentration

Nitrate concentration in plasma was measured via the colorimetric Nitrite/Nitrate Assay Kit, (Sigma-Aldrich) at Laboratory 320, Health Science 2, Bundoora, La Trobe University. Due to financial limitations, we were able to determine nitrate concentration in plasma for 11 animals in each pasture. Therefore, 11 animals from each pasture were randomly selected to measure nitrate concentration in plasma.

Plasma samples (20 µL) were centrifuged (Sigma 3-16KL) in an Amicon Ultra 0.5 mL Centrifugal Filter Unit with Ultracel 3k at 7000 × g, 4 ˚C for 20 min using Sigma Nr.12154-H rotator for deproteinisation. After centrifugation, 10 µL of deproteinised plasma were transferred into a 96-well flat-bottom plate. 10 µL Nitrate Reductase solution followed by 10 µL of Enzyme Co-factors solution were added to the plasma samples. The plate was shaken smoothly and incubated at 25˚C for 2 h. Griess Reagent A (50 µL) and Griess Reagent B (50 µL) were added to the well plate following 5 min and 10 min incubation at 25 ˚C afterwards, respectively. To make standards for colorimetric detection of nitrate, 0, 20, 40 and 80 µL of nitrate solution were added to the plate to generate 0 (control), 2, 4 and 8 nmole/well standards. Then, the well plate was read through Thermo Scientific Multiskan Go at 540 nm.

To determine the concentration of nitrate in plasma, all readings were corrected by subtracting the control value from other standard reading to plot the standard curve. After that, based on the standard curve, the concentration of nitrate in plasma was determined (see Figure 2.11).

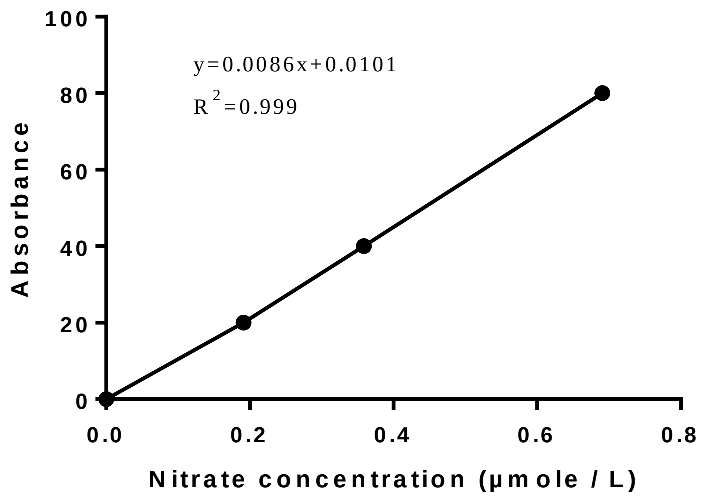


Figure 2.14: Nitrate concentration in plasma

2.9 Statistical Analysis

Descriptive analysis was performed to confirm that each set of extracted data followed a normal distribution. To check that data were normally distributed in this study, the Shapiro–Wilk test was performed in IBM SPSS (IBM Corp., Armonk, NY). To compare the average of phases, one-way analysis of variance (ANOVA; Hejcmanová et al., 2009) was performed. The Tukey post hoc test was used to compare the significance differences between averages in different phases. In one-way ANOVA, the pastures were considered the main factor (treatments) and each behaviour was considered an observation (observed response). Moreover, to assess the strength and direction of the relationship between each pair of variables, a linear regression line was fitted and R^2 (coefficient of determination) was calculated to estimate how close the data points were to the fitted line.

Chapter 3: Results

3.1 Nutritive value of the pasture

The nutritive value, including nitrate concentrations of the three pastures before and after grazing are presented in table 3.1. The CP and ME MJ/kg DM contents were higher for barley than for wheat and mixed pasture. However, the DM content of the barley crop was lower than in the mixed pasture and wheat crop. The NDF content of the three pasture types ranged between 40 and 44.5% DM, with barley having the highest NDF content. Nitrate concentration was highest in the barley and lowest in the mixed pasture.

Table 3.1 Chemical composition and characteristics of pastures before and end of grazing

Parameter	Mixed pasture		Wheat crop		Barley crop	
	PG	PSG	PG	PSG	PG	PSG
Dry matter (%)	16.9	19.3	14.9	13.7	11.2	10.1
Digestibility (% DM)	78.1	73.3	77.8	73.4	83.6	71.2
Crude protein (% DM)	28.6	23.9	30.7	28.6	35.9	29
Neutral detergent fibre (% DM)	37.2	46.1	37.3	41.6	40.1	49
Acid detergent fibre (% DM)	21.8	23.2	21.9	25.2	19.7	21.2
Metabolisable energy (MJ / kg DM)	11.8	11	11.8	11	12.8	10.6
Ash (% DM)	13.5	14.6	12.5	15.2	12.2	13.1
Nitrate (mg/kg DM)	97	81	3900	1300	8100	8700

PG: pre-grazing; PSG: post grazing

As shown in table 3.2, the FOO of the wheat crop was approximately triple that of the mixed pasture and twofold higher than the barley crop; however, the decrease in FOO following grazing was higher in the barley crop (1024 kg/ha) than in the wheat crop (734 kg/ha). Owing to differences in paddock sizes and the consistent size of the herd of cattle, the stocking rate was lowest for wheat and highest for the barley crop. Barley crop had the highest stocking rate at 92.3 DSE/ha (Table 3.2).

Table 3.2: FOO, estimated pasture height, and Stocking rate in three pasture

Parameter	Mixed pasture		Wheat pasture		Barley pasture	
	PG	PSG	PG	PSG	PG	PSG
FOO (kg DM/ha)	851	667	3162	2428	2016	992
Estimated pasture height (cm)	2.8	2.1	48.6	37.3	31.0	15.3
Stocking rate (DSE)	46.2		27.9		92.3	

DSE: Dry Sheep Equivalent, a standard unit used for comparing feed requirement to assess a paddock. DSE rate for one cow is about 8. DSE = (Total animals grazing × DSE rating)/paddock size. PG: pre-grazing; PSG: post grazing

The data in Tables 3.1 and 3.2 were provided by Mr Simon Falkiner to the author.

3.2 Classification of Behaviours

The behaviours were classified using the software developed in MATLAB environment. For example, after selecting and running data on cow 49 in phase 1 on 22/07/2016, the rumination time within 30 minutes was predicted in blue colour for 24 h (Figure 3.1).

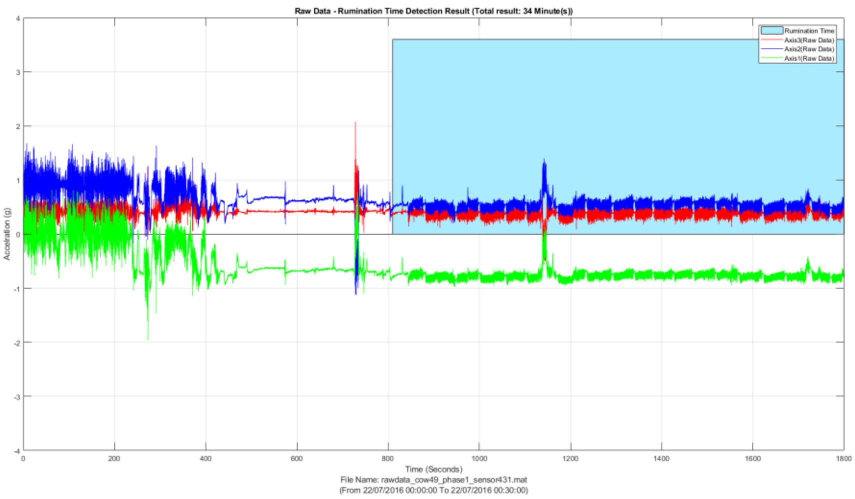


Figure 3.1: Predicted rumination time indicated in blue colour on 22/07/2016 between 00:00:00 to 00:30:00

In addition, by ticking the time budget and result table in the GUI-categoriser software (Figure 2.8), the result was generated for that specific animal and date. For instance, the rumination time was predicted for cow 49 for the whole day, 22/07/2016 (Figures 3.2 and 3.3). After extracting the data on each cow for each date and phase, all data were sorted and aggregated in an appropriate format for further analysis.

A	B	C	D
Filename:	rawdata_cow49_phase1_sensor431.mat		
Window Size (s):	30		
Algorithm:	RuminBolus_P1_P2		
Result Time Unit:	Second		
!NOTE:			
--			
Start Date and Time	End Date and Time	RuminationDuration	
22/07/2016 0:13	22/07/2016 0:41	1650	
22/07/2016 0:49	22/07/2016 0:51	150	
22/07/2016 1:23	22/07/2016 1:28	300	
22/07/2016 3:12	22/07/2016 3:44	1920	
22/07/2016 4:25	22/07/2016 4:36	690	
22/07/2016 5:12	22/07/2016 5:45	1950	
22/07/2016 6:04	22/07/2016 6:54	3000	
22/07/2016 7:30	22/07/2016 7:58	1680	
22/07/2016 9:44	22/07/2016 10:15	1860	
22/07/2016 10:56	22/07/2016 11:03	390	
22/07/2016 12:34	22/07/2016 12:44	630	
22/07/2016 15:35	22/07/2016 15:56	1290	
22/07/2016 18:22	22/07/2016 18:45	1410	
22/07/2016 19:21	22/07/2016 19:34	810	
22/07/2016 20:00	22/07/2016 20:42	2520	
22/07/2016 22:26	22/07/2016 22:32	330	
22/07/2016 22:35	22/07/2016 22:38	150	
22/07/2016 22:49	22/07/2016 23:22	1950	
22/07/2016 23:54	23/07/2016 0:00	360	
	Total:	23040	

Figure 3.2: Rumination output details for cow 49 in phase 1 on 22/07/2016 from 00:00:00 to 23:59:59 in CSV format

A	B
Filename:	rawdata_cow49_phase1_sensor431.mat
Window Size (s):	30
Sub-Window Size (s):	NaN
Algorithm:	RuminBolus_P1_P2
Unit:	Second
User Note:	Rumination minutes per day per hour
!NOTE:	
--	
Time/Date	22-Jul
0:00	1800
1:00	300
2:00	0
3:00	1920
4:00	690
5:00	1950
6:00	3000
7:00	1680
8:00	0
9:00	960
10:00	1110
11:00	180
12:00	630
13:00	0
14:00	0
15:00	1290
16:00	0
17:00	0
18:00	1410
19:00	810
20:00	2520
21:00	0
22:00	1110
23:00	1680
Total:	23040

Figure 3.3: Rumination output hourly for cow 49 in phase 1 on 22/07/2016 from 00:00:00 to 23:59:59 in CSV format

3.2.1 Bite intensity

Bite intensity was a behavioural trait that was classified from sensor data for the first time in this study. Bite intensity was a subcategory of grazing behaviour, and hence, it was predicted at the same time the bite counts were calculated. In Figure 3.4, the area of each bite was calculated by multiplying its height and width. Hence, for a larger area of each bite, the animal spent more force than for a smaller area. The unit of vertical axes of the figure is gravity defined as 9.8 m/s^2 in the metric system. Further study is required to confirm the validity and reliability of predicting bite intensity from sensor data using the method described in this thesis.

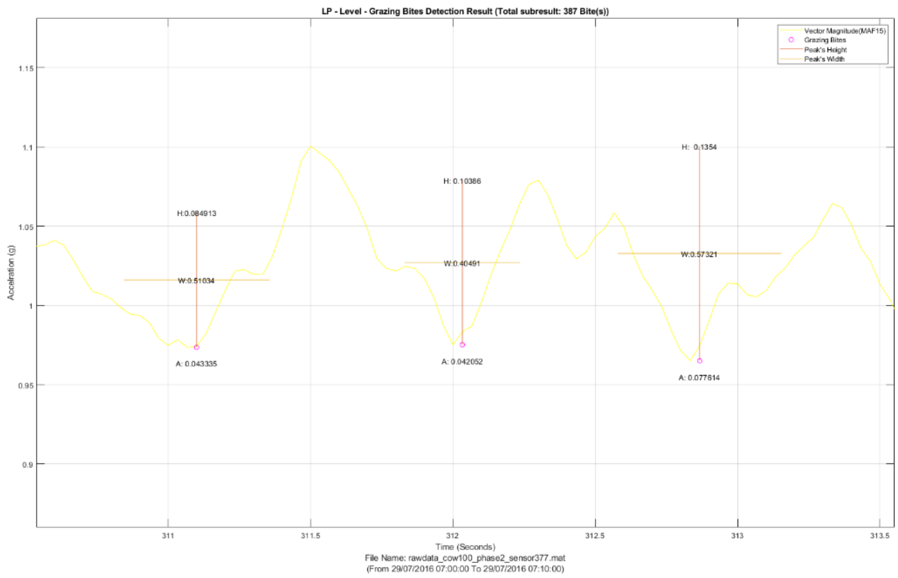


Figure 3.4: Measurement of bite intensity

3.3 Weight gain of heifers

The average weight gain of heifers is indicated in figure 3.5. Heifers did not lose weight when they were grazing on pastures. Due to the short period of adaptation on each pasture, the weight of heifers is not the reflection of that pasture.

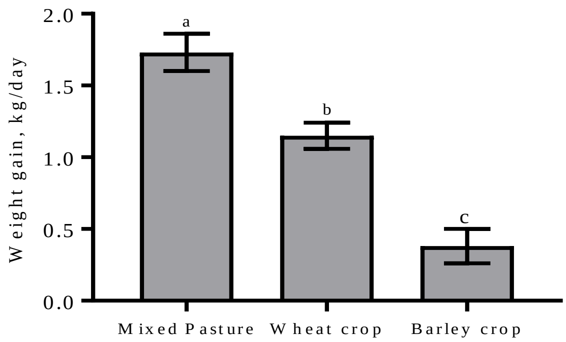


Figure 3.5: The average weight of heifers in mixed pasture, wheat crop and barley crop; the bars with different superscripts differ significantly (p < 0.05)**Feeding Behaviour and Rumination Patterns**

The data for all behaviours were normally distributed, except rumination. Therefore, a nonparametric test was used to compare rumination time in different phases.

As shown in table 3.3, when the heifers were moved to the wheat crop, they spent significantly less time grazing ($p < 0.05$) compared to when they were grazing mixed pasture or barley crop. More time was spent grazing when on the mixed pasture ($p<0.05$) compared to either of the cereal crops. Rumination times did not differ ($p > 0.05$) when the heifers grazed mixed pasture or wheat crop but was significantly lower ($p < 0.05$) when they were grazing on the barley crop. The number of boluses was also significantly lower ($p<0.05$) when they were grazing the barley crop compared to the mixed pasture and wheat crop. When grazing the wheat crop, the number of bites was significantly ($p<0.05$) less than when grazing the mixed pasture and barley crop. When grazing on the barley crop, bite intensity was significantly ($p<0.05$) lower than when grazing the wheat crop (phase 2) or mixed pasture (phase 1).

Table 3.3: Feeding behaviour and rumination duration (average ± SD)

Behaviour	Mixed pasture	Wheat crop	Barley crop	SEM (±)
Grazing (min/d)	512 ± 53.5[a]	231 ± 116.7[c]	476 ± 93.5[b]	16.2
Rumination (min/d)	357 ± 40.4[a]	343 ± 82.5[a]	192 ± 54.3[b]	10.9
Bolus (number/d)	481 ± 71.5[a]	437 ± 117.4[b]	272 ± 79.5[c]	16.1
Bite duration (sec/d)	503 ± 100[a]	202 ± 115[b]	460 ± 106[a]	18.8
Bite (number/d)	20146 ± 5767[a]	6847 ± 4489[b]	21040 ± 5832[a]	954
Bite intensity (g)*	0.38 ± 0.02[a]	0.22 ± 0.01[b]	0.15 ± 0.006[c]	0.02

[a,b,c] The values with different superscripts differ significantly (p < 0.05); SEM: standard error from the mean
*gravity (gravity = 9.8 m/s^2)

Most of the grazing time occurred in daylight (07:00 to 18:00) on all pasture types. The grazing time on wheat was the lowest (231 ± 116.7 min/day) with the highest proportion of grazing time in daylight (90%). The daytime grazing on mixed pasture and barley crop was 72 and 76% of total grazing time (Figure 3.6a). The rumination normally occurred in dark hours (from 19:00 to 06:00); 75%, 70% and 68% of rumination on barley, wheat and mixed pasture was in dark hours, respectively (Figure 3.6b).

The peak hours of grazing for the mixed pasture were 17:00 and 18:00 (approximately 50 min/h), whereas for the barley crop, the peak hours were at 07:00, 09:00, 15:00 and 17:00 (approximately 30 min/h). The heifers spent less time grazing on the wheat crops, and the peak hour was at 07:00 (approximately 30 min/h; Figure 3.6a).

The rumination peak times for the mixed pasture were prior to start of grazing at 04:00, 05:00, 06:00 and 07:00 and immediately after peak grazing time at hours 19:00, 20:00 and 21:00. The duration of rumination in peak times for mixed pasture was approximately 25 min/h, compared to approximately 15 min/h for the barley crops. The rumination peak times for wheat were at hours 04:00, 05:00, 06:00, 21:00 and 22:00. For all three pasture types, the rumination time was less than 3 min/h at hours 17:00 and 18:00 (Figure 3.6a, b).

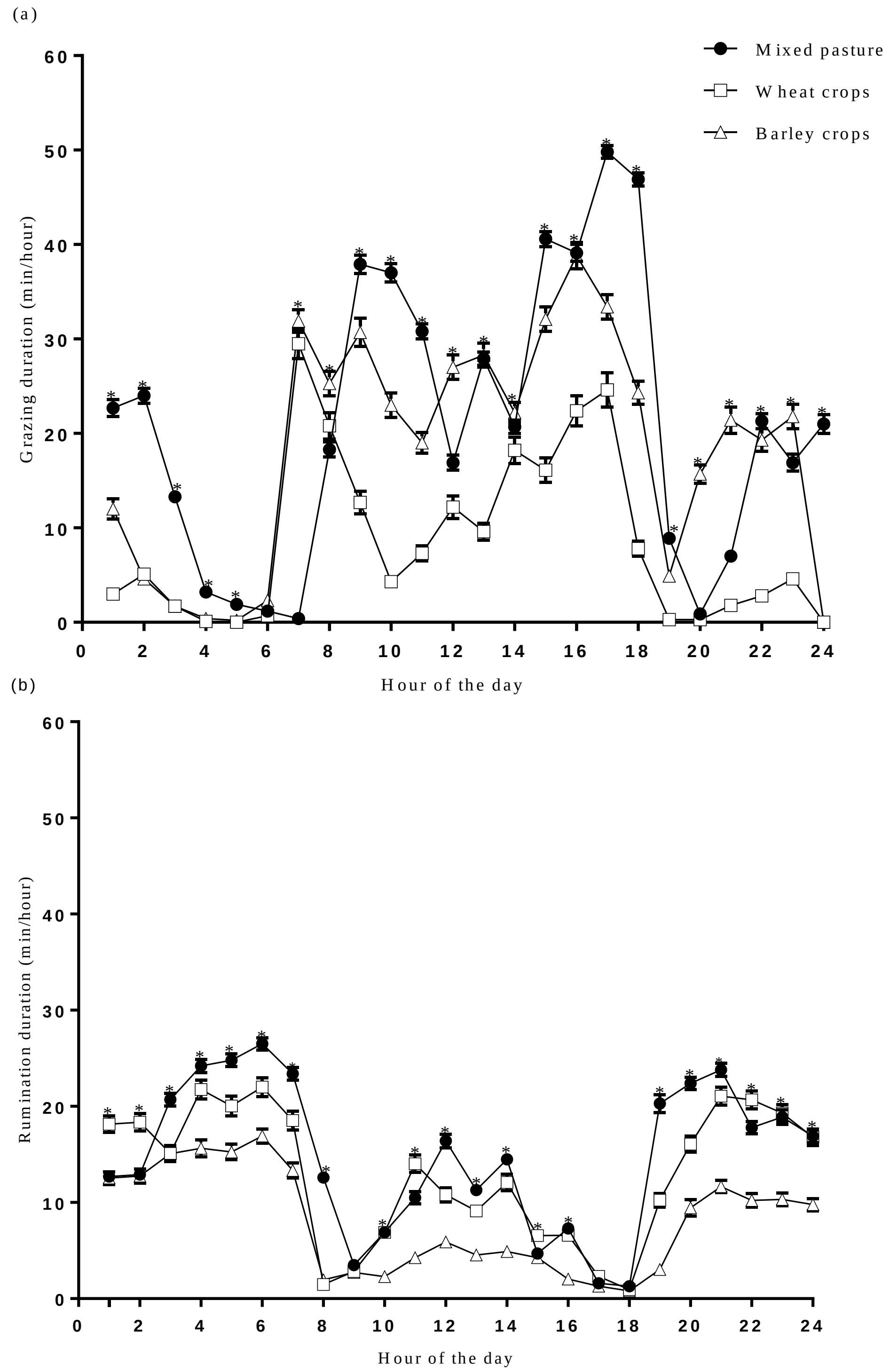


Figure 3.6: Average grazing (a) and rumination (b) time for 24 hours a day. For some points, the error bar height is less than the symbol height. In these cases, the error bars are not drawn. Hours indicated by * are different at $p < 0.05$ in different pastures.

Average daily grazing and rumination behaviours in different pastures are shown in Figure 3.7. When grazing the mixed pasture, except for day 2, the rumination time increased with an increase in the grazing time. However, there was a weak correlation between grazing and rumination ($R^2 =$ 0.03). During the last 5 d when grazing on the wheat crop, the grazing time declined gradually to

189 min/d, but the rumination time remained steady. When grazing the barley crop, the initial grazing time of 410 min/d decreased to 395 min/day on day 2, but then, with an increasing trend, reached 486 min/d on the last day of grazing. However, the rumination time initially dropped to 148 min/d on day 3 and then markedly increased to 256 min/d on day 5.

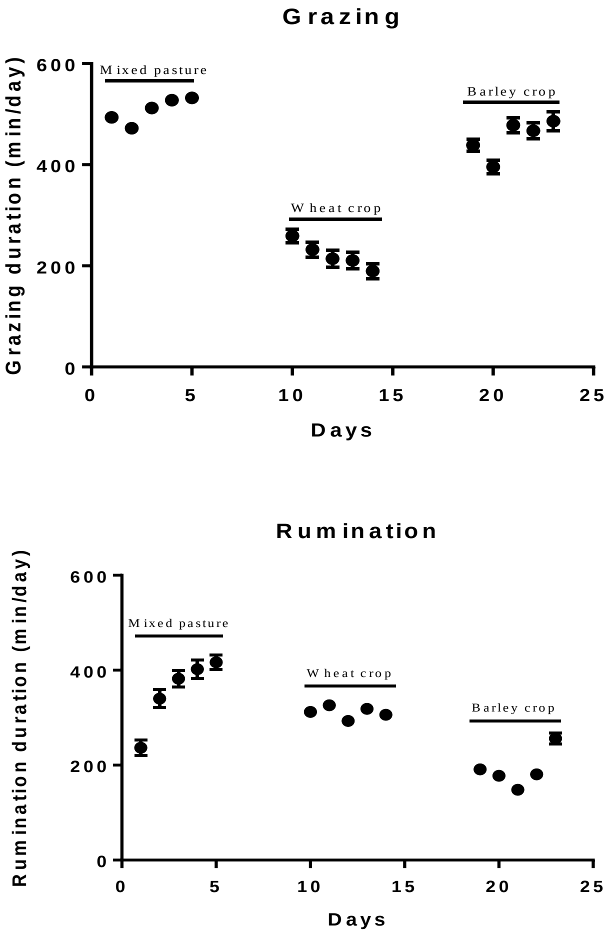


Figure 3.7: Average daily time of grazing and rumination behaviours in different pastures. For some points, the error bar height is less than the symbol height. In these cases, the error bars are not drawn.

3.5 Visits to Water Trough, Salt Lick and Straw

The animals visited drinking water sites on the barley crop more frequently than on the other pastures ($p < 0.05$). In addition, on the barley crop, they approached water troughs (2.2 ± 1.7) slightly more than when they were on the mixed pasture (1.2 ± 1.5; Figure 3.9a). Interestingly, the number of visits to salt licks on the barley crops escalated and was approximately threefold that of the wheat crops (Figure 3.9b). Heifers visited straw 15 times per day on the barley crops, whereas they approached it approximately 3 times per day on the wheat crops (Figure 3.9c).

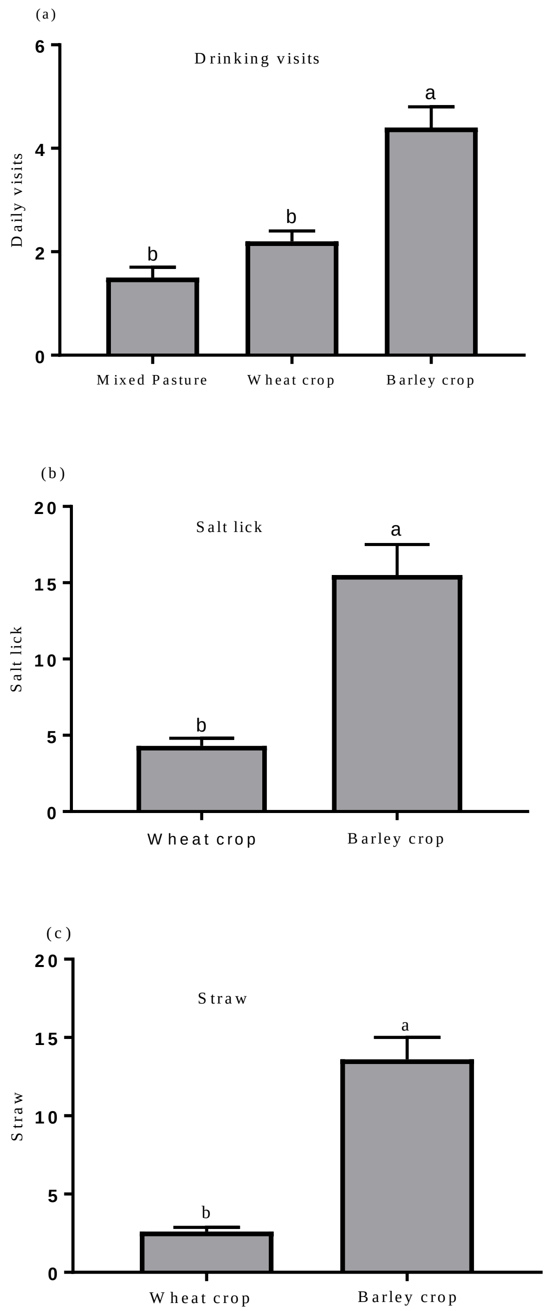


Figure 3.9: Daily access to water, salt lick and straw; bars with different superscripts differ significantly ($p < 0.05$)

3.6 Plasma Nitrate Concentration

There were no significant ($p > 0.05$) differences in plasma nitrate concentration when the heifers were grazing the mixed pastures or wheat crop, but they increased significantly ($p<0.05$) when they moved to graze on the barley crop (Figure 3.10), which contained 8.4 g nitrate/kg DM (Table 3.1). Also, the correlation between plasma nitrate concentration and grazing behaviour was examined ($r = 0.02$).

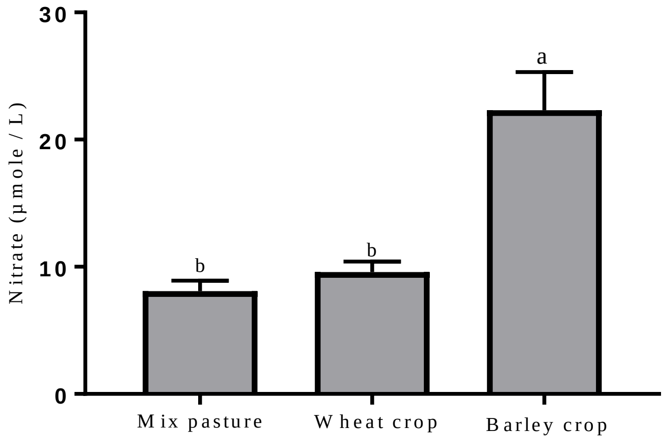


Figure 3.10: Nitrate concentration (µmole / L) in plasma; the values with different superscripts are significantly different ($p < 0.05$)

Chapter 4: Discussion

4.1 Association of Sward Height and Availability of Food with Grazing Time and Bites (Rate, Duration and Intensity)

The main aim of this thesis was to study the grazing behaviour of heifers grazing on cereal crops (wheat and barley) compared to mixed pasture. There are limited studies have evaluated the behaviour of large number of beef cattle on cereal crops. Further, these studies have mainly focused on wheat crops since it is the major dual-purpose crop sown and only a limited number of studies have evaluated livestock behaviour on barley crops as an alternative to fill the winter gap.

Most of the research for evaluating the performance of ruminants on dual-purpose crops reported no negative effect of dual-purpose crops on weight gain. In the present study, animals did not lose weight as a consequence of feeding on different pastures for short term. However, due to the short time of adaptation on each pasture, ADG was invalid in this research.

Sward surface height strongly influences bite rate and bite mass. Fonseca et al. (2013) showed that the bite mass and rate were, on average, constant for sward height between 20 cm to 50 cm but declined when the sward height exceeded 50 cm. Sward height and FOO are the major factors constraining the intake of animals grazing on the pasture. Sward height and FOO were substantially higher for the wheat and barley crops compared to the mixed pasture, which could potentially change grazing behaviours. Cazcarra, Petit and D’Hour (1995) evaluated grazing time and herbage intake of heifers for three different levels of sward height: short (7.5 cm), medium (10.2 cm) and tall (21.1 cm). They concluded that the bite rate decreased when the sward height increased. In addition, grazing time decreased while animals grazed on tall sward height. These findings are consistent with those of the present study, which showed that increased sward height and herbage mass reduced the grazing time and bite rate in the case of the wheat crop.

However, owing to reduced herbage mass of the barley compared with that of the wheat crop, the animals increased their grazing time to eat enough feed for their daily energy requirements. Cazcarra et al. (1995) showed that by increasing sward height, the harvested organic matter per bite linearly increased ($R^2 = 0.78$). In their study, when the sward height was 18 cm the ingested organic matter per bite was approximately 400 mg, even though this amount for 7 cm sward height was approximately 200 mg. In the current study, animals could ingest more feed in each bite while they grazed on tall sward heights (wheat and barley crops); therefore, as expected, they spent less time grazing on cereal crops.

When heifers were moved from wheat to barley crops, the grazing time escalated to 476 min/day, although barley crops provided in large herbage mass and high-quality composition. In a review study, it was concluded that using dual-purpose crops for grazing cattle requires greater attention to mineral imbalance in feed (Dove, 2018) because it can have a negative effect on liveweight gain. As cereal crops are low in sodium and high in potassium, providing supplementary sodium in the ration is highly recommended. This mineral imbalance also interferes with magnesium absorption which may lead to rumen dysfunction. In the current study, the heifers approached to salt lick on barley crops almost threefold more than wheat crops. This data suggests that perhaps due to the mineral imbalance on cereal crops heifers approached to salt lick more frequently. However, the heifers approached to salt lick less on wheat crops and had higher gain weight on wheat crops than barley crops. Therefore, by approaching to salt lick more frequently, they compensated to recover mineral imbalance. Most of studies focused on wheat crops as it is the major sown dual-purpose crops and only limited studies evaluated livestock performance on barley crops as an alternative to fill the gap in winter.

To the author's knowledge, the direct effect of height of forages on rumination time in the pasture-based system is yet to be determined. Nevertheless, it is assumed that lower bite intensity may be related to eating forage of larger particle size. Thus, the least rumination time, which occurred in barley pasture, could be associated with lower bite intensity. This study is the first to measure bite intensity using sensor data. Therefore, more experiments are required to validate the predicted bite intensity using sensor data.

4.2 Effects of Rumination and Nitrate

Although sensors were previously used to monitor cows' behaviour during calving (Clark et al., 2014) and to predict the health status of cattle (Marchesini et al., 2018) in a wide variety of production systems, such as TMR or pasture-based feeding, to the author's knowledge, very few studies have focused on the feeding behaviour of cattle grazing on cereal crops as against mixed pasture. In this study, the heifers grazing the barley crop decreased in their daily rumination duration to an average of 192 min/day. Marchesini et al. (2018) attached SCR collars to young Charolais bulls to measure their activity and rumination over 70 days. They found a significant relationship between daily rumination time and ADG. Bulls with minimum 157 min daily rumination had low daily weight gain, whereas the bulls with average 412 min daily rumination had high daily weight gain. They also reported that the bulls with lower daily gain had a greater variation in rumination behaviour during the experiment. Although the current study was carried out in a short time, still the rumination patterns can be used as an indicator of health of animals. The significant changes in rumination time occurs 3-6 days (Schaefer et al., 2007; Marchesini et al., 2018) prior the onset of clinical signs. Although the low rumination time can alert the farmer, the pattern of rumination during the day is important. For example, how rumination is distributed over the time. In the current study heifers had a very low rumination (less than 5 min in each hour) from hours 08:00 to 19:00. This may indicate the dysfunction of rumen and the onset of disease. However, it is necessary to compare rumination parameters over time with healthy animal (Marchesini et al., 2018). It should be noted that many complex interactions between feed and animals in the pasture-based system might influence the rumination. For instance, the length and NDF of forage are the main factors that determine rumination time (Zebeli et al., 2012).

In this study, the heifers had lower rumination time but higher grazing time in barley pasture than in wheat pasture. This result is perhaps because of availability of more food and considerably higher sward height in wheat pasture than in mixed and barley pasture. Therefore, the heifers spent less time on grazing to eat enough feed to match their requirements in wheat pasture. Moreover, DM is a major factor that influences rumination time (Beauchemin, 2018). Rumination time for the barley crop (10.6% DM) was substantially lower than for mixed pasture (18.1% DM) and wheat (13.8% DM). Marchesini et al. (2018) showed that bulls with rumination time under 180 min/d, even temporarily, were categorised in the low daily gain group. In line with their results, the heifers grazing on lush barley with average 192 min rumination/day can be grouped into the low daily gain group. Mason and Stuckey (2016) described that by rumination, a new phase of high bacterial growth is introduced to the rumen, which can balance the microbial growth in biofilm, and thus, biomass production shifts to volatile fatty acid production. Less rumination can negatively affect weight gain.

Heifers had access to the straw freely during the trial. They approached the straw more frequently in barley pasture, and hence, it can be assumed that this was a compensatory behaviour to take in more NDF content for optimising rumen function. However, the average rumination time was decreased in barley pasture although animals ate more straw. This means that consumption of more NDF did not result in more rumination time, considering DM was lower in barley pasture compared to other pastures. There are complex interaction of factors that influence rumination time, therefore the relationship between a dietary factor and rumination time could be relatively low. For example, NDF intake, particle size, hardness of feed and indigestibility of the fibre can highly affect the rumination time (Yang and Beauchemin, 2009; Nørgaard et al., 2011; Beauchemin, 2018).

In barley pasture, the level of feed nitrate largely increased to 8,300 mg/kg DM, which could affect the rumen microorganism population. Some studies reported reductions in DMI and ADG when dietary nitrate was fed to cattle (Hegarty et al., 2016a; Newbold et al., 2014). The reason that NO_3^- can change the feeding behaviour of ruminants remains unclear, but it was reported that it can supress NDF digestion in rumen. *In vitro* analysis showed that nitrite reduced the digestion of cellulose and xylanase by reducing cellulolytic, xylanolytic and total microbial population in rumen (Marais, Therion, Mackie, Kistner, & Dennison, 1988). Hence, the high nitrate level in barley pasture could negatively affect fibre digestion. By reducing cellulolytic activity in the rumen, fewer bacteria attach to the cud for further degradation; consequently, it might affect rumen fermentation characteristics, especially the profile of volatile fatty acids. Lee et al. (2015) showed that dietary nitrate (1.09% NO_3^- in DM in 10 days adaptation) reduced the acetate level and increased the butyrate level. In addition, they concluded that nitrate reduced feed intake and feed consumption rate. Their results also explain the finding in the present study that the reduced rumination time in barley pasture links to the high level of nitrate.

Hegarty et al. (2016b) reported that the cattle that were fed nitrate supplements spent more time on eating because they consumed their feed slowly (g DM ingested/min). It should be noted that ruminants, generally, can adapt to dietary nitrate by improving rumen capacity (Zhou et al., 2012). It was found that after an 18-day acclimation period, 3.04% NO_3^- of dietary DM did not affect feed intake and weight gain in sheep (Nolan et al., 2010). However, Hulshof et al. (2012) reported that nitrate at 2.2% of dietary DM (16 adaptation days) slightly reduced feed intake in beef cattle by 6% in comparison with the control group. Li et al. (2012) compared 1.5% urea and 3% calcium nitrate fed to 10 sheep in 28 days with 7 days adaptation period to the new diet. The sheep fed nitrate gained 247 g/day, but the ones fed urea gained 306 g/day. A comprehensive review by Lee and Beauchemin (2014) concluded that the potential toxicity of nitrate to ruminants can be reduced through an acclimation period. In this study, there were only 4 days of adaptation before starting the measurement of animals' behaviour since the study was performed at a commercial farm and the time for performing the experiment was limited. Therefore, the rapid change of diets could negatively affect the rumen microorganisms and consequently changes in feeding behaviour.

In this study, the range of daily rumination time when grazing mixed pasture was 273 min/d to 417 min/day, when grazing the wheat crop, it was 200 min/day to 432 min/d and when grazing the barley crop, it was 83 min/d to 311 min/day, which showed the high variability in rumination time between animals. Byskov et al., (2015) reported that 32% of variation in rumination time could be explained by variation in intakes of dietary fraction. Individual variation between cows were only accounted for 48% of the total variation in rumination time. Rumination time is highly influenced by the composition of feed, and in particular, NDF. The NDF content of the barley was higher than the mixed pasture and the wheat crop but when grazing the barley, the cows had the lowest rumination time. The possible explanation could be the elevated amount of nitrate in barley or increased passage rate. Oosting and Waanders (1993) reported that passage rate in goats significantly increased ammonia infusion. In the rumen, nitrate reduces to nitrite and ammonia. Therefore, the high amount of ammonia in the rumen could change the passage rate and consequently decrease the rumination time. Further, the feed passed through the rumen quickly before complete digestion because microorganisms did not have enough time to break down structural polysaccharides. Marais et al. (1988) found that dietary nitrate caused depression in NDF digestion, which may reduce the rumination time. By contrast, nitric oxide is produced by nitrate in the rumen, which might stimulate feed intake (Morley & Flood, 1991). This can be more complicated in the rumen environment. In addition, Hegarty et al. (2016a) pointed out that the different conditions in each trial may affect rumen fermentation as also the response of animals across studies.

Availability of nitrate in diet of ruminants may change the colour of fat. Hegarty et al. (2016b) showed that fat colour became darker in cattle supplemented with nitrate calcium, whereas for the cattle fed with urea, the fat colour was lighter (Hegarty et al., 2016b). Nitrate increases the concentration of cyclic guanosine monophosphate (cGMP) in plasma (Kapil et al., 2010) and cGMP stimulates browning of white adipose tissue (Mitschke et al., 2013). Thermogenesis is activated by developing brown tissue-initiated cellular energy stores to generate heat (Cannon & Nedergaard, 2004). Thus, the energy of cattle was possibly wasted as heat in phase 3. Moreover, the cattle wanted to cool down their body by consuming more water. This could be a possible explanation for why the cattle consumed significantly more water when grazing the barley. Also it should be noted that increased straw intake is associated with increased water intake as it has higher DM content compared to the pastures (Ammer et al., 2018).

This study has some limitations because it was conducted in a commercial farm. The measurement of weight gain was an invalid way of ADG in this study, thus the animals' performance could not be a reflection of pasture types. Also, as the duration of the study was limited, a larger adaptation period, longer assessment of performance in each pasture or other factors for improving the robustness of the results could not be implemented in the design of this experiment. Further research is required with replication for all pasture types and much longer acclimation period to link feeding behaviour to animal performance.

4.3 Conclusion

According to the results of this study: (1) Transition from mixed pasture to cereal crops in a short period led to changing the behaviours of heifers, including increasing their visits to drinking water troughs, salt licks and straw. (2) high level of nitrate can affect Angus heifer behaviour even in a short time of grazing.

Further studies are required to evaluate the performance of cattle on wheat and barley crops and mixed pasture with different levels of nitrate to have a better understanding of the effects of nitrate on cattle feeding and rumination behaviour. Also, a replication for all pasture types and much longer acclimation period to link feeding behaviour and animal performance is required. Moreover, to assess the possible negative effects of converted nitrate to nitrite on carrying oxygen molecule, the amount of methaemoglobin should be also measured during the study.

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